FORMULATION AND EVALUATION OF PRONIOSOMES FOR ANTICANCER DRUGS

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1. INTRODUCTION

Throughout history humans have been mostly afraid of certain diseases. During the 19th century, there was a great development in pharmacology and microbiology. Due to it infectious diseases do not play a major role in developed countries as they did in the past, nowadays the disease that makes fear in the hearts of most common man is cancer.[1]

Cancer is characterized by a group of cells, displaying uncontrolled growth, invasion and sometimes metastasis. All the types of cancer begin in cells, which make up the blood and other tissues of the body. Cancer is differentiated when body cell undergo changes at the molecular level resulting in loss of regulation of normal cell characteristics and functions. Therefore, although cancer is classified according to organ system *viz.* breast, lung and colorectal cancer etc., cancer is a disease of cells.[2]

1.1 Epidemiology of cancer

The leading cause of death worldwide has been reported to be cancer; it was estimated that 14.1 million cancer cases were reported around the world in 2012, and of these 7.4 million and 6.7 million cases were in men and women respectively. More than half of these 8 million occurred in economically developing countries.[3] Deaths from cancer are projected to continue to rise, with a worldwide estimated death of 12 million in 2030.[4] The four most common cancers occurring worldwide are found to be: 1) lung, 2) female breast, 3) bowel and 4) prostate cancer and these four are reported to account for around 4 in 10 of all cancers diagnosed worldwide.[5]

Cancer is the second leading cause of death in USA after cardiovascular diseases, in both males and females. It is estimated that there were about 1,437,180 new cases and 565,650 deaths from cancer in 2008, forming 23% share of all mortality. The most common sites for new cancers in males include prostate, lung and colon whereas in females, cancers of breast, lung and colon are most prevalent. The cancers of breast, prostate, lung and colon represent over 50% of cancer deaths in USA.[6] Studies have also shown that the incidence of cancer varies within different races and ethnicities and that African Americans are affected the most.[7]

Cancer remains a significant national problem, 8% of people suffer from cancer alone in India. In 2005 cancer killed approximately 8,26,000 people in India, of which 5,19,000 were under the age of 70 years.[8,]

According to National Cancer Registry Programme of the India Council of Medical Research (ICMR), more than 1300 Indians die every day due to cancer. Between 2012 and 2014, the mortality rate due to cancer increased by approximately 6%. In 2012, there are 4,78,180 deaths out of 29,34,314 cases reported. In 2013 there are 4,65,169 death out of 30,16,628 cases. In 2014, 4,91,598 people died in 2014 out of 28,20,179 cases.[9] As per Population Cancer Registry of Indian Council of Medical Research, the incidence and mortality of cancer is highest in the North Eastern region of the country.[10] Breast cancer is the most common one, with Stomach cancer the leading cause of death by cancer for the population as a whole. Breast cancer and Lung cancer kill the most women and men respectively.[11]

1.2 Types of cancer

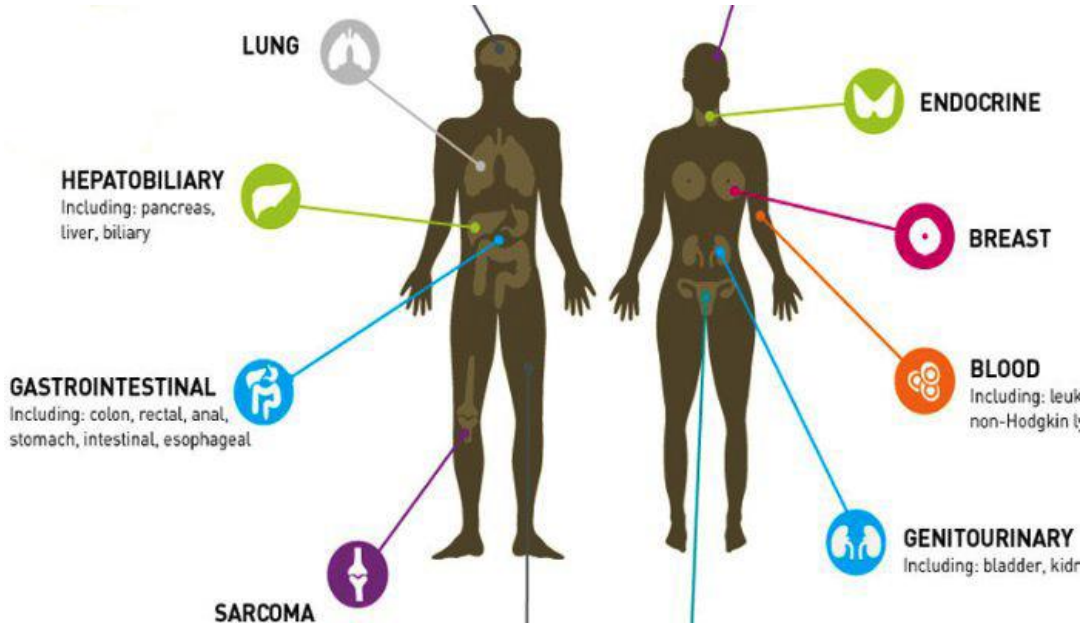

Figure 1.1 Types of cancer

1.3 Causes for cancer

Figure 1.2 Causes for cancer

1.4 Classification of anticancer drugs

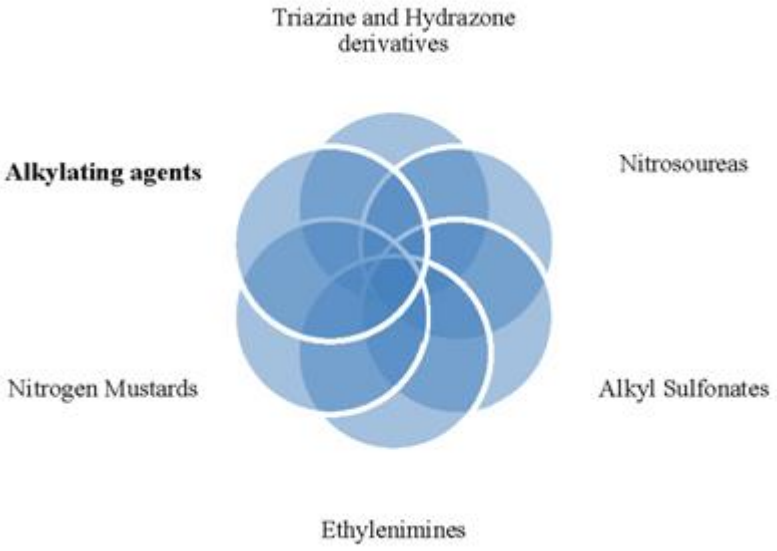

Figure 1.3 Classification of anti-cancer drug

1.5 Mechanism of action of anticancer drugs

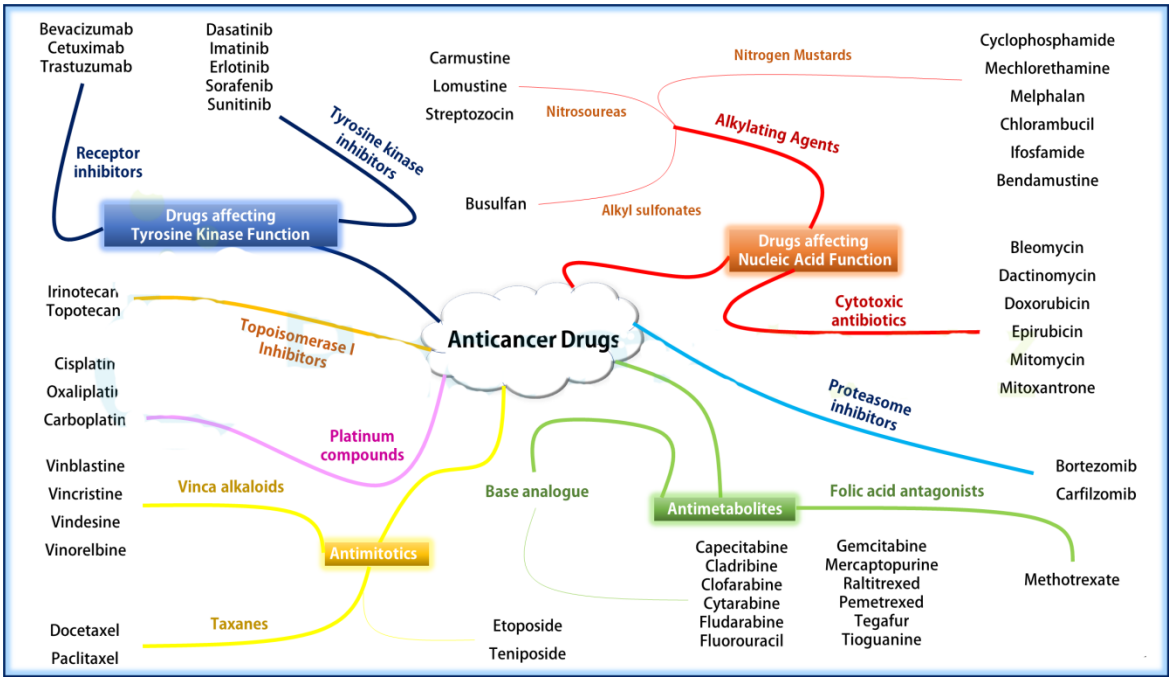

Figure 1.4 Mechanism of action of anticancer drugs

1.6 Cancer treatment

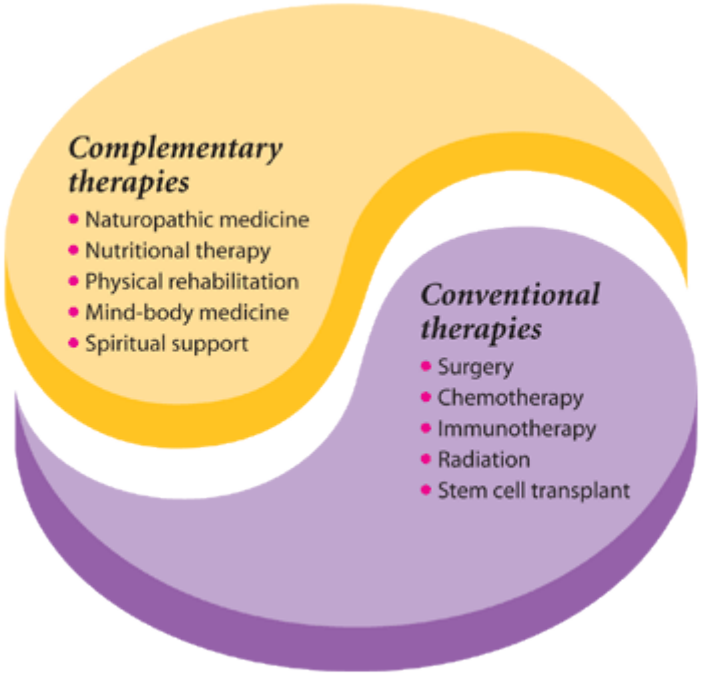

Figure 1.5 Cancer treatment

1.7 Breast cancer

Breast cancer A malignant cell growth in the breast, cancer spreads to other areas of the body, if left untreated. Accounting for one of every three cancer diagnoses, breast cancer, excluding skin cancers, is the most common type of cancer in women. Ductal carcinoma, the most common type of breast cancer, begins in the lining of the ducts. Lobular carcinoma, another type, arises in the lobules. When breast cancer spreads, is called metastatic breast cancer.

1.7.1 Early stages of breast cancer

Early Stages of Breast Cancer: Each breast is composed of up to 20 sections called "lobes" and milk is made in each lobe, made up of many smaller "lobules". Then the milk is carried to the nipples through lobes and lobules, connected by small tubes called "ducts".

1.7.1.1 Ductal carcinoma *in situ* (DCIS)

Abnormal cells in the lining of a Lp, duct. It is a non-invasive malignant tumor, and is also called intraductal carcinoma. The abnormal cells have not spread beyond the duct and have not invaded the surrounding breast tissue. However, DCIS can progress and become invasive. Tmx is sometimes used in combination with one of these two surgical treatment options. DCIS is sometimes called Stage 0 breast cancer because it is not invasive.

1.7.1.2 Lobular carcinoma in situ (LCIS

Tumor that consists of abnormal cells in the lining of a lobule. Even though "carcinoma" refers to cancer, LCIS is not a cancer and there is no evidence that the abnormal cells of LCIS will spread like cancer. Instead, having LCIS means that a woman has an increased risk of developing breast cancer in either breast. Despite the increased risk, most women with LCIS will never get breast cancer. No treatment is necessary and surgery is not usually recommended for LCIS. Some women choose to

take Tmx to decrease the likelihood of breast cancer. LCIS is sometimes called "Stage 0" breast cancer, but that is not really accurate because it is not really cancer.

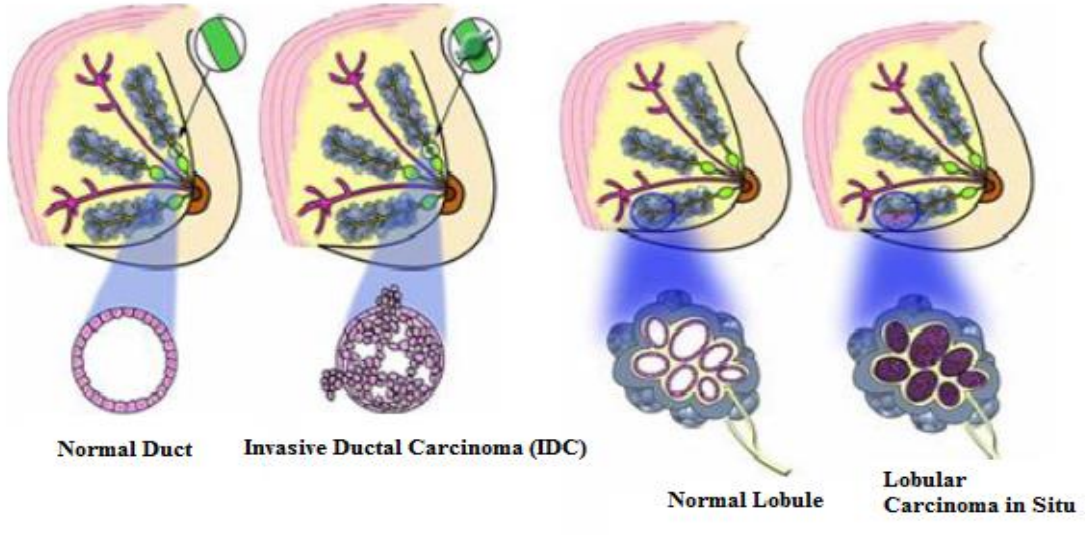

Figure 1.6 Early Stages of Breast Cancer (DCIS & LCIS)

1.7.2 Stages of breast cancer

Stage 1 The tumour measures < than 2cm across. The lymph glands in the armpit (axilla) are unaffected and there are no visible signs that the cancer has spread to other parts in the body.

Stage 2 The tumour measures about 2–5cm across and/or the lymph glands in the armpit are affected, or both. However, the tell-tale signs of further spread of cancer is absent.

Stage 3 The tumour is > than 5cm across, and may be attached to surrounding tissures organs, such as the muscle or skin. Though the lymph glands are usually affected, there are no signs of the spread of cancer beyond the breast or the lymph glands in the armpit.

Stage 4 Secondary or metastatic breast cancer: The tumour can be of any size, with the lymph glands usually affected, with the spread of cancer to other parts of the body organs, such as the bones or lungs.

Breast cancer is the most commonly occurring cancer among women, causing more deaths in the western world than any other cancer except for cancer of the lung.[12,13] The lifetime risk of breast cancer for a woman in developed countries has been calculated at around 1 in 7 to 1 in 10. Around 10% of the female population will be diagnosed with breast cancer at some point of their life. Out of these patients, around 30-40% will eventually die of this disease, mainly due to the development of metastases, an incurable condition in most types of cancer. This high incidence, the complexity and the economic costs of the treatment for this disease make breast cancer one of the most relevant health problems in our society.[14,15]

In India, with an estimated diagnosis of 80,000 new cases, annually, with an increase in the incidence of breast cancer to approximately 50% between 1965 and 1985 with the 2005 data showings that India has one of the highest cancer rates in the world.

While breast cancer incidence is still increasing, mortality from breast cancer is decreasing in many western societies, probably due to combined effect of early detection and improvements in treatment.[16,17] The risk of getting breast cancer is found to be increasing with age and for anyone living upto the age of 90, the chances of getting breast cancer has been estimated to be as high as 14% or one in seven during their lifetime. Because of the presence and composition of identical tissues in males and females, men can also develop breast cancer. The incidence of male breast cancer is < than 1% of all breast cancer cases.[18]

1.7.3 Causes of breast cancer

The majority of breast cancer cases is probably caused by lifestyle factors, environmental, and alterations in a variety of low penetrance breast cancer susceptibility genes. The major differences in breast cancer incidence between ethnic groups and geographic areas in general are assumed to be due to life style and environmental factors, probably diet, smoking, high-alcohol consumption and not differences with respect to ethnic background.[19,20,21]

1.7.4 Breast cancer treatment

Today's treatment of breast cancer includes a multi-modal approach, consisting mainly of surgery, combined to a variable degree with adjuvant chemotherapy, radiation therapy and hormone therapy depending on tumour and patient characteristics. Once diagnosed, most breast cancers are primarily treated by surgical removal of the tumour (lumpectomy) or the entire diseased breast (mastectomy) following adjuvant systemic therapy to reduce the risk of a relapse. While for most patients surgery is performed upfront to any additional therapy, use of primary or so-called neo-adjuvant chemo- or endocrine therapy is increasingly used, in particular for larger tumours.

The treatment of breast cancer depends upon many factors, including the 1) type of cancer and 2) the extent to which it has spread. The options of treatment for breast cancer may involve 1) surgery (removal of the cancer alone or, in some cases, mastectomy), 2) radiation therapy, 3) hormonal therapy, and 4) chemotherapy.

1.7.4.1 Chemotherapy

Chemotherapy employs anti-cancer drugs to kill cancer cells and for breast cancer, is usually a combination of drugs. The drugs may be administered orally or parentally into a vein.

1.7.4.2 Hormone therapy

Some breast tumors need hormones to grow. Hormone therapy keeps cancer cells from getting or using the natural hormones they need. These hormones are estrogen and progesterone. The antiestrogen tamoxifen has long been used in the treatment of pre-and postmenopausal breast cancer.[22] However some breast cancer became resistant to tamoxifen, and in some cases the drug increases the risk of endometrial cancer.[23] During these days, the aromatase inhibitors, representing a new class of agents are considered as more effective than tamoxifen in the treatment of breast cancer.[24] Letrozole (LTZ) is an oral non steroidal aromatase inhibitor approved by United States FDA and has been introduced for the adjuvant treatment of hormonally responsive local or metastatic breast cancer.[25] It decreases the amount of estrogen

produced by the body and can slow or stop the growth of some breast tumors that need estrogen to grow.

The understanding of the link between breast cancer and oestrogen, a hormone, has made a remarkable contribution to improve the cancer treatment and reduce the mortality rate. The breast cancer may be controlled by blocking the growth stimulated by oestrogen, TAM, is commonly used to treat breast cancer which directly blocks the actions of endogenous oestrogen. Breast cancer evolves in the epithelial tissue in breast. As these cells proliferate under hormonal control, breast cancer may often be treated by endocrine therapy when the tumour cells have retained the oestrogen receptor (ER).

The exception is patients with a particular low risk of relapse. Tamoxifen, an ER modulator that binds ER and obstructs estrogen from binding, is a general regime often given to premenopausal women. For postmenopausal women, aromatase inhibitors are the first-line therapy, either used alone or sequentially treatment with tamoxifen. Aromatase produces estrogen from androgens in various tissues. This reaction is the main source of estrogen after menopause.[26]

Chemotherapy or radiation therapy may also be used before or after surgery.While some local relapses may be related to residual cancer tissue deposits, the key problem relates to development of distant micro-metastases. Radiation therapy, which only has a moderate effect on overall survival; is commonly prescribed for women post lumpectomy or mastectomy surgery to reduce the risk of recurrarance of cancer and further radiation therapy wipes out microscopic cancer cells that may remain close to the area where the tumor was surgically removed. Adjuvant therapy has been found to reduce the risk of a relapse, with anthracycline based treatments most effective.[27]

Combination of cytostatic and endocrine therapy may reduce the death rate by up to 50%, compared to adjuvant therapy.Thus patients with hormone-sensitive tumors are in general treated with endocrine therapy alone. Here in the following section anticancer drugs letrozole and raloxifine are formulated as novel drug delivery system to improve the efficacy of anticancer drugs.

1.8 Novel drug delivery system

Today the challenge for the pharmaceutical formulators is to work and investigate to deliver the drug using promising drug carriers including biodegradable polymers. The systems that are capable of releasing the therapeutic agents by well defined kinetics are available at present. But in many cases these do not yet represent the ultimate therapy to needs of recipient. Hence attention should also be focused to fabricate controlled, modulated drug delivery system that are capable of receiving the physiological feedback information and adjusting the drug output and system that are capable of precisely targeting the specific tissue or cells. Current attempts to overcome these limitations include the development of novel drug delivery systems that can improve the efficacy of existing anti cancer drugs.

It is almost impossible to deliver anticancer drugs specifically to the tumour cells without damaging healthy organs or tissues.[28] Drug delivery systems using colloidal particulate carriers such as liposomes or niosomes have distinct advantages over other conventional dosage forms, acting as drug reservoirs and providing controlled release of the active substance.[29] In addition, the drug release rate and the affinity for the target site can be modified by adjusting their composition or surface on the target site. Liposomes or niosomes in dispersion can carry hydrophilic drugs by encapsulation or hydrophobic drugs by partitioning of these drugs into hydrophobic domains.[30] Colloidal drug delivery carriers are effortlessly phagocytised by macrophages and therefore, they can promote the uptake of drugs by these cells and may facilitate a considerable gain in cancer therapy.

Niosomes are non-ionic surfactant based vesicles that had been developed as alternative controlled drug delivery systems to liposomes in order to overcome the problems associated with sterilization, large scale production and stability.[31] The advantages of niosomal drug delivery systems include the structure and properties that are similar to those of liposomes, the chemical stability of niosomes and the relatively low cost of the materials that form them make niosomes more attractive than liposomes for industrial manufacturing. Many drugs, those currently available in the market and those under development, have poor aqueous solubility that results in variable bioavailability. This problem can be overcome by entrapping the drug into niosomes.[32]

Although the niosomes exhibit good chemical stability during storage, the question of physical instability in niosome dispersions may not be completely ruled out. Like liposomes, aqueous suspensions of niosomes may 1) display aggregation, 2) fusion, 3) leaking of entrapped drugs or 4) hydrolysis of encapsulated drugs and thus limiting the shelf life of the dispersion.[33] The niosomes are very difficult to sterilize, transport, distribute, store and ensure uniformity of dose and scale-up. A incomplete hydration of the lipid/surfactant film on the walls during hydration process is a high possibility.

Niosomal drug delivery system, perhaps is a useful strategy towards targeted drug delivery in cancer chemotherapy. The potential for niosomes in cancer drug delivery is perpetual with novel applications constantly being explored with niosomes playing a significant role in the delivery of cancer drugs. Earlier, various anti-cancer drug formulations were used, but were less successful and had major adverse effects. Niosomes have always fascinated the pharmaceutical formulators.

