THE USE OF RESPONSE SURFACE METHODOLOGY AND ARTIFICIAL NEURAL NETWORKS FOR THE ESTABLISHMENT OF A DESIGN SPACE FOR A SUSTAINED RELEASE SALBUTAMOL SULPHATE FORMULATION

by

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ABSTRACT

Quality by Design (QbD) is a systematic approach that has been recommended as suitable for the development of quality pharmaceutical products. The QbD approach commences with the definition of a quality target drug profile and predetermined objectives that are then used to direct the formulation development process with an emphasis on understanding the pharmaceutical science and manufacturing principles that apply to a product. The design space is directly linked to the use of QbD for formulation development and is a multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide an assurance of quality. The objective of these studies was to apply the principles of QbD as a framework for the optimisation of a sustained release (SR) formulation of salbutamol sulphate (SBS), and for the establishment of a design space using Response Surface Methodology (RSM) and Artificial Neural Networks (ANN).

SBS is a short-acting β_2 agonist that is used for the management of asthma and chronic obstructive pulmonary disease (COPD). The use of a SR formulation of SBS may provide clinical benefits in the management of these respiratory disorders. Ashtalin®8 ER (Cipla Ltd., Mumbai, Maharashtra, India) was selected as a reference formulation for use in these studies.

An Ishikawa or Cause and Effect diagram was used to determine the impact of formulation and process factors that have the potential to affect product quality. Key areas of concern that must be monitored include the raw materials, the manufacturing equipment and processes, and the analytical and assessment methods employed. The conditions in the laboratory and manufacturing processes were carefully monitored and recorded for any deviation from protocol, and equipment for assessment of dosage form performance, including dissolution equipment, balances and hardness testers, underwent regular maintenance.

Preliminary studies to assess the potential utility of Methocel® K100M, alone and in combination with other matrix forming polymers, revealed that the combination of this polymer with xanthan gum and Carbopol® has the potential to modulate the release of SBS at a specific rate, for a period of 12 hr.

A central composite design using Methocel® K100M, xanthan gum, Carbopol® 974P and Surelease® as the granulating fluid was constructed to fully evaluate the impact of these formulation variables on the rate and extent of SBS release from manufactured formulations. The results revealed that although Methocel® K100M and xanthan gum had the greatest retardant effect on drug release, interactions between the polymers used in the study were also important determinants of the measureable responses.

An ANN model was trained for optimisation using the data generated from a central composite study. The efficiency of the network was optimised by assessing the impact of the number of nodes in the hidden layer using a three layer Multi Layer Perceptron (MLP). The results revealed that a network with nine nodes in the hidden layer had the best predictive ability, suitable for application to formulation optimisation studies.

Pharmaceutical optimisation was conducted using both the RSM and the trained ANN models. The results from the two optimisation procedures yielded two different formulation compositions that were subjected to *in vitro* dissolution testing using USP Apparatus 3. The results revealed that, although the formulation compositions that were derived from the optimisation procedures were different, both solutions gave reproducible results for which the dissolution profiles were indeed similar to that of the reference formulation.

RSM and ANN were further investigated as possible means of establishing a design space for formulation compositions that would result in dosage forms that have similar *in vitro* release test profiles comparable to the reference product. Constraint plots were used to determine the bounds of the formulation variables that would result in the manufacture of dosage forms with the desired release profile. ANN simulations with hypothetical formulations that were generated within a small region of the experimental domain were investigated as a means of understanding the impact of varying the composition of the formulation on resultant dissolution profiles.

Although both methods were suitable for the establishment of a design space, the use of ANN may be better suited for this purpose because of the manner in which ANN handles data. As more information about the behaviour of a formulation and its processes is generated during the product lifecycle, ANN may be used to evaluate the impact of formulation and process variables on measureable responses.

It is recommended that ANN may be suitable for the optimisation of pharmaceutical formulations and establishment of a design space in line with ICH Pharmaceutical Development [1], Quality Risk Management [2] and Pharmaceutical Quality Systems [3] guidelines.

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STUDY OBJECTIVES

The International Conference on Harmonization (ICH) Pharmaceutical Development guideline (ICH Q8) [1] was originally published by the Food and Drug Administration (FDA) of the United States Department of Health [4] and the European Medicines Agency (EMEA) [5] in May 2006. The guideline provides direction for the pharmaceutical industry to facilitate the registration of new chemical entities in the original application and subsequently during the product lifecycle, placing emphasis on the application of pharmaceutical and manufacturing sciences to form a basis for flexible regulatory approaches [4,5]. Subsequently the guideline has been amended to include an annexure to clarify concepts discussed in the Pharmaceutical Development guideline and how to implement the principles outlined in the documentation [1].

The document recognises that a pharmaceutical product must be of appropriate quality, suitable for its intended purpose and include characteristics such as identity, strength and purity [4,5]. Pharmaceutical quality may also be considered as representing a low risk of failure in achieving the desired clinical outcomes [6]. Therefore all pharmaceutical products must satisfy both physicochemical quality control aspects and be able to elicit a desired biological response, *in vivo*.

The objectives of this study were therefore:

i. To discuss the impact of the newly recommended Pharmaceutical Development guideline on the development of pharmaceutical products,
ii. To develop and validate a suitable and sensitive High Performance Liquid Chromatographic method to accurately and precisely quantitate salbutamol sulphate (SBS) in pharmaceutical dosage forms and SBS release from dosage forms during *in vitro* release testing,
iii. To conduct preliminary formulation studies and risk assessment analysis for development of high quality sustained release (SR) formulations,
iv. To optimise a sustained release formulation of salbutamol sulphate using Response Surface Methodology (RSM),
v. To optimise a SR formulation of SBS using Artificial Neural Networks (ANN),
vi. To establish a design space using both RSM and ANN.

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LIST OF ACRONYMS

ACN	Acetonitrile
ANN	Artificial Neural Networks
ANOVA	Analysis of Variance
API	Active Pharmaceutical Ingredient
ATP	Adenosine Triphosphate
BP	British Pharmacopoeia
cAMP	Cyclic Adenosine Monophosphate
CI	Carr's Index (in context of tablet preparation)
CI	Confidence Level (in context of statistical analysis)
COPD	Chronic Obstructive Pulmonary Disease
CPP	Critical Process Parameters
CQA	Critical Quality Attributes
DSC	Differential Scanning Calorimetry
EMEA	European Medicines Agency
FDA	Food and Drug Administration
FEV_1	Forced Expiratory Volume in 1 second
FMEA	Failure Mode Effects Analysis Hazard Analysis
FTA	Fault Tree Analysis
FVC	Forced Vital Capacity (FVC),
GIT	Gastrointestinal Tract
GM-CSF	Granulocyte-Monocyte Colony-Stimulating Factor
HACCP	Hazard Analysis and Critical Control Points
HLB	Hydrophilic-Hydrophobic Balance
HPC	Hydroxypropyl Cellulose
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxypropyl Methylcellulose
ICH	International Conference on Harmonization
IL	Interleukin
IV	Intravenous
LOD	Limit of Detection
LOQ	Limit of Quantitation
MeOH	Methanol
MLP	Multilayer Perceptron
MSE	Mean Squared Errors
PAT	Process Analytical Technology
PCA	Principal Component Analysis
PEFR	Peak Expiratory Flow Rate
PEG	Polyethylene Glycol
PLS	Partial Least Squares
PRESS	Sum of Squares of the Prediction Residuals
PVP	Polyvinyl Pyrrolidone
QbD	Quality by Design
RMSE	Root Mean Square Errors

RSM	Response Surface Methodology
RSD	Relative Standard Deviation
SB	Salbutamol
SBS	Salbutamol Sulphate
SCMC	Sodium Carboxy Methylcellulose
SPC	Statistical Process Control
SR	Sustained Release
SSE	Sum of Squared Errors
SULT 1A3	Sulphotransferase 1A3
TB	Terbutaline
TBS	Terbutaline Sulphate
TNF-α	Tumour Necrosis Factor-α
USP	United States Pharmacopeia
XRD	X-ray Diffraction

CHAPTER 1
QUALITY BY DESIGN

1.1 QUALITY BY DESIGN

1.1.1 Introduction

Quality by Design (QbD) is a systematic approach that may be used for the development of pharmaceutical products and commences with predefined dosage form performance objectives. The procedure places an emphasis on understanding all aspects of product and process performance, based on sound scientific principles and quality risk management [1,6,7]. Quality products are products that ensure consistent dosage form performance and that are designed to achieve specific therapeutic outcomes with a low risk of failure in patients [6]. The principles of QbD are to ensure the production of quality pharmaceutical products and to allow for continuous product improvement throughout the lifecycle of a product. A schematic depiction of the various components of QbD and the interrelationship of related components is illustrated in Figure 1.1.

Figure 1.1 QbD graphic, adapted and redrawn from [8]

The ideal QbD approach involves a mechanistic understanding of how formulation input attributes and process variables affect the ultimate quality of a drug product [7]. Dosage form specifications and attributes that determine product performance are important considerations

in product design and an understanding of these factors is paramount to success in product development. In addition, manufacturing processes and controls must be specifically optimised to ensure that quality products are consistently manufactured.

Product performance characteristics are often referred to as critical quality attributes (CQA) and are typically those physical, chemical, biological or specific characteristics of a dosage form that should be within an appropriate or specified range or limit to ensure that a product of the desired quality and efficacy is produced [1]. CQA are used to guide product quality during pharmaceutical development and should be identified and discussed during the product development stages of research and development [6]. It is also important to understand and monitor critical process parameters (CPP), which are typically those manufacturing conditions that, if varied, have the potential to affect product quality [1].

A mechanistic understanding of how formulation and processing variables impact CQA will provide information for the development of a robust process that assures the production of pharmaceutical products with desired quality characteristics on every occasion [7]. Multivariate experiments may be conducted to enhance the understanding of interactions between product and process variables on dosage form performance. Information gained from these studies supports the development of an appropriate design space, which is a multidimensional combination and interaction of material attributes and process conditions that have been demonstrated to ensure a quality product is produced consistently [1]. In addition, the establishment of a design space facilitates the development of control and monitoring strategies that assure product quality and enables the management of information throughout the product lifecycle [7].

The objective of these studies was to apply the principles of QbD as a framework for the development of a design space for a sustained release (SR) formulation of salbutamol sulphate (SBS).

1.1.2 Traditional Formulation Approach versus Quality by Design

The view of the Food and Drug Administration (FDA) on the differences between QbD and traditional approaches to formulation development are summarised in Table 1.1.

Table 1.1 Comparison of traditional and QbD approaches to formulation development [9–11]

Aspects	Traditional approach	QbD approach
Pharmaceutical development	▪ Empirical ▪ Optimisation of formulations using a univariate approach	▪ Systematic multivariate experiments conducted ▪ Optimisation of formulations using statistical methods
Manufacturing process	▪ Fixed manufacturing conditions ▪ Validation on three initial full scale batches ▪ Focus is on reproducibility	▪ Manufacturing is adjustable within the design space ▪ Continuous verification within the design space ▪ Focus is on control strategy and robustness
Process control	▪ In process testing for decision making ▪ Offline analysis with slow response	▪ Process analytical technology (PAT) is used for feed-back, feed-forward and real-time testing of products
Product specification	▪ Primary means of quality control ▪ Based on batch data	▪ Part of overall quality control strategy ▪ Based on desired product performance, *i.e.* quality
Control strategy	▪ Mainly by intermediate and end product testing	▪ Risk based control strategy ▪ Controls shifted upstream ▪ Real-time release
Lifecycle management	▪ Reactive to problems ▪ Post-approval changes needed	▪ Continual improvement enahled within the design space ▪ Does not require post-approval changes if operating within design space

1.1.3 Advantages of Quality by Design

The application of QbD has advantages over the traditional approach to formulation development, in particular for manufacturing processes, expediting regulatory applications and saving money.

The pharmaceutical industry is set to benefit from QbD since the implementation of sound pharmaceutical and manufacturing sciences to product development ensures the design of products based on an increased understanding of the interactions between an active pharmaceutical ingredient (API), excipients, manufacturing variables and conditions. QbD has the potential to reduce the incidence of manufacturing difficulties that are often encountered during normal and routine manufacturing processes. The number of manufacturing supplements required for post-marketing dossier changes, which are usually necessary during the product lifecycle in order to maintain and/or improve quality, are also likely to decrease when QbD principles are applied. Furthermore the application of QbD has the potential to eliminate the production of "atypical" batches thereby saving pharmaceutical companies money in inventory costs [12]. QbD permits real-time quality control which potentially reduces end-product testing thereby saving the pharmaceutical industry substantial

funds [13]. QbD will therefore result in reduced overall product development and production costs for the pharmaceutical industry. It is likely that the application of QbD by pharmaceutical companies will result in a shorter time-to-market and will significantly increase success rates for new products [12,14], which in turn provides a better business model for the pharmaceutical industry as a result of improved efficiency [11].

The information presented to regulatory bodies according to QbD principles enhances the scientific foundation for the review of regulatory applications for registration of medicinal products. This allows better coordination of the review of an application, compliance and inspection, and often results in quicker approvals. Furthermore QbD may improve the consistency and transparency of regulatory applications. Regulatory considerations for post-marketing changes in manufacturing conditions and implementation of new technology are also expedited for a well defined design space when QbD is applied in development and manufacture of pharmaceutical products [10,11].

1.1.4 Application of Quality by Design

The application of QbD principles for the production of pharmaceutical products has resulted in the successful registration of products on the market including Januvia® (sitagliptin), (Merck and Co., Inc., Whitehouse Station, N.J., U.S.A.) for the treatment of Type II diabetes [12,15] and Chantix™ (varenicline tartrate) (Pfizer, New York, N.Y., U.S.A) for the promotion of smoking cessation [16].

1.1.5 The Challenges of Quality by Design

Although QbD presents a novel approach for the development of quality products, one of the biggest challenges in the implementation of QbD has been the limited understanding of the terminology and interrelationships that are described in the International Conference on Harmonization (ICH) Q8 (R2) guideline and related documents, Q9 and Q10 [17]. Communication and training are therefore essential to ensure the successful implementation of these guidelines. The potential influence of these three guidelines on older ICH quality guidelines must also be discussed [11,17].

The implementation of QbD for pharmaceutical formulation development has also been challenging since there is a need to allocate company resources to fulfil the requirements of QbD. For instance, there is a need to manage large amounts of data that are generated during

multivariate studies, in addition to managing the design space and control strategies that assure product quality [9]. Moreover, quality systems for managing process inputs must be adapted to ensure their suitability when implementing QbD [9].

1.2 PHARMACEUTICAL DEVELOPMENT GUIDELINES

1.2.1 Introduction

The core ICH Q8 (R2) [1] guideline describes the recommended contents for the pharmaceutical development section of a regulatory submission for the original marketing application of a pharmaceutical product. Core areas that are emphasised in the ICH Q8 guideline that have the potential to impact product quality include the physicochemical properties of an API and the proposed excipients, formulation development of a drug product and the impact of manufacturing processes on product quality and dosage form performance. In addition, information on the container and closure system, microbiological attributes and compatibility of a formulation with reconstitution diluents must also be included. The severity of risk on product quality when formulation variables and processing conditions are changed must be determined through risk assessment which is an important part of the formulation development process [1].

Multivariate studies, formal experimental design, process analytical technology (PAT) and prior knowledge facilitate the establishment of an appropriate design space for a pharmaceutical product. Changes to a product or a process whilst operating within a design space is not considered a change that necessitates regulatory approval, but movement out of that design space will initiate a post-approval regulatory change process [1].

1.2.2 Components of a Drug Product

The physicochemical and biological properties of an API that influence performance and manufacturability must be discussed in the application for registration or marketing authorisation of a product. These properties include, but are not limited to, the solubility, moisture content, particle size, biological activity and permeability of a molecule [1]. The choice of pharmaceutical excipients, their concentration and characteristics that influence CQA, manufacturability and dosage form performance must also be discussed. The compatibility of excipients with an API must be evaluated using suitable experimental

techniques, although prior knowledge acquired using sound scientific principles may also be applied [1].

1.2.3 Drug Product

Information that is provided relating to a drug product should encompass the evolution of formulation design in a study, starting from preliminary stages to the final optimised formulation. Pharmaceutical development studies that focus on CQA that may impact pharmacokinetic profiles and biological activity must be summarised in the application for registration. Formal experimental design may be used to identify critical formulation variables and interactions between formulation components, all of which may need to be monitored to ensure product quality [18].

Information from *in vitro* and *in vivo* comparative studies that demonstrate links between dosage form characteristics and clinical performance must also be included in the application, and where possible *in vitro/in vivo* correlations must be shown [1]. The physicochemical and biological characteristics of dosage forms that impact safety, dosage form performance or manufacturability of a dosage form must also be evaluated and discussed in detail [1].

1.2.4 Manufacturing Process Development

The manufacturing and process conditions that have the potential to impact CQA of pharmaceutical products must be explained, and therefore the selection and control of manufacturing and processing conditions should be justified in the application for market authorisation. Process development studies should also provide the basis for process improvement and validation, continuous process verification and any process control requirements that provide assurance of the quality of the product [1]. Measurement systems for monitoring CPP and process endpoints must be justified; they are often useful for enhancing the understanding of a process and to facilitate the establishment of an appropriate design space.

1.2.5 Container Closure System

The choice and rationale for the container closure system selected for a commercial product, including a justification for the intended use, suitability of the container closure system to assure product stability and quality, must be included in the application. Other considerations which are vitally important in ensuring long term product quality include the suitability of the

system for storage and transportation of a pharmaceutical product [1]. The selection of materials for primary packaging and how they impact product stability with respect to protection from light and moisture must also be considered. The appropriate nature of dosing devices such as droppers and pipettes for intended use must also be reported in the application [1].

1.2.6 Microbiological Attributes

The microbiological attributes of pharmaceutical products must be described in the application documents for product registration and must include details that relate to the use of preservatives in formulations and the effectiveness of container closure systems to prevent microbial contamination [1].

1.2.7 Compatibility

Where applicable, the compatibility of a formulation with diluents used for reconstitution must be addressed in the regulatory application. The application must include information relating to the recommended in-use shelf-life following reconstitution and appropriate storage conditions for which in-use product quality, safety and efficacy can be assured [1].

1.3 THE IMPLEMENTATION OF QbD

1.3.1 Introduction

The implementation of QbD has been recommended in the annexure to ICH Q8 (R2) [1], which provides direction of how the principles of QbD can be put into practice. The minimum requirements for a pharmaceutical development process include the definition of a quality target drug profile, the identification of potential CQA and the development of a link between material attributes and process parameters with potential CQA. A risk assessment of the impact of material attributes and CPP on product quality, the establishment of a design space using multivariate methods and selection of an appropriate manufacturing process are also important. In addition, the definition of a control strategy for which product quality can be assured is also a vital aspect of the formulation development process [1,6,19]. The steps for the development of a pharmaceutical product using QbD have been summarised in Figure 1.2 [6].

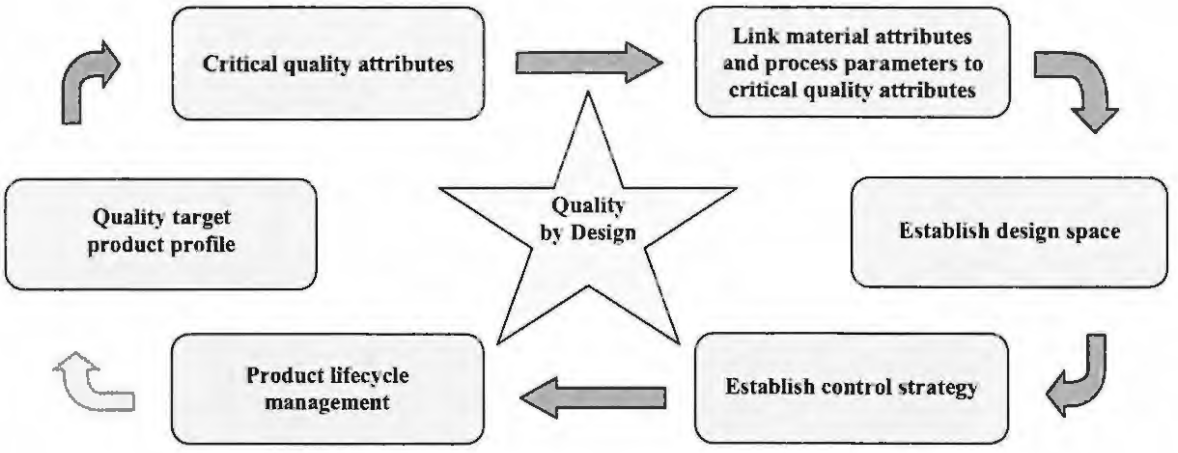


Figure 1.2 Steps in the implementation of QbD adapted and redrawn from [6,18]

These principles were used in conjunction with the ICH Q8 (R2) guideline [1] for the development of a framework for the development of a SR formulation of SBS in these studies.

1.3.2 Quality Target Product Profile

The primary focus of any pharmaceutical development process is the production of a safe and effective drug product of appropriate quality that poses a minimal risk to patients and that will achieve the desired therapeutic outcome. Appropriate dosage forms and strength, route of administration and the intended use of the product in the clinical setting must be considered during the development stages of a pharmaceutical product. An understanding of the disease state, desired drug release characteristics and resultant pharmacokinetic profile to optimise the safety and therapeutic efficacy of a product must be aligned with the product development process [1,6].

The physicochemical and biological properties of a drug molecule that may affect product quality and dosage form performance must be addressed and discussed during the development process [1,4,5]. In Chapter 2 aspects of the physicochemical properties of SBS that may affect the manufacturability and assessment of a SR dosage form are discussed. In addition, the biological activity and pharmacokinetics of SBS are used to highlight the potential benefits of using SR formulations for the administration of SBS in the clinic.

1.3.3 Critical Quality Attributes

CQA of solid oral dosage forms are typically those aspects of a formulation that affect product purity, strength, drug release characteristics and stability [1]. The primary indicator of dosage form performance for the SR formulation of SBS was considered to be the *in vitro* drug release profile over a 12 hr period. Drug release profiles of formulations that were manufactured in these studies were compared with the *in vitro* release profile of SBS from a commercially available formulation, Asthalin®8 ER (Cipla Ltd., Mumbai, Maharashtra, India) and this profile was used as the reference formulation for the optimisation procedures conducted in development studies.

Information on the development of tests that are designed to assess product quality and performance, including analytical and dissolution tests, may also be included in the application for regulatory approval [4,5]. An analytical method for the assay of dosage units that were manufactured, and for the assessment of the extent of drug release from SR dosage forms, was developed and validated and is reported in Chapter 3 of this dissertation.

1.3.4 Linkage of Material Attributes and Process Parameters to Critical Quality Attributes

Understanding the principles of formulation and manufacturing science and the control of pharmaceutical processes is crucial in mitigating the risk of producing a poor quality product [20]. Risk assessment is particularly useful for determining the impact of changing formulation variables and processing conditions on key CQA of a product [1]. The principles of risk management according to ICH Q9 [2] were used to evaluate the potential impact of formulation and manufacturing process on key CQA and is described in Chapter 5 of this dissertation.

1.3.5 Establishing the Design Space

The design space defines the relationship between process inputs and CQA that are determined during studies that are conducted during the pharmaceutical product development process [1]. A central composite design was used to determine the impact of chosen formulation variables on *in vitro* release profiles and to determine whether the interactions between input variables do exist and these are described in Chapter 6. In addition the application of Response Surface Methodology (RSM) as a tool for statistical optimisation of SR formulations of SBS is described in that chapter. The use of Artificial Neural Networks

(ANN) was also assessed as a tool for pharmaceutical formulation optimisation and these findings are summarised in Chapter 7.

A comparison of RSM and ANN was undertaken to assess their suitability as tools for the development of a design space and the relative merits and limitations of RSM and ANN for the development of the design space are evaluated and discussed in Chapter 7.

1.3.6 Establishing the Control Strategy

The control strategy should describe and justify how in-process controls and the control of input materials contribute to ultimate product quality and ensures that products of the desired quality are consistently produced [6]. Sources of variability that can impact product quality must be identified, appropriately understood and controlled during the manufacturing process. In-process control strategies were established in the study and were used to monitor product quality during the tablet manufacturing procedure.

1.3.7 Product Lifecycle

The establishment of a design space allows manufacturers to add more information to the body of knowledge about a product and its manufacturing processes and therefore continually improve on product quality [3]. QbD facilitates continual product improvement through an increased understanding of the pharmaceutical and manufacturing sciences as they pertain to the product and recommendations in this respect are made in the final chapter of this thesis.

1.4 CONCLUSIONS

QbD is a systematic approach for the development of pharmaceutical dosage forms that begins with predefined objectives, *i.e.,* as a result of quality control and manufacturing procedures, quality is built and not tested into products. The main emphasis is on understanding the factors that influence CQA, focusing on pharmaceutical and manufacturing sciences to ensure the consistent production of a safe and efficacious pharmaceutical dosage form.

The basic principles of QbD recommend the use of systematic multivariate experiments to understand the impact of formulation and process variables on product quality and for the optimisation of formulations using statistical methods. This allows for the establishment of a

design space within which product quality and dosage form specifications or criteria can be inferred and assured. The combination of the Pharmaceutical Development guideline with risk-based control strategies ensures the development of a quality product throughout the lifecycle of that product.

The Pharmaceutical Development guideline, ICH Q8 (R2), is focused on the development of quality products, specifically emphasising those key areas of the manufacturing process that must be considered when developing a pharmaceutical product. Understanding the components of a drug product, the manufacturing process and other aspects including the container closure system, compatibility issues and microbiological aspects are important considerations.

The implementation process of the principles of QbD has been recommended and was used in these studies for the establishment of a design space for a model SR formulation. The quality target product profile, CQA and a risk assessment are described and discussed in subsequent chapters. RSM and statistical methods were employed to link formulation variables with the *in vitro* release profile and for optimisation of a formulation with the desirable dissolution characteristics. In addition ANN was used for product optimisation and was compared with RSM to assess its utility in developing and presenting a design space for a SR formulation of SBS. Therefore the principles of QbD were applied to the development of a design space and for the successful development of a SR formulation of SBS.

CHAPTER 2

MONOGRAPH FOR SALBUTAMOL SULPHATE (SBS)

2.1 SALBUTAMOL SULPHATE (SBS)

2.1.1 Introduction

SBS is a water-soluble salt of salbutamol (SB), a short-acting β_2 agonist, that is used for the alleviation of bronchoconstriction and bronchospasm in patients with reversible obstructive airway disease and chronic obstructive pulmonary disease (COPD) [21,22].

2.1.2 Chemical Formula

The empirical formula of SB is $C_{13}H_{21}NO_3$ and is known as 2-*tert*-butylamino-1-(4-hydroxy-3-hydroxymethylphenyl) ethanol. SB is also known as albuterol and has a molecular weight of 239.3 g/mol. The empirical formula for SBS is $(C_{13}H_{21}NO_3)_2.H_2SO_4$ and has a molecular weight of 576.7 g/mol. Approximately 1.2 mg of SBS is equivalent to 1 mg of SB [21,23]. The chemical structure of SB is depicted in Figure 2.1 [21,23–25].

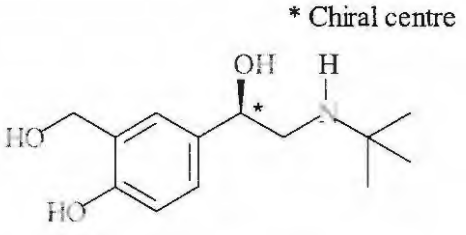


Figure 2.1 Chemical structure of SB

SB has a single asymmetric carbon atom as shown in Figure 2.1 and therefore SB exists as a pair of enantiomers. The (-)*R*-enantiomer (*levo*-SB) possesses biological activity (eutomer) and the (+)*S*-enantiomer (*dextro*-SB) has limited β_2 adrenergic activity (distomer). Despite these differences in pharmacological activity, SB is administered as a racemic mixture in most dosage forms [26].

2.1.3 Salbutamol Dosage Forms

SB is marketed commercially as Ventolin®, Vari-Salbutamol® and Venteze® in South Africa [27]. SB may be used as the free base or as the sulphate salt in aerosol products but the salt

form is used in the manufacture of solid oral dosage forms, parenteral formulations, powders for inhalation and nebuliser solutions.

2.2 PHYSICOCHEMICAL PROPERTIES

2.2.1 Appearance

SB is a white or almost white crystalline powder that is odourless and almost tasteless [21,23,25]. SBS is also a white or almost white crystalline powder that is odourless [21,23].

2.2.2 Solubility

SB is soluble 1 g in 70 ml of water, 1 g in 25 ml of ethanol and is soluble in most organic solvents [23,25]. SBS is freely soluble in water, slightly soluble in alcohol but has limited solubility in organic solvents such as dichloroform, ether and dichloromethane [21].

2.2.3 pK_a

SB is a weakly basic drug with a pK_a of 9.3 [28] and has been shown to undergo reversible protonation. SB undergoes an association-dissociation reaction when placed in an aqueous medium to produce a cation.

2.2.4 UV Absorption

SBS shows an absorption maximum at a wavelength of 276 nm when examined using UV spectroscopy [23,25] thereby indicating that UV detection may be used in analytical methods to monitor SBS.

2.3 AETIOLOGY OF ASTHMA

Asthma is a chronic inflammatory disease, in which a patient exhibits episodes of reversible obstruction in the airways due to bronchial hyperresponsiveness and which results in wheezing, dyspnoea, tightness of chest and coughing. Tachycardia and rapid respiratory rates are also common clinical symptoms observed during an acute asthma attack [29,30].

The interaction of genetic and environmental factors contributes to the development and progression of asthma in patients [29,30]. Genes that regulate the production of sensitive cell lines and mediators that result in bronchoconstriction are likely to be present in individuals

susceptible to asthma. These include genes for T-helper 1 and 2 cells, Immunoglobulin E, cytokines (Interleukin (IL)-3, -4, -5, -9 and -13), granulocyte-monocyte colony-stimulating factor (GM-CSF), tumour necrosis factor-α (TNF-α) and the ADAM33 gene. The presence of these genetic and other factors results in stimulation of the smooth muscle of the airways, the proliferation of fibroblasts or the regulation of cytokine production that ultimately results in bronchoconstriction [29].

Genetic factors may present as abnormalities in the airways of asthma patients and include increased contractility of the airway smooth muscle tissue, excessive secretions within the airway lumen and inflammatory cell infiltration which promote resistance to airflow in asthma. Furthermore, inflammation in chronic asthma causes remodelling of the airway epithelium, hypertrophy and hyperplasia of smooth muscle, which may result in the irreversible narrowing of airways over time in these patients [30].

Environmental factors that exacerbate asthma include exposure to allergens, diet and perinatal factors. Allergens including house dust mites, pets, cockroaches and diets that are low in vitamins C and E have also been linked to asthma. Perinatal factors that result in asthma include young maternal age, poor maternal nutrition, premature birth, low birth weight and lack of breastfeeding [29].

Common triggers for asthmatic attacks include exposure to allergens, infections, exercise, inhaled irritants, emotional stress and associated response, aspirin and gastroesophageal reflux [29]. Exposure to these triggers results in a reversible narrowing of the airways and uneven lung ventilation which subsequently manifests as a decrease in alveolar oxygen and an increase in alveolar carbon dioxide concentrations. Most patients respond by hyperventilating and in severe cases bronchoconstriction, this causes severe gas trapping in the lungs and results in the respiratory muscles overworking. Overworking of the muscle tissue causes hypoxemia and a consequent increase in carbon dioxide concentration in the lungs, resulting in respiratory and metabolic acidosis, which can ultimately cause respiratory and cardiac arrest if left untreated [29].

2.4 CLINICAL USE OF SALBUTAMOL SULPHATE IN ASTHMA

2.4.1 Use and Administration

The inhalation of SBS is a preferred method of administration for the management of asthma, although solid oral dosage forms, nebulisers and parenteral preparations have also been used [21]. The relief of acute bronchospasm is achieved by administration of one to two inhalations of 100 μg SB from a metered-dose aerosol up to four times daily. Prior to physical exertion two inhalations may be administered as a prophylactic measure against bronchoconstriction [21]. SBS may also be administered using inhalation capsules or discs in doses of 200 μg, which is therapeutically equivalent to the delivery of 100 μg of SB from an aerosol inhaler [21].

Oral SB may be given in doses of 2–4 mg, three to four times daily when inhalation is ineffective. However some patients may require doses of up to 8 mg at the same frequency to ensure relief of symptoms. Such high doses are generally not well tolerated nor do they provide additional clinical benefits. Alternatively, administration of 4 or 8 mg of SB delivered from SR dosage forms may be better for the management of asthma symptoms [21].

The administration of intermittent doses of 2.5–5 mg SBS up to four times daily with the aid of a nebuliser may be used to treat unresponsive bronchospasm in adults and children. The continuous use of SB at a rate of 1–2 mg/h is also possible and in severe cases where a nebuliser is not available, 4–6 inhalations of 100 μg may be given every 10–20 min using a large spacer [21].

Intravenous (IV) injections containing 250 μg of SBS may be administered for the management of severe bronchospasm, or alternatively an IV infusion of 10 μg/ml SBS may be administered at a rate of 3–20 μg/min depending on the needs of the patient [21]. Subcutaneous or intramuscular injections of 500 μg SBS may also be administered every 4 hr as required [21].

2.4.2 Mechanism of Action

SB is a β_2 receptor agonist that exhibits dose dependent effects on smooth and skeletal muscle tissue. It has been postulated that binding of SB to β_2 receptors in bronchial smooth

muscle results in the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) by adenyl cyclase which in turn activates protein kinase, an enzyme that catalyses protein phosphorylation, with a consequent increase in the concentration of bound calcium ions. The binding of calcium reduces the availability of ionised calcium in tissues which inhibits the actin-myosin linkage causing relaxation of smooth muscle tissue [31,32].

It has also been proposed that the binding of agonists to β_2 receptors increases the conductance of large calcium-sensitive potassium channels in airway smooth muscle and results in membrane hyperpolarisation and relaxation. This process is independent of the presence of adenyl cyclase and cAMP and may involve the regulation of capacitative calcium entry into muscle cells by small G-proteins [31,33,34].

β_2 agonists also bind to β_2 receptors in numerous inflammatory cell types in the airways including mast cells, basophils, eosinophils, neutrophils and lymphocytes. β_2 agonists inhibit the release of bronchoconstrictor mediators such as histamine, neutrophil chemotactive factor and prostaglandin D_2 from these cell types and therefore have an anti-allergic effect that is usually beneficial in the treatment of asthma [31,35].

2.4.3 The Clinical Benefits of Salbutamol Sulphate

The therapeutic effects of bronchodilators and anti-asthmatic medicines may be evaluated by physiological and clinical assessment criteria that are related to lung function. These criteria include peak expiratory flow rate (PEFR), forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), presence or absence of wheezing and assessment of the need for emergency or rescue bronchodilator therapy.

The administration of SBS results in an increase in FEV_1 and FVC, reduced resting and dynamic hyperinflation, increased self-paced walking distance and improved respiratory muscle function [22]. Clinically, bronchodilators cause a reduction in dyspnoea at rest and during exercise, an increased capacity to exercise and a reduction in the frequency of night-time symptoms with an ultimate improvement in the health status of the patient [22].

2.4.4 Tolerance

Regular inhalation of short-acting β_2 agonists has been reported to increase airway hyperresponsiveness which reduces the protective effects of such compounds against allergen and mediator induced bronchoconstriction [36,37]. Studies have shown that hyperresponsiveness is likely due to the presence of the *S*-enantiomer, which does not have any bronchodilatory effects [38]. It has also been proposed that the change in effect may also be due to genetic polymorphism of β_2 receptors in some individuals [39,40].

2.4.5 Adverse Effects

The non-selective binding of SB to β_1 receptors causes most of the side effects associated with the administration of SB. Commonly reported side effects include fine tremor of skeletal muscle, palpitations, tachycardia, headaches and peripheral vasodilation [21]. The oral administration of SBS is associated with a greater incidence of side effects compared with inhalation therapy since higher doses equivalent to 2–4 mg of SB are administered orally, whereas 100–200 μg doses of SB are administered by inhalation. The administration of SBS also results in a greater incidence of side effects compared with more selective β_2 agonists such as salmeterol and formoterol that have limited activity on β_1 receptors [21].

Rare side effects observed following the administration of high doses of SBS include hypokalaemia, although this is more common following use of the parenteral and nebuliser routes of delivery. Myocardial ischaemia and hypersensitivity reactions including paradoxical bronchospasm, urticaria, hypotension and collapse have also been reported [21].

The effects of administration of an overdose of SBS are related to β_1 receptor stimulation and include tachycardia, tremor, hypokalaemia and hyperglycaemia. Overdose situations have been managed successfully by the symptomatic treatment of adverse events, although activated charcoal may be considered for treating an overdose if patients are treated within an hour of ingestion of SBS [21].

The *S*-enantiomer of SB (distomer) exhibits poor binding to the β_2 receptor site and is associated with bronchial hyperresponsiveness that is associated with SBS therapy [41,42]. *In vitro* and animal studies have revealed that this effect may be due to the activation of muscarinic receptors and a phospholipase-C dependent mechanism which results in increased

levels of free intracellular calcium [43]. *S*-SB is also reported to increase the hyperresponsiveness of tissues to allergens resulting in bronchoconstriction and asthma-like symptoms [38].

2.4.6 Drug Interactions

The concomitant use of SB with corticosteroid, cardiac glycoside, diuretic and xanthene type compounds increases the risk of hypokalaemia. The monitoring of potassium concentrations in patients with severe asthma who are on combination therapy is therefore recommended [21].

Corticosteroids have been shown to modify the activity of β receptors in severe asthma, and the combination of corticosteroids with β_2 agonists may result in increased bronchodilatory effects. This effect results in a reversal of receptor desensitisation and down-regulation that is caused by β_2 agonists thereby enhancing the bronchodilator response to these compounds [44]. However, there has been little evidence to support this theory, although combination use of β_2 agonists and corticosteroids has been shown to exhibit clinical benefits in patients who suffer from severe asthma [21].

Non-cardiac selective β blockers oppose the bronchodilatory effects of β_2 agonists and are contraindicated in asthma patients since the combination may cause severe bronchoconstriction. Although this phenomenon has been reported in some patients [21], the concurrent administration of cardio-selective β blockers and β_2 agonists does not cause deleterious effects in many patients.

2.4.7 Contraindications

SB and other β_2 agonists should be given with caution in patients that present with hyperthyroidism, myocardial insufficiency, hypertension, diabetes mellitus, and who are susceptible to QT-interval prolongation [21].

2.5 CLINICAL BENEFITS OF SUSTAINED RELEASE SALBUTAMOL SULPHATE

The administration of SR dosage forms of SBS results in a significant improvement in the morning FEV of asthma patients when compared with those who received a placebo, but their use in COPD patients does not appear to have the same effect [45].

The clinical efficacy of 8 mg SBS SR tablets administered twice daily has been compared with immediate release or conventional 4 mg tablets taken four times a day for two weeks in a double-blind cross-over study [46]. It was observed that morning PEFR was significantly higher in patients who were given SBS in SR tablets compared with those on conventional tablets, although there was no significant difference in the evening PEFR. A comparison of plasma levels following administration of the SR and conventional tablets revealed that although the minimum and average plasma levels were similar in the two study groups, the maximum plasma concentration was significantly lower in patients on SR medication. It was also observed that there was less fluctuation in plasma concentrations in patients using SR therapy, and that this was associated with better management of asthma symptoms with fewer side effects [46].

SR SBS tablets may also be considered as an alternate therapy to theophylline [47,48] and aminophylline [49] for the management of reversible airways obstruction. There were no statistically significant differences in the results of lung function tests and PEFR, although the non-asthma symptom score was higher with SR theophylline [47,48]. However, patients reported a preference for SR SBS formulations compared with theophylline due to a lower incidence of side effects and better tolerance of SBS [47]. Higenbottam *et al* [49] reported similar findings when comparing the clinical effects of SR SBS and aminophylline where 68% of clinical trial participants preferred SR SBS due to a lower incidence of side effects. However no statistically significant differences in terms of lung function tests, PEFR, frequency of asthma symptoms or the need of relief medication were observed for the patient groups taking aminophylline or SR SBS [49].

SR SBS formulations are useful for the treatment of nocturnal asthma with an increase in waking PEFR being reported, which is beneficial for therapy [50]. SR tablets were also found to be effective against histamine induced bronchoconstriction, and the protection was found

to last for at least twice as long for SR formulations compared with conventional formulations [51]. Morning PEFR was higher when SR SBS formulations were administered compared with conventional formulations and there was a significant reduction in wheezing and the need for "rescue" inhalation of bronchodilators [46,52]. However, the administration of SR formulations of β_2 agonists may not necessarily reduce the side effects in all patients but may improve the quality of life of patients by improving adherence and therefore provide for better management of asthma [53].

2.6 PHARMACOKINETICS FOLLOWING ORAL ADMINISTRATION

2.6.1 Absorption

SBS is readily absorbed from the gastrointestinal tract (GIT); clinical effects occur within 15 min and may last for up to 14 hr [25,54]. The oral bioavailability of SBS is fairly low and has been reported to be 0.44 [55]. Similar values for bioavailability of 0.50 ± 0.04 [56] and 0.53 ± 0.08 [26] have also been reported.

2.6.2 Distribution

The peak plasma concentration of SBS observed following administration of a single 4 mg oral dose ranges between 5.1 and 11.7 ng/ml with a time to peak of approximately 2.5–3 hr [25]. However, when SBS is administered as a solution, peak plasma concentrations are reached much earlier, *i.e.* 1–2 hr following administration [54]. Similar values for peak concentration and time to peak for SBS ranging between 10.0 and 16.9 ng/ml observed 1–4 hr [56] and between 10 and 20 ng/ml after 1–3 hr after oral administration [55] have been reported.

The binding of SBS is negligible and approximately 7 ± 1% of circulating SB is bound to plasma proteins [56]. The apparent volume of distribution for SB following IV administration has been reported to be 156 ± 38 L [56]. SB does not cross the blood-brain barrier, although it crosses the placenta by a flow-dependent diffusion process [57].

2.6.3 Metabolism

SB undergoes first-pass metabolism in the gut wall and liver, but does not appear to be metabolised to any great extent in the lungs [21]. The rate of SBS metabolism in the gut wall is higher than that observed in the liver [58]. SB is metabolised by sulphotransferase (SULT

1A3) [59] in the gut wall to form SB 4'-O-sulphate, an inactive metabolite as shown in Figure 2.2 [60]. SULT1A3 has inter-patient phenotypic variability which results in variable drug plasma levels in patients [61,62].

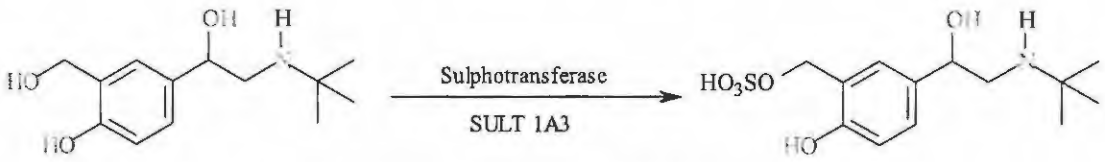


Figure 2.2 Metabolism of SB in the gut wall

2.6.4 Elimination

The half-life of SBS has been estimated to range between 4 and 6 hr [21], 4.8 and 5.5 hr [63], as 3.9 ± 0.8 hr [56], and as 3.8 hr [55]. Following oral administration approximately 50% of a dose of SB is excreted within 4 hr, and the remainder is excreted within 24 hr [25,54]. The total plasma clearance of SB following the administration of an IV dose is 480 ± 123 ml/min. The renal clearance of SBS following IV and oral administration has been reported to be 291 ± 70 ml/min and 272 ± 38 ml/min respectively [56]. The renal clearance of SB is higher than creatinine clearance (~ 118 ml/min), suggesting that active tubular secretion is an important mechanism by which SBS is excreted.

2.6.5 Multiple Dose Pharmacokinetics

The pharmacokinetics of SBS following multiple dose administration were studied using 4 mg tablets administered four times daily for five consecutive days [63]. Following the initial dose, the mean maximum plasma concentration was reported to be 8.2 ng/ml, which increased to 14.7 ng/ml at steady state. The minimum concentration at steady state was reported to be 9.9 ng/ml and the time taken to reach the peak concentration following each dose was 2.8 hr [63]. The pharmacokinetics of β agonists, including SBS, do not appear to change with continued administration, and pharmacokinetic parameters that are obtained from single dose studies can be used to predict long-term dosing requirements for this drug with a high degree of confidence [53].

2.6.6 Pharmacokinetics Following Administration of Sustained Release Dosage Forms

The pharmacokinetics of SBS following administration of SR 4 mg and 8 mg formulations to patients with bronchial asthma are summarised in Table 2.1.

Table 2.1 Single dose and steady state pharmacokinetic parameters of SR formulations [64]

	Single Dose		Steady State	
	4 mg	**8 mg**	**4 mg**	**8 mg**
C_{max} (ng/ml)	4.6 (4.1–5.1)	9.5 (8.5–10.7)	8.2 (7.4–9.1)	16.1 (14.5–17.9)
t_{max} (min)	300	360	300	240
C_{min} (ng/ml)	-	-	4.5 (4.0–5.0)	8.7 (7.8–9.7)
AUC (0–12) ng.min/ml	-	-	4507 (4094–4962)	8980 (8157–9885)

Following administration of a single 8 mg dose of SB the peak concentration is approximately double that observed following administration of a 4 mg dose. At steady state, peak plasma concentrations of SBS for both strengths are double those observed following the administration of a single dose of SBS [64]. The administration of SBS in SR formulations generally results in smooth plasma drug concentration profiles with minimal fluctuations observed at steady state [64].

2.7 SUSTAINED RELEASE SALBUTAMOL DOSAGE FORMS

2.7.1 Commercially Available Formulations

SB is marketed as Volmax® (GlaxoSmithKline, Uxbridge, Middlesex, UK), an osmotic pressure mediated SR dosage form consisting of a core of SBS surrounded by polymer that has a minute (250 μm) laser-drilled hole which allows the API to be released at a constant rate [52,65,66]. SBS is also available as Asthalin®8 ER (Cipla Ltd., Mumbai, Maharashtra, India) which was used as the reference formulation in these studies.

2.7.2 SR Salbutamol Oral Dosage Forms

The incorporation of SBS has been reported in SR formulations that include the use of lipidic [67–69] and hydrophilic matrices [70,71], acrylic acid based resins [72,73] and novel delivery systems that include the use of microcapsules [74,75].

2.7.2.1 Lipid and Wax Matrix Formulations

Lipid and wax materials such as glyceryl monostearate, stearic acid and carnauba wax have been evaluated as SR platforms for the delivery of SBS [67,68]. SBS is poorly retained in these matrices, and dosage forms exhibited a burst-type release that is commonly observed for water-soluble compounds from these matrices. For example, when glyceryl monostearate was used as the matrix material, up to 40% of the dose was released within 10 min of the commencement of the dissolution studies. Similarly the use of carnauba wax and stearic acid

produced dissolution profiles in which between 20 and 40% of the dose was released rapidly, although this was dependent on the drug: wax ratio used to manufacture the matrices [68]. Composite mixtures of wax and other excipients, such as polyethylene glycol (PEG), hydrophilic polymers and surfactants, were used in an attempt to reduce drug release rates, and a combination of carnauba wax, glyceryl monostearate and hydroxypropyl methylcellulose (HPMC) was found to be efficient in sustaining drug release for a period of up to 8 hr [67].

Murthy *et al* [69] used combinations of carnauba wax and stearyl alcohol at concentrations ranging between 60 and 70% of the tablet weight; these were found to be useful for retarding the release rate of SBS from wax matrix granulation formulations for up to 24 hr [69].

Dynasan 118, a triglyceride ester of fatty acids, has been evaluated as a potential matrix forming polymer for the manufacture of SR formulations of SBS [76]. The *in vitro* release profiles of formulations containing 14% w/w Dynasan 118 exhibited very slow and incomplete drug release, and approximately 12% of the dose had been released after 8 hr. The incorporation of different diluents, *viz.*, lactose, microcrystalline cellulose and calcium phosphate, resulted in an increase in the rate and extent of drug release from these dosage forms. It was observed that the rate and extent of release was dependent on the hydrophilicity of the diluents such that lactose-containing formulations exhibited the fastest release rate and the calcium phosphate-containing formulations resulted in formulations with the slowest release rates [76]. The application of the Higuchi [77,78] and Korsmeyer–Peppas [79] models to drug release data revealed that diffusion was the predominant factor in controlling or modulating drug release [76].

Polyglycolised glycerides such as Gelucire® have also been investigated as potential matrices for controlling or sustaining SBS release from oral delivery systems [80]. Gelucire® excipients are characterised by their hydrophilic-hydrophobic balance (HLB) and melting point which determine their physicochemical behaviour *in vitro* and *in vivo*. Studies have revealed that the use of Gelucire® with high HLB values results in drug release rates that are faster than those with low HLB values since water molecules are able to penetrate these matrices to a greater extent compared with more hydrophobic matrices, thereby promoting drug dissolution and liberation [80]. Drug release was postulated to occur according to an anomalous transport mechanism in which a combination of drug diffusion and erosion

occurs, and the value of n was reported to vary between approximately 0.6 and 0.7 when data were fitted to the Korsmeyer–Peppas model [79].

2.7.2.2 Acrylic Resin Formulations

The feasibility of manufacturing SR formulations of SBS using Eudragit® Retard in combination with PEG has been reported [72]. These studies revealed that the rate of drug release was dependent on the hydrophilic/hydrophobic nature of the Eudragit® that was used when manufacturing formulations. The use of Eudragit® RL 100, which is relatively hydrophilic, resulted in a faster release rate than that observed when Eudragit® RS 100, a more hydrophobic polymer, was used [72].

The effect of using varying proportions of Eudragit® RS in combination with different diluents was investigated as a means of retarding the rate of release of SBS from solid oral dosage forms [73]. Microcrystalline cellulose, dibasic calcium phosphate and calcium sulphate were found to be unsuitable for the manufacture of SR formulations since complete drug release was observed within 2 hr of the commencement of dissolution testing. These studies revealed that drug release was diffusion controlled; a value of $n \leq 5$ (Korsmeyer–Peppas model [79]) was observed and is more than likely due to the limited relaxation of the Eudragit® polymer resins in these systems. The incorporation of Eudragit® S100 to Eudragit® RS matrices resulted in a decrease in the burst release from such dosage forms [73].

The combination of different ratios of poorly water permeable Eudragit® RSPM and water permeable Eudragit® RLPM resulted in a significant decrease in drug release rates compared with those observed for conventional formulations of SBS, although drug release from these formulations was complete after 4–6 hr [81].

2.7.2.3 Hydrophilic Matrix Formulations

Methocel® K100M has been investigated as a rate retardant in SBS formulations in which concentrations ranging between 20 and 60% w/w of the polymer were used, and the rate of drug release was observed to decrease with an increase in polymer concentration. Hydrophilic matrix forming materials such as Methocel® K100M contain HPMC that swells in aqueous media to form gel-like structures that impede drug diffusion through that matrix [73].

Formulations of SBS manufactured using HPMC of different viscosity grades, specifically HPMC K4M, K15M and K100M at a drug to polymer ratio of 1:1, were assessed as SR platforms [70]. The resultant release rates expressed as $\%.min^{-½}$ were independent of the viscosity grade of the polymer used. The study also revealed that the mechanism of SBS release was well described using Higuchi kinetics [77,78] and that SBS was released primarily by a diffusion controlled mechanism with a minimal contribution by polymer erosion [70].

Hernández *et al* [71] manufactured two hydrophilic matrix formulations using HPMC K100M and a combination of HPMC K100M and sodium carboxy methylcellulose (SCMC). The *in vitro* release profiles in these studies indicated that the formulation that contained HPMC showed a burst release; the rate of drug release decreased in the later stages of the dissolution test and the drug release was complete after 8 hr. The combination of HPMC K100M and SCMC showed a linear rate of release for the duration of the dissolution test and drug release was also complete after 8 hr [71].

The possibility of enantioselective release of SBS from matrix tablets that were manufactured using HPMC, which is a chiral excipient, has been reported [82]. The premise of these studies related to the possibility of a preferential interaction between the polymer and one enantiomer contained in a formulation manufactured using a racemic mixture. The manufactured formulations contained approximately 40% w/w HPMC and the results indicated that the release profile of SBS was asymptotic, with the rate of drug release decreasing with time. The drug release profiles of the *R*- and *S*- entantiomers of SBS were super-imposable, and there were no significant differences between release parameters such as mean dissolution time and kinetic constants [82]. The results also showed that approximately 90% of the dose was released within 9 hr and that the mechanism of drug release was due to a combination of diffusion and erosion processes, based on the value of the exponent n [79]. Furthermore, the mechanism of drug release was independent of the enantiomer present in the formulation, and there were no significant differences observed between the exponents and constants determined for the *R*- and *S*- enantiomers of SBS [82].

Srichana and Suedee [83] also reported that the release of SB from HPMC matrices is non-stereo-selective; over 80% of both enantiomers were released from test formulations within 4 hr of the commencement of testing and there were no significant differences in drug release

rates. It was also reported that tablet matrices were completely eroded by the end of the dissolution test, and it was postulated that the penetration of water into the matrix results in dissolution of the water-soluble filler used, which resulted in the rapid release rates observed from these formulations [83].

2.7.2.4 Novel Delivery Systems

Liu *et al* [84,85] investigated the use of pH-sensitive ion-exchange resins for the development of pulsatile release formulations of SBS. Methacrylic acid, which contains carboxylic acid functional groups, was incorporated onto a styrene backbone to create a pH-sensitive resin that was used to form microspheres loaded with SBS. At acidic pH, the carboxylic acid groups are unionised, and the interior of the resin does not interact extensively with the aqueous medium. The polymer remains intact thereby hindering the liberation of drug. However, when the pH is increased, ion-exchange occurs; the methacrylic acid becomes ionised and results in an interaction of the ionised groups with water causing polymer swelling and the promotion of drug release. SBS will therefore be released in a pulsatile manner from the formulation, and drug release is delayed for a 2–3 hr period when the dosage form is in the stomach but drug is released in the intestines where the pH is alkaline [84].

Sirkiä *et al* [86] investigated the feasibility of developing a novel press-coated SR solid oral dosage form of SBS that exhibited a biphasic release profile. Each press-coated tablet was comprised of a core made of lactose, polyvinyl pyrrolidone (PVP), talc and magnesium stearate. Coats were prepared from varying ratios of HPMC, drug, talc and magnesium stearate. Increasing the content of HPMC K100M resulted in a decrease in the rate of drug release, and when high concentrations of the high viscosity grades of HPMC were used, biphasic drug release patterns were not readily attained or observed [86].

Microcapsules have also been investigated as novel delivery platforms for sustaining the release of SBS [74]. The coacervation method using ethyl cellulose as the coating agent with a core to shell ratio of 1:1 was used to manufacture microcapsules, and it was reported that microencapsulation resulted in a decrease in the rate of drug release, such that 50% of the total dose incorporated was released in 90 min when compared with drug release from immediate release formulations [74].

Beeswax and carnauba wax were compared as coating materials used to manufacture microcapsules of SBS [75]. The beeswax was ineffective in retarding the rate of drug release, although combinations of beeswax and carnauba wax were found to be suitable for this purpose. Drug release from tabletted microcapsules was significantly reduced compared with that observed for respective batches of the microcapsules, and the mechanism of release from these formulations could best be described using a first order release model [75].

β-cyclodextrin and ethylated β-cyclodextrin complexes of SB at a molar ratio of 1:1 have been investigated for retarding the release of SB from dosage forms [87]. Cylcodextrins have an amphiphilic character and consist of a hydrophobic interior and a hydrophilic exterior which interacts with water molecules in an aqueous environment. Modified cyclodextrin complexes may be useful for reducing the rate of drug liberation from pharmaceutical dosage forms [87]. The inclusion of SB in different grades of ethylated β-cyclodextrin complexes resulted in lower rates of release compared with those observed from unmodified β-cyclodextrin complexes [87].

Solinis *et al* [82] investigated the possibility of using heptakis-(2,6-di-*O*-methyl)-β-cylcodextrin, a chiral excipient, to retard the release of SBS from dosage forms. Physical and freeze dried mixtures of these dosage forms were manufactured, and complete drug release was observed after 9 hr of dissolution testing. The inclusion of HPMC in cyclodextrin formulations resulted in a further reduction in drug release rates [82]. Although drug release is generally not stereo-selective, it was observed that slight stereo-selectivity may be possible from formulations that were manufactured from freeze dried mixtures of heptakis (2,6-di-O-methyl)-β-cyclodextrin/SBS complexes [82].

Sulpho-butyl-β-cyclodextrin, heptakis (2,6-di-O-methyl)-β-cyclodextrin and γ-cyclodextrin have also been investigated as matrix forming materials for the manufacture of SR SB formulations [83]. Freeze dried mixtures of equimolar concentrations of SB and cyclodextrin were mixed with lubricant and compressed into tablets that were coated with a 20% w/v mixture of Eudragit® E-100 in acetone: isopropanol (1:1) mixture to form a 2 mm thick film. Drug release from sulpho-butyl-β-cyclodextrin formulations were not enantio-selective and over 80% of API was released within 8 hr, and almost 100% release was observed after 10 hr. Slight stereo-selectivity for release was observed from formulations containing heptakis (2,6-

di-O-methyl)-β-cyclodextrin although this was not evident in the early stages of the dissolution test. Up to 70% of the API was released from the formulation within 2 hr, and drug release was complete after approximately 6 hr. The incorporation of SB into complexes with γ-cyclodextrin resulted in stereo-selective delayed release from formulations; between 70 and 80% of the enantiomers were released within 8 hr, and drug release was complete after 12 hr [83]. The results also reveal that the rate of release of the eutomer, *R*-SB, from drug-γ-cyclodextrin complexes is higher than that of the distomer, *S*-SB [83].

Egg albumin was also investigated as a potential rate-retarding matrix-forming material for SBS formulations [83], and tablets that contained 75 mg egg albumin in an 80 mg unit were manufactured by direct compression. Over 90% of the dose was released within 4 hr, and the tablets remained intact for the duration of the 8 hr dissolution test. It was also noted that there were no significant differences between the release rates of the two enantiomers of SBS from these formulations [83].

Citric acid/disodium phosphate and sodium bicarbonate/sodium carbonate buffers have been used to create microenvironments of pH 2.5 and 10, respectively [82]. Formulations of HPMC combined with drug in its native form, freeze dried mixtures of SBS and cyclodextrin, or a physical mixture of SBS and cyclodextrin were manufactured. The results indicated that that the pH of the microenvironment can be used to alter the rate and extent of drug release. The lower pH values of the core revealed faster rates of release compared with the formulations that were micro-buffered to pH 10, as would be expected for a weakly basic compound such as SBS. This is due to the higher solubility of SBS at acidic pH and therefore faster liberation of API from tablet matrices is evident [82].

Wu *et al* [88] developed and optimised a novel osmotic pump formulation for SBS using ANN. The tablet cores were comprised of a granulation of API, SCMC and PVP that were compressed to form a tablet of 7 mm diameter. Tablets were coated using a 2.4% cellulose acetate methylene chloride – ethanol solution containing varying amounts of HPMC and PEG 1500. A 0.4 mm orifice was drilled by laser onto one side of the tablet and dissolution was performed using the paddle method over an 8 hr period. The amount of HPMC and PEG 1500 and weight of coating were used as causal factors in training the ANN, and dissolution parameters were used to assess each product. It was reported that the optimal formulation

contained 3.6 g/500 ml of HPMC and 1.5 g/500 ml of PEG 1500 in the tablet coat, which resulted in a drug release rate of 0.9360 mg/hr [88].

An elementary osmotic pump based on the pharmacokinetic parameters of SBS was designed by Sinchaipanid *et al* [89]. Tablet cores comprising 8 mg API and 272 mg sodium chloride as an osmogent, 2% talc and 0.3% colloidal silicon dioxide were manufactured by direct compression to form 8 mm tablets. The core tablets were coated with 3% w/v cellulose acetate in acetone solution to different thicknesses, and an orifice approximately 400 μm in diameter was drilled using carbon dioxide laser equipment. The rate of drug release was found to be dependent on the thickness of the coat, and the drug release rate decreased with an increase in film thickness. Increasing the amount of osmogent in each formulation resulted in an increase in drug release rates up to a maximum, after which the rate decreased as the amount of salt added was increased. This phenomenon was observed because the release of drug from elementary osmotic pump formulations is governed by two opposing forces. As the amount of salt increases, the osmotic pressure increases resulting in a greater force that expels drug from the tablet core. However, a large amount of osmogent in the core dilutes the drug concentration in the core; even though more of the saturated solution is pumped out, the concentration of API in the saturated solution decreases [89]. The mechanism of drug release from all formulations followed a zero order release pattern [89].

2.8 CONCLUSIONS

SBS is a commonly used β_2 agonist that is used for the treatment and alleviation of airways constriction as occurs in asthma and COPD. The use of SR formulations of SBS may produce clinical benefits compared with the administration of SBS in conventional immediate release dosage forms. Such benefits include an improved PEFR and FEV_1 and a reduced need for "rescue" bronchodilator therapy.

The physicochemical properties of SBS make it an ideal candidate for use in SR formulations. However the primary challenge of using SBS is that it undergoes sulphate conjugation in the gut wall, and the use of a racemic mixture may result in variable absorption and pharmacological activity patterns.

The development of several novel formulations of SBS has been reported, and these include the use of lipid and hydrophilic matrices, microcapsules and osmotic systems. Hydrophilic

matrices are an attractive choice for further study and for the investigation of a design space within which product quality can be maintained. This is because they have been extensively studied and characterised, are easy to compress into dosage units, and provide a matrix that provides SR over varying periods of time depending on levels and/or combinations of polymers used.

The use of SBS to investigate the practicality of developing a design space for a hydrophilic SR matrix formulation was therefore undertaken in these studies.

CHAPTER 3
ANALYTICAL METHOD DEVELOPMENT AND VALIDATION

3.1 INTRODUCTION

3.1.1 Introduction to HPLC

High performance liquid chromatography (HPLC) is a popular method for the quantitative analysis of SBS in pharmaceutical dosage forms [90–92] and for stability studies [93,94]. HPLC is recommended as the method of choice in official compendia including the British Pharmacopoeia (BP) [23] and the United States Pharmacopeia (USP) [95] indicating that the technique is suitable for the analysis of SBS during formulation development studies.

The objective of these studies was to develop and validate an HPLC method for the quantitation of SBS in dosage forms and assessment of the rate and extent of drug release from SR formulations. The development of a simple, rapid, accurate and precise HPLC method for the assessment of SBS was therefore undertaken.

3.1.2 Principles of HPLC Separation

Analyte retention in HPLC is a complicated and poorly understood process which has been the focus of recent reports [96–101]. An understanding of the interaction between an analyte and a stationary phase in HPLC is useful for developing an efficient and robust chromatographic separation.

Three mechanisms of solute retention in HPLC have been proposed, *viz*., analyte partitioning between a mobile and stationary phase [102]; adsorption of an analyte onto non-polar surfaces of a stationary phase [103,104]; or a combination of partitioning and adsorption, where the mobile phase is preferentially adsorbed onto a stationary phase, followed by partitioning of an analyte into the adsorbed layer of mobile phase [105].

According to early partitioning models, the stationary phase is considered to be an amorphous medium and the analyte is distributed between the mobile and stationary phases, dependent on the relative affinity of an analyte for each phase. The two primary assumptions of this model are that the stationary phase plays a passive role in the retention process and there is

instantaneous equilibrium when an analyte distributes between a mobile and stationary phase [100]. The limitation of this model is that the stationary phase is indeed important in governing mechanisms of retention, and therefore the adsorption process is an important consideration in analyte retention. Adsorption is the accumulation of an analyte in close proximity to a hydrophobic stationary surface under the influence of surface forces [100]. At equilibrium, the amount of analyte accumulated on a stationary phase is in excess of that in the mobile phase. The adsorption model is considered to be a better model when compared with the partitioning model for describing retention mechanisms in HPLC, since column packing materials are composed of solid porous particles that are impermeable to analyte or mobile phase particles and provide a large surface area for analyte adsorption [100].

Alternatively, the combination of partitioning and adsorption processes may be used to explain retention mechanisms in HPLC. An organic modifier, *e.g.* acetonitrile (ACN) or methanol (MeOH), in the mobile phase may be adsorbed onto a hydrophobic stationary phase to form a thin layer. As a result, a two-stage model of adsorption followed by partitioning may be used to describe retention mechanisms in HPLC far more effectively [100]. In an isocratic elution process, the adsorbed (organic) layer can be considered to be a stationary layer, and an analyte is therefore able to partition from the mobile phase into this layer. The analyte is subsequently adsorbed onto the surface of a non-polar (reversed-phase) stationary phase. The overall retention characteristics of an analyte in HPLC are therefore a combination of these two consecutive processes. According to this model, the accurate prediction of analyte retention is based on the assumption that there is no disturbance of the adsorbed layer by a sample injected onto the system [100].

Analyte retention in HPLC also depends on the analyte-stationary phase interactions, which are influenced by the physicochemical nature of both an analyte and a stationary phase. It is generally recognised that sample retention and column selectivity can be affected by five significant factors [97,98], *viz.*,

i) Hydrophobic interactions between a dissolved solute and stationary phase,
ii) Analyte shape selectivity,
iii) Hydrogen bonding between acidic analytes and basic functional groups of the stationary phase,
iv) Hydrogen bonding between basic analytes and acidic functional groups of the stationary phase, and

v) Cation-exchange with ionised silanol groups.

It is apparent that retention mechanisms of analytes are complex and that a basic understanding of these processes is important in developing accurate and precise HPLC methods.

3.1.3 Ion-Pair Chromatography

Basic compounds are often poorly retained on silica based stationary phases, and resultant chromatograms show peak tailing and, consequently, poor chromatographic efficiency [106]. Peak tailing occurs as underivatised silanol residues on the stationary phase interact with analyte molecules. Ionised silanol groups ($-SiO^-$) are able to retain protonated bases by cation-exchange processes, and neutral silanol groups ($-SiOH$) are able to form hydrogen bonds with proton acceptor molecules, thereby influencing the retention characteristics of analytes [107]. Ion-pair chromatography is often used to obviate the shortcomings of HPLC analysis of basic samples. In ion-pair chromatography a charged ion is added to a mobile phase to improve the retention characteristics of an analyte. Common ion-pair reagents used in HPLC include alkylsulphonate and quaternary amine compounds [106].

The mechanism of solute retention in ion-pair chromatography is based on the initial adsorption of an ion-pair reagent such as sodium heptane sulphonate onto a hydrophobic stationary phase. The hydrophobic tail of the ion-pair reagent interacts with the silica-based stationary phase and the surface becomes negatively charged due to the presence of a polar head on the ion-pair molecule. Basic molecules are positively charged at low pH; they interact with the negatively charged surface of the stationary phase by ion-exchange and therefore the retention of a basic entity is achieved [106,108,109]. The use of ion-pair reagents for the analysis of weakly basic drugs improves interactions between an analyte and stationary phase, resulting in better peak shape and symmetry, and ultimately improves the accuracy, precision and sensitivity of an analytical method [106].

3.2 LITERATURE REVIEW

A summary of the chromatographic conditions that have been used for the successful separation of SBS from a variety of matrices is shown in Table 3.1.

Table 3.1 HPLC analytical methods of SBS in the literature

Column	Mobile phase	Flow rate	Detection	Retention time	Reference
Shim-pack RP18, 4.6 µm, 250 mm × 4.6 mm	60% ACN in 0.05 M phosphate buffer, pH 4.3	1.5 ml/min	UV 243 nm	1.7 ± 0.02 min	[90]
Zorbax® Extend-C_{18}, 3.5 µm, 100 mm x 2.1 mm (Agilent)	30% MeOH in a buffer at pH 3 containing 3.15 g/l ammonium formate and 5.49 g/l 1-octanesulphonic acid, sodium salt	0.2 ml/min	Photodiode array 274 nm	8.4 min	[110]
Waters® µ-Bondapak C_{18}, 300 mm x 3.9 mm	20% MeOH in 5 mM hexanesulphonic acid sodium salt in 1% glacial acetic acid	1 ml/min	UV 280 nm	Not specified	[93]
Spherical end capped C_{18}, 5 µm, 150 mm x 3.9 mm	22% ACN in phosphate buffer containing 2.87 g sodium heptanesulphonate and 2.5 g/l potassium dihydrogen phosphate, adjusted to pH 3.65 with dilute orthophosphoric acid	1 ml/min	UV 220 nm	~ 1.9 min	[23]
L1 packing, 150 mm x 4.6 mm	40% MeOH in a solution containing 1.13 g sodium hexane sulphonate, 12 ml of glacial acetic acid in 1200 ml of water	1.5 ml/min	UV 276 nm	Not specified	[95]
Nova-Pak® C_{18}, 4 µm, 100 x 8 mm	Mobile phase A: 60% THF in distilled water containing 0.025 M Waters Pic B-8 Reagent Low UV. Mobile phase B: distilled water Mobile phase C: 50% MeOH in distilled water A mixture of 50% mobile phase A and mobile phase B were delivered up to 7.7 min, then composition changed linearly to 60% mobile phase A, 15% mobile phase B and 25% mobile phase C at 13 min	2.0 ml/min	UV	3.2 min	[91]
LiChrospher® 100 RP-18, 5 µm, endcapped, 125 mm x 4 mm	40% MeOH in in water containing 2 mM potassium hydroxide and 10 mM hexanoic acid	0.4 mm	UV 214 nm	3.5 min	[111]
LiChrosorb® RP-18, 5 µm 125 mm x 4 mm	Gradient elution with ACN: Buffer 4:96 to 9:91 step gradient. Buffer containing 40 mM sodium dihydrogen phosphate, containing 5.74 mM triethylamine, adjusted to pH 3.0 with phosphoric acid	1.5 ml/min	UV 265 nm	~ 5.5 min	[112]
YMC phenyl, 5 µm, 250 mm x 4.6 mm	5% MeOH in a 25 mM potassium dihydrogen phosphate buffer at pH = 3	1.5 ml/min	UV 225 nm	9.6 min	[92]

The analysis of SBS in dosage forms and solutions has been primarily performed on silica based C_{18} packing materials [90,91,93,110–112], and this stationary phase is recommended for use in official compendia [23,95]. The use of alternative columns, such as phenyl columns, has also been reported [92].

