Pharmaceutical Technology Division
Department of Pharmacy
Faculty of Science
University of Helsinki

Opening New Perspectives for Visual

Characterisation of Pharmaceutical Solids

by

Niklas Laitinen

Academic Dissertation

To be presented with the permission
of the Faculty of Science of the University of Helsinki,
for public criticism in Auditorium 1 at Viikki Infocentre (Viikinkaari 11A)
on June 13th, 2003, at 12 noon

Helsinki 2003

Supervisor: Professor Jouko Yliruusi
                Pharmaceutical Technology Division
                Department of Pharmacy
                University of Helsinki
                Finland

Reviewers: Docent Jyrki Heinämäki
                Pharmaceutical Technology Division
                Department of Pharmacy
                University of Helsinki
                Finland

                Docent Jorma Laaksonen
                Laboratory of Computer and Information Science
                Department of Computer Science and Engineering
                Helsinki University of Technology
                Finland

Opponent: Dr. Robert T. Forbes
                School of Pharmacy
                University of Bradford
                United Kingdom

ABSTRACT

Laitinen, N.O., 2003.

Opening New Perspectives for Visual Characterisation of Pharmaceutical Solids.

Dissertationes Biocentri Viikki Universitatis Helsingiensis 16/2003, pp. 59.
ISBN 952-10-1037-1, ISBN 952-10-1038-X (pdf), ISSN 1239-9469

The physical characterisation of pharmaceutical solids is fundamental in the drug development process. At present, the utilisation of descriptive image information in pharmaceutical technology is very limited. Subsequently, the development of this discipline creates a challenge within the characterisation of pharmaceutical solids. Research contributions aiming to improve the efficiency of pharmaceutical manufacturing processes are needed. The attempts in developing image-based particle characterisation tools for pharmaceutical powders should strive for reliable, fast, and easily usable methods. New ideas in the field of visual characterisation can broaden the scope of analytical techniques in pharmaceutical technology.

The aim of this study was to find new ways of using image information in pharmaceutical powder technology and characterisation of pharmaceutical solids. The goal was to extract relevant information from powder surfaces with the aim to broaden the use of image information compared to the commonly used image analysis (IA) approaches in pharmaceutics, which only measure properties of individual particles. Moreover, the aim was to link the information from powder surface images with functional physical properties of pharmaceutical solids and to enhance the use of surface imaging as a tool within pharmaceutical process analytical technologies.

The characterisation of particulate populations through surface images was successful, with respect to classification of powder images, measurement of the particle size of granular materials and the prediction of tabletting behaviour of granules. A new parameter for describing the particle size of granular material, the grey scale difference matrix (GSDM), was developed and used effectively in particle size measurement. The idea of using a content-based image retrieval (CBIR) technique for pharmaceutical powder images was also introduced and a basis was laid for the future use of CBIR in powder characterisation. In addition, the presented visual characterisation approach was effectively used as a process analytical tool. The utilisation of surface image information to assure acceptable end product quality at the completion of a granulation process was also possible.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

List of abbreviations and mathematical symbols

Abbreviations

ANN	Artificial neural network
API	Active pharmaceutical ingredient
BMP	Bit map picture
CBIR	Content-based image retrieval
FDA	Food and drug administration
GSDM	Grey scale difference matrix
IA	Image analysis
MCC	Microcrystalline cellulose
PAT	Process analytical technologies
PCA	Principal component analysis
PLS	Partial least squares projection to latent variables
PSD	Particle size distribution
PVP	Polyvinylpyrrolidone
QBIC	Query by image content
SEM	Scanning electron microscopy
SOM	Self-organising map
3-D	Three-dimensional

Symbols

C	Contrast, texture feature in QBIC
D	Directionality, texture feature in QBIC
O	Coarseness, texture feature in QBIC
PC	Principal component in PCA
Q^2	Predicted variation in multivariate regression and PLS modelling
R^2	Predictive power in multivariate regression and PLS modelling
t	Score values in PCA
δ	Variance factor in QBIC texture feature

List of original publications

This thesis is based on the following original papers, which are referred to in the text by the Roman numerals I-IV.

I   Laitinen, N., Antikainen, O., Mannermaa, J-P. and Yliruusi, J. 2000 Content-based image retrieval: a new promising technique in powder technology. Pharmaceutical Development and Technology , 5(2), 171-179.

II   Laitinen, N., Rantanen, J., Laine, S., Antikainen O., Räsänen, E., Airaksinen, S. and Yliruusi, J. 2002. Visualization of particle size and shape distributions using self-organizing maps. Chemometrics and Intelligent Laboratory Systems, 62(1), 47–60.

III   Laitinen, N., Antikainen, O. and Yliruusi J. 2002. Does a powder surface contain all necessary information for particle size distribution analysis? European Journal of Pharmaceutical Sciences, 17(4-5), 217-227.

IV   Laitinen N., Antikainen O., Rantanen, J. and Yliruusi, J. 2003. New perspectives for visual characterization of pharmaceutical solids. (submitted)

1. INTRODUCTION

"A picture tells more than a thousand words". Visual information and seeing is considered to be an indicator of truth and it is thus connected to reason. On the other hand seeing is linked with emotions and is therefore associated with a certain amount of subjectivity. If visual or image information is used in science, exact descriptors for this information are needed. The utilisation of descriptive image information in pharmaceutical technology is rather limited. Subsequently, the development of this discipline is a challenge within physical characterisation of pharmaceutical solids.

The physical characterisation of pharmaceutical solids is fundamental in the drug development process. Complete physical characterisation of solid materials is possible with high-resolution analytical techniques on the molecular, particulate and bulk levels (Brittain et al., 1991, Pifferi, 1999, Stephenson et al., 2001). Byrn et al. (1995) introduced decision trees for the controlling of different crystal forms to develop information on pharmaceutical solids for both scientific and regulatory purposes. Their aim was to anticipate towards direct approach in the characterisation of pharmaceutical solids and eventually to faster approval of regulatory documents containing information on pharmaceutical solids. The use of solid-state spectroscopy techniques used in the physical characterisation of the active pharmaceutical ingredient (API), excipients, physical mixtures, and the final dosage form has been recently reviewed (Bugay, 2001). Stephenson et al. (2001) have addressed the quantitative issues and methods in solid-state characterisation. The usual molecular-level properties are addressed using infrared spectroscopy and nuclear magnetic resonance spectrometry. Important particulate-level properties are particle morphology and particle size distribution, not to mention properties that are revealed using powder X-ray diffraction and thermal methods of analysis. Important bulk-level properties are e.g. surface area, porosity and pore size distribution and powder flow characteristics (Brittain et al., 1991).

The characterisation of powders and granular materials is of great interest within pharmaceutical sciences. There has been awareness for characterisation of particulate materials in the pharmaceutical sciences for a long time (Rees, 1977). As approximately 80 % of all drug products are solids i.e. tablets or capsules, the understanding of the physical characteristics of powders and granules is essential (Muzzio et al., 2001). For the

pharmaceutical industry, a comprehensive knowledge of these materials has a major economic impact. The physical characteristics of solid particulates have to be considered and studied throughout the development process of a product, from the preformulation stage to large-scale manufacturing. In development and manufacturing many powder handling steps are involved, including crystallisation, blending, granulation and compaction. Thus, different kind of interactions between particles and between particles and process equipment occur. All these interactions together with specific behaviour of bulk materials in certain unit operations may give rise to many problems.

Development of process analytical technologies aims at improving the efficiency of drug manufacturing processes. At present, the Food and Drug Administration (FDA) is establishing guidelines for process analytical technologies (PAT) to facilitate the introduction of new technologies for the pharmaceutical industry. The development aims at systems for analysis and control of manufacturing processes based on timely measurements of critical quality parameters and performance attributes of raw and in-process materials. The initiative will focus on technologies that will assure acceptable end product quality at the completion of the process.

Research contributions aiming to improve the efficiency of pharmaceutical manufacturing processes are needed. The attempts in developing image-based particle characterisation tools for pharmaceutical powders should strive for reliable, fast, and easily usable methods. New ideas in the field of visual characterisation will broaden the scope of analytical techniques in pharmaceutical technology. These new tools should be exploited comprehensively in terms of potential use in the full development cycle of pharmaceutical solids.

2. REVIEW OF THE LITERATURE

2.1 Pharmaceutical powders and granules

The fields of pharmaceutical product development and manufacturing, which mostly deal with particle technology, should focus more on physical characterisation of pharmaceutical solids, especially powders and granules (Muzzio et al., 2002). The present literature review concentrates on physical characteristics of particulate and bulk level properties of pharmaceutical powders and granules. In the next sections particle size and shape properties are discussed together with a brief insight into common particle size analysis methods. Also the fundamental phenomena of segregation, flowability, adhesion, cohesion and triboelectricity are covered. A special emphasis is put on imaging techniques and common data projection techniques are also described.

2.1.1 Classification of powders

Most often the term powder technology will cover both powders and granules. APIs or drug substances and excipients as such are often regarded as powders. Granules are usually considered to be a product of a size enlargement process i.e. granulation. According to British Standard 2955 (1991) a powder consists of dry, discrete particles with a maximum dimension of 1000 µm. A classification of pharmaceutical particles based on their size is given by Barber (1993): coarse powders >1000 µm, conventional powders 50-1000µm, fine particles 1-50 µm, very fine (submicron) particles 0.1-1 µm and ultrafine particles < 0.1 µm.

2.1.2 The complexity of powder systems

A powder is a complex system with solid–solid and solid-air interactions, since a gaseous phase exists between the solid particles. Powders are unlike other physical states of matter. Powders are related to solids since they can resist deformation. Powders also posses the ability to flow like liquids do and they can be compressed like gases (Swarbrick and Boylan, 1988, Geldhart, 1990). Particle size, shape and adsorption of the gaseous phase on the surface influence the properties of a powder or granular material (Geldhart, 1990). Consequently, the bulk behaviour or the behaviour of the collective

properties of the material is influenced by these factors. The bulk material will usually go through several powder handling situations and processes. This requires fundamental understanding of the nature of the particles, their size, shape, surface morphology, packing conditions and interparticulate forces. The handling of a bulk mass is influenced by any factor which has an effect on interactions between particles. Early reviews on particle-particle interactions of powders can be found (Hiestand, 1966). Still today, research in powder technology concentrates on relationships between particle properties and bulk behaviour of industrial powders (Hoffman et al., 1996).

2.1.3 Particle size

The bulk properties of a material depend to a great extent on its particle size distribution (Barber, 1993). The size, as a scalar measure, describes the dimensions of a particle in a population. The size of spherical and other regular particles is easy to define, but it is impossible to use linear measures for determining sizes of irregularly shaped particles. Derived diameters, so called equivalent diameters, are determined by measuring a size-dependent property of the particle, which is then related to a single linear dimension. Particle sizes of irregular particles are usually presented as equivalent spherical diameters. Typical equivalent size parameters are listed in the literature (Allen, 1990, Barber, 1993, Washington, 1992). Normally the equivalence is established on particle mass, volume, projected 2-dimensional area or sedimentation rate diameters. Examples of the most typical spherical equivalence diameters (d) are expressed with the equations 1-3.

Volume diameter d_v : $\qquad V = \dfrac{\pi}{6} d_v^3$ $\qquad\qquad\qquad$ (Eq. 1)

Surface area diameter d_s : $\quad S = \pi d_s^2$ $\qquad\qquad\qquad$ (Eq. 2)

Projected area diameter d_a : $A = \dfrac{\pi}{4} d_a^2$ $\qquad\qquad\qquad$ (Eq. 3)

The particle size measurement results are most often presented with a particle size distribution (PSD). Depending on the measurement technique employed, e.g. number,

volume and mass distributions are used. Mathematical transformations between the different kinds of distributions can always be made.

The PSD and surface area of an API are properties of great importance with regard to the bioavailability and the processing of drug products. The regulatory authorities have made international guidelines for acceptance criteria for particle size distributions of new drug substances (ICH Q6A, 1999). Within the specific guideline, specifications should be included if the particle size has an impact on the quality of the drug substance or the final product. The particle size has to be evaluated with respect to whether it has a significant effect on dissolution rates, bioavailability, stability, prosessability, drug product content uniformity and appearance. These aspects and the influence of particle size on physicochemical properties of pharmaceutical powders have been extensively studied (Atkinson et al., 1962, Lees, 1963, Kaneniwa et al., 1967, Ikekawa and Kaneniwa, 1968, Hunter and Ganderton, 1972, Walton and Pilpel, 1972, Jounela et al., 1975, York, 1978, Hintz and Johnson, 1989, Ibrahim et al., 1988, Yalkowsky and Bolton, 1990, Johnson and Swindell, 1996, Bønløkke et al., 2001, Scholz et al., 2002).

2.1.4 Particle shape

It is very difficult to define shapes of irregular particles. The usual means to describe shapes include terms such as acicular, angular, fibrous and flaky (BS 2955). These common descriptions do not quantitatively illustrate a shape. Numerous researchers have worked with defining shape parameters, such as, roundness, sphericity, circularity, flakiness, etc. (Cox, 1927, Wadell, 1932, Heywood, 1937, 1954). Also approaches based on Fourier descriptors and fractal geometry has been used in representation of particle shape and morphology (Meloy, 1977a, 1977b, Kaye, 1981, Luekens, 1982, Rösler, 1987, Carstensen and Franchini, 1993). The methods of shape characterisation have been reviewed by Kaye (1997), Allen (1990) and Barber (1993). Within pharmaceutical technology shape factors have also been used and studied in analysis of powders, granules and pellets (Ridgway and Rupp, 1969, Staniforth, 1987, Hellén, 1993, Podczeck and Newton, 1994, 1995, Podczeck et al., 1999).

2.1.5 Segregation

Differences in particle size, shape and density in a powder system may cause segregation, e.g. during mixing or the handling of a tabletting mass. The difference in particle size is the most important factor (Williams, 1990). The particle size distribution of both drugs and excipients will have consequences on the mixing phenomena and potential segregation of mixed materials (Carson, 1988, Wong and Pilpel, 1990). Segregation of coarse and fine particles can result tablet die fill variations and hence weight variation in tablets. Travers (1988) and Johanson (1996) have described the most usual mechanisms of segregation (1996). The ability of smaller particles to fit between coarser particles is critical for segregation. A size difference of three times or more in particle diameter may cause considerable segregation (Johanson, 1996).

2.1.6 Adhesion, cohesion and triboelectrification

Profound forces that have an impact on bulk behaviour are adhesion and cohesion. Cohesion occurs between similar surfaces (particle-particle) and adhesion e.g. between the particle and an instrument wall. The forces are mainly non-specific short-range van der Waal's forces. The van der Waal's forces increase when particle size decreases and diverge with changes in relative humidity. Other attractive forces in interparticulate cohesion can be electrostatic forces, which arise from contact or frictional charging (Staniforth, 1998). Generally, particles are charged statically by grinding, attrition and collision or triboelectrification. When dry particles are sieved, mixed and moved through a hopper, surface charge can also be generated. According to Staniforth (1982) excipients are normally charged negatively in contact with metal or glass surfaces, while positive charges are created with plastic surfaces. Moisture, particulate contamination and method of cleaning of processing equipment during pharmaceutical manufacturing operations may influence the electrostatic behaviour of powders (Eilbeck et al., 2000). Rowley (2001) studied pharmaceutical solid systems and found that charge acquisition was inversely related to particle size where contact surface contamination was negligible. Electrostatic studies on pharmaceutical powders have recently been made by Murtomaa (2002). Pharmaceutical compounds are cohesive in nature meaning that their flow characteristics are most likely undesirable (Brittain et al., 1991). A typical way of reducing

the cohesivity and improving flowability of materials is by different granulation techniques.