1.8.1 Structure of niosomes

The structure of niosomes are microscopically lamellar formed on the admixture of non-ionic surfactant of the alkyl or di alkyl poly glycerol-ether class and cholesterol with subsequent hydration in aqueous media.[34] Structurally, niosomes are similar to liposomes, as they are also made up of a bilayer. However, the bilayer, in the case of liposomes are made up of phospholipids in comparison to niosomes, made up of non-ionic surface active agent. Most surface active agents when immersed in water, yield micellar structures; whereas some surfactants can harvest vesicles which are niosomes. Depending on the method used to prepare niosomes, they may be uni-lamellar or multi-lamellar. The niosomes are made of a surfactant bilayer with its hydrophilic heads exposed on the outside and inside of the vesicle, while the hydrophobic chain facing each other within the bilayer. This facilitates the vesicles to hold hydrophilic drugs within the space enclosed in the vesicle, while hydrophobic drugs are embedded within the bilayer itself. This is best illustrated in the figure below of what a niosome looks like and where the drug is located within the vesicle.

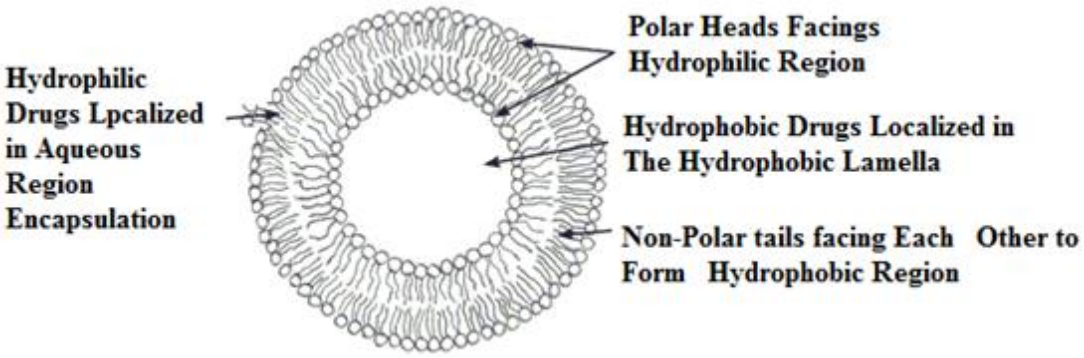

Figure1.7 Structure of niosomes

1.8.2 Advantages of niosomes[35]

The following are the advantages of use of niosomes in cosmetics, first done by L'Oreal as they offered

- The vesicle suspension being water based offers greater patient compliance over oil based systems
- They can be used for a variety of drugs as the structure of the niosome offers place to accommodate hydrophilic, lipophilic as well as amphiphilic drug moieties
- The characteristics such as size, lamellarity, etc. of the vesicle can be varied depending on the requirement
- Controlled release of the depot from the vesicles can offer slow release of the drug.

Other advantages of niosomes

- Osmotic activity and stability.
- Increase in the stability of the entrapped drug.
- Surfactant handling and storage do not require any special conditions
- The oral bioavailability of drugs can be increased.
- Can improve skin penetration of drugs.
- Can be used for topical, oral and parenteral use
- The surfactants are biodegradable, biocompatible, and non immunogenic. They improve the therapeutic performance of the drug by protecting it from

the biological environment and restricting effects to target cells, thereby reducing the clearance of the drug.

- The niosomal dispersions in an aqueous phase can be emulsified in a Non-aqueous phase to control the release rate of the drug andAdminister normal vesicles in external non-aqueous phase.

1.8.3 Salient features of niosomes

- In a manner analogous to liposomes, nNiosomes can entrap solutes.
- Niosomes have osmotic activity and stability.
- So to accommodate the drug molecules with a wide range of solubility, niosomes possess an infrastructure consisting of hydrophobic and hydrophilic mostly together.
- Niosomes exhibits flexibility in their structural characteristics (composition, fluidity and size) and can be designed according to the desired situation.
- Niosomes can improve the performance of the drug molecules by delayed clearance from the circulation.
- Improve bioavailability to the particular site, just by protecting the drug from biological environment.
- Offer controlled delivery of drug at a particular site.
- In handling and storage of Niosomes, no special conditions are required.

1.9 Proniosomes

Proniosomes are dry, free-flowing formulations of surfactant-coated carrier, which can be rehydrated by brief agitation in hot water to form a multi-lamellar niosome suspension suitable for administration by oral or other routes.[36,37]

The physical instability such as aggregation, fusion and leaking of niosomes may be minimized by proniosomes. In convenience of storage, transport, distribution and dosing, proniosome-derived niosomes are superior to conventional niosomes. The Stability of dry proniosomes is expected to be more stable than a pre-manufactured niosomal formulation. Proniosomes appear to be equivalent to conventional niosomes, in release studies. Size distributions of proniosome-derived niosomes are moderately better than those of conventional niosomes, as the release performance in more critical cases turns out to be superior.[36,38,39,40]

Proniosomes are dry powder, which makes further processing and packaging possible. The powder form provides optimal flexibility, unit dosing, in which the proniosome powder is provided in capsule could be beneficial. A proniosome formulation based on maltodextrin was recently developed that has potential applications in deliver of hydrophobic or amphiphilicdrugs. The better of these formulations used a hollow particle with exceptionally high surface area. The principal advantage with this formulation was the amount of carrier required to support the surfactant could be easily adjusted and proniosomes with very high mass ratios of surfactant to carrier could be prepared. Because of the ease of production ofproniosomes using the maltodextrin by slurry method,hydration of surfactant from proniosomes of a wide range of compositions can be studied.[36,40,41]

Non-ionic surfactants, coating carriers and membrane stabilizers are commonly used in the preparation of proniosomes.Span (20, 40, 60, 80 and 85),Tween (20, 60 and 80) are the non-ionic surfactants used. Sucrose stearate, sorbitol, maltodextrin (Maltrin M500, M700), glucose monohydrate, lactose monohydrate, spray dried lactose and membrane stabilizers like cholesterol and lecithin are the coating carriers used.[42,43,44,45]

1.9.1 Advantages of proniosome[38,42,46]

a) Drug targeting agent and provide controlled release.

b) Entrapping both hydrophilic and hydrophobic drugs

c) Greater physical and chemical stability

d) Easy of transport, distribution, measuring and storage

e) Proniosome powder can further be processed to make beads, tablets or capsule

f) Low toxicity due to nonionic nature

g) Simple method

h) Low cost

i) More uniform in size

j) It is a versatile delivery system with potential use with wide range of active compounds

1.9.2 Types of proniosomes

1. Dry granular proniosomes
2. Liquid crystalline proniosomes

1.9.2.1 Dry granular proniosomes

Dry granular proniosomes involves the coating of water soluble carrier such as sorbitol and maltodextrin with surfactant. The result of coating process is a dry formulation in which each water-soluble particle is covered with thin film of surfactant. It is essential to prepare vesicles at a temperature above the transition temperature of the non-ionic surfactant being used in the formulation. These are further categorized as follows.

Sorbitol based Proniosomes

Sorbitol based proniosomes are dry formulations involving sorbitol as carrier, further coated with non-ionic surfactant and used as niosomes within minutes by addition of hot water followed by agitation. These are usually prepared by spraying surfactant mixture prepared in organic solvent onto the sorbitol powder followed by evaporating the solvent. Till the desired surfactant coating has been achieved, the process is required to be repeated as the sorbitol carrier is soluble in organic solvent. The surfactant coating on the carrier is very thin and hydration of this coating allows multilamellar vesicles to form as the carrier dissolve.[35,38,45,46]

Maltodextrin based proniosomes

Recently, a proniosome formulation based on maltodextrin was developed that has potential application to deliver of hydrophobic or amphiphilic drugs. The ability of these formulations used to hollow particle with exceptionally high surface area. The primary advantage with this formulation was that the amount of carrier required to

support the surfactant can be simply adjusted and, proniosomes with very high mass ratios of surfactant to carrier could be prepared.[38,42]

1.9.2.2 Liquid crystalline proniosomes

There are three ways through which lipophilic chains of surfactants can be transformed into a disordered, liquid state called lytorophic liquid crystalline state (neat phase), when the surfactant molecules are kept in contact with water. These three ways are

- Increasing temperature at kraft point (Tc)
- Addition of solvent, which dissolves lipids
- Using both temperature and solvent.

Neat phase or lamellar phase contains bilayer arranged in sheets over one another within intervening aqueous layer. Under polarized microscope, these types of structures give typical X-ray diffraction and thread like bi-refringent structures.

1.9.3 Factors affecting physical nature of proniosomes

There are some factors which can affect significantly the physical nature of proniosomes[43] such as hydration temperature, choice of surfactant, nature of membrane, nature of drug, etc., (as shown in figure 1.8).

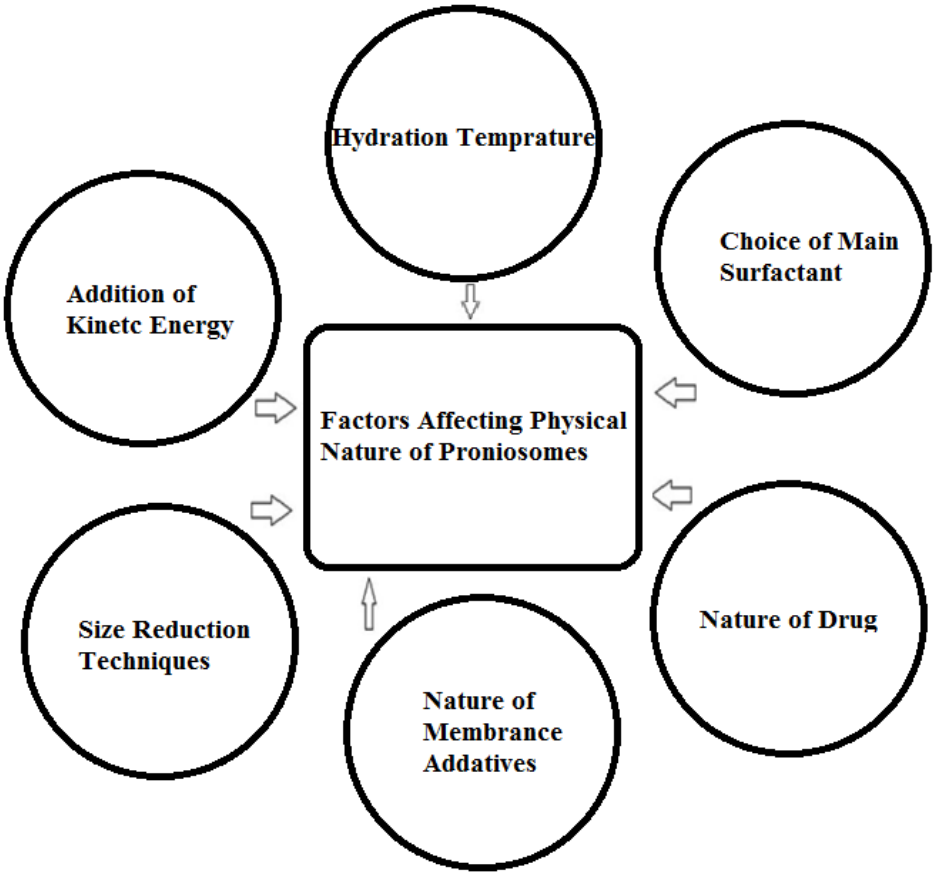

Figure 1.8 Factors affecting physical nature of proniosomes

1.9.4 Preparation of proniosomes

1.9.4.1 Necessary materials

In the preparation of proniosomes, non-ionic surfactant, different carriers and membrane stabilizers are utilized.[47] Generally using materials for formulation of proniosomes are

Non-ionic surfactants

Drug flux increase across the skin,entrapment effieiency of drug influences by the surfactant chemical structure.[48] Entrapment efficiency would be more in case of alkyl chain length increment.[49] Researchers reported that highest phase transition temperature of sorbitan esters resulted in highest entrapment efficiency. e.g., Span

(20,40 and 60), Tween(20,40,60 and 80). Surfactant selection has made based on HLB value which was a good indicator of the vesicle forming ability of any surfactant. For vesicle formation would be better while selecting HLB number of surfactant in between 4 and 8. It is also reported that the hydrophilic surfactant owing to high aqueous solubility on hydration do not reach a state of concentrated systems in order to allow free hydrated units to exist aggregates and coalesced to form lamellar structure.In presence of cholesterol, niosomes would form using water soluble detergent polysorbate20**.** This is in spite of the fact the compound;s HLB number is16.7,HLB of surfactant would affect the entrapment efficiency. Entrapment of drug also influenced by surfactant Transition temperature.[49,50]

Higher entrapment with larger vesicle could be obtained with span 40 and span 60. High phase transition and low permeability reduce the drug leaching from vesicles. Reduction in surface free energy obtained with span 40 and span 60 which has high HLB value. So it allows large vesicle size formation, there by exposing large surface area, to the dissolution medium and skin. Tween's encapsulation efficiency is relatively lower than span's. Exhaustive dialysis and freeze thawing/centrifugation could be used for determination of entrapment efficiency which was lower for former method while compared to later for both span 40 and span 60. The geometry of vesicle, which is related to critical packing parameter (CPP),[51] would be affected by the Surfactant structure. Geometry of vesicle are formed on the predication and on the basis of critical packing parameters of surfactant. Critical packing parameters are defined using following equation:

CPP 0.5 micelles form- $CPP = V/ lc \times ao$

CPP= 0.5 – 1 spherical vesicles form CPP = 1 inverted vesicles form

V – Hydrophobic group volume lc = the critical hydrophobic group length,, a_0= the area of hydrophilic head group. Span 60 is the good surfactant because it has CPP value between 0.5 and 1.

Stabilizers[52]

Lecithin

lecithin Phosphotidylcholine, major component of lecithin, has low solubility in water.[47] By Lecithin prevents the leakage of drug while added into proniosomes and improved the drug entrapment due to high phase transition temperature, by acting as a permeation enhancer. Anyway during preparation and storage addition of lecithin in formulation needs special treatment, which makes the product highly expensive and less stable. Hence, it may be better to prepare proniosomes devoid of lecithin component.[51] The vesicles composed of soya lecithin are of larger size than the vesicle composed of egg lecithin due to difference in the intrinisic componenets. The soya lecithin is considered as a good candidate as it contains unsaturated fatty acids, oleic and linoleic acid while egg lecithin contains fatty acids, on the basis of penetration capability.[53]

Cholesterol

Prevent drug leakage from formulation. For the formulation of vesicles cholesterol is an important component.[54] Stability and permeability was influenced by the addition of cholesterol. In the entrapment of drug in vesicles, concentration of cholesterol has a vital role. While adding cholesterol *in vitro* drug release will slow down.[55]. If increase the content of cholesterol and span 60 which has higher transition temperature,entrapment efficiency and permeation will increase.[56] On continuous addition of cholesterol beyond certain concentration level starts disrupting the regular bilayered structure leads to loss of drug entrapment and permeation.[57] The membrane will be more ordered above the phase transition temperature and abolish the gel to liquid phase transition of noisomal system, thus preventing drug leakage from proniosomes.[49,58]

Solvent

Selection of solvent is another important aspect as it has great effect on vesicle size and drug permeation rate.[59] e.g., Ethanol, Methanol, Propanol, Isopropanol. Different alcohols formed different size of vesicles and they follow the order: ethanol > propanol > butanol > isopropanol. Higher size and smallest size of vesicles in case of

ethanol and isopropanol due to its greater solubility in ethanol and water respectively may be due to branched chain present in it.[60] Ethanol may cause the reduction of lipid polar head interactions within the membrane, thereby increased the skin permeation.[61,62]

Aqueous phase

Entrapment efficiency of drug in vesicles would be affected by aqueous phase. e.g., Hot water, Buffer, Glycerol. Phosphate buffer 7.4, 0.1% glycerol and hot water are mainly used as aqueous phase for proniosomes. pH of the hydrating medium also play vital role in entrapment efficiency.[63,64]

Carriers[65]

Carriers provide flexibility in surfactant: other component ratio that alter the drug distribution e.g., maltodextrin, sorbitol, mannitol. Carriers selected for proniosomes preparation are supposed to display characteristics like free flow ability, non-toxicity, poor solubility in the loaded mixture solution and good water solubility for ease of hydration.

Maltodextrin

Is a flavourless, easily digested carbohydrate made from corn starch and is a mixture of glucose, disaccharides and polysaccharides, obtained by the partial hydrolysis of starch and a short chain of molecularly linked dextrose (glucose) molecules, and is manufactured by regulating the hydrolysis of starch with white or almost white, slightly hygroscopic powder or granules, freely soluble in water with minimal solubility in organic solvent. Thus, maltodextrin particles may possibly be coated by simply addition of surfactants in organic solvents.

Sorbitol

Is a sugar alcohol, slowly metabolized by the body, obtained by reduction of glucose, changing the aldehyde group to a hydroxyl group. Natural sources like apples, peaches, pears, and prunes are abundant in sorbitol. It is synthesized by the enzyme sorbitol-6-phosphate dehydrogenase, and converted into fructose by succinate dehydrogenase and then to sorbitol dehydrogenase.

Mannitol

It is a white, crystalline organic compound. This is used as an osmotic diuretic agent and a weak renal vasodilator. It was originally isolated from the secretions of the flowering ash, called manna after their resemblance to the Bibilical food.

1.9.4.2 Methods of preparation of proniosomes

Spraying method

Proniosomes were prepared by spraying the surfactant in an organic solvent into sorbitol powder and then evaporating the solvent. It is necessary to repeat the process until the desired surfactant load has been achieved because the sorbitol carrier is soluble in the organic solvent. Hydration of this coating allows multilamellar vesicles to form as the surfactant coating on the carrier comes out to be very thin.[45]

Slurry method

Using maltodextrin as a carrier, proniosomes were made by slurry method. The time required to produce proniosomes by this method is independent of the ratio of surfactant solution:carrier material. In slurry method, to maltodextrin powder in a rotary evaporator, the entire volume of surfactant solution is added, and vacuum is applied until the powder appears to be dry and free flowing. In analogous to that used for the conventional niosomes, drug containing proniosomes-derived niosomes can be prepared, by adding drug to the surfactant mixture prior to spraying the solution onto the carrier (sorbitol, maltodextrin) or by addition of drug to the aqueous solution used to dissolve hydrate the proniosomes.[66]

Coacervation phase separation method

In this method, with simple mixing, accurately weighed amount of surfactant, carrier (lecithin), cholesterol and drug are taken in a clean and dry wide mouthed glass vial (5 ml) and solvent is be added to it. The open end of the glass vial can be covered with a lid and heated over water bath at 60- 70ºC for 5 minutes, to prevent the loss of solvent, until the surfactant dissolved completely. Till the dispersion gets converted to a proniosomes, the mixture should be allowed to cool down at room temperature.[67]

1.9.5 Formation of niosomes from proniosomes[46]

The niosomes can be prepared from the proniosomes by adding the aqueous phase with the drug to the proniosomes with brief agitation at a temperature greater than the mean transition phase temperature of the surfactant i.e,

$$T > Tm$$

Where, T = Temperature Tm = Mean phase transition temperature

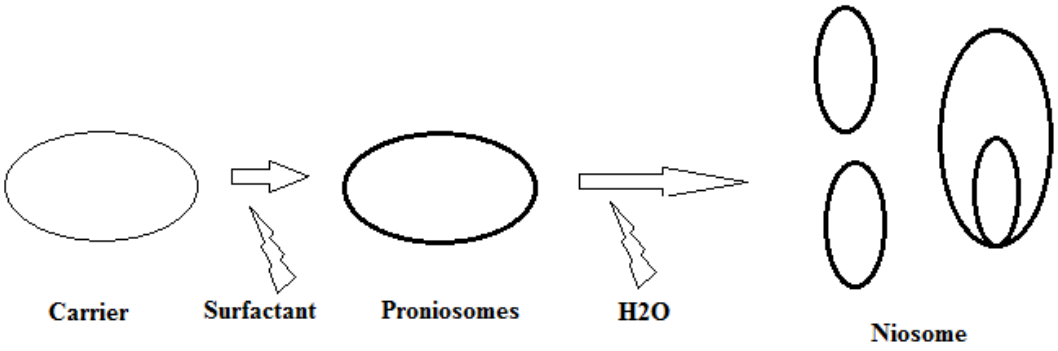

Figure1.9 Formation of niosomes from proniosomes

Maltodextrin based proniosome provides rapid reconstitution of niosomes with minimal residual carrier. Slurry of maltodextrin and surfactant was dried to form a free flowing powder, which could be rehydrated by addition of warm water.

1.9.6 Evaluation methods for proniosomes

Morphology of vesicle

Vesicle morphology comprises the measurement of size and shape of proniosomal vesicles. proniosomal vesicles size can be measured by dynamic light scattering method in two conditions: without agitation and with agitation. Largest vesicle size was obtained while doing the hydration without agitation.

Shape and surface morphology

Surface morphology demands smoothness, roundness and creation of aggregation. They are studied by scanning electron microscopy (SEM), optical microscopy (OM), transmission electron microscopy (TEM).[50]

Scanning electron microscopy

The proniosomes are sprinkled onto the double-sided tape that is to be affixed on aluminum stubs. The aluminum stub is placed in the vacuum chamber of a scanning electron microscope. The samples are observed for morphological characterization using a gaseous secondary electron detector (working pressure: 0.8 tor, acceleration voltage: 30.00 KV) XL 30.[38,39]

Optical microscopy

The niosomes are mounted on glass slides and viewed under a microscope with magnification of 1200X for morphological observation after suitable dilution. The photomicrograph of the preparation also obtained from the microscope by using a digital SLR camera.[42]

Transmission electron microscopy

The morphology of hydrated niosome dispersion is determined using transmission electron microscopy. A drop of niosome dispersion is diluted 10-fold using deionized water. A drop of diluted niosome dispersion is applied to a carbon coated 300 mesh copper grid and is left for 1 minute to allow some of the niosomes to adhere to the carbon substrate. The remaining dispersion is removed by adsorbing the drop with the corner of a piece of filter paper. After twice rinsing the grid (deionized water for 3-5 s) a drop of 2% aqueous solution of uranyl acetate is applied for 1 s. The remaining solution is removed by absorbing the liquid with the tip of a piece of filter paper and the sample is air dried. The sample is observed at 80 kv.

Angle of repose

The angle of repose of dry proniosomes powder is measured by a funnel method. The proniosomes powder is poured into a funnel which is fixed at a position so that the 13mm outlet orifice of the funnel is 5cm above a level black surface. The powder flows down from the funnel to form a cone on the surface and the angle of repose was then calculated by measuring the height of the cone and the diameter of its base.[68,69]

Rate of hydration: Neubaur's chamber.

Drug content: Drug can be quantified by a modified HPLC method.

Penetration and permeation studies: Depth of penetration in proniosomes can be visualized by confocal laser scanning microscopy (CLSM).

Encapsulation efficiency: The encapsulation efficiency of proniosomes is evaluated after separation of the unentrapped drug.

A. Separation of unentrapped drug by the following techniques:

(a) Dialysis: Against suitable dissolution medium at room temperature, the aqueous niosomal dispersion is dialyzed tubing, then the samples are withdrawn from the medium at suitable time interval centrifuged and analyzed for drug content using UV spectroscopy.[42]

(b) Gel filtration: Through a sephadex G50 column, the free drug is removed by gel filtration of niosomal dispersion and separated with suitable mobile phase and analyzed with analytical techniques. [70]

(c) Centrifugation: The surfactant is separated following centrifugation of niosomal. To obtain a niosomal suspension free from unentrapped drug, the pellet is washed and then resuspended.[71]

B. Determination of entrapment efficiency of proniosomes

Following the removal of unentraped drug by dialysis, the vesicles obtained after removal of were then resuspended in 30% v/v of PEG 200 and 1 ml of 0.1% v/v triton x-100 solution was added to solubilize vesicles. The resultant clear solution is then filtered and analyzed for drug content.[43] The percentage of drug entrapped is calculated by using the following formula Percent Entrapment = Amount of drug entrapped/total × 100

Drug release kinetics and data analysis[70]

In order to understand the kinetics and mechanism of drug release, the result of in-vitro drug release study of noisome are fitted with various kinetic equations like,

(a) Zero order, as cumulative % release vs. time,
$C = K_0t$ Where, k_0 = zero order constant expressed in units of concentration/time t = time in hours.