Organic modifiers such as ACN [23,90,112] and MeOH [92,93,95,110,111] are commonly used in analytical methods reported for the analysis of SBS. Tetrahydrofuran and Waters® Pic B-8 reagent have been used in a gradient method for the simultaneous determination of SBS and other bronchodilators in nebuliser solutions [91]. The use of ion-pair reagents in HPLC analysis of SBS has been reported, and sodium salts of alkylsulphonic acids are commonly used for this purpose [93,110]. Ion-pair reagent use is also reported in compendial methods of analysis for SBS [23,95].

SBS is a weakly basic compound and buffered systems have also been used for the successful analysis of the drug using HPLC. Phosphate buffers ranging in pH between 3 and 4.5 are commonly used for SBS analysis [23,90,92,110,112], and some reports have described separations using buffer-free mobile phases [91,93,95]. UV detection is the preferred method for monitoring SBS in sample matrices [23,90–93,95,110–112].

3.3 EXPERIMENTAL

3.3.1 Reagents and Materials

SBS standard, terbutaline sulphate (TBS) the internal standard, sodium hexane sulphonate, sodium heptane sulphonate and sodium octane sulphonate were purchased from Sigma Aldrich (St Louis, MO, USA). HPLC-grade ACN (far UV) and MeOH 215 were purchased from Romil Ltd, (Waterbeach, Cambridge, UK). Sodium hydroxide pellets and potassium dihydrogen phosphate were purchased from Merck Chemicals Ltd, (Modderfontein, Gauteng, South Africa). HPLC grade water was purified using a Milli-Ro® -15 water purification system (Millipore, Bedford, MA, USA), made up of a Super-C® carbon cartridge, two Ion-X® ion-exchange cartridges and an Organex-Q® cartridge. The water was filtered through a 0.22 mm Millipak® stack filter (Millipore, Bedford, MA, USA) prior to use. All reagents were used without further preparation and were at least of analytical reagent grade.

3.3.2 Instrumentation and Analytical Conditions

The modular HPLC system used to collect data consisted of a Model P100 dual piston pump (Thermo Separation Products, San Jose, CA, USA); a Model AS100 autosampler (Thermo Separation Products, San Jose, CA, USA), which was equipped with a Rheodyne® Model 7010 injector (Rheodyne, Reno, NV, USA) fitted with a 20 µl fixed volume loop and a 250 µl GASTIGHT® Model 1725 syringe (Hamilton Co., Reno, NV, USA); a Linear UV/VIS-500 Model 6200-9060 detector (Linear Instrument Co., CA, USA); and a Spectra Physics SP 4600 integrator (Thermo Separation Products, San Jose, CA, USA). A Phenomenex® Hyperclone® column, 5 µm, 150 mm x 4.6 mm (Phenomenex, Torrance, CA, USA) was used at ambient temperature (22 °C), and the separation was achieved under isocratic conditions with UV detection at 220 nm. The volume of injection was 20 µl and a flow rate of 1.0 ml/min was used for the separation.

3.3.3 Preparation of Stock Solutions

A standard stock solution (500 µg/ml) of SBS was freshly prepared by accurately weighing approximately 10 mg of SBS into a 20 ml A-grade volumetric flask and making up to volume with mobile phase. A 10 ml aliquot of the standard stock solution was diluted in a 100 ml A-grade volumetric flask to produce a stock solution of approximately 50 µg/ml. Calibration standards in the concentration range 0–50 µg/ml were prepared by serial dilution of the stock using A-grade volumetric flasks. Seven working standards of concentrations of 2.5, 5, 10, 20, 30, 40 and 50 µg/ml were prepared when assaying dosage forms and for the analysis of samples collected during *in vitro* dissolution testing of SBS in SR formulations.

A stock solution of TBS was prepared by accurately weighing approximately 10 mg into a 20 ml A-grade volumetric flask and making up to the volume with mobile phase. 5 ml of this solution was further diluted to 100 ml in an A-grade volumetric flask to produce a solution approximately 25 µg/ml.

3.3.4 Preparation of Buffer Solutions

An 18 mM buffer solution was prepared by accurately weighing 2.50 g of potassium dihydrogen phosphate and 3.25 g sodium octane sulphonate (15 mM) into a 1000 ml A-grade volumetric flask. Approximately 700 ml HPLC grade water was added and the pH was titrated to 4 with a dilute solution of orthophosphoric acid. The pH was monitored using a

Crison Model GLP 21 pH meter (Crison Instruments, SA, Alella, Barcelona, Spain). The solution was then made up to volume using HPLC grade water.

3.3.5 Preparation of Mobile Phase

Appropriate volumes of buffer and ACN were measured separately using A-grade measuring cylinders and the individual components were then mixed in a 1000 ml Schott® bottle (Schott Duran GmbH, Wertheim, Germany). The mobile phase was filtered and degassed under vacuum using a Millipore® HVLP 0.45 µm filter membrane (Millipore, Bedford, MA, USA) and an Eyela Aspirator A-2S vacuum pump (Rikakikai Co., Ltd, Bunkyo-ku, Tokyo, Japan), respectively.

3.4 METHOD DEVELOPMENT AND OPTIMISATION

3.4.1 Introduction

Successful analytical method development depends on the careful consideration of the nature of the sample to be analysed, a review of literature and evaluation of analytical methods for the analyte of interest [106]. Furthermore an evaluation of methods for chemically related molecules and the application of methods must be reviewed in order to set appropriate standards for acceptance criteria of a method [106].

The physical and chemical nature and properties of SB and SBS have reported in Chapter 2 of this dissertation. In summary SBS is a weakly basic water-soluble drug with a pK_a of 9.3 [28]. Therefore the retention of SBS in reversed phase HPLC is likely to require the use of buffers to modulate the pH of the mobile phase and may require the use of ion-pair reagents to improve peak shape and retention characteristics of the molecule on silica-based stationary phases. In addition the aqueous solubility of SBS would require the use of relatively low concentrations of organic modifier (<40% v/v) in the mobile phase to allow for the achievement of a suitable retention time on the hydrophobic stationary phase [106].

The analytical method that is developed will be applied to assessing SBS content in dosage forms and evaluating *in vitro* release of SBS from manufactured formulations and will require multiple analyses. Consequently the method should have a relatively short analytical run time. In general, run times of less than 20 min are acceptable but run times of between 5

and 10 min are preferable to prevent interference with un-retained compounds and to expedite analyses [106].

3.4.1.1 Column Selection

Octadecyl silica is the most commonly used stationary phase reported for the analysis of SBS (Table 3.1) and therefore a bonded phase silica column was selected for use in these studies. Specifically, a Phenomenex® Hyperclone® 150 mm x 4.6 mm (Phenomenex, Torrance, CA, USA) stationary phase with a 5 µm particle size and 100 Å pore size was selected for these studies.

3.4.1.2 Method of Detection

Sensitive and selective detector systems are required for the accurate and precise quantitation of an analyte of interest. UV and photodiode array detectors have been applied to the detection of SBS in dosage form and aqueous sample analysis (Table 3.1). SBS has an absorption maximum at a wavelength of 276 nm [23,25] indicating that successful detection can be achieved using UV spectroscopy. As shown in Table 3.1, the majority of methods used for the analysis of SBS have used wavelengths in the UV range between 210 and 280 nm.

3.4.1.3 Quantitative Measurement

Peak area and/or peak height measurements are used to construct calibration curves of detector response to solutions of standard concentrations. The amount or concentration of compounds of interest in samples of unknown concentration can be extrapolated from these curves. The peak area is determined by the sum of all individual responses of sample molecules over a given period from the start to the end of a peak, but the peak height is determined by the maximal concentration of a sample that passes through a detector flow cell [106].

Both the peak height or area of a detector response to the presence of an analyte may be influenced by prevailing experimental conditions and therefore the selection of suitable analytical conditions for the analysis of SBS was based on the possibility of variability of the environmental conditions and HPLC system used in these studies. Changes in column efficiency, composition of mobile phase and temperature are more likely to affect the peak height than peak area, whereas changes in the flow rate and imprecision in solvent flow as a

consequence of pump irregularities will have a greater impact on peak area [106]. The analytical conditions in our laboratory are such that column efficiency was monitored using test conditions as specified from the Certificate of Analysis provided with the column at the time of purchase. The mobile phase was prepared using A-grade glassware and the laboratory is air-conditioned to an ambient temperature of 22 °C. Despite this standardisation there are likely to be changes in flow rate during analysis which has a greater impact on peak area measurements compared with peak height measurements, and therefore peak height was chosen as the preferred method of quantitation in these studies.

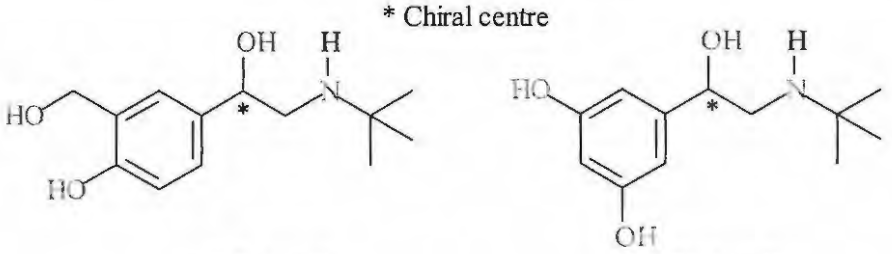
3.4.1.4 Internal Standard Selection

Calibration may be performed with the use of internal standards where a different compound with similar structural and physicochemical properties, and hence chromatographic retention characteristics, is added at a known concentration to all samples containing the analyte of interest, prior to manipulation of those samples. The compound selected for use as an internal standard must be well resolved from the peaks of interest and the ratio of the measured response of the analyte to that of the internal standard is used for the generation of the calibration curve and for subsequent quantitative measurements [106,113]. Furthermore it should be commercially available as high purity material and be chemically stable with little or no reactivity with the analyte of interest and/or the mobile phase [106].

The use of an internal standard is recommended in cases where the variability of instrument response is prevalent and its use will enhance the precision of analytical methods. Sources of variability in HPLC analyses include injection variability, flow rate variation and different detector responses for samples of the same concentration. In such cases, the use of an internal standard compensates for instrumental variability and results in an increase in the accuracy and precision of an analytical method [113].

Ethenzamide [114], fenoterol bromide [115], mepivacaine hydrochloride 1% [91] and bamethan sulphate [116] have been used as internal standards in chromatographic analyses developed for the quantitation of SBS.

Terbutaline (TB), 2-*tert*-butylamino-1-(3,5-dihydroxyphenyl) ethanol sulphate [117] is a β_2 agonist that has similar structural and chemical properties to SB as shown in Figure 3.1.



Salbutamol Terbutaline

Figure 3.1 Chemical structures of SB and TB

Both molecules possess a β-phenyl-β-ethanol amine group but have different substituents on the phenyl ring. TB has two hydroxyl functional groups on the phenyl ring whereas SB has a hydroxyl and an $-CH_2OH$ substituent on the phenyl ring. The retention characteristics of SB and TB in HPLC are therefore likely to be similar. The sulphate salt of TB was selected as an internal standard for the analytical method developed for the analysis of SBS.

3.4.2 Mobile Phase Composition

The chromatographic conditions described in the BP [23] and USP [95] were selected as the initial settings for the commencement of method development and optimisation. Mobile phase compositions of 40% v/v MeOH in a solution containing 1.13 g sodium hexane sulphonate and 12 ml of glacial acetic acid in 1200 ml of water [95], or 22% v/v ACN in phosphate buffer containing 2.87 g sodium heptane sulphonate and 2.5 g/l potassium dihydrogen phosphate adjusted to pH 3.65 with dilute orthophosphoric acid [23], were used at the start of the study. The resultant chromatograms developed using these conditions are depicted in Figure 3.2.

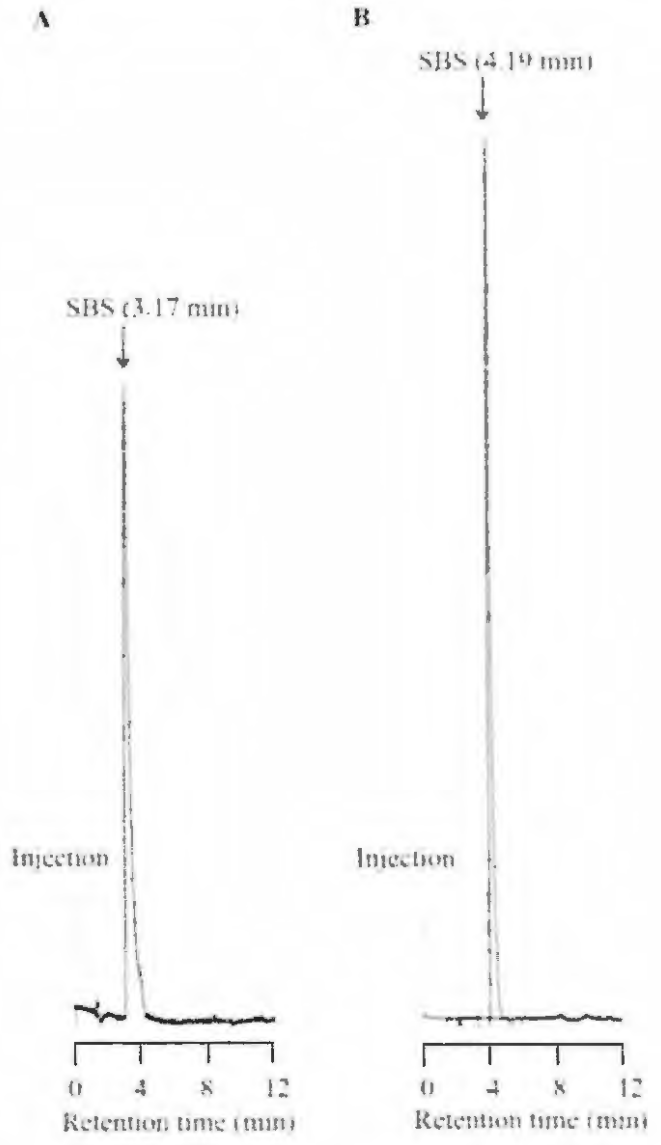


Figure 3.2 Representative chromatograms developed using USP [95] (A) or BP [23] (B) chromatographic conditions

The efficiency of each of the separations was assessed by considering the retention factor k, plate number N, peak asymmetry and peak tailing factors calculated using Equations 3.1 and 3.2 and Figure 3.3, respectively. The results of these analyses are summarised in Table 3.2.

$$k = \frac{t_R - t_0}{t_0}$$ **Equation 3.1**

$$N = 5.54\left(\frac{t_R}{W_{1/2}}\right)^2$$ **Equation 3.2**

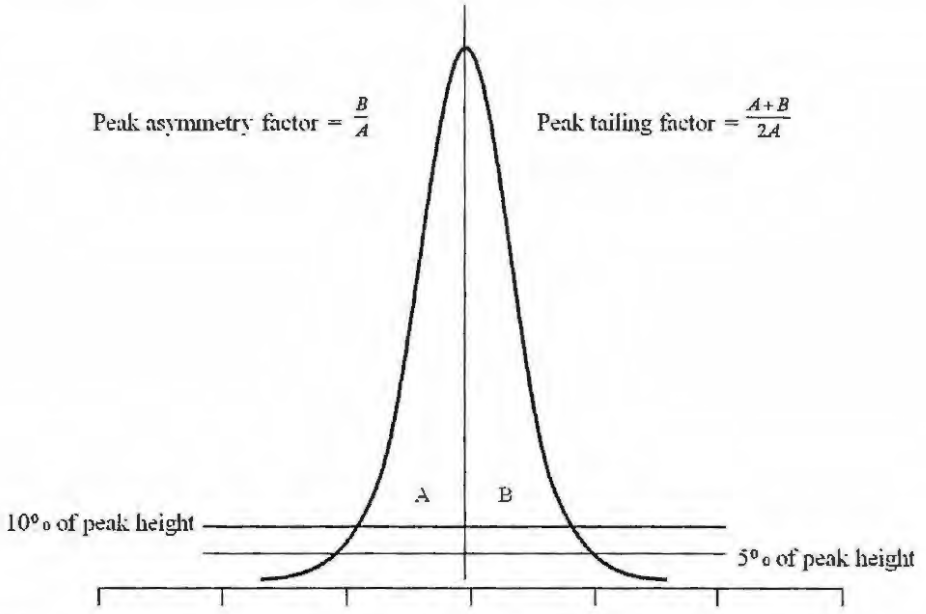


Figure 3.3 Schematic representation for the determination of peak asymmetry and peak tailing factors

Table 3.2 Chromatographic parameters calculated using compendial methods of analysis for SBS

Chromatographic parameter	USP Method	BP Method
Retention factor, k	1.11	1.79
Plate number, N	222	1556
Peak asymmetry factor	1.43	1.09
Peak tailing factor	1.24	1.14

The retention factor k refers to the position of a peak relative to the dead volume of the column or the retention of un-retained substances. If a molecule has a longer retention time than un-retained substances then the retention factor is larger than if the compound were retained for a shorter period, and therefore there is less likelihood of interference with un-retained substances. The retention factor of SBS when BP conditions are used is higher than that observed when USP analytical conditions are used. The plate number N is an indication of column efficiency which is higher when BP test conditions, as opposed to USP conditions, are used. Both peak symmetry and peak shape are better when using BP conditions compared with the USP method, showing that peaks are more regular and would therefore permit more accurate quantitation.

The differences in selectivity and efficiency of chromatographic separation observed when ACN and MeOH are used as organic modifiers are based on the ability of the organic phase to form hydrogen bonds with water. ACN is unable to accept or donate protons, whereas MeOH has the ability to form hydrogen bonds with water, and this affects the efficiency of a

separation [118]. This difference in interaction results in the production of sharper and more symmetrical peaks when ACN is used compared with MeOH as shown in Figures 3.2A and 3.2B and summarised in Table 3.2. This phenomenon will affect the percent concentration of organic modifier that is required in a mobile phase, and higher concentrations of MeOH are required for the adequate retention of SBS compared with instances when ACN is used as the organic modifier [119].

The effect of changing mobile phase composition on the retention times in both mobile phase systems are shown in Figures 3.4 and 3.5 for the USP and BP methods respectively.

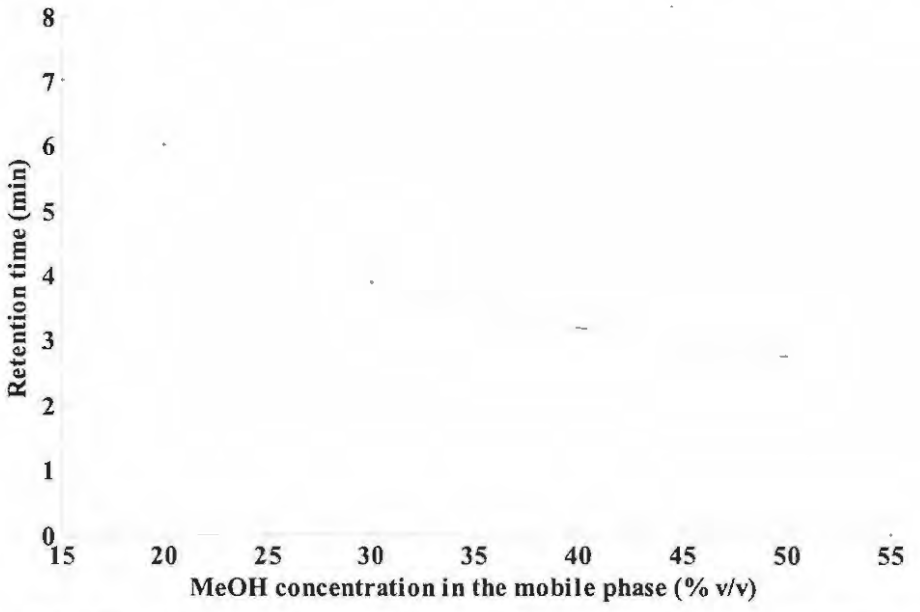


Figure 3.4 Effect of MeOH concentration on the retention time of SBS using the USP method

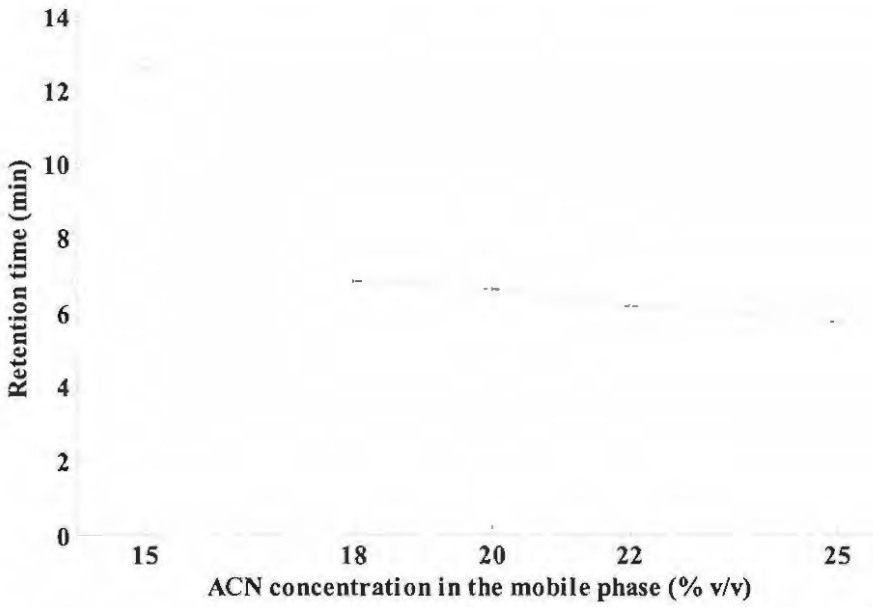


Figure 3.5 Effect of ACN concentration on the retention time of SBS using the BP method

Both figures reveal that an increase in the organic modifier content results in a corresponding decrease in the retention time for SBS. As the percent of organic modifier increases, the solubility of the drug in the mobile phase increases promoting rapid elution of SBS from the column. When the concentration of MeOH is increased from 20% v/v to 50% v/v the retention time decreases from approximately 6 min to 3 min. When ACN is used over the concentrations of organic modifier studied, a rapid decrease in retention time of approximately 7 min is observed when the percent organic modifier is increased from 15% v/v to 18% v/v (from approximately 12.2 min to 5.5 min), and thereafter, the decrease in retention time observed for ACN concentrations between 18% v/v and 25% v/v is a further 2 min.

The results indicate that a mobile phase composition of 20% v/v ACN based on the mobile phase specified in the BP [23] would provide a separation with an adequate retention time for SBS. TBS was then added to a solution of SBS and a representative chromatogram showing the separation of SBS and TBS under these chromatographic conditions is depicted in Figure 3.6.

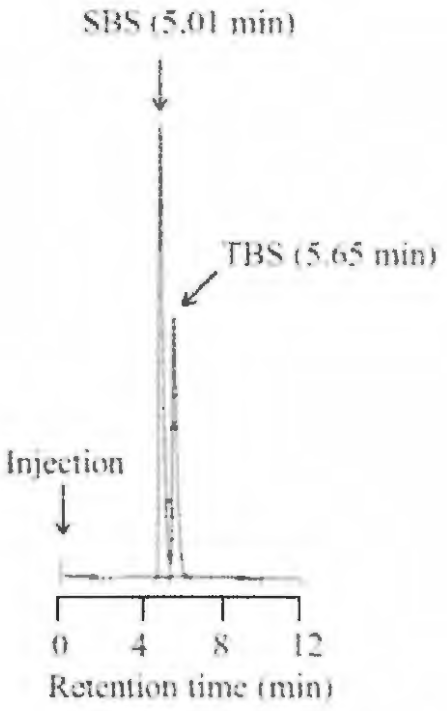


Figure 3.6 Typical chromatogram showing the separation of SBS and TBS using 20% v/v ACN in 18 mM phosphate buffer at pH 4, containing 15 mM sodium octane sulphonate

The resolution of two adjacent bands in an HPLC separation can be calculated by Equation 3.3 [106], where the resolution between two peaks is a function of the distance between the two peaks and the average of the peak width of the compounds of interest.

$$R_S = \frac{2(t_2 - t_1)}{W_1 + W_2} \qquad \textbf{Equation 3.3}$$

Where,

R_s = resolution factor
t_i = retention time for peak i
w_i = peak width of peak i

The resolution between the two peaks of interest was 0.366 which is indicative of a significant overlap, and therefore the chromatographic conditions were further optimised by changing the ion-pair reagent and adjusting the pH of the buffer used to prepare the mobile phase.

3.4.3 Effect of Ion-Pair Reagent

The effect of using ion-pair reagents of different chain length was investigated as a means of improving the resolution between SBS and TBS peaks. The impact of using reagents with an increased alkyl chain length of the ion-pair reagent by two carbons from 6–8 (*i.e.* 0.015M sodium salts of hexane, heptane and octane sulphonic acid) is depicted in Figure 3.7.

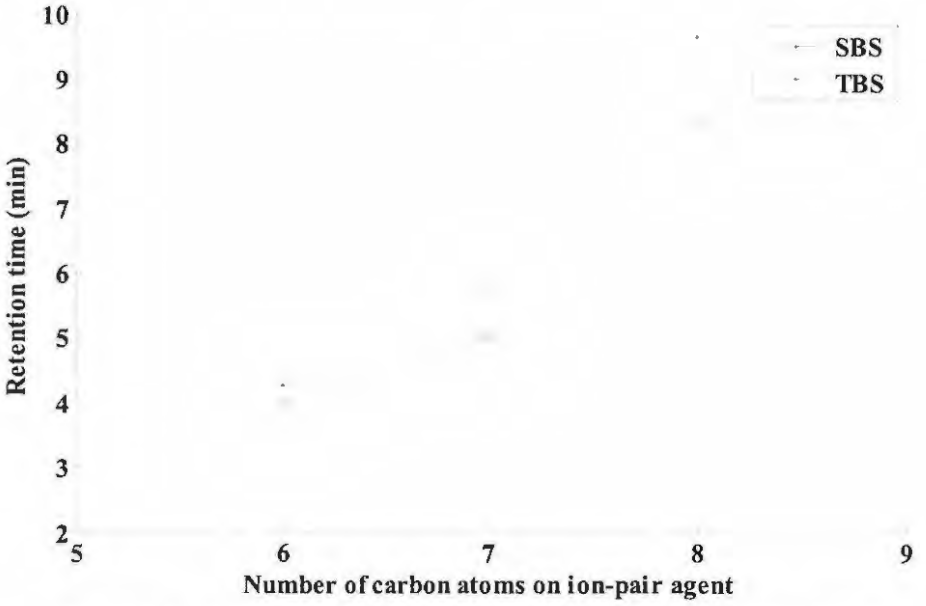


Figure 3.7 Effect of ion-pair reagent on the retention time of SBS and TBS

It is apparent that the retention times of SBS and TBS are dependent on the length of the alkyl chain of the ion-pair reagent used in the mobile phase and that the retention times of both SBS and TBS increase with an increase in alkyl chain length. Furthermore the resolution between SBS and TBS is also enhanced with the increase in alkyl chain length of the ion-pair reagent.

The resultant charge following binding of ion-pair reagents is a major factor that governs analyte retention on the surface of a stationary phase and it is not necessarily the interaction between non-polar alkyl chains and analytes that improves the separation [108]. There is a dependence of retention time on the length of the carbon chain of the ion-pair reagent but identical separations may be achieved with different ion-pair reagents. However, the concentration of ion-pair reagent required for shorter length alkyl sulphonates is higher than that required when using ion-pair reagents with longer alkyl chains [106,108]. If the same concentration of ion-pair reagent is used in a chromatographic separation, then longer length alkyl sulphonates will result in longer retention times as was observed in this study. The

separation achieved using sodium octane sulphonate was further optimised by changing the pH of the buffer used to prepare the mobile phase.

3.4.4 Effect of Buffer pH

The effect of buffer pH on the retention times of SBS and TBS in a solution containing sodium dihydrogen phosphate buffer and sodium octane sulphonate as an ion-pair reagent is shown in Figure 3.8. The pH range of the buffer tested was between 3.65 and 7 and the resultant effects on resolution and retention times are summarised in Table 3.3.

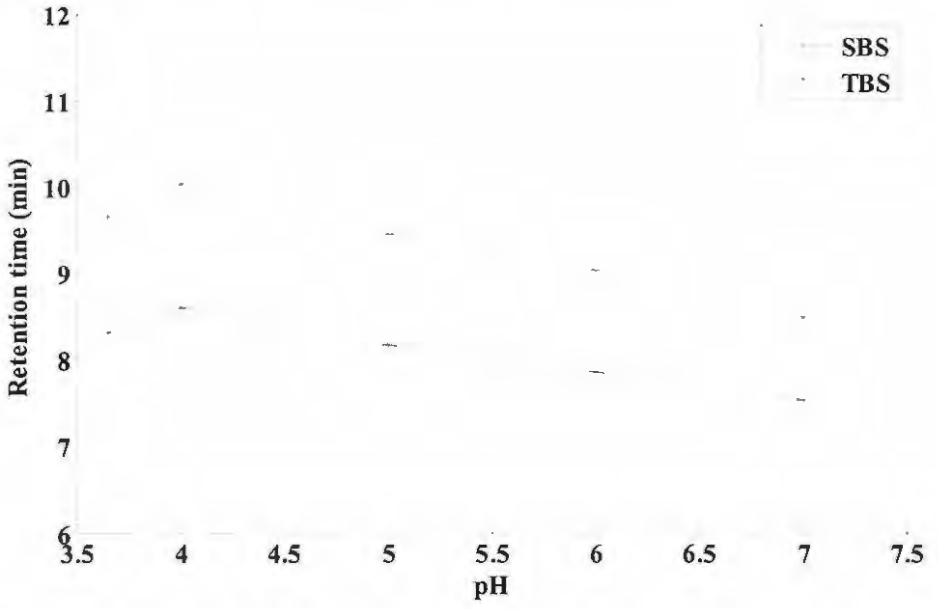


Figure 3.8 Effect of buffer pH on the retention time of SBS and TBS

Table 3.3 Retention times and resolution of SBS and TBS as a function of pH

pH	Retention time of SBS	Retention time of TBS	Resolution
3.65	8.31	9.65	0.766
4	8.61	10.0	1.014
5	8.16	9.45	0.860
6	7.85	9.04	0.680
7	7.53	8.50	0.554

These results reveal that the retention time of SBS and TBS is pH dependent and that increasing the pH from 3.65 to 4 results in an initial increase in the retention time, which declines as the pH is further increased. This phenomenon is likely due to the mechanism of retention of basic entities in ion-pair chromatography. The surface of the stationary phase in alkyl sulphonate chromatographic systems is negatively charged, and at low pH, protonated basic molecules are highly retained on the analytical column. In ion-pair chromatography,

cation-exchange is the dominant process of analyte retention [106,109], but as the pH is increased, basic molecules become neutral and reversed-phase retention characteristics become prominent; a solute will therefore have a low affinity for the charged surface [106,109]. Consequently the retention time of weakly basic drugs such as SBS and TBS decreases as the pH of the buffer used in the mobile phase is increased, as shown in Figure 3.8.

The retention characteristics of SBS and TBS follow a similar trend, and optimum resolution was achieved using a buffer of pH 4. Although higher values for resolution are often recommended, this value was considered appropriate since baseline resolution was evident and the method would be appropriate for its intended purpose.

3.4.5 Chromatographic Conditions

The optimal chromatographic conditions that were established for the quantitative determination of SBS are summarised in Table 3.4, and a typical chromatogram of a separation achieved using the optimised conditions is shown in Figure 3.9.

Table 3.4 Optimised chromatographic conditions for the quantitation of SBS in pharmaceutical dosage forms

Column	Phenomenex® Hyperclone® C_{18}, 5 µm, 150 x 4.6 mm
Mobile phase	20% v/v ACN in 18 mM phosphate buffer at pH = 4, containing 15 mM sodium octane sulphonate
Detection wavelength	220 nm
Detection sensitivity	0.005 AUFS
Injection volume	20 µl
Chart speed	2.5 mm/min
Temperature	22 °C
SBS retention time	8.10 min
TBS retention time	9.55 min

Figure 3.9 Representative chromatogram of SBS and TBS using the optimised mobile phase

3.5 METHOD VALIDATION

3.5.1 Introduction

The objective of validation of an analytical procedure is to demonstrate that the method is suitable for its intended purpose [120]. Guidelines for the validation of analytical procedures have been issued by the ICH [120] and include specific identification tests, quantitative and limit tests for impurities and assay procedures. These parameters were used for the purposes of validating an analytical procedure for application in assaying and dissolution testing in this study. According to the ICH guidelines [120], typical validation characteristics that must be

investigated include the linearity, precision, accuracy, limits of quantitation and detection, and sample stability.

3.5.2 Linearity

The linearity of an analytical procedure is defined as the ability of that procedure to produce results that are directly proportional to the concentration of analyte in samples [120]. Calibration plots were constructed using seven concentrations in the range 0–50 μg/ml and were analysed in replicates (n = 5) to test the linearity of the response of the analytical method. The ratio of peak heights of SBS to TBS was plotted against the corresponding concentrations of SBS to produce a calibration curve. Linearity was evaluated by least squares linear regression analysis of the data using the Curve Fitting Toolbox on Matlab® R2008a (Mathworks Inc., Natick, MA, USA).

The results of the linearity studies are summarised in Table 3.5 and Figure 3.10 shows a typical calibration curve generated using the analytical method developed in these studies. The linearity results indicate that the variability of the individual measurements is low and therefore error bars are not visible in Figure 3.10.

Table 3.5 Statistical analysis of calibration curve data generated during HPLC analysis of SBS (n = 15)

Regression equation, $y = mx + c$	
Slope, m (95% CI)	0.0289 ± 0.000217 (0.0286, 0.0293)
Intercept, c (95% CI)	0.00169 ± 0.00236 (-0.0110, 0.00756)
Sum square of errors (SSE)	0.000163
R^2	0.9999
$R^2_{adjusted}$	0.9999
Root mean square error (RMSE)	0.00571

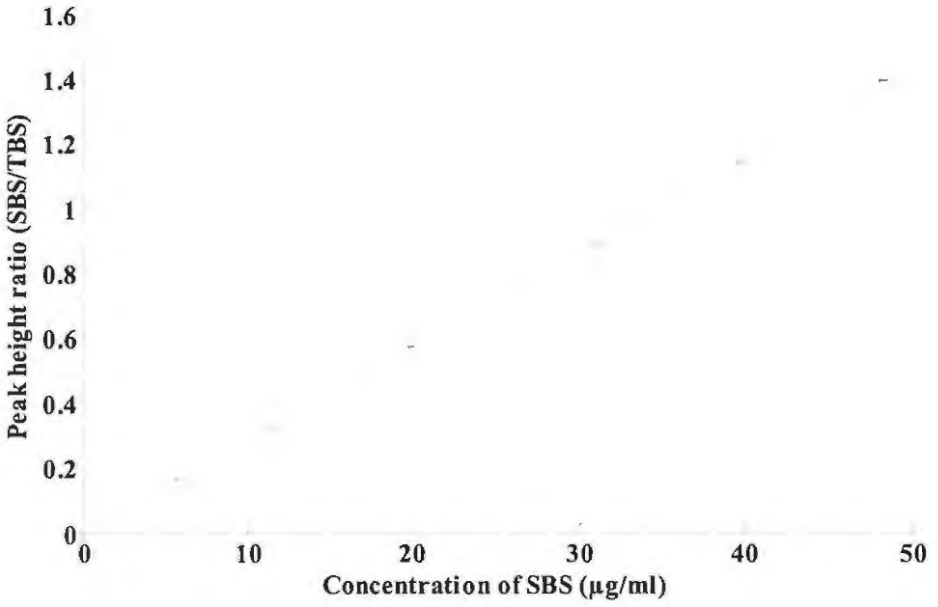


Figure 3.10 Calibration curve for the HPLC determination of SBS, $y = 0.0289x + 0.00170, R^2 = 0.9999$

3.5.3 Range

The range of an analytical method refers to the interval between the upper and lower concentrations of an analyte of interest in a sample matrix for which accuracy, precision and linearity have been demonstrated [120]. The range of the method was 0.395–50 µg/ml.

3.5.4 Precision

The precision of an analytical method refers to the closeness of agreement between a series of measurements obtained from multiple testing of the same homogenous sample under prescribed conditions. Precision may be defined at three levels, *viz.*, repeatability (intra-day precision), intermediate precision (inter-day precision) and reproducibility [120].

3.5.4.1 Repeatability (Intra-assay precision)

Repeatability refers to precision of measurements taken under the same operating conditions over a short interval of time [120]. Repeatability was calculated following replicate injections (n = 3) of freshly prepared SBS solutions at low, medium and high concentration located within the previously determined calibration range on Day 1. A summary of the intra-assay precision for the low, medium and high concentrations studied is listed in Table 3.6.

Table 3.6 Intra-assay precision data

	Low	Medium	High
1	0.242	0.712	1.20
2	0.243	0.714	1.19
3	0.243	0.712	1.19
Average	0.243	0.713	1.19
Standard deviation	0.000494	0.000737	0.000504
% RSD	0.204	0.103	0.0421

These results reveal that the method is precise and the % RSD values ranged between 0.0421 and 0.204% RSD for the samples studied.

3.5.4.2 Intermediate Precision (Inter-Day Precision)

Intermediate precision refers to the precision of a method with reference to variability within a laboratory such as running analyses on different days and/or the use of different equipment or analysts [120]. The experiment was repeated by analysing freshly prepared solutions at the same concentrations on two additional and consecutive days to determine intermediate precision of the method. Peak height ratios of SBS: TBS were calculated and the percent Relative Standard Deviation (% RSD) of the peak height ratio was reported. Analysis of variance (ANOVA) was performed to determine if there were any significant differences between the results obtained on each of the days 1, 2 and 3.

A summary of the data generated on three consecutive days of analysis, and the results of ANOVA conducted to establish whether differences between low, medium and high concentrations exist are shown in Tables 3.7 and 3.8, respectively.

Table 3.7 Intermediate precision data

	Low	Medium	High
Day 1	0.242	0.712	1.20
	0.243	0.714	1.19
	0.243	0.712	1.19
Average	0.243	0.713	1.19
Standard deviation	0.000494	0.000737	0.000504
% RSD	0.204	0.103	0.0421
Day 2	0.242	0.713	1.19
	0.243	0.713	1.19
	0.243	0.712	1.20
Average	0.243	0.713	1.19
Standard deviation	0.000577	0.000577	0.00577
% RSD	0.238	0.0810	0.484
Day 3	0.242	0.713	1.19
	0.242	0.712	1.20
	0.243	0.712	1.19
Average	0.242	0.712	1.19
Standard deviation	0.000577	0.000577	0.00577
% RSD	0.238	0.0811	0.483

Table 3.8 Results of ANOVA of intermediate precision data

	Sum of squares	Degrees of freedom	Mean square	F-ratio	Prob >F
Low					
Columns	4.51e-008	2	2.25e-008	0.26	0.782
Error	5.29e-007	6	8.82e-008		
Total	5.74e-007	8			
Medium					
Columns	3.81e-007	2	1.91e-007	0.6	0.580
Error	1.91e-006	6	3.18e-007		
Total	2.29e-006	8			
High					
Columns	1.45e-006	2	7.25e-007	0.89	0.460
Error	4.91e-006	6	8.19e-007		
Total	6.362e-006	8			

The intermediate precision of method was found to be adequate, and p values greater than 0.05 indicate that there are no significant differences between the means of the peak height ratios that were generated on different days on which the analyses were conducted.

3.5.5 Reproducibility

Reproducibility is defined as the precision of a method conducted in different laboratories, such as for collaborative studies [120]. Reproducibility was not assessed in this study since the method was used for analysis in one laboratory by the same analyst.

3.5.6 Accuracy

The accuracy of an analytical procedure is defined as the closeness of agreement between an accepted reference value and the value that is determined by use of an analytical procedure [120]. The accuracy of the method was tested by analysing samples at low, medium and high concentrations located within the calibration range and assessing the percent recovery, bias and % RSD for each level tested. Peak height ratios of SBS: TBS were calculated and accuracy was reported as the percent recovered in the samples using a freshly constructed calibration curve. The accuracy results obtained for samples at low, medium and high concentrations are summarised in Table 3.9.

Table 3.9 Results of accuracy studies

	Concentration				
	Actual	**Determined**	**RSD (%)**	**Recovery (%)**	**Bias (%)**
Low	8.48	8.69	0.397	102.4	-2.46
	8.48	8.48	0.138	99.95	0.0489
	8.48	8.51	0.103	100.3	-0.278
Medium	25.5	25.4	0.0714	99.93	0.0646
	25.5	25.4	0.0984	99.67	0.321
	25.5	25.4	0.0666	99.82	0.183
High	42.4	42.6	0.0873	100.3	-0.321
	42.4	42.0	0.0614	98.96	1.04
	42.4	42.1	0.0595	99.13	0.872

The analytical method was determined to be accurate with the percent recovery ranging between 98.9 and 102.4% and the percent bias ranging between –2.46 and 1.04% under the conditions studied.

3.5.7 Limits of Quantitation (LOQ) and Detection (LOD)

The LOQ for an analyte using an analytical procedure is the lowest amount of analyte in a sample that can be measured with suitable precision and accuracy [120]. The LOD for an analyte is the lowest amount of analyte in a sample that can be detected under the specified conditions, but not necessarily quantitated [120]. The LOQ and LOD were established by injecting progressively decreasing concentrations in the range 0–2.5 µg/ml of the working standard solution and constructing a calibration curve in this region. The LOQ and LOD were established using Equations 3.4 and 3.5.

$$LOD = \frac{3.3\sigma}{S} \qquad \textbf{Equation 3.4}$$

$$LOQ = \frac{10\sigma}{S} \qquad \textbf{Equation 3.5}$$

Where,

σ = the standard deviation of the response
S = the slope of the calibration curve

The slope was estimated from the calibration curve in the region of the LOQ and LOD, and the standard deviation of the response was determined from the residual standard deviation of the y-intercept of the regression lines in the range of the LOQ and LOD. Samples were tested in replicates (n = 6) and the % RSD was calculated from the variation in the peak height ratio of SBS to TBS. A calibration curve generated in the lower end of the concentration range for the analysis of SBS (0–3 µg/ml) is depicted in Figure 3.11.

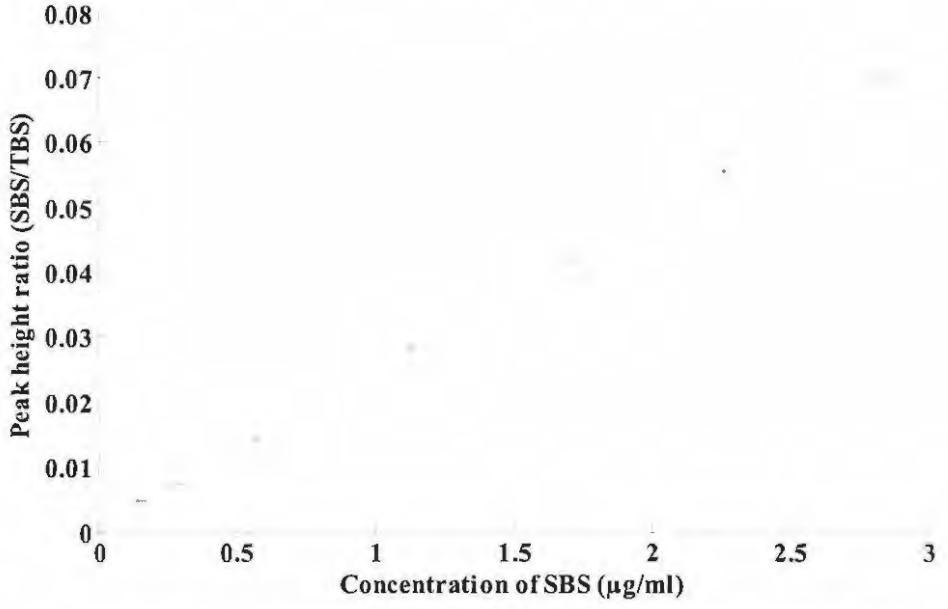


Figure 3.11 Calibration curve in the region of the LOQ and LOD for SBS, $y = 0.0244x + 0.000502, R^2 = 0.9999$

The LOQ and LOD were found to be 0.395 µg/ml (1.04 %RSD) and 0.131 µg/ml using the specified test conditions, respectively.

3.5.8 Short-Term Sample Stability

Short-term sample stability was determined over three consecutive days for drug sample solutions that were stored at ambient temperature (22 °C) (*i.e.* on the laboratory bench), in a refrigerator (4 °C) and on a windowsill in direct sunlight. The chromatograms were compared with those developed from analysing freshly prepared standards. In particular, stability was assessed by interpolating the drug content using a freshly constructed calibration curve by

computing the SBS: TBS ratio and by visually inspecting the chromatograms for the presence of additional peaks that may be attributed to degradation. The resultant chromatograms did not reveal any additional peaks compared with freshly prepared samples and there were no significant differences between the peak height ratios of the freshly prepared and stored samples. Therefore, it may be concluded that analyses may be conducted over three days with no compromise in the precision or accuracy of the results with respect to sample stability.

3.6 CONCLUSIONS

The successful application of analytical methods in the pharmaceutical sciences for different purposes including assaying solid oral dosage forms and the generation of *in vitro* dissolution profiles during formulation development studies depends on the use of an optimised and validated analytical method. An appropriate analytical method must ensure that the compounds of interest are well resolved and free of possible interference from excipients that are used in pharmaceutical formulations. Successful chromatographic resolution is a function of the combination of many factors including the use of an appropriate column, mobile phase composition, buffer molarity and pH, temperature and flow rate.

The use of silica columns for the separation of SBS is well documented and therefore a silica-based C_{18} column was chosen as an appropriate starting point for the optimisation of an analytical method for the analysis of SBS. TBS was used as an internal standard to improve the reproducibility of the method. A comparison of analytical methods that were described in the USP [95] and BP [23] revealed that the BP method resulted in chromatographic separations with better peak shape. This method was therefore optimised in terms of mobile phase composition, ion-pair reagent type and buffer pH to ensure an optimal separation.

An increase in the organic modifier content of the mobile phase resulted in a decrease in retention time and it was observed that increasing the length of the carbon chain of the ion-pair reagent resulted in better retention and resolution of SBS and the internal standard, TBS. Therefore a mobile phase comprised of 20% v/v ACN and 15 mM sodium octane sulphate was further optimised by adjustment of buffer pH to improve the resolution between the drug and internal standard. Increasing the pH of the buffer between 3.65 and 7 resulted in an initial increase in retention time at pH 4 followed by a gradual decrease in retention time between pH 5 and 7. A buffer of pH 4 was selected for the separation and therefore a mobile phase

consisting of 20% v/v ACN in 18 mM phosphate buffer at pH 4 and 15 mM octane sulphonate was used during method validation studies conducted according to ICH guidelines.

The method was found to be linear within the range 0.395–50 µg/ml and was found to have a coefficient of determination of 0.9999. The method was also precise, indicating the method was reproducible and day-to-day variation was minimal. Intra-assay precision ranged between 0.0421 and 0.203% RSD, and ANOVA revealed that there were no significant differences between SBS: TBS peak/height ratios for low, medium and high concentrations studied over three consecutive days. The method was also found to be accurate and the % bias ranged between -2.46 and 1.04%. The method can therefore be applied for the quantitation of SBS in dosage forms and to establish the rate and extent of drug release from SR dosage forms during dissolution studies.

The application of an accurate, precise and reliable analytical method is critical to the success of formulation development activities using the principles of QbD and when establishing a design space. The method developed in this study was validated and was used to assist in making formulation decisions for the establishment of a design space for a SR dosage form for SBS.

CHAPTER 4

DEVELOPMENT OF SUSTAINED RELEASE FORMULATIONS

4.1 INTRODUCTION

4.1.1 Sustained Release Dosage Forms

Immediate release formulations are designed to release the entire dose of API from a dosage form following administration, in order to ensure rapid and complete absorption. The result is that there is a rapid onset of pharmacological activity, but following completion of the absorption phase the plasma profile declines if no other dose is administered. The resultant plasma profile is dependent on the pharmacokinetic characteristics of the API and often plasma levels fall below the minimum effective concentration of that API. If a drug candidate has a short half-life due to rapid plasma clearance, repeated administration of the compound is necessary to ensure that plasma levels are maintained above a minimum effective concentration in order to ensure a constant pharmacological response [65].

In contrast, solid oral dosage forms manufactured as modified release formulations release API at a specific rate or are specifically designed to alter the time of API release in the GIT. The USP Subcommittee on Biopharmaceutics [121] defines a modified release dosage form "as one for which the drug-release characteristics of time course and/or location are chosen to accomplish therapeutic or convenience objectives not offered by conventional dosage forms such as solutions, ointments, or promptly dissolving dosage forms, as presently recognised".

Several categories of modified release dosage forms have been documented including extended release drug products (including SR and controlled release technologies) which, in general, allow for at least a two-fold reduction in dosing frequency compared with immediate release dosage forms. Additional technologies include delayed release drug products that discharge API at a time other than immediately after administration and targeted release technologies that are designed to release API at or near the site of action [65]. The term SR will be used to describe the modified release dosage forms that were developed, manufactured and assessed in these studies.

Following administration of a SR formulation via the peroral route, API is released from a dosage form at a steady rate and is continuously made available for absorption into the systemic circulation. Gradual absorption results in a steady increase in drug plasma levels that are maintained for longer periods compared with those observed following administration of immediate release dosage forms. Although there is a slow onset of action when an API is administered in a SR formulation the control of symptoms of diseases and therapeutic outcomes are often better than those observed when using treatment regimens in which immediate release dosage forms are administered [65,122].

The advantages of using SR formulations include the minimisation of fluctuations in drug plasma concentrations, which in turn provides a constant and consistent clinical response in patients, as long as plasma levels are maintained above a minimum effective concentration [65,122]. The development of SR formulations for an API that has a short half-life and/or a short duration of action may provide clinical benefits for patients since constant drug plasma concentrations may be achieved. In addition the administration of SR formulations often results in better patient adherence to therapy as a consequence of a reduced dosing frequency [65].

The use of SR formulations is not without limitations and these include variable and/or erratic drug absorption due to changes in GIT motility that may result in therapeutic failure. There is also a possibility of dose dumping of large doses of API due to dosage form failure since SR dosage forms are usually formulated with an excess of drug and such failure may result in GIT irritation and systemic toxicity. However, despite the limitations of these dosage forms, it is apparent that the use of SR formulations in therapy has significant advantages for the optimisation and achievement of appropriate therapeutic outcomes.

4.1.2 Hydrophilic Matrix Formulations

Hydrophilic monolithic matrix devices are commonly used as SR dosage forms due to their ease of manufacture and the nature of this relatively well understood technology. The rate of drug release from monolithic devices can be modulated by the level and type of polymer or combinations of polymers that are used to manufacture a formulation. The inclusion of polymeric adjuvants changes the microscopic porosity and tortuousity of matrices thereby affecting both the rate and mechanism of drug release [123].

Following immersion of hydrophilic matrix tablets into aqueous media, the polymer hydrates, swells and increases in size after which the matrix dissolves and/or erodes with time [124,125]. The penetration of an aqueous medium into the interior of the tablet matrix results in the formation of three distinct regions within the matrix, *viz.*, the inner dry polymer where water has not yet penetrated, the outer rubbery hydrated phase, and a swelling or moving front that is located between the hydrated state and the outer rubbery hydrated gel phase [126,127]. The dissolution of a drug in the swollen matrix takes place at the interface between the inner dry matrix and rubbery polymer surface, and the dissolved drug diffuses in a radial direction from the swelling front towards the bulk aqueous medium [128].

Early studies have shown that drug release from swellable hydrophilic matrices is dependent on the thickness of the hydrated gel layer that is formed during the swelling phase of polymer hydration [124,125]. The degree of swelling determines the diffusional path length of a drug and the thicker the gel layer the slower the rate of drug release from a matrix [127]. The thickness of the gel layer increases during the swelling phase and then remains constant in what is known as the synchronisation phase (*i.e.* where the rates of swelling and erosion are equal) and eventually decreases as polymer erosion becomes dominant. The relative extent of swelling and erosion is dependent on the nature and characteristics of a polymer or polymer combinations that are used to manufacture a dosage form [129].

Hydrophilic matrix formulations can be manufactured by direct compression [130–134], wet granulation [135–137], hot-melt extrusion [138] or roller compaction [139]. Direct compression is often the method of choice due to its relative simplicity, the ease of processing, and the possibility of developing a stream-lined and cost-effective manufacturing process for SR matrix systems.

4.1.3 Polymeric Materials

HPMC is one of the most commonly used hydrophilic matrix formers for SR formulations as it is non-toxic and has wide regulatory acceptance. The use of HPMC also makes it possible to incorporate large doses of drug into SR formulations and reports suggest that dosage form performance and product quality of HPMC matrices is not significantly affected by manufacturing process variables [140]. HPMC has been the polymer of choice for the manufacture of a number of SR formulations and its use and applications are well

documented [82,123,128,130,133,141,142]. The mechanism of drug release and swelling of HPMC matrices has also been extensively studied [128,131,135,143–146].

Although HPMC is a commonly used matrix forming material, other polymers that have potential use as matrix forming materials include Carbopol® [134,147,148], xanthan gum [127,149], sodium carboxy methylcellulose (SCMC) [128,150] and hydroxypropyl cellulose (HPC) [133,145,151]. Therefore the use of HPMC, as a primary excipient and other polymers as potential adjuvants, was investigated in these studies in order to evaluate their potential for the retardation of SBS release from hydrophilic matrix tablets.

The objective was to assess the suitability of HPMC in combination with different polymers for the retardation of SBS release from formulations manufactured in our laboratory. In addition, risk assessment was conducted in order to facilitate the implementation of a well controlled statistically designed experiment (Chapter 6 *vide infra*) aimed at understanding the impact of formulation variables on SBS release and to manufacture optimised matrix formulations with predetermined drug release characteristics.

4.2 MATERIALS AND METHODS

4.2.1 Materials

SBS was donated by Aspen-Pharmacare (Port Elizabeth, Eastern Cape, SA). Methocel® K100M and Methocel® K4M (HPMC) (Dow Chemical Company, Midland, MI, USA), Avicel® PH101 (FMC BioPolymer, Philadelphia, PA, USA), SCMC (Aspen-Pharmacare, Port Elizabeth, Eastern Cape, SA), Carbopol® 71G (Lubrizol, Wickliffe, OH, USA), Klucel® EF (HPC) (Aspen-Pharmacare, Port Elizabeth, Eastern Cape, SA), xanthan gum (Aspen-Pharmacare, Port Elizabeth, Eastern Cape, SA), Surelease® (Colorcon, West Point, PA, USA) magnesium stearate (Aspen-Phamacare, Port Elizabeth, Eastern Cape, SA) and colloidal silica (Aspen-Pharmacare, Port Elizabeth, Eastern Cape, SA) were used as received. All other reagents were at least of analytical reagent grade and used as received.

4.2.2 Manufacture of Formulations

Hydrophilic matrix tablets were manufactured using direct compression. Batch sizes of 1000 tablets were produced for each formulation that was manufactured. The API, SBS, Methocel®

K100M, additional polymers where required, and Avicel® PH101 were dry blended using a Saral® Rapid Mixer and Granulator (Saral Engineering Company, Mumbai, Maharashtra, India) in a 5 L bowl using a speed of 100 rpm for the main impeller for 15 min. Thereafter 1% w/w magnesium stearate and 0.5% w/w colloidal silica were added and the blend mixed at the same speed for a further 3 min. The blend was transferred to a feed hopper and tablets were compressed on a Manesty® F3 single punch tablet press tooled with 7 mm flat-faced round punches to a uniform weight of 140 mg. In-process control was achieved my monitoring environmental conditions such as temperature and humidity, and process conditions including, dry mixing time, speed of the motor, and tablet weight during the compression cycle. A summary of the composition of the batches that were manufactured is shown in Table 4.1.

Table 4.1 Unit formulae for SBS tablets

	SBS-01 (mg)	**SBS-02 (mg)**	**SBS-03 (mg)**	**SBS-04 (mg)**	**SBS-05 (mg)**	**SBS-06 (mg)**
SBS	9.6	9.6	9.6	9.6	9.6	9.6
Methocel® K100M	70	70	70	70	70	70
Methocel® K4M	-	28	-	-	-	-
SCMC	-	-	28	-	-	-
Carbopol® 71G	-	-	-	28	-	-
Klucel® EF	-	-	-	-	28	-
Xanthan gum	-	-	-	-	-	28
Colloidal silica	0.7	0.7	0.7	0.7	0.7	0.7
Magnesium stearate	1.4	1.4	1.4	1.4	1.4	1.4
Avicel® PH101	58.3	30.3	30.3	30.3	30.3	30.3

4.2.3 Tablet Analysis

Twenty tablets were weighed collectively and ground to form a homogenous powder using a mortar and pestle. An accurately weighed portion of the pooled sample, equivalent to the weight of one tablet (approximately 9.6 mg of SBS) was transferred to another mortar and mixed with approximately 70 ml of HPLC-grade water to form a paste. The paste was quantitatively transferred to a 100 ml volumetric flask and sonicated for 15 min, and the resultant solution was made up to volume with water to produce a solution of approximately 96 µg/ml of SBS. A 5 ml aliquot of the solution was then transferred to a 20 ml A-grade volumetric flask and was mixed with the internal standard, TBS, to produce a solution in which the concentration of internal standard was approximately 25 µg/ml, and the solution was made up to volume. A 2 ml aliquot of this solution was filtered through a 0.45 µm Millipore® (Millipore, Bedford, MA, USA) filter prior to injection onto the HPLC system (§

3.3.2). Tablet analysis was performed in triplicate (n = 3) and the results are expressed as the mean ± SD.

4.2.4 Weight, Diameter, Thickness and Crushing Strength

Twenty tablets of each formulation were tested for weight uniformity using a Mettler-Toledo Model AG135 electronic balance (Mettler-Toledo, Inc., Columbus OH, USA). Tablet diameter, thickness and crushing strength were assessed using a PTB 311 Automated Tablet Testing Instrument (Pharma Test Apparatebau, Hainburg, Hesse, Germany) and the results are reported as the mean ± SD.

4.2.5 *In Vitro* Dissolution Studies

A VanKel® Bio-Dis dissolution tester (VanKel Industries, Edison, NJ, USA) was used for the assessment of the *in vitro* release characteristics of these formulations. A model VK 750D digitally controlled water circulation/heater (VanKel Industries, Edison, NJ, USA) was used to maintain the temperature of the dissolution medium at 37 ± 0.5 °C. A mesh of pore size 177 µm was used to retain the dosage form in the inner tubes and a dip speed of 10 dpm was used as the agitation rate. The duration of dosage form exposure to buffers of different pH is summarised in Table 4.2.

Table 4.2 Dissolution test conditions

pH of dissolution medium	**Duration (hr)**
1.2	1
4.5	1
6.0	2
6.8	2
6.8	2
6.8	4

4.2.6 Quantitative Analysis

2 ml aliquot of media from the dissolution vessels described in § 4.2.5 was measured out using a Boeco Germany electronic pipette (Boeckel & Co (GmbH & Co), Hamburg, Hamburg, Germany) and filtered through a 0.45 µm Millipore® filter (Millipore, Bedford, MA, USA). A 1.5 ml aliquot was carefully placed in a sampling vial and 100 µl of a TBS solution at a concentration of 400 µg/ml was added to the vial such that the final concentration of the internal standard was approximately 25 µg/ml. The samples were assessed using the validated HPLC method described in Chapter 3 *vide infra* and compared

with the calibration curves prepared as described in § 3.3.3 to determine the percent drug released at different stages of the dissolution test.

4.2.7 Modelling of Dissolution Profiles

In order to determine the possible mechanism of drug release from these matrix formulations, the *in vitro* release data were fitted to the Korsmeyer–Peppas power law [79] using the Curve-Fitting Toolbox of Matlab® (MathWorks Inc., Natick, MA, USA). The goodness of fit was determined by assessing the adjusted coefficient of determination, R^2_{adj} where the closer the value is to 1 the better the data fit to the model used to describe drug release patterns. The semi-empirical model used to describe the mechanism of drug release from the hydrophilic matrix dosage forms is shown in Equation 4.1.

$$\frac{M_t}{M_\infty} = kt^n \qquad \textbf{Equation 4.1}$$

Where,

$\frac{M_t}{M_\infty}$ = fraction of drug released at time, t

k = proportionality constant which accounts for the structural and geometric properties of the matrix

n = diffusional exponent the value of which is indicative of the mechanism of drug release for a specific geometry

4.3 RESULTS AND DISCUSSION

4.3.1 Physical Properties of Tablets

The physical appearance, tablet weight, thickness and crushing strength of batches of tablets manufactured in these studies are summarised in Table 4.3.

The tablet weight, thickness and diameter ranged from approximately 140 to 142 mg, 2.98 to 3.15 mm and 7.14 to 7.22 mm, respectively for the batches tested. The manufactured tablets remained intact for the duration of the study, with no visible signs of tablet fracture, chipping or capping and therefore were suitable for their intended use.

Table 4.3 Physical properties of tablets

	SBS-01	SBS-02	SBS-03	SBS-04	SBS-05	SBS-06
Weight (mg)	141.01 ± 2.53	140.02 ± 1.59	140.68 ± 1.74	141.11 ± 1.21	141.22 ± 2.19	141.95 ± 3.67
Thickness (mm)	2.99 ± 0.06	3.07 ± 0.04	3.17 ± 0.08	3.03 ± 0.03	3.13 ± 0.05	2.98 ± 0.11
Diameter (mm)	7.16 ± 0.01	7.16 ± 0.01	7.22 ± 0.01	7.16 ± 0.01	7.14 ± 0.01	7.16 ± 0.03
Crushing strength (N)	81.82 ± 4.41	85.66 ± 5.36	55.16 ± 5.80	123.64 ± 5.63	76.34 ± 3.95	78.44 ± 8.05
Assay (mg)	9.64 ± 0.09	9.59 ± 0.11	9.62 ± 0.12	9.58 ± 0.23	9.57 ± 0.12	9.61 ± 0.20

Tablet crushing strength was consistent for each of the manufactured batches although the crushing strength values for each batch were formulation specific. The addition of other gel forming materials such as Methocel® K4M (SBS-02), Klucel® (SBS-05) and xanthan gum (SBS-06) did not result in significant changes to tablet crushing strength when compared with that of the primary formulation, SBS-01. It is apparent that the inclusion of SCMC (SBS-03) resulted in the production of relatively weak tablets and that the inclusion of Carbopol® (SBS-04) resulted in the manufacture of the hardest tablets compared with tablets of batch SBS-01.

The small standard deviations of measurements indicate that the tablets showed little variability and it can be concluded that the method of manufacture of the formulations is appropriate for the production of good quality SR matrix tablets of SBS.

4.3.2 *In Vitro* Release Studies

The *in vitro* release profiles for formulations containing Methocel® K100M as the primary matrix forming polymer are depicted in Figures 4.1 and 4.2. Batch SBS-01 contains 50% w/w Methocel® K100M and was used as the reference formulation against which all the other formulations were compared.

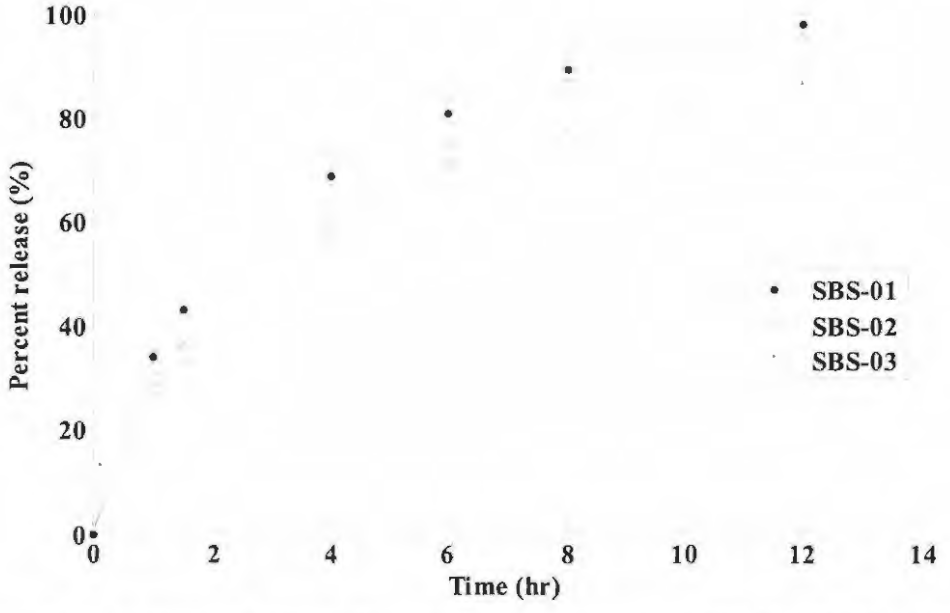


Figure 4.1 *In vitro* dissolution profiles of preliminary formulations SBS-01, SBS-02 and SBS-03

The dissolution profile for tablets from batch SBS-01 showed that the API is rapidly released from the formulation and that approximately 35% of the dose is released within 1 hr of the

commencement of testing. This is a typical characteristic observed for SR dosage forms in which water-soluble drugs are formulated and the API is usually rapidly released from hydrophilic matrix formulations. Hydrophilic matrices generally exhibit an initial burst release followed by a gradual decreasing rate of release over the course of a dissolution test [150]. The *in vitro* release profile also reveals that the release of SBS from the matrices is almost complete after 12 hr and the inclusion of additional polymers may result in a reduction in both the burst and overall release rate of SBS. HPMC contains linear hydrophilic polymeric chains which do not cross-link but form a gelatinous layer on the surface of tablets which is subsequently susceptible to erosion. When the polymer concentration is increased the linear polymer chains entangle and form what may be considered "virtual cross-linking" which impedes drug liberation initially but as the polymer eventually erodes, so drug release rates change [134,152].

Methocel® polymers are available in a variety of grades that exhibit different viscosities in solution [153]. Methocel® K100M and Methocel® K4M are high and low viscosity grades, respectively. The addition of a low viscosity grade HPMC to a high viscosity grade is a possible formulation strategy that may be used to modulate drug release from matrix formulations [153]. The addition of Methocel® K4M at 20% w/w resulted in a decrease in the drug release rate from batch SBS-02 as shown in Figure 4.1. The inclusion of additional polymer to a matrix results in a thicker gel layer being formed and consequently a greater barrier to drug diffusion is created in the matrix.

Batch SBS-03 was formulated with SCMC as an adjuvant, and the results reveal that there is an initial burst, equivalent to approximately 25% of the dose that is released within an hour of the commencement of dissolution testing. The amount of drug released as a result of the burst for this formulation is lower than that observed for batch SBS-01, which is in agreement with findings that have been previously reported [136]. The combination of an anionic polymer such as SCMC with non-ionic polymer such as HPMC has been shown to result in a synergistic increase in viscosity of the gel layer that is formed on hydration and therefore a greater barrier to drug diffusion with a consequent decrease in burst release. The change in release is attributed to the formation of hydrogen bonds between the carboxyl groups of SCMC and the hydroxyl groups of HPMC, leading to stronger physical entanglement of the polymer chains compared to either pure polymers [136]. It is also apparent that the release

rate between 1 and 8 hr of the dissolution test is linear compared with the release pattern that is observed for batch SBS-01. The linear pattern that is observed shows that during this period drug release follows a zero order mechanism.

The impact of the addition of Carbopol® 71G (SBS-04), Klucel® (SBS-05) and xanthan gum (SBS-06) at concentrations equivalent to 20% w/w of the tablet weight on drug release profiles is shown in Figure 4.2.