2.1.7 Flowability

The importance of flow properties in powder handling cannot be overemphasized (Swarbrick and Boylan, 1988). Carr (1965) has done fundamental work in evaluation and classification of the flow properties of solids. In drug product formulation work the understanding of powder flow properties is essential. Flowability influences and may cause problems in the mixing of powders, as well as tabletting and encapsulation processes. The powder flow properties are related to weight variation in tablets and capsules and consequently dose uniformity. For the pharmaceutical industry, problems with flowability can be critical and cause major economic setbacks (Prescott and Hossfield, 1994). The particle size of a material has an impact on the flow properties of a powder (Staniforth, 1988). Particles larger than 250 µm are usually relatively free flowing. Finer particles (<100 µm) with larger surface areas tend to be more cohesive, which results flow problems. The flow rate and particle size has been shown to have a strong correlation (Gold et al., 1968, Danish and Parrot, 1971). However, the particle shape also affects the flow properties. Spherical particles have least interparticle contacts and good flow properties. The flowability of elongated and irregular particles is poorer. Thus, powders with similar particle size but different shape characteristics may have very different flow properties. The effects of particle shape on particle flow have been extensively studied (Ridgeway and Rupp, 1969, Danjo et al., 1989, Cartilier and Tawashi, 1993).

2.2. Particle size measurement techniques

Various techniques for measuring the particle size distribution of powders exist. A single measurement technique cannot be used to cover the wide size range from nanometres to millimetres. Moreover, the many aspects have to be considered before making the proper choice of measurement principle e.g. the capital costs versus running costs, speed of operation, degree of skill required for operation, and most important, the end-use requirement (Show et al., 1998). Different particle size analysis techniques are well described in the literature (Allen, 1990, Washington, 1992, Iacocca and German, 1997,

Show et al., 1998). Microscopy or computer-assisted microscopy is often considered as a reference method in particle size analysis since they enable visual inspection of particles. Probably the most common particle-sizing methods for pharmaceutical dry powders and granules are sieve analysis, laser diffraction and computerised image analysis techniques. According to Brittain (1991) microscopy and sieving are normally carried out on dry powders and are truthful indicators of the actual particle size of a powdered solid. Measuring particle size distributions by sieving is a simple and inexpensive method and it gives reproducible results.

Nevertheless, there are many problems in sieving. For reaching better accuracy, great care of sieves and careful calibrations are necessary (Allen, 1997). Sieving is a useful technique in particle sizing if the goal is to produce a certain size fraction. It is also convenient for easily flowing and fairly coarse material above e.g. 100 μm with a few fines (Washington, 1992). The choice of sieving time can have considerable effects on the results and the amount of material sieved may affect reproducibility. Material cohesiveness may also cause errors in measurements and result in false size distributions. The amount of sample in sieving is relatively large, therefore, it is not suitable for expensive materials or materials of which only small quantities are available. Sieve analysis is also very time consuming.

Optical microscopy has a magnification limit approximately of 600x. Electron microscopy may be utilised at remarkably higher magnifications; it gives more information about surface structures. Simple microscopic examination is relatively slow and the information is only visual and descriptive. However, microscopy can be used in conjunction with image analysis systems. Microscopy is often used as an absolute method of particle size analysis (Allen, 1997). Yet, there are some problems encountered in microscopic examination of particles. When three-dimensional particles are studied they usually lie in their energetically most favourable position and tend to show their maximum area to the viewer. Since particles orientate in this manner particle sizes have a tendency to be somewhat larger than measured with other methods. The analysis can be time consuming. A relatively small number of particles is usually analysed, which may lead to statistical errors. The introduction of combined microscopy and computerised analysis systems has made particle studies faster, consequently more particles can be

examined. Sample preparation plays a significant role for successful particle size analysis in microscopy (Iacocca and German, 1997).

Washington (1992) lists various errors and disadvantages of laser diffraction size analysis, such as poor submicrometer performance and computing artefacts. A disadvantage is that the amount of sample is very large when using the particles in air (PIA) -method. For small particles, the particles in liquid (PIL) -method is used in order to achieve an effective dispersion. For some materials, finding a well-dispersing and suitable solvent might be difficult. There are many advantages with laser diffraction analyses, for example, the ease of operation and that the instruments usually produce very reproducible results. Light scattering methods do not produce flawless data for particles in all size ranges (Iacocca and German, 1997). Inaccuracy is created e.g. by particle aggregation, multiple particles in the measurement zone, unknown or improper relative refractive index.

Instruments that are based on measurements of a particle's aerodynamic time-of-flight have been developed and have gained a lot of popularity in particle size analysis of pharmaceutical materials, especially within aerosol sciences (Dahneke, 1973, Dahneke and Cheng, 1979, Niven, 1993, Mitchell and Nagel, 1996, Laitinen and Juppo, 2003). Other methods within pharmaceutical particle size analysis include electrical sensing zone (Beaubien and Vanderwielen, 1980), sedimentation and photon correlation spectroscopy. Comparative studies between methods have been made (Kanerva et al., 1993, Etzler and Sanderson, 1995, Andrès et al., 1996, Etzler and Deanne, 1997, Kaye et al., 1997). On-line techniques for particle size process control have been developed and reported, including image processing-based systems and light scattering methods (Lin and Miller, 1993, Watano and Miyanami, 1995, Bonifazi, 1997, Novales et al., 1998, Scott et al., 1998, Perry, 1998, Watano et al., 2000, Bordes et al., 2002). The concept of computerised image analysis is described more thoroughly below.

2.3. Image analysis

2.3.1 Digital image information

A digital image consists of picture elements called pixels. The pixels contain information about the brightness of a certain location in the image. Therefore, the term monochrome image or a black and white image refers to a two-dimensional matrix of pixels with particular levels of brightness. Only monochrome images are covered in this text since these were used in the experimental part of this thesis. The concept of digital images is well described in textbooks (Sonka et al., 1998, Gonzalez and Woods, 2001). The image and its two-dimensional pixel matrix can be presented as a light intensity function $f(x,y)$, where x and y are discrete valued spatial coordinates and f at the point (x,y) is proportional to the brightness of the image at that point. Depending on the application and imaging resolution, the size of the images i.e. size of its brightness matrix can vary. In monochrome images, for each pixel, the grey level that corresponds to the average transmitted beam from the surface of the object, is typically characterised by a number in the 0–255 range. In this range 0 is totally black and 255 completely white. Figure 1 illustrates the concept of pixels and grey level values in an image.
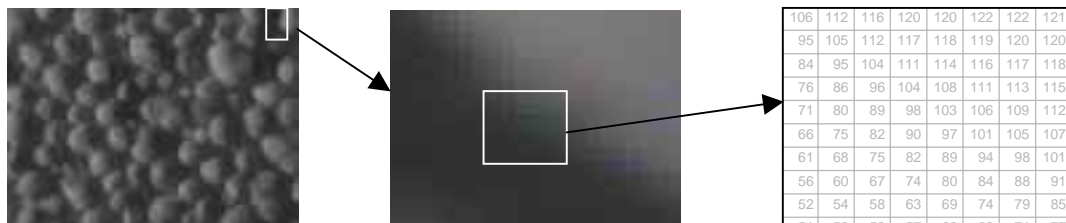
Figure 1. The concept of a digital image. Left: original digital image (resolution 800x600), Middle: top right corner of the original image with visible pixels, Right: gray level values of the white rectangular area from the previous image.

In an image the three-dimensional (3-D) reality is projected on a plane. A certain amount of 3-D characteristics is often required in order to get quantitative information about particle morphology. To acquire 3-dimensionality in images, viewing under different angles is possible (Russ, 1999). Furthermore, depending how the picture is produced different amount of 3-D features can be distinguished. For example, optical microscopy has a poorer depth of field than scanning electron microscopy. 3-D features can also be revealed using lateral illumination (Pons et al., 1999). These features are connected to

shading effects that expose the topography or the visual texture of an object or a surface (Fig. 2.). A rough structure produces an image with large grey scale variations and smoother structures generate images with smaller grey scale variations. If we consider particulate analysis, in controlled illumination conditions comparisons between materials can be made. The challenge is to find, extract and quantify the information that is produced.
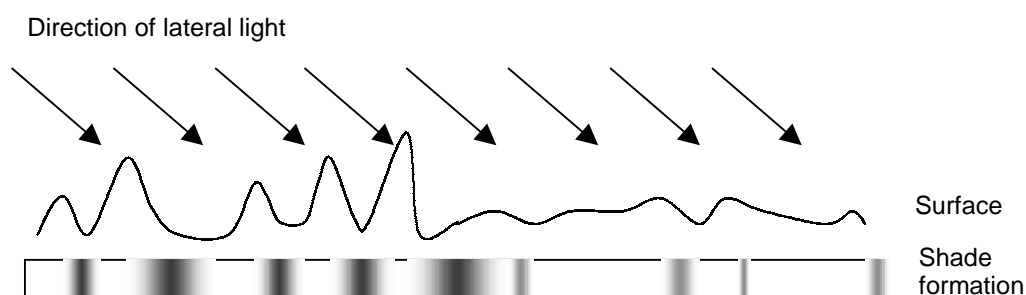
Figure 2. Shade formation using lateral illumination.

2.3.2 Computerised image analysis in powder technology

Computerised analyses of microscope pictures have gained much popularity in particle size and shape analysis since they allow relatively fast handling of a great amount of information in a picture. Computerised systems make particle size measurements faster and more practical. Image analysis (IA) systems allow the user to process images. However, Pons et al. (1999) have discussed the reluctance of the use of IA in routine analysis of particle morphology e.g. due to relative slowness of the process and the large size of the image as a dataset. Orientation effects of particles can also distort the generated IA data (Turbitt-Daoust et al., 2000).

By image processing it is possible to correct defects in the measured particles, e.g. enhance the visibility of particular structures, outline the particles from the background and perform steps to separate touching objects or select those particles to be measured (Russ, 1999). The field of image processing is used for two purposes: to improve visual appearances of images and to prepare images for measurement of characteristic features and structures. In general, image processing and analysis methods include the steps of image acquisition, preprocessing, segmentation, data extraction and data representation

(Nazar et al., 1996, Russ, 1999). Image acquisition means digitalisation of the picture, e.g. transformation of the picture to numerical values for the computer. Correcting image faults such as noise and brightness defects and enhancing distinct characteristics of images falls under preprocessing. In segmentation, the aim is to select the objects of interest. Different image analysis systems apply many operations for separating objects from each other with procedures such as skeletonisation, watershed and erosion-dilation. In traditional IA of powders and granules different particle size and shape parameters of individual particles are then measured.

Since image processing tasks are needed the IA procedure is usually performed semi-automatically and a skilled operator is usually needed. The largest source of error in optical IA is probably the sample preparation i.e. the dispersion of the powder (Iacocca and German, 1997). However, even automated on-line systems have been successfully used (Watano and Miyanami, 1995, Watano et al., 2000). In pharmaceutical technology, computerised image analysis has been widely used in particle size measurement and shape analysis (Staniforth, 1987, Hellén, 1993a, 1993b, Lindner and Kleinebudde, 1993, Podczeck and Newton, 1994, 1995, Etzler and Sanderson, 1995 Andrés et al., 1996, Eriksson et a., 1997, Hundal, 1997, Podczeck 1997, Podczeck et al., 1999, Larsen et al., 2002). According to Pons et al. (1999) there is a demand for routine morphology quantification of particles. However, they stated that operator dependence in sample preparation does not enable real –time process control.

2.3.3 Content-based image retrieval

The objective of the development of content-based image retrieval (CBIR) systems is to efficiently extract relevant feature information from images. These systems compare images based on the content information. Recent descriptions of principles of visual information retrieval have been given in literature (Del Bimbo, 1999, Lew, 2000, Castelli and Bergman, 2002). Gudivada and Raghavan (1995) have represented possible approaches that can be used in similarity retrieval: retrieval by texture, shape, spatial constraints, colour, sketch, volume, motion, objective and subjective attributes, and text. The development of CBIR systems uses ideas from areas such as knowledge-based systems, cognitive science, user modelling, computer graphics, image processing, pattern recognition, database management systems, and information retrieval.

According to Aigran et al. (1996), a system for content-based image retrieval by similarity rests on three components: 1. Extraction of features from images and an efficient system of representation and storage of this data, 2. Assortment of meaningful and effectively computable similarity measures, 3. User interface for the choice of the similarity measure to be applied and for efficient presentation of retrieved images.

In a typical content-based image retrieval system, the image features are pre-computed and indexed as the images are loaded in the system. The features include properties such as local intensity histograms, edge histograms, region-based moments. The features are stored in a database and to make a query, the user normally presents a sample image to the system. Retrieval by a user-drawn sketch is also possible. When the query image has been introduced, its features are calculated and compared against the features of the images in the database. The result of the comparisons is a score that indicates the degree of similarity and is used to rank order the images. This process can be very fast since image features are pre-computed in the image insertion phase, and distance functions have been designed to be extremely efficient at query time (Bach et al., 1996). A schematic diagram of the operation of a CBIR system is presented in figure 3.
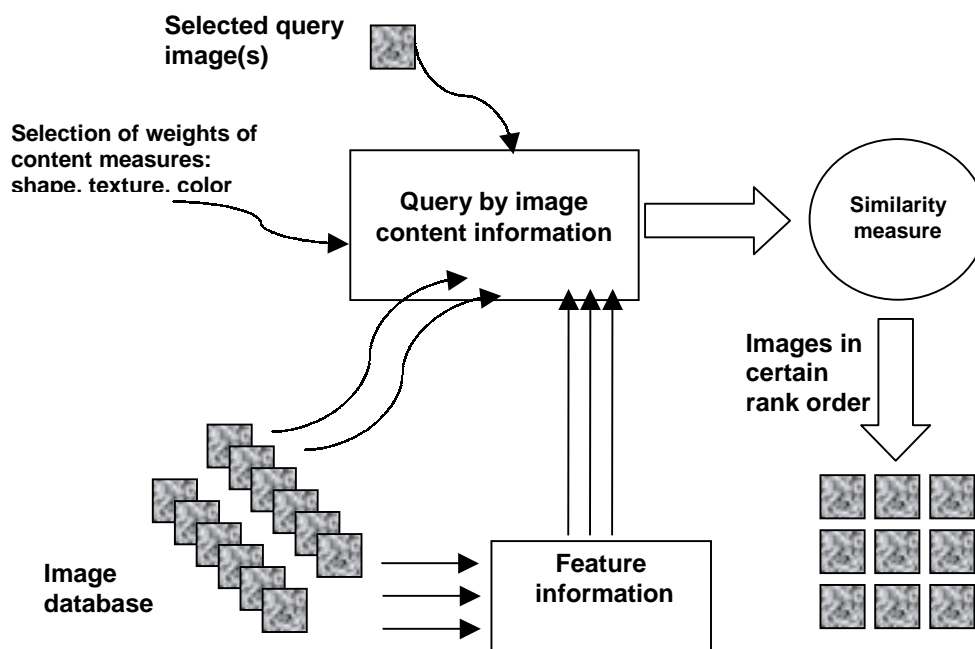
Figure 3. A diagram of a content-based image retrieval system.