(b) Higuchi's model, as cumulative % drug release vs. square root of time.
$Q = KHt_{1/2}$ Where, KH = higuchi's square root of time kinetic drug release constant.

(c) Peppa's model, as log cumulative % drug release vs. log time and the exponent 'n' was calculated through the slope of the straight line.

$M_t / M_0 = btn$ Where, M_t = amount of drug release at time t M_0 = overall amount of the drug b = constant
n = release exponent indicative of the drug release mechanism If the exponent n= 0.5 or near, then the drug release mechanism is Fickian diffusion, and if n have near 1.0 then it is Non-Fickian diffusion.

1.9.7 *In vitro* methods for assessment of drug release from proniosomes[72]

(a) Dialysis tubing

This apparatus has prewashed dialysis tubing, that can be sealed hermetically. The dialysis sac is then dialyzed against a suitable dissolution medium at room temperature; the samples were withdrawn from the medium at suitable intervals, centrifuged and analyzed for drug content using suitable method (UV spectroscopy, HPLC, etc.). It is essential to maintain the sink condition.

(b) Reverse dialysis

In this technique a number of small dialysis tubes containing 1 ml of dissolution medium are placed and then the proniosomes are displaced into the dissolution medium. Though the rapid release cannot be quantified using this method, the direct dilution of the proniosomes is possible with this method.

(b) Franz diffusion cell[73]

Franz diffusion cell is used for the *in-vitro* studies. In the donor chamber of a Franz diffusion cell fitted with a cellophane membrane, proniosomes are placed and at room temperature, are then dialyzed against suitable dissolution medium; the samples are withdrawn from the medium at suitable intervals, and analysed for drug content using suitable method (UV spectroscopy, HPLC etc.). During these process the maintenance of sink condition is essential.

(c) Zeta potential analysis[74]

Zeta potential analysis is done for determining the colloidal properties of the prepared formulations. The suitably diluted proniosomes derived niosome dispersion is determined using zeta potential analyzer based on Electrophorectic light scattering and laser Doppler Velocimetery method. The temperature is set at 25°C. Charge on vesicles and their mean zeta potential values with standard deviation of 5 measurements are obtained directly from the measurement.

Stability studies

Stability studies for the prepared proniosomes are carried out by storing them at various temperature conditions like refrigeration at 23(2°-8°C), room temperature (25°± 0.5°C) and elevated temperature (45°C ± 0.5°C) from a period of 30 days to 90 days. Drug content and variation in the average vesicle diameter are routinely monitored. ICH guidelines suggests stability studies for dry proniosomes powder meant for reconstitution should be studied for accelerated stability at 75% relative humidity as per international climatic zones and climatic conditions.[41,42,75]

1.9.8 Applications of proniosomes

Drug targeting

The ability of targeting the drugs is one of the most useful aspects of proniosomes and the reticulo-endothelial system (RES) can be used to target by proniosomes. Proniosomes vesicles are preferentially taken up by RES and the uptake of proniosomes is controlled by circulating serum factors called opsonins marking the niosomesfor clearance. Such localization of drugs is utilized to treat tumors in animals known to metastate size the liver and spleen. Parasitic infections of the liver can also be treated using this localization of the drugs. Furthermore, proniosomes can also be utilized for targeting drugs to organs other than the RES. A carrier system, such as antibodies, can be attached to proniosomes (as immunoglobin bind readily to the lipid surface of the noisome) to be targeted to the specific organs. Many cells also contain carbohydrates determinates, and this can be exploited by niosomes to direct carrier system to particular cells.[75,76]

Anti-neoplastic treatment

Most antineoplastic drugs cause severe untoward effects and these can be minimized by proniosomes by altering the metabolism; prolonging the circulation and half-life of the drug, and thereby decreasing the unwanted effects of the drugs.[77,78]

Treatment of leishmaniasis

It is a disease charecterized by the invasion of the parasite of the genus *Leishmania* in the cells of the liver and spleen. Derivatives of antimony (anti-monials) are commonly prescribed drugs for the treatment, which in higher concentrations can cause cardiac, liver and kidney damage.[79]

Delivery of peptide drugs

Bypassing the enzymes which would breakdown the peptide has long been faced with a challenge of the oral peptide drug delivery systems.[80] Investigations are underway to on the use of proniosomes to successfully protect the peptides from gastrointestinal peptide breakdown. An *in-vitro* study, oral delivery of a vasopressin derivative, entrapped in proniosomes, showed that entrapment of the drug significantly increased the stability of the peptide.

Uses in studying immune response

Due to their immunological selectivity, low toxicity and greater stability, proniosomes are used in studying immune response and these are being used to study the nature of the immune response provoked by antigens.[81]

Niosomes as carriers for haemoglobin

Proniosomes can be used as carriers for haemoglobin within the blood. The proniosomal vesicle is permeable to oxygen and hence can act as a carrier for haemoglobin in anaemic patients.[82]

Transdermal drug delivery systems

One of the most useful aspects of proniosomes is that they greatly enhance the uptake of drugs through the skin. Transdermal drug delivery utilizing proniosomal technology is widely used in cosmetics; In fact, it was one of the first uses of the niosomes. Topical use of proniosome entrapped antibiotics to treat acne is done. The

penetration of the drugs through the skin is greatly increased as compared to un-entrapped drug.[83,84] Recently, transdermal vaccines utilizing proniosomal technology is also being researched. The proniosome (along with liposomes and transferomes) can be utilized for topical immunization using tetanus toxoid. However, the current technology in proniosomes allows only a weak immune response, and thus more research to be done in this field.

Sustained release

Sustained release action of proniosomes can be applied to drugs with low therapeutic index and low water solubility because these could be maintained in the circulation *via* proniosomal encapsulation.[85]

Localized drug action

One of the approaches to achieve localized antimonials encapsulated within proniosomes drug delivery is the taking up of proniosomes by mononuclear cells resulting in localization of drug, increase in potency and hence decrease both in dose and toxicity. Still at an infancy stage, the evolution of proniosomal drug delivery technology, is but, the type of drug delivery system which has promise in cancer chemotherapy and anti-leishmanial therapy.[86]

Cosmetics or cosmeceuticals

Due to their unique properties, proniosome gel can be used as an effective delivery systems for cosmetics and cosmeceuticals. For prolong action as well as to enhances the penetration to the skin layer for applying therapeutic and cosmetic agents onto or through skin requires a non-toxic, dermatologically acceptable carrier, which not only control the release of the agent. Proniosomes gel formulation displays advantages in controlled drug delivery improved bioavailability, reduced adverse effects and entrapment of both hydrophilic and hydrophobic drugs.[71]

2. AIM AND OBJECTIVE OF THE WORK

2.1. Aim of the work

The aim of the present research work is to develop stable Letrozole loaded proniosome formulation and stable Raloxifene loaded proniosome formulation and to evaluate *in vitro* characteristics and i*n vivo* pharmacokinetic parameters of prepared formulations.

2.2. Objective of the work/Need for study

Cancer is a critical disease occurring due to progressive accumulation of epigenetic and genetic changes, leading to worldwide death. It is a category of disease characterized by uncontrolled,rapid abnormal growth of cells. The major disadvantage associated with anticancer drugs is their lack of selectivity for tumour tissue, resulting in several toxic effects leading to low cure rates. Methods available currently for cancer diagnosis and treatment are expensive and can be very harmful to the body. Presently, radiation therapy, chemotherapy, or surgery all of which can be successful are the most common cancer treatment methods based on, but have significant demerits. Though modern nanotechnology offers a possibility of materials that selectively bind to particular types of cancer cells, sensitizing them to light without affecting surrounding healthy tissues by selective targeting to tumour tissue is still a major worry. Cancer being a leading cause of death worldwide in 2012 is estimated to those 14.1 million new cases of cancer has occurred and an estimated 8.2 million people died from cancer.

The worldwide cancer deaths are projected to continue to rise, with an estimated 12 million deaths in 2030. Providing therapeutic concentrations of anticancer agents at the site of action and spare the normal tissues is the current focus in development of cancer therapy using targeted drug delivery. Next to lung cancer, breast cancer, is the second most common type of cancer worldwide and is the primary cause of death due to cancer among women globally, responsible for about 40000 US women deaths in 2001.

Aromatase inhibitors are used for the treatment of breast cancer in postmenopausal women. Letrozole, an hormonal anticancer drug can be employed to treat aromatase dependent breast cancer. Aromatase is an enzyme that catalyses the conversion of oestrogen from testosterone, an androgen. Letrozole is potent & selective inhibitor of aromatase, inhibiting the production of oestrogens in postmenopausal women. The mechanism of action of letrozole is to antagonize the conversion of the hormone androgen to small amount of oestrogen in the body by blocking cytochrome P-450 (CYP). This would make less oestrogen available to stimulate the growth of hormone receptor positive breast cancer cells. As it does not stop the ovaries from making oestrogen, aromatase inhibitors affects only on postmenopausal females.

Raloxifene hydrochloride is a non-steroidal benzothiophene, is a second- generation selective oestrogen receptor modulator that binds to oestrogen receptors possessing mixed pharmacological actions displaying oestrogen agonist effects on bone and the cardiovascular system and oestrogen antagonist effects on endometrial and breast tissue. Raloxifene hydrochloride is poorly soluble drug as it belongs to class-II category according to BCS classification.

As raloxifene hydrochloride has oral bioavailability of only 2%, owing to extensive first pass metabolism, it is necessary to increase the solubility and dissolution rate of Raloxifene hydrochloride which my lead to improvement in oral bioavailability. Enhancement in oral bioavailability can be achieved by reducing the hepatic first pass metabolism. Such problem with conventional dosage form can be minimized by any suitable novel drug delivery system. In an effort to fulfil the need of a long-term treatment with anticancer drugs, where most of them suffer from the drawbacks of frequent administration and fluctuations in plasma concentration, it is meritorious to have sustained-release drug delivery systems to improve the overall therapeutic benefit and to achieve an ideal therapy.

Using sustained delivery, it is possible to:

1) achieve effective plasma concentration without significant fluctuation,

2) avoid sub-therapeutic and toxic plasma concentrations,

3) facilitate release of the medication in a controlled manner to obtain a continuous delivery,

4) achieve an effective therapy with low dosage of the drug and

5) reduce the frequency of medication and thus to improve patient adherence.

The present study is aimed to utilize the potential of novel drug delivery system for improvement in oral bioavailability of Raloxifene hydrochloride and to minimize the exposure of normal tissues to drugs while maintaining their therapeutic concentration in tumours, which are most important goal of cancer chemotherapy. Now the pharmaceutical formulators are searching for vehicles through which drugs can be delivered to the specific target. And niosomes, as one of the vehicles can be employed to deliver the drug to specific site. Niosomes are formed from self-assembly of hydrated synthetic non-ionic surfactant monomers capable of entrapping variety of drugs. The size of these vesicles is in the nanometer range, offering decisive advantage of this class of pharmaceutical dosage forms as it allows drug targeting which often is not possible with free drug.

Niosomes can be used to vesiculize both hydrophilic and lipophilic drugs. Niosomal vesicles are composed of non-ionic surfactant with/without cholesterol or other lipids. Niosomes have lower toxicity due to non-ionic nature of the surfactant and act to improve the therapeutic index of the drug.

Proniosomes derived niosome preparation is one of the advancement in nanotechnology.Similar to those produced by more cumbersome conventional methods, proniosomes are solid colloidal particles which may be hydrated immediately before use to yield aqueous niosome dispersions. These proniosomes provide distinctive advantage in minimizing problems of niosome physical stability such as aggregation, fusion and leaking, and provide additional convenience in transportation, distribution, storage, and dosing. As they possess greater chemical stability and due to lack of many disadvantages associated with liposomes, the proniosomes are promising drug carriers.

Niosomes have always displayed advantages as drug carriers including low cost, low toxicity due to non-ionic nature and chemical stability in comparison to liposomes but are associated with problems related to physical stability such as fusion, aggregation, sedimentation and leakage and storage. Proniosomes are dry formulations of surfactant coated carrier vesicles, which can be measured out as needed and

rehydrated by brief agitation in hot water, the resulting niosomes are very similar to conventional niosomes and more uniform in size. Proniosomes, being dry, free flowing products, minimizes stability problems during storage and sterilization and it has additional advantages of ease of transfer, distribution, measuring and storage which makes them a pronouncing versatile delivery system. Proniosomes are a dry, free-flowing, granular product which, upon addition of water, disperses or dissolves to form a multilamellar niosomal suspension, suitable for administration by oral or other routes. As proniosomes are having advantages over liposome as well as niosomes, by minimizing problems of physical and chemical stability during storage and sterilization, and it have additional merits of transfer, distribution, show high entrapment efficiency, capable of releasing these drugs for the extended period of time. Hence the present study is focusing on the 6formulation and evaluation of proniosomes as drug delivery system for anticancer drug.

The main objective of the present study was to formulate and evaluate letrozole proniosome and raloxifene proniosome using sorbitan esters and slurry method.

3. REVIEW OF LITERATURE

- *Carlotta Marianecci et al.,* (2014) explained about the use of nanotechnology and during the last decades how the interest increased among the scientists regarding the formulation of surfactant vesicles, as a tool to improve drug delivery. In this review they have updated composition, preparation, characterization/evaluation, advantages, disadvantages and application of niosomes.[87]

- *Biswal S et al.,* (2008) presented an overview of theoretical concept of factors affecting niosome formation, techniques of niosome preparation, characterization of niosome, applications, limitations and market status of such delivery system. Niosomes exhibit more chemical stability than liposomes (a phospholipids vesicle) as non-ionic surfactants are more stable than phospholipids. Non-ionic surfactants used in formation of niosomes are polyglyceryl alkyl ether, glucosyl dialkyl ether, crown ether, polyoxyethylene alkyl ether, ester-linked surfactants, and steroid-linked surfactants and a spans, and tweens series.[88]

- *Mahale NB et al.,* (2012) described the history all factors affecting niosome formulation, manufacturing conditions, characterization, stability, administration routes and also their comparison with liposome and have given relevant information regarding an array of applications of niosomes in gene delivery, vaccine delivery, anticancer drug delivery. They explained as to how the vesicular systems deliver drug in controlled manner to enhance bioavailability and get therapeutic effect over a longer period of time.[89]

- *Hamdy Abdelkader et al.,* (2014) reviewed the recent advances in non-ionic surfactant vesicles, self-assembly, fabrication, characterization, drug delivery, applications and limitations.[90]

- *Joseph VM et al.,* (2010) formulated the niosomes with a versatile anticancer drug such as Etoposide by ether injection method surfactant (tween 40 or 80), cholesterol and drug in 4 different ratios by weight, 1:1:1, 2:1:1, 3:1:1, & 4:1:1. The niosomes characterized for size, shape, entrapment efficiency, stabi lity and in vivo release summary (by exhaustive dialysis).[91]

- *Sankar V et al.,* (2010) reviewed in different aspects related to proniosomes preparation, characterization, entrapment efficiency, *in vitro* drug release, applications and merits.[92]

- *Ruckmani K et al.,* (2010) encapsulated zidovudine in proniosomes which decreased drug leakage from vesicles stored at room temperature and resulted in targeted delivery of ZDV to macrophages in spleen and liver.[93]

- *Hanan El-Laithy et al.,* (2011) designed a novel sustained release proniosomal system for vinpocetine using sugar esters (SEs) as non-ionic surfactants to avoid marked first-pass effect and enhanced its absorption and dissolution.[94]

- *Sanjoy Kumar Dey et al.,* (2009) prepared and evaluated a biodegradable nanoparticulate system of Letrozole (LTZ) intended for breast cancer therapy. LTZ loaded poly (lactide-co-glycolide) nanoparticles (LTZ-PLGA-NPs) were prepared by emulsion-solvent evaporation method using methylene chloride and polyvinyl alcohol. LTZ-PLGA-NPs were characterized by particle size, zeta potential, infrared spectra, drug entrapment efficiency and *in vitro* release.[95]

- *Nita Mondala et al.,* (2010) incorporated Letrozole in nanoparticles which 120were prepared by solvent displacement technique and characterized by transmission electron microscopy, poly-dispersity index and zeta potential measurement.[96]

- *Tuan HiepTrana et al.,* (2013) using the spray-drying technique , tried to improve the physicochemical properties and bioavailability of a poorly water-soluble drug, raloxifene by solid dispersion (SD) nanoparticles. These spray-dried SD nanoparticles were prepared using raloxifene (RXF), polyvinyl pyrrolidone (PVP) and Tween 20 in water. The improved dissolution of raloxifene from spray-dried SD nanoparticles appeared to be well correlated with enhanced oral bioavailability of raloxifene in rats.[97]

- *Tara Pritchard et al.,* (2015) SMA-raloxifene was validated for the management of CRPC using a mouse xenograft model. It was reported that the biodistribution

of raloxifene was 69% higher in tumours following SMA raloxifene in comparison to free raloxifene.[98]

- *Vora B* et al., (1998) have developed and extensively characterized both *in-vitro* and *in-vivo* proniosome based transdermal drug delivery system of levonorgestrel. On the basis of *in vitro* observations the proniosomal formulation containing S 40:soya Lecithin : Cholesterol (4.5:4.5:1) and isopropanol was designated and patch having the area 0.63 cm2 (9 mm diameter) containing formulation equal to 1 mg drug was applied to the animals, in order to obtain the chosen flux (20 mg/day). *In vivo* study revealed that proniosomal gel bearing patch for levonorgestrel is tremendously superior than levonorgestrel ointment at the same dose level. [99]

- *Fang J et al.,* (2001) have investigated skin permeation of estradiol from various proniosome gel formulations across excised rat skin *in-vitro*. The EE and size of niosomal vesicles formed from proniosomes upon hydration were characterized. Proniosomes with S 3440 and S 60 increased the permeation of estradiol across skin.[44]

- *Alsarra IA et al.,* (2005) using Franz diffusion cells, have investigated permeation of ketorolac across excised rabbit skin from various proniosome gel formulations. Each of the proniosomes prepared, expressively upgraded drug permeation and reduced the lag time. Proniosomes prepared with S 60 provided a higher ketorolac flux across the skin than did those prepared with T 20 (7- and 4-fold the control, respectively). A change in the content of cholesterol was found not to affect the efficiency of the proniosomes, and the reduction in the content of lecithin did not significantly decrease the flux.[37]

- *Varshosaz J* et al., (2005) have developed proniosomal gel for transdermal drug delivery of chlorpheniramine maleate based on S 40 and broadly characterized *in-vitro*. The results exposed that lecithin fashioned more stable and larger vesicles with higher loading efficiency but lower dissolution efficiency than cholestrol and dicethyl phosphate. The ethanol produced larger vesicles (≈44 μm) and entrapped a greater amount of drug. The proniosomes that contained S 40/lecithin/ cholestrol

prepared by ethanol showed optimum stability, loading efficiency (16.7%), particle size and release kinetic suitable for transdermal delivery of chlorpheniramine maleate.[51]

- *Gupta A et al.,* (2007) have investigated the potential of proniosomes as a transdermal drug delivery system for captopril which was assured of various ratios of 54sorbitan fatty acid esters, cholesterol, lecithin prepared by coacervation-phase separation method. The method of proniosome loading resulted in an encapsulation yield of 66.7 - 78.7%.4At refrigerated conditions, higher drug retention was observed.[41]

- *Ibrahim MMA et al.,* (2008) had formulated and evaluated proniosomal transdermal carrier systems for flurbiprofen. Both S 640 and S 60 produced gel systems in presence or absence of cholesterol. Rabbit skin exposed lower drug diffusion rates compared to cellophane membrane,due to the skin permeation barrier. The proniosomal composition controlled drug diffusion rates were found to be either faster or slower than the prepared flurbiprofen suspensions in HPMC gels or distilled water, respectively.[100]

- *Chandra A et al.,* (2008) have investigated the piroxicam permeation from proniosome based reservoir type transdermal gel formulation across excised abdominal skin of rat using Keshery chein diffusion cell. It was observed that S 60 based formulations created vesicles of smallest size and higher EE while those of S 80 created vesicles of least EE. Further incorporation of lecithin was found to enhance EE and maximum flux achieved was 35.61 μg/cm2/h, an enhancement was of 7.39 times as compared to control gel.[101]

- *Azeem A et al.,* (2008) have formulated non-ionic surfactant vesicles of frusemide to enhance its skin permeation. With formulation containing S5140:soyalecithin: cholesterol (4.5:4.5:1), the rat plasma level had increased to a level of 0.42 ± 0.13 μg/ml at the sampling interval of 4 hr and stayed within the range of therapeutic concentration (1.66–0.3 μg/ml) for the next 12 hours.[102]

- *Azarbayjani AF et al.,* (2009) had studied proniosomal formulations with non-ionic surfactant. Formulations with single surfactants were observed to increase

the skin permeation of haloperidol in comparison to formulations containing two surfactants. The number of carbons in the alkyl chain of the non-ionic surfactant influenced the *in- vitro* permeation of haloperidol though the epidermis and the skin permeation was increased with an increase in hydrophilic–lipophilic balance value of the surfactant.[103]

- *Mahrous GM et al.,* (2010) have evaluated the potential of proniosomes as a carrier for transdermal delivery of meloxicam. Proniosomes prepared with S 60 provided a higher meloxicam flux (29.9μg/cm2/h) across the rat skin than those prepared with T 80 (22.30μg/cm2/h). Testing of the anti-inflammatory effect of meloxicam proniosomal gel showed improved pharmacological activity.[104]

- *Alam MI* et al., (2010) have developed low dose proniosomal gel containing celecoxib for the treatment of osteoarthritis. The entrapment was > 90%. The selected proniosomal gel (S 40: 1800 mg, cholesterol: 200 mg, soya lecithin: 900 mg) produced higher flux (0.17 mg/cm2/h) and 100% inhibition of paw oedema in rats up to 8 h after carrageenan injection. It created 95% and 92% inhibition after 12 h and 24 h, respectively.[105]

- *El Laithy HM et al.,* (2011) designed a novel sustained release proniosomal system consuming sugar esters as non ionic surfactants containing vinpocetine. All formulae revealed high EE. Vesicle size analysis exposed that all vesicles were in the range from 0.63 μm to 2.52 μm which favoured efficient transdermal delivery. The depth of drug permeation was considerably high (91%) after 48 h under occlusive conditions.[106]

- *Ammar HO et al.,* (2011) have studied transdermal drug delivery of different proniosomal gel bases containg tenoxicam. The lecithin-free proniosomes prepared from T 20: Cholesterol (9:1) proved to be stable with high entrapment and release efficiencies with flux of 0.11mg/cm2/h. The considered tenoxicam loaded proniosomal formula proved to be non-irritant, with significantly higher anti- inflammatory and analgesic effects related to that of the oral market tenoxicam tablets.[107]

- *Anindita D et al.,* (2013) formulated and evaluated tretinoin proniosomal gel. The proniosome vesicles were of LUV type and spherical shape.The proniosome vesicles prepared with S 60, S 40 and cholesterol informulation revealed maximu EE (76.77±1.54%). The *in-vitro* diffusion study carried out using sigma dialysis membrane displayed sustained release pattern of tretinoin from proniosomal gel formulation. The comparative skin irritation study carried out in 18 healthy Wister rats of either sex showed remarkable decrease in signs of skin irritation caused by tretinoin.[108]

- *Rita Muzzalupo et al., (2007)* formulated 5-FU niosomes in which biological half-life increased while administering parentally. Due to this it reduced the toxic side effects.[109]

- *Parthasarathi et al.,* (1994) formulated vincristine niosomes which showed greater tumoricidal effect in comparison to plain drug formulation.[110]

- *Zhang et al.,* (2001) developed niosomal formulation of carboplatin that exhibited greater tumoricidal effect in S-180 lung carcinoma-bearing mice as compared to plain drug solution and also less bone marrow toxic effect.[111]