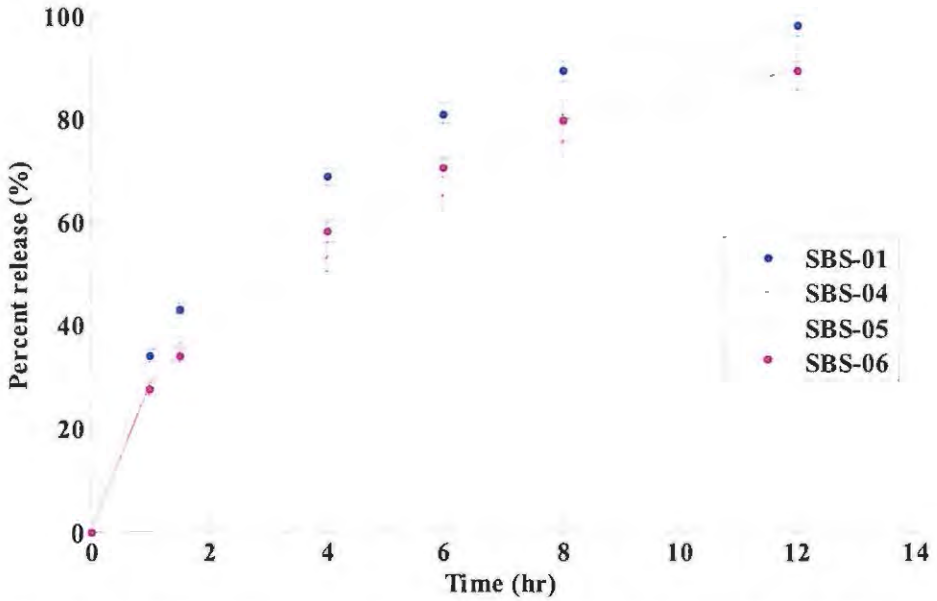


Figure 4.2 *In vitro* dissolution profiles of preliminary formulations SBS-01, SBS-04, SBS-05 and SBS-06

The inclusion of Carbopol® 71G (SBS-04) to the primary formulation (SBS-01) resulted in a decrease in both the burst and overall release rate of SBS from the formulations as shown in Figure 4.2. Carbopol® polymers are high molecular weight cross-linked synthetic polymers of acrylic acid that are insoluble in water, although they readily absorb water and swell. This property makes them ideal candidates for inclusion in the formulation of hydrophilic devices for oral SR delivery [134,152]. On hydration Carbopol® polymers form a gelatinous layer that has a significantly different structure to that formed by HPMC described previously. The gel layer is comprised of discrete microgel portions that are made up of many polymer particles within which the API is dispersed. The cross-linked nature of the network results in the entrapment of drugs in the hydrogel domains thereby hindering drug diffusion from these matrices. The addition of Carbopol® polymers therefore results in a reduction in drug release as observed in Figure 4.2 [134].

The swelling of Carbopol® is also dependent on the pH of the medium to which it is exposed. The pK_a of Carbopol® ranges between 6 ± 0.5, and at acidic pH the polymer is unionised and difficult to hydrate, whereas at pH 4.5 the polymer begins to ionise, therefore hydrates readily and subsequently swells [152]. This has important implications when designing SR formulations since pH dependent swelling kinetics present a means of controlling the initial burst release observed for water-soluble compounds during the early stages of dissolution testing.

The addition of Klucel® EF to the primary formulation results in an overall decrease in the release rate of SBS from batch SBS-05 compared with that of batch SBS-01. Klucel® is HPC, a non-ionic polymeric matrix material that has been used to manufacture monolithic hydrophilic matrix formulations [151]. HPC swells when placed in an aqueous medium and therefore retards the release of drug by hindering drug diffusion from the centre of the matrix to the bulk medium. Klucel® shows similar swelling behaviour to that observed for HPMC matrices, although the differences observed are likely due to the differences in hydrophilicity of the matrix materials [151]. The incorporation of additional Klucel® to formulations (SBS-05) may result in a decreased rate of release to that observed in Figure 4.2 due to increased hindrance to diffusion as a result of the presence of more polymer.

It is clearly evident in Figure 4.2 that the inclusion of xanthan gum (SBS-06) to the primary formulation results in a decrease in the burst effect and the overall release rate of SBS for the duration of the dissolution test. The use of xanthan gum as a drug release control material has been reported [149] and characteristics such as the compaction behaviour, *in vitro* release rate and rate of polymer hydration of xanthan gum were compared with those of HPMC. The use of xanthan gum eliminates the burst effect and results in release rates of API that are significantly lower than those observed when HPMC-based matrices were used [149]. The lower burst release following the inclusion of xanthan gum is due to the higher degree of hydration compared with HPMC which results in an increased hindrance of drug liberation that was also observed for SBS [149]. Interestingly, the addition of xanthan gum to ethylcellulose matrices has been reported to result in a proportional increase in drug release rates which has been attributed to the swelling of the xanthan gum in an aqueous medium, resulting in greater liquid uptake, polymer swelling and a consequent increase in API dissolution from polymeric matrices [138].

4.3.3 API Release Rate and Mechanism of Release

The diffusion of an API through a gelatinous layer and subsequent erosion of a polymeric matrix may contribute to the mechanism of release although one of the processes usually dominates in hydrophilic matrices [151]. The relative contribution of these processes is dependent on the specific polymer combinations and ratios that are used in formulating an SR dosage form. For example, if a durable and viscous matrix forming polymer is used to manufacture SR formulations, then water-soluble drugs such as SBS are released primarily by diffusion controlled kinetic processes and drug release rates decrease over time. In cases where an erodible polymer combination is used in a formulation, polymer erosion will contribute significantly to the mechanism of release and API release rates remain constant throughout period of use of the delivery system.

A mathematical representation of the Korsmeyer–Peppas model is shown in Equation 4.1 [79] and the model is often used to deduce release mechanisms from SR formulations. Specifically the value of the exponent n is used to infer the drug release mechanism. The dissolution data shown in Figures 4.1 and 4.2 were used to determine the mechanism of release for the batches under investigation for a percent SBS released <60% and the resultant parameters generated following modelling of the data are summarised in Table 4.4.

Table 4.4 Drug release parameters for preliminary formulations

	SBS-01	**SBS-02**	**SBS-03**	**SBS-04**	**SBS-05**	**SBS-06**
k	0.3465	0.2958	0.2699	0.2841	0.2908	0.2752
n	0.4946	0.5058	0.5434	0.4518	0.5193	0.5380
R^2_{adj}	0.9994	1	0.9996	0.9998	0.9997	1
RMSE	0.007094	0.0001538	0.004434	0.003249	0.004138	0.0009134

Drug diffusion through polymeric matrices can be described by three types of release mechanism *viz.*, Fickian, anomalous and/or swelling controlled diffusion [154]. The numerical value of the exponent n describes the mechanism of drug release from a dosage form and is specific for a particular geometry. For a cylinder such as the matrix tablets that were manufactured in this study, when $n = 0.45$, the mechanism of release is considered to be governed by Fickian diffusion and is termed Case I transport. When $0.45<n<0.89$ then mass transfer of an API from the matrix is considered to follow an anomalous transport mechanism that is a function of both drug diffusion and polymer relaxation. When the exponent $n>0.89$

the release mechanism is considered to be swelling-controlled and is referred to as Case II transport.

Fickian diffusion controlled release occurs by molecular diffusion of a drug along a concentration gradient and Case II transport is associated with stress and a state-transition in the hydrophilic glassy polymers that swell in aqueous and/or biological media [155,156]. Anomalous diffusion is used to describe API release from initially dry, hydrophilic glassy polymers that swell when added to water as a result of a relaxation of the macromolecules that make up the polymer. The thermodynamic state of a polymer determines whether Fickian or anomalous diffusion predominates. Fickian diffusion occurs at low penetrant concentrations below the glass transition temperature and anomalous diffusion occurs at higher penetrant concentrations above the glass transition temperature of the polymer [154]. Case II transport is characterised by linear drug release kinetics, a sharp diffusion front and generally occurs in polymer-penetrant systems in which the penetrant results in substantial swelling of the polymer [154].

The value of the exponent obtained following modelling the dissolution profiles generated after testing of HPMC matrices was 0.4946, indicating that drug release is primarily controlled by a diffusion mechanism and that drug transport is dependent on the concentration gradient that exists between the tablet matrix and the aqueous dissolution medium. The drug moves from a region of high concentration in the tablet matrix to a region of low concentration in the bulk aqueous medium. These results are in agreement with those previously reported [143,146] in which it was shown that the liberation of water-soluble drugs from hydrophilic matrices is primarily diffusion controlled. The inclusion of low viscosity Methocel® K4M in batch SBS-02 did not cause a significant change in the mechanism of drug release and the value for n was 0.5058.

The dissolution profile for batch SBS-03 revealed that linear release kinetics occur between 1 and 8 hr indicating that drug release is directly proportional to time. The combination of HPMC and SCMC has previously been reported to result in zero order release patterns of drug molecules from matrix formulations [130,136,157]. The exponent n, in this case, was 0.5434 indicating that an anomalous transport process predominates due to both diffusion and polymer relaxation, although diffusion is still a significant contributor to the release process.

The low dose and high aqueous solubility of SBS resulted in rapid drug release during the initial stages of dissolution testing and therefore a deviation from a true zero order release pattern was observed. By altering the ratio of HPMC: SCMC it is possible to achieve true zero order release formulations as has been previously reported [157].

The release of drug from Carbopol® matrices has been reported to exhibit both diffusion controlled [158] and zero order release profiles [147,148,159]. The inclusion of Carbopol® to matrix formulations that contain HPMC results in a shift in the mechanism of drug release as was observed for batch SBS-04. The value of n decreased from 0.4946 to 0.4518 when Carbopol® was added to the HPMC containing formulation. This is indicative that diffusion is the primary mechanism that controls drug release and there is only a small contribution from polymer relaxation. The inclusion of Carbopol® results in increased polymer hydration rates thereby increasing the molecular volume of hydrated polymer present and reducing the free volume available between the microgel structure within the matrix with the result that there is an increase in diffusion control of the drug release process.

The inclusion of Klucel® EF to the formulation did not result in a significant change in the release mechanism and a value for n of 0.5193 was generated. This is likely since Klucel® EF is similar to HPMC and undergoes a similar swelling process to that observed in HPMC matrices [151].

The inclusion of xanthan gum in the tablet matrices resulted in a change in the mechanism of drug release and a value of 0.5380 for n was observed. It has been reported that that the mechanism of drug release from xanthan matrices follows a more linear pattern compared with those observed for HPMC matrices that tend to exhibit Higuchi-type release kinetics [149]. However, in these studies the influence of HPMC as a rate retardant matrix is significant since a high proportion of HPMC was used in the formulation compared with xanthan gum.

4.4 CONCLUSIONS

Preliminary screening studies are an important part of any formulation development process as they provide a database for making scientifically sound decisions for future product

development in addition to increasing the understanding of formulation behaviour. HPMC was chosen as the primary polymer for manufacturing SR dosage forms since it is a well understood technology whose performance is not adversely affected by manufacturing processes to any great extent.

The inclusion of additional rate retarding adjuvants was investigated as a means of modulating drug release and xanthan gum and Carbopol® were found to be suitable for further investigation since the preliminary results indicate that these had a significant effect on the rate and mechanism of drug release. These polymers were therefore used in a statistically designed experiment for the optimisation of a formulation with release characteristics that would be similar to the reference formulation, Asthalin®8 ER (Cipla Ltd., Mumbai, Maharashtra, India).

CHAPTER 5
RISK ASSESSMENT OF QUALITY

5.1 INTRODUCTION

Risk is defined as "the combination of the probability of occurrence of harm and the severity of that harm" and in the context of pharmaceutical technology this is dependent on an evaluation of risk, based on sound scientific principles and knowledge, with the ultimate aim being the minimisation of risk to the patient [2].

The composition of a pharmaceutical product, manufacturing process and intended clinical use involve some degree of risk for a patient that must be managed and/or controlled to ensure patient safety. It is therefore important that pharmaceutical products of consistent quality are produced throughout the product lifecycle, and product attributes that are important to quality, *viz.*, CQA must remain consistent through clinical studies, trials and subsequent clinical use [2]. In addition to ensuring that product quality is maintained throughout the lifecycle of the product, an effective quality management approach must also provide a proactive means of identifying and controlling potential quality issues that may arise during formulation and manufacturing process development [2]. In the event that there are quality issues with respect to a medicinal product during formulation development and manufacturing, knowledge of potential risk areas facilitates intelligent problem solving and decision making [2]. The principles of risk management are intimately linked to the concepts of QbD (Chapter 1 *vide infra*).

5.2 QUALITY RISK MANAGEMENT

Quality risk management is a systematic approach for the assessment, control, communication and review of risks that impact the quality of pharmaceutical product throughout the product lifecycle [160]. An overview of a typical quality risk management process, which incorporates the consideration of elements that must be discussed during the quality risk management process, is depicted in Figure 5.1 [2].

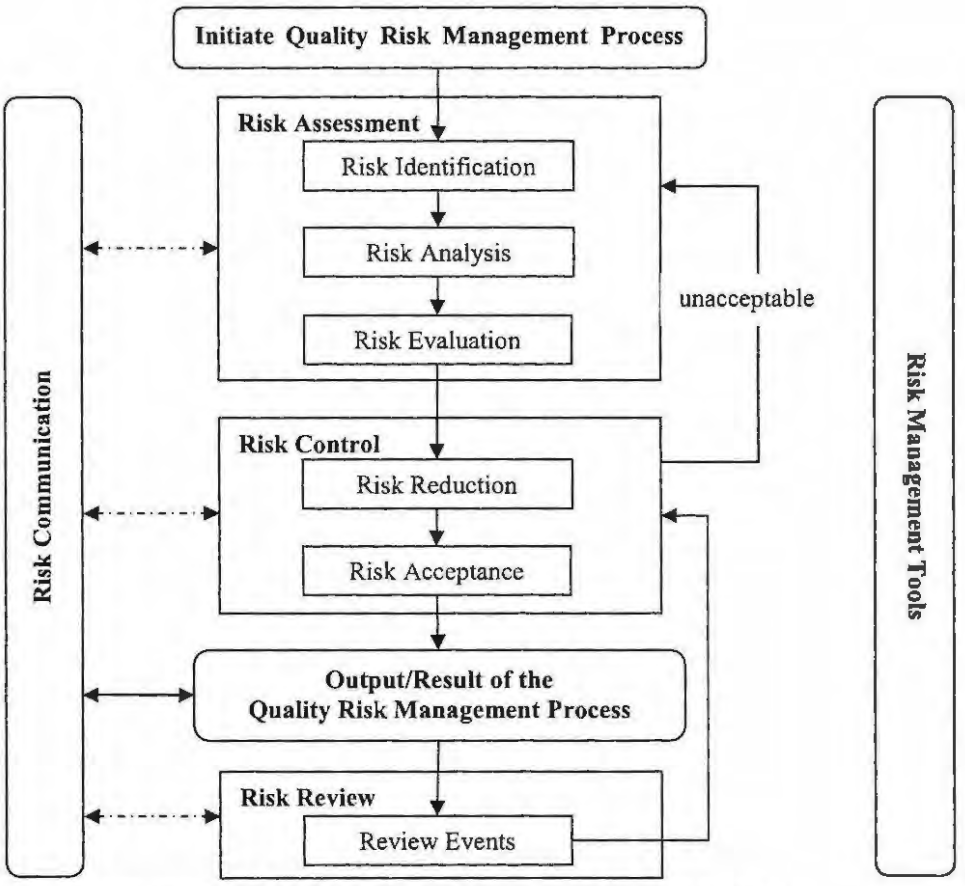


Figure 5.1 Typical risk management process, redrawn from [2]

The risk assessment process includes risk identification, subsequent analysis and evaluation. The initial stages of a quality risk management process involve identifying the potential sources of risk that may impact product quality in order to facilitate sound scientific decision making.

Risk identification is a systematic use of information to identify hazards that refer to the risk question, and risk analysis is an important aspect of product development in order to estimate the risk associated with recognised hazards, whereas risk evaluation compares the identified and analysed risks against specific risk criteria [2]. Appropriate risk assessment and management tools are used to evaluate risk following the appropriate definition of risk. The overall output of a risk assessment process is the establishment of quantitative estimates of risk or qualitative descriptions of the range of risk likely to be encountered within a system [2].

The control of risk involves decision making to reduce or accept the potential risk with the ultimate goal of reducing the risk associated with a process to an acceptable level. Risk reduction focuses on processes for mitigation or avoidance of risk if and when risk exceeds a specified level, and risk acceptance is a conscious decision to accept the risk associated with a process [2,160].

Risk must also be communicated to all relevant stakeholders and must be reviewed on a continuous basis during the lifecycle of a product [160]. The term "unacceptable" in Figure 5.1 refers to the statutory, legislative or regulatory requirements of risk management and when the control of risk is not possible the risk assessment process must be revisited [2].

5.3 RISK MANAGEMENT METHODOLOGY

Risk has been traditionally managed using informal methodologies that are empirical and/or internal procedures that are based on the compilation of observations and trends. However formal risk management tools including Failure Mode Effects Analysis (FMEA), Fault Tree Analysis (FTA), Hazard Analysis and Critical Control Points (HACCP), Cause and Effect (Ishikawa) diagrams, flowcharts and check sheets, exist and provide a more meaningful and sustainable approach to risk management [2].

A Cause and Effect diagram was selected as the tool for conducting a risk assessment in this study since it is relatively simple to use and allows for an exhaustive analysis to be conducted including all the factors that may affect a process. It is also easy to identify links and interrelationships between factors using a Cause and Effect diagram.

5.4 RISK IDENTIFICATION

Several aspects of the composition of a formulation and the associated manufacturing process may affect product quality and the aim of the risk assessment exercise was to identify these areas of concern. A Cause and Effect or Ishikawa diagram as depicted in Figure 5.2 was used to determine the formulation and process variables that are likely to affect product quality and therefore to determine areas of concern that must be controlled or monitored in statistically designed formulation development and assessment experiments.

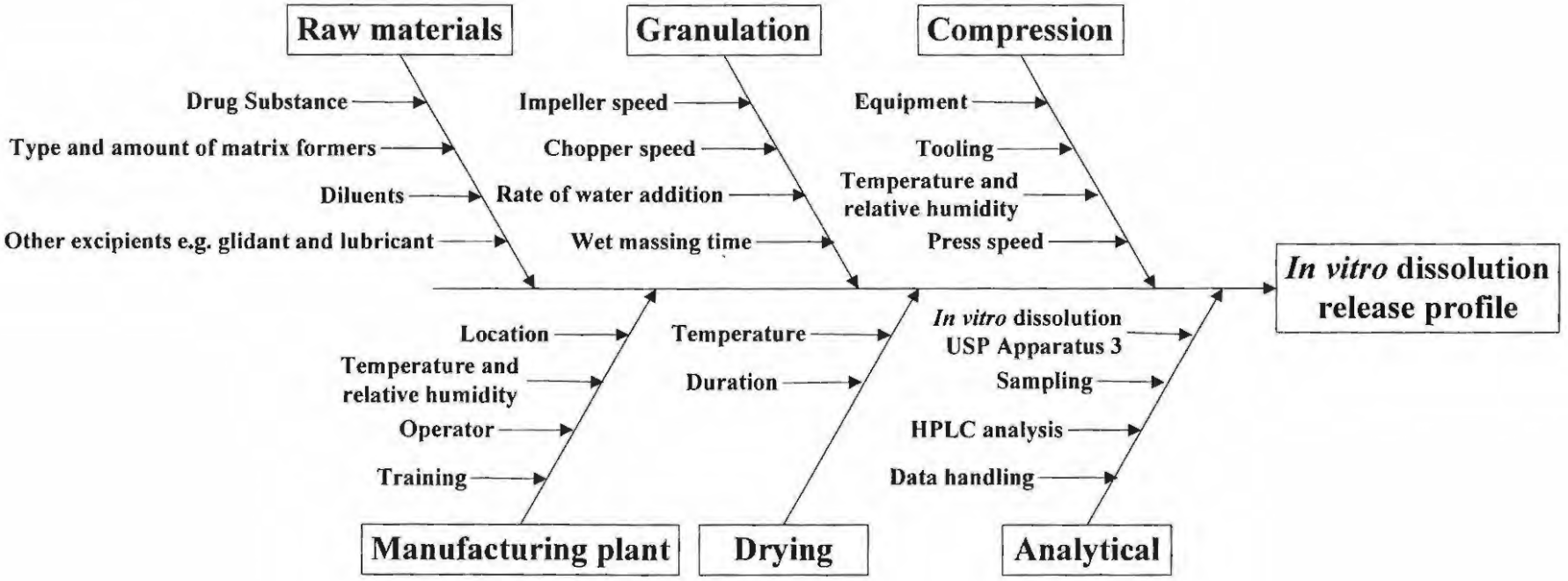


Figure 5.2 Ishikawa diagram of the sources of variability

The main sources of risk that impact the *in vitro* dissolution profile of an API from a dosage form were based on sound science and prior knowledge. The sources of product variability may include raw materials, manufacturing plants, manufacturing processes, granulation, drying and tablet compression in addition to analytical methods that are used to assess the data that is generated [161].

The synthetic procedure used to produce an API must be optimised to ensure that dosage form performance and product quality are not compromised, and steps that are likely to influence the physicochemical properties of an API must be emphasised and controlled [162]. The particle size distribution of the final product must also be well controlled as this is likely to affect *in vitro* dissolution rates and ultimately *in vivo* performance, especially for poorly water-soluble compounds [162]. Excipients can be used to control drug dissolution rates for both immediate release [163] and SR dosage forms [139], and the types, levels and quality of materials used in a formulation must be carefully controlled. Therefore the identification of critical formulation attributes that are likely to impact dosage form performance is crucial to ensure successful dosage form development [162].

The adequate control of any manufacturing process is vitally important in assuring the quality, safety and efficacy of a pharmaceutical product. The method of manufacture and all necessary processing steps must be well documented and controlled to ensure consistent dosage form manufacture and ultimately performance [162]. The conditions in a manufacturing plant and processing parameters are known to impact product quality. For example, in a wet granulation procedure, variables, such as the impeller and chopper speeds, rate of granulation fluid addition and wet massing time, can and often do affect granule quality [164–166].

Specific measures of dosage form performance including *in vitro* dissolution testing, analytical methods and data handling are also important considerations in ensuring product quality and must also be considered. It has been reported that between 20 and 38% of dosage form performance variability may be a consequence of the apparatus and related procedures used for data generation [161]. Specification tests including product assay, *in vitro* dissolution testing and analytical methods must be appropriately validated to ensure that

suitable conclusions regarding dosage form performance are made during formulation development studies [162].

5.5 RISK CONTROL

Risk assessment and analysis is important in ensuring the control of those areas of formulation development and manufacturing that must be monitored and controlled so as to ensure product quality and minimise the risk of product variability.

The same batch of each API and various excipients that were used in subsequent studies had the same batch number. For instance, the same batch of Methocel® K100M that was obtained from Dow Chemical Company (Midland, MI, USA) was used in the future studies. In this way the potential impact of different lot numbers was eliminated as a potential source of variability.

Furthermore the same manufacturing plant, granulation equipment and tablet press were used to ensure that the effect of using different equipment that would be a potential source of variation was eliminated. It was also important to ensure that the same manufacturing conditions were used and therefore careful monitoring and recording of environmental conditions was undertaken during manufacturing in order to determine if these conditions may potentially introduce variability into the batches of product. The analytical methods that were used in the study were validated to ensure that they were suitable for their intended use (see Chapter 3, *vide infra*) and the dissolution test equipment and other instruments, including weighing balances, hardness tester for analysis underwent regular maintenance procedures to ensure that they were in good working condition.

5.6 CONCLUSIONS

When conducting statistically designed experiments, external factors that do not intend to be investigated in a study but have the potential to impact the measured outcomes of that study, must be controlled. This strategy ensures that any observed changes in product performance may be fully attributed to the factors under investigation. Therefore manufacturing and processing conditions were carefully monitored to ensure that the statistically designed experiment was not affected by these factors.

Therefore, following risk analysis to ensure the control of variability and completion of preliminary studies, the impact of Methocel® K100M, xanthan gum, Carbopol® as polymeric components of hydrophilic matrices and Surelease® as a granulation fluid on *in vitro* dissolution rates were further studied using a central composite design approach and the results are discussed in Chapter 6. The well documented use of these polymers for SR applications has made them suitable for further study [134,138,149,152,153,167]. Surelease®, an aqueous dispersion of ethylcellulose, was selected as a granulating fluid as this has been shown to retard drug release from monolithic matrix formulations [137,168].

The use of a risk assessment tool, preliminary formulation studies and prior knowledge are therefore essential in designing and conducting robust statistical experiments for the establishment of an appropriate design space for a SR product of SBS.

CHAPTER 6

RESPONSE SURFACE METHODOLOGY IN FORMULATION OPTIMISATION

6.1 INTRODUCTION

6.1.1 Pharmaceutical Optimisation

The use of mathematical and statistical models for the optimisation of pharmaceutical formulations and processes has been reported from as early as 1970 [169–171]. The use of statistical methodology for the optimisation of SR formulations has been reported more recently [172–179] where the application of these methods has been used to enhance an understanding of the underlying principles of pharmaceutical formulations. In addition, the application of statistical methodology has been recommended by the ICH in the Pharmaceutical Development guideline, Q8 (R2) [1] in order to ensure the development of quality pharmaceutical products and for the establishment of an appropriate design space for a product.

Pharmaceutical optimisation has been defined as the implementation of systematic approaches to establish the best possible combination of product and/or process variables under a given set of conditions, that will result in the production of a quality pharmaceutical product with predetermined and specified characteristics each time it is manufactured [180]. There are four primary methods that can be used to optimise pharmaceutical products, *viz.*, the one-factor-at-a-time approach, a direct optimisation technique, a non-systematic approach or the use of statistical design [181].

The use of the one-factor-at-a-time approach entails a formulator changing one formulation variable at-a-time in order to determine an optimal value for that variable. This strategy continues using single factor iterations until optimal dosage form performance is achieved. This approach may not be appropriate if multiple performance objectives must be met and in situations where strong interactions between the variables being studied prevail [180,181]. Direct optimisation methods are particularly useful for establishing an experimental domain for formulation or process variables, whereas non-systematic approaches are based on knowledge and intuition of a formulator in order to produce a quality pharmaceutical product. Although the intuitive approach is often successful it is also possible that a formulator is

unsuccessful and therefore time and valuable resources may be wasted [181]. Statistically designed experiments are planned as a matrix in order to estimate coefficients from mathematical models, which relate input factors or variables to measured responses that can be used for the optimisation of formulations or manufacturing processes. Statistical methodologies are considered to be a powerful approach for use in pharmaceutical formulation and process optimisation, provided that experimental domains or zones have been correctly identified and defined [181].

The objective of this study was to develop an optimised hydrophilic matrix formulation for SBS with an *in vitro* release profile that is similar to a reference formulation, *viz.*, Asthalin®8 ER (Cipla Ltd., Mumbai, Maharashtra, India). The preliminary formulation information reported in Chapter 4 was used to define an experimental domain for a statistically designed formulation experiment approach.

6.2 RESPONSE SURFACE METHODOLOGY

6.2.1 Introduction

RSM is a combination of mathematical and statistical techniques used for developing, improving and optimising processes including industrial procedures, chemical and biological science applications, amongst others [182–184]. RSM has been applied to the optimisation of dosage forms and manufacturing processes and has been widely accepted as being suitable for these purposes [185–187]. With respect to the pharmaceutical sciences, the primary objective of RSM is to determine the optimal operational conditions or formulation compositions that will result in the development of products with appropriate CQA and performance characteristics, or the identification of an experimental region that will meet the predetermined and relevant product specifications [188].

RSM usually comprises three sequential steps, *viz.*, performing a series of statistically designed experiments, developing a mathematical model through multiple linear regression and finally determining an optimal set of experimental parameters that will produce a desired response [188].

6.2.1.1 Statistically Designed Experiments in Formulation Development

The development and optimisation of pharmaceutical formulations or manufacturing processes involves studying the variables that must be monitored and optimised to ensure the production of a quality product. The input factors or independent variables are those parameters that influence the final characteristics of a formulation and are under the direct control of formulation scientists. In the case of a SR matrix formulation, these variables typically include polymer type and composition, compression force, length of mixing time and the percent composition of a specific excipient, amongst others. The appropriate ranges for the input factors to be studied are defined from preliminary studies and are coded in such a way such that there are low (–1), medium (0) and high (+1) levels [180].

The dependent or response variables are measurable characteristics of a dosage form that are assessed following the conduction of an experimental design experiment. Typical examples of these characteristics include the dissolution rate profile, particle size distribution of a granulation and tablet crushing strength [180].

The implementation of RSM involves the use of formal statistical experimental designs and the subsequent generation of mathematical equations and graphical outcomes to depict a complete picture of the variability of a response as a function of previously defined input factors. Empirical data is fitted to statistical or mathematical models that are used to predict formulation or process performance and to optimise defined critical response variables [180].

The most common experimental techniques used for the optimisation of pharmaceutical formulations include factorial [172], central composite [173–177], Box–Behnken [178] and D-optimal designs [179].

6.2.1.2 Mathematical Models

The polynomial models that are generated from statistically designed experiments can be used to summarise empirical data and to predict the relationship between input and response variables [189]. The polynomial equations may be first, second or third order in nature although first and second order models are more commonly applied to pharmaceutical systems [181,189].

First order models as shown in Equation 6.1 are usually selected when it is expected that important variables do not vary extensively over an experimental domain and when there are no interactions between input variables that may impact a defined or measured response [181].

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \varepsilon \qquad \textbf{Equation 6.1}$$

Where,

y = the estimated response
x_i = the input factors
β_0= constant that represents the intercept
β_i= coefficients of first order terms

Second order models as shown in Equations 5.2 and 5.3 are generally used to create linear and quadratic equations for responses that vary extensively over the experimental domain and that show interactions between input variables that affect the responses. These are the most commonly used relationships in pharmaceutical optimisation processes and are used in experimental designs with up to five input variables [181].

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \cdots + \varepsilon \qquad \textbf{Equation 6.2}$$

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \cdots + \varepsilon \qquad \textbf{Equation 6.3}$$

Where,

y = the estimated response
x_i = the input factors
β_0 = constant that represents the intercept
β_i = coefficients of first order terms
β_{ii} =coefficients of second order terms
β_{ij} = coefficients of second order interaction terms

The coefficients that are defined in the models are estimated by multiple linear least square regression of empirical data, and the sign and magnitude of the coefficients indicate the impact (increase or decrease) and extent or magnitude of the effect(s) of input variables on a defined measured response [181].

A mathematical model that is dependent on at least two factors can be graphically illustrated using a response surface that depicts the effect of an independent variable(s) on a measurable response. A response surface is a three dimensional illustration of how measured response

variables change as the level of an input variable(s) is altered over the experimental domain, and which may be used to acquire knowledge of underlying relationships between input factors and a measurable response. Alternatively the geometric illustration of a response obtained by plotting one independent variable against another, while holding the magnitude of the response and other variables constant, is known as a contour plot and represents two dimensional slices of the corresponding response surface plot [180,189].

6.2.1.3 Optimisation

Optimisation aims at determining the experimental conditions that lead to the generation of a desired value for the responses that are to be measured or monitored in a study. The values are often set as either a minimum or maximum, although optimisation may also be aimed at achieving a single desired or target value. The primary objective of an optimisation procedure is to identify either a single point or an optimum zone that satisfies the criteria to be measured and is therefore defined within the experimental region or domain [180].

Response surface plots that are generated may be used to optimise a response to a desired level and to determine what regions of the input variable domain to be studied would lead to the production of a desirable product. An important assumption for the successful optimisation of a product or process is that independent variables that are studied in an experimental design are continuous and controllable in experiments with the potential for negligible error [181,189].

6.2.2 Advantages of Response Surface Methodology

Experimental design studies that use RSM are useful for conducting experiments in a rational and systematic manner thereby generating precise information from fewer experiments compared with the one-factor-at-a-time approach [180]. In addition the application of RSM has the potential to increase an understanding of the principles of pharmaceutical sciences with respect to formulation composition and is also useful for predicting dosage form performance from mathematical models that are developed.

The use of RSM is also important for the identification of input variables that are important in determining CQA that will ensure the production of a quality pharmaceutical product. Furthermore the application of RSM may be useful for detecting potential interactions and synergies between input variables that could be critical for the optimisation of formulations

[180]. An enhanced understanding of process factors is also imperative for the successful scale up of formulations from laboratory to manufacturing scale [180].

6.2.3 Limitations of Response Surface Methodology

Response surfaces are particularly useful for mapping simple relationships that exist between input factors and measured response variables and therefore are not likely to be suitable in situations where complex interactions and relationships between the variables to be studied exist. Considerable time and other resources may be necessary in order to investigate such complex relationships thoroughly, thereby limiting the potential usefulness of RSM in formulation optimisation studies. This is especially true when CQA are influenced by opposing processing and formulation variables. For instance the impact of relative humidity on a dissolution profile may be negligible for a particular formulation but may have a negative impact on the compression characteristics of the powder blend. In such cases optimisation procedures become complex and the solution may require trade-offs of one CQA in favour of another [190].

Another limitation in the use of RSM for formulation optimisation is that experimental designs such as full factorial and Box–Behnken designs are only useful for evaluating a small number of critical input factors. As the number of factors to be evaluated increases an unrealistic number of experiments are required in order to conduct a valid and reliable study. Furthermore the polynomial relationships that are estimated by linear regression become increasingly complex as the number of factors evaluated increases and this further complicates the optimisation procedure [180].

6.2.4 Central Composite Design

Factorial design experiments are frequently used for exploring a response surface(s) and for the optimisation of pharmaceutical formulations [172,191,192]. A factorial design experiment is one in which all levels of a particular factor are combined with all levels of every other important factor to be considered in that experiment [180]. Full factorial design experiments are based on studying the effect(s) of all factors (k) at all levels (x), including interactions between them, with the result that the total number of experiments to be conducted can be determined by x^k. If the number of levels to be considered is ≥ 3 then second order mathematical models can be used to estimate the non-linear or quadratic effects of that

model. However as the number of factors increases, the number of experiments that are required in a full factorial study increases to an unmanageable number. For example 81 experiments would be required to study potential interactions between 4 factors at 3 different levels.

A method proposed by Box and Wilson [193] and referred to as a central composite design can be used to minimise the number of studies to be conducted and is now considered the cornerstone of RSM. The method is commonly used to map non-linear relationships that require second order models [189]. A central composite design is constructed from three sets of data points which are described as follows [180,189,194]:

i. The 2^k vertices of a k-dimensional cube for $k \leq 4$, or a fraction of a cube for $k \geq 5$, which is a 2^k full factorial design if $k \leq 4$ or a partial factorial design if, $k \geq 5$,

ii. The 2^k vertices of a k-dimensional "star" which are pairs of points on the coordinate axes that are all located at a distance of α from the origin, where α is selected based on the rotability of a design. This is often a number between 1 and 1.5 from the centre point and is often selected as $\sqrt{2}$ distance from the centre point and,

iii. A number of centre points that permit careful estimation of coefficients with minimal error.

The star points allow for the estimation of curvature and for the establishment of new extremes of high and low settings for all factors. A diagrammatic representation of a central composite design with only two factors, x_1 and x_2 and $\alpha \pm \sqrt{2}$ is shown in Figure 6.1.

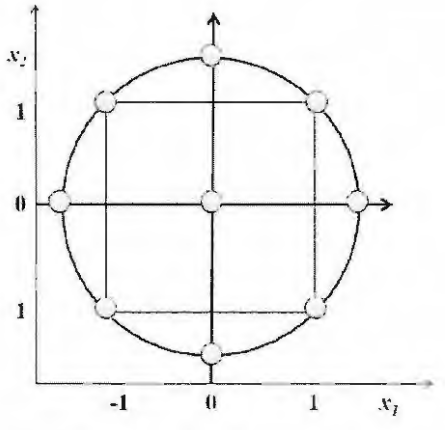


Figure 6.1 Diagrammatic representation of a central composite design with full factorial points and star points, adapted and redrawn from [180]

Due the advantages of a central composite design discussed above, this method was selected for the optimisation of a SR hydrophilic matrix formulation of SBS.

6.3 MATERIALS AND METHODS

6.3.1 Materials

Carbopol® 974P NF (Lubrizol, Wickliffe, OH, USA) and Surelease® (Colorcon, West Point, PA, USA) were used as received. All other materials and reagents are similar to those reported in § 4.2.1. All other reagents were at least of analytical grade and were used without further modification.

6.3.2 Experimental Design

A central composite design for formulation optimisation was generated and analysed using the Model Based Calibration Toolbox of Matlab® R2008a (MathWorks Inc., Natick, MA, USA). The independent variables, *viz.*, the amounts of Methocel® K100M, xanthan gum, Carbopol® 974P and percent composition of Surelease® as the granulating fluid that were assessed are given in Table 6.1, which shows the composition of the input variables at the low, medium, high and ± α levels. Tablet crushing strength, percent API released at different times of the dissolution test, *viz.*, 1, 2, 4, 6, 8 and 12 hr, and the constant and exponent generated by using the Korsmeyer–Peppas model [79] were used as the response variables. The constraints for the percent released for all formulations was determined following analysis of the dissolution profile generated for the reference product, Asthalin®8 ER (Cipla, Ltd., Mumbai, Maharashtra) using USP Apparatus 3 and the dissolution test conditions described in § 4.2.5.

Table 6.1 Factor and level of variables studied

Factors	**Levels studied**				
	-α	-1	0	+1	+α
x_1 = **Methocel® K100M**	0 mg	30 mg	60 mg	90 mg	120 mg
x_2 = **Xanthan gum**	0 mg	25 mg	50 mg	75 mg	100 mg
x_3 = **Carbopol® 974P**	0 mg	5 mg	10 mg	15 mg	20 mg
x_4 = **Surelease®**	4% w/w	8% w/w	12% w/w	16% w/w	20% w/w
Dependent variables			Constraints		
Tablet crushing strength			≥ 40 N		
y_{1hr} = **% dissolution after 1 hr**			$35 \leq y_{1hr} \leq 39$		
y_{2hr} = **% dissolution after 2 hr**			$49 \leq y_{2hr} \leq 53$		
y_{4hr} = **% dissolution after 4 hr**			$66 \leq y_{4hr} \leq 70$		
y_{6hr} = **% dissolution after 6 hr**			$77 \leq y_{6hr} \leq 81$		
y_{8hr} = **% dissolution after 8 hr**			$85 \leq y_{8hr} \leq 89$		
y_{12hr} = **% dissolution after 12 hr**			$93 \leq y_{12hr} \leq 97$		
k = **Rate of release (from Korsmeyer–Peppas model)**					
n = **Korsmeyer–Peppas release exponent**					

A summary of the different compositions that were manufactured using a central composite design approach is shown in Table 6.2. The table shows the randomised order in which the formulations were manufactured and subsequently analysed.

Table 6.2 Formulation compositions of hydrophilic matrix tablets generated using a central composite design

Formulation	Methocel® K100M (mg)	Xanthan gum (mg)	Carbopol® 974P (mg)	Surelease® (% w/w)
SAL001	120	50	10	12
SAL002	60	50	10	12
SAL003	60	50	10	4
SAL004	60	50	20	12
SAL005	90	75	15	16
SAL006	60	50	10	20
SAL007	90	25	15	16
SAL008	30	75	15	16
SAL009	60	50	10	12
SAL010	90	75	5	8
SAL011	0	50	10	12
SAL012	30	25	5	16
SAL013	60	50	10	12
SAL014	30	25	15	8
SAL015	60	50	10	12
SAL016	60	100	10	12
SAL017	90	75	5	16
SAL018	30	25	15	16
SAL019	90	25	5	8
SAL020	90	75	15	8
SAL021	30	75	5	16
SAL022	30	25	5	8
SAL023	30	75	5	8
SAL024	90	25	15	8
SAL025	60	50	0	12
SAL026	90	25	5	16
SAL027	60	0	10	12
SAL028	60	50	10	12
SAL029	60	50	10	12
SAL030	30	75	15	8

6.3.3 Manufacturing Procedure for Matrix Tablets

Surelease® is a 25% w/w aqueous dispersion of ethylcellulose; dilutions of Surelease® for use as the granulating liquid were prepared by accurately weighing the correct amount of dispersion on a top loader balanee (Mettler Toledo Inc., Columbus, OH, USA) and diluting by weight with HPLC-grade water to the required concentrations of 4, 8, 12, 16 and 20% w/w.

Batch sizes of 1000 tablets were prepared for each formulation. Matrix tablets were manufactured by dry blending SBS and the appropriate quantities of Methocel® K100M, xanthan gum, Carbopol® 974P and Avicel® PH101 in a Saral® Rapid Mixer and Granulator (Saral Engineering Company, Mumbai, Maharashtra, India) in a 5 L bowl using a speed of

100 rpm on the main impeller for 15 min. Thereafter 120 g of Surelease® diluted to the desired concentration with water (4–20% w/w) was gradually sprayed onto the powder blend using speeds of 120 rpm and 1000 rpm for the main impeller and chopper, respectively. The wet mass was further mixed for an additional 5 min at the same speed and removed from the granulator to dry on wax paper for at least 24 hr at a temperature of 22 °C until a constant weight had been achieved. Thereafter the granules were sieved and the fraction between 315 and 800 mm was collected and weighed. Prior to the addition of lubricant and glidant, the granules were characterised according to the procedures described in § 6.3.4. Thereafter, an equivalent 1% w/w magnesium stearate and 0.5% w/w colloidal silica were sieved and added to the blend that was mixed for a further 3 min at 100 rpm using the main impeller. Finally, the lubricated granules were compressed into tablets using 9 mm biconvex punches on a Manesty® B3B rotary tablet press to a uniform weight of 220 mg. In-process control was achieved my monitoring environmental conditions such as temperature and humidity, and process conditions including, dry mixing and wet massing time, speed of the motor, current on the main impeller and the chopper and tablet weight during the compression cycle.

6.3.4 Characterisation of Granules

Prior to compression the granules were evaluated to determine their suitability for compression into tablets.

6.3.4.1 Bulk Density

The bulk density of the selected fraction of the granulation was assessed by filling the granulation into a measuring cylinder up to the 100 ml mark. The graduated measuring cylinder was weighed and the bulk density, ρ_b was calculated as the ratio of the sample weight to volume.

6.3.4.2 Tapped Density

The graduated measuring cylinder (§ 6.3.4.1) was then manually tapped 50 times at a rate of 1 tap/sec on a flat surface from a constant height of 5 cm and the tapped density, ρ_t was calculated as the ratio of the sample weight to the final volume after 50 taps.

6.3.4.3 Carr's Compressibility Index

The changes that occur in powder packing were also expressed as the Carr's Index (CI) that was calculated using Equation 6.4.

$$CI = \frac{(\rho_t - \rho_b)}{\rho_t} \times 100$$ **Equation 6.4**

6.3.4.4 Angle of Repose

The angle of repose was determined using the fixed funnel method [195]. A plastic funnel was secured with its tip at a specific height (H) above a piece of Cartesian graph paper that had been placed on a flat, horizontal surface. The granulation was carefully poured into the funnel and allowed to flow until an apex had formed at the top of the conical pile of granules and that touched the tip of the funnel. The mean diameter ($2 \times R$) of the base of the powder cone was measured and the tangent of the angle of repose was calculated according to Equation 6.5.

$$tan\,\theta = \mathrm{H/R}$$ **Equation 6.5**

Where,

θ = calculated static angle of repose.

6.3.5 Characterisation of Tablets

Tablets were assayed for SBS content as previously described in § 4.2.3 and were characterised for tablet weight, thickness, diameter and crushing strength as previously described in § 4.2.4. *In vitro* dissolution studies and quantitative analysis of drug release were conducted according to the procedures described previously in § 4.2.5 and § 4.2.6 respectively.

6.3.6 Data Analysis

6.3.6.1 Response Surface Analysis

The Model Browser in the Model-Based Calibration Toolbox of Matlab® R2008a (MathWorks Inc., Natick, MA, USA) was used to assess the response models that depict relationships between input variables and the resultant responses that were considered appropriate and that are listed in Table 6.1.

The Root Mean Square Error (RMSE) was used to determine the goodness and degree of fit for the linear and quadratic models used to fit the data generated. The RMSE estimates the standard deviation associated with any experimental error and measures the average variance between each experimental data point that is generated by the model used to fit the data. The lower the value for RMSE, the closer the model fits the data and if the fitted data passes

through each experimental data point then the value for RMSE will be zero [196]. The limitation of using only the RSME as a means of model evaluation is that minimisation of the RMSE can result in over-fitting of the data, which leads to poor model performance at regions distant to the experimentally derived data points. Therefore the Predicted Error Sum of Squares (PRESS) was also used to determine which model best fits the experimental data generated in these studies [196].

Mathematical models were used to fit the experimental data and were subsequently assessed to determine which of the input factors had a significant impact on measured responses by minimising the PRESS for each model. The use of PRESS is appropriate for working towards a regression model that provides good predictive capabilities for an experimental domain. When PRESS is evaluated in data modelling, if there are n runs in the data set, then the model equation is assessed by fitting $n-1$ runs to the model under investigation. The difference between a recorded experimental data value and the value predicted by the model with $n-1$ data points is termed the prediction residual and PRESS is the sum of the squares of the prediction residuals for each point in a data set [196]. Models were evaluated using stepwise regression by minimising the PRESS statistic for each coefficient in the model. A Student's *t*-test was used to evaluate whether a coefficient was statistically different from zero with a 5% level of significance [196] and only significant factors were included in the evaluation of models selected for further investigation.

Two types of model diagnostic plots, *viz.*, the actual data *vs.* predicted data and studentised residuals *vs.* predicted data graphs, were constructed to evaluate the goodness of fit of the proposed models to experimental data and these results are shown in Appendix 3. The plot of experimental data *vs.* predicted response data generated from a model is particularly useful for detecting outliers [180]. The studentised residual values provide an indication of the magnitudinal differences between observed and predicted responses from a mathematical model and these are plotted *vs.* the predicted values of the response parameters under investigation [180].

6.3.6.2 Curve Fitting

In order to elucidate the possible mechanism of SBS release from matrix formulations, the dissolution profile was fitted to the Korsmeyer–Peppas power law [79] as discussed in § 4.2.7 using the Curve-Fitting Toolbox of Matlab® R2008a (MathWorks, Inc., Natick, MA, USA).

6.3.6.3 Optimisation of Formulations

The Calibration Generation Browser on the Model-Based Calibration Toolbox of Matlab® R2008a (MathWorks, Natick, MA, USA) was used to optimise the formulation to achieve a release profile similar to the reference product. A Normal Boundary Intersection method was used to solve the multi-objective problem using the constraints listed in Table 6.1 as the limits for the optimisation.

6.3.6.4 Comparison of Formulations

Moore and Flanner [197] proposed the use of the similarity factor f_2 for the comparison of dissolution profiles; this parameter can be calculated by fitting dissolution data from a test and reference product to Equation 6.6.

$$f_2 = 50 \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n} w_t(R_t - T_t)^2\right]^{-0.5} \times 100\right\} \qquad \textbf{Equation 6.6}$$

Where,

n = is the number of dissolution sample times,
t = the time sample index
R_t = the mean percent dissolved at time t for the reference dissolution profile
T_t = the mean percent dissolved at time t for the test dissolution profile

The similarity factor f_2 is a logarithmic transformation of the sum-squared error of the differences between a test and reference product over all time points. The factor falls between 0 and 100 and a value of 100 indicates that the dissolution profiles of a test and reference product are identical. At least three or four dissolution time points are needed to calculate the similarity factor f_2 [197], and only one point may be used after 85% dissolution has occurred. In general an f_2 value that falls between 50 and 100 is indicative that the two dissolution profiles being compared are similar [197]. The similarity factor was used to determine the similarity of drug release between the reference and optimised formulations.

6.4 RESULTS AND DISCUSSION

6.4.1 Micromeritic Analysis of Granules

The characterisation of granules is an important step in the development of high quality pharmaceutical dosage forms that conform to physical quality testing criteria such as uniformity of weight, thickness, diameter and crushing strength. For instance the packing geometry of materials to be compressed must be evaluated by determining parameters such as Carr's Index prior to compression of a granulation into tablets. In general, tightly packed powders require a larger driving force to ensure uniform powder flow compared with more loosely packed particles of the same powder [198]. This is an important consideration during the tablet compression cycle since uniform flow of powders from a hopper into a die cavity will result in the production of tablets with minimal weight variation. The micromeritic characteristics of the granules manufactured in these studies, *viz.*, batches SAL001–SAL030, were evaluated and the results are summarised in Table 6.3.

Table 6.3 Granule characteristics of manufactured formulations

Formulation	Bulk density (g/cm^3)	Tap density (g/cm^3)	Carr's Index (%)	Angle of repose (°)
SAL001	0.404	0.441	8.41	33.9
SAL002	0.413	0.462	10.6	33.7
SAL003	0.453	0.487	7.06	34.8
SAL004	0.511	0.527	3.13	33.0
SAL005	0.486	0.534	9.09	33.2
SAL006	0.478	0.519	8.00	32.4
SAL007	0.424	0.464	8.64	37.7
SAL008	0.525	0.552	5.00	33.3
SAL009	0.368	0.421	12.5	43.6
SAL010	0.389	0.432	10.0	36.0
SAL011	0.563	0.614	8.33	38.7
SAL012	0.379	0.400	6.54	37.1
SAL013	0.476	0.510	6.67	35.8
SAL014	0.437	0.483	9.59	34.8
SAL015	0.474	0.501	5.36	33.9
SAL016	0.409	0.436	6.19	32.9
SAL017	0.486	0.514	5.45	35.0
SAL018	0.428	0.474	9.88	35.4
SAL019	0.378	0.407	7.06	35.3
SAL020	0.456	0.540	15.4	34.1
SAL021	0.446	0.486	8.16	32.9
SAL022	0.361	0.395	8.60	34.3
SAL023	0.483	0.530	8.93	35.0
SAL024	0.440	0.474	7.27	19.3
SAL025	0.420	0.065	9.09	35.5
SAL026	0.354	0.394	10.1	36.5
SAL027	0.329	0.364	9.57	36.0
SAL028	0.460	0.510	9.68	33.0
SAL029	0.454	0.481	5.63	20.1
SAL030	0.478	0.520	8.08	32.9

Carr's Index is an indication of the compressibility of a powder, expressed as a percentage, which is a direct measure of the potential powder arch or bridge strength and stability and is particularly useful for predicting the flow properties of a powder. In general powders that have a compressibility index between 5 and 15% exhibit excellent flow properties whereas powders with indices in excess of 21% tend to flow poorly and inconsistently [198]. Carr's Index is a one point determination and does not always reflect the ease with which a powder blend consolidates into compacts, as some powders that have a high index can and do consolidate readily. Rapid consolidation is essential for uniform die filling on tablet presses as the powder flows into a die cavity [199]. Carr's Indices for the batches manufactured in these studies ranged between approximately 3 and 15%, indicating that the granules have relatively good flow properties and that they were suitable for compression into solid oral dosage forms.

The static angle of repose is also an important indicator of powder flow and is related to the nature of inter-particle cohesion. In general powders that have an angle of repose greater than 50° tend to exhibit poor and unsatisfactory flow properties, whereas powders that have minimum angles of repose close to 25° generally exhibit very good flow properties [198]. The granules manufactured in these studies appear to have acceptable flow properties and are suitable for compression into tablets as the experimentally determined angles of repose ranged between 30 and 40° for most of the formulations that were manufactured as shown in Table 6.3.

6.4.2 Physical Properties of Tablets

The manufactured tablets were small and cream coloured with no evidence of chipping, cracking or capping and remained intact following compression. No visible signs of breaking were observed throughout the study. The tablet weight, thickness and diameter data are summarised in Table 6.4.

Table 6.4 Physical characteristics of tablets

Formulation	Tablet weight (mg)	Tablet thickness (mm)	Tablet diameter (mm)	Tablet crushing strength (N)	Tablet assay (mg)
SAL001	221.25 ± 1.07	4.93 ± 0.08	8.73 ± 0.20	33.27 ± 2.13	9.34 ± 0.36
SAL002	223.23 ± 2.37	4.71 ± 0.10	8.81 ± 0.02	31.53 ± 3.91	9.57 ± 0.16
SAL003	221.72 ± 1.26	4.67 ± 0.06	8.71 ± 0.01	30.18 ± 1.95	9.72 ± 0.32
SAL004	221.85 ± 1.50	4.80 ± 0.10	8.82 ± 0.06	25.53 ± 2.11	9.64 ± 0.07
SAL005	222.53 ± 1.15	5.05 ± 0.04	8.81 ± 0.05	16.40 ± 3.21	9.58 ± 0.17
SAL006	220.83 ± 1.47	4.81 ± 0.09	8.77 ± 0.01	21.82 ± 1.83	9.61 ± 0.16
SAL007	224.15 ± 2.19	4.95 ± 0.16	8.83 ± 0.04	36.57 ± 3.95	9.54 ± 0.39
SAL008	223.12 ± 1.39	4.86 ± 0.05	8.87 ± 0.06	28.97 ± 1.91	9.59 ± 0.14
SAL009	223.42 ± 0.64	4.84 ± 0.15	8.79 ± 0.01	29.08 ± 4.38	9.48 ± 0.15
SAL010	223.45 ± 0.82	4.85 ± 0.08	8.82 ± 0.03	14.20 ± 2.14	9.43 ± 0.35
SAL011	224.63 ± 1.14	4.66 ± 0.09	8.84 ± 0.03	13.68 ± 3.04	9.53 ± 0.15
SAL012	221.12 ± 1.39	4.50 ± 0.05	8.82 ± 0.03	48.88 ± 5.09	9.61 ± 0.12
SAL013	221.20 ± 0.60	4.84 ± 0.12	8.74 ± 0.17	24.93 ± 3.23	9.47 ± 0.11
SAL014	221.90 ± 1.15	4.73 ± 0.07	8.77 ± 0.07	42.22 ± 4.23	9.71 ± 0.23
SAL015	221.93 ± 1.38	4.84 ± 0.11	8.80 ± 0.02	24.78 ± 4.10	9.55 ± 0.77
SAL016	210.07 ± 2.89	5.04 ± 0.04	8.85 ± 0.08	13.45 ± 6.43	9.61 ± 0.13
SAL017	220.53 ± 1.40	4.88 ± 0.05	8.85 ± 0.05	11.22 ± 1.49	9.57 ± 0.39
SAL018	221.95 ± 1.71	4.66 ± 0.04	8.84 ± 0.04	30.00 ± 4.20	9.50 ± 0.21
SAL019	221.88 ± 1.16	4.77 ± 0.03	8.78 ± 0.01	43.67 ± 1.36	9.51 ± 0.20
SAL020	220.63 ± 3.44	5.12 ± 0.31	8.84 ± 0.03	16.73 ± 8.45	9.71 ± 0.19
SAL021	222.57 ± 0.89	4.70 ± 0.08	8.84 ± 0.03	31.82 ± 1.88	9.41 ± 0.45
SAL022	222.40 ± 1.31	4.58 ± 0.05	8.98 ± 0.39	59.48 ± 6.83	9.74 ± 0.15
SAL023	221.45 ± 1.29	4.65 ± 0.03	8.80 ± 0.04	23.27 ± 2.50	9.55 ± 0.09
SAL024	222.00 ± 0.84	4.81 ± 0.06	8.83 ± 0.06	34.33 ± 4.00	9.50 ± 0.24
SAL025	222.70 ± 1.21	4.75 ± 0.09	8.81 ± 0.02	32.85 ± 2.71	9.61 ± 0.10
SAL026	221.90 ± 1.20	4.85 ± 0.04	8.79 ± 0.05	44.62 ± 2.44	9.64 ± 0.15
SAL027	220.77 ± 1.04	4.59 ± 0.06	8.80 ± 0.03	70.40 ± 2.26	9.72 ± 2.04
SAL028	222.30 ± 1.12	4.76 ± 0.09	8.85 ± 0.05	23.58 ± 2.97	9.58 ± 0.13
SAL029	221.95 ± 1.11	4.69 ± 0.03	8.83 ± 0.03	29.97 ± 3.51	9.49 ± 0.22
SAL030	221.12 ± 2.26	4.77 ± 0.07	8.75 ± 0.03	31.95 ± 14.63	9.69 ± 0.13

The tablet weight ranged between approximately 220 and 225 mg for all batches manufactured and the tablet thickness and diameter ranged between 4.50 and 5.12 mm, and 8.71 and 8.85 mm, respectively. The low standard deviation values for the measurements are an indication that the tablets showed little variability, and it may therefore be concluded that the method of manufacture of the formulations is suitable for the manufacture of SR matrix tablets of SBS.

The tablets that were produced were fairly soft and forces ranging between 15 and 70 N were required to fracture the dosage forms. Although the tablets were fairly soft, they remained intact for the duration of all tests conducted in these studies and were therefore deemed suitable for their intended purpose.

6.4.3 Tablet Crushing Strength

The impact of formulation variables on tablet crushing strength were investigated using RSM to establish which of the input factors had a significant effect on the measured response, *i.e.* crushing strength. The quadratic model shown in Equation 6.7 was used to describe the relationship between selected input factors and tablet crushing strength.

$$y_H = 27.5 - 23.3x_2 - 4.55x_3 + 15.9x_2^2 + 16.8x_2x_3 \qquad \textbf{Equation 6.7}$$

The equation represents the quantitative effect of significant independent variables such as Carbopol® 974P and xanthan gum content and their effect on tablet crushing strength. It is clear that increasing the content of xanthan gum and Carbopol® 974P results in the production of softer tablets, whereas the interaction between these polymers results in an increase in tablet crushing strength.

The crushing strength of tablet compacts that are manufactured using xanthan gum has been reported [149,200]; it was shown that the use xanthan gum resulted in the production of soft tablets with crushing strength values equivalent to approximately 75 N when compacts were prepared from pure polymer, but values were as low as 15 N for ternary mixtures for compacts that contained 50% w/w of xanthan gum [149]. Similar results were reported for tablets that contained 12–28% w/w of xanthan gum in combination with an API, microcrystalline cellulose and with or without PEG 6000 [200]. These results suggest that the use of xanthan gum in tablet matrices results in the formation of mechanically weak tablets.

The results generated in these studies reveal that Methocel® K100M does not have a statistically significant effect on tablet crushing strength whereas xanthan gum does. HPMC-based compacts with and without API and adjuvants result in the formation of harder tablets compared with those manufactured using xanthan gum, ranging in crushing strength from 65–180 N compared with only 15–75 N for xanthan gum matrix compacts [149]. HPMC and xanthan gum undergo plastic deformation and polymer fragmentation when they are compressed into tablets. However, both plastic deformation and polymer fragmentation are higher for HPMC matrices compared with those observed in xanthan gum matrices, which results in increased bonding when HPMC is compressed into compacts. This ultimately results in the formation of harder tablets when HPMC is used as a matrix forming polymer as opposed to those manufactured using xanthan gum [149]. The consequent elastic recovery

that follows tablet compression of both polymers is relatively high, however the bonds that are formed are strong enough to keep the tablets intact following manufacture [149].

A three dimensional response surface that depicts the relationship between xanthan gum and Carbopol® 974P and tablet crushing strength is depicted in Figure 6.2. In this case the composition of Methocel® K100M and Surelease® was maintained at the medium level for the purposes of this comparison.

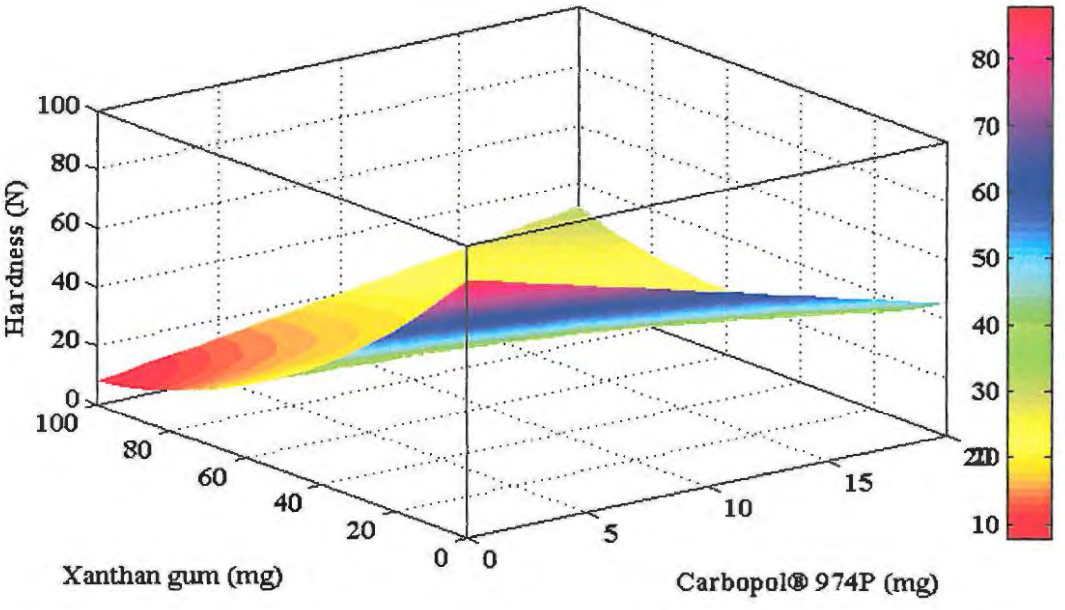


Figure 6.2 Response surface plot depicting the effect of and xanthan gum and Carbopol® 974P on tablet crushing strength with Methocel® K100M and Surelease at the centre levels

The response surface plot reveals that the hardest tablets are produced when the amount of both polymers in the formulation are at their lowest levels and increasing the concentration of xanthan gum at low levels of Carbopol® 974P results in the formation of weaker tablets. When the Carbopol® 974P content is high, increasing the content of xanthan gum results in an initial decrease in tablet crushing strength followed by a slight increase at even higher levels. The response surface also shows that at low levels of xanthan gum, increasing the content of Carbopol® 974P results in the formation of mechanically weaker tablets, whereas at high xanthan gum levels increasing the content of Carbopol® 974P has the opposite effect. These results suggest that there is a significant interaction between the two polymers that have an impact on tablet crushing strength. Evidence of an interaction between Carbopol® polymers and xanthan gum has not been previously reported, but may be linked to the chemical structure of the two polymeric materials. Carbopol® polymers are high molecular

weight acrylic acid polymers that contain free carboxylic acid groups and xanthan gum contains repeating sugar residue units, including glucose, mannose and glucuronic acid [201]. It is possible that the free carboxylic acid residues located on the Carbopol® backbone can react with the free hydroxyl functional groups located on the sugar residues. This may be evident in formulations containing high levels of xanthan gum, where an increase in the content of Carbopol® can result in stronger bonds being formed and which therefore will increase the integrity of the matrix and hence result in the production of harder and/or stronger tablets. This effect however is not apparent at low concentrations of xanthan gum due to the possible formation of weaker bonds and interactions between the reactive groups of the polymer chains.

The contour plot shown in Figure 6.3 shows the relationship between the impact of xanthan gum and Carbopol® 974P content on tablet crushing strength.

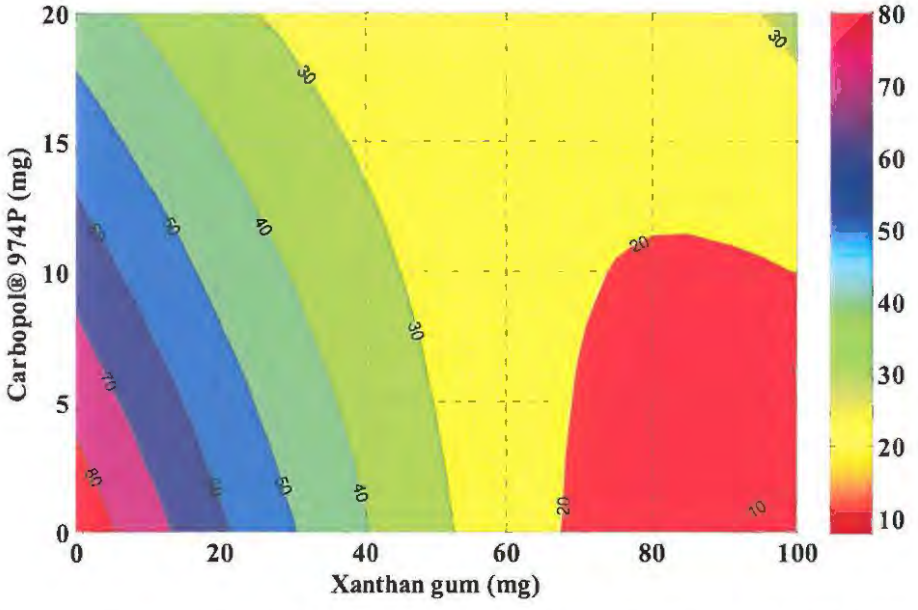


Figure 6.3 Contour plot depicting the effect of and xanthan gum and Carbopol® 974P on tablet crushing strength
with Methocel® K100M and Surelease at their centre levels

The contour plot shown in Figure 6.3 reveals that as the levels of both polymers are increased, there is initially an almost proportionate and linear decrease in tablet crushing strength. The decline in tablet crushing strength becomes non-linear after the incorporation of approximately 50 mg of xanthan gum per tablet. Tablets that are crushed by a force of less than 30 N will be produced after incorporating at least 40 mg xanthan gum in formulations in which high concentrations of Carbopol® 974P (>10 mg) are included. The weakest tablets

will be produced when the content of xanthan gum is at least approximately equivalent to 70 mg and that of Carbopol® 974P is less than approximately 12 mg per tablet.

6.4.4 *In Vitro* Dissolution Results for Manufactured Formulations

The *in vitro* release profiles generated for manufactured formulations derived using experimental design are depicted in Figures 6.4–6.9. The formulations have been classified according to similar composition for ease of comparison and to gain an insight into qualitative factors that can impact *in vitro* release of SBS from these hydrophilic matrix tablets. The notation used to depict the formulation composition uses ratios of Methocel® K100: xanthan gum: Carbopol® 974P: Surelease®, and this convention has been adopted in the following discussion.

The effect of extremes of Methocel® K100M and xanthan gum concentration compared with a formulation with a composition that represents the centre points in the experimental design, *viz.*, 60:50:10:12, is shown in Figure 6.4. A comparison of the *in vitro* release profile of formulations located at the centre points with those generated at the low and high extremes of other formulation variables provides an insight into the qualitative relationships, if any exist, between the input factors and measured responses for the formulations under investigation.

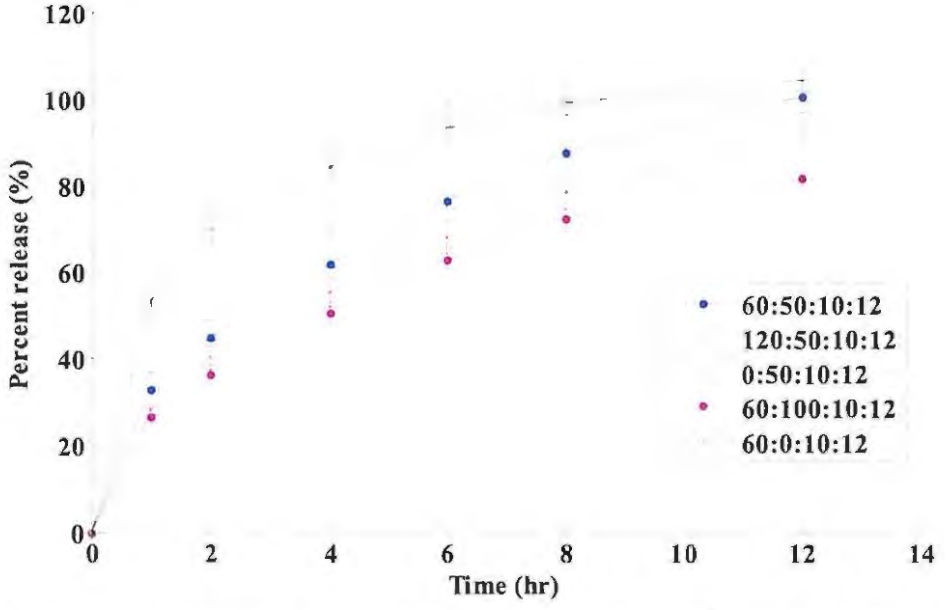


Figure 6.4 *In vitro* release profiles for formulations that contain Methocel® K100M and xanthan gum at at the lower and upper limits of composition compared with the centre formulation

The *in vitro* release profile of the centre point formulation shows that approximately 35% of the dose is released within the first hour of dissolution testing and that the balance of the dose is released gradually over the following 11 hr. It is evident that the rate of release decreases as the *in vitro* release dissolution test progresses. This type of dissolution profile is typical of water-soluble API release from hydrophilic matrix formulations in which HPMC is used as the matrix forming polymer and in which diffusion controlled kinetics are displayed [149].

It is evident from the *in vitro* dissolution profiled depicted in Figure 6.4 that the addition of twice the amount of Methocel® K100M in the formulation, 120:50:10:12 resulted in a marked decrease in both the burst and overall drug release rate from these matrix tablets compared with that observed for the centre point formulation. At the other extreme, the exclusion of Methocel® K100M from the formulation results in a rapid release of SBS initially and complete drug release is observed within 6 hr of commencement of the test compared with that observed for formulations that contained 60 mg of Methocel® K100M. These results indicate that the inclusion of Methocel® K100M as a rate controlling polymer is essential for modulating SBS release rates from hydrophilic matrix formulations.

Similarly the inclusion of 100 mg xanthan gum per tablet (60:100:10:12) indicated that a significant reduction in the initial burst and overall drug release rate was possible. The exclusion of xanthan gum from the matrix formulation, 60:0:10:12 resulted in a marked increase in the burst effect and drug release rates thereby validating the potential usefulness of xanthan gum in controlling the rate of SBS release from hydrophilic matrix formulations.

The dissolution profiles depicted in Figure 6.4 suggest that xanthan gum has a better retardant effect on the SBS release rates compared with when Methocel® K100M is used in these formulations. Increasing the xanthan gum content by 100% resulted in a greater decrease in overall drug release rate compared with that observed when the content of Methocel® K100M in the formulation was doubled. In the absence of these matrix forming polymers, the percent SBS released after 1 hr is approximately 38% and 53% for formulations that did not contain Methocel® K100M and xanthan gum, respectively. It is apparent from these results that the inclusion of Methocel® K100M and xanthan gum is important in controlling the initial release of SBS from these formulations. However, the exclusion of xanthan gum from the matrix formulations resulted in a greater burst release than that observed when Methocel® K100M was excluded from the formulations. This implies that the initial rate of hydration of xanthan

gum is faster than that for Methocel® K100M and results in a better barrier to drug liberation from these matrix formulations. These results are similar to those observed by Talukdar *et al* [149] who reported that the incorporation of xanthan gum in matrix formulations results in a lower burst effect compared with that observed when HPMC was used in matrix formulations.