Content-based image retrieval systems create an abstraction of the raw information of images in the form of features. Current approaches to CBIR differ in terms of choice of image features extracted, level of abstractions manifested in the features, similarity functions used and the degree of desired domain independence. Two major categories of features are primitive and logical (Gudivada and Raghavan, 1995). Primitive image features include, e.g. object centroids and boundaries. Logical features are abstract representations of images at various levels of detail. The logical features express the deeper domain semantics represented in an image (Gudivada and Raghavan,1995). For image information, features may be regarded as belonging to five abstract data types presented by Bach et al. (1996): values, indexed values, distributions, indexed distributions, and graphs. A value is usually a set of vectors that presents a global property of the image. An indexed value is an indexed set of vectors local to a region of an image. A distribution, like a colour histogram, is a multi-dimensional vector. An indexed distribution is a local pattern like the intensity profile of a region of interest. A graph represents relational information like spatial relations of objects in an image. Queries of the image data include comparisons of feature vectors of a query image with the stored database images.

Colour, texture and shape are the most commonly acknowledged and utilised feature categories and the data type grouping in the previous paragraph suits them all. The grey level images used in this study are a special example of colour features. A number of researchers have proposed methods for image indexing based on colour (Ng et al., 1995, Smith and Chang, 1996), texture (Pentland et al., 1994, You et al., 1997, Aksoy and Haralick, 1998) and shape (Mehrotra and Gary, 1995). Caelli and Reye (1993) and Niblack et al. (1993) have described methods for indexing combining all these three features.

CBIR systems may be developed with emphasis on automatic and dynamic feature extraction or with weight on semi-automatic extraction (Gudivada and Raghavan 1995). Ideally all object and feature identifications would be done automatically, but automatic methods to identify and outline objects are not yet adequate enough. Naturally, manual or semi-automatic methods are more time-consuming, but they are often necessary for satisfactory feature extraction. Colour and texture features are often extracted without manual intervention. One of the most difficult aspects of content-based image retrieval is

retrieval by shape (Faloutsos, 1994). Ashley et al. (1995) describe two methods, enhanced floodfill and snake-based edge following, for semi-automatic object shape identification. As shapes can be arbitrary, exact descriptions of shape are very difficult. There is no mathematical definition that would accurately match the human conception of shape (Del Bimbo, 1996). Many practical shape description methods exist, but there is no generally accepted methodology of shape description (Sonka et al., 1998). In a CBIR system, described by Faloutsos et al. (1994), the primary shape features are based on the combination of area, circularity, eccentricity and major axis orientation, in addition to a set of algebraic moment invariants.

CBIR systems for general use do not usually meet the same level of performance as systems which are tuned for special applications. Therefore, almost every application requires special development of a CBIR system. The aim is often to develop systems which can distinguish the objects in an image and which have the capability to learn, e.g. by employing relevance feedback given by the user. Several image retrieval applications, such as, QBIC (Niblack et al., 1993, Flickner et al., 1995), Photobook (Pentland et al., 1994), VisualSEEk (Smith and Chang, 1996), Virage (Bach et al., 1996), and PicSOM (Laaksonen et al., 2000, 2001, 2002) have been developed and described in literature.

CBIR systems have been used with applications in various fields. In medicine, magnetic resonance chest images were retrieved by certain proposed spatial features of images (Hou et al., 1992). Gupta et al. (1996) described a prototype content-based retrieval system for ophthalmologic images. Medical applications for CBIR are useful since image archives are large, and certain people need to use the image databases effectively. Medical images are often not only retrieved by looking at the image content but also in terms of other information associated with the images, such as text describing treatment and diagnosis (Gudivada and Raghavan, 1995). Retrieval systems have been developed also, e.g. for fingerprint identification (Manjunath and Ma, 1996) and for geographical images (Ratha et al., 1996, Soffer and Samet, 1996).

2.3.4 Surface and bulk imaging

Optical techniques for measurement of surface characteristics are widely used in different industrial inspection applications (Cielo, 1988). Often, these industrial techniques are

used to characterise roughness properties of different kinds of surfaces in process control and quality control. Russ (1999) has described the concept of surface imaging.

The inspection of surface information can be made in terms of qualitative or quantitative properties. To obtain quantitative information exact descriptors for the image information is required. In order to receive qualitative data generalisation of the image information is possible.

A key property of a bulk particulate material is a typical pattern of the image field-of-view called texture (Fig. 4.). Texture is related to distribution of the spatial variation in grey scale levels (or colour levels in colour images) and can be connected to general bulk-particle characteristics (Bonifazi, 2002). Global measurements of the texture that is observed in an image can portray information about the size of the particles (Novales et al., 1998). Smaller particles lead to finer textures and larger particles to coarser textures. Apparently the presentation geometry, e.g. the magnification and resolution used will affect the outcome. Standardised imaging conditions for this kind of textural comparisons are therefore needed. An advantage of textural methods is that particles do not have to be identified individually. Bonifazi (2002) has shown that particulate overall pictorial characteristics influenced by grain size, shape and colour distribution can be correlated to the chemical composition of powders.
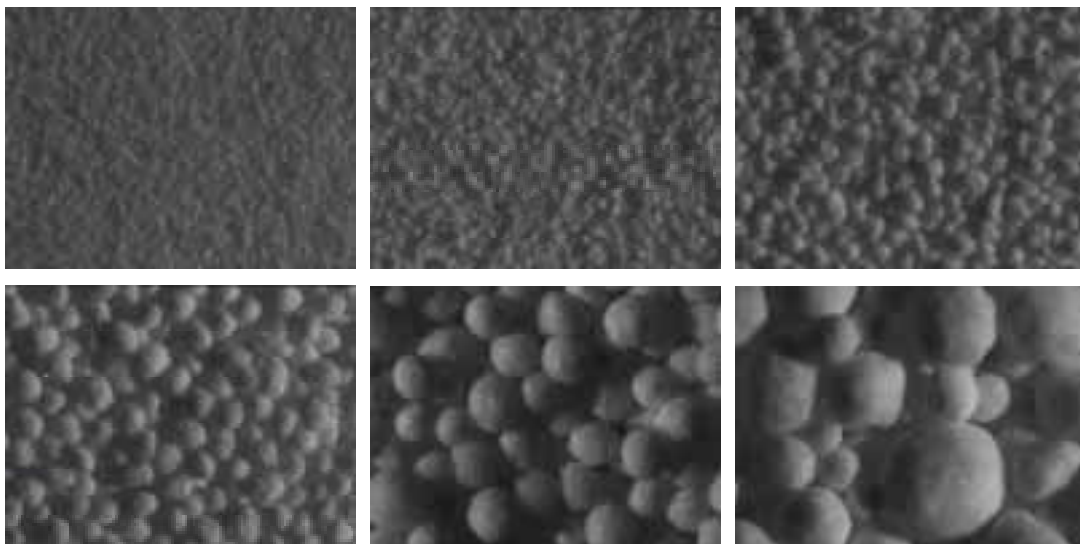

Figure 4. Demonstration of textural differences in a set of six different granules with dissimilar size characteristics. Smaller particles have finer textures and larger particles coarser textures.

Bertrand et al. (1991) characterised the texture of pea samples by the grey level run lengths method and found it to be useful in powder classification. A grey level run length

is a set of subsequent pixels having the same grey scale value. In this approach, for each grey level, the number of runs that have a certain length are counted for a given direction. Novales et al. (1998) successfully used global features of images in particle size characterisation of a cereal milling process. The methods applied were based on textural analysis, such as grey level run lengths and grey level co-occurrence. Gray level co-occurrence describes the spatial relationships between grey level values. Russ (1999) has covered the general concept of texture and different texture operators.

Bonifazi and co-workers (1997, 2002) discussed the new perspectives in the field of particulate solids control of bulk or collection of particles instead of single particles. Huang and Esbensen (2000) introduced a method of IA that does not deal with individual particles and acquires images directly from in-situ powders. They addressed that this type of images of the entire field-of-view of powders also contain some information which relates to individual particles, but mainly about the bulk powder. They presented that this information is the reflection of complex bulk properties, such as flowability and fluidisation velocity. In a later study with seven reference powders Huang and Esbensen (2001) established quantitative predictive models between multivariate images and particle size, density, minimum fluidisation velocity, wall friction angle and angle of repose. They applied Angle Measure Technique (AMT) in image analysis of a variety of powders. In the AMT approach a special camera with red, green and near infrared channels was used with low-angle unilateral illumination. AMT has been developed to describe signal complexity as a function of geometrical scale from local to global. In the described application the images of powders were unfolded to produce one-dimensional measurement series, which AMT transforms to multivariate scale characterisations.

2.4 Data projection methods

Commonly, any produced measurement data is multidimensional. In order to understand, interpret and summarise a large data set, visualisation of its structure with projection methods is needed. A functional projection tool compresses the original data while preserving relevant features in the original data structure, and allows its visualisation. Different projection techniques, linear and nonlinear, exist. When chemically relevant information is extracted from data produced in chemical experiments

the term chemometrics is often used (Wold, 1995). Chemometrics offers a large variety of compression techniques, which by data dimensionality reduction allow data visualisation. Daszykowksi and co-workers (2003) have recently reviewed different chemometric projection methods. There are two dominant projection methods in chemistry. The most popular linear projection method is principal component analysis (PCA). If PCA is not suitable for efficient compression of the data, then usually the nonlinear self-organising maps (SOM) are used. Other projection methods are Sammon mapping, generative topographic mapping (GTM), principal curves, multidimensional scaling and auto-associative neural networks (Daszykowksi et al., 2003). Here, the methods used in this thesis, PCA and SOM, are presented.

2.4.1 Principal component analysis

Pearson (1901), Hotelling (1933) and Karhunen (1947) were pioneers with principal component analysis. Today, PCA is the most popular linear projection method (Daszykowksi et al., 2003). PCA is used to examine observations, structures, similarities and trends of large data tables. It projects multidimensional data into a few orthogonal features, called principal components (PCs).

The PCs are constructed as a linear combination of the original variables to maximise the preservation of the data variance. The PCs of a data set are found by calculating the eigenvectors and eigenvalues of the data covariance matrix. These vectors give the directions in which the data cloud is stretched in the data space. The projections of the data on the eigenvectors are the PCs. The corresponding eigenvalues give an indication of the amount of variance the respective PCs stand for, i.e. large eigenvalues signify a large amount of variance in the data set. Consequently, principal components with large eigenvalues inform more about the relations between the data points than any other directions.

The principal components are also known as latent variables which sum up the variation of the original dataset. The reduction of the dimensionality of the original variables to latent variables makes the data visualisation possible. The main property of PCA is that it reaches the best linear map by minimising the squared error of the data reconstruction from the latent variables back to the original high-dimensional space. Significant principal

components are often used as a starting point in other methods, such as Sammon projection, SOM or GTM (Daszykowksi et al. 2003).

The formation of PCs is illustrated in figure 5. The N observations are each characterised by a point in the K-dimensional space, i.e. K is the dimensionality of the raw data space. The raw data matrix X is a group of points in this space. The first principal component (PC_1) is a line in the X data space which makes the best approximation of the raw data (least squares fit). The approximation of X is improved by a line PC_2 as much as possible with the restriction that PC_2 is orthogonal to PC_1. The two principal components are represented by a plane in the X space. The original data points can be projected on the plane, on which the scores, (t_1 and t_2), summarise the relationship between the observations.
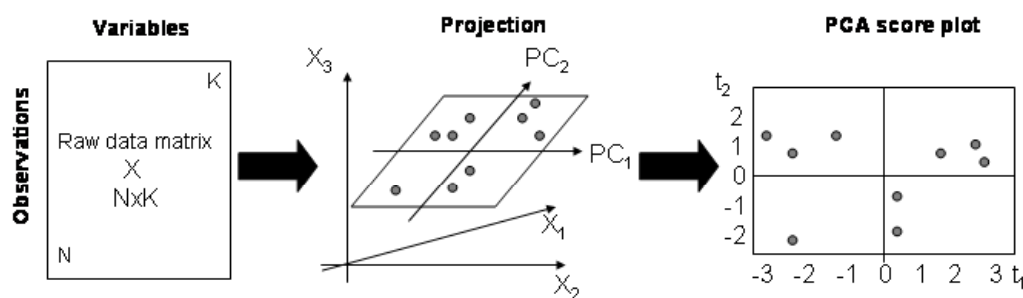
Figure 5. Data projection in PCA. Group of data points (observations) in K-dimensional space (K=3, including variables X_1, X_2, X_3) and the projection of the points onto a plane.

Partial least squares projection to latent structures (PLS) relates two data matrices, X and Y, to each other by a multivariate model. PLS is a regression extension of PCA. The PLS method enables modelling of data in which the number of variables exceeds the number of observations (Wold, 1995).

In pharmaceutical technology PCA has been used in formulation development (Benkerrour et al., 1994, Gabrielsson et al., 2000), optimisation of manufacturing of solid dosage forms (Bergman et al., 1998) and process monitoring (Rantanen et al., 2000). It has been widely used in pharmaceutical research including different investigations of formulation and response variables, particle characterisation and composition design. Recently, Ålander et al. (2003) applied PCA in the evaluation of shape and size factors of paracetamol agglomerates measured with image analysis. They concluded that visually observed differences in crystallised products of paracetamol can be characterised by

image analysis in combination with PCA. Lindberg and Lundstedt (1995) have reviewed applications of multivariate analysis in pharmaceutical development work.

2.4.2 Self-organising maps

Artificial neural networks (ANNs) became quite widely used as a data analysis method in the 1990s in pharmaceutical development (Hussain et al., 1991, Murtoniemi et al., 1994, Watano et al., 1997, Bourquin et al., 1997). A specific method that is based on neural networks is the self-organising map (SOM) (Kohonen, 1982, 1997). The SOM is an unsupervised ANN. While typical ANNs are often used for data modelling the SOM can be applied for data visualisation. Supervised techniques (e.g. back-propagation algorithm (Haykin, 1994)) create a model for the relationships between training inputs and the required responses. Unsupervised techniques discover similarity in the input vectors without a training target. The purpose of SOM is to perform nonlinear mapping of input data objects typically on a two-dimensional grid with a distinct topology, i.e. a layer of active neurons is arranged into a grid of squares or hexagons on a two-dimensional plane. The SOM is particularly advantageous in visualisation of high-dimensional, nonlinear data.