- *Mohamed Nasr et al.,* (2010) formulated a new proniosomal delivery system for a poorly water-soluble drug such as celecoxib and further was established and exposed to *in vitro* and *in vivo* studies.[112]

- *Kapil Kumar et al.,* (2011) developed a proniosomal carrier system of curcumin for transdermal delivery in which one of the formulations indicated prolonged *in vitro* drug release of 61.8% over a period of 24 hours.[113]

- *Abd Elbary A et al.,* (2008) developed a preparation of controlled release proniosome- derived niosomes, using sucrose stearates as non-ionic biocompatible surfactants for the nebulisable delivery of cromolyn sodium and compared it with conventional niosomes and reported a successful retardation of the drug release attained with proniosome derived niosomes.[40]

- *Ranjan et al.,* (2014) fabricated maltodextrin based proniosomes of nateglinide and their potential as controlled delivery system for the therapy of diabetes. New Zealand Albino male rabbits was used as animal model for *in vivo* study in evaluating the bioavailability of nateglinide proniosome by a rapid, simple and sensitive HPLC method.[114]

- *Nilufer Yuksel* et al., (2016) developed novel proniosomal tablets of candesartan cilexetil. Drug loaded proniosomes were prepared as dry powder by slurry method. The critical parameters of the production process were the type of the carrier (sorbitol, maltodextrin, and lactose), addition of charge inducers, rotationspeed of the rotavapor and solvent evaporation temperature. The proniosomes demonstrated good flowability, compressibility and consolidation properties both alone and together with the tableting agents (microcrystalline cellulose and cross-linked poly vinylpyrrolidone. [115]

- *Ashish kute et al.,* (2012) using coaservation phase separation method, the authors prepared perindopril erbumine proniosome gel, with various surfactant in different ratios. The vesicle size determined was in between 15.13±4.65µm to 24.05µ. Optimized formulation gave 75.26% cumulative drug release.[116]

- *Rita B et al.,* (2012) formulated a modified hydroxyzine hydrochloride using various combinations of tween and span by coacervation phase separation technique. Tween60:span 40 ratio was given higher entrapment efficiency and *in vitro* release.[117]

- *Ram et al.,* (2012) studied about the hydralazine proniosomes as transdermal drug delivery system. It indicated drug entrapment efficiency was very high (93.08%) while formulated as proniosomes.[118]

- *Patil et al.,* (2011) developed the carvedilol proniosome formulation with better bioavailability and controlled release.[119]

- *Walve et al*., (2012) developed Diclofenac transdermal gel in the form of proniosome using span 60 and tween. Span 60shown better encapsulation efficiency while compared with other surfactants.[120]

- *Mahmoud Mokhtara et al.,* (2008) investigated about various surfactants effect in the formulation of flurbiprofen proniosomes. These parameters influence the formulation, entrapment efficiency and release rate.[36]

- *Tank Chintankumar et al.,* (2009) prepared aceclofenac proniosome using maltodextrin as carrier with different cholesterol and surfactant ratio by slurry method.97.12% cumulative achieved for F4 formulation and follows zero order kinetics.[121]

- *Viviane F et al.,* (2012) studied about the acyclovir proniosome and *in vitro* and *in vivo* studies were carried out. Higher encapsulation was attained with high cholesterol content. Vesicle shape is spherical and increase in particle size leads to increase in entrapment.[122]

- *Kumar et al.,* (2012) investigated about flurbiprofen proniosome gel. Encapsulation capacity varying in the range of 30.6-75.4%.Prolonged release observed for best formulation.[123]

- *Sankar et al.,* (2009) developed hydrocortisone proniosome gel using various combinations of cholesterol and spans and tweens which have given high entrapment and extended action of drug.[124]

- *Shamseer Ahamad S et al.,* (2011) investigated about transdermal patch loaded with lisinopril using cholesterol as encapsulating agent and permeation enhancer span. Zero order release was observed for formulations and the products were stable at 4 to 8°C and 25°.[125]

- *Mishra et al.,* (2013) optimized the formulation of naproxen proniosome gel with different concentration of chlolesterol and nomionic surfactants by coacervation phase separation technique.[126]

- *Mohammad Reza Saboktakin et al.,* (2010) developed biodegradable nanoparticular system for letrozole which was used in breast cancer treatment.

Emulsion solvent evaporation was used as method of preparation. Sustained release was obtained.[127]

- *Nair et al.,* (2011) investigated the nanoparticles formulated by binding the letrozole with hyaluronic acid using nanoprecipitation method. Biodegradable co polymer PLGA-PEG used in the preparation tested on MCF-7 breast cancer cells.[128]

- *Azizi et al.,* (2015) incorporated letrozole in pegylated niosomes by reverse phase evaporation technique and characterized. Drug release followed first order and Hixson Crowell models.[129]

- *Mondal et al.,*(2008) using direct precipation technique, formulated and evaluated biodegradable nanoparticles with poly (D, L-lactide co glycolide). Entrapment efficiency observed in between 37% to 79% and FESEM indicated that the particles are spherical with smooth surface.[130]

- *Mehbuba Hossain S et al.,* (2014) investigated about effect of formulation variables on the release of letrozole prepared using bidegradable polymer in the form of implants. letrozole release nearly 19 days in the *in vitro* study.[131]

- *David Awotwe Otoo et al.,* (2012) characterized letrozole nanoparticles using non-invasive techniques like near infrared spectroscopy and near infrared chemical imaging which was prepared by solvent evaporation technique.[132]

- *Li Lia et al.,* (2010) developed and characterized letrozole transdermal patch using permeation enhancers and pharmacokinetic parameters were carried out in rats. In vivo report indicated that plasma concentration predicted in vivo was matched with *in vitro* data.[133]

- *Archana Nerella et al.,* (2014) developed letrozole solid lipid nanoparticles using hot homogenization continued by ultrasonication. Surfactant mixture Tween80 and soyphosphatidyl choline and solid core trimyristin used in this study and characterized mean particle size, poly dispersity index (PDI) and release study was carried out for 24 hours.[134]

- *Gopal Venkatesh Shavi et al.,* (2015) formulated peglyated liposomesloaded with anastrozole using various lipids like cholesterol, methoxy polyethylene glycol, soya phosphatidyl choline in different ratio. Cytotoxicity study was carried out for AL07 &AL09 and long circulation and sustained release was attained.[135]

- *Li Li et al.,* (2011) investigated about permeation study of letrozole using rat skin. Ethanol and IPM solvent mixture was used in this work which was a good permeation enhancer.[136]

- *Julie C Dougty et al.,* (2011) completed a survey among the women for whom the surgery for breast cancer and also the work allocated risk of recurrence and distance metastases for aromatic inhibitor.[137]

- *Paul E Goss et al.,* (2004) reported on the role for estrogen in both the initiation and promotion of breast cancer. Aromatase inhibitors were found to be superior to tamoxifen in terms of both efficacy and toxicity in advanced disease.[138]

- *Sidharth M Patil et al.,* (2013) estimated letrozole in pure and pharmaceutical dosage form by validated UV spectrophotometric method. While using acetonitrile as solvent it was shown maximum absorbance at 240 nm.[139]

- *Ganesh M et al.,* (2008) developed a UV spectrophotometric method for the determination of letrozole in bulk and solid dosage form. Letrozole obeyed Beers law in the range of 1-10µg/ml 161with regression slope and intercept 0.9998,-0.016, 0.1164 respectively. Percentage recovery result is 100.63±0.4215 which indicated it was free from interference of additives.[140]

- *Mani Ganesh et al.,* (2010) developed RPHPLC method for letrozole in bulk and in tablets using a new mobile phase [acetonitrile: water (50:50)]. Eluent was monitored at 265nm.[141]

- *Sophia Yui Kau Fong et al.,* (2015) described about phospholipid based solid drug formulations for oral drug delivery. The current review performed a systemic search of references in three evidence based English databases,

medline, embase and scifinder from the year of 1985 up till march2015 and highlighted about proliposomes for oral bioavailability enhancement which overcome the solubility, permeability and bioavailability issues of orally delivered drugs.[142]

- *Punna Rao Ravi et al.,* (2014) optimized raloxifene lipid nanoparticles for oral delivery and deteremined oral bioavailability using wistar rats and shown high encapsulation efficiency.[143]

- *Aditya N et al., (2014)* formulated polycaprolactone based nanocapsules by double emulsion method.Plackett Burman design used to arrive the optimized formulation. Obtained nanoparticles were observed to be spherical in shape with particle size less than 200nm and high encapsulation efficiency.[144]

- *Deepa Saini et al.,* (2015) prepared and characterized raloxifene loaded chitosan nanoparticles to enhance the bioavailability. 74CS-NPs were prepared by ionic gelation method. The particle size, entrapment efficiency and loading efficiency varied from 216.65 to 1890nm, 32.84 to 97.78% and 23.89 to 62.46%, respectively.[145]

- *Sudipta Senapati et al.,* (2016) investigated layered double hydroxides as a carrier for raloxifene prepared by ion exchange technique for controlled drug delivery. To analyse the absence of crystalline form FTIR, DSC and PXRD studies carried out and enhance the dissolution performance liquisoli systems were formulated.[146]

- *Devender Reddy Komala et al.,* (2015) developed a RLX loaded liquid-solid compacts for enhanced dissolution and permeation of intestine. Formulations were prepared by allowing liquid vehicles with varying concentrations of drug to get absorbed onto carrier and various ratios of coating materials used given good liquid retention potential values.[147]

- *Manal Elsheikh et al.,* (2014) reviewed the rational of appraisal of nanocarriers employed in cell culture vs. conventional techniques in pharmaceutical. The crucial process for approving the therapeutics outcome is the assessment of major quality attributes of nanocarriers. Emphasis on the imperative pharmaceutical

considerations demanding proper application for this technique were mentioned.[148]

- *Vijaykumar Nekkantia et al.,* (2013) developed, characterized and evaluated raloxifene hydrochloride solid lipid nanoparticles. Wistar rats were used for in vivo study.malver zeta siozer was used to determine particle size and zeta potential.More than 90% entrapment efficiency was found with raloxifene SLN.[149]

- *Douglas Muchmore,* (2000) reviewed about the selective estrogen receptor modulator raloxifene, characterized by estrogenic agonist activity in some tissues and estrogen antagonist activity in other tissues. SERM was developed to avoid some undesirable estrogen agonist action and to improve drug safety profile.[150]

- *Sathyaraj A et al.,* (2011) developed gradient RP-HPLC method for the determination of raloxifene hydrochloride using 280nm for UV. Mobile phase used was acetonitrile and water (30:70).[151]

- *Buridi Kalyanaramu et al.,* (2011) For the determination of raloxifene hydrochloride in formulations based on Charge- Transfer complex, a visible spectrophotometric model was developed by the authors. It was obtained in the presence of pH 7 buffer solution at a maximum wavelength 555nm.[152]

- *Fernanada Rodrigues Salazar et al.,* (2015) developed three methods like UV, HPLC, MEKC for the determination of raloxifene hydrochloride. These methods were developed and statistically analysed. HPLC carried out using water: acetonitrile: triethyl amine mobile phase.[153]

- *Pavithra DC et al.,* (2006) developed HPLC method to quantify raloxifene hydrochloride in tablets using methanol and water as mobile phase. The linearity range was 10- 60 µg /ml for raloxifene hydrochloride.[154]

- *Jaya Prakash et al.,* (2014) optimized the raloxifene loaded gellan gum nanoparticles using emulsion cross linking method. The optimum concentration of gellan gum was 1.31% and the preparation showed 97% encapsulation.[15]

- *Nirmal Shah et al.,* (2016) improved the bioavailability of raloxifene using nano structured lipid carriers prepared by solvent diffusion method. A full factorial design was used to study the impact of following parameters: solid lipid to liquid lipid ratio and concentration of stabilizer on the entrapment efficiency.[156]

- *Jaya Prakash et al.,* (2014) formulated and evaluated novel gellan gum raloxifene hydrochloride nanoparticles. *In vitro* release studies showed an initial burst release followed by a consistent release for 24 hours.[157]

3.1 Drug Profile

3.1.1 Letrazole[158]

Letrozole is a Non Steroidal 107 aromatase inhibitor(inhibitorof estrogens synthesis). It is chemically described as 4,4'-(1H-1,2,4-Triazol 1ylmethylene) dibenzonitrile. The structural formula is

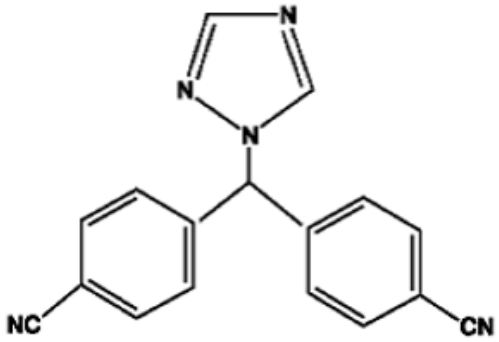

Figure 3.1 Structure of Letrozole

Empirical formula: $C_{17}H_{11}N_5$

Description: Letrozole is a white to yellowish crystalline powder, practically odourless.

Solubility: Freely soluble in dichloromethane, slightly soluble in ethanol, and practically insoluble in water.

Molecular weight : 285.31dl

Melting point : 184°C to 185°C

Mechanism of action/Effect on Pharmacokinetics Absorption

Letrozole is a nonsteroidal competitive inhibitor of aromatase and thus, in postmenopausal women, inhibits conversion of adrenal androgens (primarily androstenedione and testosterone) to estrogens (estrone and estradiol) in peripheral

tissues and cancer tissue. As a result, Letrozole interferes with estrogens-induced stimulation or maintenance of growth of hormonally responsive (estrogens and/or progesterone receptor positive or receptor unknown) breast cancer.

Pharmacokinetics

Absorption : Rapidly and completely absorbed. Absorption is not affected by food.
Distribution: Letrozole is weakly protein bound hence the volume of distribution (Vol_D) is approximately 1.9 liters per kg of body weight (high volume of distribution)
Biotransformation: Hepatic, by the CYP isoenzymes 3A4 and 2A6 (CYP 3A4 and CYP 2A6), to an inactive carbinol metabolite and its ketone analog.

Half-life: 2 days

Time to steady-state concentration: Plasma 2 to 6 weeks

Note: Steady-state plasma concentrations are 1.5 to 2 times higher than would be predicted on the basis of single-dose measurements, indicating some nonlinearity in Letrozole pharmacokinetics with daily administration. However, steady-state concentrations are maintained for extended periods, without further accumulation of Letrozole.

Elimination: Renal, approximately 90% of a dose (approximately 75% as the glucuronide conjugate of the inactive metabolite, 9% as two unidentified metabolites, and 6% unchanged).

Recommended Dose: The recommended dose 2.5 mg administered once a day, without regard to meals.

3.1.2 Raloxifene[159]

Raloxifene is a Selective estrogen-receptor modulator (SERM); high affinity for estrogen receptor. It is chemically described as (2-(4-Hydroxyphenyl)-6-hydroxybenzo(b)thien-3-yl) (4-(2-(1-piperidinyl)ethoxy)phenyl) methanone. The structural formula is

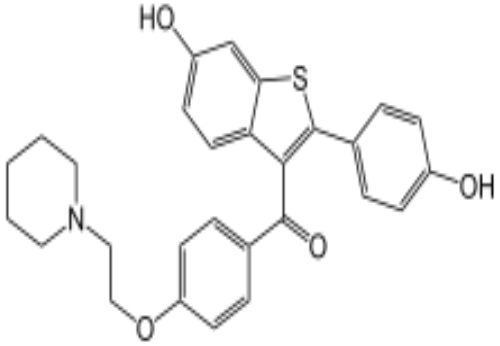

Figure 3.2 Structure of Raloxifene

Empirical formula : $C_{28}H_{27}NO_4S$

Description : Crystalline powder, odourless.

Solubility: Soluble in organic solvents like ethanol, DMSO and dimethyl formamide (DMF)

Molecular weight : 473.583g/mol

Melting point : 143-147 °C

Category

Antineoplastic Agent

Endocrine-Metabolic Agent

Indication

For the prevention and treatment of osteoporosis in post-menopausal women, as well as prevention and treatment of corticosteroid-induced bone loss. Also for the reduction in the incidence of invasive breast cancer in postmenopausal women with osteoporosis or have a high risk for developing breast cancer.

Pharmacodynamics

Raloxifene, a selective estrogen receptor modulator (SERM) of the benzothiophene class, is similar to tamoxifen in that it produces estrogen-like effects on bone and lipid metabolism, while antagonizing the effects of estrogen on breast and uterine tissue. The subsequent activation or repression of the target gene is mediated through 2 distinct transactivation domains of the receptor: AF-1 and AF-2. The estrogen receptor also mediates gene transcription using different response elements and other signalling pathways. The role of estrogen as a regulator of bone mass is well established. In postmenopausal women, the progressive loss of bone mass is related to decreased ovarian function and a reduction in the level of circulation estrogens. Estrogen also has favourable effects on blood cholesterol.

Mechanism of action

Raloxifene by binding to estrogen receptors, results in differential expression of multiple estrogen-regulated genes in different tissues. It produces estrogen- like effects on bone, reducing resorption and increasing bone mineral density in postmenopausal women, thus slowing the rate of bone loss. The maintenance of bone mass by raloxifene and estrogens is, in part, through the regulation of the gene-encoding transforming growth factor-β3 (TGF-β3), which is a bone matrix protein with antiosteoclastic properties.

Pharmacokinetics

Absorption : Approximately 60% of an oral dose is absorbed, but presystemic glucuronide conjugation is extensive. Absolute bioavailability of raloxifene is 2.0%.

Distribution : 2348 L/kg [oral administration of single doses ranging from 30 to 150 mg]

Biotransformation : Hepatic, by the CYP isoenzymes 3A4 and 2A6 (CYP 3A4 and CYP 2A6), to an inactive carbinol metabolite and its ketone analog.

Half-life : 27.7 hours

Metabolism:Hepatic, raloxifene undergoes extensive first-pass metabolism to the glucuronide conjugates: raloxifene-4'-glucuronide, raloxifene-6-glucuronide, and

raloxifene-6, 4'-diglucuronide. No other metabolites have been detected, providing strong evidence that raloxifene is not metabolized by cytochrome P450 pathways.

Elimination : Raloxifene is primarily excreted in feces, and less than 0.2% is excreted unchanged in urine.

Recommended Dose : The recommended dose 60 mg orally daily.

3.2 Excipient profile[75]

3.2.1 Cholesterol

Synonym : Cholesterin

Chemical Name : Cholest-5-En 3β-O1 (57-88-5)

Empirical Formula : $C_{27}H_{46}O$

Molecular Weight : 386.67

Structural Formula :

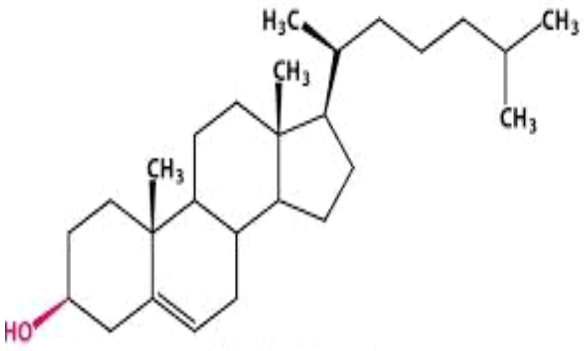

Figure 3.3 Structure of Cholesterol

Functional Category: Emollient; emulsifying agent.

Application in pharmaceutical formulation:

- Cholesterol is used in cosmetics and topical pharmaceutical formulation at concentration between 0.3-0.5% w/w as an emulsifying agent.
- It imparts water-absorbing power to an ointment and has emollient activity.
- Cholesterol additionally has a physiological role

Description: white or faintly yellow, odourless, pearly leaflets, needles, powder or granules. Prolonged exposure to light, air cholesterol acquires a yellow to tan colour.

Table 3.1 Typical properties of Cholesterol

Properties	**Values**
Boiling Point	360
Density	1.052 g/cm3 for anhydrous form
Dielectric Constant D	5.41
Melting Point	147 – 150^{o}C
Solubility	Insoluble in water, Ethanol – 1 in 29 at 40^{o}C, ether: 1 in 2.8 Chloroform is 1 in 4

Stability and storage conditions*:* Cholesterol is stable and should be stored in a well closed container, protected from light. Precipitated b digitonin at the level employed as excipient. Cholesterol is often derived from animal sources and must be done so in accordance with the regulation for human consumption.

Handling precautions*:* May be harmful following inhalation or ingestion of large quantities, or over prolonged period of time, due to the possible involvement of cholesterol in arteriosclerosis and gallstone. May be irritant to the eyes. Rubber or plastic gloves, eye protection, and a respiratory are recommended.

3.2.2 Span 20

Synonym : Sorbitan laurate, Sorbitan monolaurate

Chemical Name : Octadecanoic acid [2-[(2R,3S,4R)-3,4-dihydroxy-2-tetrahydrofuranyl]-2- hydroxyethyl]ester

Empirical Formula: $C_{18}H_{34}O_6$

Molecular Weight : 346.46

Structural Formula:

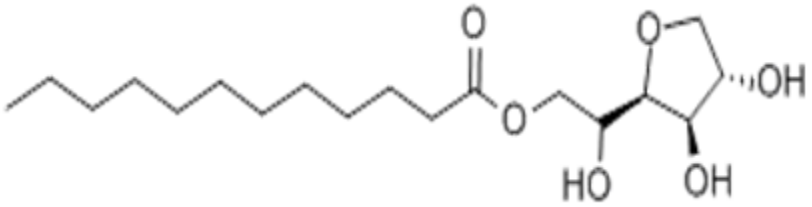

Figure 3.4 Structure of Span 20

Functional Category: Emulsifier, wetting agent and lubricant

Application in pharmaceutical formulation:

- Sorbitan monolaurate is used in the manufacture of food and healthcare products and is a non-ionic surfactant with emulsifying, dispersing, and wetting properties**.**
- It is also employed to create synthetic fibers, metal machining fluid, and brighteners in the leather industry, and as an emulsifier in coatings, pesticides, and various applications in the plastics, food and cosmetics industries.

Table 3.2 Typical properties of span 20

Properties	Values
Boiling Point	$>250^0$C
Density	1.03 g/ mL at 20 °C
Dielectric Constant D^{20}	5.41
Melting Point	$>200^o$C
Solubility	Soluble in water (partly), 2-ethoxyethanol, ethanol, and methanol.

Stability and storage conditions: Store in a dry, cool and well-ventilated place, away from direct sunlight. Keep containers closed when not in use.

Handling precaution: All chemicals should be considered hazardous. Avoid direct physical contact. Use appropriate, approved safety equipment. Untrained individuals should not handle this chemical or its container. Handling should occur in a chemical fume hood.

3.2.3 Span 60

Synonym : Sorbitan stearate, Sorbitane monostearate

Chemical Name : Octadecanoic acid [2-[(2R,3S,4R)-3,4-dihydroxy-2-tetrahydrofuranyl]-2-hydroxyethyl] ester

Empirical Formula : $C_{24}H_{46}O_6$

Molecular Weight : 430.62

Structural Formula:

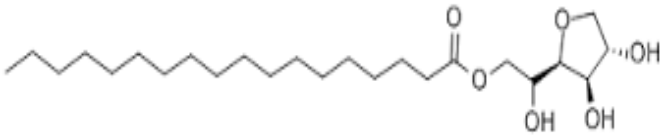

Figure 3.5 Structure of Span 60

Functional Category: Emulsifier, wetting agent and lubricant.

Application in pharmaceutical formulation*:*

- Sorbitan monostearate is used in the manufacture of food and healthcare products and is a non-ionic surfactant with emulsifying, dispersing, and wetting properties**.**
- It is also employed to create synthetic fibers, metal machining fluid, and brighteners in the leather industry, and as an emulsifier in coatings, pesticides, and various applications in the plastics, food and cosmetics industries

Table3.3 Typical properties of span 60

Properties	Values
Density	1.0 g/cm3 (20 C)
Dielectric Constant D^{20}	5.41
Melting Point	$52+3^{o}C$
Solubility	Soluble in water (partly), 2-ethoxyethanol, ethanol, and methanol.