The impacts of Carbopol® 974P and Surelease® on SBS release when included at the lower and upper extreme of composition, *i.e.* ±α, are shown in Figure 6.5.

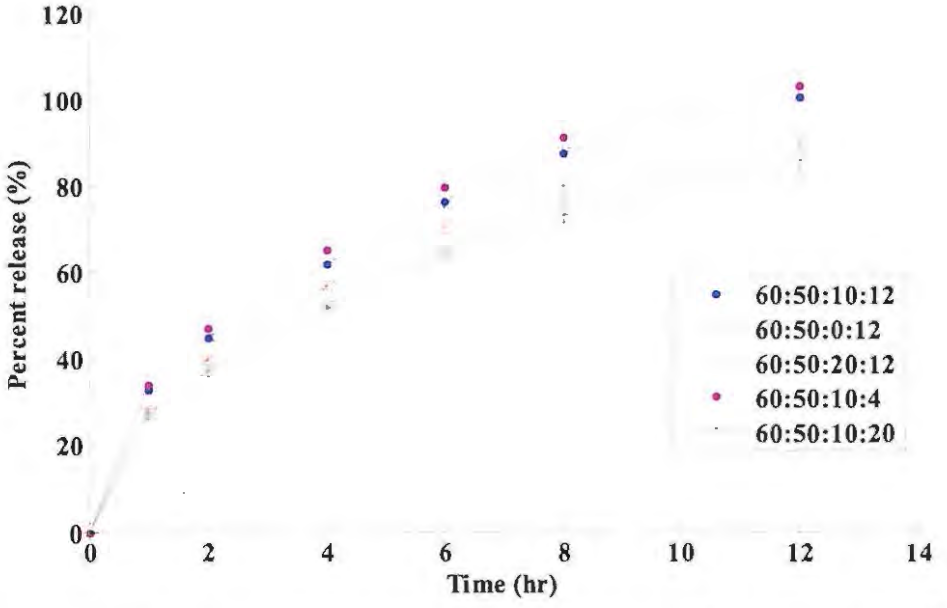


Figure 6.5 *In vitro* release profiles for formulations that contain Carbopol® 974P and Surelease® at the lower and upper limits of composition compared with the centre formulation

The resultant *in vitro* dissolution profiles reveal that the exclusion of Carbopol® 974P from a formulation (60:50:0:12 *vs.* 60:50:10:12) results in a decrease in the overall rate of SBS release. This finding is contrary to what was expected because the exclusion of a polymer generally results in an increased rate of API release. Furthermore, the use of Carbopol® 974P at an extreme level (60:50:20:12) resulted in a further decrease in the rate of SBS release, which implies that the relationship between the level of Carbopol® 974P and the *in vitro* release profile is a rather complex one. The relationship may possibly be described by a non-linear model that is characterised by an initial increase in the drug release rate to a maximum as the polymer level is increased, followed by a subsequent decrease in SBS release rates as the polymer content in the formulation is further increased. These relationships show that there is a possible interaction between Carbopol® 974P and the other polymers used in the

manufacture of these hydrophilic matrix formulations. The impact of Carbopol® 974P on the initial burst release shows the same trend as that observed for the overall drug release rate.

The addition of Surelease® has an impact on the both the initial burst and overall SBS release rates as shown in Figure 6.5. Decreasing the concentration of Surelease® in the formulation does not appear to have a significant effect on the initial release (up to 1 hr) of SBS with an equivalent of approximately 38% SBS released when formulations 60:50:10:4 and 60:50:10:12 were tested. However the overall drug release rate is slightly higher for formulations that were manufactured with more dilute dispersions of Surelease®. It is also apparent that using more concentrated dispersions of Surelease® resulted in a decrease in both the burst and overall rate of SBS release. This is because when Surelease® is used for coating or is sprayed onto a powder blend, a water-insoluble rate controlling membrane which controls drug diffusion is formed [202,203]. It is apparent that the thicker the membrane, the greater the retardant effect on drug release [202,203].

The effect of Carbopol® 974P and Surelease® on formulations that contain Methocel® K100M and xanthan gum at their high levels, *i.e.* 90 mg and 75 mg respectively, is illustrated in Figure 6.6.

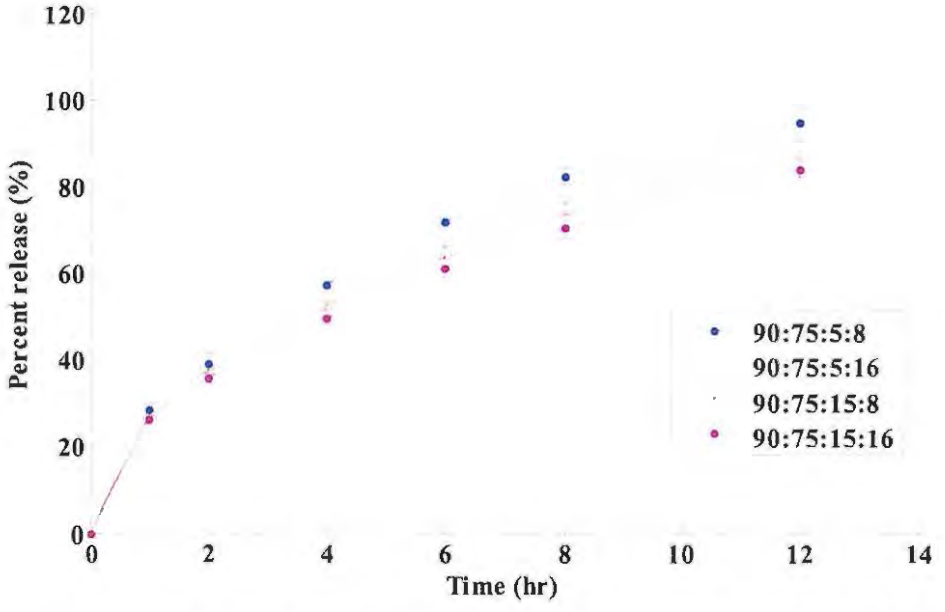


Figure 6.6 *In vitro* release for formulations that contain high levels of Methocel® K100M and high levels of xanthan gum

It is evident from Figure 6.6 that using a higher concentration of Surelease® results in an overall decrease in the release rate of SBS when comparing formulations 90:75:5:8 *vs.* 90:75:5:16. Similarly, the use of higher levels of Carbopol® 974P at these levels of Methocel® K100M and xanthan gum results in a decrease in the overall release rate of SBS. The combination of Carbopol® 974P and Surelease® at their high levels (90:75:15:16) resulted in an additive reduction of the extent of drug release and which is lower than that observed when low concentrations of both polymers are used in the matrix formulations.

Examination of the *in vitro* release profiles reveals that the initial burst release, *i.e.* the percent drug released within an hour of the dissolution test, does not appear to be affected by the presence of Carbopol® 974P and Surelease® when included in these formulations when high levels of Methocel® K100M and xanthan gum are used.

The *in vitro* release profile of SBS from matrix formulations that contain high levels of Methocel® K100M and low levels of xanthan gum with varying levels of Carbopol® 974P and Surelease® is shown in Figure 6.7.

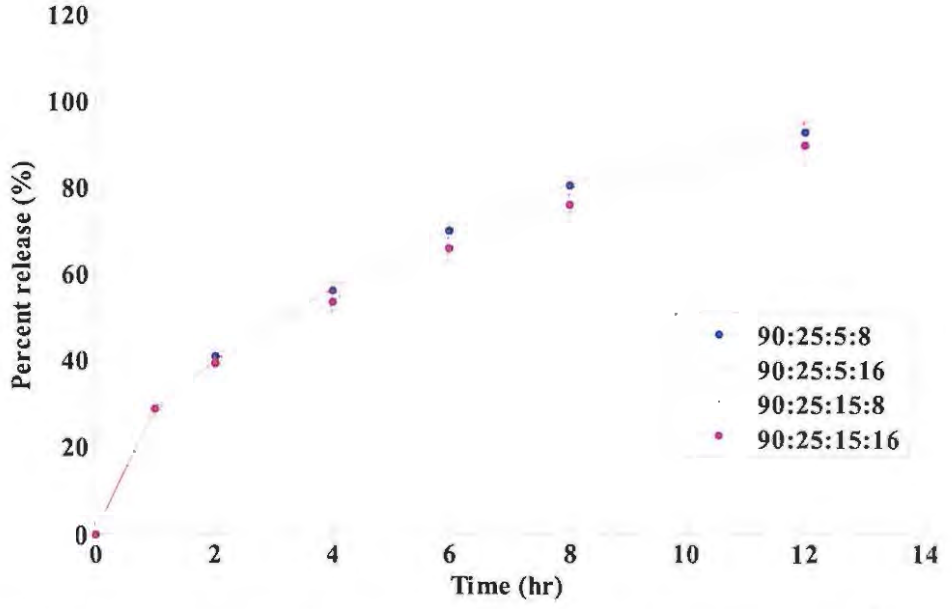


Figure 6.7 *In vitro* release of formulations that contain high levels of Methocel® K100M and low levels of xanthan gum

It is evident in Figure 6.7 that, at the levels of Methocel® K100M and xanthan gum used, increasing the concentration of Surelease® at both high and low levels of Carbopol® (974P, 90:25:5:8 *vs.* 90:25:5:16 and 90:25:15:8 *vs.* 90:25:15:16) did not result in a significant change

in the *in vitro* release profiles for SBS, and the resultant dissolution curves are almost superimposable. However, it is apparent that the use of higher levels of Carbopol® at both low (90:25:5:8 *vs.* 90:25:15:8) and high (90:25:5:16 *vs.* 90:25:15:16) levels of Surelease® resulted in a decrease in the extent of SBS release from the formulations. Further examination of the dissolution profiles shown in Figure 6.7 indicates that there are no observable changes to the initial burst release from the formulations and approximately 30% of the dose is released within 1 hr of the commencement of dissolution testing for all formulations. This is in agreement with the results shown in Figure 6.6 where the incorporation of Carbopol® 974P and Surelease® did not appear to have an impact on the initial release of SBS during the first hour of testing.

The *in vitro* release profiles of formulations that contain low levels of Methocel® K100M and high levels of xanthan gum are shown in Figure 6.8.

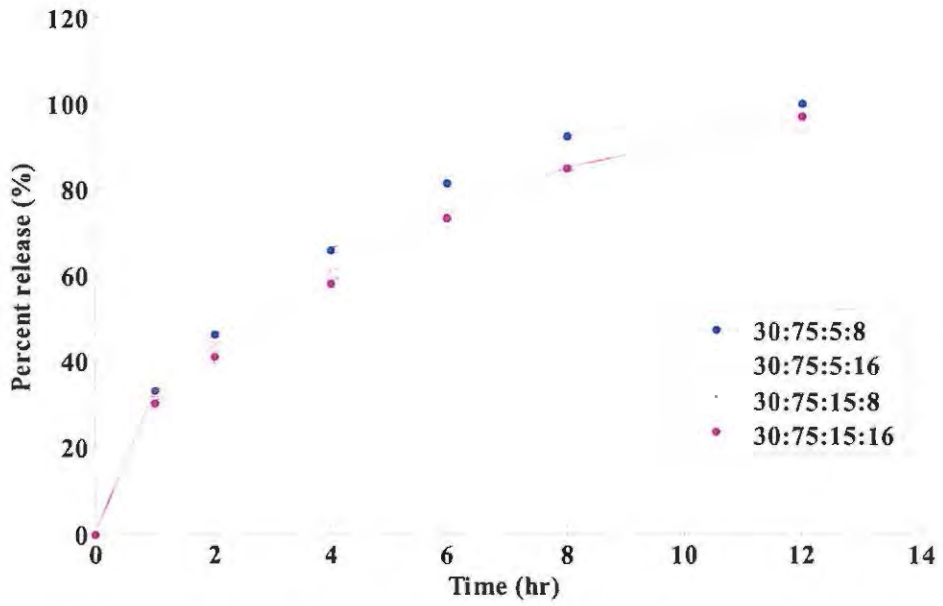


Figure 6.8 *In vitro* release of formulations containing low levels of Methocel® K100M and high levels of xanthan gum

It is clear from the *in vitro* release profiles depicted in Figure 6.8 that the use of different levels of Carbopol® 974P and Surelease® does not have a significant impact on the initial burst release of SBS from all formulations. However, the rate and extent of *in vitro* release of SBS from these formulations is highest for the formulation that contains low levels of both Carbopol® and Surelease® (30:75:5:8). Increasing the content of both Carbopol® 974P and Surelease® to their high levels, *i.e.* 30:75:15:8 and 30:75:5:16, resulted in a decrease in the

extent of drug release from these matrix formulations compared with the formulation 30:75:5:8. However, when Carbopol® 974P and Surelease® are increased to their high levels, *i.e.* 30:75:15:16, a further and significant decline in the extent of drug release is not observed. Since this curve is nearly superimposable with the *in vitro* release profile obtained for formulations 30:75:15:8 and 30:75:5:16, there is evidence that at these levels of Methocel® K100M and xanthan gum, the impact of Carbopol® 974P and Surelease® on the dissolution rate of SBS is not enhanced.

The *in vitro* release profiles of formulations that contain low levels both Methocel® K100M and xanthan gum are depicted in Figure 6.9.

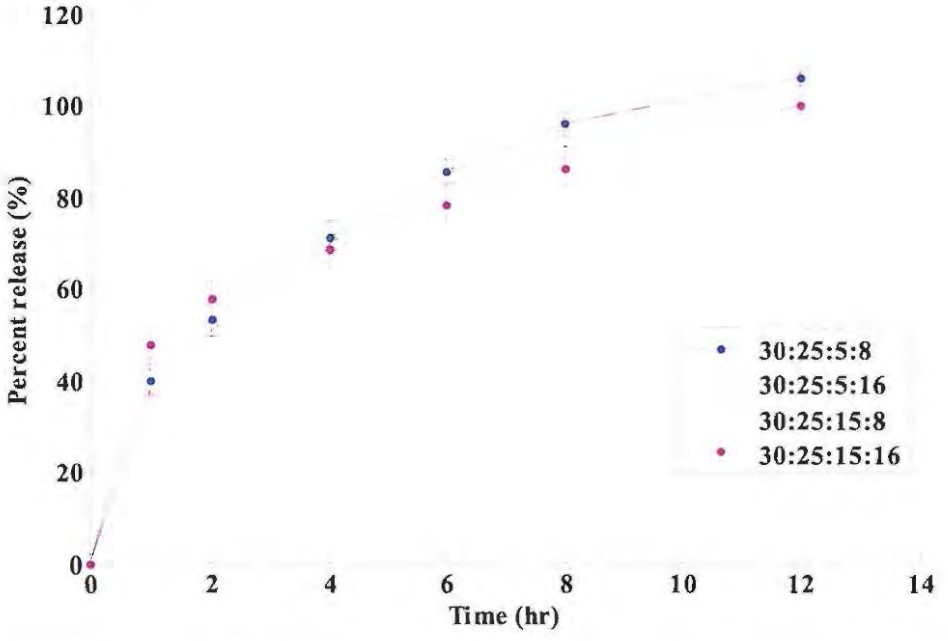


Figure 6.9 *In vitro* release profiles of formulations containing low levels of Methocel® K100M and low levels of xanthan gum

The *in vitro* dissolution profiles for the formulations shown in Figure 6.9 reveal that there is a high initial burst release within the first hour of the dissolution test and approximately 35–45% of the dose is released within that hour. Interestingly, formulations that contain high levels of Surelease® and low levels of Carbopol® 974P (30:25:5:16) showed the lowest burst effect for SBS and increasing the concentration of Carbopol® (30:25:15:16) resulted in an increase in the burst release from these formulations. The complexity of the relationship between Carbopol® 974P and the rate and extent of SBS release has been previously noted and will be further investigated using RSM.

The overall rate and extent of drug release from formulations that contain high and low levels of Surelease® (30:25:5:8 and 30:25:5:16) are similar indicating that the impact of Surelease® on the extent of drug release at low levels of Methocel® K100M and xanthan gum is limited. When Carbopol® 974P is used at high levels (30:25:15:8 and 30:25:15:16), the use of a higher concentration of Surelease® resulted in an initial faster rate of release, but the overall extent of drug release in the latter stages of the *in vitro* release test decreased.

It is apparent from Figures 6.4–6.9 and the preceding discussion that the relationship between the levels of polymer and the rate and extent of drug release is complicated and is further confounded by interactions between the formulation variables and their relative compositions. The use of RSM may be applied to investigate and understand the nature of the complex relationships that exist between the input variables and resultant responses selected for investigation in these studies.

6.4.5 Analysis of *In Vitro* Release Data and Response Surface Modelling

The extent of drug release following the early stages of *in vitro* release testing, *i.e.* between 1 and 8 hr, is best described by use of quadratic equations (Equations 5.8 to 5.12), whereas a linear model was best suited to describe the relationship between the input factors and the extent of drug release after 12 hr of testing (Equation 6.13). The polynomial models used to investigate these relationships are summarised in Table 6.5.

Table 6.5 Polynomial models that depict the relationships between input variables and responses

$y_{1hr} = 30.5 - 7.67x_1 - 8.43x_2 + 9.55x_1x_2 + 9.78x_2^2 - 5.66x_2x_3 - 2.88x_2^3$	**Equation 6.8**
$y_{2hr} = 41.8 - 8.52x_1 - 10.2x_2 - 2.25x_4 + 9.49x_1x_2 + 11.0x_2^2 - 4.67x_2x_3 - 3.17x_3^2$	**Equation 6.9**
$y_{4hr} = 60.3 - 10.2x_1 - 9.45x_2 - 2.81x_3 - 3.46x_4 - 7.41x_1x_2 - 4.90x_3^2$	**Equation 6.10**
$y_{6hr} = 72.1 - 13.5x_1 - 8.08x_2 - 4.01x_3 - 4.43x_4 + 10.4x_1^2 + 4.49x_1x_2 - 5.29x_3^2$	**Equation 6.11**
$y_{8hr} = 82.7 - 12.0x_1 - 7.06x_2 - 4.26x_3 - 4.91x_4 + 6.37x_1^2 + 4.70x_3^2$	**Equation 6.12**
$y_{12hr} = 94.3 - 7.71x_1 - 6.87x_2 - 3.21x_3 - 3.98x_4$	**Equation 6.13**

Coefficients with more than one factor represent interactions between factors, whereas coefficients with second order terms are indicative of the quadratic nature of relationships. A positive value for a coefficient represents an effect that will favour a measured response whereas a negative value indicates that an inverse relationship exists between a factor and a measured response.

It is apparent from the equations listed in Table 6.5 that the inclusion of both Methocel® K100M and xanthan gum results in a significant decrease in the extent of drug release at all stages of the dissolution test. The relative contribution of both polymers to the *in vitro* release profile depends on the stage of the dissolution test, where it is apparent that xanthan gum has a higher retardant effect in the early stages of the dissolution test (y_{1hr} and y_{2hr}), whereas Methocel® K100M has a greater impact on the extent of drug release as observed in the latter stages of the dissolution test ($>y_{4hr}$). These results are corroborated by those observed by Talukdar *et al* [149] who showed that the use of xanthan gum resulted in a lower burst release compared with that observed when HPMC was used as a matrix forming polymer. These results are also in agreement with those depicted the *in vitro* release profiles shown in Figure 6.4 where the exclusion of xanthan gum resulted in a higher burst effect compared with that from formulations that contained no Methocel® K100M. The impact of these polymers on the extent of drug release is due to the formation of a hydrophilic gel layer when these matrices are immersed in an aqueous medium. The thickness of the gel layer hinders drug diffusion from the interior of the matrix to the bulk dissolution medium, and therefore increasing the relative content of these polymers in a formulation results in an overall decrease in the extent of drug release at all stages of the dissolution test.

Close inspection of the equations listed in Table 6.5 reveals that both Carbopol® 974P and Surelease®, at the levels that were studied in the experimental domain under investigation, do not appear to have a significant impact on drug release during the initial stages of the dissolution test, *i.e.* y_{1hr}.

The addition of Carbopol® 974 to the matrix has in fact a negative effect on the extent of drug release in the latter stages of the dissolution test. Carbopol® polymers on hydration form a gelatinous layer composed of discrete microgels that are made up of many polymer particles within which SBS is dispersed. This cross-linked network entraps SBS in the hydrogel domains and hinders drug diffusion from the matrix. It is also apparent that the interaction between Carbopol® and other matrix forming materials is significant in the early stages of the dissolution test.

The use of Surelease® as a granulating fluid also results in a reduction in the extent of drug release at the latter stages of dissolution testing *i.e.* from at least 2 hr from commencing *in*

vitro dissolution testing. Surelease® is an aqueous dispersion of ethylcellulose that contains fractionated coconut oil, dibutyl sebacate and ammonium hydroxide and, when Surelease® is sprayed onto a powder blend, a water-insoluble rate controlling polymer, which controls the passage of an API through a membrane, may be formed [202,203]. The concentration of the dispersion used when manufacturing the formulation determines the thickness of a membrane and in general, the thicker the membrane, the slower the rate of drug release [202,203].

The impact of formulation variables on the rate and extent of drug release at different stages of the dissolution test is shown as response surface and contour plots in Figures 6.10–6.30.

The response surface and respective contour plots for the *in vitro* release data in the early stages of the dissolution test with Carbopol® 974P and Surelease® held at the centre levels are depicted in Figures 6.10–6.13.

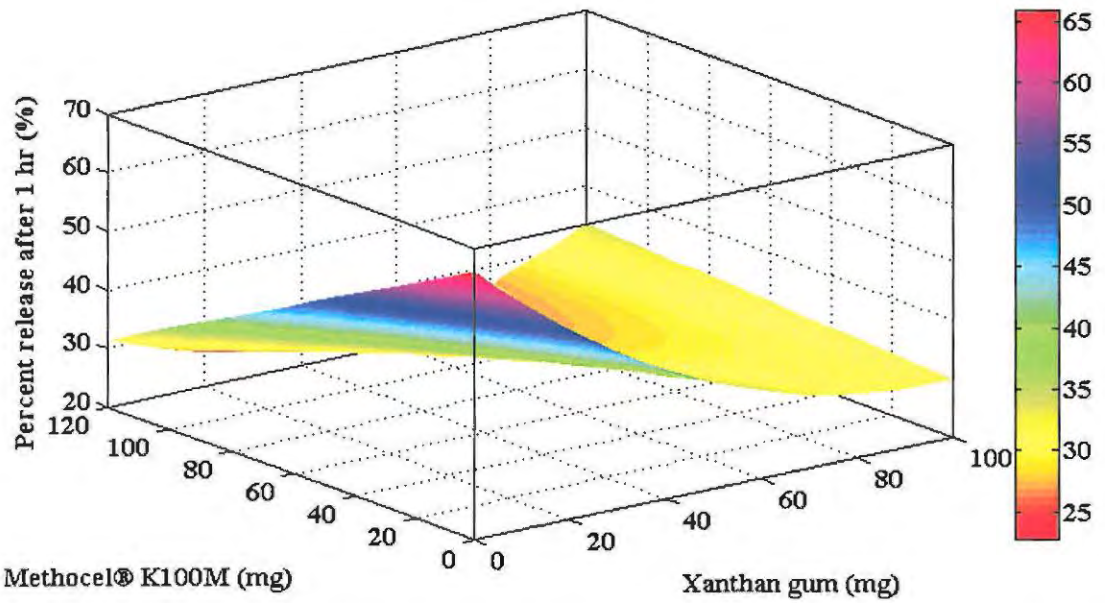


Figure 6.10 Response surface plot depicting the effect of Methocel® K100M and xanthan gum on percent SBS released after 1 hr with Carbopol® 974P and Surelease® concentrations at the centre level

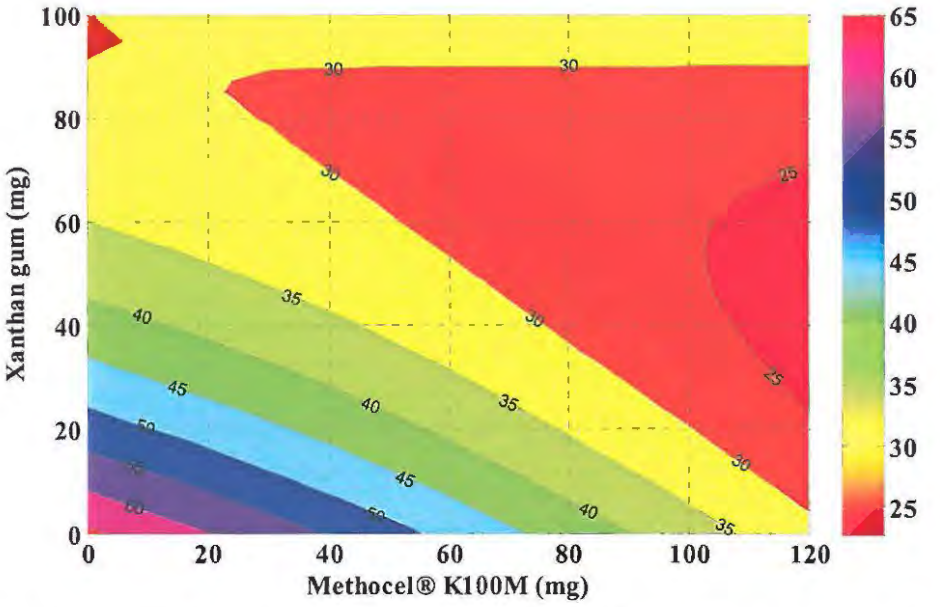


Figure 6.11 Contour plot depicting the effect of Methocel® K100M and xanthan gum on percent SBS released after 1 hr with Carbopol® 974P and Surelease® concentrations at the centre level

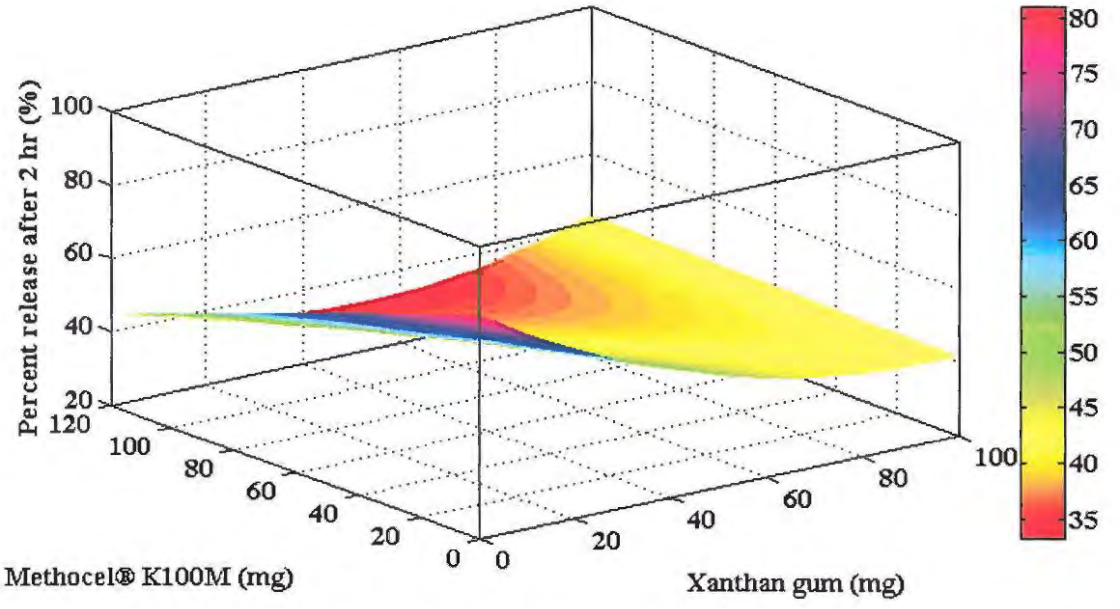


Figure 6.12 Response surface plot depicting the effect of Methocel® K100M and xanthan gum on percent SBS released after 2 hr with Carbopol® 974P and Surelease® concentrations at the centre level

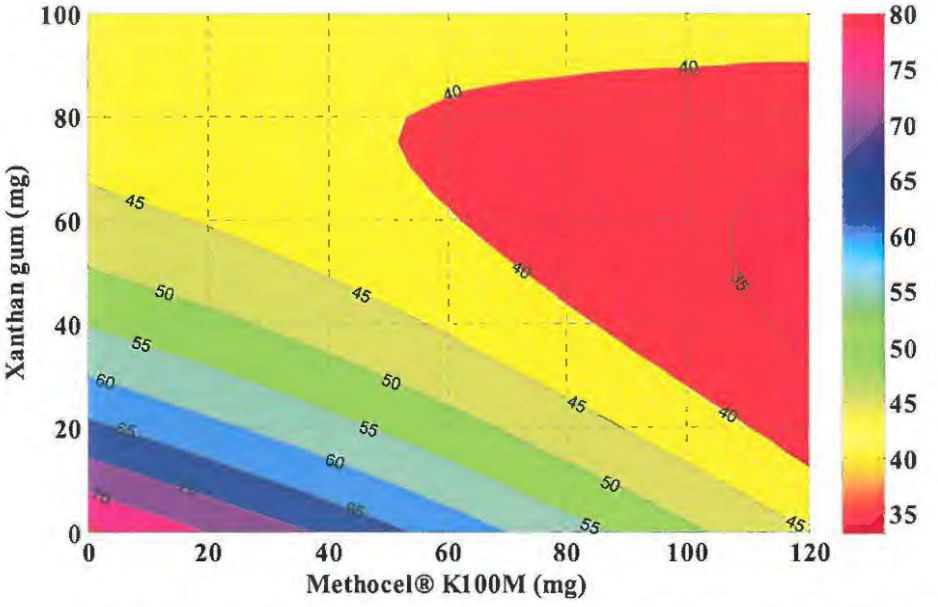


Figure 6.13 Contour plot depicting the effect of Methocel® K100M and xanthan gum on percent SBS released after 2 hr with Carbopol® 974P and Surelease® concentrations at the centre level

The response surface plots shown in Figures 6.10 and 6.12 illustrate that the percent SBS released is highest when low levels of the two polymers are used in formulations. It is evident that at low levels of Methocel® K100M the inclusion of xanthan gum in progressively increasing concentrations results in a consequent decline in the extent of drug release. Similarly at low levels of xanthan gum, increasing the concentration of Methocel® K100M also results in a decrease in the extent of SBS release at the initial stages of dissolution testing.

An interesting observation is there is evidence of an interaction between Methocel® K100M and xanthan gum during the early stages of dissolution testing, *i.e.* at times of 1 and 2 hr as shown in Figures 6.10 and 6.12. The addition of Methocel® K100M to formulations that contain high concentrations of xanthan gum resulted in a slight increase in the extent of SBS release from these formulations. This observation is in contradiction to the assumption that using high concentrations of polymer in pharmaceutical formulations results in a decrease in the extent of drug release. It is apparent from examining both the topographical surface plots and the respective equations for y_{1hr} (Equation 6.8) and y_{2hr} (Equation 6.9) that the interaction between Methocel® K100M and xanthan gum results in an increase in the extent of drug release, when the polymers are used in combination.

The contour plots that represent the relationship between Methocel® K100M and xanthan gum and the extent of SBS release (Figures 6.11 and 6.13) also depict the complex nature of the relationship that exists between the input factors and measured responses under investigation. In the early stages of the dissolution test, *i.e.* at times 1 hr and 2 hr, it is apparent that the addition of increasing quantities of polymer results in a progressive decline in SBS release rates and the decline occurs in an almost linear fashion. However as the polymer levels are increased, drug release follows a quadratic pattern that is evidence of the inherent complexities of the interaction and relationships that exist in this formulation. The lowest extent of SBS release would be observed when the xanthan gum content used is >80 mg and that of Methocel® K100M is >20 mg and >50 mg in order to minimise drug release at times 1 hr and 2 hr, respectively.

The response surface and contour plots that depict the relationship between Methocel® K100M and xanthan gum in the latter stages of the dissolution testing are shown in Figures 6.14–6.19.

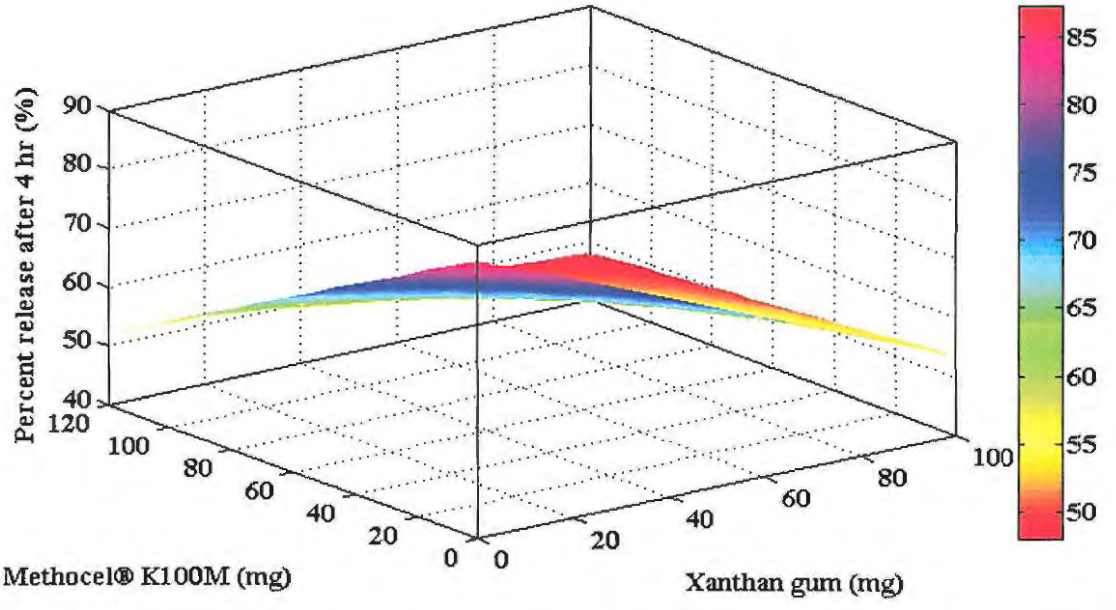


Figure 6.14 Response surface plot depicting the effect of Methocel® K100M and xanthan gum on percent SBS released after 4 hr with Carbopol® 974P and Surelease® concentrations at the centre level

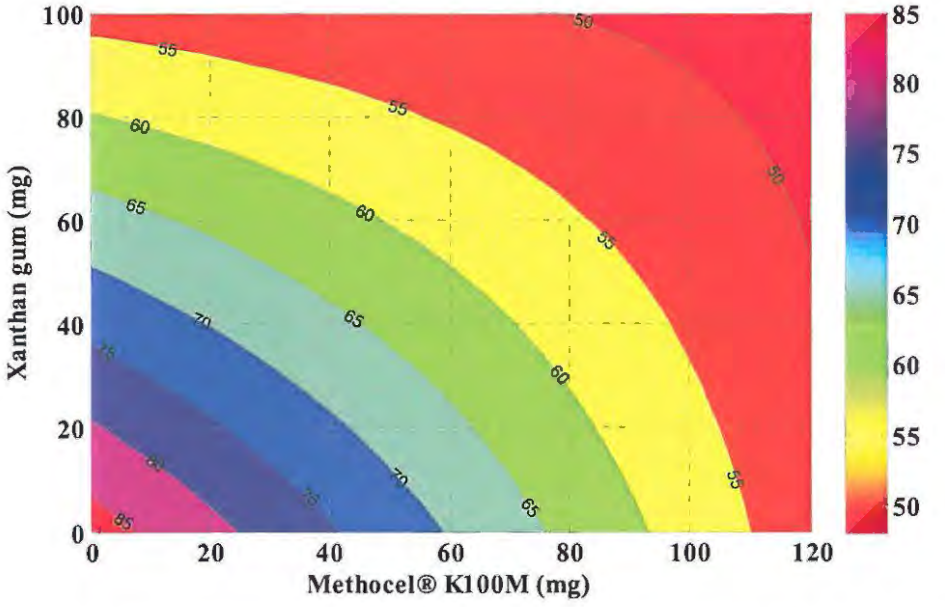


Figure 6.15 Contour plot depicting the effect of Methocel® K100M and xanthan gum on percent SBS released after 4 hr with Carbopol® 974P and Surelease® concentrations at the centre level

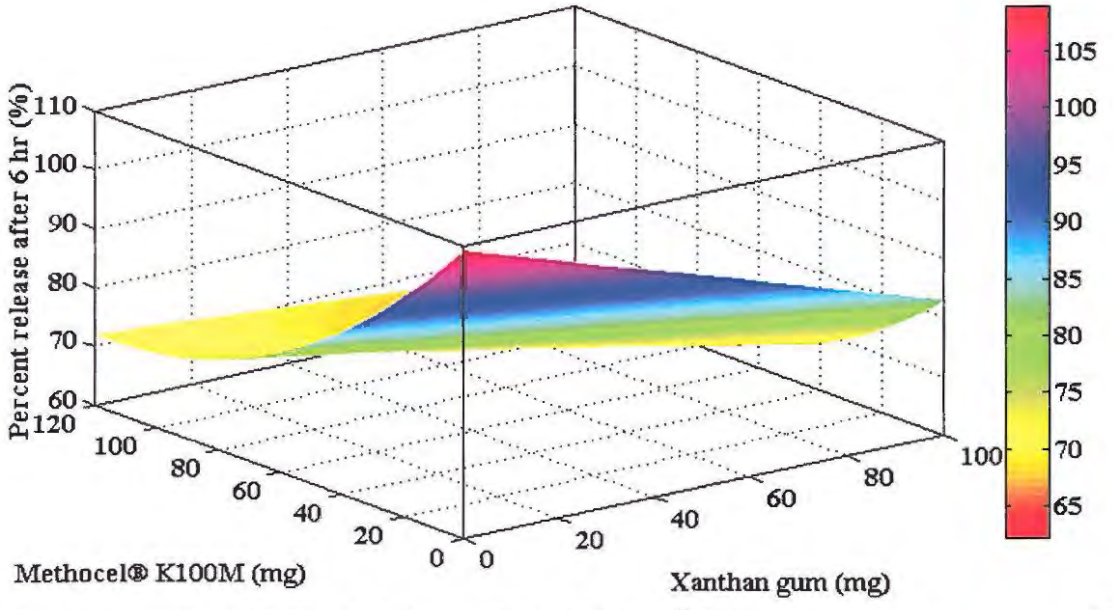


Figure 6.16 Response surface plot depicting the effect of Methocel® K100M and xanthan gum on percent SBS released after 6 hr with Carbopol® 974P and Surelease® concentrations at the centre level

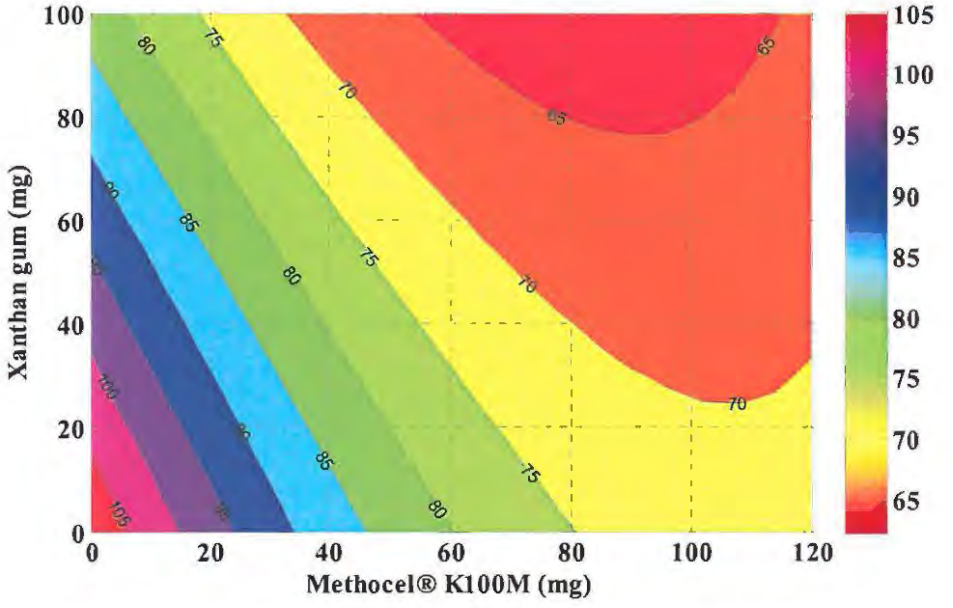


Figure 6.17 Contour plot depicting the effect of Methocel® K100M and xanthan gum on percent SBS released after 6 hr with Carbopol® 974P and Surelease® concentrations at the centre level

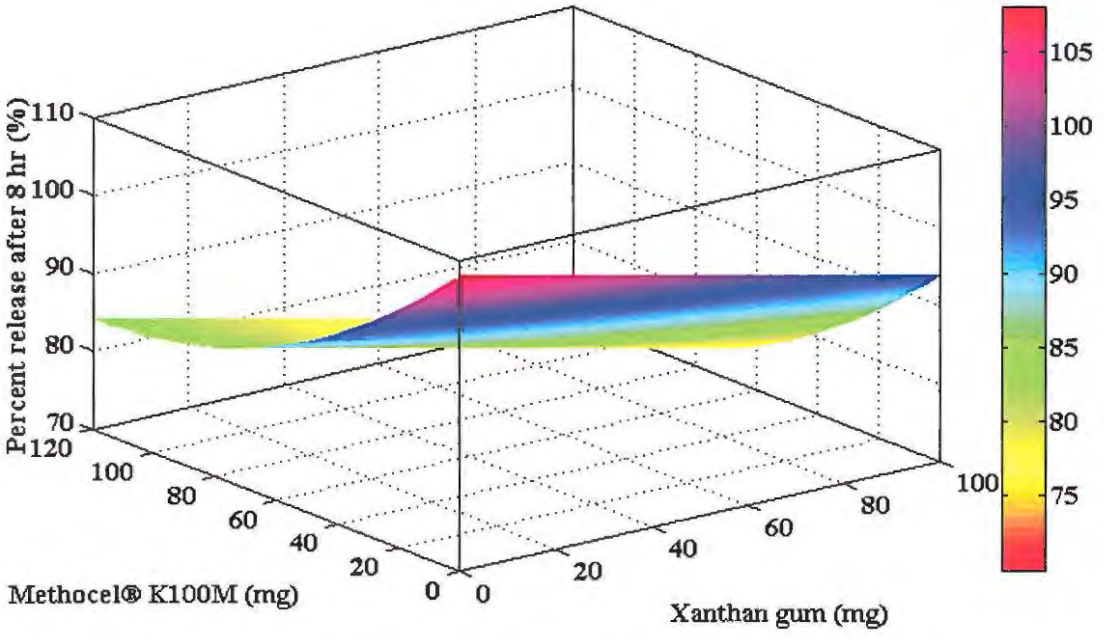


Figure 6.18 Response surface plot depicting the effect of Methocel® K100M and xanthan gum on percent SBS released after 8 hr with Carbopol® 974P and Surelease® concentrations at the centre level

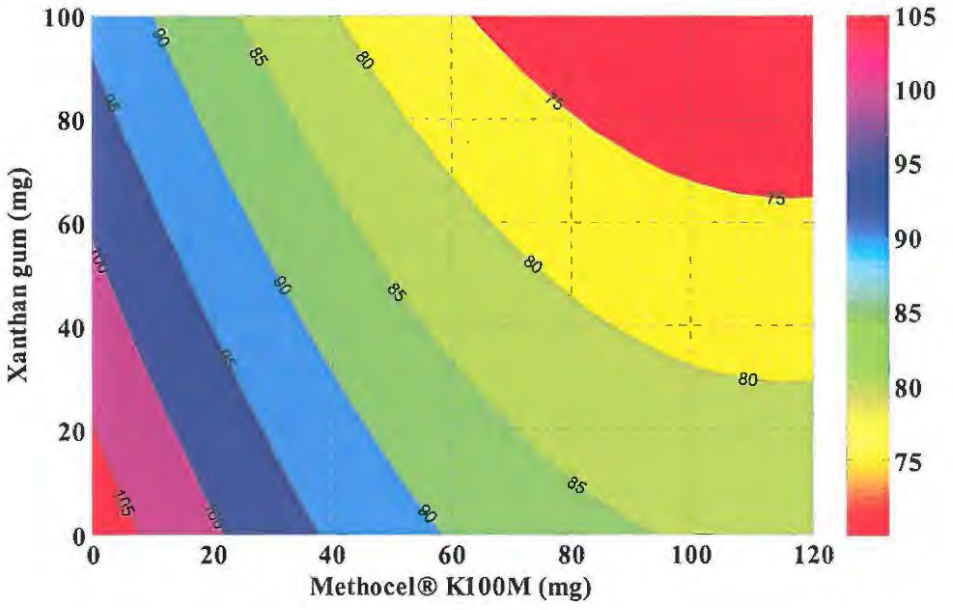


Figure 6.19 Contour plot depicting the effect of Methocel® K100M and xanthan gum on percent SBS released after 8 hr with Carbopol® 974P and Surelease® concentrations at the centre level

In general the response surface plots for these data reveal that percent SBS released is highest when low levels of the polymers are used in the formulations and this is the case for the latter stages of dissolution testing. It is apparent that, at low and high levels of Methocel® K100M, the inclusion of xanthan gum in progressively increasing concentrations results in a synergistic decline in the extent of SBS release at all dissolution time points. Similarly, after 4 hr of dissolution testing (Figure 6.14), increasing the Methocel® K100M content at low levels of xanthan gum results in a decrease in the extent of drug release. However, after 6 hr and 8 hr of dissolution testing, the inclusion of Methocel® K100M gum in formulations that contain both low and high levels of xanthan results in an initial decrease in extent of release followed by a slight increase in the extent of SBS as is seen in the topographical surface plots depicted in Figures 6.16 and 6.18.

The impact of xanthan gum on the extent of SBS dissolution decreases as the dissolution test progresses as is apparent in the topographical surface plots depicted in Figures 6.14, 6.16 and 6.18. In addition the equations listed in Table 6.5 further support this conclusion. The magnitude of the coefficient related to xanthan gum decreases, *i.e.* x_2, which is mirrored by a decreasing gradient in the response surface plots in the region associated with increasing xanthan gum content. These findings are slightly different to those in which the impact of Methocel® K100M on the extent of dissolution is depicted in the equations shown in Table

6.5; these show drug release to increase to a maximum value at 6 hr after which the magnitude of the effect of this polymer decreases as the dissolution test progresses.

The contour plots (Figures 6.15, 6.17 and 6.19) that depict the relationship between the levels of Methocel® K100M and xanthan gum in the latter stages of the dissolution test, *i.e.* at ≥4 hr, also show the same relationships as described above. In general, it is apparent that increasing the polymer content results in a progressive decrease in the extent of drug release at these stages of the dissolution test. However, closer examination of the contour plots reveals that there is a shift in the relationship between the polymer content and extent of drug release. The extent of drug release tends to become less quadratic and more linear in nature as the dissolution test progresses.

Evaluation of Figure 6.20 reveals that the percent SBS released after 12 hr of dissolution testing has a linear relationship with the input factors that were studied. Furthermore the extent of drug release decreases with increasing levels of polymers, and the lowest overall drug release percent will be observed when at least 80 mg and 60 mg of Methocel® K100M and xanthan gum, respectively are used in formulations.

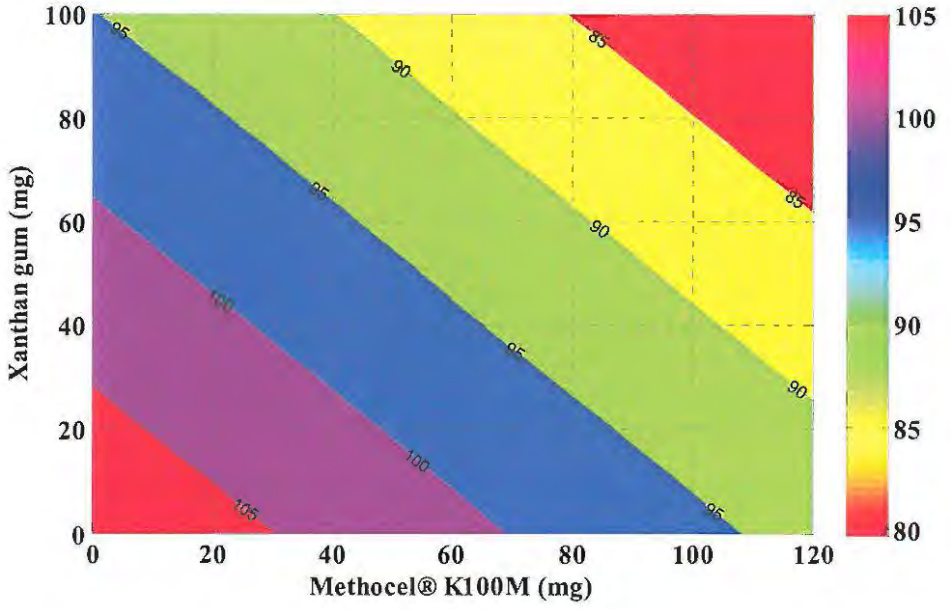


Figure 6.20 Contour plot depicting the effect of Methocel® K100M and xanthan gum on percent SBS released after 12 hr with Carbopol® 974P and Surelease® concentrations at the centre level

The relationships between the input variables and responses are summarised in Table 6.5 which shows that there is an interaction between xanthan gum and Carbopol® 974P in the

early stages of the dissolution test. The response surface and contour plots that illustrate the effect of xanthan gum and Carbopol® 974P with Methocel® K100M and Surelease® concentrations at the centre level are shown in Figures 6.21–6.24.

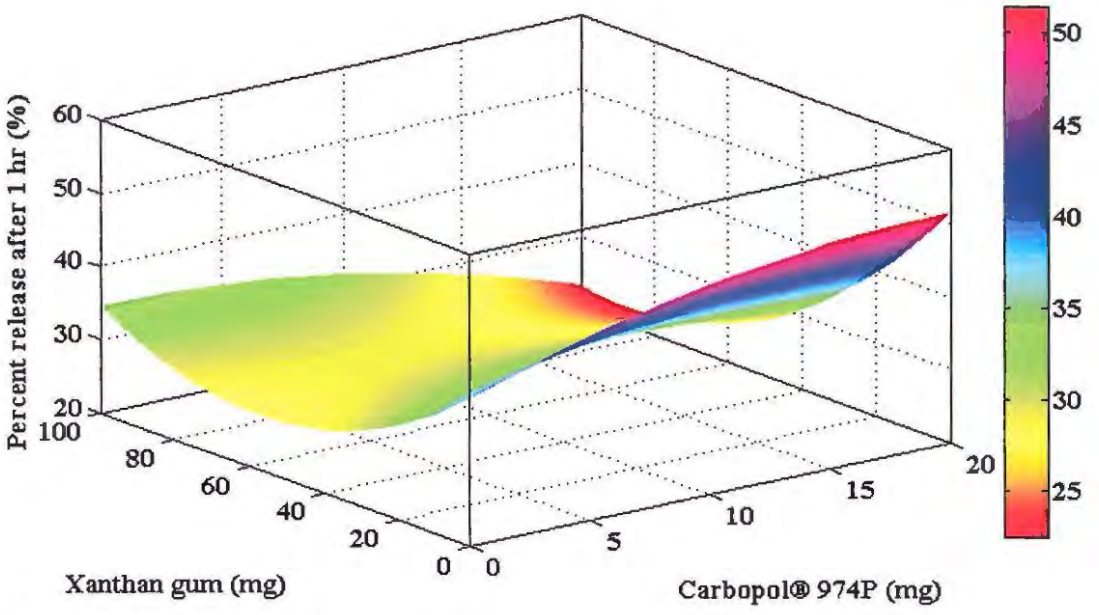


Figure 6.21 Response surface plot depicting the effect of xanthan gum and Carbopol® 974P on percent SBS released after 1 hr with Methocel® K100M and Surelease® concentrations at the centre level

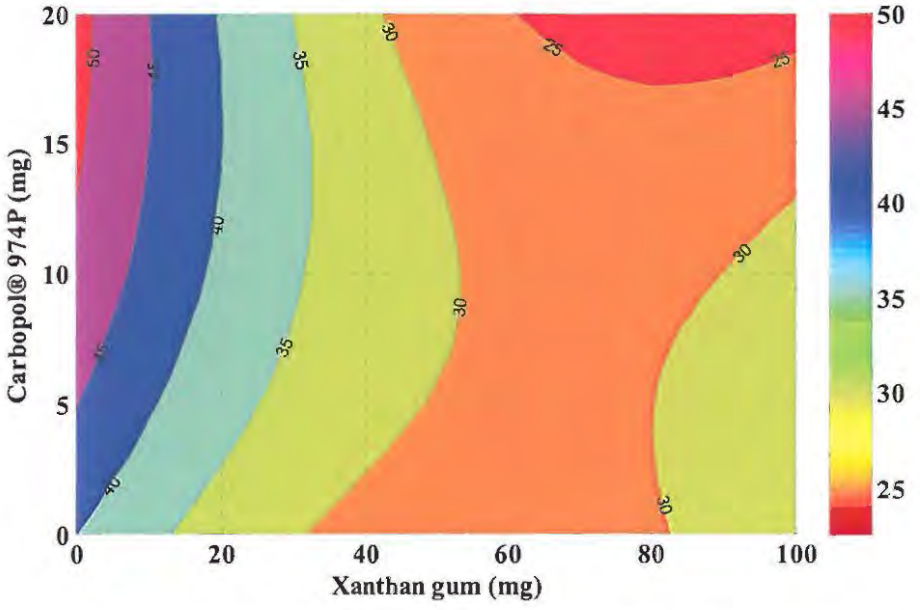


Figure 6.22 Contour plot depicting the effect of xanthan gum and Carbopol® 974P on percent SBS released after 1 hr with Methocel® K100M and Surelease® concentrations at the centre level

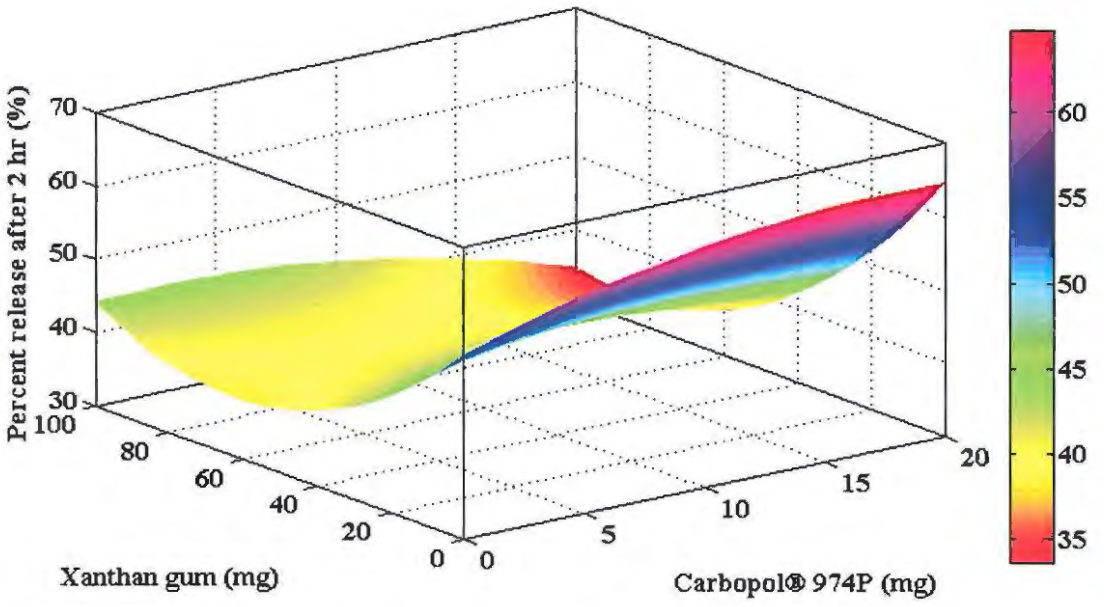


Figure 6.23 Response surface plot depicting the effect of xanthan gum and Carbopol® 974P on percent SBS released after 2 hr with Methocel® K100M and Surelease® concentrations at the centre level

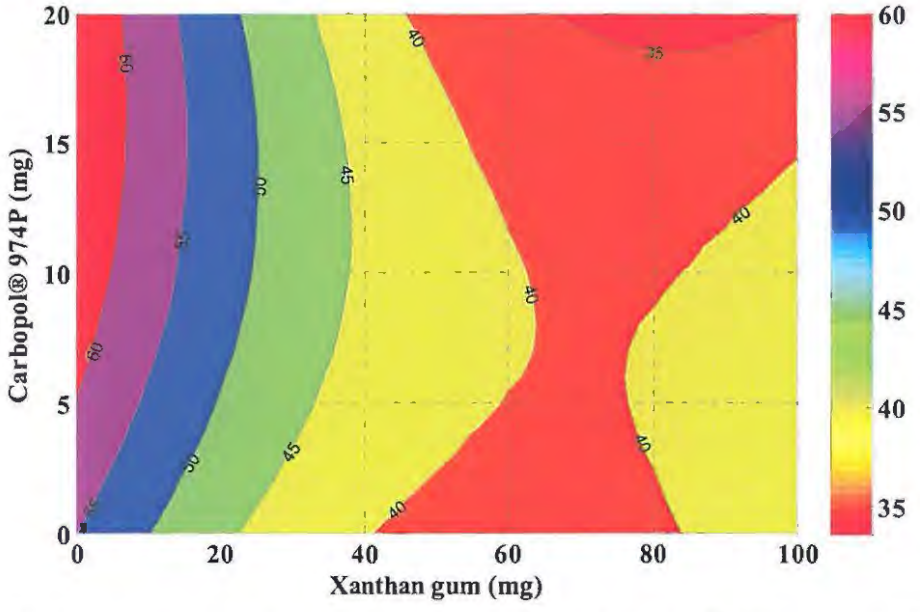


Figure 6.24 Contour plot depicting the effect of xanthan gum and Carbopol® 974P on percent SBS released after 2 hr with Methocel® K100M and Surelease® concentrations at the centre level

The topographical surface plots depicted in Figures 6.21 and 6.23 show that when low levels of xanthan gum are used, increasing the amount of Carbopol® 974P in the formulation results in an increase in the overall extent of SBS release. These results are surprising since the inclusion of additional polymer will often result in a decrease in the overall rate and extent of

drug release. However in cases where the xanthan gum content is high, increasing Carbopol® 974P content results in a decrease in the extent of drug release. The response surface plots depicted in Figures 6.21 and 6.23 show evidence of an interaction between the two polymers since at both low and high levels of Carbopol® 974P, increasing the xanthan gum content results in an initial decrease followed by increase in the extent of drug release.

Inspection of the equations listed in Table 6.5 reveals that there is no interaction between xanthan gum and Carbopol® 974P in the latter stages of dissolution testing. This observation may be related to the difference in the pH of the dissolution media used in the early and latter stages of dissolution testing. During the first two hours, the pH is acidic and is then increased to at least pH 6 over the next 10 hours. The interaction may be direct consequence of the ionisation state of Carbopol® 974 at acidic pH where the polymer is unionised and difficult to hydrate, but at pH 4.5 the polymer begins to ionise, hydrates and begins to swell [152]. Unionised carboxylic acid functional groups may interact with the hydroxyl groups on the sugar moieties of the xanthan gum backbone by forming hydrogen bonds that either weaken or strengthen the gel layer depending on the concentration of either polymer in the formulation. When the xanthan gum content is low, increasing the Carbopol® 974P content results in the formation of a gel phase that has little integrity and that permits rapid drug liberation from the matrices. However at high xanthan gum concentrations it is possible that the interaction results in the formation of a gel layer that has greater integrity and that hinders drug diffusion as the concentrations of Carbopol® 974P are increased.

The contour plots depicted in Figures 6.22 and 6.24 depict an interaction between the two polymers and show a curvature, evident from the response surface plots, that indicates an initial decrease and a subsequent increase in the extent of drug release as xanthan gum is added to formulations that contain Carbopol® 974P.

The impact of Surelease® on *in vitro* dissolution profiles is not evident in the first hour of dissolution testing but becomes significant after 2 hr, shown during *in vitro* release studies and in the equations summarised in Table 6.5. The response surface and contour plots depicted in Figures 6.25–6.30 show that at the later stages of dissolution testing *i.e.* ≥4 hr an interaction between Surelease® and Carbopol® 974P, exists.

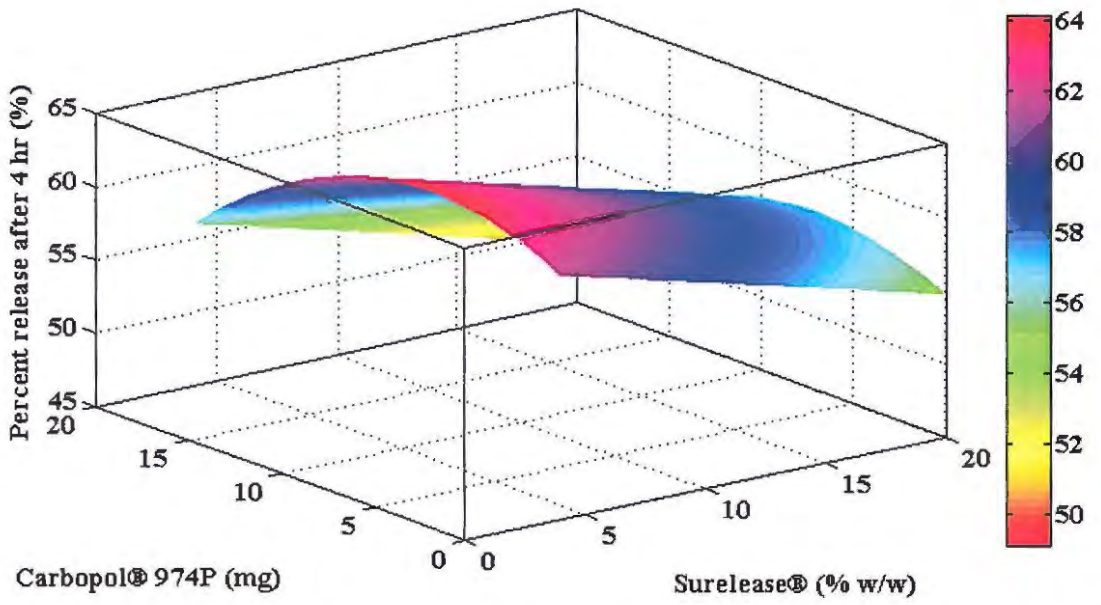


Figure 6.25 Response surface plot depicting the effect of Carbopol® 974P and Surelease® on percent SBS released after 4 hr with Methocel® K100M and xanthan gum concentrations at the centre level

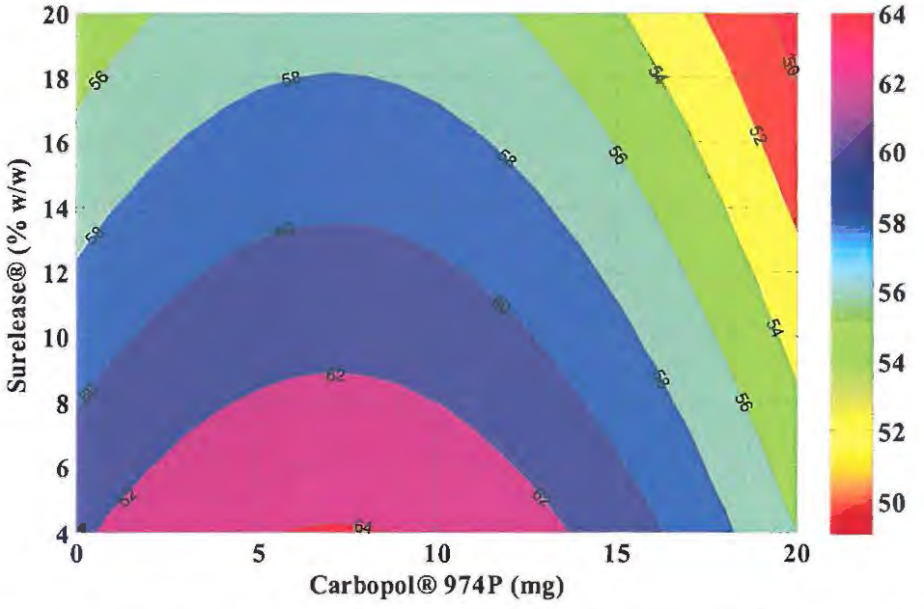


Figure 6.26 Contour plot depicting the effect of Carbopol® 974P and Surelease® on percent SBS released after 4 hr with Methocel® K100M and xanthan gum concentrations at the centre level

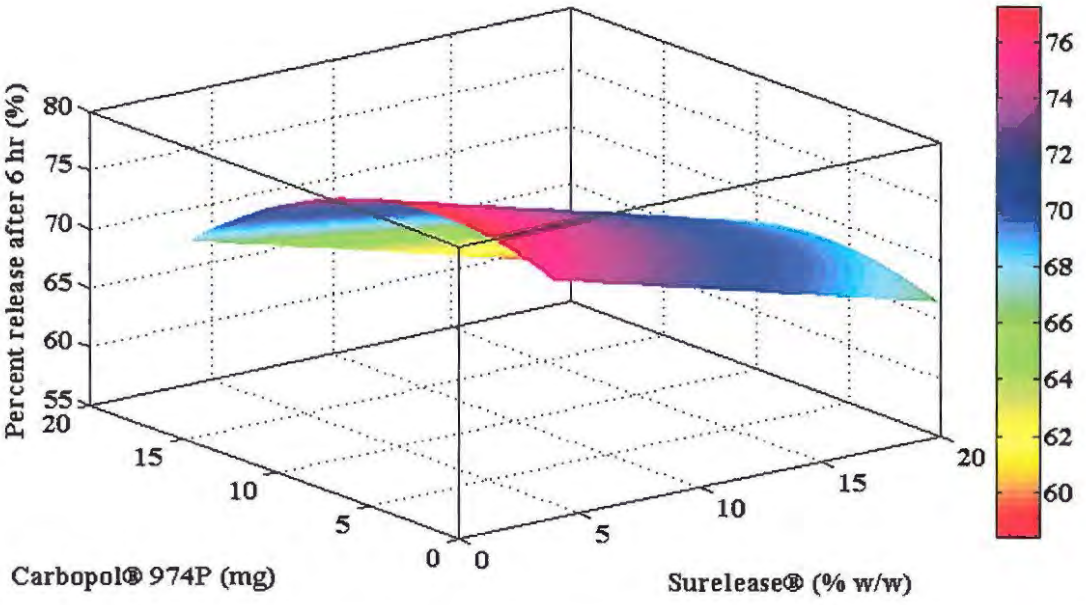


Figure 6.27 Response surface plot depicting the effect of Carbopol® 974P and Surelease® on percent SBS released after 6 hr with Methocel® K100M and xanthan gum concentrations at the centre level

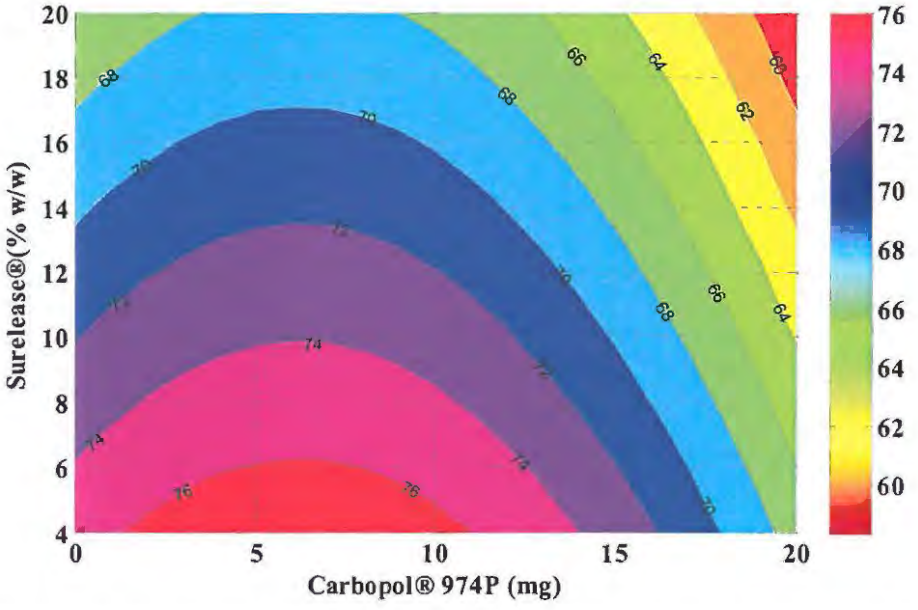


Figure 6.28 Contour plot depicting the effect of Carbopol® 974P and Surelease® on percent SBS released after 6 hr with Methocel® K100M and xanthan gum concentrations at the centre level

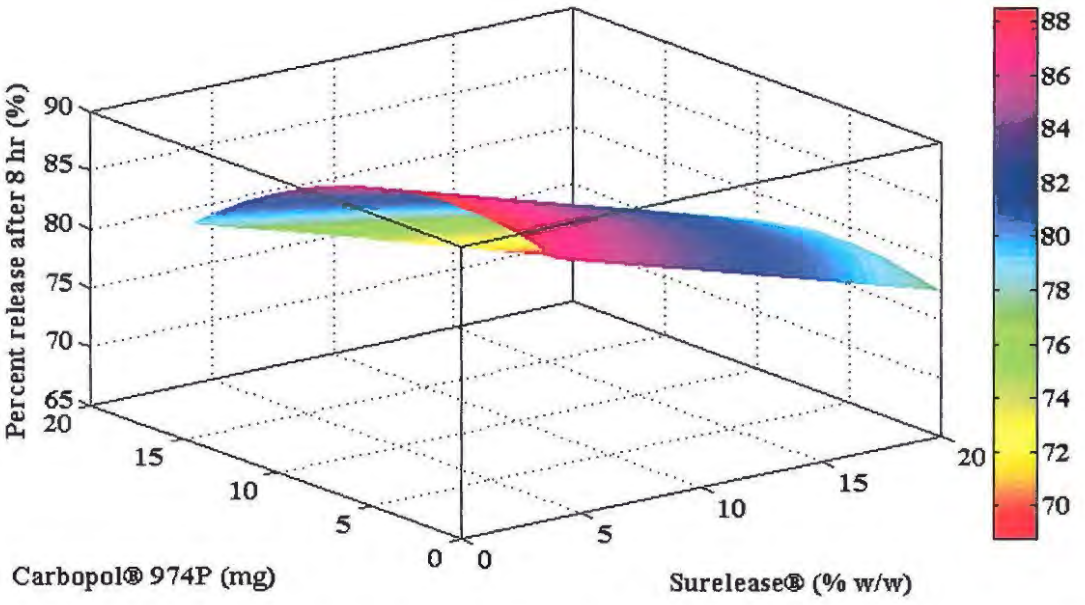


Figure 6.29 Response surface plot depicting the effect of Carbopol® 974P and Surelease® on percent SBS released after 8 hr with Methocel® K100M and xanthan gum concentrations at the centre level

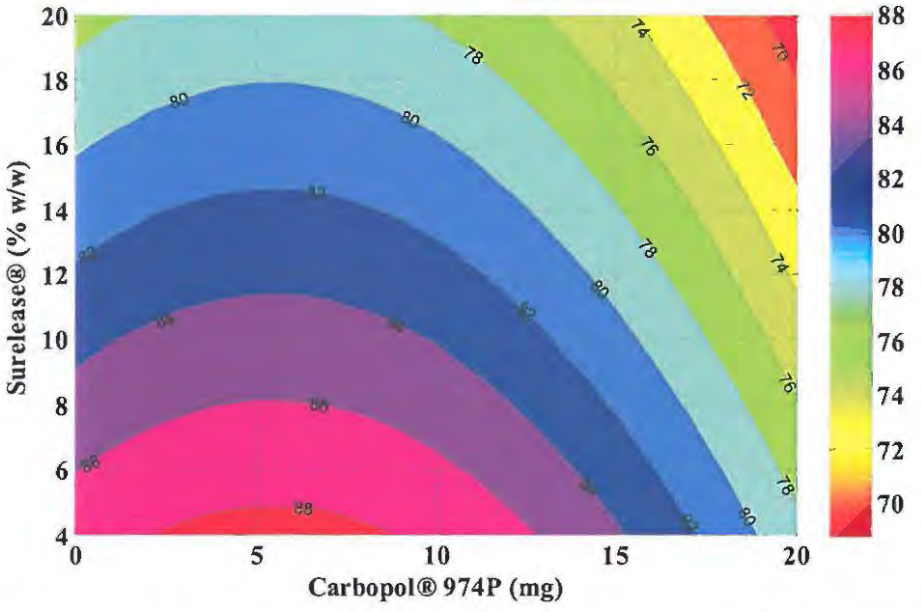


Figure 6.30 Contour plot depicting the effect of Carbopol® 974P and Surelease® on percent SBS released after 8 hr with Methocel® K100M and xanthan gum concentrations at the centre level

The topographical surface plots depicted in Figures 6.25, 6.27 and 6.29 show that at low and high levels of Carbopol® 974P an increase in the concentration of the aqueous ethylcellulose dispersion results in a decrease in the extent of SBS release. However, the addition of increasing concentrations of Carbopol® 974P at both high and low levels of Surelease®

results in an initial increase and then a subsequent decrease in the extent of SBS release. The curvature in the plot is indicative of the presence of an interaction between these factors. Surelease® contains ethylcellulose, fractionated coconut oil, dibutyl sebacate and ammonium hydroxide which may interact with the ionisable groups on the Carbopol® 974P backbone and result in the patterns that are observed in the response surface and contour plots. The contour plots shown in Figures 6.26, 6.28 and 6.30 also show similar responses and relationships.