The SOM is an elastic net with a grid of nodes connected to their neighbours with elastic bands. Input observations are presented in random order for training and for each observation the winning node is located on the map. The winning node is the one best matching the presented observation. The standard method is to represent the observations and SOM nodes with vectors. The vectors of the SOM nodes are often called model vectors. Thus, the winning node is the one whose model vector has the shortest distance to the presented vector. In the training the model vectors of the winner and its neighbouring nodes are modified to represent the input signals more closely. Such training causes the net to stretch through the densely populated areas of the input data space. This net can be straightened back to two dimensions from the K-dimensional space and investigated with different visualisation methods. This gives information with regard to how the data resides in the K-dimensional space, which is the principal advantage of the SOM. The architecture of the SOM is illustrated in figure 6.
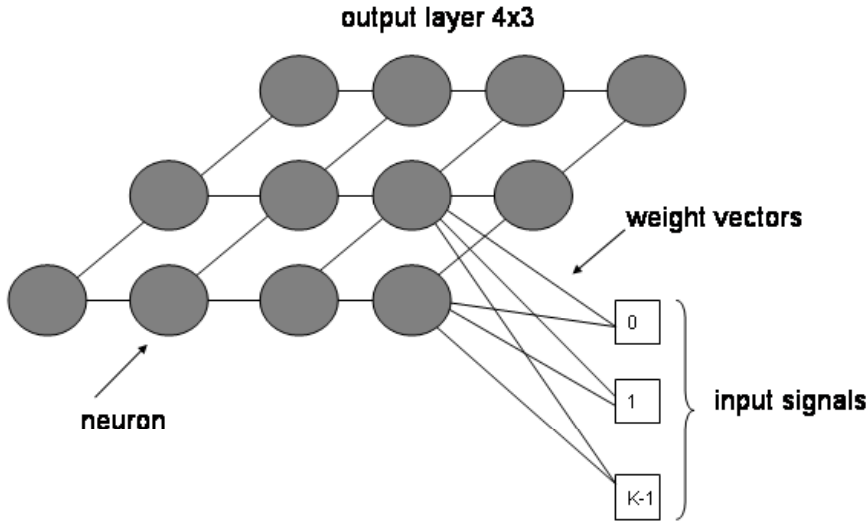
Figure 6. The architecture of the self-organizing map (SOM). A topological map of the input vector signals is created during the training phase. Each input vector finds a winning neuron. Similar vectors become arranged in same regions of the SOM during the training cycle.

If the input vector is denoted by $x=[\,x_0,\,x_1,...,x_{K-1}]^T$ and the model vector of a map node by $m_i =[m_{i,0},\,m_{i,1},...,m_{i,K-1}]^T$, then the algorithm which explains the self-organising operation can be presented:

A. Model vectors of the nodes are initialised with random values.

B. Steps C and D are computed for each vector _x(t)_ in the training data.

C. The SOM node _mc_ (winning neuron) that matches best to the data vector _x(t)_ is found by searching all nodes by:

$$\| x(t) - m_c(t) \| = \min_i \{ \| x(t) - m_i(t) \| \} \qquad\qquad \text{(Eq. 4)}$$

D. The model vectors of the nodes are adjusted by

$$m_i(t+1) = \begin{cases} m_i(t) + \alpha(t)\{x(t) - m_i(t)\}, \, for \; i \in N_c(t), \\ m_i(t), \, for \;\; all \;\; other \;\; indices \end{cases} \qquad \text{(Eq. 5)}$$

where the parameter _α(t)_ is a coefficient which determines to what extent the winning node and the neighbourhood are moved in the direction of vector _x(t)_. During training, the parameter _α(t)_ is decreasing with time. _Nc_ is a neighbourhood set of map points around node _c_ and it can be defined _Nc(t)_ as a decreasing function of time. Euclidean metric can be used as the distance measure $\| \cdot \|$ in equation 4.

Zupan and Gasteiger (1993) and Melssen et al. (1994) have discussed the practical issues concerning the use of self-organising maps in solving chemical problems. In pharmaceutical technology Antikainen et al. (2000) have used the SOM to predict the flowability of powders with satisfactory results. Recently, Kachrimanis et al. (2003) applied SOMs for visualisation of the correlation of certain micromeritic properties of powders. The SOM was also successfully used for visualisation of fluid bed granulation by Rantanen et al. (2001). Bourquin et al. (1997) modeled data sets from tablet compression with different ANN models, including self-organising maps.

3. AIMS OF THE STUDY

The purpose of this study was to find new ways of using image information in pharmaceutical powder technology and characterisation of pharmaceutical solids. The goal was to extract relevant information from powder surfaces with the aim to broaden the use of image information compared to the commonly used image analysis (IA) approaches in pharmaceutical technology, which only measure properties of individual particles. The specific aims of the study were:

1. to characterise undispersed particulate populations through surface images.

2. to extract relevant information from powder surfaces.

3. to link the information from powder surface images with functional physical properties of pharmaceutical solids.

4. to enhance the use of surface imaging as a tool for pharmaceutical process analytical technologies (PAT).

5. to develop a useful and reliable method for particle size analysis with emphasis on uncomplicated sample preparation.

6. to evaluate different chemometric methods in data visualisation.

4. EXPERIMENTAL

In this chapter the materials and methods used in this thesis are covered. A more detailed description of materials and methods is given in the original publications which are here referred to by their respective Roman numerals I-IV.

4.1 Materials

4.1.1 Excipient mixtures

Microcrystalline cellulose (MCC), Avicel grades PH 101, PH 102, and PH 200 (FMC International, Ireland), was used as test material in the first stage of the study (I). Sixteen mixtures of the different MCC grades were prepared. A mixture design was used.

4.1.2 Model particles

In the second phase of the study a series of model particles with a range of different shapes and sizes were used (II). A pilot batch of pellets was chosen to represent large round particles (Pharmaceutical Technology Division, University of Helsinki, Finland). Sodium phosphate (Riedel de Häen, Seelze, Germany) particles had sharp edges and many were diamond-shaped with a large size distribution. The salt (Jozo, Amersfoort, The Netherlands) and sugar (Finnsugar, Finland) particles represented cube-shaped particles with smaller particles and a narrower size distribution, lactose monohydrate (Pharmatose 200 M, DMW International, Veghel, The Netherlands) was chosen to represent a finer material with a smaller particle size distribution. Finally, dry black tea (Twinings Ltd, London, England) represented elongated particles with large size and shape variations.

4.1.3 Granules

Seventeen different granulations were made in study II using verapamile hydrochloride (Orion Pharma, Finland) as a model drug substance with varying amounts of three excipients: MCC (Emcocel 50M, Penwest Pharmaceuticals, Nastola, Finland), lactose monohydrate (Pharmatose 200 M, DMW International, The Netherlands) and

pregelatinised starch (Starch 1500, Colorcon, Indianapolis, IN, USA). A mixture design was used. In study III granules from 40 different pilot batches (Pharmaceutical Technology Division, University of Helsinki, Finland) with varying particle sizes and compositions prepared with fluidised bed granulation were analysed.

In the final stage of the study (IV), a model formulation (batch size 3500 g) consisting of 5% wt/wt of caffeine (Orion Pharma, Espoo, Finland), 475 g MCC (Emcocel 50M, Penwest Pharmaceuticals, Nastola, Finland), 2200 g lactose monohydrate (Pharmatose 200M, DMV Pharma, Veghel, The Netherlands), and 500 g pregelatinised starch (Starch 1500, Colorcon, Indianapolis, IN, USA). Polyvinylpyrrolidone (PVP) (Kollidon K25, BASF, Ludwigshafen, Germany) was used as a binder in the formulation (5 % wt/wt). Solutions in purified water were prepared using 8.75 % wt/wt of PVP. In total, 34 granulations were prepared.

4.2 Unit operations

4.2.1 Mixing (I)

200 grams of each powder mixture was mixed at 14 rpm for 8 min using glass jars in a laboratory mixer (Turbula mixer T10B, Willy A. Bachofen AG Maschinenfabrik, Basel, Switzerland).

4.2.2 Fluidised bed granulation (II,III,IV)

All granulations were made in a bench-scale fluidised bed granulator (Glatt WSG 5, Glatt GmbH, Binzen, Germany). The granulation setup has been described in detail by Rantanen et al. (2000). In study IV 34 granulations were made with a bench-scale fluidised bed granulator (Glatt, WSG 5, Glatt Gmbh, Binzen, Germany). The process conditions were planned using an experimental design. Three process variables were altered on three levels: inlet air temperature (30°, 40°, 50°C), nozzle spraying pressure (1, 1.5, 2 bar) and granulation liquid flow rate (160, 175 and 190 g/min). A series of 17 granulations was performed two times in randomised order.

4.2.3 Tablet compression (IV)

Tablets of 17 batches were compressed using an instrumented Korsch (EK-0, Korsch AG, Berlin, Germany) eccentric tablet machine. Flat-faced 9 mm punches were utilised. The target crushing strength of tablets was 100 N and the target weight was 250 mg. Prior to tabletting the granules were mixed with 0.5 w-% magnesium stearate (Ph.Eur.) in a Turbula mixer for 5 minutes. The tabletting was carried out in a condition-controlled room (55 ± 3 RH%, 24 ± 2°C). In total, 500 tablets of a selection batches were compressed. The weight variation of the tablets was determined using the relative standard deviation of the upper punch force profiles for the 500 tablets. The weight variation (wv) of the tablets was calculated according to equation 6:

$$wv = r.s.d._{fv} / average_{fv} \cdot 100 \%, \qquad\qquad (Eq.\ 6)$$

where $r.s.d._{fv}$ is the relative standard deviation in the upper punch force profile and $average_{fv}$ is the average of the upper punch force measurements for the 500 tablets/batch. The tablet weight variation was also measured by weighing 50 tablets from a random selection of 10 tabletted batches.

4.3 Characterisation of materials

4.3.1 Laser diffraction (I- III)

The particle size of the MCC mixtures (I) and 40 granule batches (III) were measured with a Fraunhofer laser diffraction particle sizer (Malvern 2506 LC Droplet and Particle Size Analyser, Malvern Instruments Ltd., Worcestershire, UK) using a dry powder feeder. The focal lens lengths used were 600 mm (I) and 800 mm (III). Three replicate samples were measured (n=3).

4.3.2 Sieve analysis (III, IV)

The particle size distributions were measured with sieve analysis (Fritsch analysette, Idar-Oberstein, Germany) using the following sieves: 0.045 mm; 0.071 mm; 0.090 mm; 0.125

mm; 0.180 mm; 0.250 mm; 0.355 mm; 0.500 mm; 0.710 mm; 1.000 mm; 1.400 mm and 2.000 mm. The sample size in sieve analysis was 20 grams (5 min with amplitude 6)(n=3).

4.3.3 Scanning electron microscopy (II)

A scanning electron microscope, SEM, (Zeiss DSM 962, Zeiss, Germany) was used to take the micrographs of the model particles.

4.3.4 Optical microscopy (I)

An optical microscope (Leica MZ6, Leica Mikroskopie und Systeme Gmbh, Bensheim, Germany) was used to take surface images of the mixtures of MCC. The magnification was 0.8x. The microscope was operated in a dark room with the absence of any background illumination. The sample was illuminated with two light fibers and the light intensity was constant during the imaging. The positioning of the lights was made in a way that the illumination created a good contrast and shadows could be distinguished when the sample was inspected through the microscope. Three grey scale images of each MCC mixture were captured and stored in bmp (bit map picture) format.

4.3.5 Image analysis of size and shape parameters (II)

An optical microscope (Leica MZ6, Leica Microskopie und Systeme GmbH, Germany) which was connected to an image analysis (IA) software (Leica Qwin, Leica Imaging Systems Ltd, Cambridge, England) was used to determine the size and shape parameters of the granules and model particles in study II. The parameters were: Convex area, Aspect Ratio, Equivalent diameter, Roundness and Fullness ratio. Approximately 1000 particles of the 6 model particles and the 17 granule batches were measured. The description of the shape parameters is given in table 1 (II).

4.4 Content-based image retrieval (I)

A web version of the QBIC system, Query by Image Content (IBM, Almaden Research Center, CA, USA), was used as the content-based image retrieval system. This QBIC version included an indexing and a retrieval engine, a user interface, and APIs (Application Programming Interface). The QBIC version supported queries based on

colour histogram, colour layout, and texture. The image database created consisted of 64 images. The images were indexed, and thereafter each image was used as a sample image for a query. The texture feature was used as the search criterion. The texture feature in QBIC is based on mathematical representations of coarseness, contrast, and directionality. Coarseness measures the scale of texture (pebbles vs. boulders) and is calculated by using moving windows of different sizes. Contrast describes the vividness of pattern. It is a function of the variance of the grey level histogram. Directionality describes whether the image has a favored direction (e.g. grass) or is isotropic (e.g. a smooth object), and it is a measure of the peakedness of the distributions of gradient directions in the image (Faloutsos et al., 1994). In QBIC, similarity queries are performed against the database of pre-extracted features using distance functions between the features (Flickner et al., 1995). The normalisation factors are the inverse variances of each feature component. In a texture query, the distance d between object i and object j is calculated as

$$d_{ij} = (O_i - O_j)^2 / \delta^2_O + (C_i - C_j)^2 / \delta^2_C + (D_i - D_j)^2 / \delta^2_D, \qquad \text{(Eq. 7)}$$

where O, C, and D represent the features coarseness, contrast, and directionality, respectively. The δ stands for the variance factor (Faloutsos et al., 1994).

The rank order of images and the image similarity values were compared to the particle size of the different mixtures. The similarity value of each image shows how the extracted image features differ from the features of the sample image. The similarity value for the query image is 0.000000 and the more dissimilar the compared images are the higher the value. The program used (Modde 3.0, Umetri AB, Umeå, Sweden) calculated the predictive power according to cross validation. The original model was a quadratic mixture model from which the least significant terms were removed as long as the predictive power was increasing. A statistical analysis (Spearman test) of the correlation of the similarity value and the particle size was made using the Windows version of Systat 5.0. Figure 7 shows the course of the use of QBIC in analysis of the images of MCC.

Image acquisition of MCC powders	→	Extraction of texture features	→	Determi-nation of similarity measure	→	Correlation with particle size data
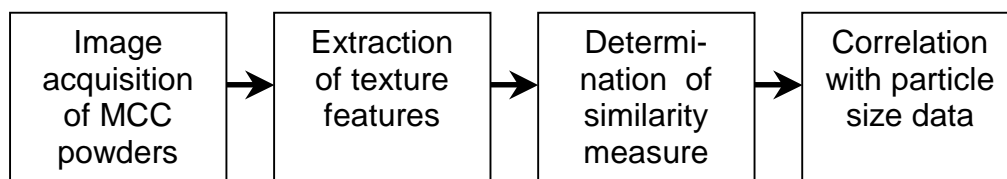
Figure 7. The flow of the use of QBIC texture features in analysis of surface images of MCC powders.

4.5 Surface imaging of powders and granules

4.5.1 The imaging setup (III, IV)

In order to improve imaging conditions and reproducibility, a new optical setup was constructed during the study. This optical instrument consisted of the following elements. The imaging unit, with a light source, a monochrome CCD camera (JAI, CV-M50, Copenhagen, Denmark) and a lens objective, is connected to a frame grabber (WinTV, Hauppauge Computer Works Inc., Hauppauge, NY, USA) and a Personal Computer. The symmetrically positioned, bilateral light sources, on opposite sides of the sample, stand on rails, on which they can be accurately positioned. The illumination system includes two lamp housings, 100 W quartz tungsten halogen lamps, and two collimating lens assemblies (Oriel Instruments, Stratford, CT, USA). The collimated output beam can be turned 90 degrees with a beam turning assembly. The light sources are connected to stabilised DC power supplies (Oriel Instruments, Stratford, CT, USA). A sketch of the imaging setup is presented in figure 8.
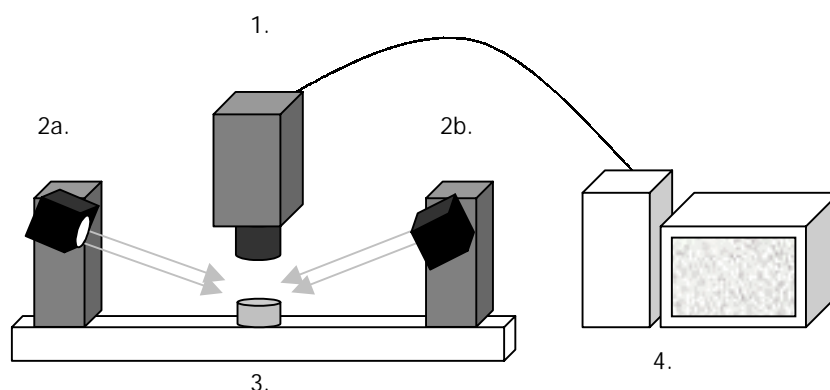
Fig 8. Imaging setup. 1: CCD camera with optics. 2a and 2b: light sources on rails with collimated light beams. 3: Powder sample in sample cup 4: PC and frame grabber.