Stability and storage conditions: Store in a dry, cool and well-ventilated place, away from direct sunlight. Keep containers closed when not in use.

Handling precaution: All chemicals should be considered hazardous. Avoid direct physical contact. Use appropriate, approved safety equipment. Untrained individuals should not handle this chemical or its container. Handling should occur in a chemical fume

3.2.4 Maltodextrin

Synonyms: Maltrin

Empirical Formula and Molecular Weight: (C6H10O5)nH2O 900–9000

The USP32–NF27 describes maltodextrin as a nonsweet, nutritive saccharide mixture of polymers that consist ofD-glucose units, with a dextrose equivalent (DE) less than 20;see alsoSection, TheD-glucose units are linked primarily bya-(1!4) bonds but there are branched segments linked by a-(1!6) bonds. It is prepared by the partial hydrolysis of a food-grade starch with suitable acids and/or enzymes.

Structural Formula

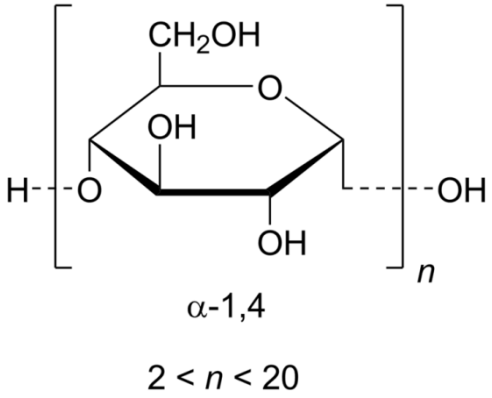

Figure 3.6 Structure of Maltodextrin

Functional Category: Coating agent; tablet and capsule diluent; tablet binder; viscosityincreasing agent.

Applications in Pharmaceutical Formulation or Technology

Maltodextrin is used in tablet formulations as a binder and diluent in both direct-compression and wet-granulation or agglomeration processes. Maltodextrin appears to have no adverse effect on the rate of dissolution of tablet and capsule formulations; magnesium stearate 0.5–1.0 % may be used as a lubricant. It has been used as a carrier in a spray-dried redispersible oil-in-water emulsion to improve the bioavailability of poorly soluble drugs.Maltodextrin may also be used as a tablet film former in aqueous film-coating processes. Maltodextrin grades with a high DE value

are particularly useful in chewable tablet formulations. Maltodextrin may also be used in pharmaceutical formulations to increase the viscosity of solutions and to prevent the crystallization of syrups. Therapeutically, maltodextrin is often used as a carbohydrate source in oral nutritional supplements because solutions with a lower osmolarity than isocaloric dextrose solutions can be prepared. At body osmolarity, maltodextrin solutions provide a higher caloric density than sugars. Maltodextrin is also widely used in confectionery and food products, as well as personal care applications.

Uses of Maltodextrin

- Aqueous film-coating 2–10
- Carrier 10–99
- Crystallization inhibitor for lozenges and syrups 5–20
- Osmolarity regulator for solutions 10–50
- Spray-drying aid 20–80
- Tablet binder (direct compression) 2–40
- Tablet binder (wet granulation) 3–10

Description

Maltodextrin occurs as a nonsweet, odorless, white powder or granules. The solubility, hygroscopicity, sweetness, and compressibility of maltodextrin increase as the DE increases. The USP32– NF27 states that it may be physically modified to improve its physical and functional characteristics.

4. SCOPE AND PLAN OF WORK

4.1 Scope of The Present Work

To provide an ideal drug delivery system of anticancer drugs in order to maintaining the therapeutic plasma concentration for a required period of time.

To provide the proniosomal drug delivery system

a) For the patient compliance

b) Effectiveness of anticancer therapy

c) Reduction of adverse effect.

This is achieved by maintaining the plasma drug concentration at the level with in therapeutic range for the required period of time. Hence it is absolute necessity to develop effective drug delivery system with minimum dose for reducing undesired side effects.

4.2 Plan of Work

Phase I: Literature review.

Phase II: Selection of raw materials including bulk drug for proniosome formulation.

Pre-formulation study

- Carrier-Drug interaction by FTIR spectroscopy and DSC study
- Calibration Curve of anticancer drug by UV spectrophotometer and HPLC.

Phase III:

- Formulation of anticancer drug loaded proniosome
- Characterization of proniosome
  - Optical microscopy
  - Measurement of angle of repose
  - Number of vesicles per cubic mm
  - Vesicle size
  - Zeta potential
  - Scanning Electron Microscopy
  - Drug entrapment studies

Phase IV:

In vitro drug release from proniosome vesicle. Selection of best formulation based on drug entrapment, *in vitro* drug release and vesicle size. Kinetic data analysis

Phase V: Stability study

Phase VI: *In vivo* pharmacokinetic study for best formulation in animal model.

5. MATERIALS AND METHODS

Materials and instruments used

List of materials and instruments used in the research work has given in Table 5.1 and Table 5.2 respectively

Table 5.1 Materials used for research work

S.no	Name	Company name
1.	Letrozole	Sun pharmaceuticals Advanced Research Centre, Vadodara, India
2.	Raloxifene	Cipla Ltd,Mumbai,India
3.	Cholesterol	S.D. Fine Chem Ltd, Mumbai ,India
4.	Span-20	S.D. Fine Chem Ltd, Mumbai,India
5.	Span 60	S.D. Fine Chem Ltd, Mumbai,India
6.	Maltodexrin	Himedia,Mumbai,India
7.	HCL	S.D. Fine Chem Ltd, Mumbai,India
8.	Ethanol	Merck, Germany
9.	Dimethyl formamide	Loba Chemie pvt.ltd Mumbai
10.	Methyl chloride	Universal Laboratories
11.	DMSO	Universal Laboratories
12.	Methanol HPLC Grade	Merck, Germany
13.	Acetronitrile HPLC Grade	Merck, Germany
14.	Chloroform	S.D. Fine Chem Ltd, Mumbai,India
15.	Triton X-100	Merck, Germany
16.	Sodium Hydroxide	Universal Laboratories
17.	Potassium di hydrogen phosphate	Merck, Germany

Table 5.2 Instruments used for the research work

S.No	Name	Company name
1.	Rotary flash evaporator	Super fit, India
2.	Electronic digital balance	Shimadzu, Japan
3.	Dialysis membrane 50	Hi media, India
4.	Digital pH meter	ELICO, India
5.	Double beam UV/ Visible spectrophotometer	Lab India
6.	Zetasizer	Malvern, England
7.	Probe sonicator	Electro sonic Industries, India
8.	Trinocular Optical microscope	Olympus, Japan
9.	Scanning Electron Microscope	Hitachi, Japan
10.	Refrigirated Centrifuge	Plasto Crafts Industries Private Limited, India
11.	FTIR Spectrophotometer	Bruker Alpha-E
12.	Differential Scanning Calorimeter	Shimadzu, Japan
13.	High Performance Liquid Chromatography	Shimadzu, Japan
14.	Magnetic Stirrer	Remi Instruments Private Limited,Mumbai
15.	Vortex mixer	Thermo Scientific
16.	Microscope	Almicro
17.	Digital Camera	Nikon D810 Nikon FX, DX
18.	Melting point apparatus	Sigma Scientific
19.	Stability chamber	Rolex
20.	Refrigerator	Voltas

Methods

5.1 Preformulation study

5.1.1 Organoleptic properties

The organoleptic character of the drug like colour odour, and appearance play an important role in the identification of the sample and hence they should be recorded in an descriptive terminology.[160]

Colour: a small quantity of pure drug was taken in a butter paper and viewed in well illuminated place.

5.1.2 Solubility studies

Solubility is one of the most important physicochemical properties studied. Accurate solubility data are essential to ensure the robustness of the finished product for the development of liquid dosage forms. Solubility data are important in determining, if an adequate amount of drug is available for absorption *in vivo*, for solid dosage forms. It may be subject to dissolution rate-limited or solubility-limited absorption within the gastrointestinal (GI) residence time, if a compound has a low aqueous solubility.[161]

The sample was qualitatively tested for its solubility in various solvents. It was determined by taking 2 mg of drug sample in 5 ml of solvent as water, methanol, ethanol, phosphate buffer P^H7.4, di chloro methane ,DMSO, dimethyl formamide etc., in small test tubes and well solubilized by shaking.

5.1.3 Melting point

The melting point of Letrozole and Raloxifene was determined by capillary method, using small quantity of drug which was taken and placed in apparatus and determined the melting point and matched with standards.[161]

5.1.4 Angle of repose[69]

Funnel method was used to measure the angle of repose of dry proniosome powder. The proniosomes free flowing powder was poured into a funnel which was fixed at a position so that the 13mm outlet orifice of the funnel is 5cm above a level black surface. The powder flows down from the funnel to form a cone on the surface and

the angle of repose was then calculated by measuring the height of the cone and the diameter of its base with the help of calibrated scale.

5.2 Drug -Excipient compatability studies[162]

5.2.1 Fourier Transform Infrared (FTIR) spectroscopy

The drug-excipient compatibility is holding the major role for a formulator to prepare therapeutically effective formulations. This pre formulation study was carried out using FTIR spectroscopic determination of drug molecule alone and combination of drug molecule with all excipients used in the formulation process.

FT-IR spectra of drug, excipients, physical mixture and optimized formulation were obtained using Bruker Alpha FT-IR spectrophotometer. Drug and excipients ,physical mixture and optimized formulation were analysed by KBr pellet technique.In this method drug and KBr were mixed at the ratio of 1:100.Then the mixtures were pressed into a pellet. The FT-IR spectra were recorded in the region 500-4000cm^{-1}

5.2.2 Differntial Scanning Calorimetry(DSC)

Differential Scanning Calorimetry was performed to study the thermal behavior of drug alone, selected proniosome powder formulations.

5.3 Construction of calibration curve

5.3.1 Calibration curve of letrozole

5.3.1.1 UV/Visible spectrophotometry

The weighed quantity 50 mg of Letrozole was placed in 50ml standard measuring flask. Letrozole was dissolved by using 10ml of ethanol and the volume was made up to the mark. The aliquots were prepared to obtain the concentrations of 10,20,30,40,50, and 60µg/ml using the above solution. The samples were analysed using UV/Visible spectrophotometer at 240 nm.

5.3.1.2 HPLC method

The analysis was carried out on a reversed-phase C18 (250 mm × 4.6 mm, 5 µm) column with an isocratic mobile phase of methanol-water (70:30,v/v), at a flow rate of 1.0 ml/ min^{-1}. Detection was carried out at 239 nm with a UV-visible spectrophoto-metric detector.

5.3.2Calibration curve of raloxifene hydrochloride

5.3.2.1 UV/Visible spectrophotometry

The weighed quantity 50 mg of Raloxifene hydrochloride was placed in 50ml standard measuring flask. Raloxifene hydrochloride was dissolved by using 10ml of methanol and the volume was made up to the mark. The aliquots were prepared to obtain the concentrations of 10,20,30,40,50 and 60 µg/ml using the above solution. The samples were analysed using UV/Visible spectrophotometer at 289 nm.

5.3.2.2 HPLC method

The mobile phase consisted of a mixture of buffer (pH7.4)-acetonitrile (60:40 v/v). The flow rate was set to 0.8 ml /$minutes^{-1}$, Injection volume 20µl, The Column used is C18 (150mm). The detection wavelength was set to be at 287 nm. RP-HPLC analysis was performed isocratically at room temperature.

5.4 Formulation of proniosomes

5.4.1. Formulation of letrozole loaded proniosomes

Based on the composition given in Table 5.3 and Table 5.4, using slurry method, proniosomes were formulated. Carrier maltodextrin should be taken in a round bottom flask. Then necessary amount of span20,span 60, cholesterol and drug should be added according to the formulae. These were dissolved by addition of chloroform. Further to make slurry, some more chloroform should be added in case of lower surfactant loading. Then in a rapid rotating flash evaporator, the round bottom flask was fitted and the solvent was evaporated at 60 rpm under reduced pressure at a temperature of 45±2°C , until the product become free flowing, dry condition. After that the obtained product were dried overnight at room temperature in a desiccator

under vacuum. The obtained final preparation was termed as proniosomes which was stored in a sealed container at 5°C and kept it for evaluation process.

Table 5.3 Composition of letrozole proniosome using span20

Sl.No	Formulation Code	Letrozole	Span 20 in mg	Cholesterol in mg	Molar ratio	
					Span 20 in mm	Cholesterol in mm
1	LS201	2.5 mg	346.46	0	1.000	0.000
2	LS202	2.5 mg	311.814	38.665	0.900	0.100
3	LS203	2.5 mg	277.168	77.33	0.800	0.200
4	LS204	2.5 mg	242.522	115.995	0.700	0.300
5	LS205	2.5 mg	207.876	154.66	0.600	0.400
6	LS206	2.5 mg	173.23	193.325	0.500	0.500
7	LS207	2.5 mg	138.584	231.99	0.400	0.600
8	LS208	2.5 mg	103.938	270.655	0.300	0.700
9	LS209	2.5 mg	69.292	309.32	0.200	0.800
10	LS210	2.5 mg	34.646	347.985	0.100	0.900

1 mole = 1000 milli mole 1 mole = weight in grams/molecular weight

Molecular weight of span 20 346.46, Molecular weight of Cholesterol 386.65

Table 5.4 Formulation of letrozole proniosomes using span 60

Sl.No	Formulation Code	Letrozole	Span 60 in mg	Cholesterol in mg	Molar ratio	
					Span 60 in mm	Cholesterol in mm
1	LS601	2.5 mg	430.62	0	1.000	0.000
2	LS602	2.5 mg	387.558	38.665	0.900	0.100
3	LS603	2.5 mg	344.496	77.33	0.800	0.200
4	LS604	2.5 mg	301.434	115.995	0.700	0.300
5	LS605	2.5 mg	258.372	154.66	0.600	0.400
6	LS606	2.5 mg	215.31	193.325	0.500	0.500
7	LS607	2.5 mg	172.248	231.99	0.400	0.600
8	LS608	2.5 mg	129.186	270.655	0.300	0.700
9	LS609	2.5 mg	86.124	309.32	0.200	0.800
10	LS610	2.5 mg	43.062	347.985	0.100	0.900

Molecular weight of span 60 430.62

5.4.2 Formulation of raloxifene proniosomes

Slurry method was used for the preparation of proniosomes. Table 5.5 represents the composition of different proniosomal formulations. Lipid mixture comprising of span20/span 60 and cholesterol were accurately weighed at various molar ratios and drug (60 mg) was dissolved in ethanol. Then maltodextrin was added to the resultant solution to form slurry and was then transferred into a round bottom flask. Attached to a rotary flash, the organic solvent was then evaporated under reduced pressure, at a temperature of 45±2 °C, and thereafter to ensure complete removal of solvent. Product the resultant powders were further dried overnight in a vacuum oven at room temperature to obtain dry, free-flowing and stored in a tightly closed container at 5 °C for further evaluation.[35,38]

Table 5.5 Formulation of raloxifene proniosomes using span 60

Sl. No	Formulation Code	Raloxifene	Span 20 in mg	Cholesterol in mg	Molar ratio	
					Span 20 in mm	Cholesterol in mm
1	RS201	60 mg	346.46	0	1.000	0.000
2	RS202	60 mg	311.814	38.665	0.900	0.100
3	RS203	60 mg	277.168	77.33	0.800	0.200
4	RS204	60 mg	242.522	115.995	0.700	0.300
5	RS205	60 mg	207.876	154.66	0.600	0.400
6	RS206	60 mg	173.23	193.325	0.500	0.500
7	RS207	60 mg	138.584	231.99	0.400	0.600
8	RS208	60 mg	103.938	270.655	0.300	0.700
9	RS209	60 mg	69.292	309.32	0.200	0.800
10	RS210	60 mg	34.646	347.985	0.100	0.900

Table 5.6 Formulation of raloxifene proniosomes using span 60

Sl.No	Formulation Code	Raloxifene	Span 60 in mg	Cholesterol in mg	Molar ratio	
					Span 60 in mm	Cholesterol in mm
1	RS601	60 mg	346.46	0	1.000	0.000
2	RS602	60 mg	311.814	38.665	0.900	0.100
3	RS603	60 mg	277.168	77.33	0.800	0.200
4	RS604	60 mg	242.522	115.995	0.700	0.300
5	RS605	60 mg	207.876	154.66	0.600	0.400
6	RS606	60 mg	173.23	193.325	0.500	0.500
7	RS607	60 mg	138.584	231.99	0.400	0.600
8	RS608	60 mg	103.938	270.655	0.300	0.700
9	RS609	60 mg	69.292	309.32	0.200	0.800
10	RS610	60 mg	34.646	347.985	0.100	0.900

5.5 Evaluation of proniosomes

5.5.1 Morphological evaluation

After a suitable dilution, proniosomes derived niosomes were mounted on glass slides and viewed under a microscope for morphological observation. By using digital camera the photomicrograph of the preparation was obtained from the microscope. proniosome powder was transformed to niosomes by hydrating with phosphate buffer (pH 7.4) at 80^{o}C using vortex mixer for 2 min. The niosome dispersion was placed over a glass slide and the vesicles formed were observed at a magnification of 450 through an optical microscope.[43,50]

5.5.2 Measurement of angle of repose

Funnel method was used to measure the angle of repose of dry proniosome powder. The proniosomes free flowing powder was poured into a funnel which was fixed at a position so that the 13mm outlet orifice of the funnel is 5cm above a level black surface. The powder flows down from the funnel to form a cone on the surface and the angle of repose was then calculated by measuring the height of the cone and the diameter of its base with the help of calibrated scale.[69]

5.5.3 Vesicle size

The vesicle dispersions were diluted about 100 times in the same solvent medium used for their preparation. Vesicle size was measured on a particle size analyzer.[50]

5.5.4 Zeta potential

The particle charge was one of the most important parameter in assessing the physical stability of any colloidal dispersion. The large number of particles was equally charged, then electrostatic repulsion between the particles was increased and thereby physical stability of the formulation was also increased. Zeta potential analysis was done for determining the colloidal properties of the prepared formulations. The diluted proniosome derived niosome dispersion was determined using zeta potential analyzer based on electrophoretic light scattering and laser Doppler velocimetry method. The temperature was set at 25°C. Charge on vesicles and their mean zeta potential values were obtained directly from the measurement.[162]

5.5.5 Entrapment efficiency

Entrapment efficiency of the proniosomes derived niosomal dispersion was be done by separating the unentrapped drug by dialysis method and the drug remained entrapped in niosomes was determined by complete vesicle disruption using 0.1% Triton X-100 and analyzed spectro photometrically for the drug content after suitable dilution with pH7.4 phosphate buffer and filtered through what mann filter paper.[43] The percentage of drug encapsulation (EE (%)) was calculated by the following equation:

$$EE\ \% = [(C_t - C_r / C_t)] \times 100\%$$

Where C_t is the concentration of total drug

C_r is the concentration of free drug

5.5.6 Number of vesicles per cubic mm

One of the important parameter to evaluate the proniosome powder is the number of vesicles formed after hydration. The proniosome powder was subjected to hydration with phosphate buffer (P^H 7.4) and the formed niosomes were counted by optical microscope using a haemocytometer.The niosomes in 80 small squares were counted and calculated by using the following formula.[162]

Number of niosomes per cubic mm = Total number of niosomes counted x dilution factor x 4000/Total number of small squares counted

5.5.7 Scanning Electron Microscopy (SEM)

The scanning electron microscopy (SEM) is one of the most important instrument used for analysis of surface morphology. The particle size of proniosomes is a very important characteristic. The surface morphology such as roundness, smoothness, and formation of aggregates and the size distribution of proniosomes were studied by Scanning Electron Microscopy (SEM). Proniosomes were sprinkled onto the double-sided tape that was affixed on aluminum stubs. The aluminum stub was placed in the vacuum chamber of a scanning electron microscope.[38,39]

5.5.8 *In vitro* drug release study

This study was carried out using open end cylinder method. One end of the tube is tightly covered with a Himedia dialysis membrane. The proniosome powder was placed over the membrane in the donar chamber. The donar chamber is then lowered to the vessels of the glass beaker containing 100 ml of phosphate buffer (P^H 7.4) which act as a receptor compartment so that the dissolution medium outside and the vesicles preparation inside were adjusted at the same level. The release study was carried out at 37±0.5ºC, and the stirring shafts were rotated at a speed of 50 rpm. Five millilitre samples were withdrawn periodically at predetermined time intervals. Every withdrawal was followed by replacement with fresh medium to maintain the sink condition. The samples were analysed spectro photometrically.[72]

5.5.9 Drug release kinetic data analysis

The release data obtained from various formulations were studied further for their fitness of data in different kinetic models like Zero order, first order, Higuchi's and korsmeyer-peppa's. In order to understand the kinetic and mechanism of drug release, the result of *in vitro* drug release study of the prepared proniosome was fitted with various kinetic equation like zero order (cumulative % release vs. time), first order (log cumulative % remain vs time) and higuchi's model (cumulative % drug release vs. square root of time).[70]

To understand the release mechanism *in vitro* data was analyzed by korsmeyer-peppa's model (log cumulative % drug release vs. log time) and the exponent n was calculated through the slope of the straight line. $M_t / M_\infty = btn$, Where M_t is amount of drug release at time t, M_∞ is the overall amount of the drug, b is constant, and n is the release exponent indicative of the drug release mechanism. If the exponent n = 0.5 or near, then the drug release mechanism is Fickian diffusion, and if n have value near 1.0 then it is non-Fickian diffusion. R^2 values were calculated for the curves obtained by regression analysis of the above plots.

5.5.10 Stability of proniosomes

The optimized batch was stored in airtight sealed glass vials at different temperatures. Surface characteristics and percentage drug retained in proniosomes and parameters for evaluation of the stability, since instability of the formulation would reflect in drug leakage and a decrease in the percentage drug retained. The proniosomes were sample at regular intervals of time (0, 1, 2 and 3months), observed for colour change,vesicle size ,percentage drug retained and *in vitro* release after being hydrated to form niosomes and analyzed by spectrophotometer.

5.5.11 *In vivo*pharmacokinetic study in animal model

Female rabbits (weighing 1.5-2 kg) were used for the bioavailability study. Animals were housed in the standardized conditions at the animal house of the J.K.K. Nattraja College of Pharmacy Kumarapalayam, Tamil Nadu, India. All animals were acclimatized and kept under constant temperature (25°C $\pm$ 2°C). All animal procedures were performed in accordance to the approved protocol for use of experimental animals set by the standing committee on animal care of the J.K.K.Nattraja College of Pharmacy. Animals were divided into three groups of three rabbits in each group. The study was designed as a single oral dose.

- Group 1not received any drug (Control)
- Group 2 received an equivalent of 1mg Letrozol/kg body weight of rabbits.
  (Or)
- Group 2 received an equivalent of 30mg of Raloxifene kg body weight of rabbits.
- Group 3 received drug loaded proniosomal powders (the best formulation that exhibited the maximum EE% and the slowest release rate).

Blood samples (about 1 ml) were withdrawn from the sinus orbital into heparinized tubes at 0, 2,4,12,18,24,30,36,40,48,52,56,60 and 72 hours after each administration. The blood samples were centrifuged immediately at 3000 rpm for 10 minutes to obtain the plasma samples and were stored at -20°C for subsequent assay using HPLC.

Pharmacokinetic analysis Maximum plasma drug concentration (Cmax), area under the plasma drug concentration-time profile (AUC), the area under first moment curve

(AUMC), the elimination half life ($t_{½}$) and other pharmacokinetic parameters were evaluated using PK solver MS Excel Add-in programme.