The response surfaces and contour plots that have been shown in Figures 6.10–6.30 demonstrate the complex nature of the relationships that exist between the levels of input factors used and the extent of SBS release at different stages of dissolution testing. In order to undertake formulation optimisation experiments to develop a pharmaceutical product with optimal *in vitro* release characteristics it is essential to have an understanding of the interactions that may exist between formulation factors.

6.4.6 Drug Release Rate and Mechanism of Release

6.4.6.1 Overview

A summary of the release parameters for SBS derived from fitting drug release data to the Korsmeyer–Peppas model *viz.*, the exponent n that describes the nature of SBS release kinetics and the rate of release k for the manufactured formulations are shown in Table 6.6.

Table 6.6 Kinetic parameters for SBS release derived from the Korsmeyer–Peppas model

Formulation	k	n	R^2_{adj}
SAL001	0.2840	0.4833	0.9996
SAL002	0.3083	0.4725	1
SAL003	0.3394	0.4678	1
SAL004	0.2644	0.5022	0.9995
SAL005	0.2571	0.4799	0.9997
SAL006	0.2691	0.4784	0.9996
SAL007	0.2852	0.4622	0.9997
SAL008	0.2979	0.4799	0.9996
SAL009	0.3075	0.4699	0.9999
SAL010	0.2785	0.5137	0.9994
SAL011	0.3704	0.4334	1
SAL012	0.3727	0.4469	1
SAL013	0.3258	0.4623	1
SAL014	0.4461	0.3517	1
SAL015	0.2748	0.4858	0.9994
SAL016	0.2623	0.4842	0.9992
SAL017	0.2680	0.4789	0.9997
SAL018	0.4764	0.3482	1
SAL019	0.2915	0.4745	0.9999
SAL020	0.2640	0.5044	0.9996
SAL021	0.3224	0.4538	0.9997
SAL022	0.3982	0.4166	1
SAL023	0.3299	0.4945	0.9999
SAL024	0.2749	0.4718	0.9999
SAL025	0.2843	0.4999	1
SAL026	0.2795	0.5066	1
SAL027	0.5337	0.3482	1
SAL028	0.2671	0.5036	1
SAL029	0.2864	0.4647	1
SAL030	0.2987	0.4727	0.9997

6.4.6.2 Drug Release Rate

The relationship between polymer levels and interactions that impact the overall release rate of SBS from the manufactured formulations is shown in Equation 6.14.

$$k = 0.304 - 0.0764x_1 - 0.0876x_2 + 0.0953x_1x_2 + 0.0955x_2^2 - 0.0555x_2x_3 - 0.0281x_3^2$$

Equation 6.14

It is clearly evident that the inclusion of Methocel® K100M and xanthan gum results in a significant decrease in SBS release rates, although an interaction between xanthan gum and Carbopol® 974P and the quadratic term for Carbopol® 974P are important factors that must be considered. The equation also shows that the magnitude of the effect of xanthan gum on the overall rate of SBS release is greater than that for Methocel® K100M in the formulations that were studied.

The relationship and impact of the levels of Methocel® K100M and xanthan gum on the rate of drug release from the hydrophilic matrix formulations that were manufactured are shown in Figures 6.31 and 6.32.

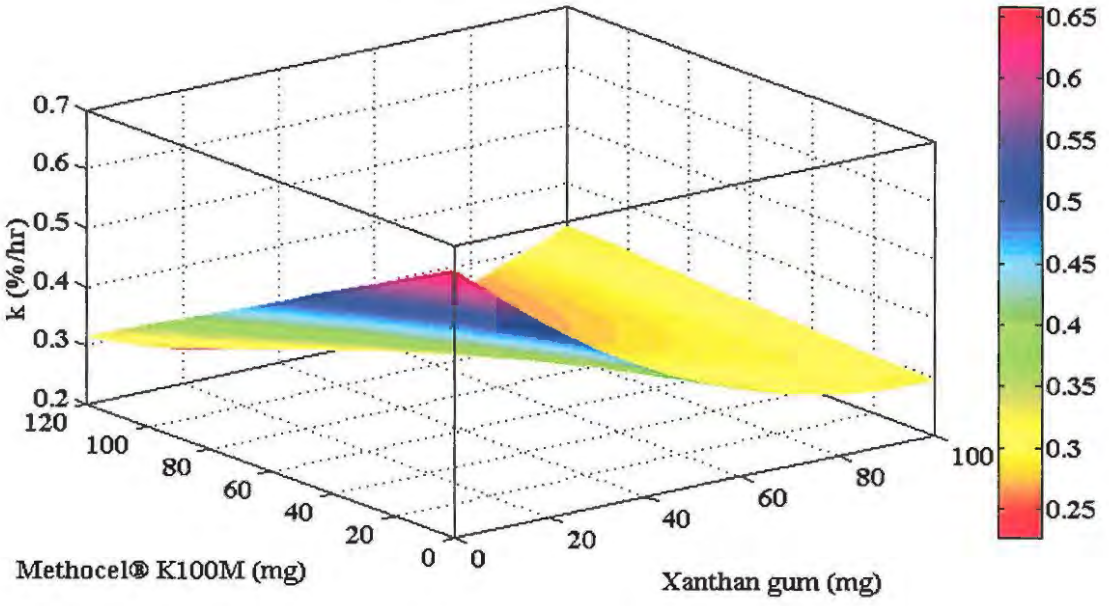


Figure 6.31 Response surface plot depicting the effect of Methocel® K100M and xanthan gum on SBS release rate with Carbopol® 974P and Surelease® concentration at the centre level

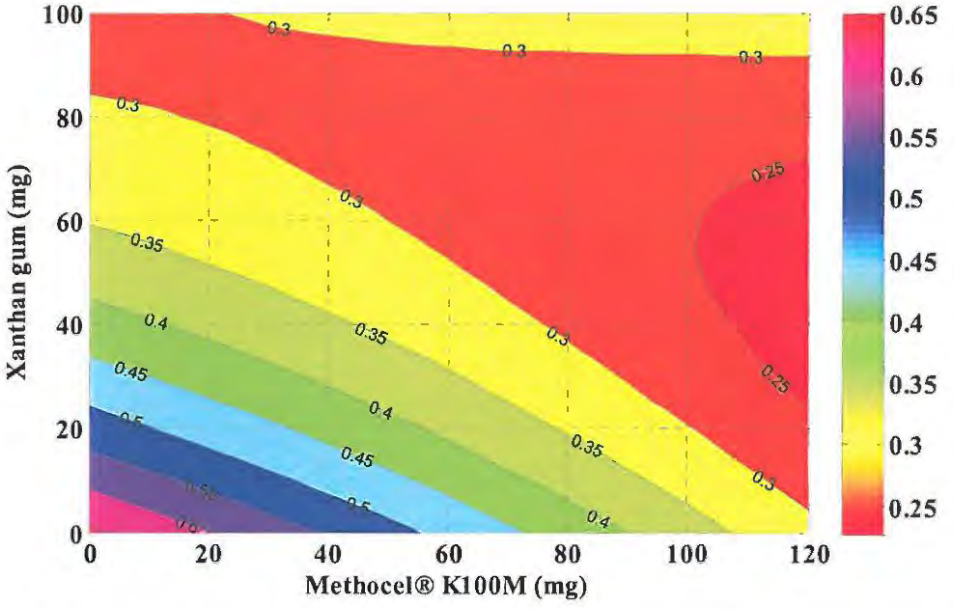


Figure 6.32 Contour plot depicting the effect of Methocel® K100M and xanthan gum on SBS release rate with Carbopol® 974P and Surelease® concentrations at the centre level

The topographical surface plot depicted in Figure 6.31 reveals that, at low levels of Methocel® K100M, increasing levels of xanthan gum result in a decrease in the rate of SBS release. However at high levels of Methocel® K100M the inclusion of xanthan gum results in an initial decrease and then a subsequent increase in the release rate of SBS. Similarly the addition of Methocel® K100M to formulations that contain low levels of xanthan gum results in a decrease in the release rate of SBS, but at high concentrations of xanthan gum the addition of Methocel® K100M results in a slight increase in the rate of SBS release.

The interaction between xanthan gum and Carbopol® 974P that impacts on the release rate of SBS is shown in Figures 6.33 and 6.34.

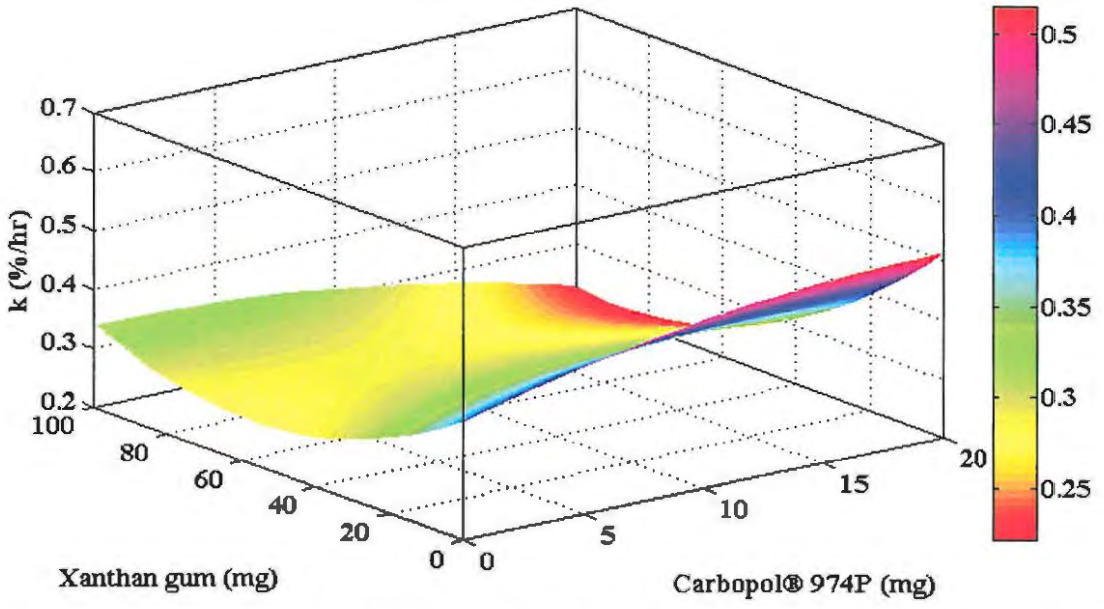


Figure 6.33 Response surface plot depicting the effect of xanthan gum and Carbopol® 974P on the rate of SBS release with Methocel® K100M and Surelease® concentrations at the centre level

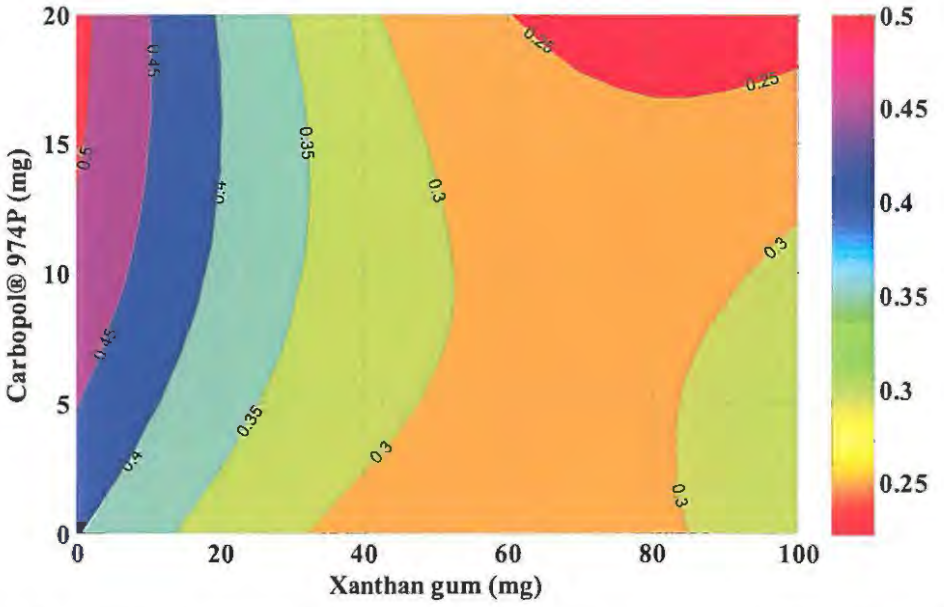


Figure 6.34 Contour plot depicting the effect of xanthan gum and Carbopol® 974P on the rate of SBS release with Methocel® K100M and Surelease® concentrations at the centre level

It is apparent from Figures 6.33 and 6.34 that at low levels of xanthan gum, increasing the levels of Carbopol® 974P results in an increase in the release rate of SBS. However the opposite is observed when high levels of xanthan gum are used in the dosage forms. At both low and high levels of Carbopol® 974P, the addition of xanthan gum results in a slight decrease and subsequent increase in the release rate of SBS. The potential interaction between xanthan gum and Carbopol® 974P has been described in § 6.4.5.

6.4.6.3 Drug Release Kinetics

The release kinetics of SBS have been summarised in Table 6.6. The relationship between the formulation variables investigated and the mechanism of drug release, *i.e.* the exponent *n*, is mathematically represented by Equation 6.15.

$$n = 0.476 + 0.0440x_1 + 0.0599x_2 - 0.0175x_3 - 0.06689x_1x_2 - 0.0617x_2^2 + 0.0517x_2x_3 - 0.0355x_2x_4$$

Equation 6.15

Evaluation of Equation 6.15 reveals that Methocel® K100M, xanthan gum and Carbopol® 974P content have a significant impact on the mechanism of drug release from the manufactured formulations. The use of Methocel® K100M and xanthan gum results in an increase in the value of the exponent when the polymer levels are increased, whereas the use

of Carbopol® 974P results in a decrease in the value of the exponent. It is apparent that the inclusion of xanthan gum results in the largest deviation from a truly diffusion controlled matrix system and that the interaction between Methocel® K100M and xanthan gum results in a decrease in the value of the exponent.

The response surface and contour plots that depict the relationship between Methocel® K100M and xanthan gum on the mechanism of drug release are shown in Figures 6.35 and 6.36.

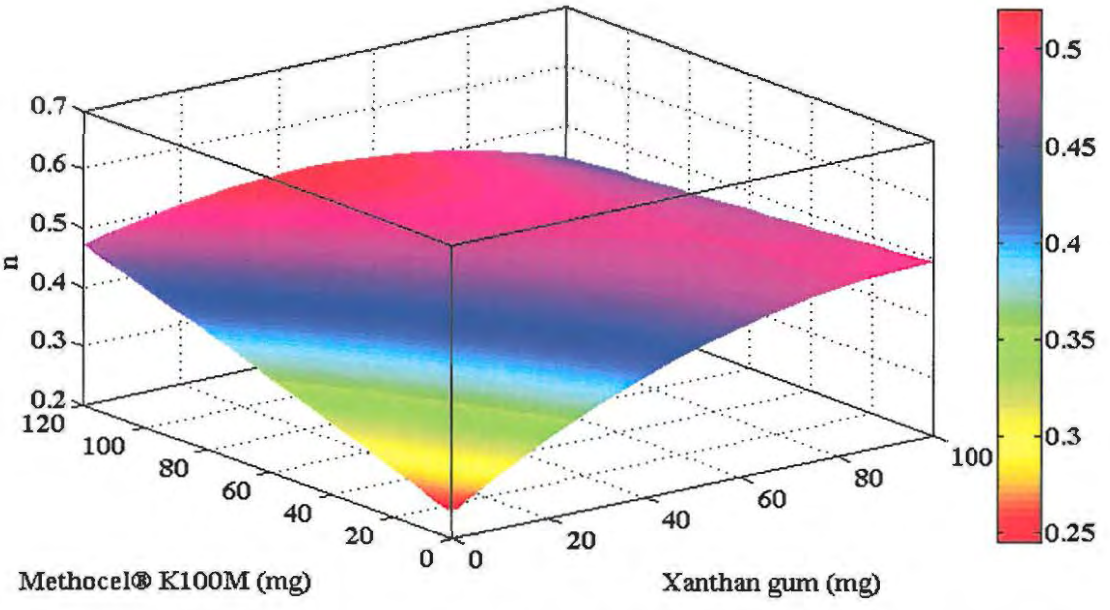


Figure 6.35 Response surface plot depicting the effect of Methocel® K100M and xanthan gum on the mechanism of SBS release with Carbopol® 974P and Surelease® concentrations at the centre level

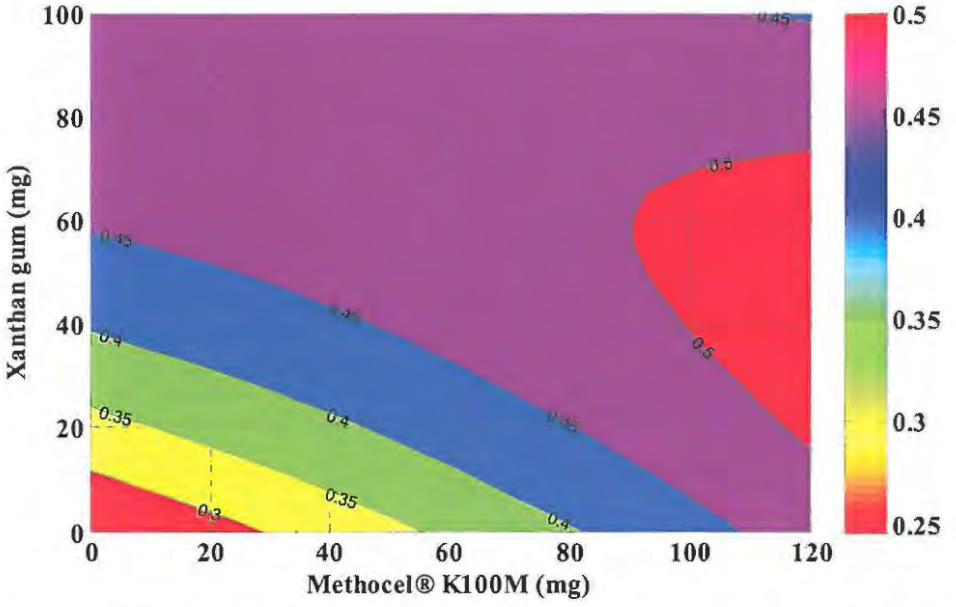


Figure 6.36 Contour plot depicting the effect of Methocel® K100M and xanthan gum on the mechanism of SBS release with Carbopol® 974P and Surelease® concentrations at the centre level

The relationship between Methocel® K100M and xanthan gum and their impact on drug release mechanisms is shown in Figure 6.35. It is apparent, and supported by the contour plot in Figure 6.36, that increasing the concentration of both polymers results in an increase in the value of the exponent from 0.45 towards 0.50, indicating the dominance of diffusion controlled mechanisms in these systems. The inclusion of Methocel® K100M and xanthan gum results in an increase in the value of the exponent towards a value that is indicative of a diffusion controlled mechanism (*i.e.* as n approaches 0.45).

It is also apparent from Equation 6.15 that the inclusion of Carbopol® 974P in the formulation results in a decrease in the value for the exponent, and the interaction between xanthan gum and Carbopol® 974P is depicted in Figures 6.37 and 6.38.

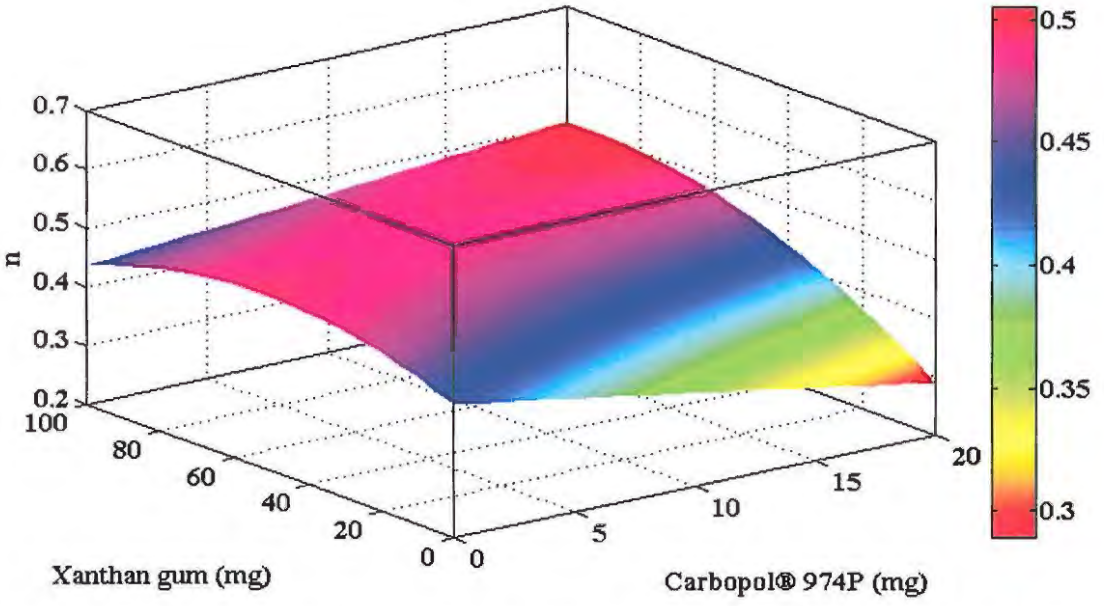


Figure 6.37 Response surface plot depicting the effect of xanthan gum and Carbopol® 974P on the mechanism of SBS release with Methocel® K100M and Surelease® concentrations at the centre level

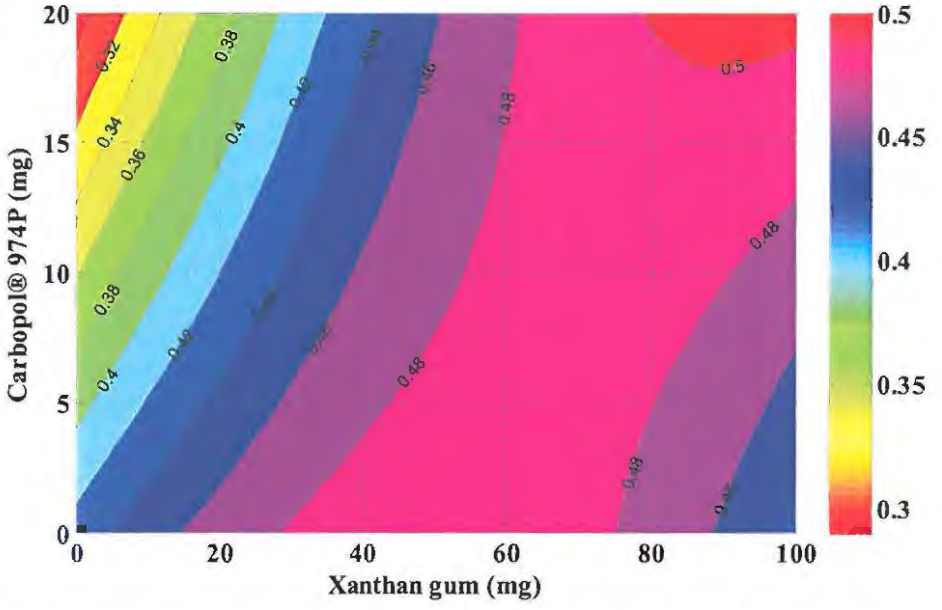


Figure 6.38 Contour plot depicting the effect of xanthan gum and Carbopol® 974P on the mechanism of SBS release with Methocel® K100M and Surelease® concentrations at the centre level

As shown in Equation 6.15, it is clear that Surelease® does not appear to have to a significant impact on the mechanism of SBS release from the manufactured formulations. However, an interaction between xanthan gum and Surelease® impacts the mechanism of release as shown in Figures 6.39 and 6.40.

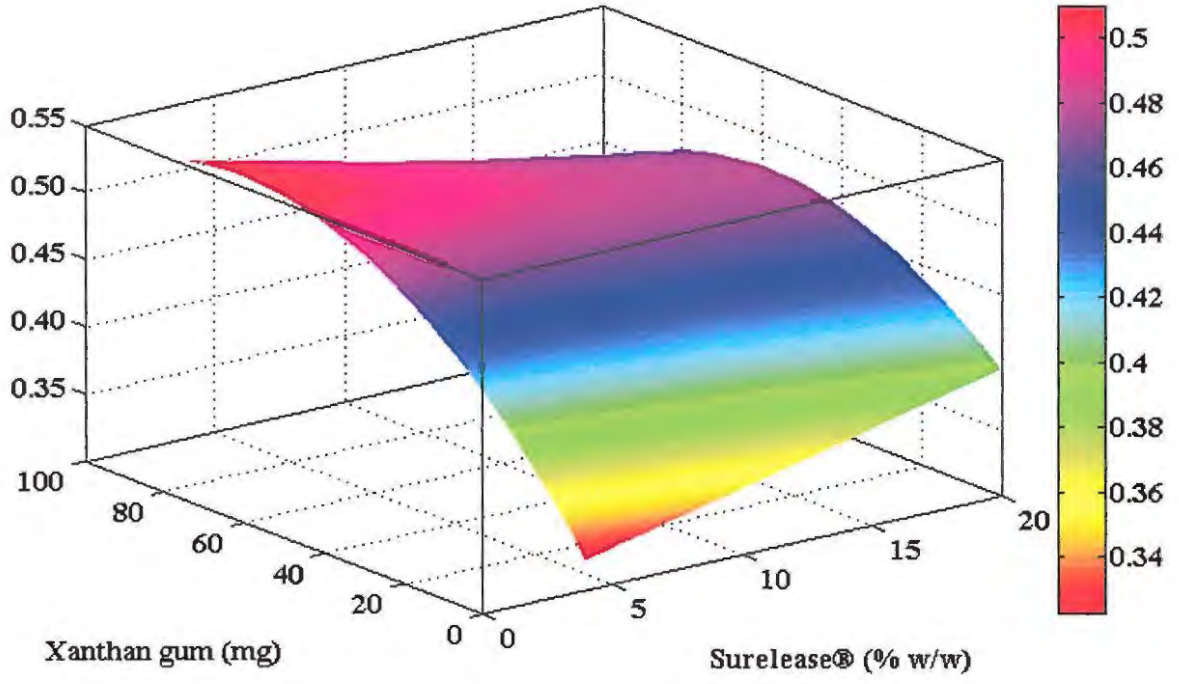


Figure 6.39 Response surface plot depicting the effect of xanthan gum and Surelease® on the mechanism of SBS release with Methocel® K100M and Carbopol® 974P concentrations at the centre level

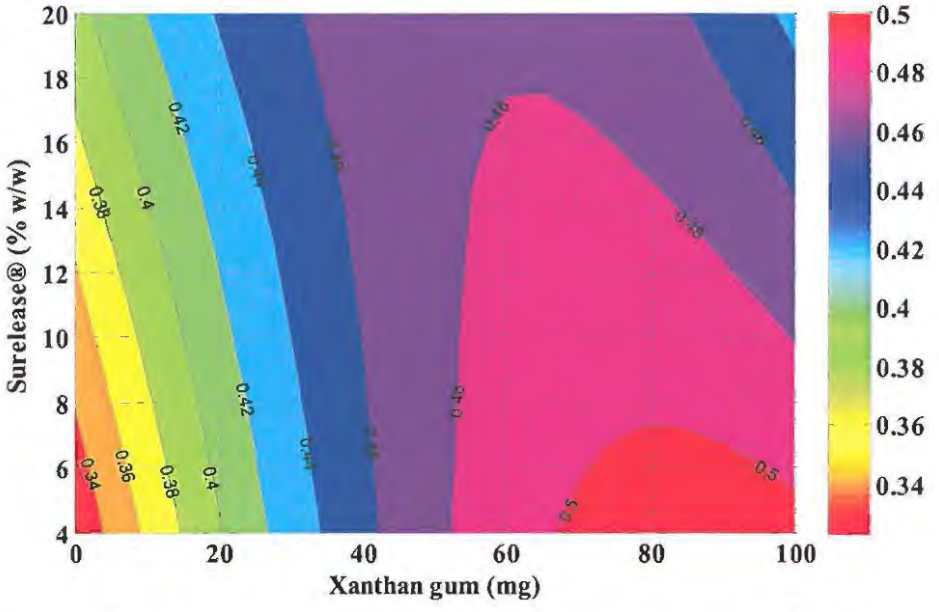


Figure 6.40 Response surface plot depicting the effect of xanthan gum and Surelease® on the mechanism of SBS release with Methocel® K100M and Carbopol® 974P concentrations at the centre level

It is apparent when evaluating Figures 6.39–6.40 that lowering the concentration of xanthan gum results in a decrease in the value of the exponent and that a maximum value between 0.45 and 0.5 is obtained when these formulation compositions are used to prepare hydrophilic

matrix formulations. In general, increasing the concentration of Surelease® in the formulation results in an increase in the value of the exponent indicating that a diffusion controlled mechanism predominates. The interaction that exists between xanthan gum and Surelease® is evident since the impact of increasing the xanthan gum concentration at low and high levels of Surelease® is different, and this is clearly evident from the different gradients in these regions of the topographical surface plot.

6.4.7 Formulation Optimisation

Objective slice graphs for the optimisation of the hydrophilic matrix formulation of SBS with desirable *in vitro* release characteristics are shown in Figure 6.41.

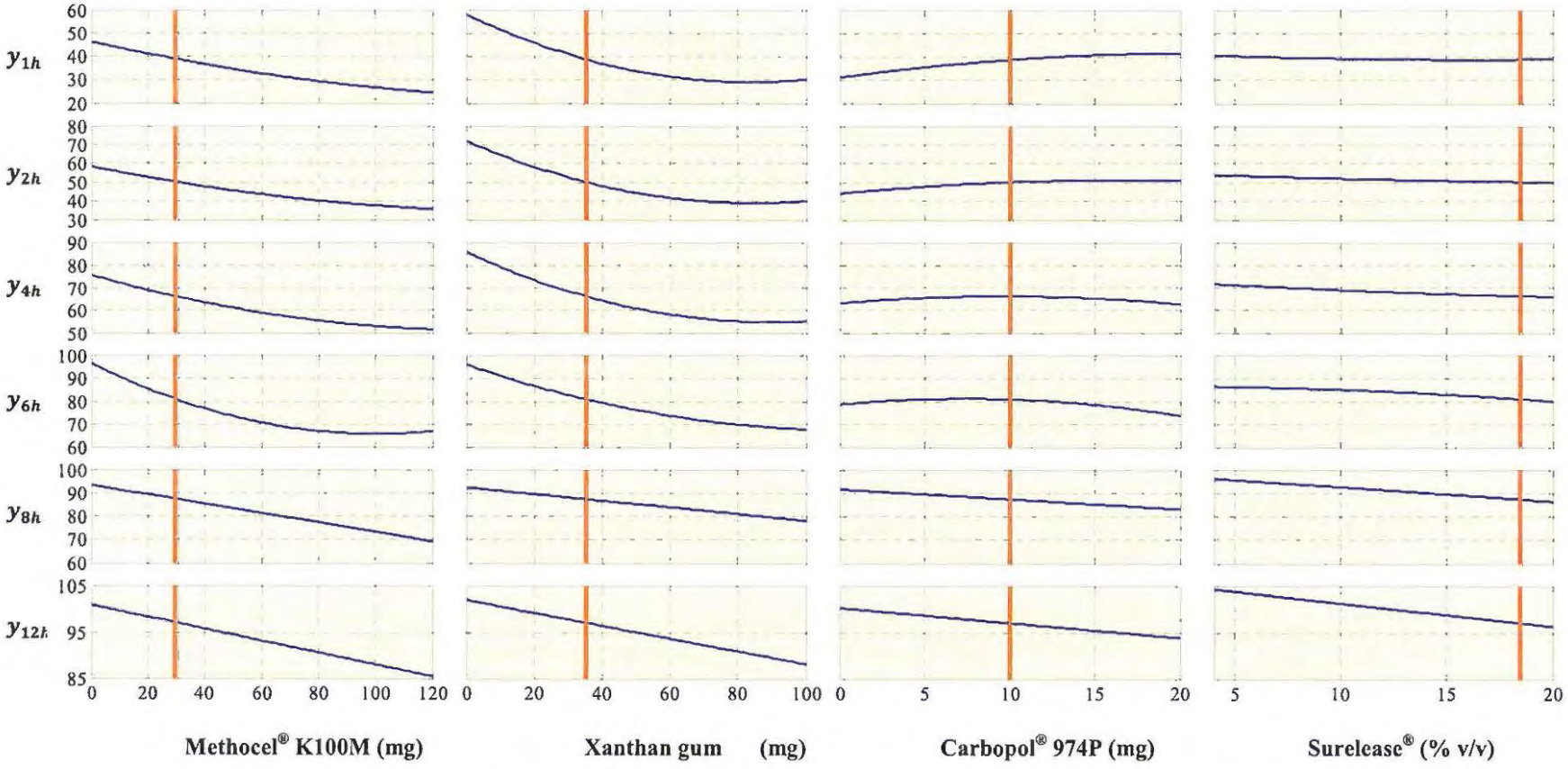


Figure 6.41 Objective slice graph for the optimisation of a hydrophilic matrix formulation for SBS

The objective graphs show the relationship between input factors and each response characterised using a curve that depicts the change in each response as the level of the input factor is changes. The vertical orange line depicts the amount of excipient that is required in a formulation to produce tablets with the desired release characteristics for SBS. The composition of the optimised formulation that was evaluated using this approach is described in § 6.3.6.3 and the predicted *in vitro* release data are shown in Table 6.7. The f_2 similarity factor was used to compare the dissolution profile of the predicted formulation to that of the reference formulation; the results show that the f_2 = 89.7 indicating that the predicted *in vitro* release profile was indeed similar to that of the reference formulation.

Table 6.7 Optimised formulation

Formulation		**Predicted dissolution profile**	
Methocel® K100M	29.77 mg	y_{1h}	38.73%
Xanthan gum	35.31 mg	y_{2h}	50.05%
Carbopol® 974P	10.01 mg	y_{4h}	66.00%
Surelease®	18.5% w/w	y_{6h}	80.73%
Avicel®	91.32 mg	y_{8h}	87.09%
Colloidal silica	0.5% w/w	y_{12h}	97.00%
Magnesium stearate	1% w/w	f_2 **factor**	89.7

The physical characteristics of the manufactured formulations were evaluated and are summarised in Table 6.8. It is apparent that the tablets that were produced had satisfactory performance qualities and were deemed suitable for their intended use.

Table 6.8 Physical properties of tablets manufactured using the optimised formulation

Granule characteristics		**Physical characteristics**	
Bulk density	0.437 g/cm^3	**Tablet weight**	222.58 ± 0.94 mg
Tap density	0.466 g/cm^3	**Tablet thickness**	4.73 ± 0.09 mm
Carr's Index	6.25%	**Tablet diameter**	8.84 ± 0.04 mm
Angle of repose	43.9°	**Tablet crushing strength**	43.90 ± 2.70 N
		Assay	9.59 ± 0.04 mg

The *in vitro* dissolution profile generated following testing of the optimised formulation compared with the target and predicted formulations is shown in Figure 6.42. It is clearly evident that there is a degree of similarity that exists between the manufactured formulation and that of the reference formulation, Asthalin®8 ER (Cipla Ltd., Mumbai, Maharashtra, India). The f_2 similarity factor of the manufactured formulation compared with the reference formulation was 83.0.

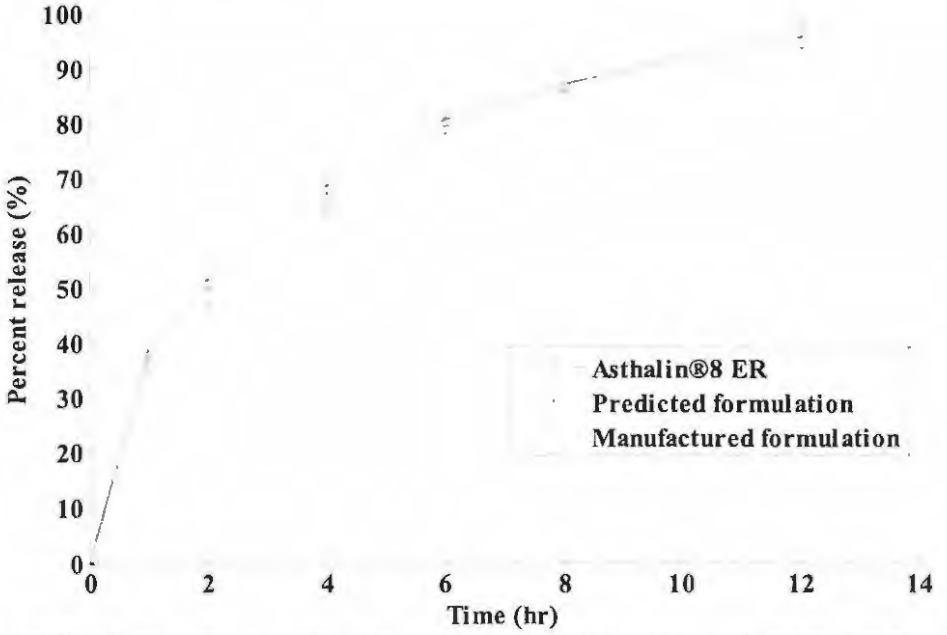


Figure 6.42 *In vitro* release profile of the optimised formulation compared with that of the predicted formulation and the reference formulation, Asthalin®8 ER

The relationship between the results for the optimised formulation that was manufactured and that predicted from the optimisation procedure is shown in Figure 6.43.

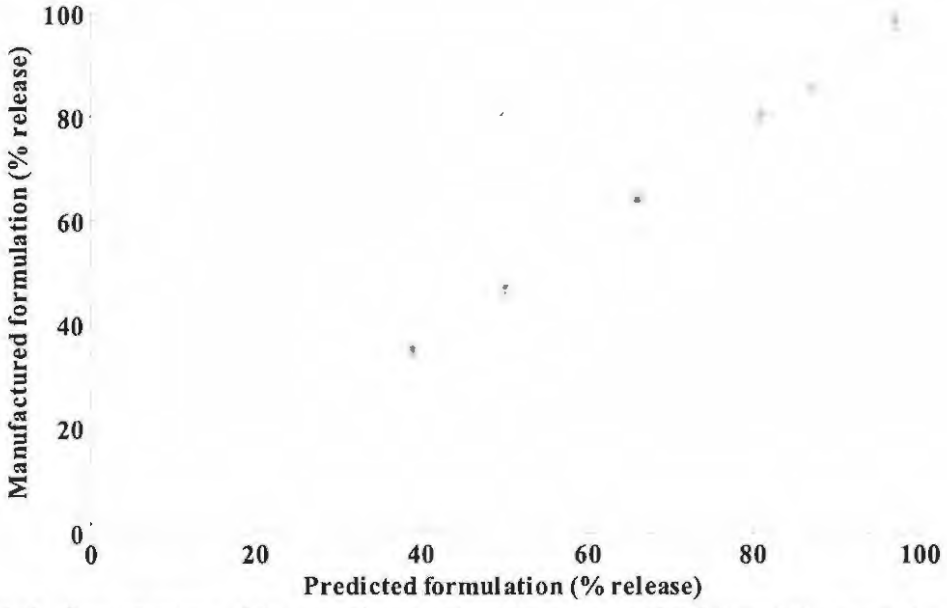


Figure 6.43 Percent release of the manufactured formulation *vs.* predicted formulation, $y = x - 2.6, R^2 = 0.9928$

The results show that the relationship between the predicted and manufactured formulations was nearly linear showing excellent predictability for the optimisation by use of RSM.

6.5 CONCLUSIONS

The optimisation of a pharmaceutical formulation is complicated by the need for a precise combination of input factors (formulation components) and the relationships between the levels of polymers that are used in a formulation. Furthermore, the nature of these effects on measurable responses must also be investigated. Multi-objective optimisation is a more complicated procedure compared with single-objective optimisation since trade-offs may be necessary to produce an optimal formulation composition.

The selection of a central composite design to optimise pharmaceutical formulations in these studies reduces the number of experiments that are required to estimate quadratic surface effects compared with the use of full factorial design procedures. In addition the use of a central composite design allows for the establishment of high and low limits to expand the experimental domain under investigation. The compositions of Methocel® K100M, xanthan gum, Carbopol® 974P and Surelease® were chosen as formulation variables to determine how these may impact tablet crushing strength, *in vitro* release characteristics, and both the rate and mechanism of drug release from these formulations.

Response surface and contour plots were used to examine the nature of these complex relationships. It is apparent that the incorporation of xanthan gum and Carbopol® 974P had an impact on tablet crushing strength, and using high levels of xanthan gum in tablet formulations resulted in the production of soft tablets. Although the handling and transport of soft tablets is a major quality concern, the formulations remained intact during these studies and therefore were deemed suitable for use in these experiments.

The *in vitro* release profile of SBS at different stages of dissolution testing was characterised by quadratic relationships and, in general, Methocel® K100M and xanthan gum had the greatest impact on the extent of drug release at all stages of dissolution testing. It is evident that increasing the content of these polymers in formulations results in retardation of drug release from the prepared solid oral dosage forms. The content of Carbopol® 974P and Surelease® had a lower impact on drug release, but it was apparent that interactions between these formulation variables were important in controlling the extent of SBS release from manufactured formulations.

The rate and mechanism of drug release was assessed using the Korsmeyer–Peppas model and it was apparent that Methocel® K100M and xanthan gum had the greatest impact on the overall rate and mechanism of SBS release from these formulations. The mechanism of drug release from the formulations is primarily a diffusion controlled process, and increasing the concentration of Methocel® K100M and xanthan gum resulted in the value of the Korsmeyer–Peppas exponent ranging between 0.45 and 0.50.

The Calibration Generation Browser in Matlab® R2008a (Mathworks Inc., Natick, MA, USA) was used to determine the composition of an optimised formulation that would have satisfactory physical characteristics and the desired *in vitro* release profile. The positive result is an indication of the potential application of RSM for pharmaceutical formulation optimisation in drug product development studies.

The use of RSM has been recommended for the establishment of a design space within which product quality can be assured. Consequently the RSM outputs that were generated were used to establish a design space that could be used to ensure that SR dosage forms of SBS satisfy the *in vitro* release and quality standards required, and are consistently produced. This approach will be described in Chapter 8 of this dissertation.

Furthermore, the *in vitro* release profile data that were generated from the central composite design were subsequently used to train an ANN for application in formulation optimisation. The ANN approach is described in Chapter 7 of this dissertation. The output generated by the ANN was compared with that from RSM studies for the optimisation of formulations and for the establishment of an appropriate and suitable design space for SR SBS matrix tablets.

CHAPTER 7
THE APPLICATION OF ARTIFICIAL NEURAL NETWORKS FOR FORMULATION OPTIMISATION

7.1 INTRODUCTION

Pharmaceutical formulations are complex systems in which the properties and performance characteristics are influenced by numerous formulation and process factors that may not be easy to understand. The use of statistical methodologies for the development of pharmaceutical formulations with desirable physical characteristics and optimal dosage form performance was discussed in Chapter 6 *vide infra*.

The use of statistical methodologies for studying the impact and interaction of factors that affect dosage form performance often requires simplification and reduction of the problem from many possible causes to a few factors in order to be able model the situation adequately. Although these approaches are often successful for pharmaceutical optimisation, the application of statistical tools is limited by the assumption that the measurable attributes of pharmaceutical systems can be defined by mathematical models. As a result this approach may not always be appropriate for modelling complex input-output or factor-response relationships. Furthermore the use of RSM for optimisation experiments limits the number of factors that can be realistically studied to not more than five, since an increased number of experiments would be required to investigate all factors completely [190].

The use of artificial intelligence is a rapidly growing field in knowledge discovery and data mining and has been applied in the pharmaceutical sciences for the development and optimisation of dosage forms [190,204–208]. The complex relationships between input and output factors may be inferred from data mining methods such as ANN, Neurofuzzy Logic and Fuzzy Logic that are used to map associations between input or causal factors and responses that may not be obvious when only statistical methodologies are used [190]. These methods can be used for dosage form development to identify and learn correlative patterns that exist between formulation and process variables with predetermined CQA [190].

Therefore the development of an ANN model for the optimisation of a hydrophilic matrix formulation for SBS was undertaken. In addition, the use of an ANN model for constructing a design space was also investigated and will be reported in Chapter 8 of this dissertation.

7.2 ARTIFICIAL NEURAL NETWORKS

7.2.1 Introduction

ANN are computational tools that emulate the interconnected neurological structures of the human brain and the ability of the human brain to learn and solve problems through pattern recognition [190]. ANN simulate the learning behaviour of the human brain by modelling data and recognising patterns for complicated multi-dimensional relationships that exist between input and output sets of data. Once trained an ANN can be used to predict and forecast outputs for a given a set of input conditions and may therefore be used to optimise both formulation and process variables in order to engineer and manufacture high quality, safe and effective dosage forms [209]. This approach has the potential to streamline resource allocation and to facilitate formulation development thereby reducing the cost and overall time associated with drug product development.

ANN are constructed in such a way so as to model real neurons in the human brain and the basic processing unit is referred to as a neuron or a node. Analogous to biological neurons, mathematical neurons take a range of inputs, weight and add them together in order to produce an output signal which may be passed onto adjacent neurons in the network if the resultant signal is strong enough. In a similar way to biological neurons, mathematical neurons are only effective when they are interconnected in a network [190].

The interconnections between neurons in a network can be feed-forward or feed-back connections: in feed-back models, connections are characterised by cycles between the neurons but these connections do not form cycles in feed-forward models [207]. An example of a basic feed-forward artificial neuron which takes a range of inputs, weights and adds them together and finally performs a transformation which results in an output is shown in Figure 7.1. This neuron may also be referred to as an elementary perceptron [210].

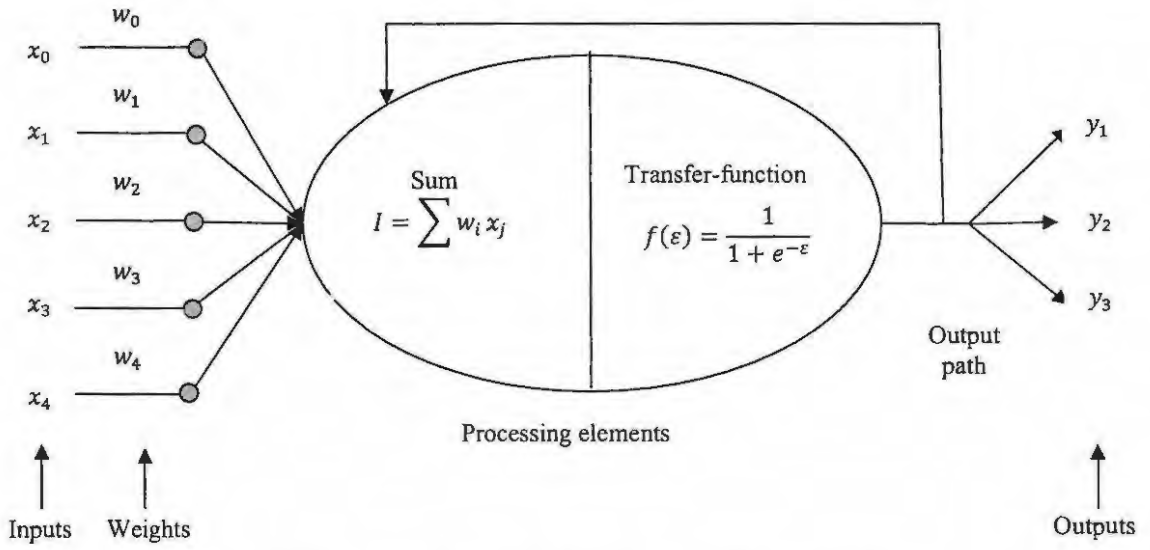


Figure 7.1 A simple artificial neuron, adapted from [209,211]

Each input to an ANN is associated with a weight that defines its significance to the model that is being constructed. The neuron computes the sum of weights of all inputs through a summation function and then calculates an output which is further modified via a transfer function prior to being forwarded to another neuron [209].

For a standard neural network, such as a single unit perceptron, the relationship between the input, $x = x_1 \dots x_p$, and the output y is represented by a multiple linear regression model as shown in Equation 7.1.

$$y = w_0 + \sum_{j=1}^{p} w_j x_j \qquad \textbf{Equation 7.1}$$

Where,

w_0 = bias term
$w_j, j = 1 \dots p$ = connection weights or strengths

Neural computations occur in three basic phases, *viz.*, learning, recall and generalisation. During the learning phase an ANN is presented with a series of input and output or target data sets derived from empirical experiments and the network attempts to determine and recognise patterns between variables, should they exist. In the recall phase, a trained ANN will generate responses from the input data that was used in the training phase. Finally, in the generalisation phase the ANN is used to generate responses from new input data and can be used to predict outputs for a given set of input information [209].

ANN are generally characterised by network architecture, transfer function and learning paradigms. These are all important elements of an ANN that must be understood in order to optimise the utility of these models [212].

7.2.2 Neural network architecture

Neural network architecture describes the way neurons in an ANN are organised and shows the interconnections between individual processing elements and their counterparts. The multilayer perceptron (MLP) configuration is a commonly used neural network architecture in the pharmaceutical sciences and is composed of a series of highly interconnected layers of neurons or nodes. It has been reported that when a MLP ANN is given sufficient processing elements it can approximate any linear function with reasonable accuracy [213].

A MLP consists of three layers, *viz.*, an input, hidden and output layer. Each layer has a number of neurons or nodes that are fully interconnected with neurons in the neighbouring layers as shown in Figure 7.2.

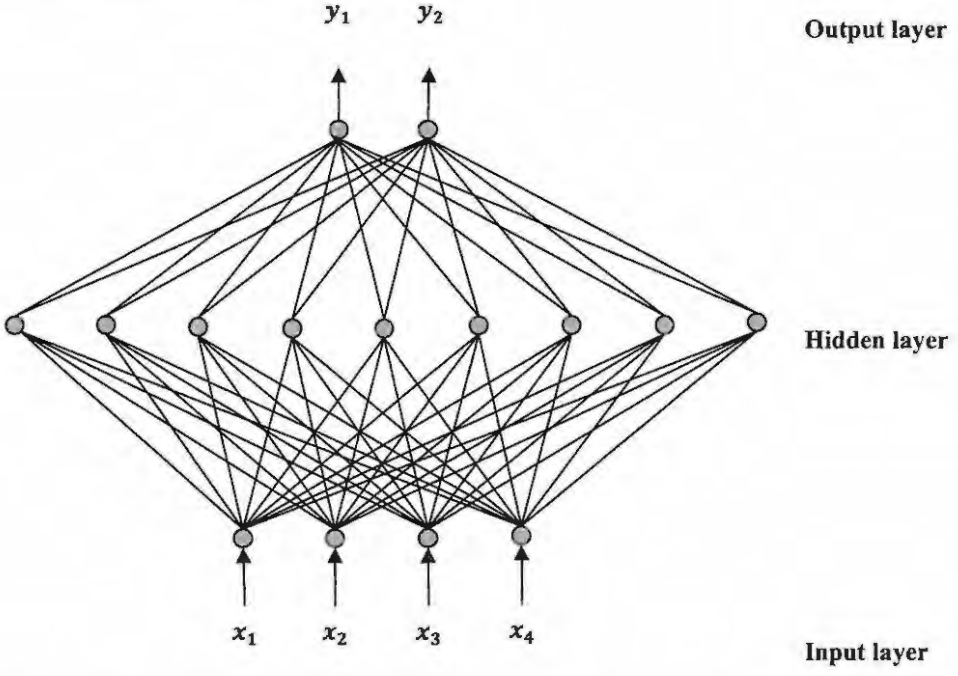


Figure 7.2 MLP architecture for ANN where $x_1 - x_4$ are inputs and y_1 and y_2 represent response factors, adapted and redrawn from [209,211]

The input layer consists of one or more input nodes or processing elements that distribute input data to nodes located in the hidden layer of the ANN. Each node in the input layer can represent an independent variable such as for example, an amount of polymer in a

formulation, machine operating conditions, temperature or humidity. The input layer does not process any information but serves as a distribution point for information to be delivered to the hidden layer.

The hidden layer can be made up of one of more layers of parallel nodes (Figure 7.2Figure 7.2 shows one layer). The nodes in the hidden layer perform a weighted summation of the inputs followed by a non-linear transformation as shown in Figure 7.1, which then relays that data to the output layer as shown in Figure 7.2. The number of nodes in the hidden layer is critical to the efficiency of a network and if the hidden layer has too few nodes, the ANN will lack the power needed to classify the data provided to it. Conversely if there are too many nodes, patterns in the input data will be memorised and therefore the ability of the network to interpolate data will be diminished [211,212].

The output nodes represent measureable properties of pharmaceutical formulations and may include parameters such as, for example, tablet crushing strength and percent drug released at different stages of a dissolution test [211,212,214].

7.2.3 Transfer function

The transfer function computes the output value determined by the nodes in the hidden layer based on the summation of weighted inputs (Figure 7.1). The transfer function can control or scale the output value via the use of a threshold specification. Examples of transfer functions include sigmoid, sine and hyperbolic tangent functions [211] although the sigmoid function is most commonly used in ANN for pharmaceutical applications [209,211,212]. The sigmoid function output delivered from each node is shown in Equation 7.2.

$$f(\Sigma) = \frac{1}{1+e^{-\Sigma}} \qquad \textbf{Equation 7.2}$$

Where,

Σ = sum of the input to the node.

The output from a hidden layer is usually limited to values between 0 and 1. The advantage of using a sigmoidal function is that a normalisation process may be used for non-linear outputs and their use prevents domination or overload effects in a network that may result from a single large input value [212]. Furthermore the use of a sigmoidal function facilitates rapid network learning [211,212].

7.2.4 Training Paradigm

ANN are presented with a sequence of stimuli or input factors and a set of expected responses or targets during the training phase. The training of an ANN begins by randomly assigning weights for each input node and a bias term, after which an output from the last layer of the network is generated. Each iteration performed by the ANN is made up of two steps, a feed-forward step and a back-propagation step. In the feed-forward step, training data is presented to the ANN model, and in the back-propagation step, the weights are adjusted during successive iterations and are determined by minimising the sum of squares of the deviations between experimental data and the output values generated by the network. During training the difference between the actual target values and model predicted outputs are propagated back through the architectural structure of the network. Therefore new interconnected weights are computed to minimise or reduce the total error until a minimum total error value is calculated [190,207,209,212]. This process is known as back-propagation of errors since the process returns the error information backward from the output nodes to the hidden nodes so as to allow modification of the weights and bias of the network [215].

Pharmaceutical applications commonly use multilayer back-propagation neural networks since they are ideal for generating general purpose, flexible non-linear models that can predict response values with a high degree of accuracy. Furthermore they are also capable of handling complicated non-linear patterns [211]. The back-propagation network is the most common type of network used in pharmaceutical applications [209,212,214].

The usual procedure for training a network is to partition experimental data sets into three subsets, *viz.*, a training, test and validation set. Training data sets can be presented to a neural network using an incremental training technique where the data set is presented on an example-by-example basis, or by using the entire data set which is then handled by a process known as batch training [216]. The weights used in the network are subsequently adjusted after processing each sample for incremental training or following use of the entire data set for batch training. During the training phase of a network, each cycle in which input data is fed through a network is called an epoch.

The errors of the training, test and validation sets are monitored when developing a neural network. Convergence is the process of searching for a set of weight factors such that there is a reduction in the prediction of errors of an ANN model. The most common criterion for

establishing that convergence has occurred is based on the sum of squared errors (SSE), which can be calculated using Equation 7.3.

$$SSE = \frac{1}{N}\sum_{p=1}^{N}\sum_{i=1}^{M}\left(t_{pi} - o_{pi}\right)^2 \qquad \textbf{Equation 7.3}$$

Where,

o_{pi} = actual output of the ith node, from the p^{th} sample
t_{pi} = target output of the ith node, from the p^{th} sample
N = number of input nodes
M = number of output nodes

During the training phase of an ANN, the SSE of the training data set reduces indefinitely with an increasing number of epochs and the number of hidden nodes that are used in a network. The initial rapid decrease in the SSE is due to rapid learning by the network from the input data and the subsequent gradual decline of the SSE can be attributed to memorisation or over-fitting of the data provided to the network, due to excessive numbers of hidden nodes or the use of a large numbers of training cycles [207]. The training error is not a reliable indicator of the ability of a network to generalise, therefore test and cross-validation error must be monitored independently of the training error.

The error of the test data initially decreases but subsequently increases due to memorisation and over-fitting of data in an ANN model. The training phase of an ANN should therefore be stopped when the test error starts to increase and an optimal number of nodes for a network must be selected so as to minimise the test error [207]. In general, if the test error is low then there is a good chance that the network will be able to produce reliable simulations for data that are within the limits of the network that has been trained.

Cross-validation is performed to evaluate how well the network classifies the data provided to it from outside the training set and to ensure that a network does not memorise solutions to solve problems. Although the training error decreases monotonically during training, the cross-validation error reaches a minimum early in training while the training error is still decreasing. The cross validation error can be calculated using Equation 7.4 [211].

$$\delta_{cv} = \left[\left(\frac{1}{N}\right)\Sigma_i(T - A)^2\right]^{0.5} \qquad \textbf{Equation 7.4}$$

Where,

δ_{cv} = cross-validation error
N = number of cases/facts in the data
A = network output $0<A<1$

Neural networks can learn by two methods *viz.*, supervised and unsupervised learning. In the supervised learning approach a network is presented with a set of input variables and their resultant outputs or targets which are then compared with the outputs generated by the network. The error is computed from the network and is back-propagated through the system. Weights are then adjusted and the network attempts to regenerate an output: this process continues over several iterations until an output with minimal error is generated after which training ceases. In the unsupervised learning approach a network is provided with a set of input factors and targets but there is no feed-back on what the outputs mean or whether they are correct or not. The network is expected to discover patterns and correlations between the input and output variables without assistance. Back-propagation supervised learning is the most commonly used method used for training ANN for pharmaceutical applications [211,212].

7.2.5 Formulation Optimisation of Sustained Release Formulations using Artificial Neural Network Approaches

Several authors have reported the use of formulation variables such as the level of excipients used in a formulation as input or causal factors and the percent drug released at different stages of a dissolution test as response factors for ANN [206,217,218]. However, Takayama *et al* [206] and Ibric *et al* [219] used response factors from dissolution models to train and optimise pharmaceutical formulations. In these studies the predicted values from an ANN model were found to be in close agreement with those of experimentally generated data.

Ibric *et al* [219] used a generalised regression neural network to optimise SR formulation compositions for aspirin. The amount of polymer and the compression pressure were used as causal factors and the *in vitro* dissolution test sampling time points and Korsmeyer–Peppas model parameters were used as response factors. The optimised generalised regression neural network was used to predict the formulation composition of an optimal formulation for aspirin.

Formulation optimisation using ANN models has also been performed using a generalised distance function with optimal drug release parameters being used as response factors [206]. The f_1 or difference and f_2 or similarity fit factors are typically used for the comparison of experimentally generated and predicted *in vitro* dissolution profiles when conducting optimisations with ANN models [217,219].

7.2.6 Advantages of Artificial Neural Networks

The advantages of using an ANN include the fact that they can handle multiple independent and dependent variables in one model, and functional relationships between independent and dependent variables do not need to be known prior to constructing an ANN model [207]. ANN can also be paired with other artificial intelligence methods including genetic algorithms and fuzzy logic to improve the data mining capability of this tool [190].

Although a basic understanding of network architecture and function may be important in constructing an ANN model, the inner working of an ANN model does not need to be well understood by end users and therefore the use of ANN models may be considered a black box approach to formulation optimisation [207].

The ability of an ANN model to predict appropriate data is highly dependent on the data set that is used to train the network, and the predictive ability of an ANN is usually defined within the limits of the input and output data originally provided to the network. In general, interpolation within a model is usually accurate but extrapolation outside the experimental domain may result in the generation of highly erratic results [207].

Data from experimental design methodologies are often used to construct ANN models since the experimental design approach usually ensures independency of the formulation factors used to evaluate a system [207]. The use of experimental design for developing training data for ANN has been reported and includes the use of a three factor, three level, central composite design [220], two-factor spherical second order composite experimental design [221] and a four component simplex centroid mixture design [209]. However it has been shown that the use of experimental design approaches such as Box–Behnken and central composite designs are not appropriate for modelling highly curved responses and extensive internal mapping of the experimental domain is required to construct a highly predictive ANN model [222].

7.2.7 Limitations of Artificial Neural Networks

Although ANN can be used to recognise patterns between input and output data they cannot be used to elucidate mechanistic correlations between the input and response factors [207]. The combination of RSM described in Chapter 6 and ANN may be important in depicting interactions between input and output factors. Furthermore, in order to obtain reliable data when using ANN, a large data set may be required to fully study relationships between the input variables and responses. It is also important to realise that although ANN provide an efficient method for the optimisation of formulations, not all challenges may be solved using mathematical algorithms and/or computer software and the intuition and instinct of the formulation scientist must not be ignored [207].

7.3 METHODS

7.3.1 Approach to ANN

Commercially available software, Matlab® R2008a (MathWorks Inc., Natick, MA, USA) was used to write mathematical code for training and evaluating the ANN developed and used for formulation optimisation.

Four input factors corresponding to different levels of Methocel® K100M (x_1), xanthan gum (x_2), Carbopol® 974P (x_3) and Surelease® (x_4) were used as units in the input layer of the ANN. The data generated from the central composite design reported in Chapter 6 was used as the training data set for the ANN.

The response factors used from the central composite design, *i.e.* percent drug dissolved at different stages of the dissolution test, were used as output layers or the target data during ANN training. Specifically, $y_{i\,hr}$ = % SBS released, such that i = 1, 2, 4, 6, 8 and 12 hr.

7.3.2 Neural Network Training

An ANN designed in Matlab® R2008a (MathWorks Inc., Natick, MA, USA) can be trained for function approximation, pattern association or for the classification of patterns. A set of input and output data pairs are used to train the network and during the training process, weights and biases are adjusted iteratively to minimise the network performance function, *viz.*, SSE or MSE (Mean Square Errors). The manner in which the network performance

function is minimised is dependent on the training algorithm used to train the network. The Levenberg–Marquardt back-propagation algorithm was chosen to determine the adjustment of weights during training of the network. The Levenberg–Marquardt algorithm is often selected for use, despite the fact that it is computer-memory intensive, since it operates rapidly.

The percent SBS released at different stages of *in vitro* dissolution testing of formulations that were manufactured for the central composite design studies (Chapter 6) ($n = 6$) were used as the input and target data for the purposes of training the ANN. There were 180 (30 X 6) input-target data pairs that were used to train, test and validate the neural network. Although there are no rules as to the distribution of training, testing and validation data sets, it has been proposed that 65% of a data set should be used for training, 25% for testing and 10% for validation purposes [223]. For the purposes of this study, the data was split into three categories, *viz.*, the training, test and validation data sets, where 67% of the data set was used for training and 33% as the test data. The validation set was selected from the test data and constituted 67% the test data set.

The input and target data from *in vitro* dissolution testing was initially randomised to allow for efficient training of the network. Prior to training, the `mapminmax` function was used to scale the inputs and targets so that the values for these fell within a range of -1 to +1 based on the highest and lowest values in the data sets. Scaling the factors to this range is useful for efficient training of the network since it prevents bias in training if some of the values are significantly bigger or smaller than the other values in the training data set. If `mapminmax` is used to scale the target value, then the output of the network will be trained to produce outputs in the range -1 to +1 and these must then be converted back to original values by using the reverse of this function.

A neural network composed of an input and output layer with one hidden layer was chosen for the purposes of this study and a `logsig` function was used as the transfer function to the hidden layer and a `purelin` function was used for the output layer. The Levenberg–Marquardt algorithm for back-propagation with a gradient descent and momentum weight and bias learning function, was used to train the network. The MSE was used as the

performance function and training was terminated after either 200 validation failures or 800 epochs or iterations of the network, whichever came first.

Several training sessions with different numbers of nodes (3–10) in the hidden layer were conducted in order to determine the optimal ANN structure and each of these models were evaluated as described in § 7.3.3.

7.3.3 Artificial Neural Network Model Evaluation

Activation of the neural network was determined by simulating the neural network using normalised training data. A post-process activation on the predicted data set was then required to convert it back to the original input range. In the final step, the predicted data was then compared with the original data set by plotting the predicted *vs.* original values and computing the correlation coefficient for each of the responses in the output layer. These results were then compared and the closer the value was to 1 then the better the predictive capability of the model. ANN models with different numbers of nodes in the hidden layer were compared in this way and the optimal network architecture was then selected for further application. This final model was then retrained with the entire data set to obtain a network model that can be used for formulation optimisation and further simulations.

7.3.4 Formulation Optimisation using Artificial Neural Networks

Formulation optimisation was conducted using the f_2 similarity factor [197] for the comparison of dissolution profiles. Functions for formulation optimisation using simulation of the trained ANN and the f_2 similarity factor were written in the Matlab® editor (Mathworks Inc., Natick, MA, USA). All possible permutations of the formulation variables, *viz.*, Methocel® K100M, xanthan gum, Carbopol® 974P and Surelease®, within the experimental domain were generated using a brute force method and were then simulated using the trained ANN. The resultant simulated profiles were compared with the dissolution profiles of the reference formulation, Asthalin®8 ER (Cipla Ltd., Mumbai, Maharashtra, India). The function was used to determine the formulation that had the highest value for similarity. This formulation was subsequently manufactured and assessed as described in § 7.3.5.

7.3.5 Manufacture and Assessment of Optimised Formulation

An optimised hydrophilic matrix formulation of SBS was manufactured using the wet granulation method that was described in § 6.3.3. The tablet formulations were assessed and analysed as previously described in § 6.3.4 and § 6.3.5, respectively.