Extensive optimising studies concerning the illumination and imaging conditions were performed. Consequently, the following imaging settings were established for studies III and IV. A 50 mm lens objective with additional 40 mm extension tube was used. The light source distance from the sample was 20 cm. The angle of illumination was 30° with referral to the horizontal line. The used power source voltage was 5.5 V and the image resolution in the frame grabber was 600 x 800 pixels. The dimensions of each sample surface in the taken images were 8.2 mm x 6.1 mm. All images were taken in a dark room with no disturbing light sources. The calibration of the imaging conditions was made with a smooth white calibration board (Xerox Premier, batch 11/DD/YKD/1, Xerox Corporation, CONN, USA).

4.5.2 Grey scale difference matrix (III,IV)

In studies III and IV a parameter, grey scale difference matrix (GSDM), for calculations of the particle size from surface images was developed and used. The subsequent steps were taken in the creation of the GSDM. The two light sources were used to illuminate the sample from opposite sides. Two images of a sample surface were taken. A digital image of the sample was first captured by using one light source. Then, another image was taken by illuminating the sample with the other light source. Consequently, two digital images were received and two matrices of their grey scale values were formed. The difference of these two matrices was then calculated. The operation of matrix subtraction is explained by equation 8 using a 2x2 example matrix.

$$GSDM = M1 - M2 = \begin{bmatrix} 4 & 6 \\ 9 & 5 \end{bmatrix} - \begin{bmatrix} 8 & 6 \\ 5 & 11 \end{bmatrix} = \begin{bmatrix} -4 & 0 \\ 4 & -6 \end{bmatrix}, \quad \text{(Eq. 8)}$$

where M1 is the grey scale matrix of image 1 and M2 is the grey scale matrix of image 2. The difference is thus calculated for each corresponding pixel in M1 and M2.

For an ideal completely smooth surface the difference of the two matrices consist of zeros. For a real surface the difference matrix gets values between −255 and +255. In the next step a distribution of the difference matrix is formed: i.e. how many matrix cells holds each of the possible 511 values. Figure 9 presents two different example granule sample surfaces with two images illuminated from the opposite sides for each material. Subsequently, the formation of the difference distributions from the difference matrices

is shown. One can notice that the difference distribution is characteristic for the different kind of surfaces. The particle size distributions in studies III and IV was derived from the GSDM .
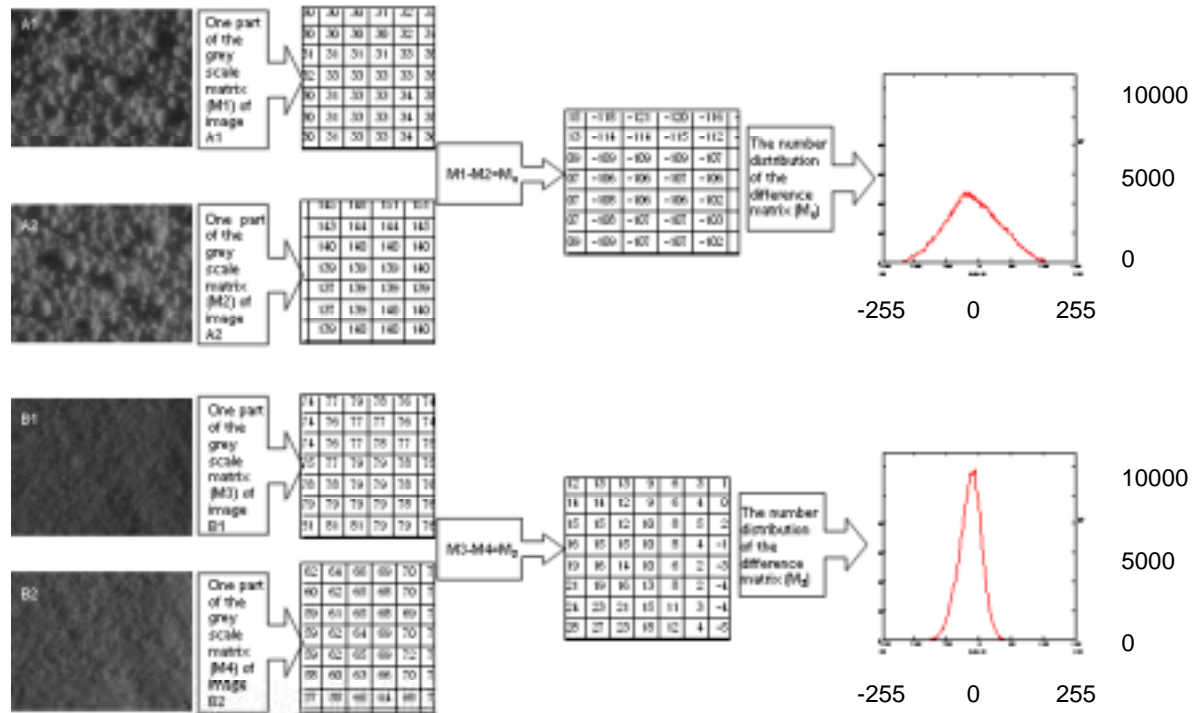
Figure 9. Generation of the grey scale difference distribution from granule surface images.

4.6 Data analysis

4.6.1 Correlation analysis (I, III, IV)

A Spearman test to find out the correlation of the similarity value and the particle size was performed (I). In the later part of the work (III, IV) Pearson correlation analysis was made between the particle sizes modelled from image information and particle median size values measured with sieve analysis and laser diffraction.

4.6.2 Multivariate regression (I)

In the first study (I) a regression model between the image similarity values and the particle size of the different mixtures was created using Modde software (Modde v. 3.0, Umetri AB, Umeå, Sweden). The program calculated the predictive power according to

cross validation. The original model was a quadratic mixture model from which the least significant terms were removed as long as the predictive power (R^2) was increasing. Modelling can be used to find quantitative relations between predictors and responses. The model explains the variation in the responses with R^2 and Q^2. R^2 is the fraction of the variation which is explained by the model and Q^2 indicates the fraction of the variation that can be predicted by the model. Possible values will be in the range of 0-1.0 where 1.0 represents a model with excellent predictive power.

4.6.3 Principal component analysis (II, IV)

The measured image analysis data was evaluated using principal component analysis (PCA) employing Simca-P Software version 8.0 (Simca-P v. 8.0, Umetrics, Umeå,Sweden) software.

4.6.4. Self-organising maps (II)

The SOM was used for training and visualisation of the IA data matrix. The work was performed using a Matlab (Matlab, v. 5.3, MathWorks Inc., USA) SOM toolbox, which is available on a public domain (Alhoniemi et al., 1997). Figure 10 shows a simplified illustration of the steps in data reduction and visualisation.
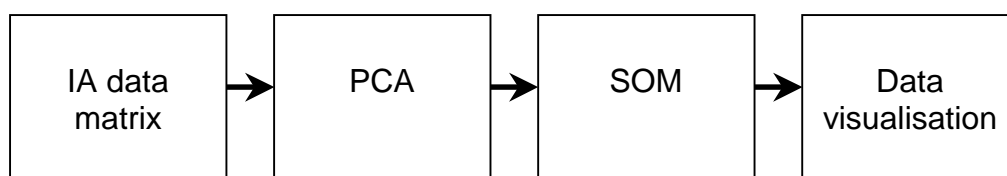
Figure10. The reduction of dimensionality and visualisation of the IA data in paper II.

4.6.5 Partial least squares modelling (III, IV)

PLS models were created in studies III and IV. The vector with 511 values consisting of GSDM distribution data was used as explanatory variables (predictors) and size fractions were used as the response variables in the creation of the model (Fig. 11.). In both studies (III and IV) the percent mass proportion of sieve fractions of the analysed

granule batches were used as the response variables. In the former study a model was also created for laser diffraction data using the percent volume proportions of size fractions as response variables. In the former study, the models were evaluated using nine granule batches as test data. In the later study, the created model was evaluated by inspecting the goodness of fit (R^2) and the predicted variation (Q^2). The terms R^2 the Q^2 are explained above in paragraph 4.6.2.

```
┌─────────────┐      ┌──────────┐      ┌──────────────┐
│    X=       │      │   PLS    │      │     Y=       │
│ Predictors= │ ───▶ │  model   │ ───▶ │  responses=  │
│  GSDM data  │      │          │      │ Particle size│
│             │      │          │      │ distribution │
│             │      │          │      │     data     │
└─────────────┘      └──────────┘      └──────────────┘
```

Figure 11. A simplified graph showing the idea of the creation of a PLS model between the gray scale distribution data and the measured size distributions.

5. RESULTS AND DISCUSSION

In this chapter the results of this thesis are summarised. A more detailed discussion is presented in the original publications which are referred to in the text by their respective Roman numerals I-IV.

5.1 Visualisation of traditional image analysis data

5.1.1 Principal component analysis (II)

The two principal components form a plane in the original image analysis data space. The differently shaped and sized model part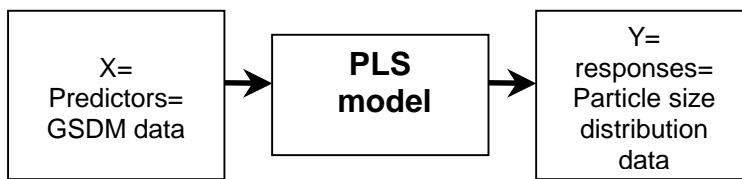icles are projected on different areas on the score plot (II, Fig. 6a-b.). PCA indicated that two components explained about 83 percent of the variance of the data investigated. The first component accounted for 50 % of the variance and was characterised by the size factors. The second component described the shape parameters. Similar PCA plots for the IA for the granules were formed (Fig. 7a-b.). The first two components accounted for 85.2 % of the variation, with the first component explaining 54.9 % of the variance. The results indicated that PCA is a good technique to study the variance within samples of different kind of particles.

5.1.2 Self-organising maps (III)

Self-organising maps were also used for visualisation and reduction of the IA data. The results of the trained SOM for the model particles are visualised in Figures 8 – 9 (II). In figure 8 the values of the 5 different variables of the particles are visualised. The colour of the node indicates the level of the individual variable in the specific region of the map. The high values are indicated with red colour. When the size parameters (equivalent diameter and convex area) are studied we can notice that the particles with the largest values for these variables are organised in the lower left corner of the map with maximum values in the middle on the left side edge. The particles with large values for the shape factors roundness and aspect ratio are located in the upper left corner of the SOM. The particles with highest values of the fullness ratio shape parameter are organised in the bottom of the map, around the left side corner.

Figures 9a-f (II) show the organisation of the particle data on the map for each sample of model particles. The SOM visualises clearly the differences between the particles. For example, when studying the size variables one can notice that the particles with larger values for these variables are organised on the lower side of the map, more specifically, in the left-hand corner. Particles with a wider size distribution are spread more widely on the map, such as sugar (II, Fig. 9 a, yellow colour) and salt (II, Fig. 9 d, cyan colour) particles. The smallest particles (lactose, II, Fig. 9 b, red colour) could also be distinguished. The variation and the differences in the data for the 5x17330 data matrix for the granules were also clearly visualised by the SOM. The results of the trained SOM are visualised in figures 10-11(II). Figure 11a-c (II) shows the visualisation of the particle data on the map for the granule formulations A, B, C, which represented the corner points of the mixture design used.

5.1.3 Comparison of data visualisation with PCA and SOM

Multivariate data analysis methods are capable of creating a perceptive presentation of various data sets. Both methods used, SOM and PCA, enable the lowering of the dimensionality of multivariate data. In this study (II) SOM was capable of creating intuitive presentation of the differences between the examined particles. If more than two principal components are needed to capture the variation in data with PCA, the visualisation of the projection is more complicated. Subsequently, 3D or parallel 2D plots are needed. The use of the unsupervised SOM enables the 2D plotting, and all the information is in the same plot. The SOM illustrates structures in the data in a different way than PCA multivariate data analysis by focusing to preserve the topological neighbourhood relations in the data instead of trying to preserve the distances between the data items. In a majority of cases, PCA, as a linear projection method, allows efficient compression of the data and produces profound insight into the data structure. When PCA is inefficient, nonlinear projection methods, such as the SOM, should be applied (Daszykowski et al. 2003). For inexperienced persons in data analysis the interpretation of SOMs can be somewhat more difficult than the analysis with PCA plots.

5.2 Data extraction from powder surface images

5.2.1 Content-based image retrieval

Surface images of powders and granules were evaluated with respect to particle size of the materials (I, III, IV). The results from first the phase studies showed that the content-based image retrieval system used, QBIC, extracted applicable information connected to the particle size of the excipient mixtures (I). The texture feature used was capable of extracting relevant information of the appearance of the powder surfaces, which could be connected to particle size (I, Fig. 4.).

With large image databases and a lot of image information, efficient image retrieval systems become a necessity. Image retrieval technology combined with text querying can lead to powerful retrieval of image data, which may effectively be used in different fields of pharmaceutical research and industry. Possible application areas include: measurement of size and shape distributions of samples, analysis of crystalline habit and the examination of batch quality of raw materials.

Applications of CBIR are usually automatic and do not take into account user feedback during queries. Interactivity in a CBIR system could be an advantage in image retrieval of pharmaceutical powder images. Laaksonen et al. (2000, 2001, 2002) have developed a content-based image retrieval system using the self-organising map (SOM) as the image similarity scoring method. The system employs user feedback for supervised learning to adjust following queries. We applied PicSOM successfully in classification of granules using certain shape and texture features (Laitinen et al., 2002). To achieve the goal of developing a CBIR tool, which is efficient and has a high precision rate for powder technology, requires that the algorithms for measurement of image content should be essential.

This study (I) was a fundamental step towards further advances in the use surface image information of pharmaceutical solids. It established the basis of the development of the GSDM algorithm and evolved the sample preparation and the methods of imaging.