6. RESULTS AND ANALYSIS

6.1 Results and analysis of letrozole formulation

6.1.1 Preformulation

Preformulation is the science of the physicochemical characterization of the solid and solution properties of compounds. Before starting prototype formulation of Letrozole proniosome preformulation studies for letrozole pure drug was performed. Following were the results of preformulation studies carried out for letrozole pure drug, which were carried out as per methods, explained in the methodology section.

6.1.1.1 Identification of letrozole

Drug :Letrozole
Colour: yellow
Odour :Odourless
Appearance: Crystalline Powder

6.1.1.2 Solubility study

Letrozole was slightly soluble in chloroform slightly soluble in ethanol, freely soluble in dichloromethane and insoluble in water.

6.1.1.3 Melting point

The procedure for determination is same as section 5.1.3. The melting point of Letrozole was found to be 183-185 °C. This matches with the standard melting temperature range 184-185 °C indicating the identity of letrozole .

6.1.1.4 Angle of repose

- Letrozole - 32°38'±0.64
- Maltodextrin - 30°06'±0.14

6.1.2 Drug -Excipient compatability studies

6.1.2.1 FT-IR spectroscopy of letrazole

Compatibility studies were performed using FT-IR spectrophotometer. The IR spectrum of pure drug physical mixture of drug ,excipents and optimized formulation were studied by making a KBr pellet.

The spectral details for all types of formulations are shown as follows

Table 6.1 FT IR spectrum peaks in letrazole

Wave Number in cm^{-1}		**Functional group**
Range	**Observed**	
2260-2240 cm^{-1}	2378.07 cm-1	C≡N
1690-1640 cm-1	1170.64 cm-1	C=N
1350-1000 cm-1	1058.35 cm-1	C-N
1600-1475 cm-1	1460.18 cm-1	C=C, Aromatic

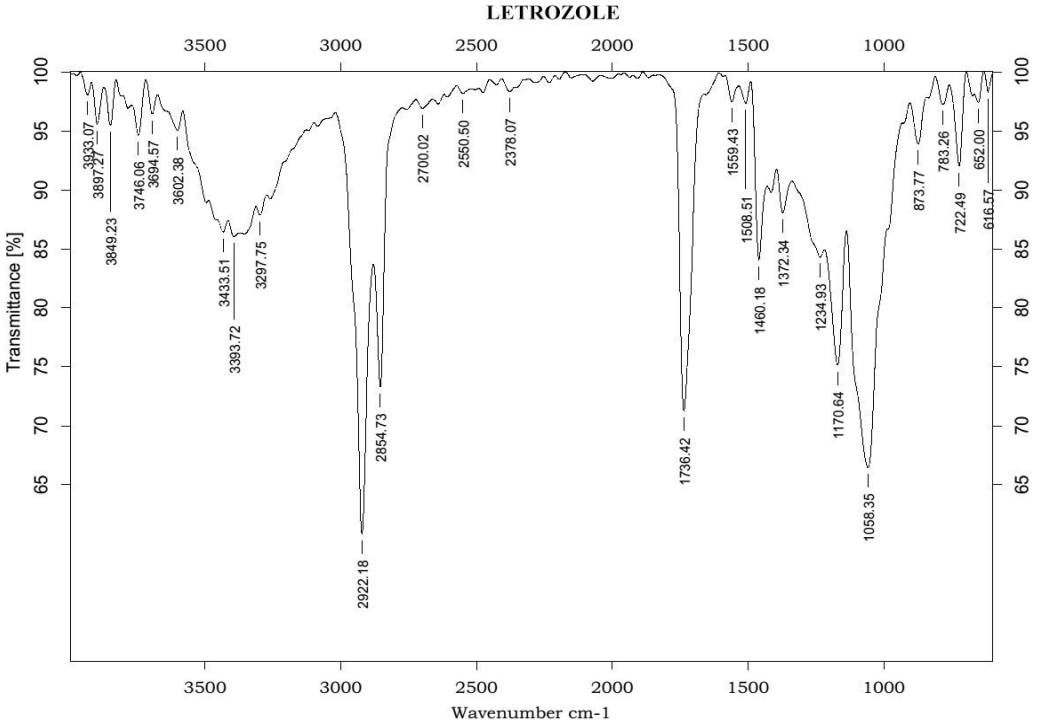

Figure 6.1 FT IR spectrum of letrozole

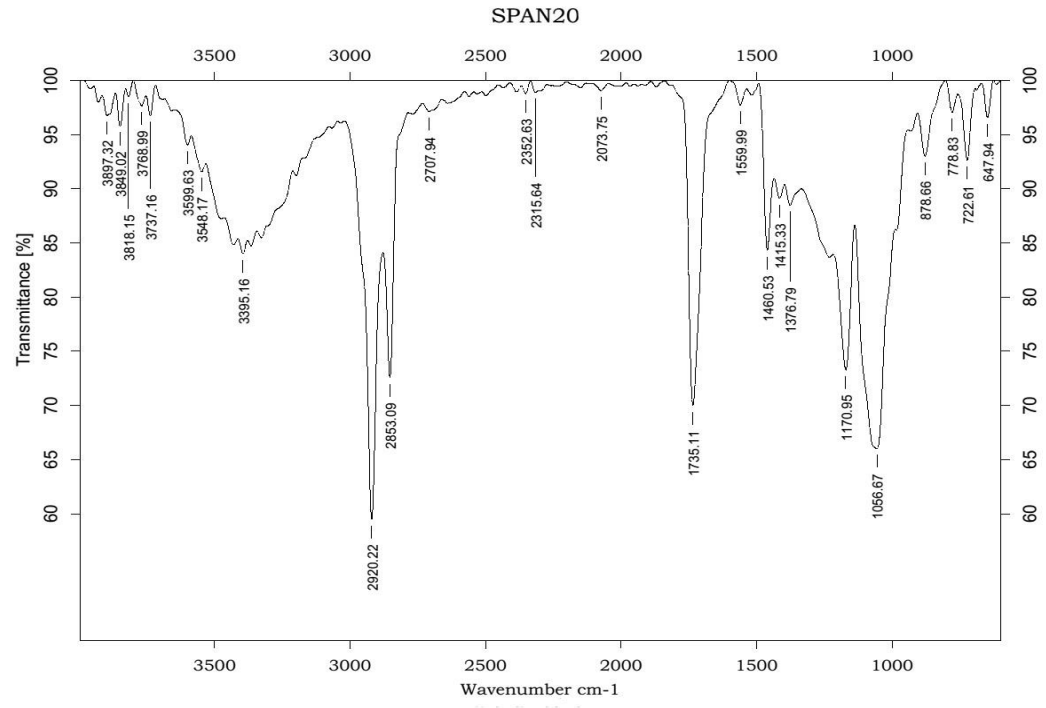

Figure6.2 FT IR spectrum of span20

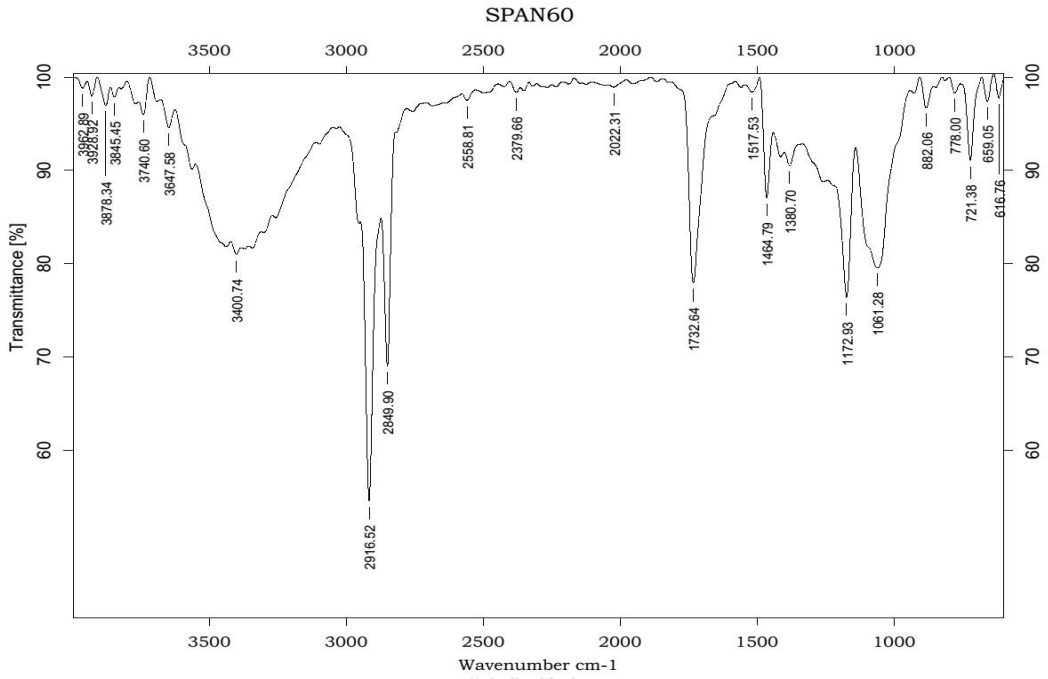

Figure 6.3 FT IR spectrum of span 60

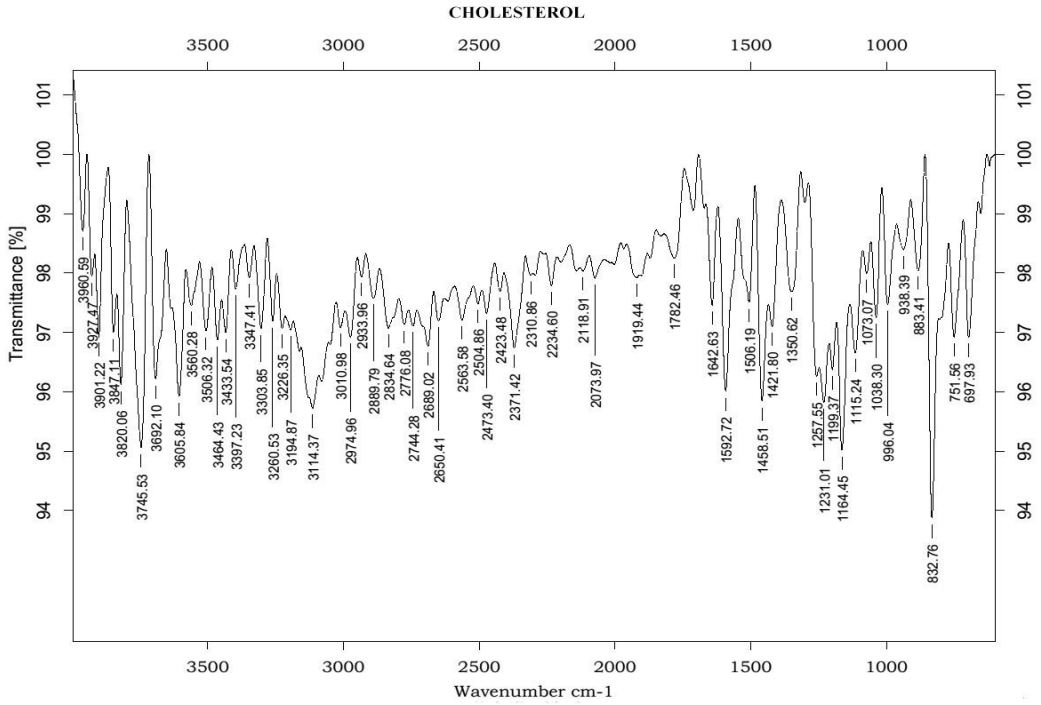

Figure 6.4 FT IR spectrum of cholesterol

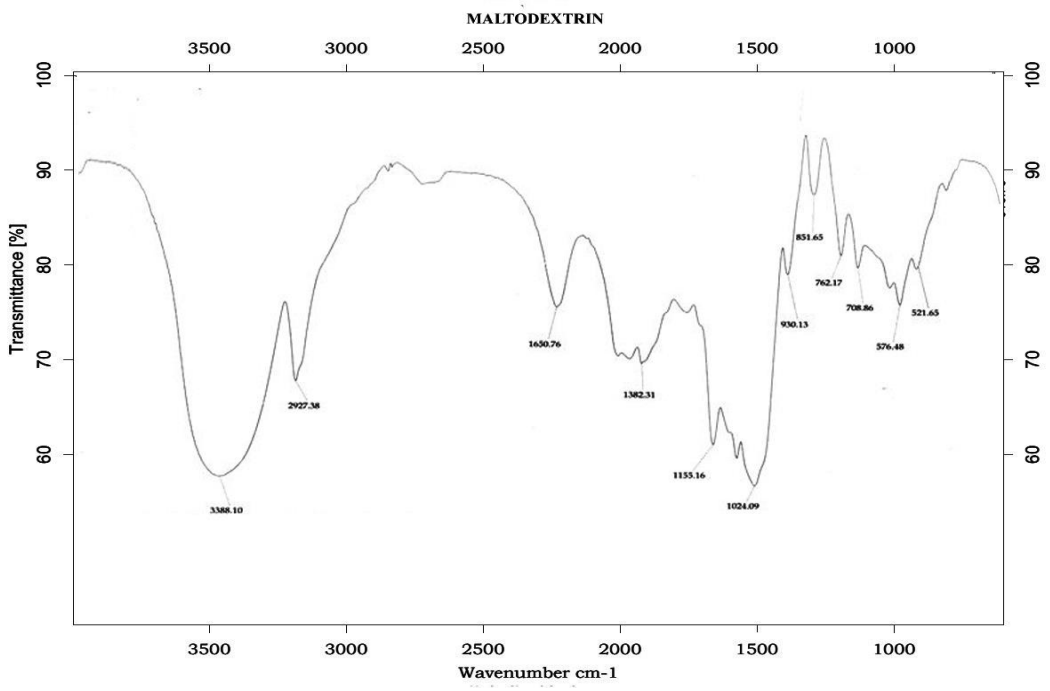

Figure 6.5 FT IR spectrum of maltodextrin

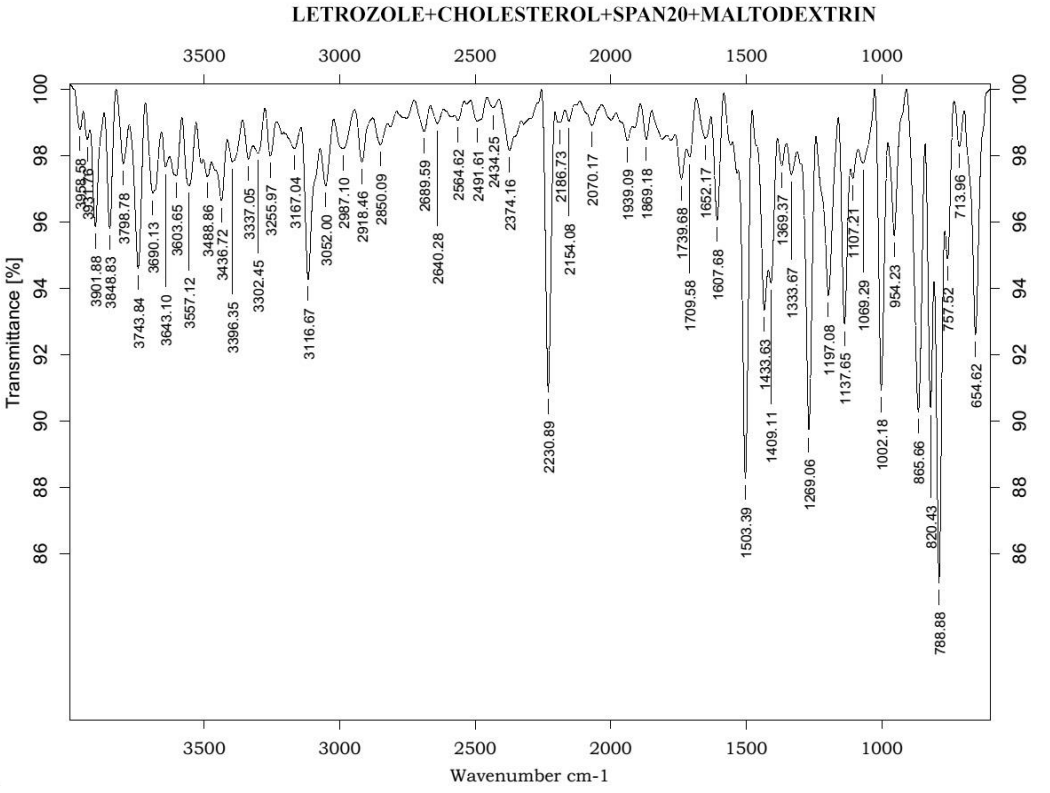

Figure 6.6 FT IR spectrum of physical mixture of letrozole+cholesterol+span20+maltodextrin

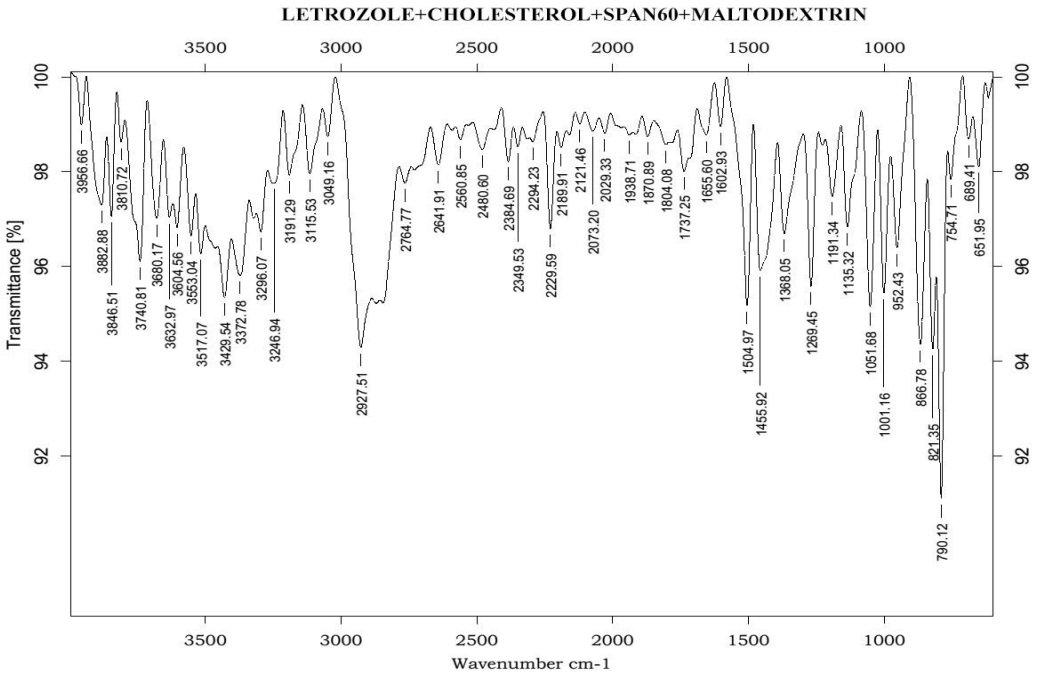

Figure 6.7 FT IR spectrum of physical mixture of Letrozole+cholesterol+span 60+maltodextrin

FT IR Spectrum of optimized formulation:

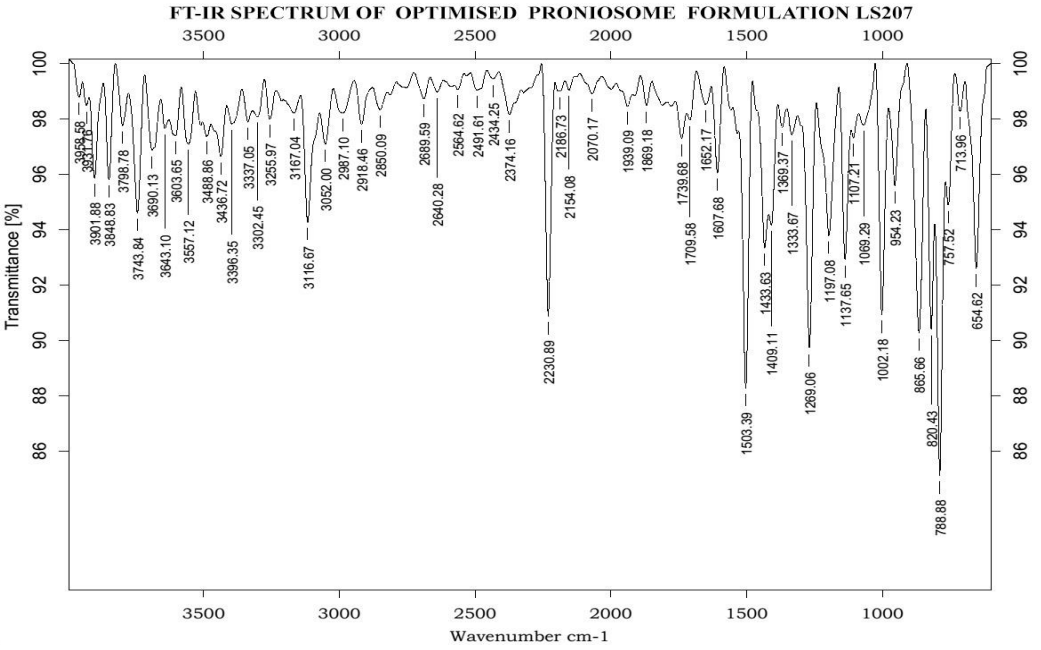

Figure 6.8 FT IR spectrum of optimised proniosome formulation LS207

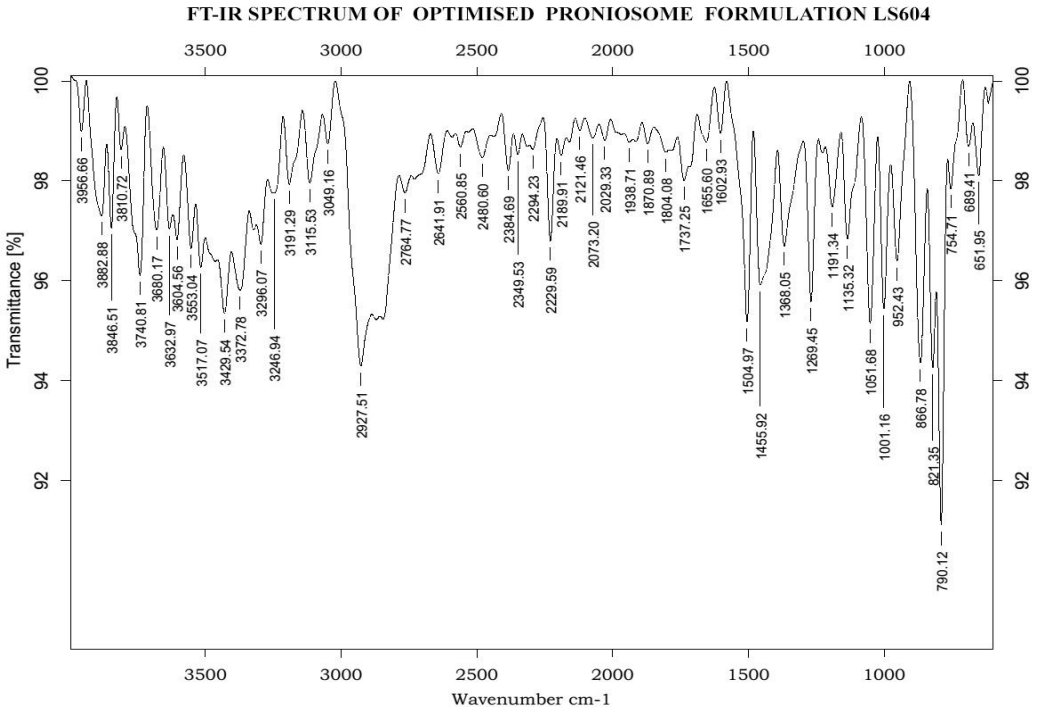

Figure 6.9 FT IR spectrum of optimised proniosome formulation LS604

Table 6.2 Characteristic absorption peaks in FT IR spectrum

(KBr Disc) peaks at					Indications
Pure Drug Letrazole	**Physical Mixture**		**Formulation**		
	Letrozole+Cholesterol+ Span20+Maltodextrin	**Letrozole+Cholesterol+ Span 60+Maltodextrin**	**LS207**	**LS604**	
2378.07 cm-1	2374.16 cm-1	2474.06 cm-1	2274.26 cm-1	2574.16 cm-1	C≡N
1170.64 cm-1	1191.34 cm-1	1091.14 cm-1	1171.24 cm-1	1071.14 cm-1	C=N
1058.35 cm-1	1051.68 cm-1	1061.68 cm-1	1041.48 cm-1	1081.58 cm-1	C-N
1460.18 cm-1	1455.92 cm-1	1565.72 cm-1	1435.12 cm-1	1545.22 cm-1	C=C, Aromatic