7.4 RESULTS AND DISCUSSION

7.4.1 Training and Testing the Neural Network Model

The number of nodes in the hidden layer that are required to produce a good predictive network depends on the complexity of the problem to be solved, the number of nodes in both the input and output layers, and the size of the training data set. Furthermore, the amount of noise in the target data, network architecture, the required accuracy of the prediction and the training algorithm that is used are also important factors that determine the number of nodes that are required in the hidden layer [207]. The number of nodes in the hidden layer is of paramount importance when constructing an ANN model: having too few hidden nodes decreases the learning ability of a network, and too many hidden nodes results in over-fitting or memorisation of the training data set and a reduced ability of a network to generalise and predict accurately [207].

Although several approaches, including Kolmogorov's theorem [224] and Carpenter and Hoffman's Equation [225], have been proposed as suitable to determine the number of nodes to be included in a hidden layer of an ANN, a trial and error approach is often selected [207,226]. The trial and error approach was used to determine the optimal number of nodes for inclusion in the network, and the situation that produced the highest range of R^2 values for each of the response factors was selected as the optimal architecture to use.

The impact of changing the number of nodes in the hidden layer of an ANN is depicted in Figure 7.3. Inspection of this diagram reveals that the efficiency of the network is dependent on the number of nodes in that network. It is clearly evident that the optimal number of nodes in the hidden layer for this system is nine since this architecture produces the highest value of R^2 for all stages of the dissolution test data. As the number of nodes is increased above 9 it is also apparent that there is a decrease in the efficiency of the network which is likely due to overtraining of the network.

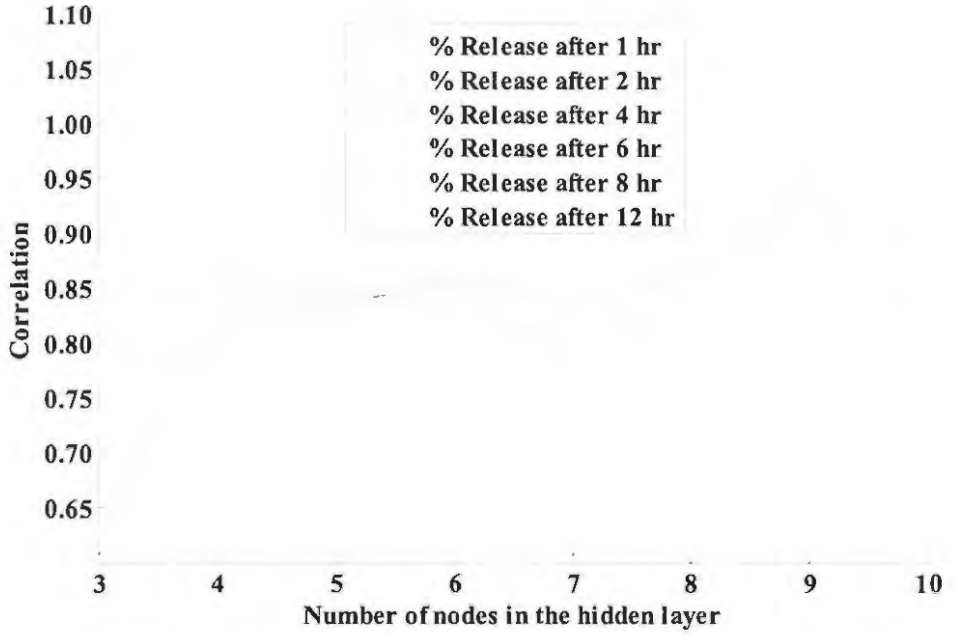


Figure 7.3 The impact of the number of nodes in the hidden layer on the correlation coefficient at different stages of the dissolution test

The training, validation and test error during training of a network that has nine nodes in the hidden layer is shown in Figure 7.4.

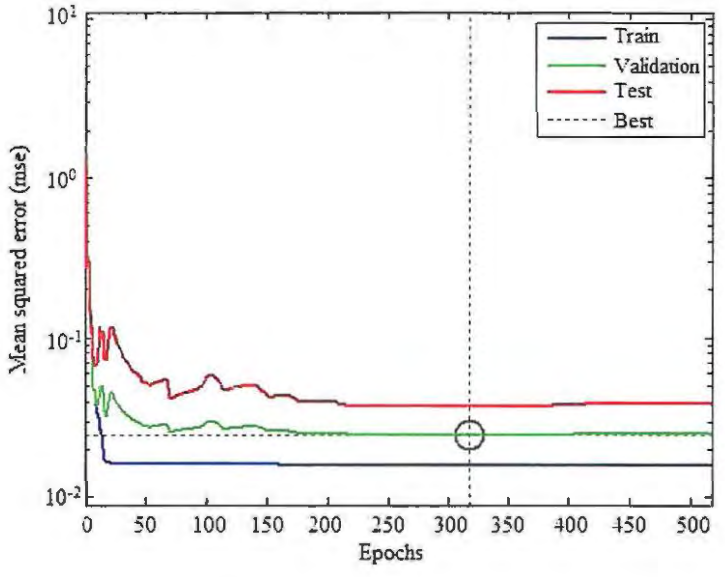


Figure 7.4 Plot of changes in training, validation and test error observed while training the network

The training error rapidly decreases during the first few epochs, after which it continues to decrease during the rest of the training phase (Figure 7.4). The validation and test error were

more variable and later decreased until a minimum was reached. The lowest or best validation error was observed at epoch 317 and was equivalent to 0.024861. The low error indicates that the model may be used to accurately predict the relationship between input and target data pairs and may therefore be used for prediction of outputs given a set of inputs.

The correlation data for each of the different data points are summarised in Table 8.1.

Table 7.1 Correlation of output factors

Output factor	R^2
% Release after 1 hr	0.9366
% Release after 2 hr	0.9501
% Release after 4 hr	0.9366
% Release after 6 hr	0.9508
% Release after 8 hr	0.9181
% Release after 12 hr	0.8323

The results from the prediction of test data generated by the ANN *vs.* the experimentally determined or observed values in the test data set are shown in Figure 7.5.

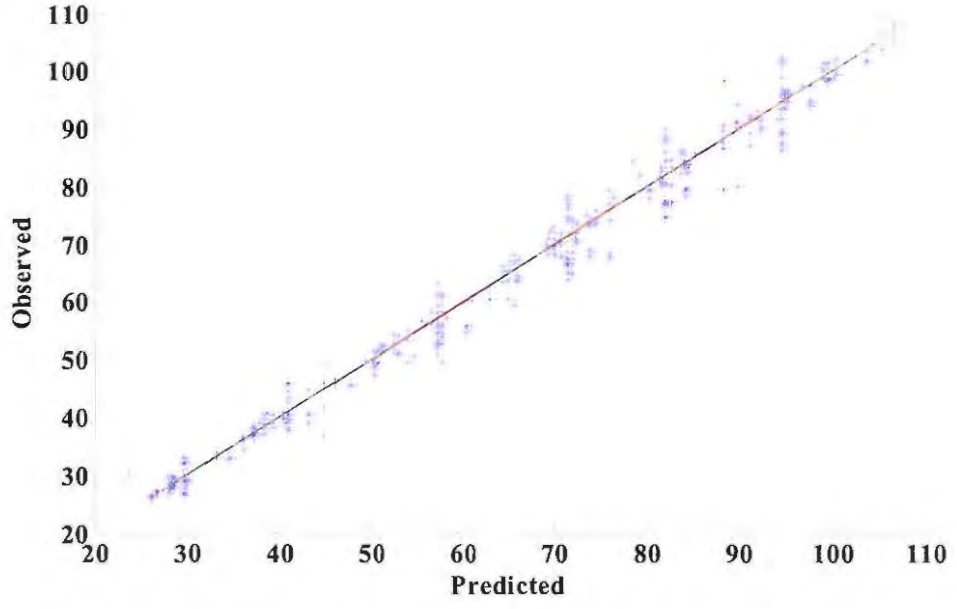


Figure 7.5 Comparison of correlation coefficients for predicted *vs.* observed data

These results show that an ANN that has nine nodes in the hidden layer has good predictive capabilities and could therefore be used for formulation optimisation and simulation of the impact of formulation variables on the dissolution rate of SBS from hydrophilic matrix formulations. The network was therefore trained with all data and was used for a formulation optimisation exercise as explained in § 7.3.4.

7.4.2 Formulation Optimisation

The brute force method and program were used to predict the composition of a hydrophilic matrix formulation with the desirable release characteristics similar to the reference formulation, Asthalin®8 ER (Cipla Ltd., Mumbai, Maharashtra, India). Table 7.2 lists a summary of the proposed formulation composition, including the predicted *in vitro* release data and the f_2 similarity factor for that hypothetical formulation.

Table 7.2 Optimisation formulation

Formulation		**Predicted dissolution profile**	
Methocel® K100M	45 mg	y_{1h}	38.38%
Xanthan gum	30 mg	y_{2h}	49.95%
Carbopol® 974P	5 mg	y_{4h}	65.87%
Surelease®	10% w/w	y_{6h}	80.00%
Avicel®	105.1 mg	y_{8h}	87.00%
Colloidal silica	0.5% w/w	y_{12h}	95.00%
Magnesium stearate	1% w/w	f_2 **factor**	90.5

The physical characteristics of the manufactured formulation were evaluated and are summarised in Table 7.3. It is apparent that the tablets that were produced had satisfactory performance qualities and attributes and were deemed suitable for their intended use.

Table 7.3 Physical properties of the optimised formulation following manufacture

Granule characteristics		**Physical characteristics**	
Bulk density	0.437 g/cm^3	Tablet weight	221.53 ± 1.36 mg
Tap density	0.442 g/cm^3	Tablet thickness	4.54 ± 0.04 mm
Carr's Index	6.98%	Tablet diameter	8.83 ± 0.03 mm
Angle of repose	37.9°	Tablet crushing strength	60.83 ± 6.01 N
		Assay	9.61 ± 0.05 mg

The *in vitro* dissolution profile generated for the optimised formulation following manufacture is shown in comparison with the predicted and reference formulations in Figure 7.6.

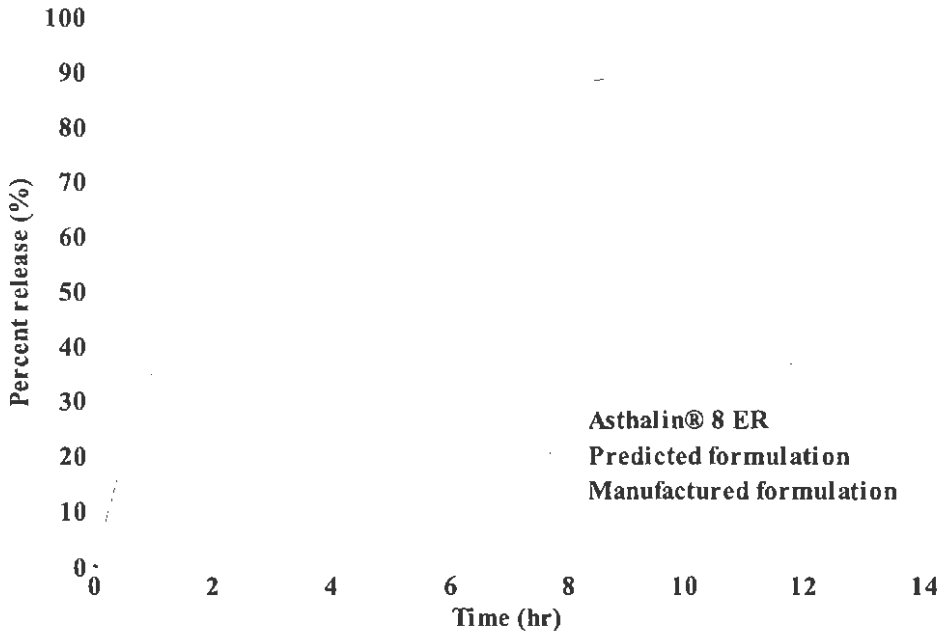


Figure 7.6 *In vitro* release profile of the optimised formulation compared with the reference formulation, Asthalin® 8 ER

It is evident that there is a high degree of similarity between the manufactured formulation and that of Asthalin®8 ER (Cipla Ltd., Mumbai, Maharashtra, India) tablets. The f_2 similarity factor was calculated to be 86.0. The relationship between the manufactured formulation derived from the optimisation procedure using ANN and that for the predicted formulation is shown in Figure 7.7. The results show that the relationship between the predicted and observed formulations was nearly linear showing excellent predictability for the optimisation by use of ANN.

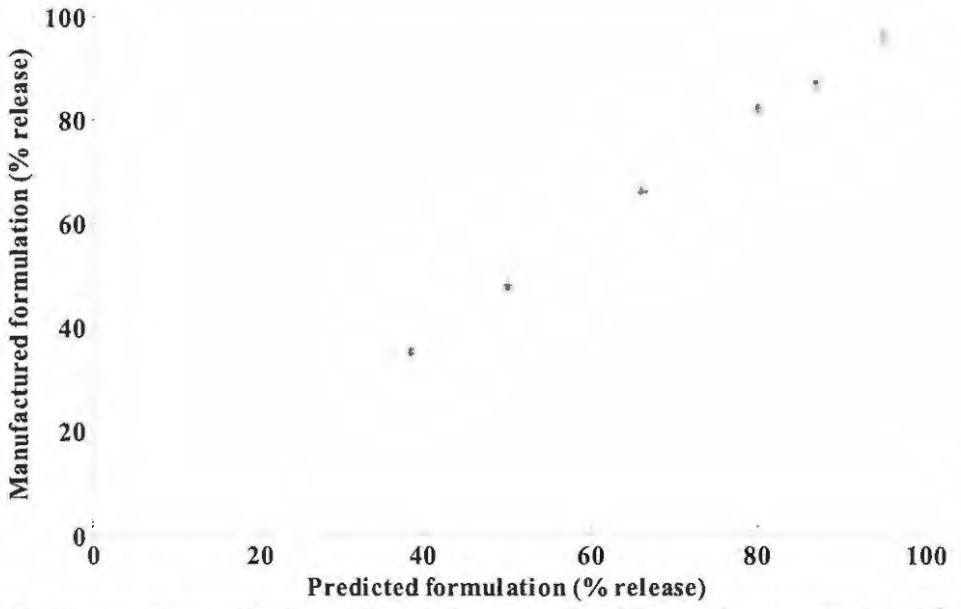


Figure 7.7 Percent release of optimised formulation *vs.* predicted formulation, $y = x - 2.5, R^2 = 0.9964$

7.5 CONCLUSIONS

ANN simulate the learning behaviour of the human brain by modelling data and recognising patterns for complicated multi-dimensional relationships between sets of input and output data pairs. ANN may be used to predict the output for a specific system given a set of input patterns once they have been trained. ANN may also be used for the optimisation of pharmaceutical processes and systems.

The primary advantage of ANN is that they may be used to map relationships between causal and response factors without prior information from input data sets and once a network has been trained, simulations may be generated from the model. Although ANN are simple to use once they have been developed, they are not useful for extrapolation of data that is not within the bounds of the training data set that was used.

Matlab® R2008a (Mathworks Inc., Natick, MA, USA) was used to write code for the training and evaluation of a neural network. Formulation variables and *in vitro* dissolution profiles from a central composite study were used for training, testing and validating the network models that were developed.

The efficiency of the network is dependent on the number of nodes in the hidden layer and the numbers of nodes were tested by means of a trial and error approach using between three and ten neurons. The optimal number of nodes that produced a good predictive model was nine. Once this was established, the data was once again used to train a network that was then applied to the optimisation of a SR matrix formulation for SBS.

The f_2 similarity factor was used to determine whether formulation optimisation had been successful by comparing ANN predicted dissolution profiles to that generated from the reference formulation, Asthalin®8 ER (Cipla Ltd., Mumbai, Maharashtra, India). A brute force method was applied to generate permutations for simulation into the model and the combination of formulation variables that resulted in the highest f_2 value was selected. The resultant model formulation was then manufactured using the wet granulation procedure described in Chapter 6. The resultant formulation was found to perform satisfactorily and an f_2 value of 86.0 was calculated for the comparison, clearly indicating that the dissolution profiles were similar.

These results demonstrate the potential utility of ANN models for formulation development and optimisation. Defining CQA from the outset is vitally important in developing a model formulation with the desired physical and quality characteristics.

The ANN model that was developed will be investigated for use in the development of a design space for the production of a high quality SR matrix tablet of SBS as described in Chapter 8 *vide infra*.

CHAPTER 8
DEVELOPMENT AND ESTABLISHMENT OF A DESIGN SPACE

8.1 DESIGN SPACES

8.1.1 Introduction

According to the ICH Q8 (R2) Pharmaceutical Development guideline [1] a design space is "the multidimensional combination and interaction of input variables (e.g. material attributes) and process parameters that have been demonstrated to provide an assurance of quality". The premise of establishing a design space for formulation development is to define formulation and processing variables within which the quality of a product can be maintained with an associated reduction in the statistical variation of quality [227].

The establishment of a design space is an important driver of efficiency since an operational adjustment within an approved design space is not considered by Regulatory Agencies as a change that requires post-approval supplementary filing [228]. Furthermore operating within a design space allows for the continual improvement of pharmaceutical products during the product lifecycle [229].

The establishment of a design space commences with the definition of a target product profile that identifies the desired performance characteristics of a product, including dissolution profile, quality attributes such as crushing strength, friability and other physical characteristics. The attributes that are likely to impact product quality, *i.e.* CQA are identified during the initial stages of formulation development experiments, during which multivariate experiments and modelling are often used to study complex relationships that exist between variables and CQA which must be subsequently linked to a design space [230].

One of the key challenges in developing a design space in product development is that the concept of a design space in a pharmaceutical context is fairly new and therefore not well understood. Consequently, the interpretation of this concept and how best to present usable and relevant information that relates to a design space has not yet been standardised.

There are many scientifically justifiable approaches that can be used to develop a design space and the approach used is dependent on the combination of tools available, technologies to be investigated and internal corporate experience [231]. Although the use of formal experimental design has been proposed as a means of generating design spaces [1,230] there is a dearth of information with respect to the manner of presentation of the actual design spaces, although graphical, interaction, response surface and contour plots have been proposed as useful techniques for depicting such spaces [1,231].

The purpose of this research is to identify and present perspectives on the presentation of design spaces by focusing on the application of RSM and ANN for this purpose. A hydrophilic SR matrix formulation of SBS was used to evaluate these methods on a comparative basis. The *in vitro* dissolution performance of a reference formulation, Asthalin®8 ER (Cipla Ltd., Mumbai, Maharashtra, India) was used as a CQA to define appropriate dosage form performance.

8.1.2 Related Concepts

There are two vital concepts that are intimately related to a design space, *viz.*, knowledge and control spaces. A conceptual visualisation of the relationship and interaction between the knowledge, design and control spaces that need to be identified and managed when developing and optimising formulations is depicted in Figure 8.1.

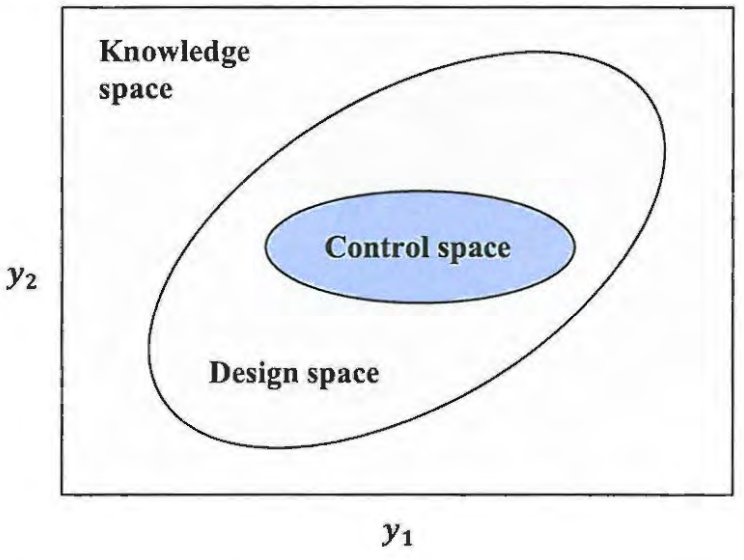


Figure 8.1 The interrelationship between knowledge, design and control spaces with respect to CQA, redrawn and adapted from [227]

The knowledge space is a summation of all information about the impact of formulation and process variables on measured CQA and the design space is a subset of the knowledge space. A design space must be defined in terms of product CQA, y_1 and y_2 and represents a multidimensional combination of the variables within which the CQA are acceptable. In other words product quality, in terms of safety and efficacy, is assured [227].

Although product quality is assured within a design space, most pharmaceutical companies operate within control spaces that are located within a specified design space. Control spaces are defined in the CQA space as $\pm 3\sigma$ limits about the target, where the variance σ^2 is based on the common cause or inherent variability of a manufacturing process [227].

8.1.3 Approaches to the Development of Design Spaces

Although the concept of design spaces in the pharmaceutical industry is relatively new there have been several approaches reported for establishing a design space. A design space may be developed from mechanistic models if the scientific principles relating to a process are well known. For example, if the thermodynamic and rate phenomena associated with chemical processes are well known, a design space can be defined relatively easily. However, complex pharmaceutical formulations are not easily defined by simple models and therefore may not easily be described using mechanistic models and for that reason empirical research and models such as RSM must be used to map any relationships that exist between input variables and resultant responses [231].

Traditionally, design spaces for raw materials were defined by setting specifications for individual materials separately and identifying the limits within which each material attribute (e.g. physical and chemical properties, NMR spectra, *etc.*) must fall. However the application of this approach can be limiting and may lead to the establishment of a poorly defined design space for those raw materials [232]. Alternatively a design space for a raw material may be defined using Principal Component Analysis (PCA). PCA is used to extract dominant patterns in a data matrix using a complementary set of score and loading plots [233]. To demonstrate the utility of PCA, the measurable properties of raw materials for manufacturing polymer films were defined in a study undertaken by Duchesne and MacGregor [232]. Prior to the commencement of the studies, ten properties of raw materials were identified with the objective of specifying a design space for each attribute that would result in the production of polymer films of acceptable quality. A model that defined the regions, which result in

polymer films with acceptable properties by identifying statistically significant terms for the production of the films was constructed using PCA. The advantage of using PCA for this application is that the method results in the development of a design space that can be effectively used to consistently produce quality films [232].

Statistical Process Control (SPC) concepts have also been suggested as a means of defining pharmaceutical control spaces [227]. The use of this methodology permits the establishment of design spaces for a manufacturing process and raw material variables simultaneously, so as to ensure the manufacture of pharmaceutical products that satisfy predetermined objectives with minimal variability [227].

Cogdill *et al* [228] used a risk-based strategy for the quality assessment of pharmaceutical products that applies a model-based simulation approach to link variations in drug product parameters with clinical performance. Taguchi's concept of quality loss, pharmaceutical quality and risk, in conjunction with a simulation framework for assessing pharmaceutical quality based on probabilistic risk assessment, was used to investigate the concept of QbD [228]. One of the key advantages of using risk-spaces to define product quality in implementing QbD is that drug product parameters such as API content may be linked to *in vitro* dosage form performance and the possible risk of failing to meet therapeutic objectives for that product [228].

RSM specifically the use of a Box–Behnken design has been implemented as a strategy for understanding formulation variables that impact the quality of a self-nanoemulsified drug delivery system [234]. Although the impact of formulation components on the measured responses was evaluated using a response surfaces approach, no explicit description of what constitutes a design space for this product was reported.

Takayama *et al* [235,236] proposed the use of computer-aided design for establishing design spaces for drug products. More specifically, a central composite design with multiple linear regression was used to develop quadratic relationships that depicted the interaction between input and output factors that affected product performance. However a major limitation of using predictions from quadratic polynomials is that the result may be a poor estimation of product performance. To overcome these limitations a multivariate spline interpolation was investigated to generate smooth response surfaces. In addition a bootstrap re-sampling

technique and Kohonen's self-organising map were used to evaluate the reliability of the optimal solution to a formulation design that was established using these approaches [235,236]. Furthermore, the use of a leave-one-factor-out approach to understand factors that significantly affect optimal formulation performance and therefore to establish a region within which product quality can be assured, was proposed [236].

A multivariate statistical approach that used a simplex lattice design in combination with a non-linear response surface method incorporating thin-plate spline interpolation was used as a tool for establishing a design space for a SR formulation of diltiazem hydrochloride [237].

Other multivariate methods that combine the use of experimental design approaches and RSM with PCA and Partial Least Squares (PLS) analysis to develop a powerful means to elucidate complex multivariate relationships between input factors and outputs have also been suggested as possible means of establishing design spaces [238]. Furthermore, concepts using Bayesian models [239], multivariate chemometrics, engineering process control, design of experiments and SPC [227] have been used to establish design spaces.

8.1.4 Presentation of Design Spaces

Although there is no set way to represent a proposed pharmaceutical design space, the ICH Pharmaceutical Development guideline [1] recommends the use of interaction, contour and response surface plots for presenting the design space.

Interaction plots that were generated from a Box–Behnken experimental design were used to highlight the relationship between input factors and the measured responses for a self-nanoemulsified drug delivery system of cyclosporine A [234]. Alternatively, surface plots [240] and design space trajectories [241], a combination of interaction and contour plots [242], PCA plots [243] or even a multivariate combinations of interaction profiles and response surfaces from formal experimental designs, Partial Least Squares (PLS) and PCA plots [238] may be used to fully characterise and understand a specific design space.

The limitation of using response surface and contour plots for visualising response surfaces is that they cannot be used to display the effect of more than two variables on a measured response simultaneously. Yamashita *et al* [244] proposed the use of a novel visualisation technique referred to as Hyper-Dimensionality Embedded Cuboids which are useful for

understanding design space variables comprehensively. Data from experimental design approaches including polynomial models may be used as the input parameters for this data visualisation tool. Hyper-Dimensionality Embedded Cuboids are used to map N-dimensional data on a two-dimensional rectangular region defined by a recursive slice and dice subdivision of the $x-y$ plane. However the use of this technique is limited since the computer code to display the resultant data must be written in the Java programming language, although there is a graphical user interface which can be used to make the software user friendly.

8.2 METHODS

8.2.1 Response Surface Methodology

The Calibration Generation tool in the Model-Based Calibration Toolbox in Matlab® R2008a (Mathworks Inc. Natick, MA, USA) was used for the formulation optimisation described in Chapter 6 *vide infra.* The *in vitro* dissolution profile of Asthalin®8 ER (Cipla Ltd., Mumbai, Maharashtra, India) was used as the constraints to determine the levels of polymers which would result in the manufacture of similar and acceptable formulations. The impact of the levels of Methocel® K100M, xanthan gum, Carbopol® 974P and Surelease® on the extent of drug release at all stages of dissolution testing was determined from the resultant constraint plots.

8.2.2 Artificial Neural Network Simulation

The optimisation procedure described in Chapter 7, *vide infra* revealed that an optimal composition with the desired *in vitro* release characteristics can be manufactured. The impact of varying formulation composition within the limits of ± 5 mg for solid polymers and ± 5% w/w for the composition of the liquid dispersion used to granulate the formulation compared with the optimised formulation is summarised in Table 8.1.

Table 8.1 ANN limits related to the optimised formulation

	Lower limit	Optimised formulation	Upper limit
Methocel® K100M	40 mg	45 mg	50 mg
Xanthan gum	25 mg	30 mg	35 mg
Carbopol® 974P	0 mg	5 mg	10 mg
Surelease®	5% w/w	10% w/w	15 % w/w

A function for simulating the *in vitro* release profile for hypothetical formulations within the constraints listed in Table 8.1, using the ANN model described in Chapter 7, was written in Matlab® code (Mathworks Inc., Natick, MA, USA) and used to simulate different *in vitro* release profiles.

The *in vitro* dissolution profiles that were generated using the simulation function were then plotted and compared, using the f_2 similarity factor, to those generated for the reference formulation [197], and these data were used to study the design space for a SR formulation of SBS.

8.3 ESTABLISHMENT OF A DESIGN SPACE

8.3.1 Response Surface Methodology

Constraint plots were used to determine the regions of the experimental domain which correspond to limits of the dissolution profile of the reference formulation Asthalin®8 ER (Cipla Ltd., Mumbai, Maharashtra, India). The impact of each of the formulation variables that were investigated and described in Chapter 6 on the different stages of the dissolution test are shown in Figures 8.2–8.7. The blue curve in these figures is an indication of the change in the extent of drug release at each stage of dissolution test and the red curves represent the constraints within which the quality of the product will be maintained.

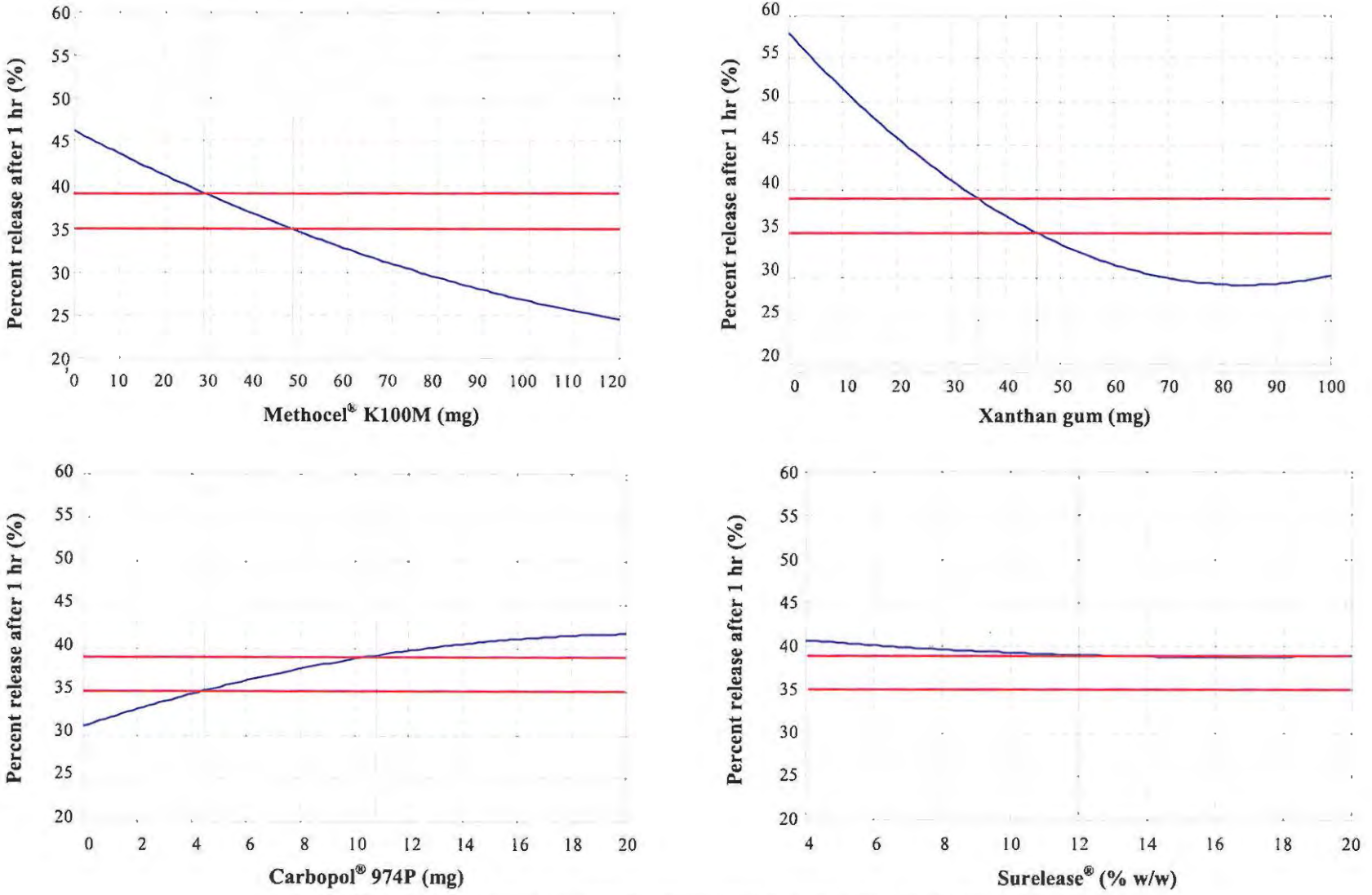


Figure 8.2 Constraints plots for percent SBS released after 1 hr of dissolution testing

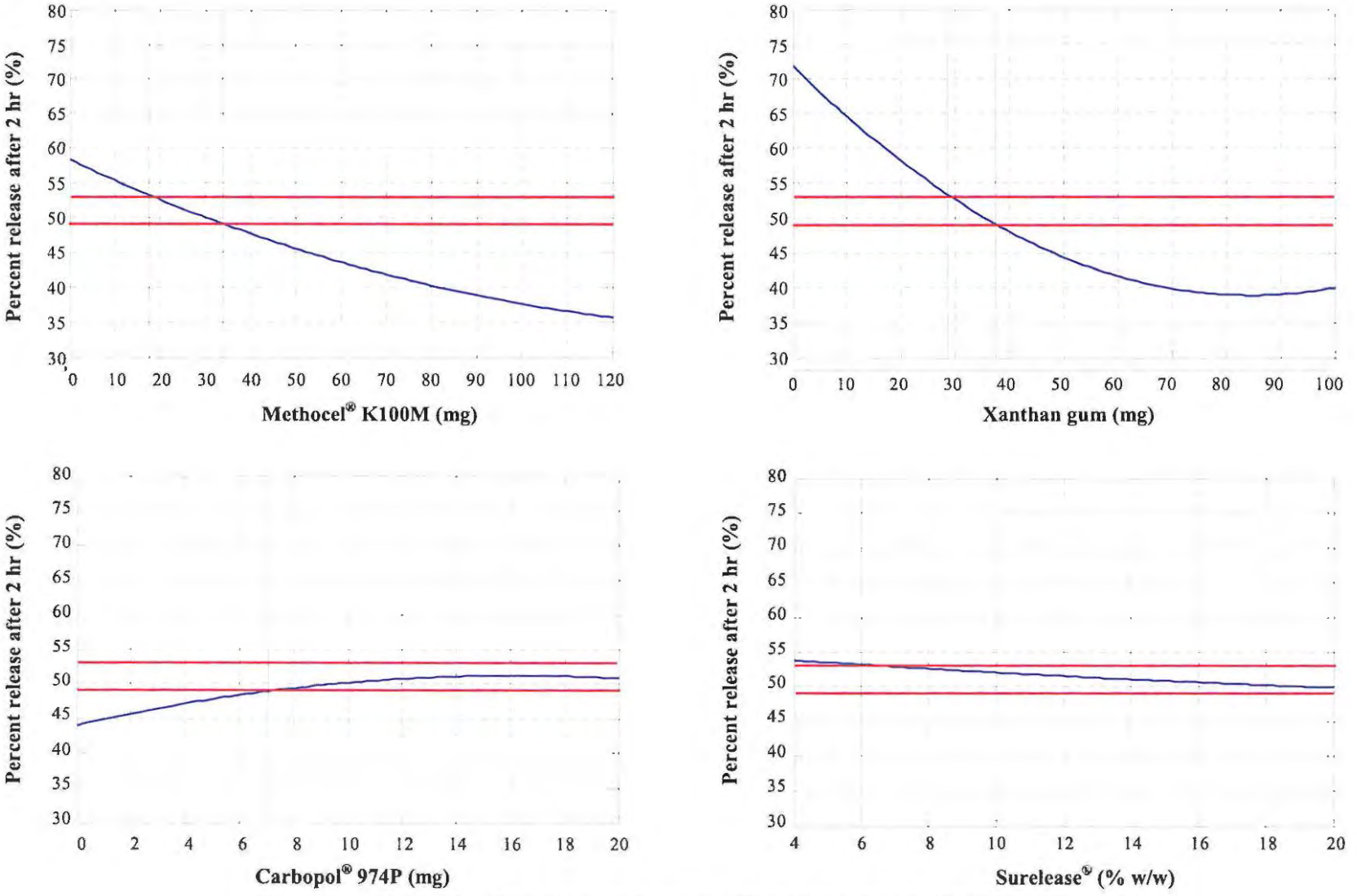


Figure 8.3 Constraint plots for percent SBS released after 2 hr dissolution testing

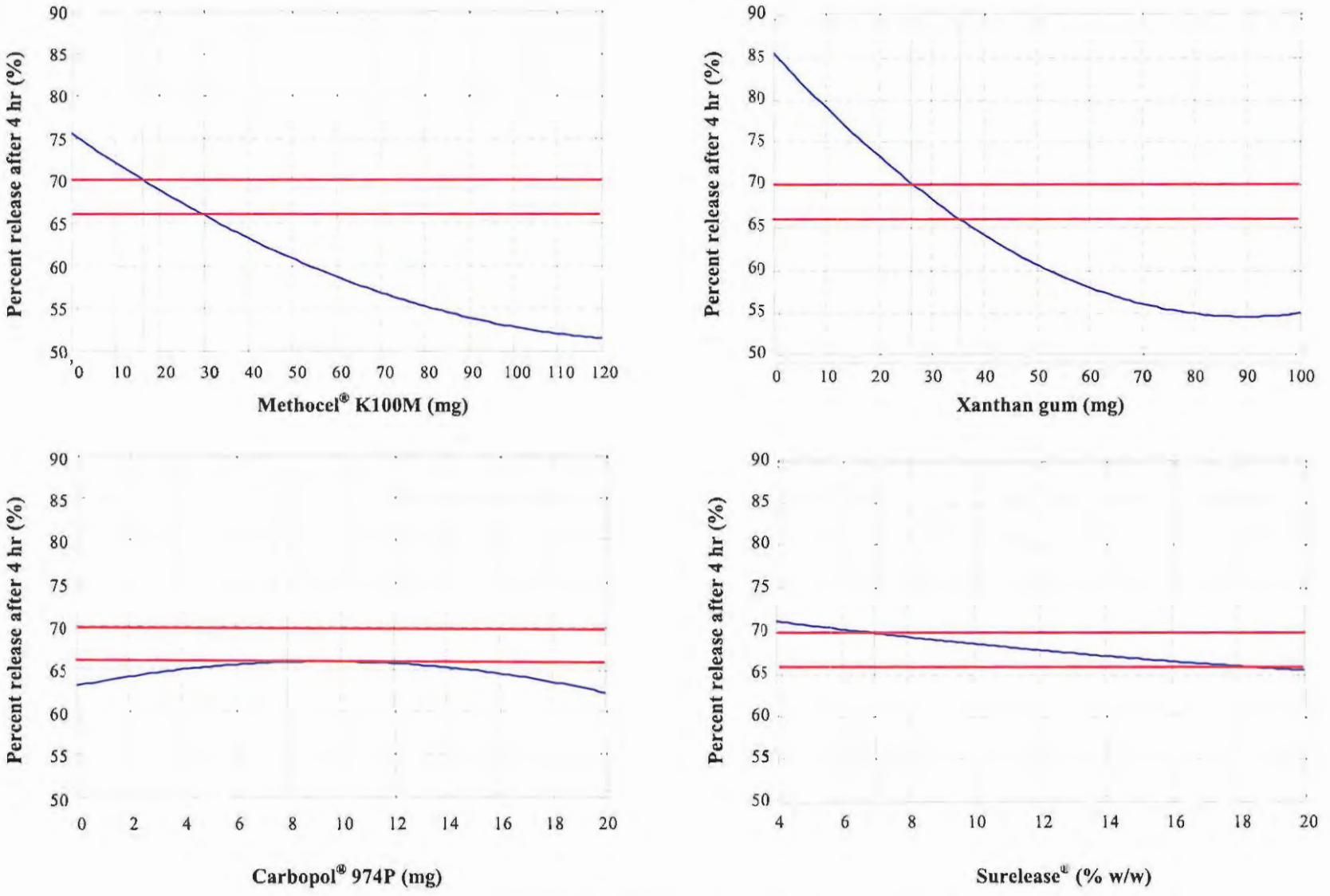


Figure 8.4 Constraint plots for percent SBS released after 4 hr of dissolution testing

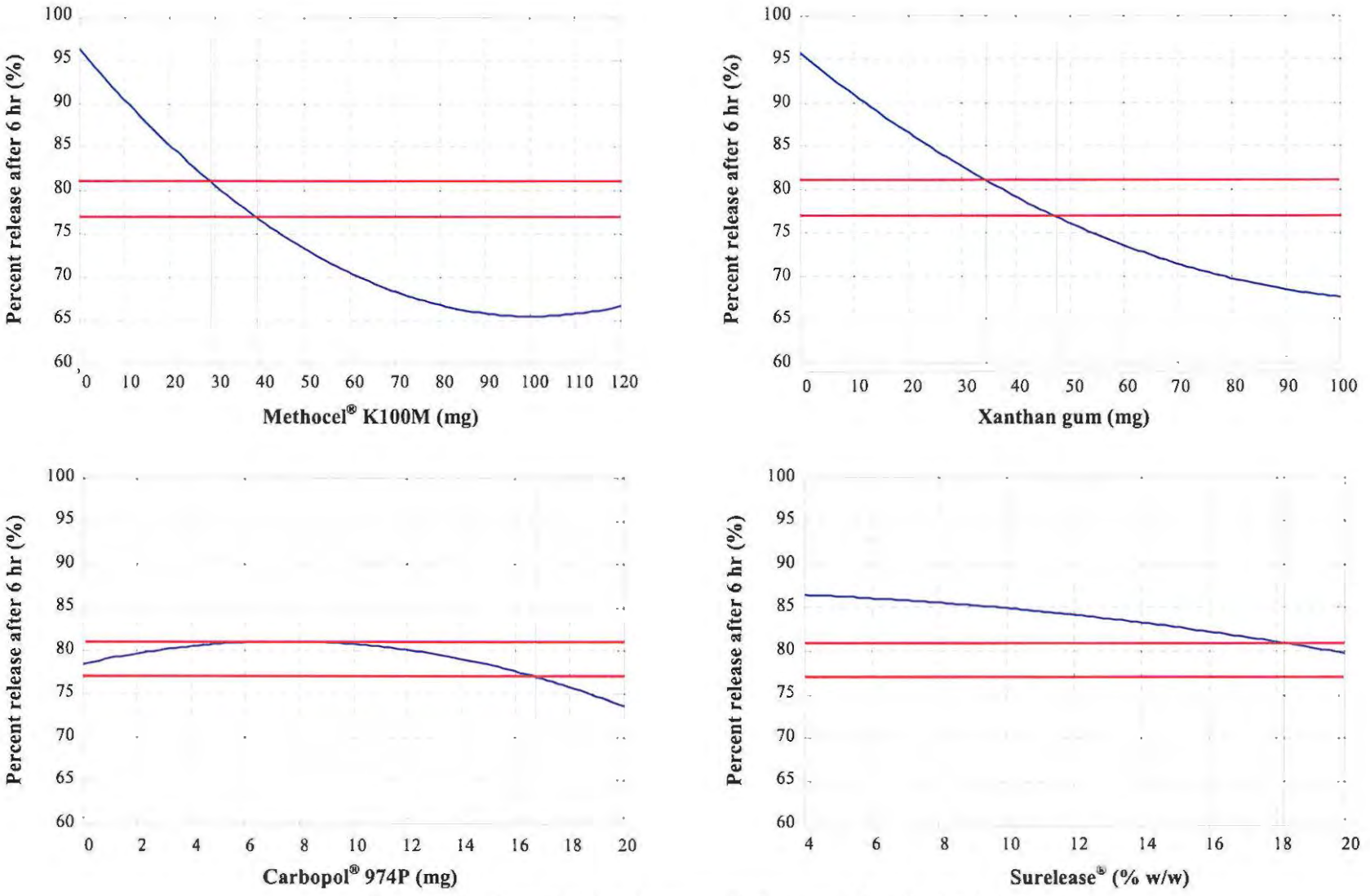


Figure 8.5 Constraint plots for percent SBS released after 6 hr dissolution testing

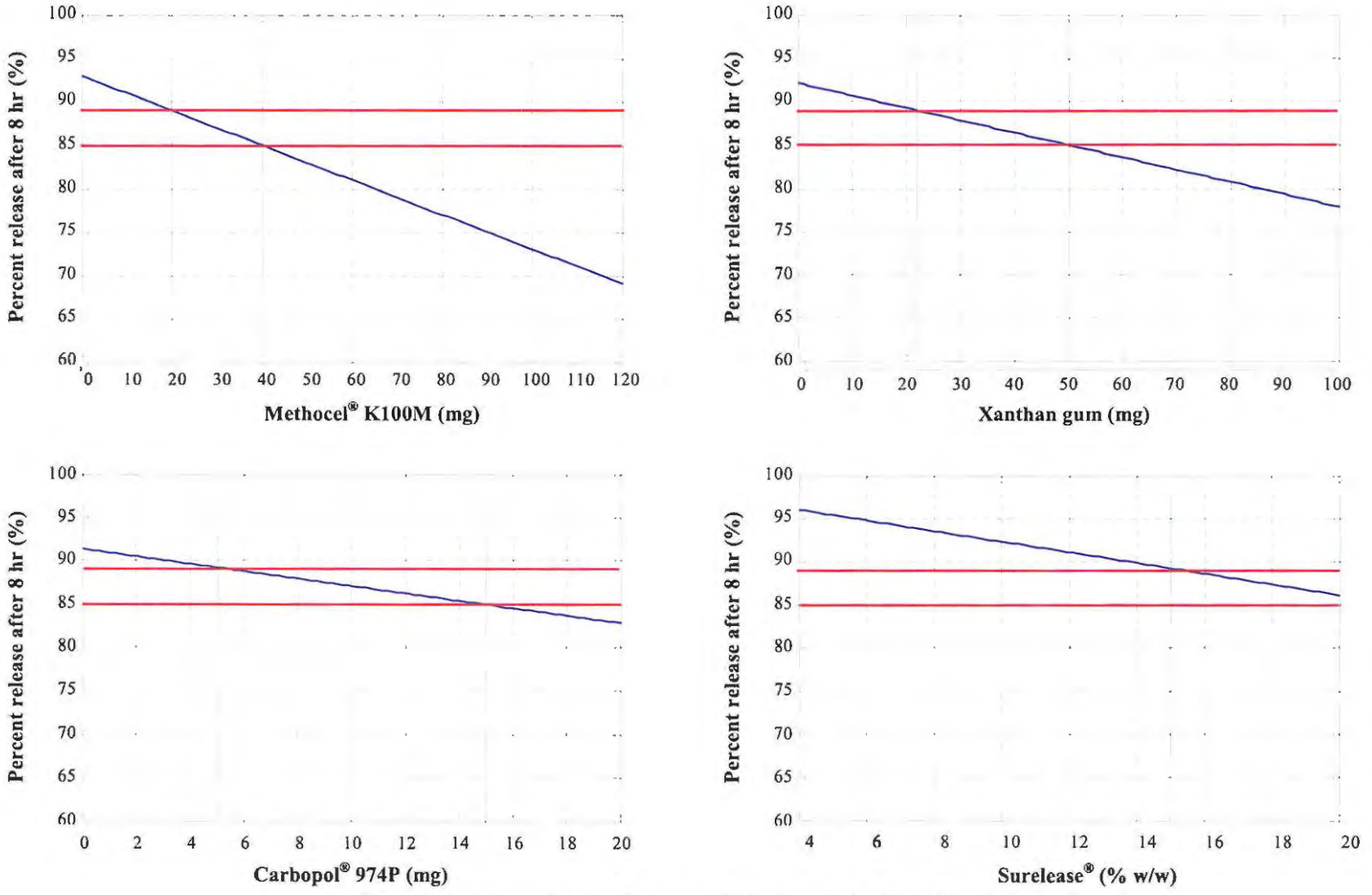


Figure 8.6 Constraint plots for percent SBS released after 8 hr of dissolution testing

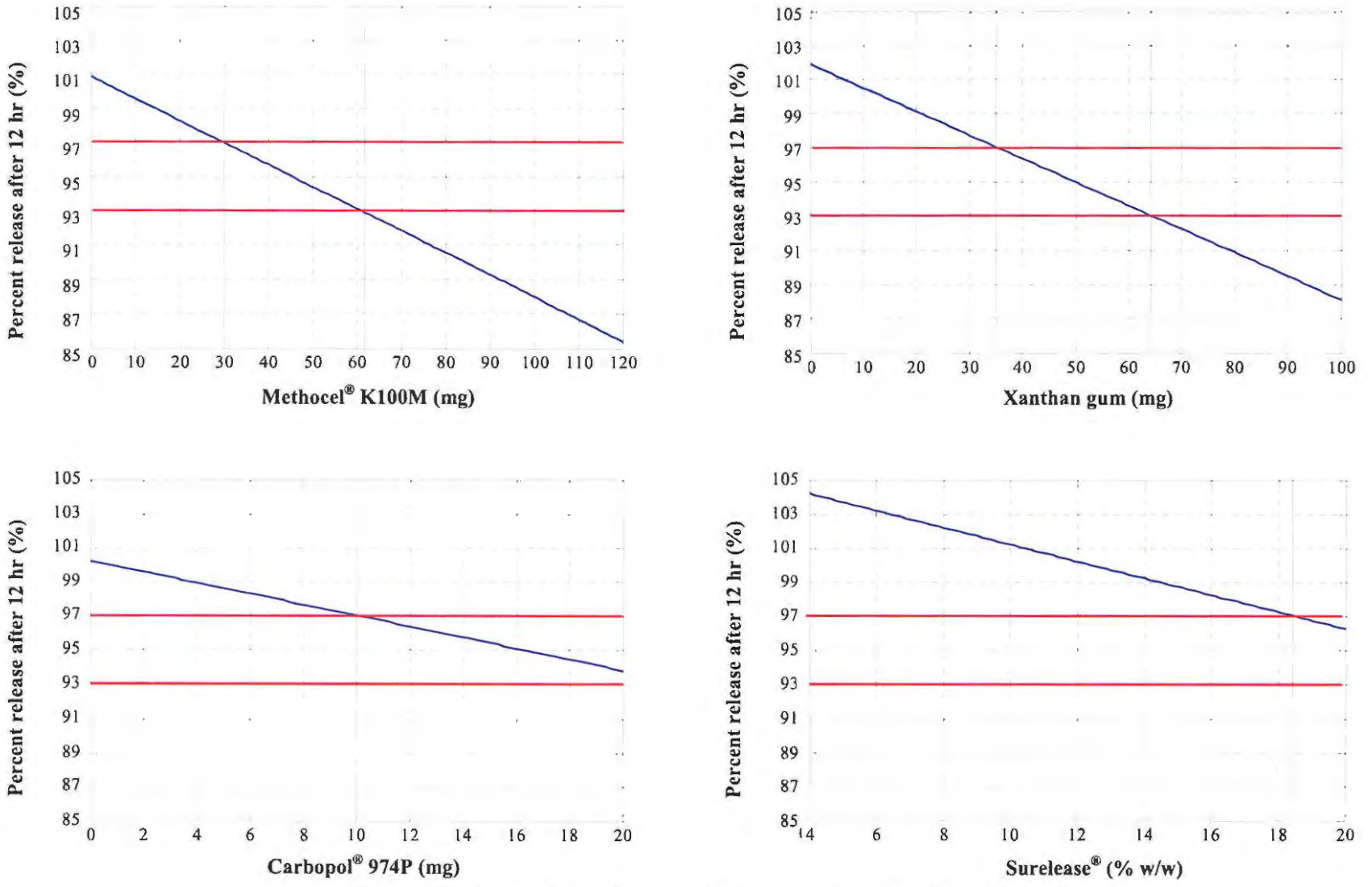


Figure 8.7 Constraint plots for percent SBS released after 12 hr of dissolution testing

A summary of the formulation constraints for the optimised formulation that was developed from assessment of Figures 8.2–8.7 is listed in Table 8.2. The impact of the different formulation variables of the rate and extent of drug release has been described and discussed in Chapter 6, *vide infra*. It is apparent that the limits for each tested formulation change as the dissolution test progresses. However it is apparent that there is a region in which the impact of formulation variables on SBS release at different stages of the dissolution test, overlap. Consequently formulation limits can only be maintained within certain ranges of excipient composition in the formulation design.

Table 8.2 Design space limits obtained from RSM evaluation

	Methocel® K100M (mg)		Xanthan gum (mg)		Carbopol® 974P (mg)		Surelease® (% w/w)	
	Lower limit	**Upper limit**	**Lower limit**	**Upper limit**	**Lower limit**	**Upper limit**	**Lower limit**	**Upper limit**
y_{1hr}	28.5	47.0	34.0	45.5	4.5	10.5	10.0	20.0
y_{2hr}	17.5	34.0	28.5	37.5	7.5	20.0	6.0	20.0
y_{4hr}	16.0	30.0	26.0	36.0	8.0	10.0	7.0	20.0
y_{6hr}	28.5	40.0	34.0	47.5	0.0	17.0	18.0	20.0
y_{8hr}	20.0	40.0	22.0	50.0	5.5	15.5	15.0	20.0
y_{12hr}	30.0	62.0	35.0	65.0	10.0	20.0	18.5	20.0
Limits	30.0	30.0	35.0	36.0	10.0	10.0	18.5	20.0

Although the range of composition for each of the excipients required to satisfy the design space criteria are wide in some instances, it is apparent that the limits within which formulation performance can be maintained at all stages of the dissolution test are somewhat narrow in others.

The limits that have been identified can therefore be considered to be the design space for the SR hydrophilic matrix formulation of SBS that was manufactured in this study. These limits can therefore be considered to satisfy the design space for the model formulation.

8.3.2 Simulation Using Artificial Neural Networks

ANN simulations can also be used to establish the impact of changing or altering formulation variables on CQA and therefore to evaluate if changes in formulation composition produce dissolution profiles that differ for hypothetical formulations.

The formulation compositions described in Table 8.1 were selected and used to assess the feasibility of using ANN simulations for developing and monitoring a design space for

pharmaceutical formulations. The design space permutations that were investigated are shown in Table 8.3.

Table 8.3 Simulated compositions for hypothetical formulations

Formulation	Methocel® K100M	Xanthan gum	Carbopol® 974P	Surelease®	f_2 fit factor
1	40	25	0	5	31.6012
2	40	25	0	10	31.6525
3	40	25	0	15	33.6231
4	40	25	5	5	56.2400
5	40	25	5	10	40.3193
6	40	25	5	15	40.6134
7	40	25	10	5	48.6557
8	40	25	10	10	48.1637
9	40	25	10	15	47.2785
10	40	30	0	5	36.5141
11	40	30	0	10	32.5460
12	40	30	0	15	33.8473
13	40	30	5	5	59.4408
14	40	30	5	10	48.6317
15	40	30	5	15	39.3311
16	40	30	10	5	49.3272
17	40	30	10	10	53.0141
18	40	30	10	15	51.8354
19	40	35	0	5	45.5320
20	40	35	0	10	35.1357
21	40	35	0	15	34.6290
22	40	35	5	5	58.4069
23	40	35	5	10	58.0327
24	40	35	5	15	42.2794
25	40	35	10	5	48.9379
26	40	35	10	10	58.5845
27	40	35	10	15	48.4519
28	45	25	0	5	32.0438
29	45	25	0	10	31.3923
30	45	25	0	15	33.4131
31	45	25	5	5	60.2388
32	45	25	5	10	43.4958
33	45	25	5	15	41.9055
34	45	25	10	5	46.7753
35	45	25	10	10	47.5839
36	45	25	10	15	47.4910
37	45	30	0	5	38.1946
38	45	30	0	10	32.5949
39	45	30	0	15	32.5948
40	45	30	5	5	62.0819
41	45	30	5	10	54.0798
42	45	30	5	15	41.5888
43	45	30	10	5	48.8460
44	45	30	10	10	54.1409
45	45	30	10	15	52.2857
46	45	35	0	5	47.5462
47	45	35	0	10	35.9310

Formulation	Methocel® K100M	Xanthan gum	Carbopol® 974P	Surelease®	f_2 fit factor
48	45	35	0	15	34.6761
49	45	35	5	5	60.2652
50	45	35	5	10	60.6438
51	45	35	5	15	46.0947
52	45	35	10	5	50.3023
53	45	35	10	10	62.4939
54	45	35	10	15	49.0600
55	50	25	0	5	32.7315
56	50	25	0	10	32.7315
57	50	25	0	15	33.1909
58	50	25	5	5	63.9643
59	50	25	5	10	47.6710
60	50	25	5	15	43.5129
61	50	25	10	5	49.7792
62	50	25	10	10	49.1474
63	50	25	10	15	49.6309
64	50	30	0	5	40.2822
65	50	30	0	10	32.7639
66	50	30	0	15	33.5742
67	50	30	5	5	64.4999
68	50	30	5	10	60.3595
69	50	30	5	15	44.3797
70	50	30	10	5	51.6535
71	50	30	10	10	58.3930
72	50	30	10	15	53.2213
73	50	35	0	5	48.6955
74	50	35	0	10	37.0437
75	50	35	0	15	34.7775
76	50	35	5	5	60.8569
77	50	35	5	10	61.4447
78	50	35	5	15	51.2066
79	50	35	10	5	54.7737
80	50	35	10	10	69.1416
81	50	35	10	15	69.1415

* shaded areas represent formulation compositions that produced f_2 values >50

The simulated *in vitro* dissolution profiles for hypothetical formulations that were generated using ANN and that were compared with the *in vitro* dissolution profile of the reference formulation and the manufactured formulation are depicted in Figures 8.8–8.17.

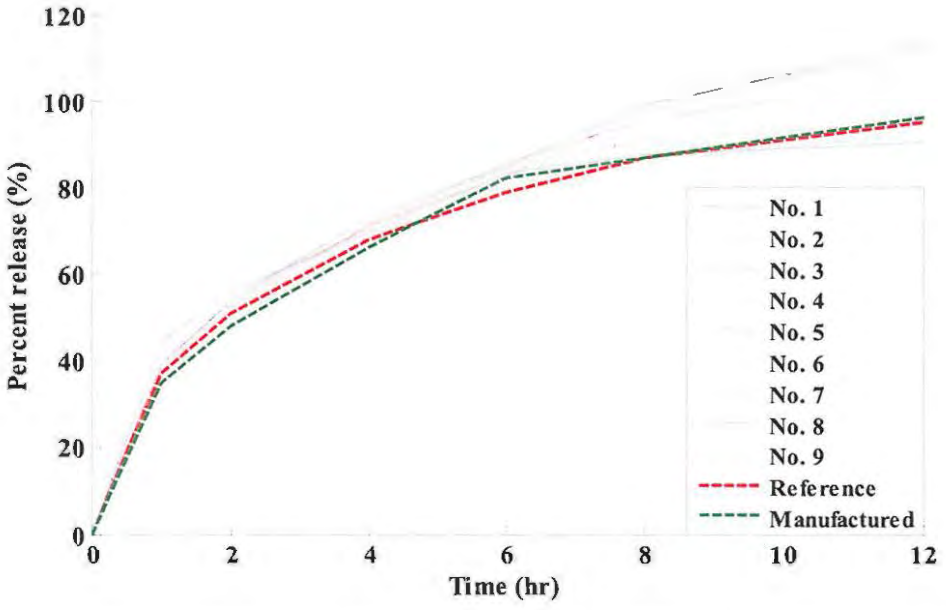


Figure 8.8 Dissolution profile simulation for hypothetical formulations 1–9 compared with the reference and manufactured formulations

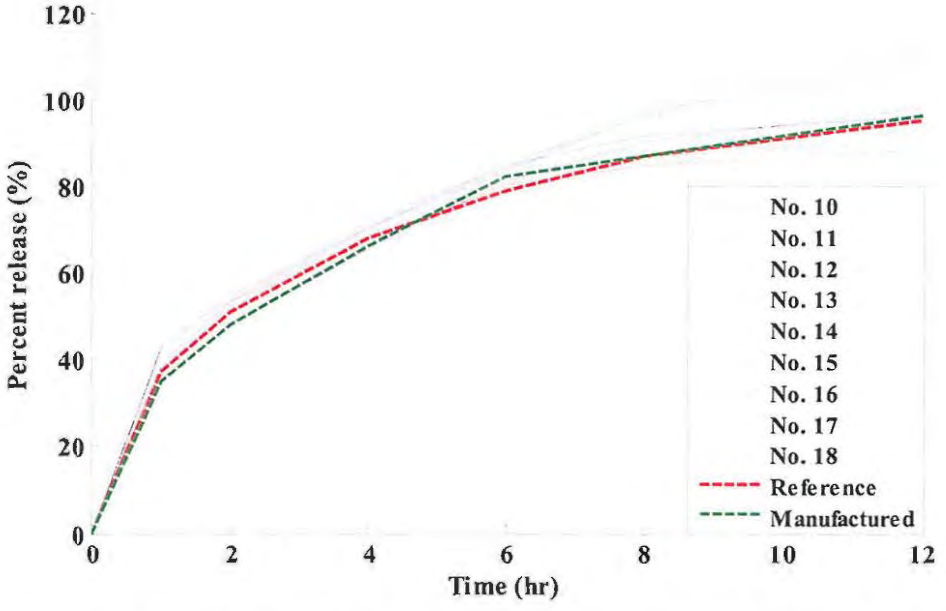


Figure 8.9 Dissolution profile simulation of hypothetical formulations 10–18 compared with the reference and manufactured formulations

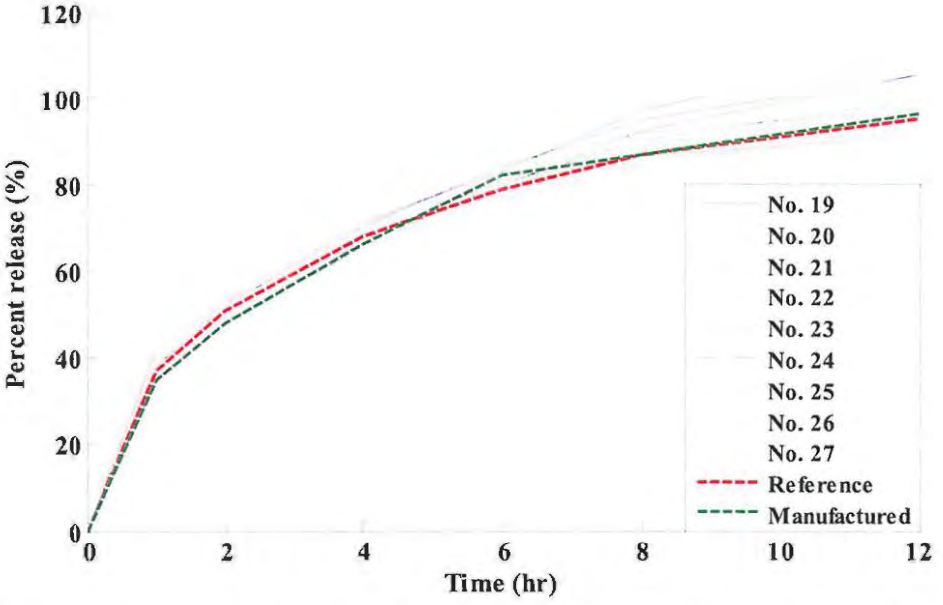


Figure 8.10 Dissolution profile simulation of hypothetical formulations 19–27 compared with the reference and manufactured formulations

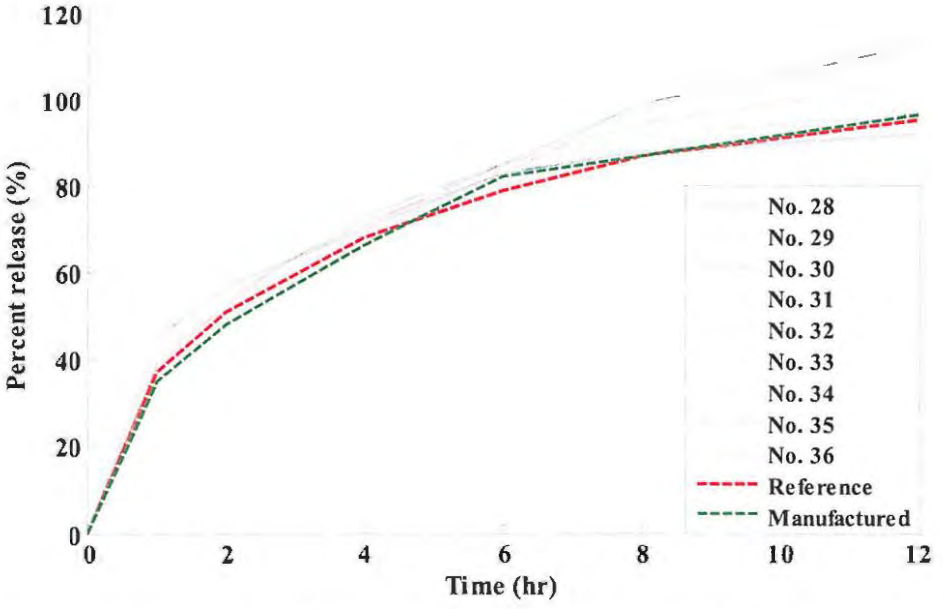


Figure 8.11 Dissolution profile simulation of hypothetical formulations 28–36 compared with the reference and manufactured formulations

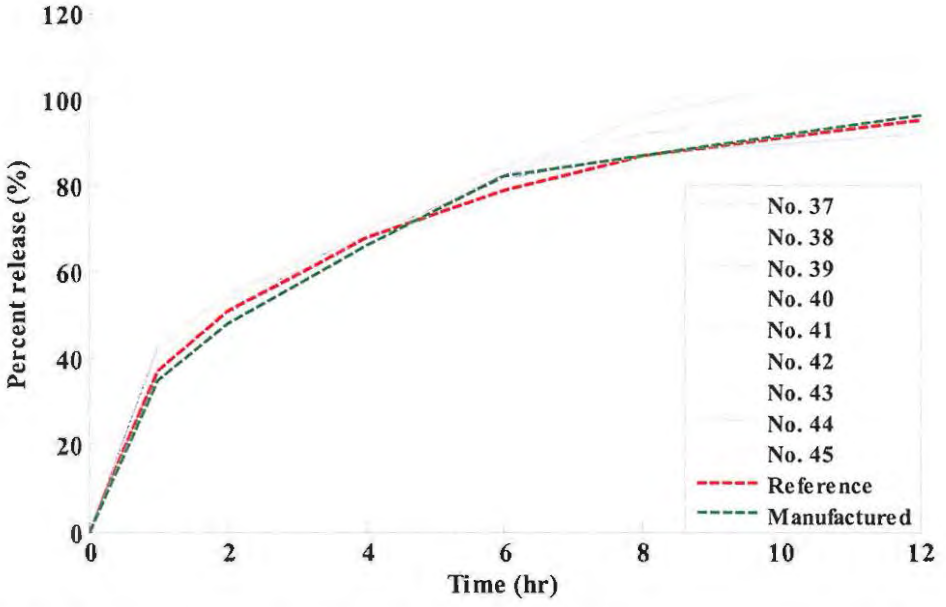


Figure 8.12 Dissolution profile simulation of hypothetical formulations 37–45 compared with the reference and manufactured formulations

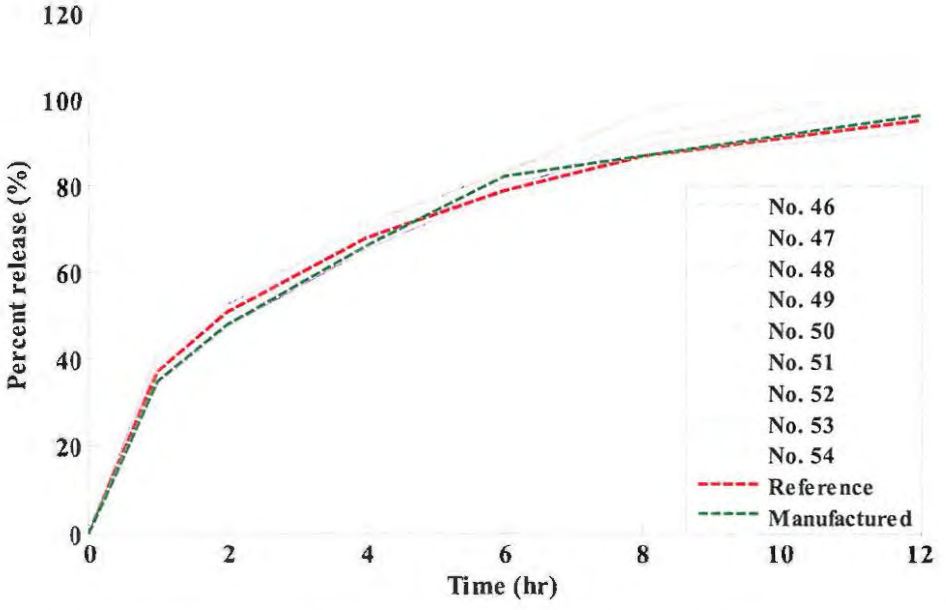


Figure 8.13 Dissolution profile simulation of hypothetical formulations 46–54 compared with the reference and manufactured formulations

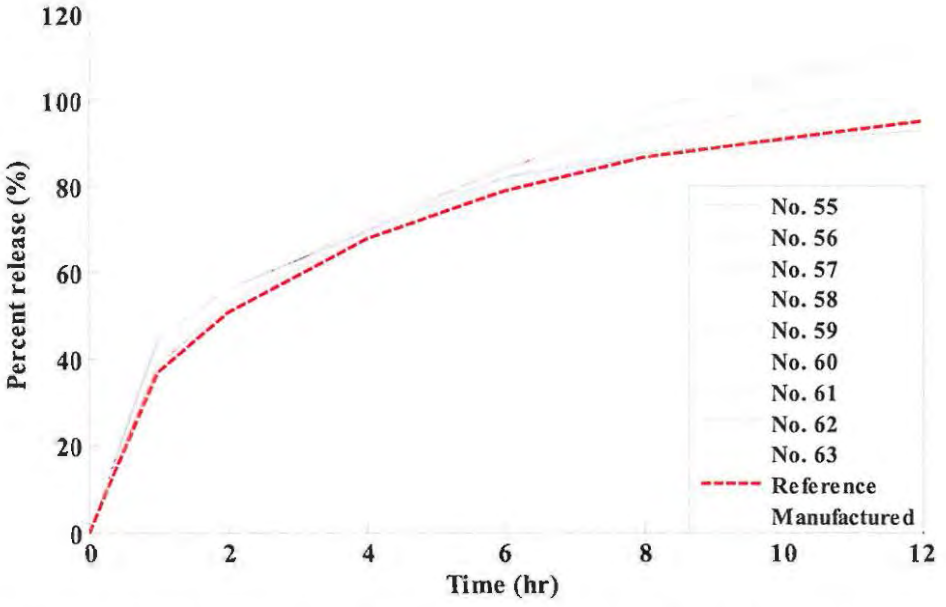


Figure 8.14 Dissolution profile simulation of hypothetical formulations 55–63 compared with the reference and manufactured formulations

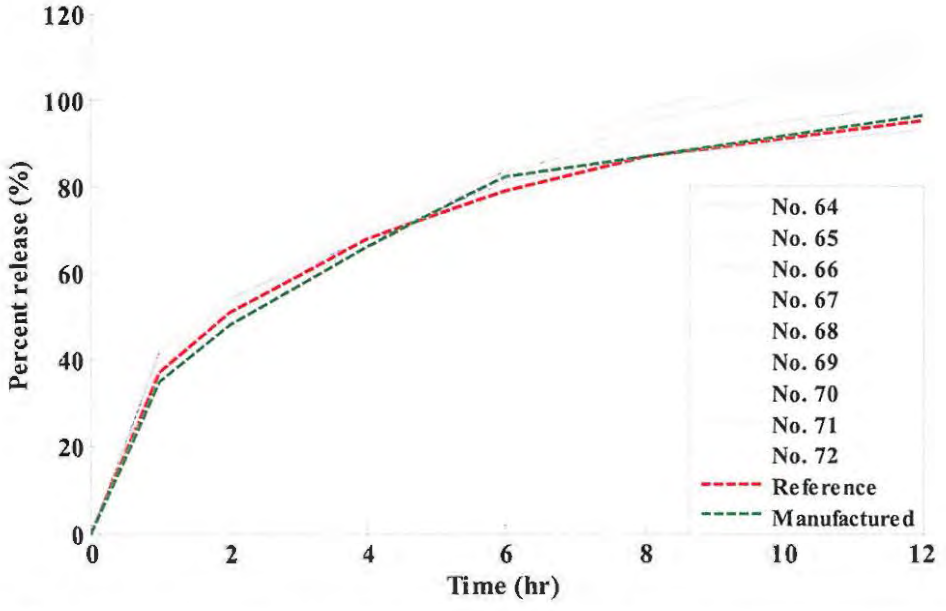


Figure 8.15 Dissolution profile simulation of hypothetical formulations 64–72 compared with the reference and manufactured formulations

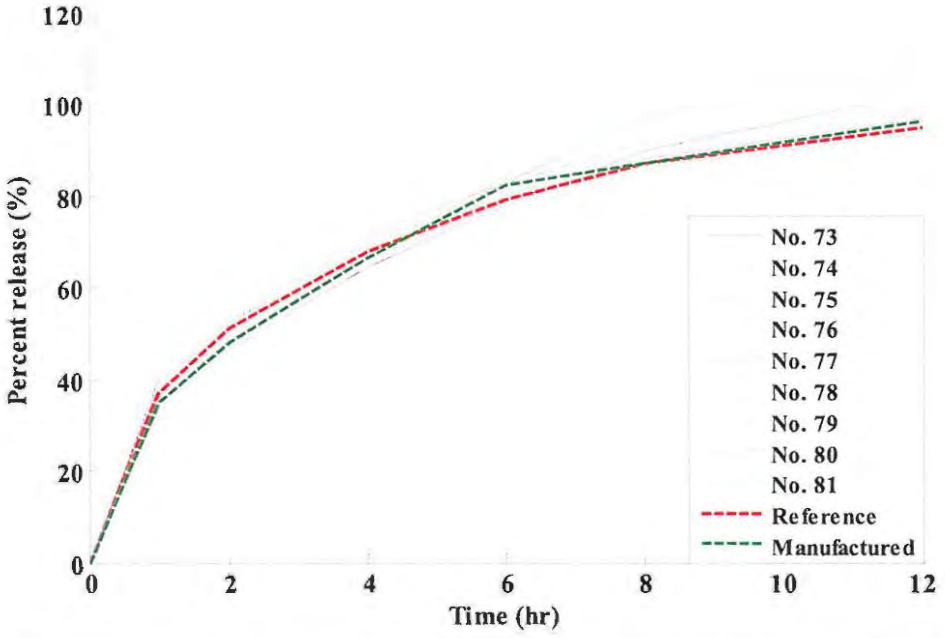


Figure 8.16 Dissolution profile simulation of hypothetical formulations 73–81 compared with the reference and manufactured formulations

It is apparent from the data summarised in Table 8.3 and investigation of the f_2 factor values that dissolution profile simulation of formulations that contain no Carbopol® 974P resulted in release profiles that are not similar to that of the reference formulation. It can therefore be concluded that the inclusion of Carbopol® 974P in the formulations is essential for the production of hydrophilic matrix formulations that will release SBS at a rate and extent that is equivalent to that of the reference formulation.