5.2.2 GSDM in particle size measurements

Models between surface image information of granules and their respective particle size distributions were formed (III, IV). The results indicate that the GSDM feature can be used in particle size measurements. The modelled particle size distributions of 5 test batches with respect to particle size measurements with sieve analysis are presented in figure 5 (III) and one example batch is shown in figure 12. The figures indicate that the created models corresponded fairly well with the particle size distributions measured with sieve analysis. The correlation coefficient between the median particle size of the results from the optical measurements and sieve analysis was 0.82 (P<0.0001). The distributions are more similar for particles in the size range from 100 to 200 micrometers (mean granule size with sieve analysis). Granule batches with size fractions with smaller sizes were over-represented in the training data compared to granules with fractions containing larger sizes. This is an explanation for the better models for the batches with smaller particles. This is clearly indicated in the cumulative graphs (III, Fig. 5.). When the batches with the largest particle sizes were omitted from the test data the correlation coefficient improved significantly to 0.915 (P< 0.0001).
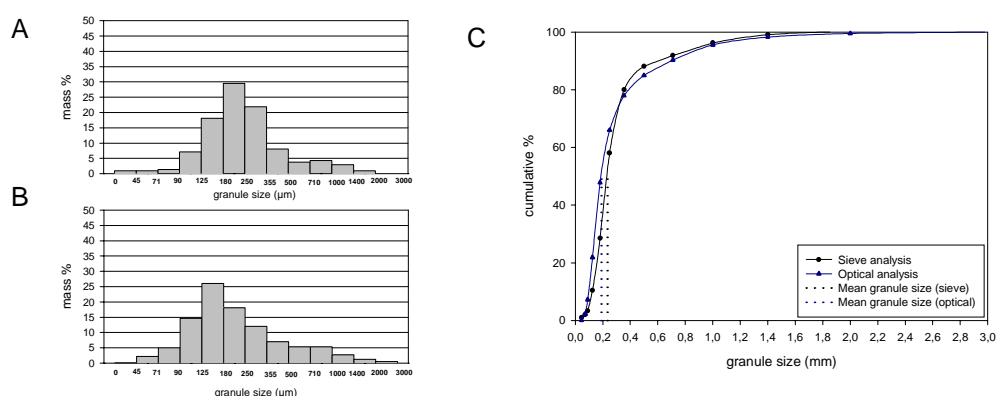
Figure 12. An example of the particle size distributions measured with sieve analysis (A) and modelled using the GSDM (B). Figure C shows the cumulative graphs (sieve analysis= ●, GSDM (image information) = ▲) with the mean granule size (dotted lines). Figure taken from publication III.

Comparable models were made between the GSDM data and laser diffraction measurements (III, Fig. 6a-e). The correlation coefficient between the median particle size of the results from the optical measurements and laser diffraction was 0.92 (P<0.0001). These figures show that the created models correspond well with the particle size distributions measured with laser diffraction. However, there are some differences

compared to the models of sieve analysis. The differences of the models for sieve analysis and laser diffraction result from the different nature of the particle sizing methods. Sieve analysis measures the percent mass proportions and laser diffraction measures the percent volume proportion of the size fractions.

An improved model between the GSDM features and sieve analysis was created in paper IV. The Pearson correlation coefficient between the mean sizes measured from surface images and the mean size measured by sieving was 0.97. The degree of explanation or goodness of fit (R^2) for the PLS-model was 0.91 and the predicted variation (Q^2) was 0.87.

5.2.3 Imaging Conditions

Digital images taken from powder surfaces contain substantial information that is needed for particle size distribution analysis. To obtain this information reproducibly from images, careful consideration has to be given on the imaging conditions. In this work (III, IV) two stabilised collimated illumination units were used, but the illumination could also be made with one light source and by rotating the sample. The main issue is that the sample has to be unilaterally illuminated and the optical axis has to remain the same. By taking several images while rotating the sample round its optical axis, 3–D spatial information of the surface could be revealed (Russ, 1999). Lateral illumination reveals distinct details of the analysed material (Pons et al. 1999, Huang and Esbensen, 2000). A controlled and reproducible imaging environment enables inspection of differences between batches. As we are examining surface images from identically-sized areas we are in fact inspecting textural properties of materials. The texture of a surface is formed by shading effects, which in turn expose the topographic properties of the surfaces (Pons et al. 1999). When the GSDM is calculated the means of image subtraction is used. Subtraction is primarily a way to discover differences between images (Russ 1999). In the present approach, the combining of information from two images strengthens the shading effects.

The measurement sensitivity and the particle size range depend on the magnification used. In studies III and IV the approach was used for granules in the size range of approximately 20 – 2500 µm. In future studies the use of this optical technique with

larger magnifications and a smaller particle size range has to be investigated. The limiting factor for very small particles will be the wavelength of electromagnetic light. Larger particles can be measured representatively by taking several images of a powder surface. Some improvements in the imaging can be made with increased resolution of the system. Likewise, using more advanced cameras can increase the number of grey scale levels distinguished at each point. On the other hand, these improvements would mean slower image acquisition, digitisation and overall data processing time. Naturally, with high-resolution imaging the costs of equipment rise (Russ, 1999).

5.2.4 Comparison of methods used in particle size analysis

The introduced approach is very fast including the sample preparation, imaging and achieving the results, compared with sieve analysis. The time of analysis for replicate samples was 3 minutes for the introduced optical method and approximately 120 min for sieve analysis (III). In practice, the analysis of three samples with one sieve took approximately 8 hours if the time for cleaning and drying of equipment was included. When comparing to particle size determination with traditional image analysis, the examination is easier as the sample does not have to be dispersed. However, lately IA system providers have introduced relatively effective sample dispersers for measurements of individual particles (Hammond 1999). Extensive image pre-processing is also avoided when analysis is made directly from a surface image of undispersed particles. Such image processing steps, including noise reduction, binarisation and filtering, are needed to obtain results when measuring single particles.

An advantage of the GSDM method described in this paper is that the analysed sample can be utilised for other purposes after the analysis, since the sample is not destroyed. This is an advantage when the analysed particles are brittle. Sometimes a particle sizing method, such as sieve analysis might break larger granules to smaller units and give erroneous results. In sieving the blockage or blinding of the sieves is often encountered (Iacocca and German, 1997). In addition, static attraction of particles on sieves may create a problem.

The sample size, when using the introduced imaging method can be very small (a few milligrams). However, it has to be enough to completely cover the field-of-view of the employed imaging equipment. Samples for especially sieve analysis but also most often for laser diffraction as well have to be much larger. This makes the technique suitable for samples and materials which are expensive or available in small quantities only. Wet samples e.g. analysis of granules during granulation, can also be measured (IV). In this work (III, IV) models were calculated for sieve-analysis and laser diffraction, but similar models can be created for other particle size analysers as well. If viable models are created between the powder surface information and different sizing instruments, a particle size distribution that corresponds to all methods can be determined from powder surface data simultaneously. While the models are created using a reference method they can only be as good as the quality of measurements with the reference method.

Since the function of the GSDM approach has only been studied with granular materials in this work, future studies should focus on a wide range of materials including relatively small particles with different properties, e.g. highly cohesive powders. Moreover, studies on the effects of particle shape and the relation of shape on shade formation in particle size measurement from powder surface images have to be made. This will enable the evaluation of the possibilities and limitations of the introduced approach in powder characterisation. Naturally, also issues of qualification and validation have to be addressed. This aspect has been taken up by Bell et al. (1999) in the case of new analytical techniques for particle size measurement.

5.2.5 Surface images in process monitoring

Particle size measurements from a selection of 10 batches with different kind of granule growth behaviour are presented in figure 2 (IV). Three different growth kinetics curves (one from each distinct size range from figure 2, IV) were chosen to show how the process can be controlled and monitored visually by images and data points. These batches, R2, R13, and R23 with one image from each data point during processing and three end-point images are shown in Figs 3a-c (IV). Batch R23 is also visualised in figure 13 below. By inspecting the images, it is clearly seen how the particle growth is evolving.
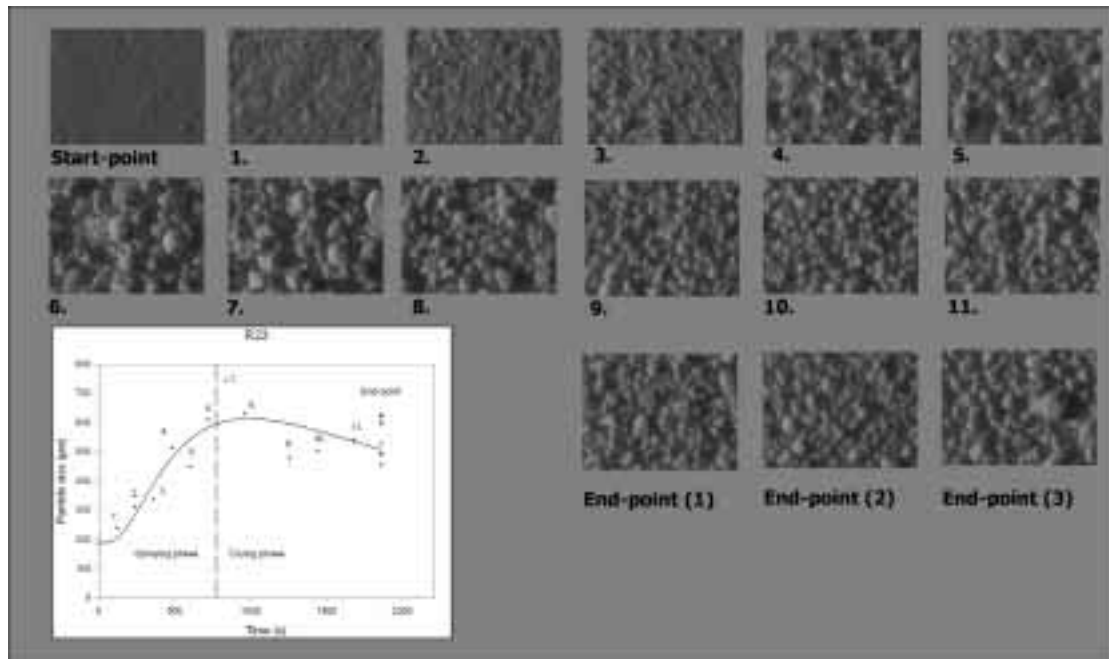
Figure 13. The granule growth of example batch R23. Each dot (•) shows the data point for the particle mean size measured from the surface image information. Each numbered dot corresponds to the numbered surface images. Additionally, three end-point data points and images are shown together with three replicate data points from end-point sieve analysis (▪). The spraying and drying phases of the process are separated with the dashed line. Figure taken from publication IV.

As images from only one sample per data point were captured and used in particle size measurement, the particle size might be slightly over- or underestimated. The sizes of the samples that are taken from the process are also relatively small with respect to total mass in the granulator. However, as approximately 14 samples were collected from one process run both over- and underestimations occur evenly. In order to create a growth kinetic curve, a log-normal curve fit was made, which travels as close to the centre of data points as possible (IV, Eq. 2).

Non-destructive at-line particle sizing methods can be advantageous to use if the applied method provides close to real time results and the analysis of material can be made during all process phases from both moist and dry samples. When samples are taken out of the process during granulation, it may be beneficial for the operator to be able to inspect the material visually. If real time monitoring of the particle size can be made at-line leaving the sample undamaged, physical characterisation of the sample with other techniques is also possible. The method used was suitable in the measurement of granule samples during all process phases. It was possible to measure both dry and wet samples with a wide particle size range. We used a prototype of the imaging instrument that was

built in our laboratory, which was not located in the same room with the granulator. This did not enable real time monitoring of particle size. However, if the optical instrument would be located next to the granulator the analysis of a sample within 5-10 s would be possible. Automatic sampling would enable on-line measurements. A pilot scale fluidised bed granulation process is usually relatively short (15-25 min). The monitoring of granule growth by the introduced method is more suitable for large-scale granulations, where processing times are longer. The operator has more time to use the visual information of the images and the particle data generated as a tool in process control decision-making. One advantage of the presented approach is that inspection of different granule samples can be made visually without difficulty and batch-to-batch comparisons of these images can be helpful. Optimally the visual inspection can be made first in real time and then in addition as a control after the completed process.

Process monitoring can often be established on visual observations. The use of acoustic methods in monitoring of particle fluidisation has been investigated successfully (Tsujimoto et al., 2000). An interesting aim would be to try to mimic human perception in process control situations. By combining acoustic and visual methods together with multivariate modelling one could open further perspectives in process control.

5.2.6 Prediction of compression behaviour

Principal component analysis was capable of visualising the differences in the particle size distribution information generated from the images. The observations are arranged into a plane (IV, Fig 4a-b.). The granule batches with different kind of particle size distributions are projected on different areas on the score plot. PCA indicated that two components explained about 85 percent of the variance of the data investigated. The first component accounted for 59.8 % of the variance. The loadings plot shows how the different size fractions reside on the plane. The area with the tablets with the smallest weight variation (r.s.d. between 1.8 and 4.2 %) is marked with a circle on the score plot. Characteristic for this area is that size fractions 0.250-0.355 mm and 0.355-0.500 mm dominate. The batches with a large amount of particles in this size range are situated here. This is confirmed by table 3 (IV). It can clearly be seen from the score plot that the granule batches that have the largest weight variation are situated outside the circle. In the case of size fractions with the largest particles the larger weight variation is caused by

the fact that large particles do not fill the die completely and a varying amount of empty space is left between the granules. The weight variation of the batches containing smaller particle fractions is most likely explained by poor flowability of smaller particles.

There has to be a balance between fine and coarser particles. A critical increase in the amount of fines results in poorer flowability (Danish and Parrot, 1971). The results of this study show that granules with a larger amount of particles in size fractions 0.250-0.355 mm and 0.350-0.500 mm have the best behaviour to fill the tablet machine die causing the smallest weight variation in tablets. This valuable information could be directly extracted from the images of granule surfaces.

Principal component analysis as a data projection method was capable of efficiently visualise the variation in the data. PCA was useful in the prediction of tabletting behaviour of granules in question when using particle size data generated from surface images. It has always been difficult to give a definition for good granules. From the tabletting point of view granules are good when the bulk behaviour results in minimal weight variation of tablets. The introduced method gives one example how to define good granules by using information extracted from images.

6. SUMMARY AND CONCLUSIONS

In this study, the use of image information from surfaces of pharmaceutical powders was exploited in a novel fashion. The characterisation of particulate populations through surface images was successful, with respect to classification of images, measurement of the particle size of granular materials and the prediction of tabletting behaviour of granules.

The novel surface-imaging instrument was successfully used in surface imaging of undispersed powder surfaces. A new descriptor for describing the particle size of granular material, the grey scale difference matrix (GSDM), was developed and used effectively in particle size measurement.

The idea of using a content-based image retrieval (CBIR) technique for pharmaceutical powder images was introduced and a basis was laid for the future use of CBIR in powder technology.

It was also found that the current visual characterisation approach could be used as an effective process analytical tool. The utilisation of surface image information to assure acceptable end product quality at the completion of a granulation process was also possible. Fast screening of properties can be made using the combination of automatically processed image information and multivariate visualisation.

This work promotes the use of image information more comprehensively in pharmaceutical powder technology and physical characterisation. In general, the idea of characterisation of bulk surface images opens new perspectives for visual characterisation of pharmaceutical solids.

References

Aigrain, P., Zhang, H. and Petkovic, D. 1996. Content-based representation and retrieval of visual media: A state-of-the-art review. Multimedia Tools and Applications 3, 179-202.

Aksoy, S. and Haralick, R.M. 1998. Textural features for image database retrieval Proceedings. IEEE Workshop on Content-Based Access of Image and Video Libraries. P. 45 –49.

Allen, T. 1988. Particle size measurement, 4 th Ed., Chapman & Hall, London, New York.