6.1.2.2 DSC analysis

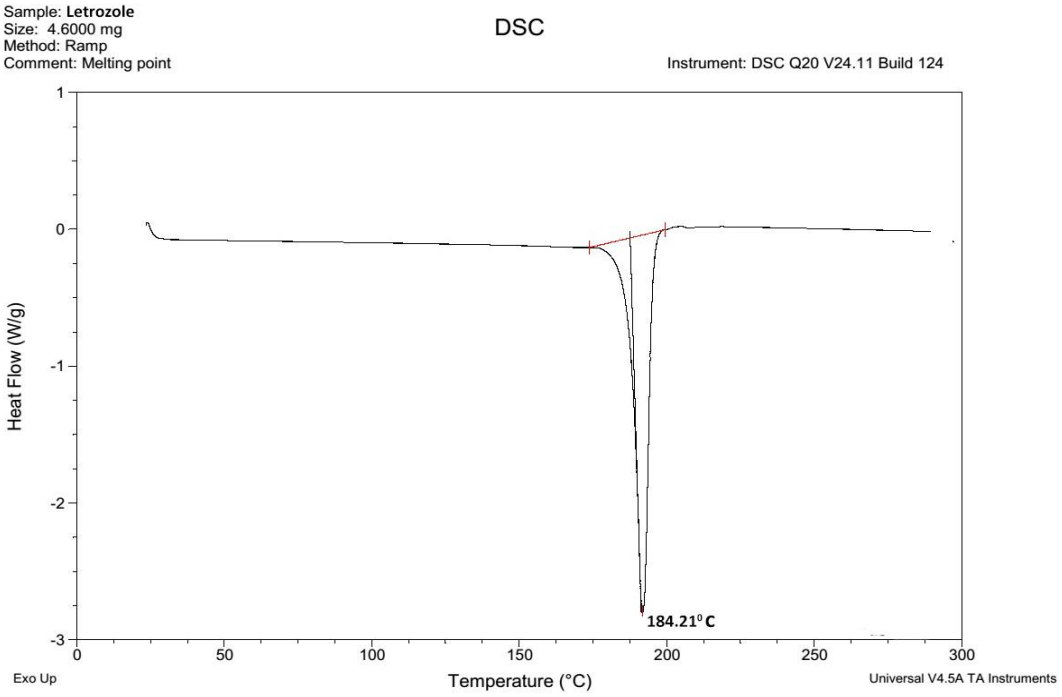

Figure 6.10 DSC Thermogram of letrozole

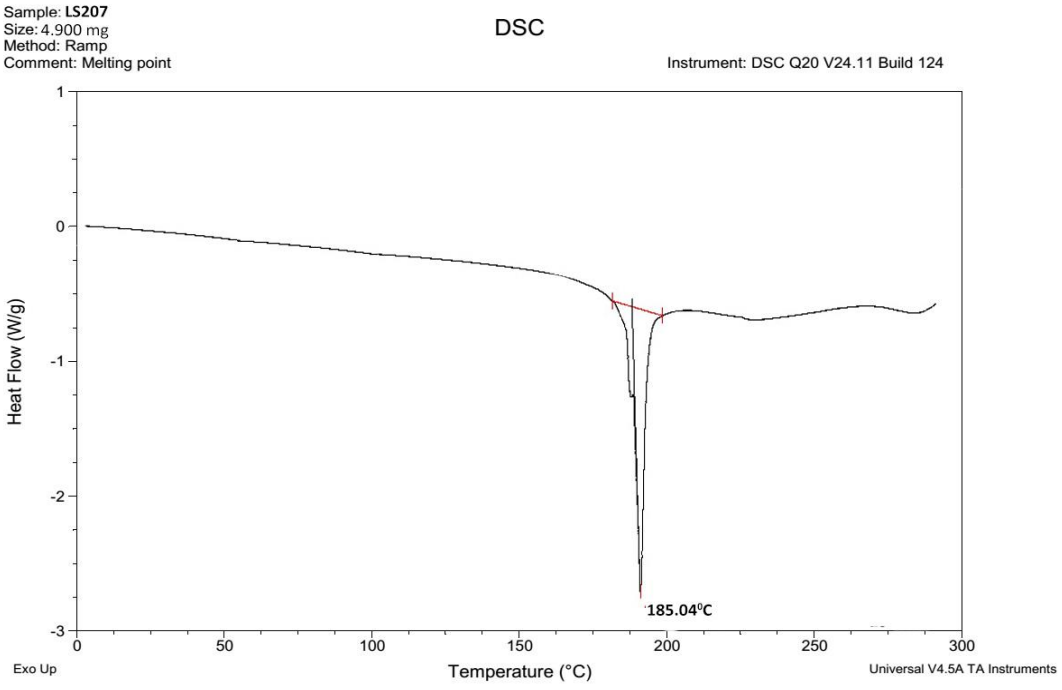

Figure 6.11 DSC Thermogram of LS207

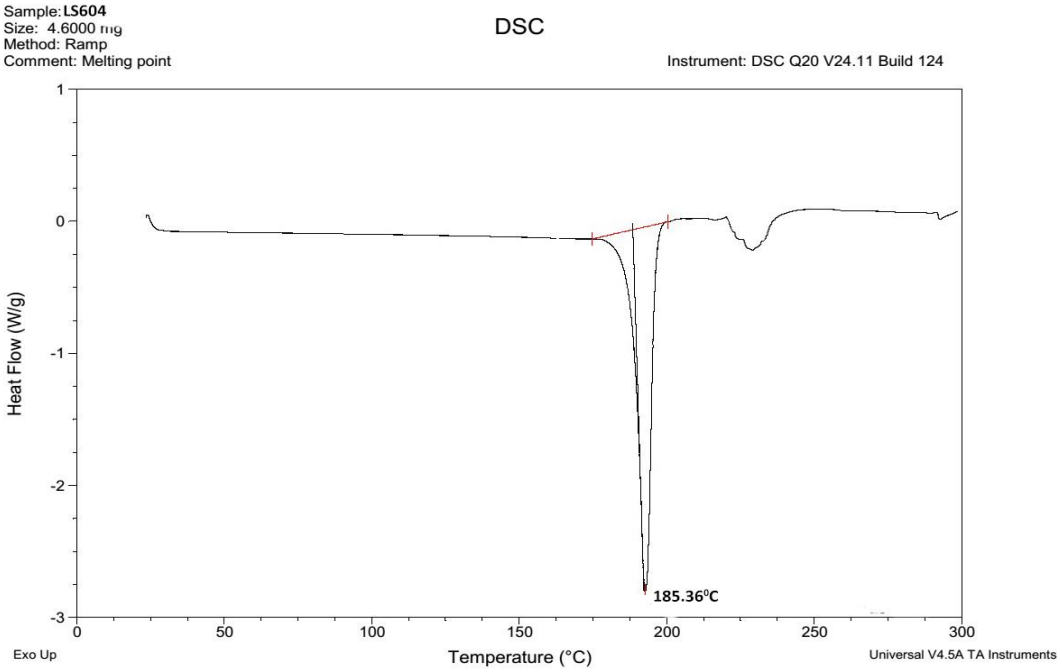

Figure 6.12 DSC Thermogram of optimised formulation LS604

6.1.3 Construction of calibration curve

6.1.3.1 Calibration curve of letrozole

6.1.3.1.1 Construction of letrozole calibration curve by UV Spectroscopy

Calibration curve of letrozole was constructed by plotting concentration (µg/ml) vs absorbance(nm). The results were optained as follows

Table 6.3 Calibration curve of letrozole in P^H 7.4 phosphate buffer at 240nm

S.No	Concentration in (µg/ml)	Absorbance Trial 1	Absorbance Trial 2	Absorbance Trial 3	Mean Absorbance
1.	0	0	0	0	0
2.	2	0.167	0.164	0.166	0.1657±0015
3.	4	0.328	0.321	0.321	0.3233±0040
4.	6	0.493	0.496	0.484	0.4910±0062
5.	8	0.664	0.658	0.662	0.6613±0031
6.	10	0.847	0.844	0.836	0.8423±0057
7.	12	0.987	0.978	0.984	0.9830±0046

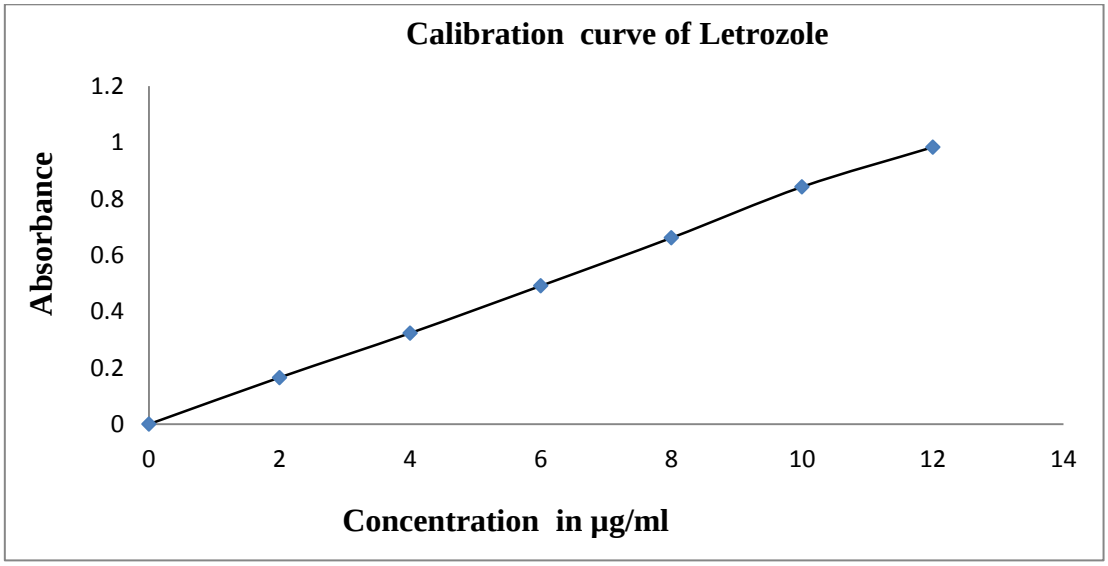

Figure 6.13 Calibration curve of letrozole by UV Spectroscopy

Slope	0.0829
Intecept	-0.0019
Regression	0.9997

6.1.3.1.2 Construction of letrozole calibration curve by HPLC

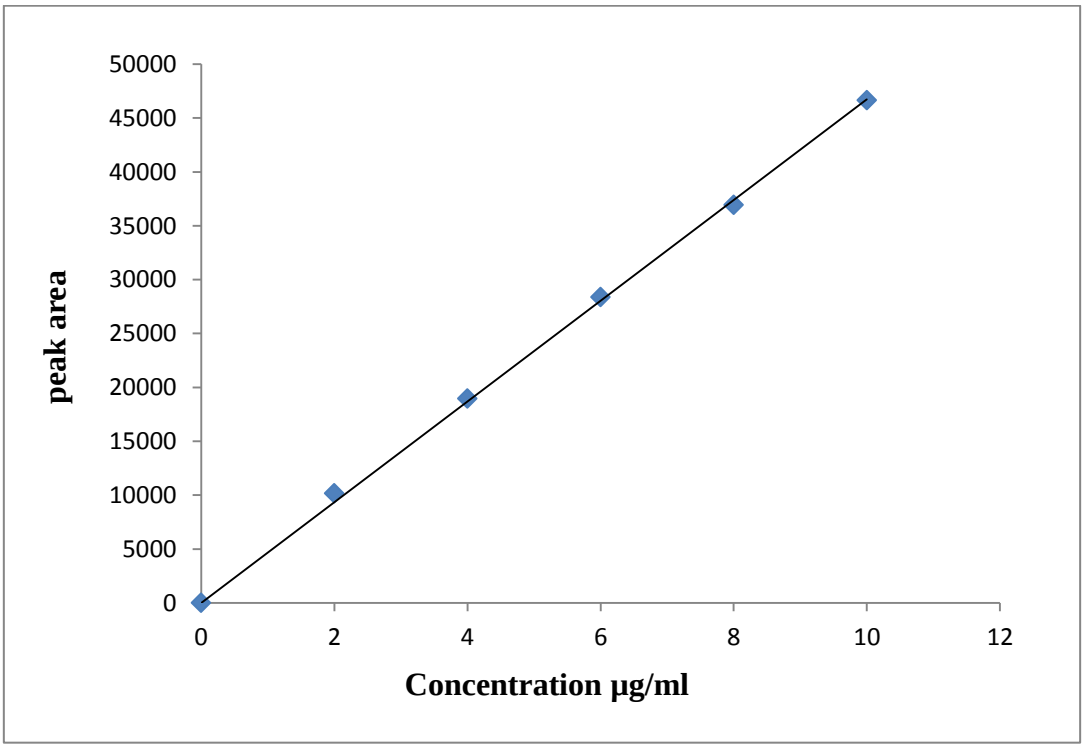

Figure 6.14 Calibration curve of letrozole by HPLC

Slope	4613.81
Intercept	447.8413
Regression	0.999766

6.1.4 Formulation of proniosome

6.1.4.1 Formulation of letrozole loaded proniosomes

According to the procedure given in the methodology proniosomes were formulated using sapn 20 and span 60, stored in a sealed container and used it for evaluation.

6.1.5 Evaluation of proniosomes

6.1.5.1 Morphological evaluation

After hydrating the proniosome using phosphate buffer, proniosome derived niosomes were obsrved under microscope and the photomicrograph was given as follows

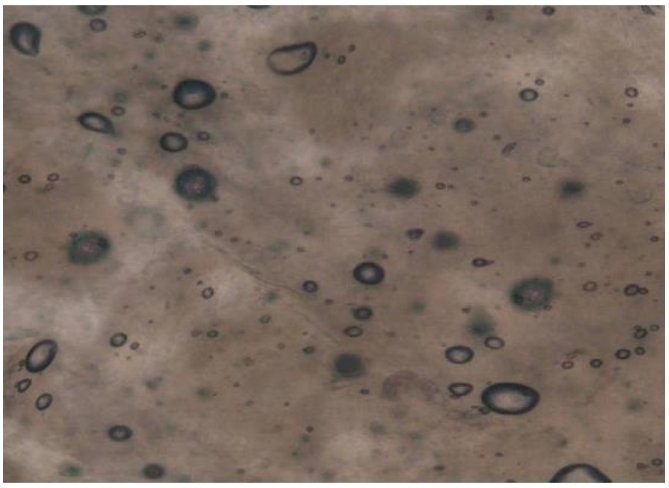

Figure 6.15 Photomicrograph of pronisome LS207

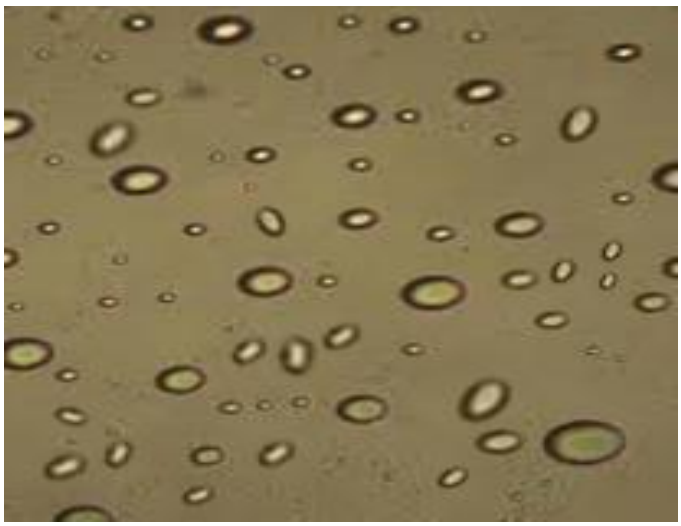

Figure 6.16 Photomicrograph of pronisome LS 604

6.1.5.2 Characteristics of letrozole loaded proniosomes

Angle of Repose ,Vesicle Size , Zeta Potential, Entrapment Efficiency, Number of Vesicles Per Cubic mm were determined as per procedure given in methodology and the results were summarised as follows

Table 6.4 Physical characteristics of formulation LS201-LS210

Formulation	***Angle of Repose in Θ** ± STD	**Vesicle size in nm**	**Zeta Potenial in *mv***	***Entrapment Efficiency in %** ± STD	**Number of vesicle per mm^3X10^5**
LS201	30°45'±0.52	110	+17	42.26 ± 0.77	3.8
LS202	31°27'±0.46	190	+21	48.04 ± 1.09	3.6
LS203	31°33'±2.26	240	+21	52.02 ± 0.36	3.4
LS204	31°50'±2.08	330	+24	56.34 ± 0.55	2.4
LS205	32°58'±0.64	470	+26	64.46 ± 0.90	1.6
LS206	31°17'±0.48	540	+30	71.54 ± 0.43	1.2
LS207	30°16'±0.73	690	+32	76.32± 0.93	1
LS208	30°58'±0.94	630	+21	70.44± 0.34	1.2
LS209	33°27'±0.41	670	+22	63.66± 0.08	1.2
LS210	33°25'±0.43	450	+16	61.06± 0.31	1.6

Table 6.5 Physical characteristics of formulation LS601-LS610

Formulation	***Angle of Repose in Θ ± STD**	**Vesicle size in nm**	**Zeta Potenial in *mv***	***Entrapment Efficiency in % ± STD**	**Number of vesicle per mm^3X10^5**
LS601	31°43'±0.44	490	+18	57.70±1	2.6
LS602	31°58'±0.35	510	+26	67.20±0.95	1.2
LS603	30°50'±1.13	502	+28	77.56±1	1
LS604	29°11'±0.31	650	+36	83.64±0.42	0.9
LS605	30°19'±0.39	645	+28	75.42±0.67	1.2
LS606	30°15'±0.73	631	+26	71.22±0.80	1.3
LS607	32˚17'±0.71	614	+24	64.78±0.60	1.5
LS608	32°58'±0.64	611	+21	61.92±0.68	1.7
LS609	32°20'±0.63	607	+20	59.32±0.80	2.6
LS610	31°54'±0.42	604	+16	54.18±0.36	2.8

6.1.5.3 Vesicle size

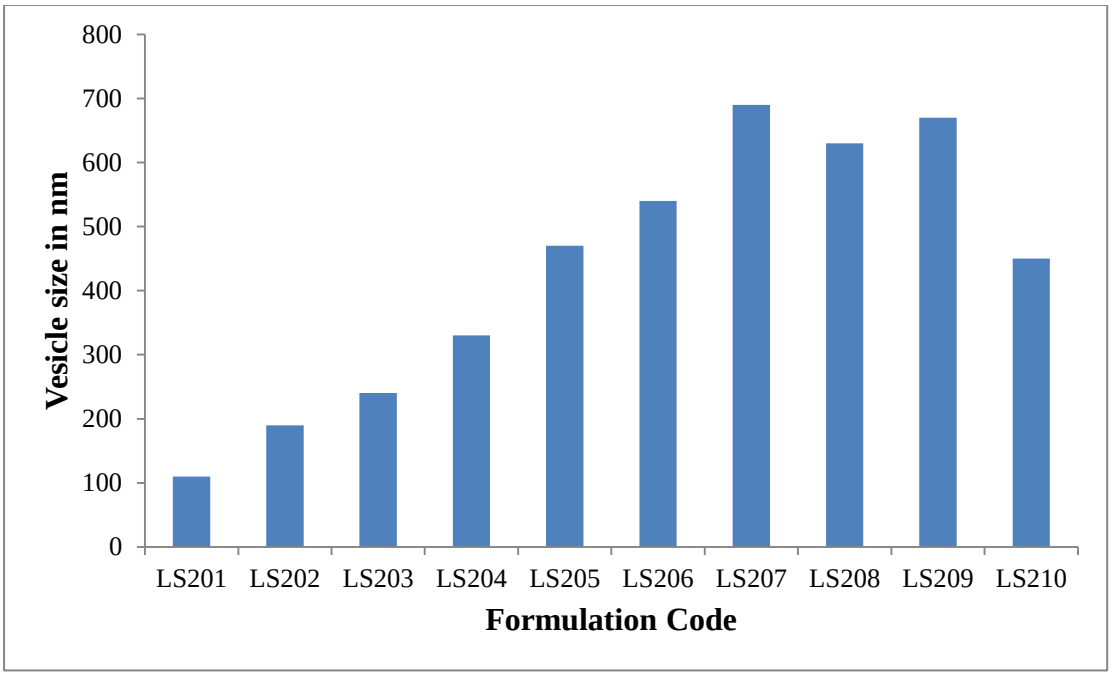

Figure 6.17 Vesicle size of formulation LS201- LS210

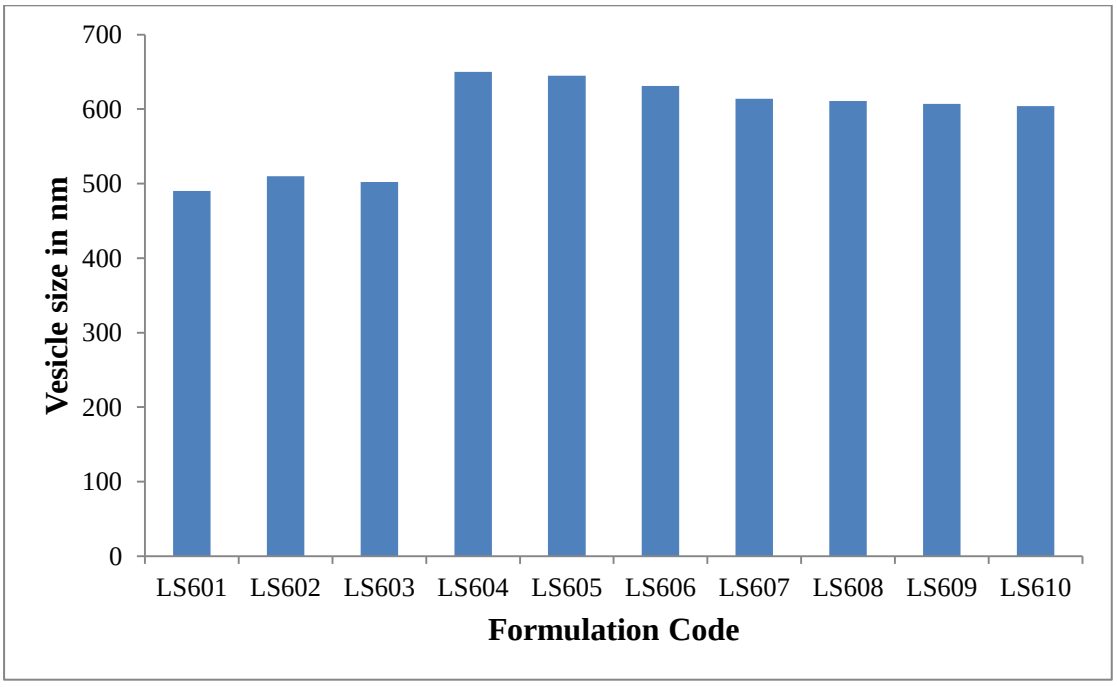

Figure6.18 Vesicle size of formulation LS601- LS610

LS207

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 630	**Peak 1:**	630	100.00	864.12
PdI: 0.67	**Peak 2:**	0.000	0.0	0.000
Intercept: 0.747	**Peak 3:**	0.000	0.0	0.000