Furthermore, it is apparent that formulations that contain low levels of Methocel® K100M and xanthan gum, *i.e.* 40 and 25 mg respectively, are generally dissimilar to the target formulation. However, formulations that contain either 45/50 mg and 30/35 mg of Methocel® K100M and xanthan gum, respectively, result in the production of formulations that have dissolution profiles that are similar to that of the reference formulation, provided that the concentrations of Carbopol® 974P and Surelease® that are used in the formulation are also optimised.

The simulated dissolution profiles for all hypothetical formulations that may result in dosage form performance similar to that of the reference formulation are depicted in Figure 8.17.

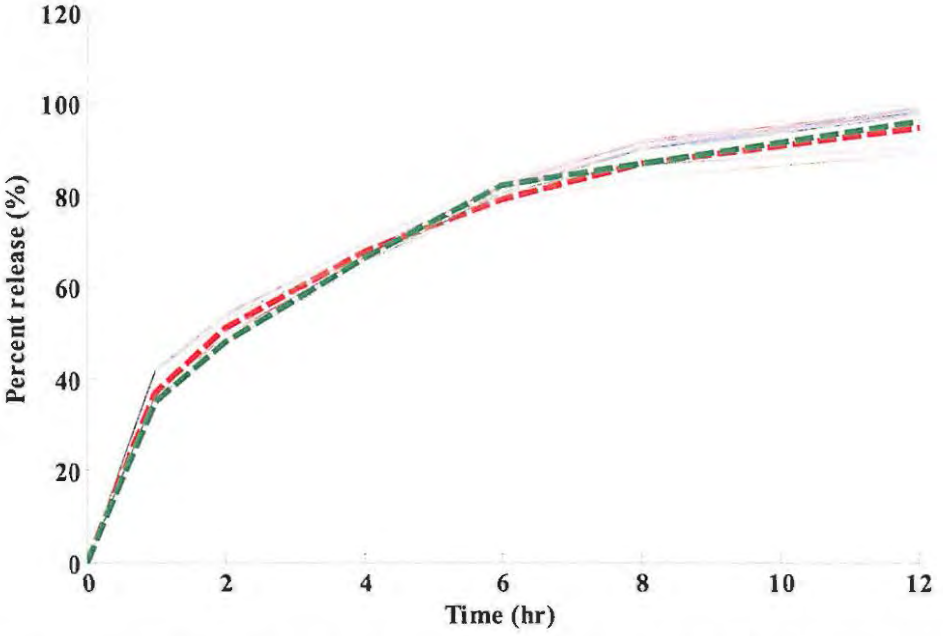


Figure 8.17 Dissolution profile simulations for formulations that are similar to the reference (red line) and manufactured (green line) formulations

8.3.3 Response Surface Methodology *vs.* Artificial Neural Network Methodology for Establishing a Design Space for Hydrophilic Matrix SR Formulations of SBS

The studies were conducted to demonstrate whether RSM and ANN could be used to explore and establish a design space for pharmaceutical product development. Although both approaches have specific advantages that make each methodology potentially useful for establishing a design space, the challenges with respect to using these techniques require further investigation.

The use of experimental design methodologies allows for the generation of mathematical equations that are then used to depict graphical relationships between input factors and output responses that can be used to describe the performance of a specific formulation. This approach provides a mechanistic understanding of relationships between input variables and output responses that may not be identifiable initially, thereby enhancing the understanding of the underlying pharmaceutical principles that impact on formulation performance. This approach has the advantage of allowing a formulation scientist to predict the impact of changes in formulation performance from a sound scientific basis.

However, a major limitation of using an experimental design approach to establish a design space is that, as more information is added to the knowledge base through augmentation

studies, mathematical relationships may shift significantly as the design space becomes more complicated and complex. Clearly this approach has the potential to produce a dynamic design space as the response surface evolves and will in all likelihood necessitate changes to the research strategy as the experimental domain broadens. The use of experimental design is further limited by the fact that realistically the technique is only suitable for studying the impact of no more than five factors at any one time. This is because as the domain expands, mathematical relationships and interactions become more complex, and the information generated becomes more difficult to manage effectively.

The quality attributes of a pharmaceutical product are also determined by processing conditions that are linked, in part, to the levels of formulation composition and the excipients that are used. It is also desirable that the impact of processing conditions on product quality are studied and that a design space which includes the manufacturing process be established. When a RSM is used to establish a design space, it is imperative that at least two separate studies are conducted if both the formulation and manufacturing variables are to be fully understood. However, this may not be possible since the number of variables to be studied increases and therefore the number of experiments to be conducted would increase. Consequently it is difficult to rely solely on a tool such as RSM to explore an extensive design space for pharmaceutical products.

The application of ANN overcomes some of the limitations that may be encountered when using RSM for establishing and exploring a design space for a pharmaceutical product. An advantage of using ANN is that this strategy is an active means of investigating the impact of changing variables on a measured outcome for a product. For instance in the RSM study described in this Chapter, the impact of changing formulation variables within the experimental domain was studied, and it was possible to generate a realistic visual display for the impact of formulation compositions on the ultimate *in vitro* dissolution profiles.

Furthermore, as information is generated through additional studies during the lifecycle of a product, data can be added to the information pool to improve the predictive capability of ANN network models. This does not necessarily result in changes in the visual outcome for the models compared with those generated when RSM is used but will permit a formulation scientist to consider the nature and impact of a specific outcome of a proposed change to a formulation as and when they are made.

Due to the manner in which ANN deal with the information presented to the network, and because ANN can achieve pattern recognition, the impact of process factors and formulation variables on CQA can be studied simultaneously, without information being discarded or becoming unmanageable. This is also due to ANN's ability to deal with a large number of input factors at any one time in order to ensure that accurate predictions are achieved. Furthermore, additional information can be added to an ANN following initial model parameter allocation; meaningful conclusions can be drawn from the additional data studied, which is an important advantage of using ANN models to evaluate data during product lifecycle management.

However, the use of ANN methodology does not permit a mechanistic understanding of the scientific principles of a specific formulation composition to be elucidated for a formulation scientist. This disadvantage means that there are likely to be instances where a mechanistic understanding of formulations and intuition are important for the effective optimisation of a dosage form. Furthermore, no clear relationships can be drawn between input variables and output factors when using ANN, thereby limiting their use for formulation and manufacturing process definition.

Another limitation of using ANN is that the networks do not necessarily produce a graphical output which permits visual mapping of a design space, and therefore it is conceptually difficult to visualise a design space without the use of ANN software. Therefore human resource training in the use of ANN methodologies and software use is essential when attempting to use these approaches for the purposes of establishing a design space for formulation optimisation strategies.

8.4 CONCLUSIONS

One of the key challenges to developing appropriate design space(s) is that this concept is relatively new and is not well understood, and therefore there is wide scope for interpretation of the data generated using these concepts.

Several methods, including an experimental design approach such as central composite design and the Box–Behnken strategy, and multivariate methods such as SPC, PLS and PCA, have been explored for their utility in identifying and defining a design space for

pharmaceutical formulations. Design spaces have been presented as interactive contour and response surface plots in the past.

In this study, RSM methodology and constraint plots generated from statistical optimisation processes were used to establish the design space limits for formulation variables that were investigated. In addition, the use of ANN simulations was investigated to establish the potential for this technique to establish a design space for a pharmaceutical formulation.

The results reveal that both methods can be used to define a design space and that the use of RSM methodologies is also able to produce graphical outcomes. However, simulations using ANN produce an outcome that represents the overall effect of all variables on the simulated dissolution profiles. Recommendations for establishing design spaces are discussed in Chapter 8 *vide infra*.

CHAPTER 9
CONCLUSIONS AND RECOMMENDATIONS

9.1 CONCLUSIONS

QbD is a systematic approach for the development of quality pharmaceutical dosage forms that begins with predefined objectives based on the premise that quality must be built into a product and not tested into it. With respect to formulations, quality products are products that ensure consistent dosage form performance and that are designed to achieve specific therapeutic outcomes with a low risk of failure in patients.

The ICH [1] has recommended the use of multivariate experiments such as RSM to understand the impact of formulation and process variables on product quality and for the optimisation of pharmaceutical formulations. An important aspect of QbD is the establishment of a design space which is a multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide an assurance of quality for a specific dosage form. The overall objective of this research was to investigate the feasibility of using RSM and ANN for the optimisation of a SR hydrophilic matrix formulation of SBS that had desirable dissolution test characteristics and for the establishment of a design space for that formulation.

SBS is a short-acting β_2 agonist that is used for the alleviation of bronchoconstriction and bronchospasm in patients with reversible obstructive airway disease and COPD. The use of SR formulations of SBS may result in clinical benefits compared with the administration of SBS in conventional and/or immediate release dosage forms. SR formulations of SBS have been manufactured using lipidic and hydrophilic matrices, acrylic acid based resins and novel delivery systems including the use of osmotic pumps and microcapsules.

A sensitive, accurate, and precise HPLC method for the quantitation of SBS in pharmaceutical dosage forms and for assessing *in vitro* release was developed and validated according to ICH guideline Q2 (R1) [120]. The HPLC method was found to be linear over the concentration range 0–50 μg/ml and precise with % RSD values that were <5%. The

method was also found to be accurate with a % Bias that was determined to be <5% in the range that was studied.

Hydrophilic monolithic matrix devices are commonly used as SR dosage forms due to their ease of manufacture and the nature of this well understood technology. HPMC is one of the most commonly used hydrophilic matrix forming polymers for SR formulations as it is non-toxic and has wide regulatory acceptance. There is a wealth of information on the characterisation of HPMC as a SR matrix material, and it was therefore chosen as the primary matrix polymer for developing an optimised dosage form and for investigating the concept of a design space. The addition of polymeric adjuvants to HPMC matrices may be used to modulate both the rate and mechanism of drug release and was therefore investigated as a potential strategy for formulation development. The results demonstrated the potential use of xanthan gum and Carbopol® polymers as matrix polymers and therefore combinations of HPMC, Carbopol® 974P and xanthan gum in varying proportions were thought to be potentially useful for the retardation of SBS release. These polymers were therefore used in a statistically designed experiment for the optimisation of a formulation with release characteristics that would be similar to that of a reference formulation, Asthalin®8 ER (Cipla Ltd., Mumbai, Maharashtra, India).

When conducting statistically designed experiments, external factors, which are not necessarily investigated in a study but have the potential to impact the measured outcomes of that study, must be controlled. An Ishikawa or Cause and Effect diagram was used to determine these factors and to ensure that the manufacturing method and the analysis of dosage forms would produce reproducible and reliable results.

A central composite design was selected to optimise a SBS formulation. The composition of Methocel® K100, xanthan gum, Carbopol® 974P and Surelease® were selected as variables to establish how these may impact tablet crushing strength, *in vitro* dissolution and both the rate and mechanism of drug release from these formulations. Response surface and contour plots were used to examine the nature of the relationships between the levels of input factors and the measured responses.

The results revealed that incorporation of xanthan gum and Carbopol® 974P had an impact on tablet crushing strength and that using high levels of xanthan gum in tablet formulations

resulted in the production of soft tablets. In general Methocel® K100M and xanthan gum had the greatest impact on the extent of drug release at all stages of dissolution testing, and increasing the content of these polymers in formulations results in retardation of drug release. The content of Carbopol® 974P and Surelease® had less of an impact on drug release, although interactions between formulation variables were important in controlling the extent of SBS release. Similarly Methocel® K100M and xanthan gum had the greatest impact on the overall mechanism of SBS release from the formulations tested, and the results revealed that drug release from the formulations is primarily diffusion controlled.

The Calibration Generation Browser in Matlab was used to determine the composition of an optimised formulation that would have the appropriate physical characteristics and *in vitro* release profile. The f_2 similarity factor was used to compare the dissolution profile of the predicted formulation to that of the reference formulation, and a value of $f_2 = 89.7$ was generated indicating that the predicted *in vitro* release profile would be similar to that of the reference formulation. The f_2 similarity factor of a formulation manufactured according to the composition from the optimisation process compared with the reference formulation was 83.0, thereby indicating that statistical optimisation was successfully applied to the development of an optimised delivery system for SBS.

Matlab® R2008a was used to write code for the training and evaluation of a neural network. Formulation variables and *in vitro* dissolution profiles from the central composite study were used for training, testing and validating the network models that were developed. A three layer MLP with nine nodes in the hidden layer was found to have the highest predictive ability and was therefore deemed suitable for the simulation of dissolution profiles for formulation optimisation and establishment of a design space.

A brute force method was used to generate permutations for simulation using the ANN model, and the combination of formulation variables that resulted in the highest f_2 value was selected for further evaluation. The predicted formulation resulted in an f_2 value of 90.5. The manufactured formulation was found to perform satisfactorily and an f_2 value of 86.0 was calculated, clearly indicating the dissolution profile was indeed similar to that of the reference formulation.

The optimisation outputs from RSM and ANN are summarised in Table 9.1.

Table 9.1 Comparison of optimised formulation compositions derived from RSM and ANN

Formulation	RSM	ANN
Methocel® K100M (mg)	29.77	45
Xanthan gum (mg)	35.31	30
Carbopol® 974P (mg)	10.01	5
Surelease® (% w/w)	18.5	10

The results indicate that the two procedures resulted in different compositions for optimised formulations and demonstrate that the optimisation procedure that is used when developing formulations has the potential to impact the outcome of these experiments. The optimisation method that was used was a Normal Boundary Intersection method for multi-objective optimisation, whereas randomised weighting procedures through iterations are used for pattern recognition when training ANN. The two methods therefore do not result in the generation of a similar formulation composition. Although the formulation compositions, which are used to produce a formulation with the release characteristics similar to the reference product, are different both approaches result in the production of formulations with similar dissolution profiles under the conditions used in this research. The resultant dissolution profiles are shown in Figure 9.1.

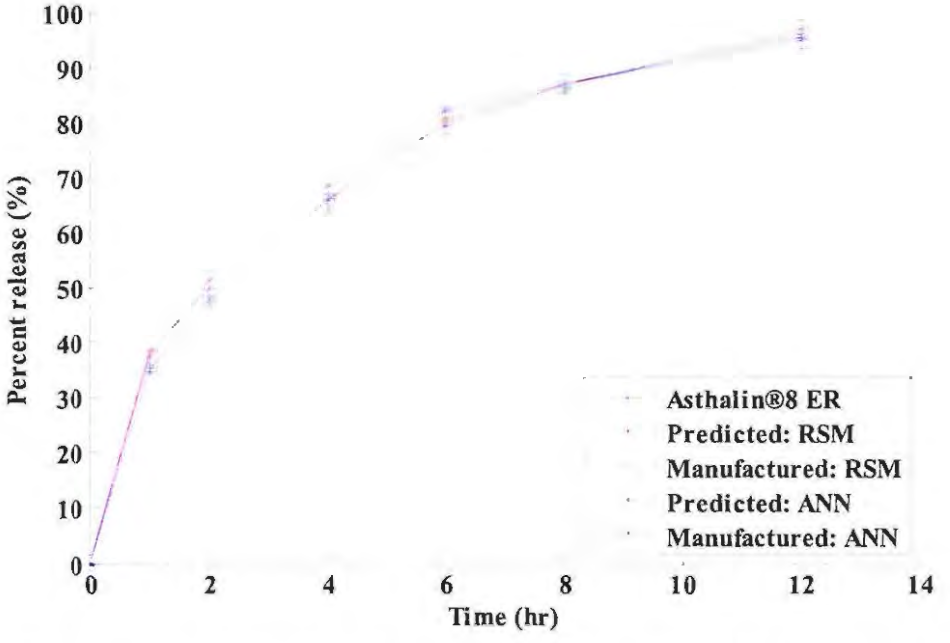


Figure 9.1 Comparison of formulations predicted and manufactured from RSM and ANN compared with the reference formulation

ANN are particularly useful for studying systems where there is little known information about the relationship between causal and response factors whereas such information needs to be explicitly expressed when using statistical optimisation techniques [211,214]. The RSM data was used to train an ANN model that had a good predictive capability for this research.

It has been reported that ANN give better predictions to solutions than RSM [209], although when RSM analysis is used the surface is described by a continuous function, and a visual representation of the relationship between the input variables and outputs can be easily mapped [209]. When using RSM the relationship between an input variable and the rate of change of a response may be easily determined by plotting the first derivative of the response as a function of the input variable [209]. The output for ANN is a discrete set of points, and extensive simulations are required to visualise the relationship between an input variable and output. In addition, in order to evaluate the rate of change of a response with respect to that of an input variable for an ANN model, numerical differentiation of the input variables is required [209].

It has also been reported that the application of ANN models results in the calculation of smaller error values between predicted and experimentally observed responses compared with those generated when using RSM analysis [209]. However from the data generated in this research, RSM and ANN produced satisfactory results.

ANN have been shown to model non linear relationships better than classical RSM [245] since non-linear functions are typically difficult to describe using classic polynomial regression equations. This statement is based on the low value for correlation coefficients generated when using classical RSM to model non-linear data patterns [245]. However, RSM is reported to be more robust than ANN when dealing with outliers and this must be considered when developing ANN models [246].

It is clearly evident that SR formulations are inherently complex systems for which the performance is affected by the type and levels of polymer combinations that are used to manufacture the formulations. Although the research did not focus on the impact of manufacturing variables of dosage form characteristics, the impact of processing conditions of product quality cannot be overlooked. It is therefore clear from these observations that the design and development of SR systems and dosage forms is a multivariate optimisation

process and is very challenging. The applications of statistical approaches such as RSM that is based on polynomial regression can be used to obtain a mechanistic understanding of the complex relationships between formulation variables and measurable characteristics of dosage form performance.

The impact of four formulation variables on the *in vitro* release profile of SBS using a central composite design was investigated. However, 30 experiments are required to completely study the experimental domain and as the number of variables to be studied increases, the number of experiments becomes unmanageable and therefore unrealistic to evaluate all factors entirely. In addition, as the number of input variables increases, the number of coefficients for the polynomial equation increase and thereby limiting the application of polynomial models for multi-objective optimisation that is necessary when developing SR formulations. In general, RSM works well for the low dimensionality or for approximating simple functions and is only be practically suited to one dependent variable or a low order polynomial such as second order polynomials. When conducting multi-objective optimisation, an appropriate response surface model for each response variable that is being studied needs to be built and a set of independent variables that optimise all the responses or keep them in the desired ranges need to be obtained. This makes the application of RSM for formulation optimisation very cumbersome.

The application of ANN for formulation development overcomes these concerns and therefore makes them more suitable for multi-objective optimisation. They are particularly useful in incidences when the functional relationships or dependence between input and output factors is not clear. A well trained ANN model can handle multiple independent and dependent variables simultaneously in one model thereby negating the need for multiple polynomial models. For instance, in the current study, it was possible to construct an ANN model that was useful for predicting the impact of the four studied formulation variables on the extent of *in vitro* release dissolution at all stages of the dissolution test. This is in comparison to the RSM model that was developed, where a separate polynomial model was required for each response variable. This made the ANN less cumbersome to use in comparison to the RSM model although the disadvantage was that it was not possible to infer interactions between input factors from the ANN model that was constructed. When ANN models are used to depict relationships between input and output factors, it is also not easy to obtain a graphical output of the experimental domain without the use of complicated

software. A primary advantage of ANN for developing formulations is that ANN can be uploaded with new data that will not necessarily affect the model, but will enhance the quality of predictions that can be made from ANN models. It is also possible to increase the efficiency of ANN models when dealing with multi-dimensional problems because searching algorithms such as genetic algorithms may be integrated in the model and augment the predictive ability of ANN models.

It was established that the optimisation of a SR formulation was possible using both RSM and ANN and the selection of either method depends on the expertise available to researchers and formulators although previous studies have shown better predictive ability of ANN compared to RSM.

RSM and ANN were further used to establish a design space for the formulation that would result in a product with desirable *in vitro* release characteristics that was similar to that of the reference formulation. The impact of the levels of Methocel® K100M, xanthan gum, Carbopol® 974P and Surelease® on the extent of drug release at all stages of dissolution testing was determined from the resultant constraint plots generated from the RSM procedures. The results revealed that the target formulation could be obtained within certain limits of each formulation variable to ensure the production of a matrix formulation with the desired dissolution profile.

The impact of varying formulation composition within the limits of ± 5 mg for solid polymers and ± 5% w/w for the composition of the liquid dispersion used to granulate the formulation compared with the optimised formulation was used to simulate resultant dissolution profiles using ANN. This is useful for establishing which compositions would result in the generation of formulations with similar performance to that of the reference formulation. Using this approach it was possible to simulate the impact of varying the composition of the formulation on the resultant dissolution profiles.

The limitations of both these methods have resulted in the recommendation that perhaps these methods should be used in combination to explore a design space effectively. However this does not appear possible since the compositions that were generated from the optimisation procedure were not similar (Table 9.1).

9.2 RECOMMENDATIONS

9.2.1 Overview

The QbD system integrates product development and process design, with risk assessment and control, which are considered to be crucial for the development of quality pharmaceutical products. The interrelationship between these elements is summarised in Figure 9.2.

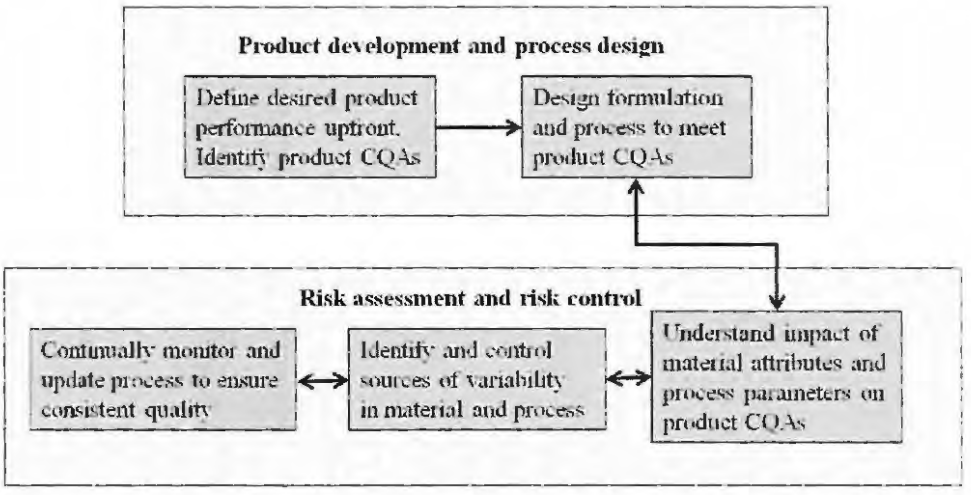


Figure 9.2 Interrelationship between product development and process design with risk assessment and risk control, adapted and redrawn from [10]

The ICH Q8 (R2) Pharmaceutical Development [1], ICH Q9 Quality Risk Management [2] and ICH Q10 Pharmaceutical Quality System [3] guidelines form the ICH Harmonised Tripartite Guidelines which direct QbD. The interrelationship between the guidelines and how they ultimately impact product quality is shown in Figure 9.3.

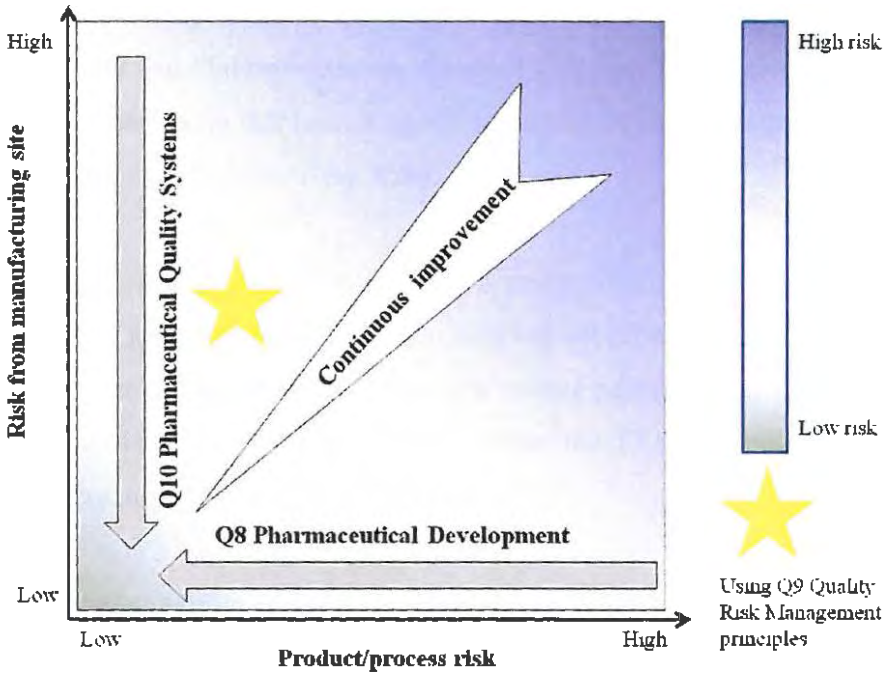


Figure 9.3 Relationship between pharmaceutical development, quality risk management and development of pharmaceutical quality systems, adapted and redrawn from J. Ramsbotham [160,247]

The application of Pharmaceutical Development principles and Pharmacy Quality Systems may result in a decreased risk to patients that may otherwise occur as consequence of product and processing variability and manufacturing site, respectively.

The Pharmaceutical Development guideline places an emphasis on the application of pharmaceutical and manufacturing sciences to facilitate the production of high quality pharmaceutical products [1]. As such, an extensive understanding of the formulation variables and manufacturing processes that impact product quality is essential [8] and may be enhanced by use of RSM and ANN models as completed in the current study. However the potential impact of the ICH Q9 [2] and the ICH Q10 [3] guidelines and how this can be used with RSM and ANN must also be considered.

9.2.2 Pharmaceutical Development Guideline

Preliminary studies are an integral part of formulation development and provide a mechanistic understanding of the variables under investigation and a database from which a formulator may continually draw in future research. Conducting carefully designed screening studies may also identify variables that do not significantly affect measured responses and these may be eliminated from future optimisation work, thereby potentially increasing

efficiency and saving valuable and costly resources. Screening studies such as two-level factorial designs and Plackett–Burman designs [181] may be suitable for preliminary studies to establish those factors that have a significant effect on the measured responses and that are useful for future optimisation using RSM.

The RSM approach described in Chapter 6 revealed that there are potential interactions between certain formulation variables that may impact physical quality characteristics such as tablet crushing strength and affect the *in vitro* release performance. Tools such as Differential Scanning Calorimetry (DSC) and X-ray Diffraction (XRD) would be useful to identify potential interactions between excipients and API.

The quality target profile of an API encompasses physical quality and *in vitro* and *in vivo* dosage form performance characteristics. Although the current study has only focused on the *in vitro* dissolution profile of a reference product as the primary CQA, other elements including tablet crushing strength, friability *etc.* must also be considered in formulation development studies. In order to study the impact of input variables on the CQA, RSM may be used to gain a mechanistic understanding of these factors. However, as the number of measurable attributes increases, the information may become unmanageable as the number of models that are developed increases. It may therefore become difficult to contextualise the information in order to understand the impact of variables on measurable responses. The use of ANN for expanding the design space may become suitable as more information is generated since the output from an appropriately trained ANN is linked to all measured responses.

Formulation development strategies that use tools such as RSM and ANN provide invaluable information regarding the complex relationship between formulation and/or process factors and how these may impact predetermined CQA. The result of this research demonstrates that it is possible to gain a conceptual understanding of the inter-relationships between formulation factors and the *in vitro* dissolution profile. Therefore this information serves a platform for controlling those factors that may be deemed to be high-risk factors and ensuring that such conditions are always met when developing pharmaceutical formulations. The use of ANN as a tool for understanding the impact of formulation changes on dosage form performance can be used to perform iterations on the potential impact of the criticality of

formulation and/or process factors on dosage form performance. In this way, this research serves to provide a potential platform for the QbD process.

The design space for a pharmaceutical product is best described by three interacting spaces, *viz.*, the raw material property space, CPP (Critical Process Parameters) space and CQA spaces. It is imperative that the design and control spaces are carefully controlled and managed since any changes in at least one of these domains may adversely affect the other domain spaces. As such, when there are changes in any one of these spaces, the impact on overall product quality must be addressed or managed. Strategies to control operating and/or process variables must be established when defining a design space [227]. It is vitally important that operating and/or control strategies for pharmaceutical processes are defined prior to defining additional design spaces since changes in procedures have the potential to dramatically alter and affect ultimate product quality and performance [227].

The quality attributes of a pharmaceutical product are also determined by processing conditions that are linked, in part, to the levels of formulation composition and the excipients that are used. It is also desirable that the impact of processing conditions on product quality are studied and that a design space that includes the manufacturing process must be established. Although the focus of the research was limited to understanding the impact of formulation variables on *in vitro* release performance, process information may be added at a later stage to an ANN model to enhance understanding of the design space.

The impact of formulation variables on the *in vitro* dissolution profile, specifically that of the 1000-tablet batches manufactured were the focus of this work. The ICH Q8 guideline [1] recommends that, when describing a design space for a pharmaceutical formulation, the size of the scale must be reported in the application for regulatory approval. The relationship between a design space at small or pilot scale to that observed at production scale during the manufacturing process must be discussed, and potential risk to product quality must be appropriately determined. It may often therefore be desirable that the design space should be applicable to multiple operational scales. Although the flexibility of the design space with respect to scale-up was not assessed, the use of relevant scale-independent parameters may alleviate the risks that are usually associated with scale up. This may often be the case with manufacturing processes such as wet granulation procedures that are affected by batch sizes, and the use of a shear rate and other dimensionless parameters for scale-up may therefore be

included in the determination of a design space [1]. In general, semi-empirical approaches, based on the application of sound pharmaceutical and manufacturing sciences to determine the relationships between operating conditions when different scales or pieces of equipment are used, may be necessary when establishing a design space for a pharmaceutical product [10].

To overcome the limitations of RSM and ANN when establishing a design space for a pharmaceutical product it may be possible to use the different methods in combination. In this manner, the use of RSM will permit the development of a mechanistic understanding of the underlying pharmaceutical principles that apply to a specific formulation and will generate graphical outcomes that can be used to enhance understanding of the design space that is established. ANN can be used to evaluate the impact of formulation changes at the limits of the design space using simulation models to evaluate the impact of formulation composition and manufacturing conditions on CQA.

A recommendation is that RSM and ANN be used in combination to develop an effective and applicable pharmaceutical design space. However this approach may not be feasible for all dosage forms as it is evident from these outcomes that optimisation using the same data set generated from a central composite design (Chapter 6) resulted in different sets of outcomes when the data was evaluated using RSM and ANN. Therefore the use of the information from RSM and ANN must be thoroughly interrogated in order to prevent misinterpretation of the resultant data that is generated.

Recommendations for developing a design space for pharmaceutical product also include considering independent variations from the manufacturing process that are likely to impact product quality. This would allow setting specifications for operating variables to conditions that have been demonstrated to provide an assurance of quality.

9.2.3 Quality Risk Management

The ICH Q9 guideline [2] provides a systematic approach to pharmaceutical quality risk management and facilitates the assessment and control of risk in those areas of pharmaceutical development that are likely to impact product quality. Quality risk management is based on scientific knowledge with the core objective of protecting the patient

by ensuring product performance, safety and efficacy and minimising the level of risk that is associated with pharmaceutical formulations or processes [248].

Risk assessment tools are an important aspect of optimising pharmaceutical formulations and processes. Various tools including FMEA, FTA, HACCP (Failure Mode Effects Analysis, Fault Tree Analysis, Hazard Analysis and Critical Control Points) and Cause and Effect (Ishikawa) diagrams may be used for this purpose. Understanding the scope of information that can be generated from these tools may be important in assessing which method will result in usable information. In this way, potential sources of risk are fully understood, managed or eliminated as sources of variability that may impact product quality.

A recommendation for the Quality Risk Management process is that risk assessment tools must be used to identify and rank the variables and control parameters that are related to both manufacturing and formulation variables and how these are likely to impact product quality. These variables and control parameters must therefore be monitored and controlled when developing and optimising pharmaceutical dosage forms. The use of experimental design and ANN may be used for evaluating these aspects of control and used to enhance understanding of the criticality of these factors and therefore useful to ensure the production of safe and effective dosage forms. The risk assessment process must also be linked to the established design space to ensure that process and formulations variables will ensure that the design space is always maintained. Another potential area for consideration when establishing a design space is the impact of different sources of excipients on product quality, which is an important variable when working with polymers.

9.2.4 Product Lifecycle

The ICH Q10 guideline [3] describes the development of a model that assures product quality throughout the lifecycle of that product and promotes continual improvement in product and manufacturing processes that assure that there is minimal risk on product quality [13]. This allows for the development of quality control strategies that are useful in monitoring and controlling quality [248]. Moreover this guideline complements and facilitates the implementation of the Pharmaceutical Development [1] and Quality Risk Management [2] guidelines.

As information is generated through additional studies during the lifecycle of a product, data can be added to the information pool to improve the predictive capability of an ANN network model, thereby increasing both the experimental domain and the understanding of the design space.

This research has demonstrated the potential utility of RSM and ANN as tools for developing quality pharmaceutical formulations and that the establishment and presentation of a design space may be conducted using a combination of both these methods.

APPENDIX 1

SAMPLE BATCH MANUFACTURING RECORD

Only one of each direct compression and one wet granulation batch manufacturing record templates, indicating the quantities of polymers that were used, procedures that were followed and information that was recorded, are included in the thesis. The records for all formulation batches that were manufactured are available on request.

Rhodes University
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Department of Pharmaceutics
Grahamstown 6140

BATCH MANUFACTURING RECORD DIRECT COMPRESSION

Date of manufacture	
Temperature and humidity	
Formulator	Faith Chaibva
Product	9.6 mg salbutamol sulphate matrix tablets
Batch number	SBS-01
Batch Size	1000 tablets
Target weight	140 mg

MANUFACTURING DIRECTIONS

Step	Procedure	Formula	Weight	Time	Done	Check
1	Separately screen the following materials through a 20 mesh screen					
	Salbutamol sulphate	9.6 g				
	Methocel® K100M	70 g				
	Avicel® PH101	58.3 g				
2	Place the weighed powders in the Saral® Rapid mixer and granulator and blend at 100 rpm for 15 min					
3	Separately screen the following materials using a 44 mesh screen					
	Colloidal silicone dioxide	0.7 g				
	Magnesium stearate	1.4 g				
4	Add the above to the blend and mix for a further 3 min at the same speed					
5	Compress the powder blend using a Manesty® F3 single punch tablet press to produce tablets of the desired weight					

OBSERVATIONS

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Faculty of Pharmacy
Department of Pharmaceutics
Grahamstown 6140

BATCH MANUFACTURING RECORD WET GRANULATION

Date of manufacture	
Temperature and humidity	
Formulator	Faith Chaibva
Product	9.6 mg salbutamol sulphate SR matrix tablets
Batch number	SAL001CCD
Batch Size	1000 tablets
Target weight	220 mg
Granulating fluid	12% Surelease®

MANUFACTURING DIRECTIONS

Step	Procedure					
1	Separately screen the following materials through a 20 mesh screen					
		Formula	Weight	Time	Done	Check
	Salbutamol sulphate	9.6 g				
	Methocel® K100M	120 g				
	Xanthan gum	50 g				
	Carbopol® 974P NF	10 g				
	Avicel® PH101	12.7 g				
2	Place them in the Saral® Rapid mixer and granulator and blend at 100 rpm for 15 min					
3	Set the blade to 120 rpm and chopper to 1000 rpm and add 120 g of granulating fluid					
4	Weight of container before Weight of container after Amount of granulating fluid added Time taken to add fluid Current measured	Impeller Chopper				
5	Continue to mix granules for a further 5 min					
6	Remove granules from the mixer and weigh them before drying Weight of wet granules					
7	Dry the granules at room temperature for 24 hr					
8	Weight of dried granules					
9	Weight of granules used					
10	Separately screen the following materials using a 44 mesh screen and add to the blend and mix for a further 3 min at 100 rpm Cab-O-Sil® M5 (0.5% w/w) Magnesium stearate (1% w/w)					
11	Compress the granules blend using a Manesty® B3B rotary tablet press to produce tablets of the desired weight					

OBSERVATIONS

APPENDIX 2

SAMPLE BATCH RECORDS SUMMARY

Only one of each direct compression and one wet granulation batch record summary, indicating the quantities of polymers that were used, physical tests that were conducted and any other information that was recorded are included in the thesis. The records for all formulation batches that were manufactured are available on request.

Rhodes University
Faculty of Pharmacy
Department of Pharmaceutics
Grahamstown 6140

RHODES

BATCH RECORD SUMMARY

Date of manufacture	1 May 2008
Formulator	Faith Chaibva
Product	9.6 mg SBS SR matrix tablets
Batch number	SBS-01
Batch Size	1000 tablets
Target weight	140 mg

FORMULATION

	Formula	Working formula	Rhodes batch number
Salbutamol sulphate	9.6 mg	9.6 g	RM000188
Methocel® K100M	50% w/w	70 g	RM000062
Colloidal silica	0.5% w/w	0.7 g	X052098
Magnesium stearate	1.0% w/w	1.4 g	X052015
Avicel® PH101 *qs*	100% w/w	140 g	RM000087

PHYSICAL ASSESSMENT

	Mean ± SD
Assay (mg)	9.64 ± 0.09
Weight (mg)	141.01 ± 2.53
Crushing strength (N)	81.82 ± 4.41
Thickness (mm)	2.99 ± 0.06
Diameter (mm)	7.16 ± 0.01

IN VITRO RELEASE

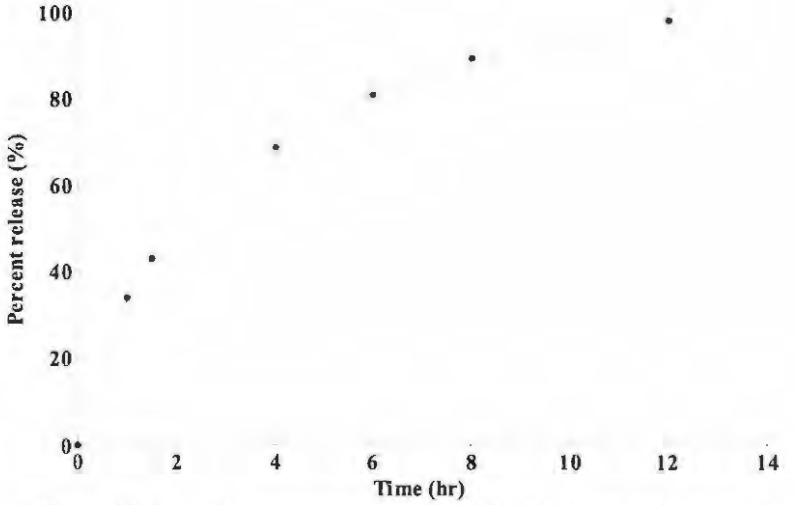

Figure 10.1 In vitro release profile of SBS-01 over a 12 hr period

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Grahamstown 6140

BATCH RECORD SUMMARY

Date of manufacture	5 November 2008
Formulator	Faith Chaibva
Product	9.6 mg SBS SR matrix tablets
Batch number	SAL001CCD
Batch Size	1000 tablets
Target weight	220 mg
Granulating fluid	12% w/w Surelease®

FORMULATION

	Formula	Rhodes batch number
Salbutamol sulphate	9.6 g	RM000188
Methocel® K100M	120 g	RM000062
Xanthan gum	50 g	RM000063
Carbopol® 974P	10 g	RM000121
Avicel® PH101 *qs*	12.7 g	RM000087
Colloidal silica	0.5% w/w	X052098
Magnesium stearate	1.0% w/w	X052015

GRANULE ASSESSMENT

Granule characteristics	Mean ± SD
Bulk density (g/cm^3)	9.34 ± 0.36
Tap density (g/cm^3)	221.25 ± 1.07
Carr's Index (%)	33.27 ± 2.13
Angle of repose (°)	4.93 ± 0.08

PHYSICAL ASSESSMENT

Tablet characteristics	Mean ± SD
Assay (mg)	9.34 ± 0.36
Weight (mg)	221.25 ± 1.07
Crushing strength (N)	33.27 ± 2.13
Thickness (mm)	4.93 ± 0.08
Diameter (mm)	8.73 ± 0.20

IN VITRO RELEASE

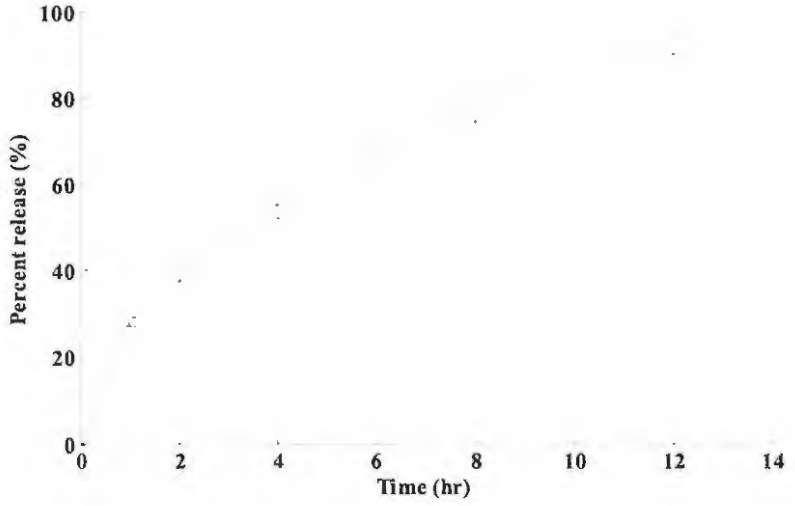

Figure 10.2 *In vitro* release profile of SAL001 over a 12 hr period

APPENDIX 3

RESPONSE SURFACE METHODOLOGY STATISTICS

11.1 TABLET CRUSHING STRENGTH

Table 11.1 Model fit comparison for tablet crushing strength

Model	PRESS	RMSE
Linear	8.8513	8.2691
Quadratic	7.6350	6.6676

Table 11.2 Stepwise regression results quadratic model for tablet crushing strength

Coefficient	Value	Standard deviation	t	Significance
Intercept	**27.47**	**1.571**	**17.48**	**Significant**
x_1 – **Methocel® K100M**	-3.3236	2.6940	-1.2337	Not significant
x_2 – **Xanthan gum**	**-23.26**	**2.7220**	**-8.5445**	**Significant**
x_3 – **Carbopol® 974P**	**-4.5514**	**2.7220**	**-1.6721**	**Significant**
x_4 – **Surelease**	-2.8431	2.7169	-1.0464	Not significant
x_1^2	-2.9547	5.0680	-0.5830	Not significant
x_1x_2	-9.0125	6.5517	-1.3756	Not significant
x_1x_3	5.1625	6.7230	0.7679	Not significant
x_1x_4	4.2792	6.7488	0.6341	Not significant
x_2^2	**15.87**	**4.9697**	**3.1941**	**Significant**
x_2x_3	**16.77**	**6.6676**	**2.5153**	**Significant**
x_2x_4	5.4708	6.7128	0.8150	Not significant
x_3^2	3.5328	5.05526	0.6992	Not significant
x_3x_4	-2.3042	6.7888	-0.3394	Not significant
x_4^2	-0.05781	5.1038	-0.01133	Not significant

Table 11.3 ANOVA summary table

Source	SS	df	MS
Regression	4105.189	4	1026.297
Error	1111.41	25	44.456
Total	5216.598	29	0

$$y_H = 27.5 - 23.3x_2 - 4.55x_3 + 15.9x_2^2 + 16.8x_2x_3 \qquad \textbf{Equation 11.1}$$

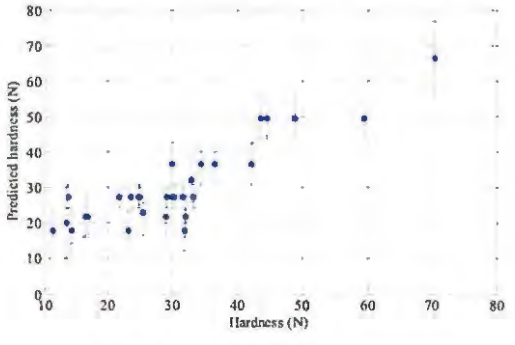

Figure 11.1 Predicted *vs.* observed

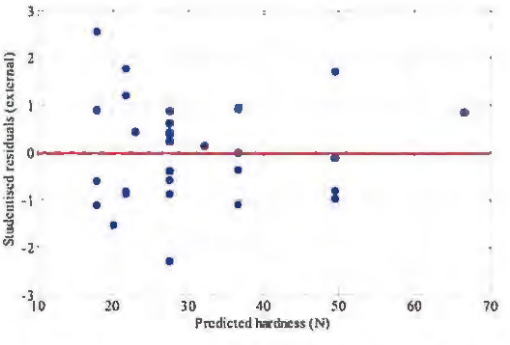

Figure 11.2 Studentised residuals *vs.* observed

11.2 PERCENT RELEASE AFTER 1 HR

Table 11.4 Model fit comparison for percent drug released after 1 hr

Model	PRESS	RMSE
Linear	4.8646	4.4295
Quadratic	4.0944	3.0088

Table 11.5 Stepwise regression results quadratic model for percent release after 1 hr

Coefficient	Value	Standard deviation	*T*	Significance
Intercept	**30.50**	**0.8686**	**32.11**	**Significant**
x_1 – Methocel® K100M	**-7.6720**	**1.2283**	**-6.2459**	**Significant**
x_2 – Xanthan gum	**-8.4306**	**1.2283**	**-6.8635**	**Significant**
x_3 – Carbopol® 974P	0.1926	1.2553	0.1535	Not significant
x_4 – Surelease	-1.2723	1.2263	-1.0375	Not significant
x_1^2	2.5455	2.2613	1.1257	Not significant
x_1x_2	**9.5520**	**3.0088**	**3.1747**	**Significant**
x_1x_3	-3.2283	2.9984	-1.0767	Not significant
x_1x_4	-0.3566	3.0754	-0.1160	Not significant
x_2^2	**9.7755**	**2.2566**	**4.3320**	**Significant**
x_2x_3	**-5.6587**	**3.0088**	**-1.8807**	**Significant**
x_2x_4	-0.7667	3.0720	0.2496	Not significant
x_3^2	**-2.8861**	**2.2566**	**-1.2790**	**Significant**
x_3x_4	2.2365	3.0392	0.7359	Not significant
x_4^2	0.4233	2.3238	0.1822	Not significant

Table 11.6 ANOVA summary table

Source	SS	df	MS
Regression	1101.156	6	183.526
Error	208.21	23	9.053
Total	1309.366	29	0

$$y_{1h} = 30.5 - 7.67x_1 - 8.43x_2 + 9.55x_1x_2 + 9.78x_2^2 - 5.66x_2x_3 - 2.88x_2^3 \quad \textbf{Equation 11.2}$$

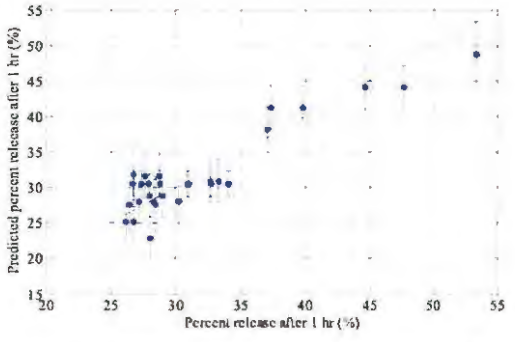

Figure 11.3 Predicted *vs.* observed

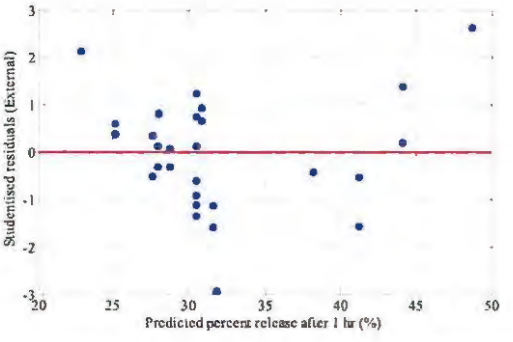

Figure 11.4 Studentised residuals *vs.* observed

11.3 PERCENT RELEASE AFTER 2 HR

Table 11.7 Model fit comparison for percent drug released after 2 hr

Model	PRESS	RMSE
Linear	5.5492	5.0172
Quadratic	5.1703	3.6146

Table 11.8 Stepwise regression results quadratic model for percent release after 2 hr

Coefficient	Value	Standard deviation	t	Significance
Intercept	**41.83**	**1.0435**	**40.09**	**Significant**
x_1 – Methocel® K100M	**-8.5218**	**1.4757**	**-5.7749**	**Significant**
x_2 – Xanthan gum	**-10.20**	**1.4757**	**6.9102**	**Significant**
x_3 – Carbopol® 974P	-0.6528	1.5037	-0.4342	Not significant
x_4 – Surelease	**-2.2459**	**1.4757**	**-1.5220**	**Significant**
x_1^2	3.3200	2.7012	1.2291	Not significant
x_1x_2	**9.4949**	**3.6146**	**2.6268**	**Significant**
x_1x_3	-2.2304	3.6675	-0.6082	Not significant
x_1x_4	0.09924	3.6996	0.02682	Not significant
x_2^2	**11.00**	**2.7110**	**4.0952**	**Significant**
x_2x_3	**-4.6734**	**3.6146**	**-1.2929**	**Significant**
x_2x_4	-1.1203	3.6916	-0.3035	Not significant
x_3^2	**-3.1663**	**2.7110**	**-1.1680**	**Significant**
x_3x_4	1.9065	3.6762	0.5186	Not significant
x_4^2	-0.1796	2.7964	-0.06421	Not significant

Table 11.9 ANOVA summary table

Source	SS	df	MS
Regression	1451.833	7	207.405
Error	287.442	22	13.066
Total	1739.275	29	0

$$y_{2h} = 41.8 - 8.52x_1 - 10.2x_2 - 2.25x_4 + 9.49x_1x_2 + 11.0x_2^2 - 4.67x_2x_3 - 3.17x_3^2$$

Equation 11.3

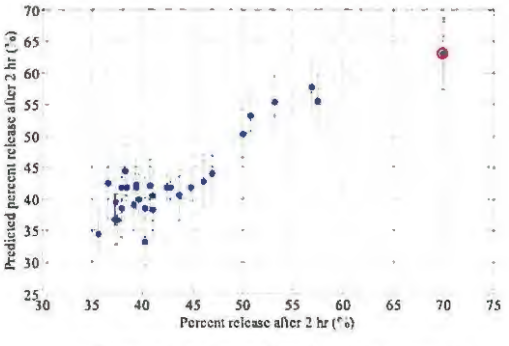

Figure 11.5 Predicted *vs.* observed

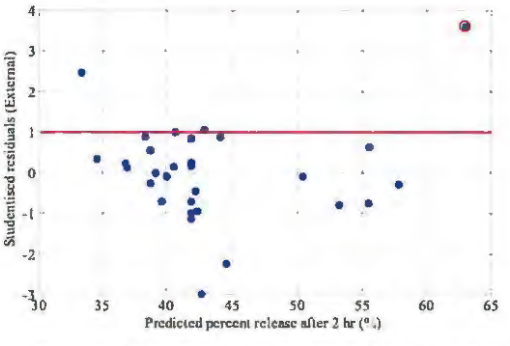

Figure 11.6 Studentised residuals *vs.* predicted

11.4 PERCENT RELEASE AFTER 4 HR

Table 11.10 Model fit comparison for percent drug released after 4 hr

Model	PRESS	RMSE
Linear	5.4987	4.9461
Quadratic	5.1843	4.7248

Table 11.11 Stepwise regression results quadratic model for percent release after 4 hr

Coefficient	Value	Standard deviation	*T*	Significance
Intercept	**60.26**	**1.1136**	**54.11**	**Significant**
x_1 – **Methocel® K100M**	**-10.15**	**1.9389**	**-5.2611**	**Significant**
x_2 – **Xanthan gum**	**-9.4504**	**1.9389**	**-4.8994**	**Significant**
x_3 – **Carbopol® 974P**	**-2.8128**	**1.9389**	**-1.4583**	**Significant**
x_4 – **Surelease**	**-3.4614**	**1.9389**	**-1.7945**	**Significant**
x_1^2	3.4738	3.5467	0.9794	Not significant
x_1x_2	**7.4074**	**4.7248**	**1.5678**	**Significant**
x_1x_3	-0.4601	4.8300	-0.09525	Not significant
x_1x_4	0.3270	4.8305	0.06770	Not significant
x_2^2	8.9463	3.0806	2.9041	Not significant
x_2x_3	-2.355	4.8048	-0.4902	Not significant
x_2x_4	-2.6437	4.7980	-0.5510	Not significant
x_3^2	**-4.9034**	**3.5217**	**-1.3923**	**Significant**
x_3x_4	2.0110	4.8119	0.4179	Not significant
x_4^2	-1.2617	3.6132	-0.3492	Not significant

Table 11.12 ANOVA summary table

Source	SS	df	MS
Regression	1371.271	6	228.545
Error	513.447	23	22.324
Total	1884.7171	29	0

$$y_{4h} = 60.3 - 10.2x_1 - 9.45x_2 - 2.81x_3 - 3.46x_4 - 7.41x_1x_2 - 4.90x_3^2 \quad \textbf{Equation 11.4}$$

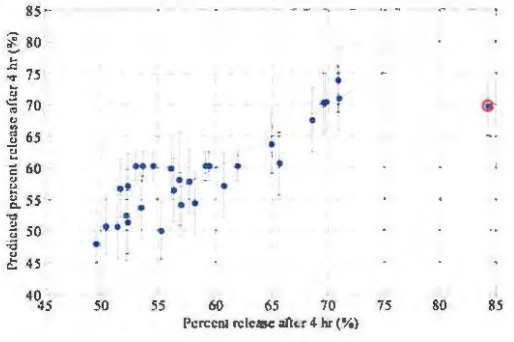

Figure 11.7 Predicted *vs.* observed

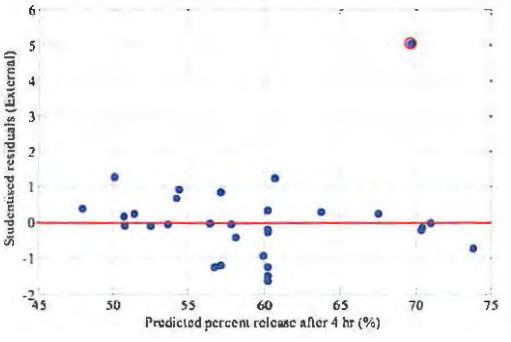

Figure 11.8 Studentised residuals *vs.* predicted

11.5 PERCENT RELEASE AFTER 6 HR

Table 11.13 Model fit comparison for percent drug released after 6 hr

Model	PRESS	RMSE
Linear	5.9872	5.3826
Quadratic	5.1152	4.4342

Table 11.14 Stepwise regression results quadratic model for percent release after 6 hr

Coefficient	Value	Standard deviation	*t*	Significance
Intercept	**72.07**	**1.2800**	**56.30**	**Significant**
x_1 – Methocel® K100M	**-13.49**	**1.8103**	**-7.4537**	**Significant**
x_2 – Xanthan gum	**-8.0773**	**1.8103**	**-4.4620**	**Significant**
x_3 – Carbopol® 974P	**-4.0048**	**1.8103**	**-2.2123**	**Significant**
x_4 – Surelease	**-4.4294**	**1.8103**	**-2.4469**	**Significant**
x_1^2	**10.39**	**3.3257**	**3.1267**	**Significant**
x_1x_2	**4.9922**	**4.4342**	**1.1258**	**Significant**
x_1x_3	0.09546	4.5385	0.02103	Not significant
x_1x_4	0.2184	4.5383	0.04813	Not significant
x_2^2	5.6356	3.2028	1.7595	Not significant
x_2x_3	0.3365	4.5380	0.07414	Not significant
x_2x_4	-4.2177	4.4443	-0.9490	Not significant
x_3^2	**-5.2877**	**3.3257**	**1-5900**	**Significant**
x_3x_4	2.1363	4.5145	0.4732	Not significant
x_4^2	-1.8580	3.4068	-0.5454	Not significant

Table 11.15 ANOVA summary table

Source	SS	df	MS
Regression	1989.514	7	284.216
Error	432.567	22	19.662
Total	2422.081	29	0

$$y_{6h} = 72.1 - 13.5x_1 - 8.08x_2 - 4.01x_3 - 4.43x_4 + 10.4x_1^2 + 4.49x_1x_2 - 5.29x_3^2$$

Equation 11.5

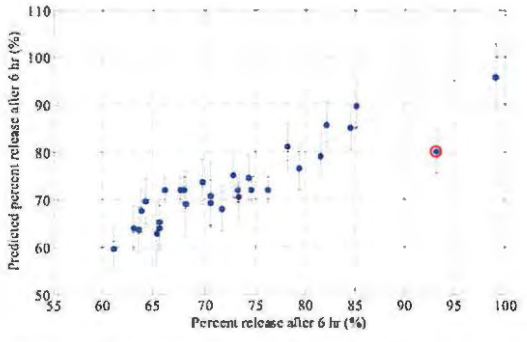

Figure 11.9 Predicted *vs.* observed

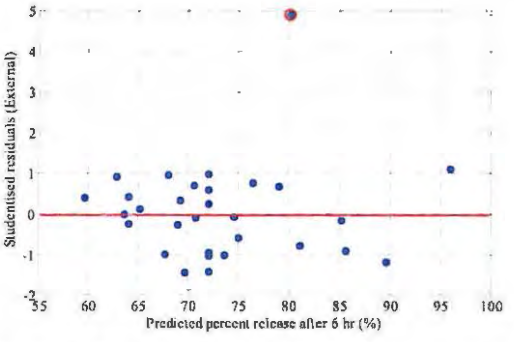

Figure 11.10 Studentised residuals *vs.* predicted

11.6 PERCENT RELEASE AFTER 8 HR

Table 11.16 Model fit comparison for percent drug released after 8 hr

Model	PRESS	RMSE
Linear	5.0768	4.6084
Quadratic	4.7586	4.2015

Table 11.17 Stepwise regression results quadratic model for percent release after 8 hr

Coefficient	Value	Standard deviation	*t*	Significance
Intercept	**82.66**	**1.2129**	**68.15**	**Significant**
x_1 – Methocel® K100M	**-11.95**	**1.7153**	**-6.9679**	**Significant**
x_2 – Xanthan gum	**-7.0601**	**1.7153**	**-4.1160**	**Significant**
x_3 – Carbopol® 974P	**-4.2601**	**1.7153**	**-2.4836**	**Significant**
x_4 – Surelease	**-4.9151**	**1.7153**	**-2.8655**	**Significant**
x_1^2	**6.3764**	**3.1512**	**2.0235**	**Significant**
x_1x_2	3.1545	4.2430	0.7435	Not significant
x_1x_3	0.7125	4.2933	0.1659	Not significant
x_1x_4	-0.1416	4.2959	-0.03296	Not significant
x_2^2	3.4475	3.1632	1.0899	Not significant
x_2x_3	2.0969	4.2726	0.4908	Not significant
x_2x_4	-4.4066	4.1920	-1.051	Not significant
x_3^2	**4.6993**	**3.1512**	**-1.4913**	**Significant**
x_3x_4	1.9661	4.2755	0.4599	Not significant
x_4^2	-0.8054	3.2429	-0.2484	Not significant

Table 11.18 ANOVA summary table

Source	SS	df	MS
Regression	1534.91	6	255.818
Error	406.018	23	17.653
Total	1940.928	29	0

$$y_{8h} = 82.7 - 12.0x_1 - 7.06x_2 - 4.26x_3 - 4.91x_4 + 6.37x_1^2 + 4.70x_3^2 \quad \textbf{Equation 11.6}$$

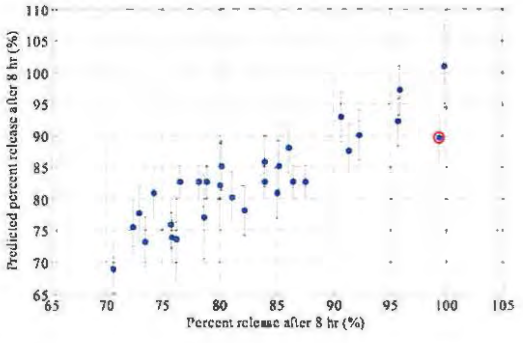

Figure 11.11 Predicted *vs.* observed

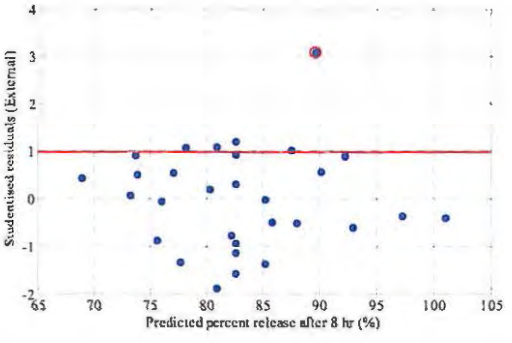

Figure 11.12 Studentised residuals *vs.* observed

11.7 PERCENT RELEASE AFTER 12 HR

Table 11.19 Model fit comparison for percent drug released after 12 hr

Model	PRESS	RMSE
Linear	4.9752	4.5572
Quadratic	4.9752	4.5572

Table 11.20 Stepwise regression results quadratic model for percent release after 12 hr

Coefficient	Value	Standard deviation	*t*	Significance
Intercept	**94.33**	**0.8321**	**113.4**	**Significant**
x_1 – Methocel® K100M	**-7.7059**	**1.8606**	**-4.1417**	**Significant**
x_2 – Xanthan gum	**-6.8699**	**1.8606**	**-3.6924**	**Significant**
x_3 – Carbopol® 974P	**-3.2194**	**1.8606**	**-1.7304**	**Significant**
x_4 – Surelease	**-3.9763**	**1.8606**	**-2.1372**	**Significant**

Table 11.21 ANOVA summary table

Source	SS	df	MS
Regression	796.519	4	199.13
Error	519.254	25	20.77
Total	1315.772	29	0

$$y_{12h} = 94.3 - 7.71x_1 - 6.87x_2 - 3.21x_3 - 3.98x_4 \qquad \textbf{Equation 11.7}$$

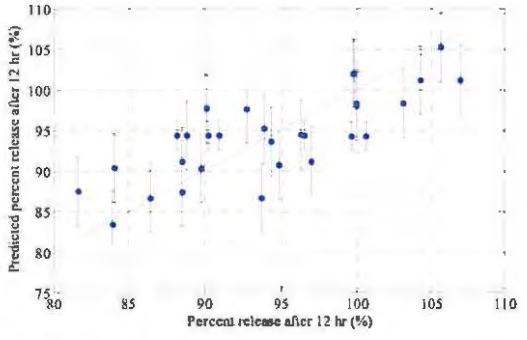

Figure 11.13 Predicted *vs.* observed

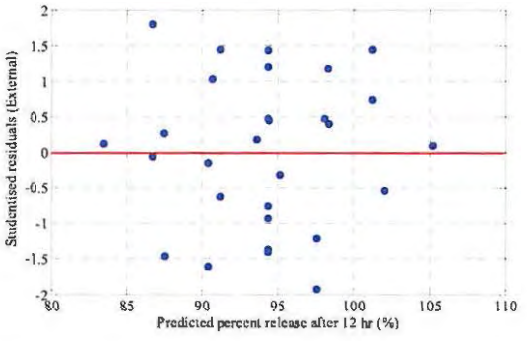

Figure 11.14 Studentised residuals *vs.* predicted

11.8 DRUG RELEASE RATE

Table 11.22 Model fit comparison for percent drug release rate, k

Model	PRESS	RMSE
Linear	0.048535	0.044203
Quadratic	0.041361	0.030710

Table 11.23 Stepwise regression results quadratic model for drug release rate, k

Coefficient	Value	Standard deviation	T	Significance
Intercept	**0.3038**	**0.008865**	**34.27**	**Significant**
x_1 – Methocel® K100M	**-0.07637**	**0.01254**	**-6.0911**	**Significant**
x_2 – Xanthan gum	**-0.08757**	**0.01254**	**-6.9844**	**Significant**
x_3 – Carbopol® 974P	0.001650	0.01281	0.1288	Not significant
x_4 – Surelease	-0.01360	0.01249	-1.0891	Not significant
x_1^2	0.02824	0.02296	1.2301	Not significant
x_1x_2	**0.09525**	**0.03071**	**3.1016**	**Significant**
x_1x_3	-0.03305	0.03060	-1.0801	Not significant
x_1x_4	-0.003900	0.03139	-0.1242	Not significant
x_2^2	**0.09551**	**0.02303**	**4.1468**	**Significant**
x_2x_3	**-0.05545**	**0.03071**	**-1.8056**	**Significant**
x_2x_4	-0.007200	0.03136	-0.2296	Not significant
x_3^2	**-0.02814**	**0.02303**	**-1.2216**	**Significant**
x_3x_4	0.02210	0.03105	0.7119	Not significant
x_4^2	0.002014	0.02373	0.08487	Not significant

Table 11.24 ANOVA summary table

Source	SS	Df	MS
Regression	0.112	6	0.019
Error	0.022	23	9.431×10^{-4}
Total	0.134	29	0

$$k = 0.304 - 0.0764x_1 - 0.0876x_2 + 0.0953x_1x_2 + 0.0955x_2^2 - 0.0555x_2x_3 - 0.0281x_3^2$$

Equation 11.8

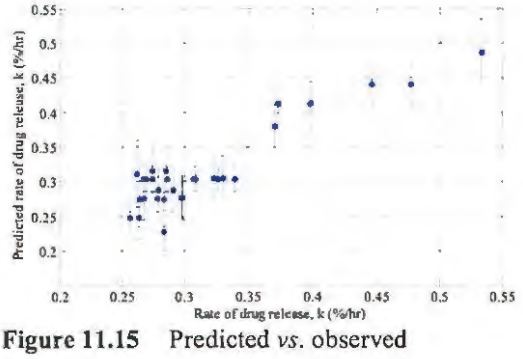

Figure 11.15 Predicted *vs.* observed

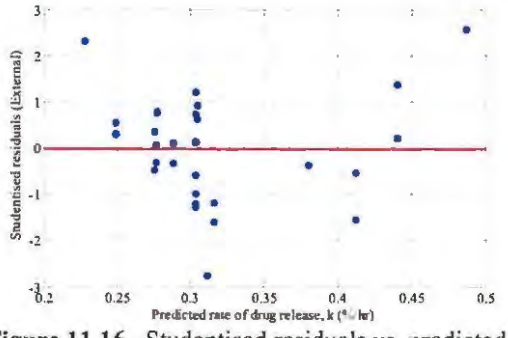

Figure 11.16 Studentised residuals *vs.* predicted

11.9 MECHANISM OF RELEASE

Table 11.25 Model fit comparison for exponent of drug release, *n*

Model	PRESS	RMSE
Linear	0.033794	0.031250
Quadratic	0.024526	0.020222

Table 11.26 Stepwise regression results quadratic model for exponent of drug release, *n*

Coefficient	Value	Standard deviation	*t*	Significance
Intercept	**0.4761**	**0.004766**	**99.89**	**Significant**
x_1 – Methocel® K100M	**0.04396**	**0.008256**	**5.3247**	**Significant**
x_2 – Xanthan gum	**0.05594**	**0.008256**	**6.7763**	**Significant**
x_3 – Carbopol® 974P	**0.01751**	**0.008256**	**-2.1208**	**Significant**
x_4 – Surelease	-0.001858	0.008440	-0.2202	Not significant
x_1^2	-0.02197	0.01476	-1.4882	Not significant
x_1x_2	**-0.06892**	**0.02022**	**-3.4085**	**Significant**
x_1x_3	0.02597	0.01991	1.3049	Not significant
x_1x_4	-0.007527	0.02063	-0.3647	Not significant
x_2^2	**-0.06168**	**0.01507**	**-4.0923**	**Significant**
x_2x_3	**0.05167**	**0.02022**	**2.5554**	**Significant**
x_2x_4	**0.03552**	**0.02022**	**-1.7568**	**Significant**
x_3^2	-0.02607	0.01444	1.8046	Not significant
x_3x_4	-0.04325	0.02068	-0.2092	Not significant
x_4^2	-0.05378	0.01548	-0.3475	Not significant

Table 11.27 ANOVA summary table

Source	SS	Df	MS
Regression	0.048	7	6.82×10^{-3}
Error	8.996×10^{-3}	22	4.089×10^{-4}
Total	0.057	29	0

$$n = 0.476 + 0.0440x_1 + 0.0599x_2 - 0.0175x_3 - 0.06689x_1x_2 - 0.0617x_2^2 + 0.0517x_2x_3 - 0.0355x_2x_4$$

Equation 11.9

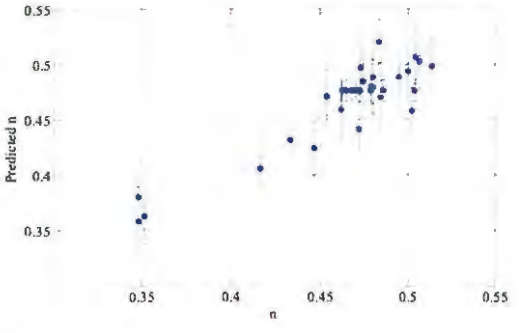

Figure 11.17 Predicted *vs.* observed

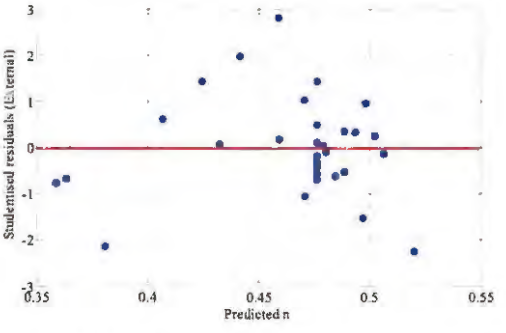

Figure 11.18 Studentised residuals *vs.* predicted

REFERENCES

1. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, "Pharmaceutical Development Q8 (R2)," 2009, http://www.ich.org/LOB/media/MEDIA4986.pdf [cited 30 November 2009].

2. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, "Quality Risk Management Q9," 2005, http://www.ich.org/LOB/media/MEDIA1957.pdf [cited 5 August 2009].

3. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, "Pharmaceutical Quality System Q10," 2008, http://www.ich.org/LOB/media/MEDIA3917.pdf [cited 5 August 2009].

4. U.S.Food and Drug Administration (FDA), "Guidance for Industry: Q8 Pharmaceutical Development," 2006, http://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm128029.pdf [cited 5 August 2009].

5. European Medicines Agency, "ICH Topic Q8, Pharmaceutical Development. Note for the Guidance on Pharmaceutical Development (EMEA/CHMP/167068/2004)," 2006, http://www.emea.europa.eu/pdfs/human/ich/16706804en.pdf [cited 5 August 2009].

6. Sam, T. and Khan, M., "Pre-Symposium Workshop 1.Quality by Design," *14th International Pharmaceutical Technology Symposium*, Antalya, Turkey, 2008.

7. Drennen III, J. K., "Quality by design – What does it really mean?," *Journal of Pharmaceutical Innovation*, Vol. 2, No. 3, 2007, pp. 65–66.

8. U.S.Food and Drug Administration (FDA), "Pharmaceutical Quality for the 21st Century. A risk-based approach. Progress Report," 2007, http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm128080.htm [cited 2 April 2009].

9. Mazzocchi, B. and Perotti, G., "Practical aspects of implementation of a design space to support real time release for a pharmaceutical product," 2007, http://www.pda.org/Presentation/2008-Conference-on-Quality-by-Design/Perotti-Mazzocchi.aspx [cited 9 August 2009].

10. Nasr, M. M., "Quality by Design (QbD) – A modern system approach to pharmaceutical development and manufacturing – FDA perspective," 2007, www.aaps-ispe.org/pdfs/070227-QbD-Nasr.ppt [cited 9 August 2009].

11. Winkle, H. N., "Implementing Quality by Design," *PDA/FDA Joint Regulatory Conference: Evolution of the Global Regulatory Environment: A Practical Approach to Change* [online], 2007, http://www.fda.gov/Cder/OPS/ImplementingQualitybyDesign.pdf [cited 24 September 2007].

12. Tway, P., "Why QbD worked for Januvia," 2009, http://www.pharmaqbd.com/?q=node/133 [cited 9 August 2009].

13. Graffner, C., "ICH Q8 Pharmaceutical Development," 2006, http://www.emea.europa.eu/Inspections/docs/Presentations/SEMPA-ICHQ8PharmaceuticalDevelopment.pdf [cited 9 August 2009].

14. Cerulean Associates LLC, "Why quality by design? An executive's guide to the FDA's quality by design," 2008, http://www.ceruleanllc.com/Booklets/Downloads_Private/Cerulean_QbD_Executive_Guide.pdf [cited 20 September 2009].

15. U.S.Food and Drug Administration (FDA), "FDA approves new treatment for diabetes: First in a new class of diabetes drugs," 2006, http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2006/ucm108770.htm [cited 5 August 2009].

16. Blumenstein, J., "QbD pilot experience," 2006, http://www.fda.gov/ohrms/dockets/ac/06/briefing/2006-4241B1-02-15-FDA-QbD%20ONDQA2%20130_Blumenstein.pdf [cited 9 August 2009].

17. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, "Final Concept Paper. ICH Implementation Working Group (IWG) on ICH Q8, Q9 and Q10," 2007, http://www.ich.org/LOB/media/MEDIA4457.pdf [cited 5 August 2009].

18. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, "Pharmaceutical Development Q8 (R1)," 2008, http://www.ich.org/LOB/media/MEDIA4986.pdf [cited 3 August 2009].

19. Van Arnum, P., "A FDA perspective on Quality by Design," 2007, pp. 22, http://pharmtech.findpharma.com/pharmtech/Article/A-FDA-Perspective-on-Quality-by-Design/ArticleStandard/Article/detail/469915 [cited 5 August 2009].

20. Hussain, A. S., "Pharmaceutical Quality by Design: Improving Emphasis on Manufacturing Science in the 21st Century," *FDA Pharmaceutical Inspectorate* [online], 2004, http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/ucm089025.pdf [cited 5 August 2009].

21. "Salbutamol," *Martindale: The Complete Drug Reference* [online]. Pharmaceutical Press, London, Electronic Version 2009, http://www.medicinescomplete.com/mc/martindale/current/ms-2104-z.htm [cited 21 February 2009].

22. Calverley, P. M. A., "Modern treatment of chronic obstructive pulmonary disease," *European Respiratory Journal*, Vol. 18, Suppl. 34, 2001, pp. 60s–66s.

23. Salbutamol/Salbutamol Sulphate. British Pharmacopoeia, Volume 2. The Stationary Office, London, 2008, pp .1916–1920.

24. Albuterol. O'Neil, M. J., Heckelman, P. E., Koch, C. B., and Roman, K. J. The Merck Index, An Encyclopedia of Chemical, Drugs and Biologicals, 14th Edition, Merck, New Jersey, 2004, pp. 40.

25. Aboul-Enein, H. Y., Al-Badr, A. A., and Ibrahim, S. E., "Salbutamol," *Analytical Profiles of Drug Substances*, Vol. 10, 1981, pp. 665–689.

26. Boulton, D. W. and Fawcett, J. P., "Enantioselective disposition of salbutamol in man following oral and intravenous administration," *British Journal of Clinical Pharmacology*, Vol. 41, No. 1, 1996, pp. 35–40.

27. "Vari-Salbutamol/Venteze/Ventolin," *Monthly Index of Medical Specialities (MIMS)*, Vol. 48, No. 10, 2008, pp. 164–165.

28. Katzung, B. G., "Introduction to Pharmacology," *Basic & Clinical Pharmacology*, edited by B. G. Katzung, 10 ed. McGraw Hill's Access Pharmacy, New York, 2009, http://www.accesspharmacy.com.wam.seals.ac.za/content.aspx?aID=2500000 [cited 14 February 2009].

29. "Asthma," *The Merck Manual Online* [online], No. 48, 2009, http://www.merck.com/mmpe/sec05/ch048/ch048a.html [cited 25 April 2009].

30. "Asthma," *Martindale: The Complete Drug Reference* [online] Pharmaceutical Press, London, Electronic Version 2009, http://www.medicinescomplete.com/mc/martindale/current/9795-a3-1-e.htm [cited 21 February 2009].

31. Dollery, C. T., *Therapeutic Drugs*, 1 ed., Vol. 2, Churchill Livingstone, Edinburgh, 1992, pp. S1–S4.

32. Sylvester, J. T., "The tone of pulmonary smooth muscle: ROK and Rho music?," *American Journal of Physiology - Lung Cellular and Molecular Physiology*, Vol. 287, No. 4, 2004, pp. L624–L630.

33. Koike, K., Yamashita, Y., Horinouchi, T., Yamaki, F., and Tanaka, Y., "cAMP-independent mechanism is significantly involved in beta(2)-adrenoceptor-mediated tracheal relaxation," *European Journal of Pharmacology*, Vol. 492, No. 1, 2004, pp. 65–70.

34. Kume, H., Hall, I. P., Washabau, R. J., Takagi, K., and Kotlikoff, M. I., "Beta-Adrenergic agonists regulate K-Ca channels in airway smooth-muscle by cAMP-dependent and cAMP-independent mechanisms," *Journal of Clinical Investigation*, Vol. 93, No. 1, 1994, pp. 371–379.

35. Barnes, P. J., "Effect of beta-agonists on inflammation cells," *Journal of Allergy and Clinical Immunology*, Vol. 104, No. 2, 1999, pp. S10–S17.

36. Cockcroft, D. W., McParland, C. P., Britto, S. A., Swystun, V. A., and Rutherford, B. C., "Regular inhaled salbutamol and airway responsiveness to allergen," *Lancet*, Vol. 342, No. 8875, 1993, pp. 833–837.

37. Perrin-Fayolle, M., "Salbutamol in the treatment of asthma," *Lancet*, Vol. 346, No. 8982, 1995, pp. 1101.

38. Handley, D., "The asthma-like pharmacology and toxicology of (S)-isomers of beta agonists," *Journal of Allergy and Clinical Immunology*, Vol. 104, No. 2, 1999, pp. S69–S76.

39. Israel, E., Chinchilli, V. M., Ford, J. G., Boushey, H. A., Cherniack, R., Craig, T. J., Deykin, A., Fagan, J. K., Fahy, J. V., Fish, J., Kraft, M., Kunselman, S. J., Lazarus, S. C., Lemanske, J., Liggett, S. B., Martin, R. J., Mitra, N., Peters, S. P., Silverman, E., Sorkness, C. A., Szefler, S. J., Wechsler, M. E., Weiss, S. T., and Drazen, J. M., "Use of regularly scheduled albuterol treatment in asthma: Genotype-stratified, randomised, placebo-controlled cross-over trial," *The Lancet*, Vol. 364, No. 9444, pp. 1505–1512.

40. Tattersfield, A. E. and Hall, I. P., "Are β_2-adrenoceptor polymorphisms important in asthma - an unravelling story," *The Lancet*, Vol. 364, No. 9444, 2004, pp. 1464–1466.

41. Page, C. P. and Morley, J., "Contrasting properties of albuterol stereoisomers," *Journal of Allergy and Clinical Immunology*, Vol. 104, No. 2, 1999, pp. S31–S41.

42. Templeton, A. G. B., Chapman, I. D., Chilvers, E. R., Morley, J., and Handley, D. A., "Effects of S-salbutamol on human isolated bronchus," *Pulmonary Pharmacology & Therapeutics*, Vol. 11, No. 1, 1998, pp. 1–6.

43. Mitra, S., Ugur, M., Ugur, O., Goodman, H. M., McCullough, J. R., and Yamaguchi, H., "(S)-albuterol increases intracellular free calcium by muscarinic receptor activation and a phospholipase C dependent mechanism in airway smooth muscle," *Molecular Pharmacology*, Vol. 53, No. 3, 1998, pp. 347–354.

44. Taylor, D. R., Wilkins, G. T., Herbison, G. P., and Flannery, E. M., "Interaction between corticosteroid and beta-agonist drugs: Biochemical and cardiovascular effects in normal subjects," *Chest*, Vol. 102, No. 2, 1992, pp. 519–524.

45. Veale, D., Cooper, B. G., Griffiths, C. J., Corris, P. A., and Gibson, G. J., "The effect of controlled-release salbutamol on sleep and nocturnal oxygenation in patients with asthma and chronic obstructive pulmonary-disease," *Respiratory Medicine*, Vol. 88, No. 2, 1994, pp. 121–124.

46. Maesen, F. P. V. and Smeets, J. J., "Comparison of a controlled-release tablet of salbutamol given twice daily with a standard tablet given 4 times daily in the management of chronic obstructive lung-disease," *European Journal of Clinical Pharmacology*, Vol. 31, No. 4, 1986, pp. 431–436.

47. Vyse, T. and Cochrane, G. M., "Controlled release salbutamol tablets versus sustained release theophylline tablets in the control of reversible obstructive airways disease," *Journal of International Medical Research*, Vol. 17, No. 1, 1989, pp. 93–98.

48. Pierson, W. E., Laforce, C. F., Bell, T. D., Maccosbe, P. E., Sykes, R. S., and Tinkelman, D., "Long-term, double-blind comparison of controlled-release albuterol versus sustained-release theophylline in adolescents and adults with asthma," *Journal of Allergy and Clinical Immunology*, Vol. 85, No. 3, 1990, pp. 618–626.

49. Higenbottam, T. W., Khan, M. A., Williams, D. O., Mikhail, J. R., Peake, M. D., and Hughes, J., "Controlled release salbutamol tablets versus aminophylline in the control of reversible airways obstruction," *Journal of International Medical Research*, Vol. 17, No. 5, 1989, pp. 435–441.

50. Fairfax, A. J., McNabb, W. R., Davies, H. J., and Spiro, S. G., "Slow-release oral salbutamol and aminophylline in nocturnal asthma: Relation of overnight changes in lung function and plasma drug levels," *Thorax*, Vol. 35, No. 7, 1980, pp. 526–530.

51. Maconochie, J. G. and Fowler, P., "Plasma concentrations of salbutamol after an oral slow release preparation," *Current Medical Research and Opinion*, Vol. 8, No. 9, 1983, pp. 634–639.

52. Milroy, R., Carter, R., Carlyle, D., and Boyd, G., "Clinical and pharmacological study of a novel controlled release preparation of salbutamol," *British Journal of Clinical Pharmacology*, Vol. 29, No. 5, 1990, pp. 578–580.

53. Morgan, D. J., "Clinical pharmacokinetics of beta-agonists," *Clinical Pharmacokinetics*, Vol. 18, No. 4, 1990, pp. 270–294.

54. Martin, L. E., Hobson, J. C., Page, J. A., and Harrison, C., "Metabolic studies of salbutamol-^{3}H: A new bronchodilator, in rat, rabbit, dog and man," *European Journal of Pharmacology*, Vol. 14, No. 2, 1971, pp. 183–199.

55. Goldstein, D. A., Tan, Y. K., and Soldin, S. J., "Pharmacokinetics and absolute bioavailability of salbutamol in healthy adult volunteers," *European Journal of Clinical Pharmacology*, Vol. 32, No. 6, 1987, pp. 631–634.

56. Morgan, D. J., Paull, J. D., Richmond, B. H., Wilson-Evered, E., and Ziccone, S. P., "Pharmacokinetics of intravenous and oral salbutamol and its sulfate conjugate," *British Journal of Clinical Pharmacology*, Vol. 22, No. 5, 1986, pp. 587–593.

57. Nandakumaran, M., Gardey, C. L., Richard, M. O., Panigel, M., and Olive, G., "Transfer of salbutamol in the human placenta in vitro," *Developmental Pharmacology and Therapeutics*, Vol. 3, No. 2, 1981, pp. 88–98.

58. Pacifici, G. M., Giulianetti, B., Quilici, M. C., Spisni, R., Nervi, M., Giuliani, L., and Gomeni, R., "(-)-salbutamol sulphation in the human liver and duodenal mucosa: Interindividual variability," *Xenobiotica*, Vol. 27, No. 3, 1997, pp. 279–286.

59. Boulton, D. W. and Fawcett, J. P., "The pharmacokinetics of levosalbutamol: What are the clinical implications?," *Clinical Pharmacokinetics*, Vol. 40, No. 1, 2001, pp. 23–40.

60. Lin, C., Li, Y., McGlotten, J., Morton, J. B., and Symchowicz, S., "Isolation and identification of the major metabolite of albuterol in human urine," *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, Vol. 5, No. 3, 1977, pp. 234–238.

61. Price, R. A., Cox, N. J., Spielman, R. S., Vanloon, J. A., Maidak, B. L., and Weinshilboum, R. M., "Inheritance of human-platelet thermolabile phenol sulfotransferase (Tl Pst) activity," *Genetic Epidemiology*, Vol. 5, No. 1, 1988, pp. 1–15.

62. Price, R. A., Spielman, R. S., Lucena, A. L., Vanloon, J. A., Maidak, B. L., and Weinshilboum, R. M., "Genetic-polymorphism for human-platelet thermostable phenol sulfotransferase (Ts Pst) activity," *Genetics*, Vol. 122, No. 4, 1989, pp. 905–914.

63. Powell, M. L., Chung, M., Weisberger, M., Gural, R., Radwanski, E., Symchowicz, S., and Patrick, J. E., "Multiple-dose albuterol kinetics," *Journal of Clinical Pharmacology*, Vol. 26, No. 8, 1986, pp. 643–646.

64. Lipworth, B. J., Clark, R. A., Dhillon, D. P., Charter, M. K., Palmer, J. B. D., and McDevitt, D. G., "Single dose and steady state pharmacokinetics of 4 mg and 8 mg oral salbutamol controlled-release in patients with bronchial asthma," *European Journal of Clinical Pharmacology*, Vol. 37, No. 1, 1989, pp. 49–52.

65. Shargel, L., Wu-Pong, S., and Yu, A. B. C., "Modified-release drug products," *Applied Biopharmaceutics and Pharmacokinetics*, edited by L. Shargel, S. Wu-Pong, and A. B. C. Yu, 5 ed. McGraw Hill's Access Pharmacy, New York, 2009, pp. http://0-www.accesspharmacy.com.wam.scals.ac.za/content.aspx?aID=2481984 [cited 21 February 2009].

66. "Controlled release salbutamol tablets," *Drug and Therapeutics Bulletin*, Vol. 28, No. 15, 1990, pp. 58–59.

67. Lalla, J. K. and Kapadnekar, K. S. G., "Sustained release salbutamol tablets - Formulation engineering and evaluation composite wax matrix and influence of additives. Part IIB," *Research and Industry (New Dehli)*, Vol. 30, No. 3, 1985, pp. 192–198.

68. Lalla, J. K. and Kapadnekar, K. S. G., "Sustained release salbutamol tablets: Formulation engineering and evaluation use of fat and wax matrix: Part IIA," *Research and Industry (New Dehli)*, Vol. 30, No. 3, 1985, pp. 189–191.

69. Murthy, R. S. R., Malhotra, M., and Miglani, B. D., "Sustained-release formulation of salbutamol sulfate," *Drug Development and Industrial Pharmacy*, Vol. 17, No. 10, 1991, pp. 1373–1380.

70. Baveja, S. K., Ranga Rao, K. V., Singh, A., and Gombar, V. K., "Release characteristics of some bronchodilators from compressed hydrophilic polymeric matrices and their correlation with molecular geometry," *International Journal of Pharmaceutics*, Vol. 41, No. 1-2, 1988, pp. 55–62.

71. Hernández, R. M., Gascón, A. R., Calvo, M. B., Caramella, C., Conte, U., Dominguez-Gil, A., and Pedraz, J. L., "Correlation of 'in vitro' release and 'in vivo' absorption characteristics of four salbutamol sulphate formulations," *International Journal of Pharmaceutics*, Vol. 139, No. 1-2, 1996, pp. 45–52.

72. Hashem, F. and Eldien, E. E. Z., "Controlled release salbutamol sulfate molded tablets using Eudragit Retard," *Drug Development and Industrial Pharmacy*, Vol. 16, No. 3, 1990, pp. 541–549.

73. Sanghavi, N. M., Bijlani, C. P., Kamath, P. R., and Sarwade, V. B., "Matrix tablets of salbutamol sulfate," *Drug Development and Industrial Pharmacy*, Vol. 16, No. 12, 1990, pp. 1955–1961.

74. Yazan, Y., Demirel, M., and Guler, E., "Preparation and in-vitro dissolution of salbutamol sulfate microcapsules and tabletted microcapsules," *Journal of Microencapsulation*, Vol. 12, No. 6, 1995, pp. 601–607.

75. Raghuvanshi, R. S., Tripathi, K. P., Jayaswal, S. B., and Singh, J., "Release kinetics of salbutamol sulfate from wax coated microcapsules and tabletted microcapsules," *Journal of Microencapsulation*, Vol. 9, No. 4, 1992, pp. 449–455.

76. El-Sayed, G., El-Said, Y., Meshali, M., and Schwartz, J. B., "A wax as a potential matrix for sustained release salbutamol tablets," *Pharmazeutische Industrie*, Vol. 39, No. 2, 1997, pp. 179–182.

77. Higuchi, T., "Rate of release of medicaments from ointment bases containing drugs in suspensions," *Journal of Pharmaceutical Sciences*, Vol. 50, No. 10, 1961, pp. 874–875.

78. Higuchi, T., "Theoretical analysis of rate of release of solid drugs dispersed in solid matrices," *Journal of Pharmaceutical Sciences*, Vol. 52, No. 12, 1963, pp. 1145–1149.

79. Korsmeyer, R. W., Gurny, R., Doelker, E., Buri, P., and Peppas, N. A., "Mechanisms of solute release from porous hydrophilic polymers," *International Journal of Pharmaceutics,* Vol. 15, No. 1, 1983, pp. 25–35.

80. San Vicente, A., Hernández, R. M., Gascón, A. R., Calvo, M. B., and Pedraz, J. L., "Effect of aging on the release of salbutamol sulfate from lipid matrices," *International Journal of Pharmaceutics*, Vol. 208, No. 1-2, 2000, pp. 13–21.

81. Lin, S.-Y. and Hou, S.-J., "Preparation and evaluation of directly compressible controlled-release salbutamol tablets with Eudragit RLPM/RSPM and calcium phosphate," *Acta Pharmaceutica Zagreb*, Vol. 42, No. 2, 1992, pp. 99–108.

82. Solinís, M. A., Lugará, S., Calvo, B., Hernández, R. M., Gascón, A. R., and Pedraz, J. L., "Release of salbutamol sulfate enantiomers from hydroxypropylmethylcellulose matrices," *International Journal of Pharmaceutics*, Vol. 161, No. 1, 1998, pp. 37–43.

83. Srichana, T. and Suedee, R., "Evaluation of stereo-selective dissolution of racemic salbutamol matrices prepared with commonly used excipients and H-1-NMR study," *Drug Development and Industrial Pharmacy*, Vol. 27, No. 5, 2001, pp. 457–464.

84. Liu, H. F., Zhang, S., Nits, S. F., Zhao, X., Sun, X. Y., Yang, X. G., and Pan, W. S., "Preparation and characterization of a novel pH-sensitive ton exchange resin," *Chemical & Pharmaceutical Bulletin*, Vol. 53, No. 6, 2005, pp. 631–633.

85. Liu, H. F., Sun, T. H., Yu, F. Q., Zhao, X., Guo, H., and Pan, W. S., "The investigation of the pharmacokinetics of pulsatile-release salbutamol sulfate with pH-sensitive ion-exchange resin as the carriers in beagle dogs," *Chemical & Pharmaceutical Bulletin*, Vol. 55, No. 3, 2007, pp. 480–481.

86. Sirkiä, T., Mäkimartti, M., Liukkosipi, S., and Marvola, M., "Development and biopharmaceutical evaluations of a new press-coated prolonged-release salbutamol sulfate tablet in man," *European Journal of Pharmaceutical Sciences*, Vol. 1, No. 4, 1994, pp. 195–201.

87. Lemesle-Lamache, V., Wouessidjewe, D., Chéron, M., and Duchêne, D., "Study of [beta]-cyclodextrin and ethylated [beta]-cyclodextrin salbutamol complexes, in vitro evaluation of sustained-release behaviour of salbutamol," *International Journal of Pharmaceutics*, Vol. 141, No. 1-2, 1996, pp. 117–124.

88. Wu, T., Pan, W., Chen, J., and Zhang, R., "Formulation optimization technique based on artificial neural network in salbutamol sulfate osmotic pump tablets," *Drug Development and Industrial Pharmacy*, Vol. 26, No. 2, 2000, pp. 211–215.

89. Sinchaipanid, N., Pongwai, S., Limsuwan, P., and Mitrevej, A., "Design of salbutamol EOP tablets from pharmacokinetics parameters," *Pharmaceutical Development and Technology*, Vol. 8, No. 2, 2003, pp. 135–142.

90. El-Gindy, A., Emara, S., and Shaaban, H., "Development and validation of chemometrics-assisted spectrophotometric and liquid chromatographic methods for the simultaneous determination of two multicomponent mixtures containing bronchodilator drugs," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 43, No. 3, 2007, pp. 973–982.

91. Jacobson, G. A. and Peterson, G. M., "High-performance liquid chromatographic assay for the simultaneous determination of ipratropium bromide, fenoterol, salbutamol and terbutaline in nebulizer solution," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 12, No. 6, 1994, pp. 825–832.

92. Erram, S. V., Fanska, C. B., and Asif, M., "Determination of albuterol sulfate and its related substances in albuterol sulfate inhalation solution, 0.5% by RP-HPLC," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 40, No. 4, 2006, pp. 864–874.

93. Basly, J. P., Duroux, J. L., and Bernard, M., "The effect of gamma radiation on the degradation of salbutamol," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 15, No. 8, 1997, pp. 1137–1141.

94. Mälkki-Laine, L., Purra, K., Kähkönen, K., and Tammilehto, S., "Decomposition of salbutamol in aqueous solutions. II. The effect of buffer species, pH, buffer concentration and antioxidants," *International Journal of Pharmaceutics*, Vol. 117, No. 2, 1995, pp. 189–195.

95. Albuterol/ Albuterol Sulphate/ Albuterol Tablets. The United States Pharmacopeia 32/ The National Formulary 27, United States Pharmacopeial Convention, Rockville, MD, 2009, pp. 1437–1439.

96. Marchand, D. H. and Snyder, L. R., "Anion-exchange behavior of several alkylsilica reversed-phase columns," *Journal of Chromatography A*, Vol. 1209, No. 1-2, 2008, pp. 104–110.

97. Marchand, D. H., Snyder, L. R., and Dolan, J. W., "Characterization and applications of reversed-phase column selectivity based on the hydrophobic-subtraction model," *Journal of Chromatography A*, Vol. 1191, No. 1-2, 2008, pp. 2–20.

98. Snyder, L. R., Dolan, J. W., and Carr, P. W., "The hydrophobic-subtraction model of reversed-phase column selectivity," *Journal of Chromatography A*, Vol. 1060, No. 1-2, 2004, pp. 77–116.

99. Kazakevich, Y. V., "2 HPLC theory," *Separation Science and Technology, HPLC Method Development for Pharmaceuticals*, edited by Satinder Ahuja and Henrik Rasmussen, Volume 8 ed. Academic Press, Boston, 2007, pp. 13–44.

100. Kazakevich, Y. V., "High-performance liquid chromatography retention mechanisms and their mathematical descriptions," *Journal of Chromatography A*, Vol. 1126, No. 1-2, 2006, pp. 232–243.

101. Subirats, X., Bosch, E., and Rosés, M., "Retention of ionisable compounds on high-performance liquid chromatography: XV. Estimation of the pH variation of aqueous buffers with the change of the acetonitrile fraction of the mobile phase," *Journal of Chromatography A*, Vol. 1059, No. 1-2, 2004, pp. 33–42.

102. Ying, P. T., Dorsey, J. G., and Dill, K. A., "Retention mechanisms of reversed-phase liquid-chromatography: Determination of solute solvent interaction free-energies," *Analytical Chemistry*, Vol. 61, No. 22, 1989, pp. 2540–2546.

103. Wang, H. L., Duda, J. L., and Radke, C. J., "Solution adsorption from liquid chromatography," *Journal of Colloid and Interface Science*, Vol. 66, No. 1, 1978, pp. 153–165.

104. Riedo, F. and Kováts, E. sz., "Adsorption from liquid mixtures and liquid chromatography," *Journal of Chromatography*, Vol. 239, 1982, pp. 1–28.

105. Knox, J. H. and Pryde, A., "Performance and selected applications of a new range of chemically bonded packing materials in high-performance liquid chromatography," *Journal of Chromatography A*, Vol. 112, 1975, pp. 171–188.

106. Snyder, L. R., Kirkland, J. J., and Glach, J. L., *Practical HPLC method development*, John Wiley, New York, 1997.

107. Kimata, K., Iwaguchi, K., Onishi, S., Jinno, K., Hosoya, K., Araki, M., and Tanaka, N., "Chromatographic characterization of silica C_{18} packing materials. Correlation between a preparation method and retention behaviour of stationary phase," *Journal of Chromatographic Science*, Vol. 27, No. 12, 1989, pp. 721–728.

108. Knox, J. H. and Hartwick, R. A., "Mechanism of ion-pair liquid chromatography of amines, neutrals, zwitterions and acids using anionic hetaerons," *Journal of Chromatography A*, Vol. 204, 1981, pp. 3–21.

109. Goldberg, A. P., Nowakowska, E., Antle, P. E., and Snyder, L. R., "Retention-optimization strategy for the high-performance liquid chromatographic ion-pair separation of samples containing basic compounds," *Journal of Chromatography A*, Vol. 316, 1984, pp. 241–260.

110. Yamini, Y., Reimann, C. T., Vatanara, A., and Jönsson, J. A., "Extraction and preconcentration of salbutamol and terbutaline from aqueous samples using hollow fiber supported liquid membrane containing anionic carrier," *Journal of Chromatography A*, Vol. 1124, No. 1-2, 2006, pp. 57–67.

111. Ackermans, M. T., Beckers, J. L., Everaerts, F. M., and Seelen, I. G. J. A., "Comparison of isotachophoresis, capillary zone electrophoresis and high-performance liquid chromatography for the determination of salbutamol, terbutaline sulphate and fenoterol hydrobromide in pharmaceutical dosage forms," *Journal of Chromatography A*, Vol. 590, No. 2, 1992, pp. 341–353.

112. Mälkki-Laine, L. and Hartikainen, E., "Electrokinetic behaviour of salbutamol and its decomposition products and determination of salbutamol by micellar electrokinetic capillary chromatography," *Journal of Chromatography A*, Vol. 724, No. 1-2, 1996, pp. 297–306.

113. Hammarstrand, K., "Internal standard in gas chromatography," *Varian Instrument Applications*, Vol. 10, No. 1, 1976, pp. 10–11.

114. Kurosawa, N., Morishima, S., Owada, E., and Ito, K., "Reversed-phase high-performance liquid chromatographic determination of salbutamol in rabbit plasma," *Journal of Chromatography B: Biomedical Sciences and Applications*, Vol. 305, 1984, pp. 485–488.

115. Tan, Y. K. and Soldin, S. J., "Determination of salbutamol in human serum by reversed-phase high-performance liquid chromatography with amperometric detection," *Journal of Chromatography B: Biomedical Sciences and Applications*, Vol. 311, 1984, pp. 311–317.

116. Gupta, R. N., Fuller, H. D., and Dolovich, M. B., "Optimization of a column liquid chromatographic procedure for the determination of plasma salbutamol concentration," *Journal of Chromatography B: Biomedical Sciences and Applications*, Vol. 654, No. 2, 1994, pp. 205–211.

117. "Terbutaline Sulphate," *Martindale: The Complete Drug Reference* [online]. Pharmaceutical Press, London, Electronic Version, 2009, http://www.medicinescomplete.com/mc/martindale/current/2107-a.htm [cited 22 March 2009].

118. Tan, L. C. and Carr, P. W., "Study of retention in reversed-phase liquid chromatography using linear solvation energy relationships: II. The mobile phase," *Journal of Chromatography A*, Vol. 799, No. 1-2, 1998, pp. 1–19.

119. Kromidas, S., *Practical problem solving in HPLC*, Wiley-VCH, Weinheim, New York, 2000.

120. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, "Validation of Analytical Procedures: Text and Methodology: Q2(R1)," 2006.

121. United States Pharmacopeia, "USP Subcommittee on Biopharmaceutics. In vitro/in vivo correlation for extended release oral dosage forms," *Pharmacopeial Forum*, Vol. 14, No. 4, 1988, pp. 4160–4161.

122. Hoffman, A., "Pharmacodynamic aspects of sustained release preparations," *Advanced Drug Delivery Reviews*, Vol. 33, No. 3, 1998, pp. 185–199.

123. Escudero, J. J., Ferrero, C., and Jiménez-Castellanos, M. R., "Compaction properties, drug release kinetics and fronts movement studies from matrices combining mixtures of swellable and inert polymers: Effect of HPMC of different viscosity grades," *International Journal of Pharmaceutics*, Vol. 351, No. 1-2, 2008, pp. 61–73.

124. Peppas, N. A., Gurny, R., Doelker, E., and Buri, P., "Modelling of drug diffusion through swellable polymeric systems," *Journal of Membrane Science*, Vol. 7, No. 3, 1980, pp. 241–253.

125. Lee, P. I., "Diffusional release of a solute from a polymeric matrix: Approximate analytical solutions," *Journal of Membrane Science*, Vol. 7, No. 3, 1980, pp. 255–275.

126. Kiil, S. and Dam-Johansen, K., "Controlled drug delivery from swellable hydroxypropylmethylcellulose matrices: Model-based analysis of observed radial front movements," *Journal of Controlled Release*, Vol. 90, No. 1, 2003, pp. 1–21.

127. Sujja-areevath, J., Munday, D. L., Cox, P. J., and Khan, K. A., "Relationship between swelling, erosion and drug release in hydrophillic natural gum mini-matrix formulations," *European Journal of Pharmaceutical Sciences*, Vol. 6, No. 3, 1998, pp. 207–217.

128. Conti, S., Maggi, L., Segale, L., Ochoa Machiste, E., Conte, U., Grenier, P., and Vergnault, G., "Matrices containing NaCMC and HPMC: 2. Swelling and release mechanism study," *International Journal of Pharmaceutics*, Vol. 333, No. 1-2, 2007, pp. 143–151.

129. Harland, R. S., Gazzaniga, A., Sangalli, M. E., Colombo, P., and Peppas, N. A., "Drug polymer matrix swelling and dissolution," *Pharmaceutical Research*, Vol. 5, No. 8, 1988, pp. 488–494.

130. Baveja, S. K., Ranga Rao, K. V., and Padmalatha Devi, K., "Zero-order release hydrophilic matrix tablets of [beta]-adrenergic blockers," *International Journal of Pharmaceutics*, Vol. 39, No. 1-2, 1987, pp. 39–45.

131. Ford, J. L., Mitchell, K., Rowe, P., Armstrong, D. J., Elliott, P. N. C., Rostron, C., and Hogan, J. E., "Mathematical modelling of drug release from hydroxypropylmethylcellulose matrices: Effect of temperature," *International Journal of Pharmaceutics*, Vol. 71, No. 1-2, 1991, pp. 95–104.

132. Ju, R. T. C., Nixon, P. R., and Patel, M. V., "Drug release from hydrophilic matrices. 1. New scaling laws for predicting polymer and drug release based on the polymer disentanglement concentration and the diffusion layer," *Journal of Pharmaceutical Sciences*, Vol. 84, No. 12, 1995, pp. 1455–1463.

133. Vueba, M. L., de Carvalho, L. A. E. B., Veiga, F., Sousa, J. J., and Pina, M. E., "Role of cellulose ether polymers on ibuprofen release from matrix tablets," *Drug Development and Industrial Pharmacy*, Vol. 31, No. 7, 2005, pp. 653–665.

134. Khan, G. M. and Jiabi, Z., "Formulation and in vitro evaluation of ibuprofen-carbopol® 974P-NF controlled release matrix tablets III: Influence of co-excipients on release rate of the drug," *Journal of Controlled Release*, Vol. 54, No. 2, 1998, pp. 185–190.

135. Tahara, K., Yamamoto, K., and Nishihata, T., "Overall mechanism behind matrix sustained release (SR) tablets prepared with hydroxypropyl methylcellulose 2910," *Journal of Controlled Release*, Vol. 35, No. 1, 1995, pp. 59–66.

136. Madhusudan Rao, Y., Krishna Veni, J., and Jayasagar, G., "Formulation and evaluation of diclofenac sodium using hydrophilic matrices," *Drug Development and Industrial Pharmacy*, Vol. 27, No. 8, 2001, pp. 759–766.

137. Majid Khan, G. and Bi Zhu, J., "Ibuprofen release kinetics from controlled-release tablets granulated with aqueous polymeric dispersion of ethylcellulose II: Influence of several parameters and coexcipients," *Journal of Controlled Release*, Vol. 56, No. 1–3, 1998, pp. 127–134.

138. Verhoeven, E., Vervaet, C., and Remon, J. P., "Xanthan gum to tailor drug release of sustained-release ethylcellulose mini-matrices prepared via hot-melt extrusion: *In vitro* and in *vivo evaluation*," *European Journal of Pharmaceutics and Biopharmaceutics*, Vol. 63, No. 3, 2006, pp. 320–330.

139. Hariharan, M., Wowchuk, C., Nkansah, P., and Gupta, V. K., "Effect of formulation composition on the properties of controlled release tablets prepared by roller compaction," *Drug Development and Industrial Pharmacy*, Vol. 30, No. 6, 2004, pp. 565–572.

140. Ranga Rao, K. V. and Padmalatha Devi, K., "Swelling controlled-release systems: Recent developments and applications," *International Journal of Pharmaceutics*, Vol. 48, No. 1–3, 1988, pp. 1–13.

141. Murthy, S. N. and Hiremath, S. R. R., "Formulation and evaluation of controlled-release transdermal patches of theophylline-salbutamol sulfate," *Drug Development and Industrial Pharmacy*, Vol. 27, No. 10, 2001, pp. 1057–1062.

142. Baveja, S. K. and Rao, K. V. R., "Sustained release tablet formulation of centperazine," *International Journal of Pharmaceutics*, Vol. 31, No. 1-2, 1986, pp. 169–174.

143. Siepmann, J. and Peppas, N. A., "Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC)," *Advanced Drug Delivery Reviews*, Vol. 48, No. 2-3, 2001, pp. 139–157.

144. Siepmann, J. and Peppas, N. A., "Hydrophilic matrices for controlled drug delivery: An improved mathematical model to predict the resulting drug release kinetics (the "Sequential Layer" Model)," *Pharmaceutical Research*, Vol. 17, No. 10, 2000, pp. 1290–1298.

145. Ferrero, C., Massuelle, D., Jeannerat, D., and Doelker, E., "Towards elucidation of the drug release mechanism from compressed hydrophilic matrices made of cellulose ethers. I. Pulse-field-gradient spin-echo NMR study of sodium salicylate diffusivity in swollen hydrogels with respect to polymer matrix physical structure," *Journal of Controlled Release*, Vol. 128, No. 1, 2008, pp. 71–79.

146. Siepmann, J., Kranz, H., Bodmeier, R., and Peppas, N. A., "HPMC-matrices for controlled drug delivery: A new model combining diffusion, swelling and dissolution mechanisms and predicting the release kinetics," *Pharmaceutical Research*, Vol. 16, No. 11, 1999, pp. 1748–1756.

147. Durrani, M. J., Andrews, A., Whiteker, R., and Banner, S. C., "Studies on drug release kinetics from carbomer matrices," *Drug Development and Industrial Pharmacy*, Vol. 20, No. 15, 1994, pp. 2439–2447.

148. Huang, L. L. and Schwartz, J. B., "Studies on drug release from a carbomer tablet matrix," *Drug Development and Industrial Pharmacy*, Vol. 21, No. 13, 1995, pp. 1487–1501.

149. Talukdar, M. M., Michoel, A., Rombaut, P., and Kinget, R., "Comparative study on xanthan gum and hydroxypropylmethyl cellulose as matrices for controlled-release drug delivery I. Compaction and in vitro drug release behaviour," *International Journal of Pharmaceutics*, Vol. 129, No. 1-2, 1996, pp. 233–241.

150. Conti, S., Maggi, L., Segale, L., Ochoa Machiste, E., Conte, U., Grenier, P., and Vergnault, G., "Matrices containing NaCMC and HPMC 1. Dissolution performance characterization," *International Journal of Pharmaceutics*, Vol. 333, 2007, pp. 136–142.

151. Sinha Roy, D. and Rohera, B. D., "Comparative evaluation of rate of hydration and matrix erosion of HEC and HPC and study of drug release from their matrices," *European Journal of Pharmaceutical Sciences*, Vol. 16, No. 3, 2002, pp. 193–199.

152. Lubrizol Advanced Materials, "Lubrizol pharmaceutical polymers for controlled release tablets and capsules," Vol. 30, 2009, http://www.lubrizol.com/WorkArea/linkit.aspx?LinkIdentifier=id&ItemID=33648 [cited 28 September 2009].

153. Colorcon, "Using METHOCEL cellulose ethers for controlled release of drugs in hydrophilic matrix systems," 2000, http://www.colorcon.com/literature/marketing/mr/Extended%20Release/METHOCEL/English/hydroph_matrix_broch.pdf [cited 28 September 2009].

154. Mitchell, K., Ford, J. L., Armstrong, D. J., Elliott, P. N. C., Rostron, C., and Hogan, J. E., "The influence of concentration on the release of drugs from gels and matrices containing Methocel®," *International Journal of Pharmaceutics*, Vol. 100, No. 1–3, 1993, pp. 155–163.

155. Ritger, P. L. and Peppas, N. A., "A simple equation for description of solute release I. Fickian and non-Fickian release from non-swellable devices in the form of slabs,

spheres, cylinders or discs," *Journal of Controlled Release*, Vol. 5, No. 1, 1987, pp. 23–36.

156. Ritger, P. L. and Peppas, N. A., "A simple equation for description of solute release II. Fickian and anomalous release from swellable devices," *Journal of Controlled Release*, Vol. 5, No. 1, 1987, pp. 37–42.

157. Dabbagh, M. A., Ford, J. L., Rubinstein, M. H., Hogan, J. E., and Rajabi-Siahboomi, A. R., "Release of propranolo hydrochloride from matrix tablets containing sodium carboxymethylcellulose and hydroxypropylmethylcellulose," *Pharmaceutical Development and Technology*, Vol. 4, No. 3, 1999, pp. 313–324.

158. Pérez-Marcos, B., Iglesias, R., Gómez-Amoza, J. L., Souto, C., and Concheiro, A., "Mechanical and drug release properties of atenolol-carbomer hydrophilic matrix tablets," *Journal of Controlled Release*, Vol. 17, No. 3, 1991, pp. 267–276.

159. Pérez-Marcos, B., Gutiérrez, C., Gómez-Amoza, J., Martínez-Pacheco, R., Souto, C., and Concheiro, A., "Usefulness of certain varieties of carbomer in the formulation of hydrophilic furosemide matrices," *International Journal of Pharmaceutics*, Vol. 67, No. 2, 1991, pp. 113–121.

160. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, "Quality Risk Management. ICH Q9," 2009, www.ich.org/MediaServer.jser?@_ID=3173&@_MODE=GLB [cited 9 August 2009].

161. Qureshi, S. A. and McGilveray, I. J., "Assessment of pharmaceutical quality of furosemide tablets from multinational markets," *Drug Development and Industrial Pharmacy*, Vol. 24, No. 11, 1998, pp. 995–1005.

162. DiFeo, T. J., "Safety and efficacy: The role of chemistry, manufacturing, and controls in pharmaceutical drug development," *Drug Development and Industrial Pharmacy*, Vol. 30, No. 3, 2004, pp. 247–257.

163. De Castro, W. V., Pires, M. A. S., Oliveira, M. A., Vianna-Soares, C. D., Nunan, E. A., Pianetti, G. A., Moreira-Campos, L. M., De Castro, W. V., Mertens-Talcott, S. U., and Derendorf, H., "The influence of formulation on the dissolution profile of diclofenac sodium tablets," *Drug Development and Industrial Pharmacy*, Vol. 32, No. 9, 2006, pp. 1103–1109.

164. Badawy, S. I. F., Menning, M. M., Gorko, M. A., and Gilbert, D. L., "Effect of process parameters on compressibility of granulation manufactured in a high-shear mixer," *International Journal of Pharmaceutics*, Vol. 198, No. 1, 2000, pp. 51–61.

165. Voinovich, D., Campisi, B., Moneghini, M., Vincenzi, C., and Phan-Tan-Luu, R., "Screening of high shear mixer melt granulation process variables using an asymmetrical factorial design," *International Journal of Pharmaceutics*, Vol. 190, No. 1, 1999, pp. 73–81.

166. Vojnovic, D., Chicco, D., and El Zenary, H., "Doehlert experimental design applied to optimization and quality control of a granulation process in a high shear mixer," *International Journal of Pharmaceutics*, Vol. 145, No. 1-2, 1996, pp. 203–213.

167. Fyfe, C. A. and Blazek, A. I., "Investigation of hydrogel formation from hydroxypropylmethylcellulose (HPMC) by NMR spectroscopy and NMR imaging techniques," *Macromolecules*, Vol. 30, No. 20, 1997, pp. 6230–6237.

168. Tsai, T., San, Y. P., Ho, H. O., Wu, J. S., and Sheu, M. T., "Film-forming polymer-granulated excipients as the matrix materials for controlled release dosage forms," *Journal of Controlled Release*, Vol. 51, No. 2-3, 1998, pp. 289–299.

169. Fonner, D. E. Jr., Buck, R., and Banker, G. S., "Mathematical optimization techniques in drug product design and process analysis," *Journal of Pharmaceutical Sciences*, Vol. 59, No. 11, 1970, pp. 1587–1596.

170. Schwartz, J. B., Flamholz, J. R., and Press, R. H., "Computer optimization of pharmaceutical formulations I: General procedure," *Journal of Pharmaceutical Sciences*, Vol. 62, No. 7, 1973, pp. 1165–1170.

171. Schwartz, J. B., Flamholz, J. R., and Press, R. H., "Computer optimization of pharmaceutical formulations II: Application in troubleshooting," *Journal of Pharmaceutical Sciences*, Vol. 62, No. 9, 1973, pp. 1518–1519.

172. Bodea, A. and Leucuta, S. E., "Optimization of propranolol hydrochloride sustained release pellets using a factorial design," *International Journal of Pharmaceutics*, Vol. 154, No. 1, 1997, pp. 49–57.

173. Gil, E. C., Colarte, A. I., Bataille, B., Pedraz, J. L., Rodríguez, F., and Heinämäki, J., "Development and optimization of a novel sustained-release dextran tablet formulation for propranolol hydrochloride," *International Journal of Pharmaceutics*, Vol. 317, No. 1, 2006, pp. 32–39.

174. Vijayalakshmi, P., Devi, V. K., Narendra, C., and Srinagesh, S., "Development of extended zero-order release gliclazide tablets by central composite design," *Drug Development and Industrial Pharmacy*, Vol. 34, No. 1, 2008, pp. 33–45.

175. Mcleod, A. D., Lam, F. C., Gupta, P. K., and Hung, C. T., "Optimized synthesis of polyglutaraldehyde nanoparticles using central composite design," *Journal of Pharmaceutical Sciences*, Vol. 77, No. 8, 1988, pp. 704–710.

176. Boza, A., De la Cruz, Y., Jordan, G., Jauregui-Haza, U., Aleman, A., and Caraballo, I., "Statistical optimization of a sustained-release matrix tablet of lobenzarit disodium," *Drug Development and Industrial Pharmacy*, Vol. 26, No. 12, 2000, pp. 1303–1307.

177. Chopra, S., Patil, G. V., and Motwani, S. K., "Release modulating hydrophilic matrix systems of losartan potassium: Optimization of formulation using statistical experimental design," *European Journal of Pharmaceutics and Biopharmaceutics*, Vol. 66, No. 1, 2007, pp. 73–82.

178. Bodea, A. and Leucuta, S. E., "Optimization of propranolol hydrochloride sustained-release pellets using Box–Behnken design and desirability function," *Drug Development and Industrial Pharmacy*, Vol. 24, No. 2, 1998, pp. 145–155.

179. Bodea, A. and Leucuta, S. E., "Optimization of hydrophilic matrix tablets using a D-optimal design," *International Journal of Pharmaceutics*, Vol. 153, No. 2, 1997, pp. 247–255.

180. Singh, B., Kumar, R., and Ahuja, N., "Optimizing drug delivery systems using systematic "design of experiments." Part I: Fundamental aspects," *Critical Reviews in Therapeutic Drug Carrier Systems*, Vol. 22, No. 1, 2004, pp. 27–105.

181. Lewis, G. A., "Optimization methods," *Encyclopedia of pharmaceutical technology*, edited by J. Swarbrick. Informa Healthcare, London, 2002, pp. 1922–1937.

182. Aslan, N., "Application of response surface methodology and central composite rotatable design for modeling and optimization of a multi-gravity separator for chromite concentration," *Powder Technology*, Vol. 185, No. 1, 2008, pp. 80–86.

183. Yadav, R., Devi, A., Tripathi, G., and Srivastava, D., "Optimization of the process variables for the synthesis of cardanol-based novolac-type phenolic resin using response surface methodology," *European Polymer Journal*, Vol. 43, No. 8, 2007, pp. 3531–3537.

184. Théodore, K. and Panda, T., "Application of response surface methodology to evaluate the influence of temperature and initial pH on the production of [beta]-1,3-glucanase and carboxymethylcellulase from Trichoderma harzianum," *Enzyme and Microbial Technology*, Vol. 17, No. 12, 1995, pp. 1043–1049.

185. Chang, J. S., Huang, Y. B., Hou, S. S., Wang, R. J., Wu, P. C., and Tsai, Y. H., "Formulation optimization of meloxicam sodium gel using response surface methodology," *International Journal of Pharmaceutics*, Vol. 338, No. 1-2, 2007, pp. 48–54.

186. Huang, Y. B., Tsai, Y. H., Lee, S. H., Chang, J. S., and Wu, P. C., "Optimization of pH-independent release of nicardipine hydrochloride extended-release matrix tablets using response surface methodology," *International Journal of Pharmaceutics*, Vol. 289, No. 1-2, 2005, pp. 87–95.

187. Miyamoto, Y., Ogawa, S., Miyajima, M., Matsui, M., Sato, H., Takayama, K., and Nagai, T., "An application of the computer optimization technique to wet granulation process involving explosive growth of particles," *International Journal of Pharmaceutics*, Vol. 149, No. 1, 1997, pp. 25–36.

188. Myers, R. H. and Montgomery, D. C., *Response surface methodology : Process and product optimization using designed experiments*, J. Wiley, New York, 2002.

189. Draper, N. R. and Lin, D. K. J., "Response surface designs," *Design and analysis of experiments*, edited by S. Ghosh and C. R. Rao Handbook of statistics, v. 13, North-Holland, Amsterdam, 1996, pp. 343–375.

190. Colbourn, E. A., "Neural computing in pharmaceutical formulation," Vol. 4, 2004, http://www.pharmainfo.net/reviews/neural-computing-pharmaceutical-formulation [cited 21 April 2009].

191. Paterakis, P. G., Korakianiti, E. S., Dallas, P. P., and Rekkas, D. M., "Evaluation and simultaneous optimization of some pellets characteristics using a 3^3 factorial design and the desirability function," *International Journal of Pharmaceutics*, Vol. 248, No. 1-2, 2002, pp. 51–60.

192. Chuo, W. H., Tsai, T. R., Hsu, S. H., and Cham, T. M., "Development of nifedipine-loaded albumin microspheres using a statistical factorial design," *International Journal of Pharmaceutics*, Vol. 134, No. 1-2, 1996, pp. 247–251.

193. Box, G. E. P. and Wilson, K. B., "On the experimental attainment of optimum conditions," *Journal of Royal Statistics Society Series B*, Vol. 13, No. 1, 1951, pp. 1–45.

194. Mead, R., "Response surface exploration," *The design of experiments: Statistical principles for practical applications* Cambridge University Press, Cambridge [England], 1988, pp. 538–573.

195. Zhao, W., Song, L., Deng, H., and Yao, H., "Hydration, erosion, and release behavior of guar-based hydrophilic matrix tablets containing total alkaloids of *Sophora alopecuroides*," *Drug Development and Industrial Pharmacy*, Vol. 35, No. 5, 2009, pp. 594–602.

196. Mathworks, I., "Model-Based Calibration Toolbox™ 3. Model Browser User's Guide," 2009, http://www.mathworks.com/access/helpdesk/help/pdf_doc/mbc/mbcmodel.pdf [cited 20 November 2009].

197. Moore, J. W. and Flanner, H. H., "Mathematical comparison of dissolution profiles," *Pharmaceutical Technology*, Vol. 20, No. 6, 1996, pp. 64–74.

198. Staniforth, J., "Powder flow," *Pharmaceutics : The science of dosage form design*, edited by M. E. Aulton, 2 ed. Churchill Livingstone, Edinburgh, 2002, pp. 197–210.

199. Wells, J., "Pharmaceutical preformulation: The physicochemical properties of drug substances," *Pharmaceutics : The science of dosage form design*, edited by M. E. Aulton, 2 ed. Churchill Livingstone, Edinburgh, 2002, pp. 113–138.

200. Yeole, P., Galgatte, U., Babla, I., and Nakhat, P., "Design and evaluation of Xanthan gum-based sustained release Matrix tablets of diclofenac sodium," *Indian Journal of Pharmaceutical Sciences*, Vol. 68, No. 2, 2006, pp. 185–189.

201. Rowe, R. C., Sheskey, P. J., Owen, S. C., and American Pharmacists Association., *Handbook of pharmaceutical excipients*, Pharmaceutical Press; American Pharmacists Association, Greyslake, IL, 2006.

202. Sadeghi, F., Ford, J. L., Rubinstein, M. H., and Rajabi-Siahboomi, A. R., "Comparative study of drug release from pellets coated with HPMC or Surelease," *Drug Development and Industrial Pharmacy*, Vol. 26, No. 6, 2000, pp. 651–660.

203. Rekhi, G. S., Porter, S. C., and Jambhekar, S. S., "Factors affecting the release of propranolol hydrochloride from beads coated with aqueous polymeric dispersions," *Drug Development and Industrial Pharmacy*, Vol. 21, No. 6, 1995, pp. 709–729.

204. Wu, P. C., Obata, Y., Fujikawa, M., Li, C. J., Higashiyama, K., and Takayama, K., "Simultaneous optimization based on artificial neural networks in ketoprofen hydrogel formula containing O-ethyl-3-butylcyclohexanol as percutaneous absorption enhancer," *Journal of Pharmaceutical Sciences*, Vol. 90, No. 8, 2001, pp. 1004–1014.

205. Takayama, K., Fujikawa, M., Obata, Y., and Morishita, M., "Neural network based optimization of drug formulations," *Advanced Drug Delivery Reviews*, Vol. 55, No. 9, 2003, pp. 1217–1231.

206. Takayama, K., Morva, A., Fujikawa, M., Hattori, Y., Obata, Y., and Nagai, T., "Formula optimization of theophylline controlled-release tablet based on artificial neural networks," *Journal of Controlled Release*, Vol. 68, No. 2, 2000, pp. 175–186.

207. Sun, Y., Peng, Y., Chen, Y., and Shukla, A. J., "Application of artificial neural networks in the design of controlled release drug delivery systems," *Advanced Drug Delivery Reviews*, Vol. 55, No. 9, 2003, pp. 1201–1215.

208. Bourquin, J., Schmidli, H., van Hoogevest, P., and Leuenberger, H., "Comparison of artificial neural networks (ANN) with classical modelling techniques using different experimental designs and data from a galenical study on a solid dosage form," *European Journal of Pharmaceutical Sciences*, Vol. 6, No. 4, 1998, pp. 287–300.

209. Hussain, A. S., Yu, X. Q., and Johnson, R. D., "Application of neural computing in pharmaceutical product development," *Pharmaceutical Research*, Vol. 8, No. 10, 1991, pp. 1248–1252.

210. Rowe, R. C. and Roberts, R. J., "Artificial intelligence in pharmaceutical product formulation: Neural computing and emerging technologies," *Pharmaceutical Science & Technology Today*, Vol. 1, No. 5, 1998, pp. 200–205.

211. Ebube, N. K., "Intelligent preformulation design and predictions using artificial neural networks," *Preformulation solid dosage form development*, edited by M. C. Adeyeye and H. G. Brittain Drugs and the pharmaceutical sciences, 178, Informa Healthcare USA, New York, 2008, pp. 81–114.

212. Erb, R. J., "Introduction to backpropagation neural network computation," *Pharmaceutical Research*, Vol. 10, No. 2, 1993, pp. 165–170.

213. Peh, K. K., Lim, C. P., Quek, S. S., and Khoh, K. H., "Use of artificial neural networks to predict drug dissolution profiles and evaluation of network performance using similarity factor," *Pharmaceutical Research*, Vol. 17, No. 11, 2000, pp. 1384–1388.

214. Achanta, A. S., Kowalski, J. G., and Rhodes, C. T., "Artificial neural networks: Implications for pharmaceutical sciences," *Drug Development and Industrial Pharmacy*, Vol. 21, No. 1, 1995, pp. 119–155.

215. Beale, R. and Jackson, T., *Neural computing : An introduction*, Hilger, Bristol, 1990.

216. Wythoff, B. J., "Backpropagation neural networks: A tutorial," *Chemometrics and Intelligent Laboratory Systems*, Vol. 18, No. 2, 1993, pp. 115–155.

217. Chen, Y., McCall, T. W., Baichwal, A. R., and Meyer, M. C., "The application of an artificial neural network and pharmacokinetic simulations in the design of controlled-release dosage forms," *Journal of Controlled Release*, Vol. 59, No. 1, 1999, pp. 33–41.

218. Zupancic Bozic, D., Vrecer, F., and Kozjek, F., "Optimization of diclofenac sodium dissolution from sustained release formulations using an artificial neural network," *European Journal of Pharmaceutical Sciences*, Vol. 5, No. 3, 1997, pp. 163–169.

219. Ibric, S., Jovanovic, M., Djuric, Z., Parojcic, J., and Solomun, L., "The application of generalized regression neural network in the modeling and optimization of aspirin extended release tablets with Eudragit® RS PO as matrix substance," *Journal of Controlled Release*, Vol. 82, No. 2-3, 2002, pp. 213–222.

220. Takahara, J., Takayama, K., and Nagai, T., "Multi-objective simultaneous optimization technique based on an artificial neural network in sustained release formulations," *Journal of Controlled Release*, Vol. 49, No. 1, 1997, pp. 11–20.

221. Takayama, K., Takahara, J., Fujikawa, M., Ichikawa, H., and Nagai, T., "Formula optimization based on artificial neural networks in transdermal drug delivery," *Journal of Controlled Release*, Vol. 62, No. 1-2, 1999, pp. 161–170.

222. Plumb, A. P., Rowe, R. C., York, P., and Doherty, C., "The effect of experimental design on the modeling of a tablet coating formulation using artificial neural networks," *European Journal of Pharmaceutical Sciences*, Vol. 16, No. 4-5, 2002, pp. 281–288.

223. Looney, C. G., "Advances in feedforward neural networks: Demystifying knowledge acquiring black boxes," *IEEE Transactions on Knowledge and Data Engineering*, Vol. 8, No. 2, 1996, pp. 211–226.

224. Hecht-Nielsen, R., "Kolmogorov's mapping neural network existence theorem," *First IEEE International Joint Conference of Neural Networks*, 1987, pp. 11–14.

225. Carpenter, W. C. and Hoffman, M. E., "Understanding neural network approximation and polynomial approximations helps neural network performance," March, 1995, pp. 31–33.

226. Plumb, A. P., Rowe, R. C., York, P., and Brown, M., "Optimisation of the predictive ability of artificial neural network (ANN) models: A comparison of three ANN programs and four classes of training algorithm," *European Journal of Pharmaceutical Sciences*, Vol. 25, No. 4-5, 2005, pp. 395–405.

227. MacGregor, J. F. and Bruwer, M.-J., "A framework for the development of design and control spaces," *Journal of Pharmaceutical Innovation*, Vol. 3, No. 1, 2008, pp. 15–22.

228. Cogdill, R. P. and Drennen, J. K., "Risk-based quality by design (QbD): A Taguchi perspective on the assessment of product quality, and the quantitative linkage of drug

product parameters and clinical performance," *Journal of Pharmaceutical Innovation*, Vol. 3, No. 1, 2008, pp. 23–29.

229. U.S.Food and Drug Administration (FDA), "Pharmaceutical cGMPs for the 21st Century: A risk-based approach," 2009, http://www.fda.gov/Drugs/DevelopmentApprovalProcess/Manufacturing/QuestionsandAnswersonCurrentGoodManufacturingPracticescGMPforDrugs/ucm137175.htm [cited 5 August 2009].

230. Garcia, T., Cook, G., and Nosal, R., "PQLI key topics: Criticality, design space and control strategy," *Journal of Pharmaceutical Innovation*, Vol. 3, No. 2, 2008, pp. 60–68.

231. Lepore, J. and Spavins, J., "PQLI Design space," *Journal of Pharmaceutical Innovation*, Vol. 3, No. 2, 2008, pp. 79–87.

232. Duchesne, C. and Macgregor, J. F., "Establishing multivariate specification regions for incoming materials," *Journal of Quality Technology*, Vol. 36, No. 1, 2004, pp. 78–94.

233. Wold, S., Esbensen, K., and Geladi, P., "Principal component analysis," *Chemometrics and Intelligent Laboratory Systems*, Vol. 2, No. 1–3, 1987, pp. 37–52.

234. Zidan, A. S., Sammour, O. A., Hammad, M. A., Megrab, N. A., Habib, M. J., and Khan, M. A., "Quality by design: Understanding the formulation variables of a cyclosporine A self-nanoemulsified drug delivery systems by Box–Behnken design and desirability function," *International Journal of Pharmaceutics*, Vol. 332, No. 1-2, 2007, pp. 55–63.

235. Takayama, K., "Computer-aided design and optimisation for pharmaceutical formulations," *European Journal of Pharmaceutical Sciences*, Vol. 34, No. 1, Supplement 1, 2008, pp. S10.

236. Kikuchi, S. and Takayama, K., "Reliability assessment for the optimal formulations of pharmaceutical products predicted by a non-linear response surface method," *International Journal of Pharmaceutics*, Vol. 374, No. 1-2, 2009, pp. 5–11.

237. Kikuchi, S. and Takayama, K., "Multivariate statistical approach to optimizing sustained-release tablet formulations containing diltiazem hydrochloride as a model highly water-soluble drug," *International Journal of Pharmaceutics*, Vol. 386, No. 1-2, 2010, pp. 149–155.

238. Huang, J., Kaul, G., Cai, C., Chatlapalli, R., Hernandez-Abad, P., Ghosh, K., and Nagi, A., "Quality by design case study: An integrated multivariate approach to drug product and process development," *International Journal of Pharmaceutics*, Vol. 382, No. 1-2, 2009, pp. 23–32.

239. Peterson, J. J., "A Bayesian approach to the ICH Q8 definition of design space," *Journal of Biopharmaceutical Statistics*, Vol. 18, No. 5, 2008, pp. 959–975.

240. Harms, J., Wang, X. Y., Kim, T., Yang, X. M., and Rathore, A. S., "Defining process design space for biotech products: Case study of *Pichia pastoris* fermentation," *Biotechnology Progress*, Vol. 24, No. 3, 2008, pp. 655–662.

241. Lipsanen, T., Antikainen, O., Räikkönen, H., Airaksinen, S., and Yliruusi, J., "Novel description of a design space for fluidised bed granulation," *International Journal of Pharmaceutics*, Vol. 345, No. 1-2, 2007, pp. 101–107.

242. Verma, S., Lan, Y., Gokhale, R., and Burgess, D. J., "Quality by design approach to understand the process of nanosuspension preparation," *International Journal of Pharmaceutics*, Vol. 377, No. 1-2, 2009, pp. 185–198.

243. Maltesen, M. J., Bjerregaard, S., Hovgaard, L., Havelund, S., and van de Weert, M., "Quality by design - Spray drying of insulin intended for inhalation," *European Journal of Pharmaceutics and Biopharmaceutics*, Vol. 70, No. 3, 2008, pp. 828–838.

244. Yamashita, F., Itoh, T., Yoshida, S., Haidar, M. K., and Hashida, M., "A novel multi-dimensional visualization technique for understanding the design parameters of drug formulations," *Computers & Chemical Engineering*, In Press, Corrected Proof.

245. Bourquin, J., Schmidli, H., van Hoogevest, P., and Leuenberger, H., "Advantages of artificial neural networks (ANNs) as alternative modelling technique for data sets showing non-linear relationships using data from a galenical study on a solid dosage form," *European Journal of Pharmaceutical Sciences*, Vol. 7, No. 1, 1998, pp. 5–16.

246. Bourquin, J., Schmidli, H., van Hoogevest, P., and Leuenberger, H., "Pitfalls of artificial neural networks (ANN) modelling technique for data sets containing outlier measurements using a study on mixture properties of a direct compressed dosage form," *European Journal of Pharmaceutical Sciences*, Vol. 7, No. 1, 1998, pp. 17–28.

247. Gough, P., "ICH Q8 pharmaceutical development and ICH Q9 quality risk management: An industry view," 2009, http://www.iob.org/userfiles/File/1030.pdf [cited 9 August 2009].

248. Gough, P., "ICH Q8, 9 and 10. The history and overview," 2009, http://www.iob.org/userfiles/ICH%20Q8%209%2010%20The%20Story%20So%20Far%20-%20Peter%20Gough(1).pdf [cited 9 August 2009].