Andersson, M., Holmquist, B., Lindquist, J., Nilsson, O., and Wahlund, K-G. 2000. Analysis of film coating thickness and surface area of pharmaceutical pellets using fluorescence microscopy and image analysis. J. Pharm. Biomed. Anal. 22, 325-339.

Andrès, C., Bracconi, P., Réginault, P., Blouquin, P., Rochat, M.H., and Pourcelot, Y. 1998. Assessing the particle size of a broadly dispersed powder by complementary techniques. Int. J. Pharm. 167 (1-2), 129-138.

Andrés, C., Réginault, P., Rochat, M.H., Chaillot, B. and Pourcelot, Y. 1996. Particle size distribution of a powder: comparison of three analytical techniques. Int. J. Pharm. 144, 141-146.

Antikainen, O.K., Rantanen, J.T. and Yliruusi, J.K. 2000. Use of Kohonen self-organising map to predict the flowability of powders. S.T.P. Pharma Sci. 10 (5), 349- 354.

Ashley, J., Barber, R., Flickner, M, Hafner, D., Lee,D., Niblack, W. and Petkovic, D. 1995. Automatic and semi-automatic methods for image annotation and retrieval in QBIC. Storage and retrieval for image and video datbases III, Proc. SPIE -Int. Soc. Opt. Eng. 2420, 24-35.

Atkinson, R.M., Beford, C., Child, K.J., and Tomich, E.G. Effect of particle size on blood griseofulvin levels on man. Nature. 193, 588-589.

Bach, J.R., Fuller, C., Gupta, A., Hampapur, A., Horowitz, B., Humphrey, R., Jain, R.and Shu, C. 1996. The Virage image search engine: An open framework for image management. Storage and retrieval for image and video datbases, Proc. SPIE -Int. Soc. Opt. Eng. 2670, 76-87.

Barber, T.A.(editor). 1993. Pharmaceutical particulate matter -Analysis and control, Interpharm Press, Buffalo Grove, IL.

Bell, R., Dennis, A, Hendriksen, B., North, N. and Sherwood, J. 1999. Position paper on particle sizing, Sample preparation, method of validation and data presentation. Pharm. Technol. Europe. Nov. 1-3.

Benkerrour, L., Duchêne, D., Puisieux, F. and Maceario, J. 1984. Granule and tablet formulae study by principal component analysis. Int. J. Pharm. 19(1), 27-34.

Bergman, R, Johansson, M.E., Lundstedt, T., Seifert, E. and Åberg, J. 1998. Optimisation of a granulation and tabletting process by sequential design and multivariate analysis. Chemom. Intell.Lab.Syst. 44(1-2), 271-286.

Bertrand, D., Robert, P., Melcion J.P. and Sire, A. 1991. Characterisation of powders by video image analysis. Powder Technol. 66(2), 171-176.

Bonifazi, G. 1997. Particulate solids control in bulk by image analysis. Proceedings in Powder and bulk solids conference, Rosemont III, May 5-8, 337-348.

Bonifazi, G., La Marca, F. and Massacci, P. 2002. Characterisation of bulk particles in real time. Part. Part. Syst. Charact. 19, 240-246.

Bønløkke, L., Hovgaard, L., Kristensen H.G., Knutson, L. and Lennernäs, H. 2001. Direct estimation of the in vivo dissolution of spironolactone, in two particle size ranges, using the single-pass perfusion technique (Loc-I-Gut®) in humans. Eur. J. Pharm. Sci., 12(3), 239-250.

Bordes C, Garcia, F. Snabre, P. and Frances C. 2002. On-line characterisation of particle size during ultrafine wet grinding process. Powder Technol. 128, 218-228.

Bosquillon, C., Lombry, C., Preat, V., and Vanbever, R. 2001. Comparison of particle sizing techniques in the case of inhalation dry powders. J. Pharm.Sci. 90(12), 2032-3041.

Bourquin, J., Schmidli, H., van Hoogevest, P. and Leuenberger, H. 1997. Application of artificial neural networks (ANN) in the development of solid dosage forms. Pharm. Dev. Tech. 2(2), 111-121.

Bourquin, J., Schmidli,H., van Hoogevest, P. and Leuenberger, H. 1997. Basic concepts of artificial neural networks (ANN) modelling in the application to pharmaceutical development. Pharm. Dev. Tech. 2(2), 95-109.

Brewer, E., and Ramsland, A. 1995. Particle size determination by automated microscopical imaging analysis with comparison to laser diffraction. J. Pharm. Sci. 84(4), 499-501.

British Standard BS 2955. 1991. Glossary of Terms Relating to Particle Technology, British Standards Institution, London.

Brittain H.G., Bogdanowich S.J., Bugay D.E., DeVincentis J., Lewen G., Newman A.W. 1991. Physical characterisation of pharmaceutical solids. Pharm. Res. 8(8), 963-73.

Brown R.L. and Richards J.C. 1970. Principles of Powder Mechanics, Oxford Pergamon Press, UK.

Bugay D.E. 2001.Characterisation of the solid-state: spectroscopic techniques. Adv. Drug Deliv. Rev. 48 ,43–65.

Byrn S, Pfeiffer R, Ganey M, Hoiberg C. and Poochikian G. 1995. Pharmaceutical solids: a strategic approach to regulatory considerations. Pharm.. Res. 12(7), 945-54.

Caelli, T. and Reye, D. 1993. On the classification of Image regions by colour, texture and shape. Pattern Recognition 26, 461-470.

Carr R.L. 1965. Evaluating flow properties of solids. Chem. Eng. (Jan.) 163-168.

Carr R.L. 1965. Classifying flow properties of Solids. Chem. Eng (Feb.) 69-72.

Carson J.W. 1988. Overcoming particle segregation in the pharmaceutical and cosmetics industries. Drug Dev. Ind. Pharm. 14, 2749-2758.

Carstensen J.T. and Franchini M. 1993. The use of fractal geometry in pharmaceutical systems. Drug Dev. Ind. Pharm.19(1-2), 85-100.

Cartilier, L.H., and Tawashi R.1993. Effect of particle morphology on the flow and packing properties of lactose. S.T.P. Pharma Sciences, 3(3), 213-220.

Castelli, V. and Bergman, L.D. (Editors). 2002. Image Databases – Search and Retrieval of Digital Imagery. John Wiley & Sons Inc. New York.

Cielo, P. 1988. Optical Techniques for Industrial Inspection, Academic Press, London.

Coppens, P., Deriemaker, L. and Finsy,R. 2000. Shape and size determination by laser diffraction: feasibility of data analysis by neural networks. Part. Part. Syst. Charact., 17, 111 -125.

Cox, E.P. 1927 A method of assigning numerical and percentage values on the degree of roundness of sand grains. J. Paleont. 1, 179-183.

Dahneke, B.E. 1973. Aerosol beam spectrometry. Nature Phys. Sci. 244, 54-55.

Danish F.Q. and Parrot E.L. 1971. Flow rates of solid particulate pharmaceuticals. J.Pharm. Sci. 60, 548-554.

Danjo K., Kinoshita, K., Kitagawa, K, Iida K. Sunada, H and Otsuka, A. 1989. Effect of particle shape on the compaction and flow properties of powders. Chem. Phar. Bull. 11, 3070-3073.

Dasykowski, M., Walczak, B. and Massart, D.L. 2003. Projection methods in chemistry. Chemom Intell. Lab Syst. 65, 97-112.

Del Bimbo. A. 1996. Image and video databases: Visual browsing, querying and retrieval. J. Visual Lang. Comput. 7, 353-359.

Del Bimbo. A. 1999. Visual iinformation retrieval, Morgan Kaufmann Publishers Inc. San Fransisco. Eilbeck, J., Rowley, G., Carter, P.A., Fletcher, E.J. 2000. Effect of contamination of pharmaceutical equipment on powder triboelectrification. Int. J. Pharm. 195, 7–11.

Eriksson, M., Alderborn, G., Nyström, C., Podczeck F. and Newton, J. M. 1997. Comparison between and evaluation of some methods for the assessment of the sphericity of pellets. Int. J. Pharm.. 148(29), 149-154.

Etzler F.M. and Sanderson, M.S. 1995. Particle size analysis: A comparative study of various methods. Part. Part. Syst. Charact. 12(5), 217-224.

Etzler F.M. and Deanne, R. 1997. Particle size analysis: A comparative study of various methods. Part 2. Part. Part. Syst. Charact. 14(6), 278-282.

Faloutsos, C. , Barber, R., Flickner, M., Hafner, J., Niblack, W. and Petkovic, D. 1994. Efficient and effective querying by image content. J. Intell. Inf. Syst. 3, 231-262.

Faure, A., York, P. and Rowe, R.C. 2001. Process control and scale-up of pharmaceutical wet granulation processes: review. Eur .J. Pharm. Biopharm. 52, 269-277.

Flickner, M., Sawhney, H., Niblack, W., Ashley, J. Huang, Q., Dom, B., Gorkani, M., Hafner, J., Lee, D., Petkovic, D., Steele,D., Yanker, P. 1995. Query by image and video content: The QBIC system. Computer. 28(9), 23-31.

Geldhart, D.1990. Powder processing the overall view, In Principles of Powder Technology, M. Rhodes (editor). John Wiley & Sons, New York. P.1-7.

Gold, G. Duvall, R.N. Palermo, B.T. and Slater, G. 1968. Powder flow studies III, Factor affecting the flow of lactose granules. J. Pharm. Sci. 57, 667-671.

Gonzalez and Woods 2001. Digital Image Processing. Prentice-Hall, Inc. Upper Saddle River, New Jersey.

Grasa, G. and Abanades, J.C. 2001. A calibration procedure to obtain solid concentrations from digital images of bulk powders. Powder Technol. 114, 125-128.

Gudivada, V.N. and Raghavan, V.V. 1995. Content-based image retrieval systems. Computer 28(9), 18-21.

Gupta, A. , Moezzi, S., Taylor, A., Chatterjee, S., Jain, R., Goldbaum, M and Burgess, S. 1996. Content-based retrieval of opthalmological images, IEEE –International Conference on Image Processing, 3, 703-706.

Hammond, S. and Egelberg, P. 1999. Particle size and shape measurement by automated microscopy. Eur. Pharm. Rev. 4(4), 16-20.

Hassel, D. and Bowman, E. 1998. Process analytical chemistry for spectrosopists. Appl. Spectrosc. 52, 18A-29A .

Haykin, S. 1994. Neural Networks. A Comprehensive Foundation. Macmillan Press, New York.

Hellén, L., Yliruusi, J. and Kristofferson, E. 1993a Process variables of instant granulator and spheroniser: II. Size and size distributions of pellets. Int. J. Pharm. 96(1-3), 205-216.

Hellén, L. and Yliruusi, J. 1993b. Process variables of instant granulator and spheroniser: III. Shape and shape distributions of pellets. Int. J. Pharm. 96(1-3), 217-223.

Heywood, H. 1937. Numerical definitions of particle size and shape. Chem. Ind. 15, 149-154.

Heywood, H. 1954. Particle shape coefficients. J. Imp.Coll. Eng .Soc. 8, 25-39.

Hoffmann, A.C, Finkers, H.J. and Seegers, J.A. 1996. Relationships between particle properties and bulk behaviour for some industrial powders. Part. Part. Syst. Charact. 13, 354-361.

Hiestand E.N. 1966. Powders: particle-particle interactions. J. Pharm. Sci. 55(12), 1325-1344.

Hintz, R.J. and Johnson, K. C. 1989. The effect of particle size distribution on dissolution rate and oral absorption. Int. J. Pharm. 51(1), 9-17.

Hörter, D. and Dressman, J. B. 2001. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. Adv. Drug Del. Rev. 46(1-3), 75-87.

Hotelling, H., 1933. Analysis of a complex of statistical variables into principal components. J. Educat. Psych. 24, 417-441.

Hou, T.-Y., Liu, P., Hsu, A. and Chiu, M.-Y. 1992. Medical Image Retrieval by Spatial Features. Proc. IEEE -International Conference on System, Man and Cybernetics, 1364-1369.

Houghton M.E. and Amidon, G.E. 1992. Microscopic characterisation of particle size and shape: an inexpensive and versatile method. Pharm. Res. 9(7), 856-859.

Huang, J. and Esbensen, K.H. 2000. Applications of Angle Measure Technique (AMT) in image analysis. Part I. A new methodology for in situ powder characterisation. Chemom. Intell. Lab. Syst. 54, 1-19.

Huang, J. and Esbensen, K.H. 2001. Applications of Angle Measure Technique (AMT) in image analysis. Part I. Prediction of powder function properties and mixing components using Multivariate AMT regression (MAR). Chemom. Intell. Lab. Syst. 57, 37-56.

Hundal, H.S., Rohani, S. , Wood, H.C. and Pons, M.N. 1997. Particle shape characterisation using image analysis and neural networks. Powder Technol. 91, 217-227.

Hunter, B.M. and Ganderton, D. 1972. Effect of particle size on the granulation of lactose by massing and screening. J. Pharm. Pharmac. 24, 17-24.

Hussain, A.S., Yu, X., Johnson, R.D. 1991. Application of neural computing in pharmaceutical product development. Pharm. Res. 8, 1248-1252.

Ibrahim, H., Sallam, E., Takieddin, M., Shamat, M., Abu. 1988. Dissolution characteristics of interactive powder mixtures. Part one: Effect of solubility and particle size of excipients. Drug Dev. Ind. Pharm. 14(9), 1249-76.

Iacocca, R.G. and German, R.M. 1997. A comparison of powder particle size measuring instruments. Int. J. Powder Metall. 33(8), 35-48.

Ikekawa, A. and Kaneniwa, N. 1968. Influence of particle size on physicochemical properties of pharmaceutical powders. IV. On packing of pharmaceutical powders 2. Chem. Pharm. Bull. 16(7), 1174-1180.

International Conference on Harmonisation (ICH).1999. ICH 6QA - Specifications: Test procedures and acceptance criteria for new drug substances and new drug products: Chemical substances.

Iveson, S.M., Litster, J.D., Hapgood, K. and Ennis, B.J. 2001. Nucleation, growth and breakage phenomena in agitated wet granulation processes: a review, Powder Technol. 117, 3-39.

Johanson, J.R. 1996. Predicting segregation of bimodal particle mixtures using the flow properties of bulk solids, Pharm. Technol. Europe. 8(1), 38-44.

Johnson K.C.and Swindell A.C. Guidance in the setting of drug particle size specifications to minimize variability in absorption. Pharm.Res. 13(12), 1795-1798.

Jounela, A., Pentikäinen P. and Sothmann, A. 1975. Effect of particle size on the bioavailability of digoxin. Eur. J. Clin. Pharmacol.. 8, 365-370.

Kachrimanis, K. Karamyan, V. and Malamataris, S. 2003. Artificial neural networks (ANNs) and modelling of powder flow. Int. J. Pharm. 250(1,) 13-23.

Kaneniwa, N.; Ikekawa A.; and Aoki, H. 1967. Influence of particle size on physicochemical properties of pharmaceutical powders. I. On fluidity of sodium borate and boric acid powders 1. Chem. Pharm. Bull. 15(10), 1441-1445.

Karhunen, K. 1947. Uber lineare methoden in der Wahrscheinlichkeitsrechnung. Annales Academiae Scientiarum Fennicae, Seried A1: Mathematica-Physica, 37, 3-79.

Kaye B.H. 1978. Specifications of the ruggedness and/or texture of a fine particle profile by its fractal dimension. Powder Technol. 1-16.

Kaye B.H., Alliet D. Switzer, L. and Turbitt-Daoust, C. 1997. The effect of shape intermethod correlation of techniques for characterizing the size distribution of powder. Part 1: correlating the size distribution measured by sieving, image analyis and diffractometer methofs. Part. Part. Syst. Charact. 14, 219-224.

Kennedy J.P. and Niebergall, P.J. 1997. Preliminary assessment of an image analysis method for the evaluation of pharmaceutical coatings. Pharm. Dev.Tech. 2, 205-212.

Kohonen, T. 1982. Self-organised formation of topologically correct feature maps. Biol. Cybern. 43, 56-69.

Kohonen,T. 1997. Self-organizing Maps. In: Series in Information Sciences, vol. 30, Springer Heidelberg, Germany.

Laaksonen, J.T. , Koskela, J.M., Laakso, S.P. and Oja, E. 2000. PicSOM - content-based image retrieval with self-organizing maps. Pattern Recogn. Lett. 21(13-14), 1199-1207.

Laaksonen, J., Koskela, M. Laakso, S. and Oja, E. 2001. Self-organizing maps as a relevance feedback technique in content-based image retrieval. Pattern Anal. Appl. 4(2+3), 140-152.

Laaksonen, J., Koskela, M. and Oja, E. 2002. PicSOM –self organizing image retrieval with MPE-7 contetnt descriptor. IEEE Transactions on Neural Networks 13(4), 841-853.

Laitinen, N., Rantanen, J., Laaksonen, J., Antikainen, O., and Yliruusi, J. 2002. Major advances in the use of digital image information in pharmaceutical powder characterisation. Proceedings in 4th World meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, 8-11.4.2002, Florence, Italy. p. 33-34.

Laitinen, N. and Juppo, A.M. 2003. Measurement of pharmaceutical particles using a time-of-flight particle sizer. Eur.J.Pharm.Biopharm. 55(1), 93-98.

Larsen, C.C., Sonnergaard, J.M. Bertelsen, P., and Holm, P. 2003. Validation of an image analysis method for estimating coating thickness on pellets. Eur.J.Pharm.Sci 18, 191-196.

Lees, K.A. 1963. Pharmaceutical aspects of fine particles and their evaluation. Pharm. J. 191(5211):289-291.

Lin, C.L. and Miller, J.D. 1993. The development of a PC, image based, on-line particle size anlyzer. Miner Metall. Proc. (Feb), 29-35.

Lindberg, N-O. and Lundstedt, T. 1995. Application of multivariate analysis in pharmaceutical development work, Drug Dev. Ind. Pharm. 21 (9), 987-1007.

von Lindener, H. and Kleinebudde P. 1993. Anwendung der automatischen Bildanalyse zur Charkteriserung von Pellets. Pharm. Ind. 55(7) 694-701.

Luekens, D.W., Beddow, J.K. and Vetter, A.F. 1982. Morphological fourier descriptors. Powder Technol. 31, 209-215.

Manjunath, B.S. and Ma, W.Y. 1996. Browsing large satellite and aerial photographs. IEEE - International Conference on Image Processing, 2, 765-767.

Mehrotra, R. and Gary, J.E. 1995. Similar-shape retrieval in shape data mangement. Computer. 28(9), 57-62.

Meloy, T.P. 1977a. A hypothesis on morphological characterisation of particles shape and physicochemical properties. Powder Technol. 16, 233-253.

Meloy, T.P. 1977b. Fast fourier transorms applied to shape of particle silhouettes to obtain morphological data. Powder Technol. 17, 27-35.

Melssen, W.J. Smits, J.R.M, Buydens, L.M.C. and Kateman, G. 1994. Using artificial neural networks for solving chemical problems, Part II,. Kohonen self-organising feature maps and Hopfield networks. Chem. Intell. Lab. Syst. 23, 267-291.

Mitchell, J.P. and Nagel, M.W. 1995. An assessment of the API Aerosizer® for the real-time measurement of medical aerosols from pressurised metered-dose inhaler (pMDI) systems. Aerosol Sci. Technol. 25, 411-423.

Murtomaa, M. 2002. Electrostatic studies on pharmaceutical powders and aerosols, Doctoral Thesis, Annales Universitatis Turkuensis, Turku, Finland.

Murtoniemi, E., Yliruusi, J., Kinnunen, P., Merkku, P., and Leiviskä, K. 1994. The advantages by the use of neural networks in modelling the fluidised bed granulation process. Int. J. Pharm. 108,155-164.

Muzzio, F.J., Shinbrot, T. and Glasser, B.J. 2002. Powder technology in the pharmaceutical industry: The need to catch up fast. Powder Technol. 124(1-2), 1-7.

Nazar, A.M., Silca, F.A. and Amman, J.J. 1996. Image processing for particle characterisation. Mater. Charact. 36, 165-173.

Ng, V., Cheung, D. and Fu, A. 1995. Medical image retrieval by colour content. Proc. IEEE International Conference on System, Man and Cybernetics: Intelligent Systems for the 21st century, 3:1980-1985.

Niblack, W., Barber, R. Equitz., W., Flickner, M.B., Glasman, E., Petkovic, D., Yanker, P., Faloutsos, C. and Taubin, G. 1993.The QBIC project: querying images by content using colour, texture and shape. Storage and Retrieval for Image and Video Databases, Proc. SPIE -Int. Soc. Opt. Eng. 1908, 173-181.

Niven, R.W.. 1993. Aerodynamic particle size testing using a time-of-flight aerosol beam spectrometer. Pharm. Technol. 17, 72-78.

Novales, B., Guillaume, S. Devaux, M.F. and M. Chaurand. 1998. Particle size characterisation of in-flow milling products by video image anlysis using global features. J.Sci. Food Agric. 78, 187-195.

Passerini, N., Albertini, B., González-Rodríguez, M.L., Cavallari, C. and Rodriguez, L. 2002. Preparation and characterisation of ibuprofen-poloxamer 188 granules obtained by melt granulation. Eur. J. Pharm. Sci. 15, 71-78.

Pearson, K. 1901. On lines and planes of closest fit to systems of points in space. Philos.Mag. Ser. 6, 2 559-572.

Pentland, A., Picard, R.W. and Scarloff, S. 1994. Photobook: tools for content-based manipulation of image databases. Storage and Retrieval for Image and Video Databases II, Proc. SPIE -Int. Soc. Opt. Eng. 2185, 34-47.

Pifferi, G, Santoro,P. and Pedrani, M. 1999. Quality and functionality of excipients, Farmaco. 54(1-2), 1-14.

Podczeck ,F., Rahman, S.R., and Newton, J.M. 1999. Evaluation of a standardised procedure to assess the shape of pellets using image analysis. Int. J. Pharm. 192(2), 123-138.

Podczeck, F. 1997. A shape factor to assess the shape of particles using image analysis. Powder Technol. 93, 47-53.

Podczeck, F. and Newton, J.M . 1995. Evaluation of a three-dimensional shape factor for the quantitative assessment of the sphericity and surface roughness of pellets. Int J Pharm. 124, 253-259.

Podczeck, F., Newton J.M. 1994. A shape factor to characterise the quality of spheroids. J. Pharm. Pharmacol. 46(2), 82-95.

Pons, M.N., Vivier, H., Belaroui,K., Bernard-Michel,B., Cordier,F., Oulhana, D., and Dodds, J.A. 1999. Particle morphology: from visualisation to measurement. Powder Technol. 103, 44-57.

Pons, M.N., Vivier, H. Belaroui, K., Bernard-Michel, B., Cordier, F., Oulhana, D., and Dodds J.A. 1999. Particle morphology: from visualisation to measurement. Powder Technol. 103, 44-57.

Pons, M-N., Vivier, H., Delcour, V., Authelin J-R. and Paillères-Hubert, L. 2002. Morphological analysis of pharmaceutical powders. Powder Technol., 128(2-3), 276-286.

Prescott, J.K. and Hossfield, R.J. 1994. Maintaining product uniformity and uninterrupted flow to direct-copmpression tabletting presses. Pharm.Technol. June, 99-114.

Rantanen, J. , Känsäkoski, M., Suhonen, J., Tenhunen, J., Lehtonen,S., Rajalahti, T., Mannermaa J.-P. and Yliruusi, J. 2000. Next generation fluidised bed granulator automation. AAPS Pharm. Sci. Tech. 1 (2) article 10 (http://www.pharmscitech.com).

Rantanen, J.T., Laine, S.J., Antikainen, O.K., Mannermaa, J-P. , Simula, O.E. and Yliruusi, J.K. 2001. Visualisation of fluid-bed granulation with self-organizing maps, Pharm. Biomed. Anal. 24, 343-352.

Ratha, N., Karu, K., Chen, S. and Jain, A. 1996. A real-time matching system for large fingerprint databases. IEEE Trans. PAMI, 18(8), 799-812.

Rees, J.E. 1977. Characterisation of pharmaceutical particulate materials. Boll Chim. Farm. 116(3), 125-41.

Ridgway K, and Rupp R. 1969. The effect of particle shape on powder properties. J. Pharm. Pharmacol. 21, Suppl. 30-39.

Ros,. F., Guillaume, S., Bellon-Maurel, V., and Bertrand, D. 1997. Building and preprocessing image data using indices of representativeness and classification applied to granular product characterisation. J. Chemometrics. 11, 469-482.

Rowley, G. 2001. Quantifying electrostatic interactions in pharmaceutical solid systems, Int.J. Pharm. 227, 1-2(4), 47-55.

Russ, J. 1999. The Image Processing Handbook. 3rd Ed., CRC Press, Boca Raton, FL.

Rösler, R. Schneider, H.A. and Schuberth R. 1987. Relation between particle shape and profile Fourier coefficients. Powder Technol. 49, 255-260.

Scholz, A., Abrahamsson, B., Diebold, S.M., Kostewicz, E., Polentarutti, B.I, Ungell, A-L., and Dressman, J.B. 2002. Influence of hydrodynamics and particle size on the absorption of felodipine in labradors. Pharm. Res. 19(1), 42-46.

Scott, D.M., Boxman, A. and Davies. R. 1998. Industrial applications of in-line particle characterisation. Proc. in World Congress on particle Technology 3, Brighton, UK, July 6-9.

Smith, J.R. and Chang, S.-F. 1996. Visual SEEk: A fully automated content-based image query system. Proc. ACM –Int. Conf. Multimedia. 96, 87-96.

Smith, J.R. and Chang, S.-F. 1996. Tools and techniques for colour image retrieval, Storage and retrieval for image and video databases, Proc. SPIE -Int. Soc. Opt. Eng. 2670, 426-437.

Snow, R.H., Allen, T., Ennis, B.K. and Litster, J.D. 1997. Size reduction and size enlargement. In Perry's Chemical Engineers' Handbook, McGraw-Hill, New York, p. 20-56 – 20-89.

Soffer, A. and Samet, H. 1996. Retrieval by content in symbolic-image databases Storage and retrieval for image and video databases IV, Proc. SPIE -Int. Soc. Opt. Eng. 2670, 144-155.

Sonka, M., Hlavac, V. and Boyle, R. 1998. Image Processing, Analysis and Machine Vision, Brooks/Cole Publishing company., Pacific Grove, CA.

Staniforth, J.N. and Rees J.E. 1982. Electrostatic charge interactions in ordered powder mixes. J. Pharm. Pharmacol. 34(2), 69-76.

Staniforth, J.N 1988. Particle size anlaysis, In Pharmaceutics - the science of dosage form design, M.E. Aulton (editor).Churchill Livingstone, London, UK. p. 566-558.

Stephenson,G.A, Forbes R.A. and Reutzel-Edens., S.M. 2001. Characterisation of the solid state: quantitative issues, Adv. Drug Deliv. Rev. 48(1), 67-90.

Swarbrick J. and Boylan, J.C. 1992. Encyclopedia of Pharmaceutical Technology: Film coatings and film forming materials: Evaluation to generic drugs and generic equivalency, Marcel Decker Inc., New York.

Travers, D.N. 1988. Mixing, In Pharmaceutics - the science of dosage form design, M.E. Aulton (editor). Churchill Livingstone, London, UK. p. 550-565.

Tsujimoto, H, Yokoyama, T., Huang, T.T., Sekiguchi, I. 2000. Monitoring particle fluidisation in a fludized bed granulator with an acoustic emission sensor. Powder Technol. 113, 88-96.

Turbitt-Daoust, C., Alliet, D.F., Kaye, B.H., and Matchett, A.J., 2000. Orientation effects in the evaluation of populations of irregularly shaped particles by image analysis, Part. Part. Syst .Charact. 17, 159-166.

Wadell, H. 1933. Sphericity and roundness of rock particles. J.Geol. 41, 310-331.

Walton, C. A. and Pilpel, N. 1972. Effects of particle size, shape, and moisture content on the tensile properties of procaine penicillin powders. J. Pharm. Pharmac. 24, 10-16.

Washington, C. 1992. Particle Size Analysis in Pharmaceutics and Other Industries: Theory and Practice, Ellis Horwood Limited, UK.

Watano, S. and Miyanami, K. 1995. Image processing for on-line monitoring of granule size distribution and shape in fluidised bed granulation. Powder Technol. 83:55-60.

Watano, S. Numa, T., Miyanami, K., and Osako, Y.2000. On-line monitoring of granule growth in high shear granulation by an image processing system. Chem. Pharm. Bull. 48(8), 1154-1159.

Watano, S., Sato, Y. and Miyanami, K. 1997. Application of neural network to granulation scale-up. Powder Technol. 90, 153-59.

Williams, D.1990. Mixing and segregation of powders, In Principles of Powder Technology, M. Rhodes (editor). John Wiley & Sons, New York. p.71-90.

Wold S. Chemometrics. 1995. What do we mean with it, and what do we want from it? Chemom .Intell. Lab. Syst. 42, 175-185.

Wold S., Trygg,J., Berglund,A. and Antti, H. 2001. Some recent developments in PLS modelling. Chemom. Intell. Lab. Syst. 58, 131–150.

Wold, S. 1995. PLS for multivariate linear modelling, In: QSAR: Chemometric methods in molecular design, Vol. 2. H. van de Waterbeemd. Ed. VCH, Weinheim.

Wold, S. Sjöström, M.and Eriksson L. 2001. PLS-regression: a basic tool of chemometrics. Chemom. Intell. Lab. Syst. 58, 109-130.

Wong, L.W. and Pilpel, N. 1990. Effect of particle shape on the mixing of powders. J. Pharm. Pharmac. 42(1), 1-6.

Yalkowsky and Bolton. 1990. Particle size and content uniformity. Pharm. Res. 7(9), 962-966.

York, P. 1978. Particle slippage and rearrangement during compression of pharmaceutical powders. J. Pharm. Pharmac. 30(1), 6-10.

You, J., Shen, H. and Cohen, H.A. 1997. An efficient parallel texture classification for image retrieval. J Visual Lang. Comput. 8, 359-372.

Zupan, J. and Gasteiger, J. 1993. Neural Networks for Chemists: An introduction, VCH, Wienheim, 1993, Chapters 10, 16, 18 and 19.

Ålander, E.M., Uusi-Penttilä, M.S. and Rasmuson, Å.C. 2003. Characterisation of paracetamol agglomerates by image analysis and strength measurement. Powder Technol. 130, 298-306.