Result quality : **Refer to quality report**

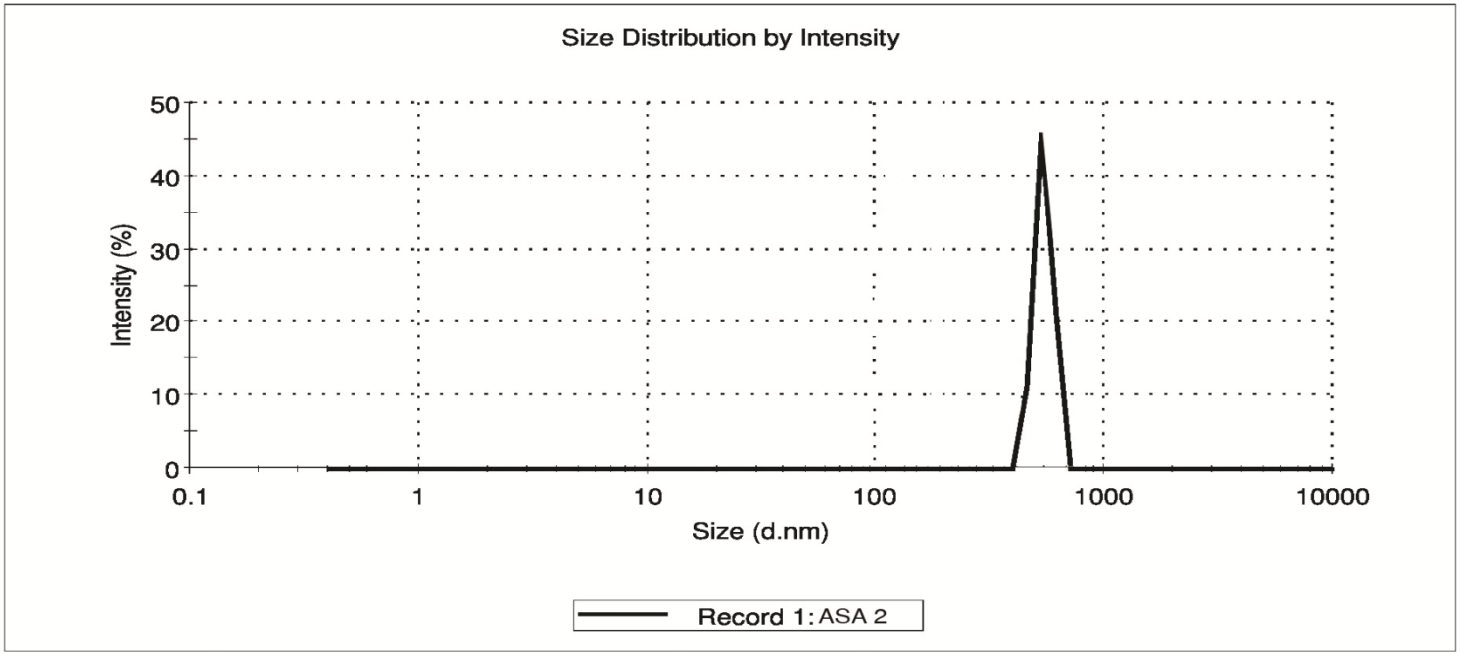

Figure 6.19 Size distribution by intensity of formulation LS207

LS604		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 650	**Peak 1:**	650	100.00	889.74
PdI: 0.67	**Peak 2:**	0.000	0.0	0.000
Intercept: 0.747	**Peak 3:**	0.000	0.0	0.000

Result quality : **Refer to quality report**

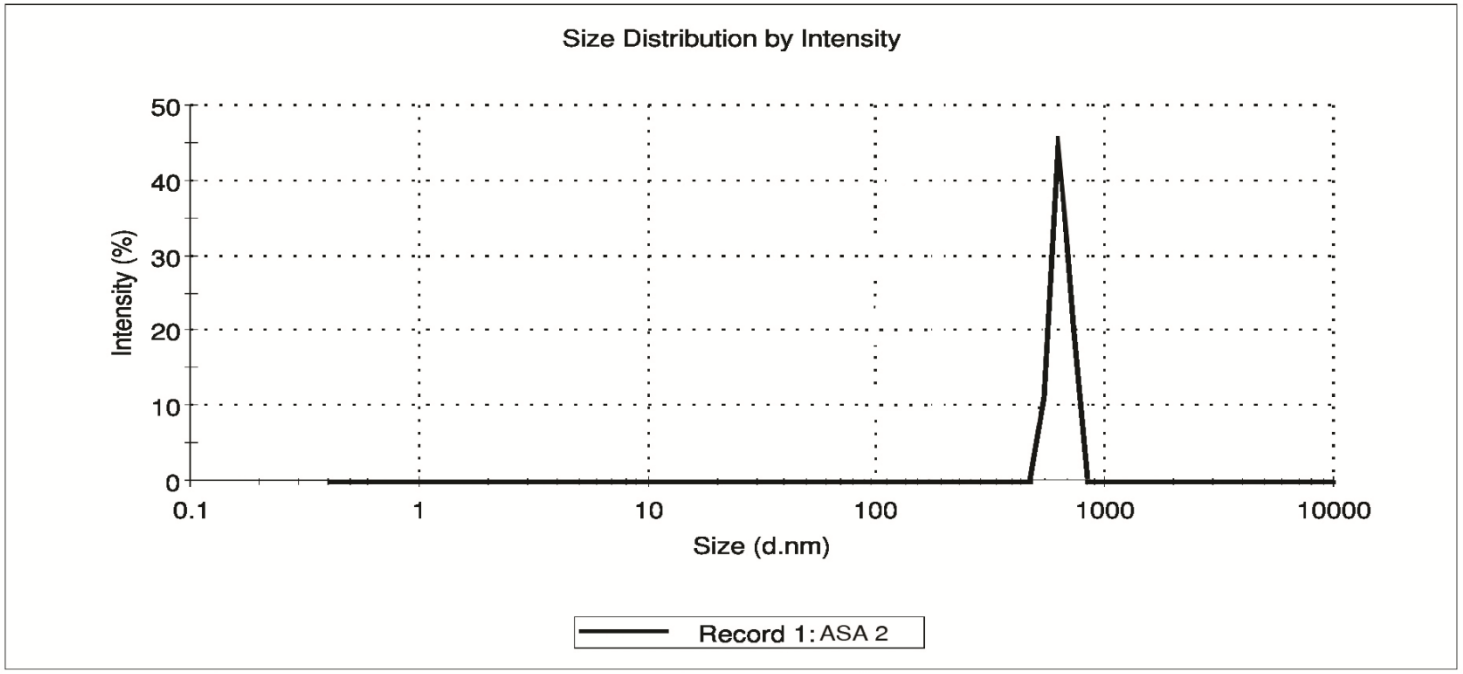

Figure 6.20 Size distribution by intensity of formulation LS604

6.1.5.4 Zeta potential

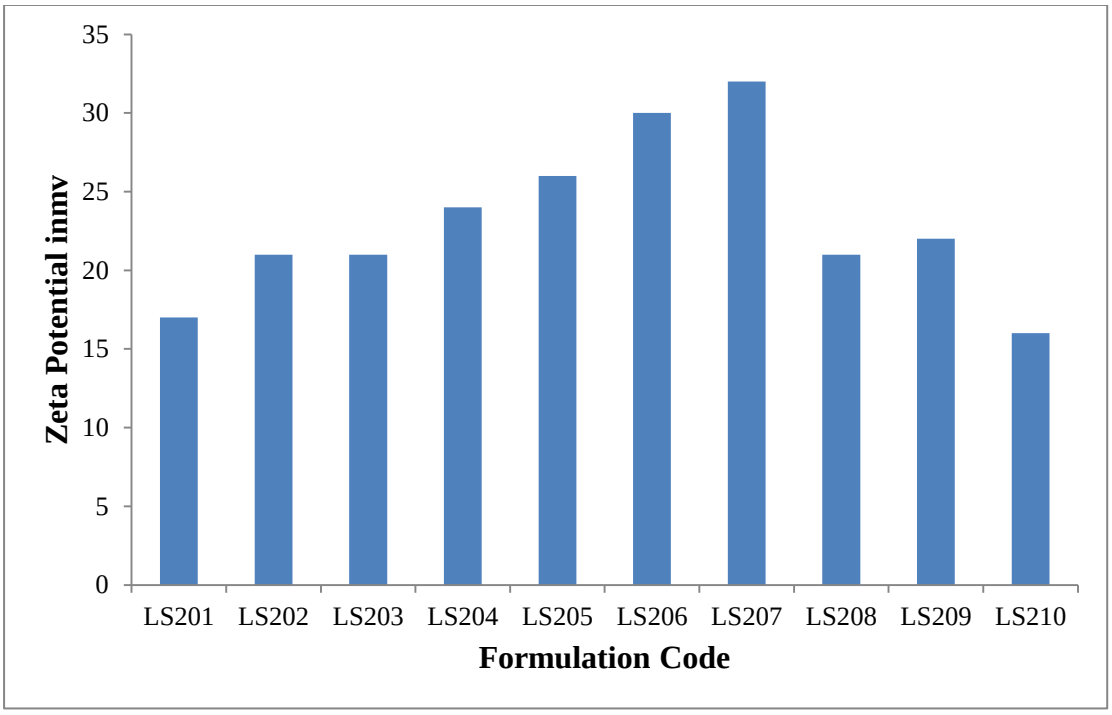

Figure 6.21 Zeta potential of formulation LS201- LS210

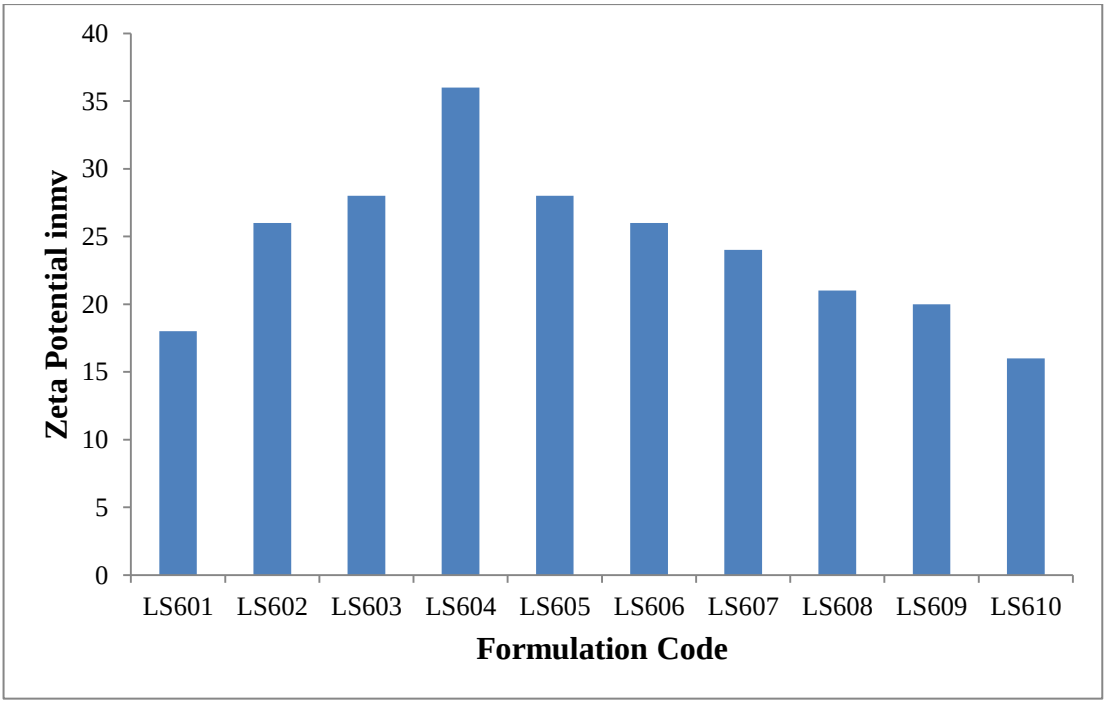

Figure 6.22 Zeta potential of formulation LS601-LS610

6.1.5.5 Entrapment efficiency

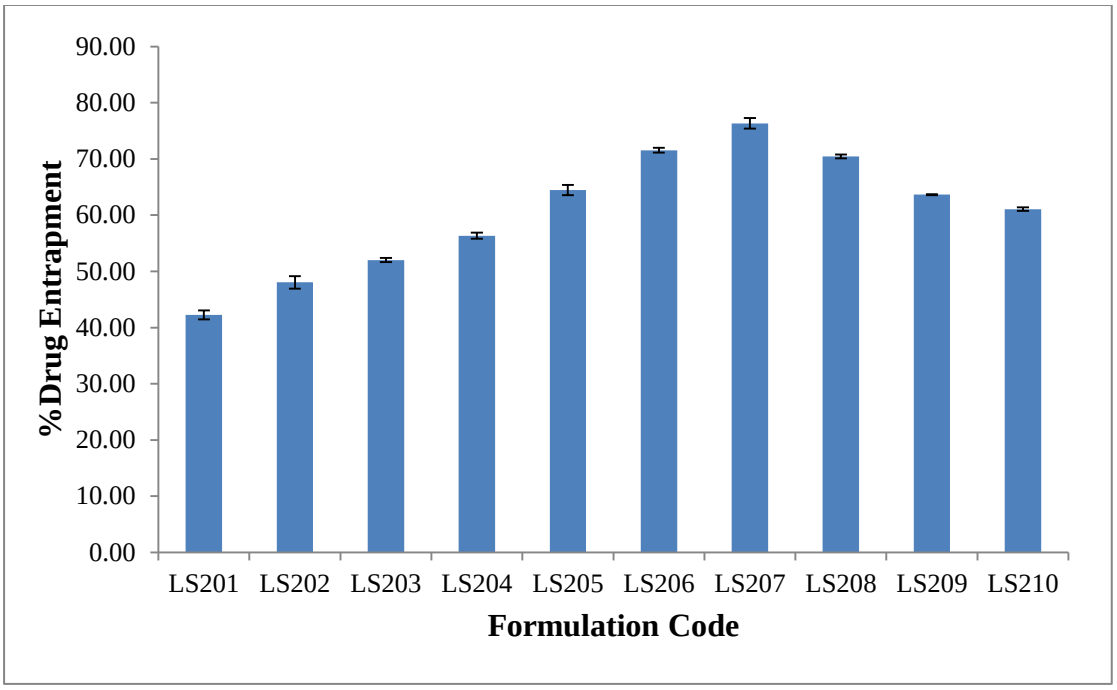

Figure 6.23 Entrapment efficiencyof formulations LS201-LS210

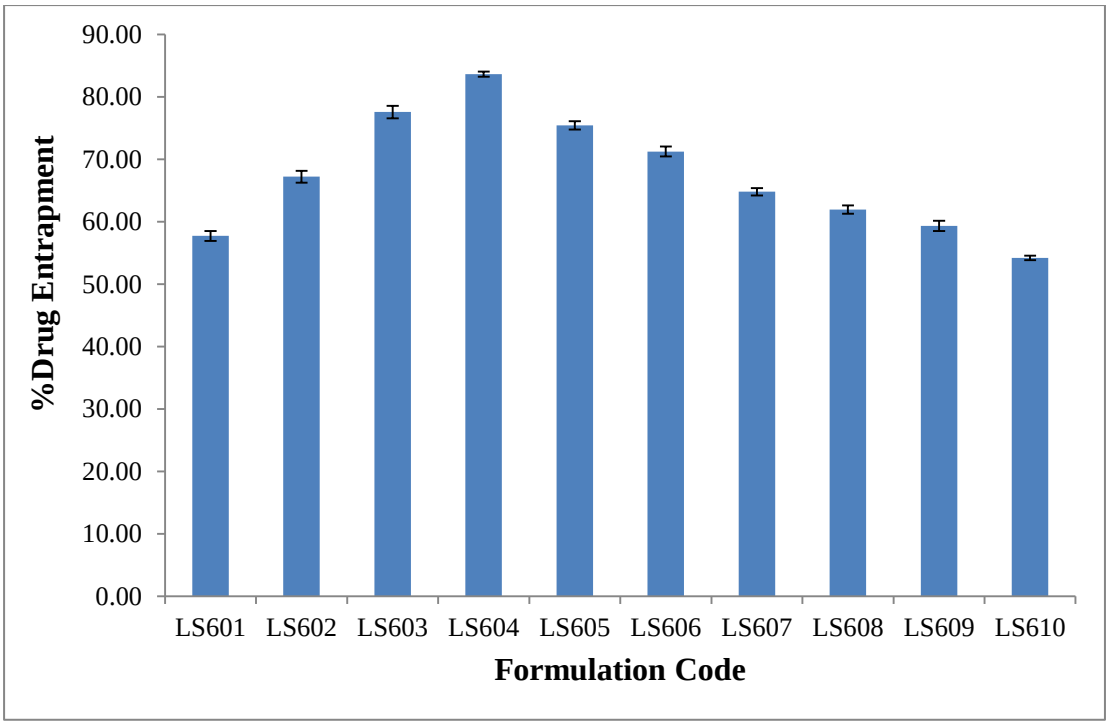

Figure 6.24 Entrapment efficiencyof formulation :LS601-LS610

6.1.5.6 Number of vesicles Per mm^3

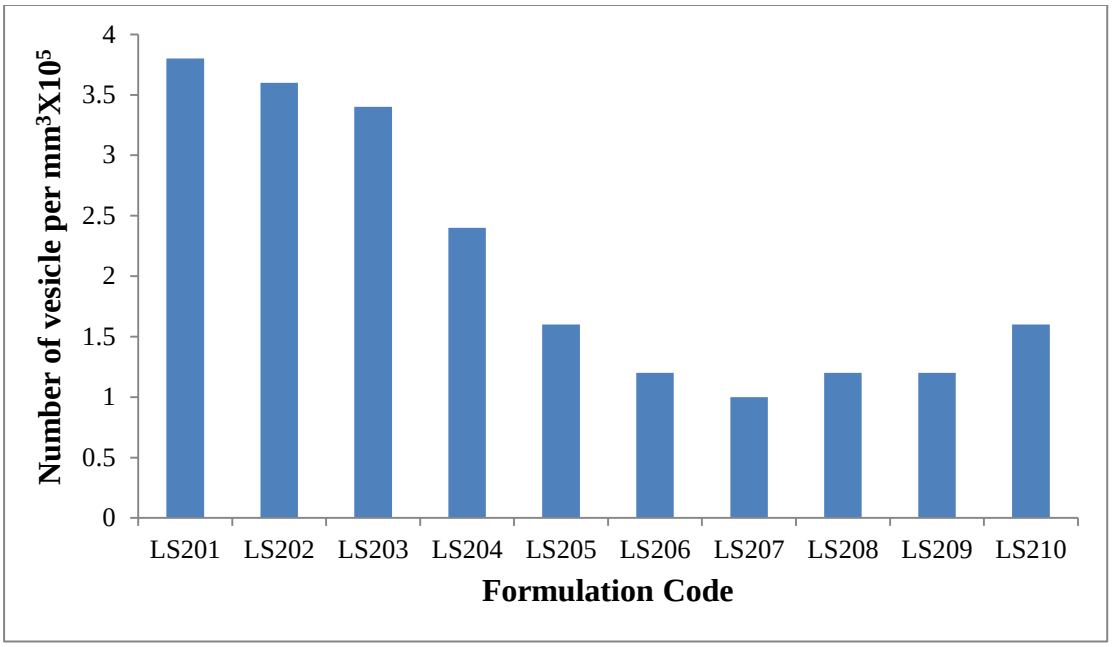

Figure 6.25 Number of vesicles per cubic mm of formulation LS201-LS210

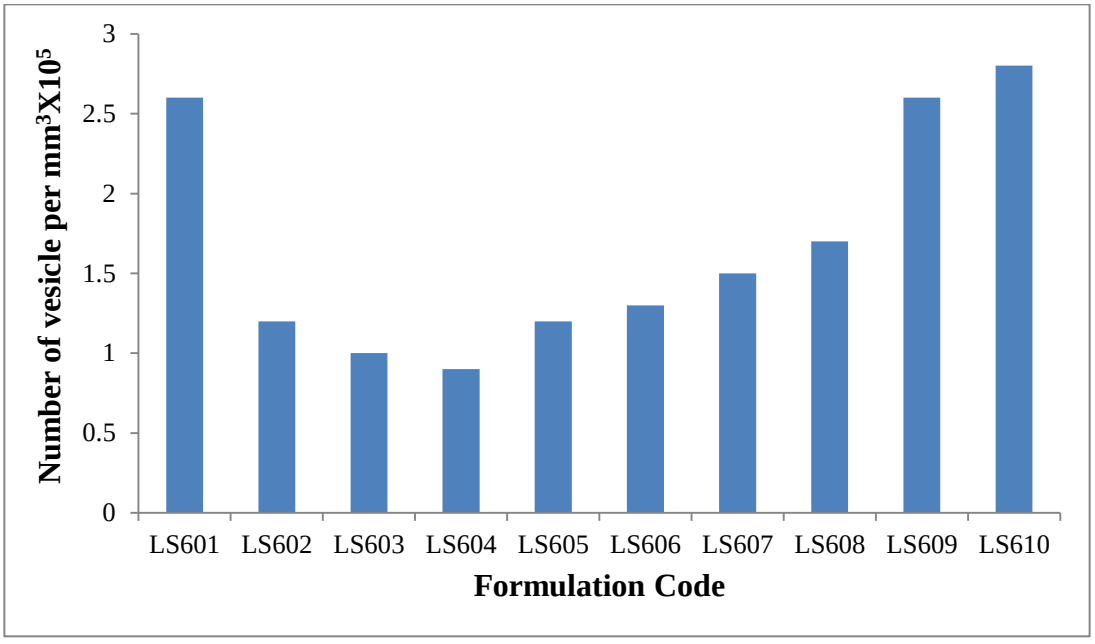

Figure 6.26 Number of vesicles per cubic mm of formulation LS601-LS610

6.1.5.7 Scanning Electron Microscopy (SEM)

Shape and surface charctertics of proniosome examined by Scanning Electron Microscopy shown in figures

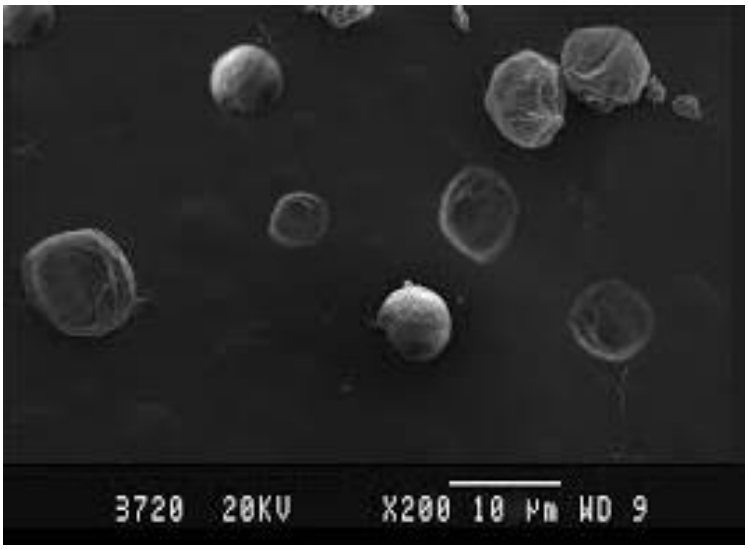

Figure 6.27 Scanning electron image of proniosomal formulation LS207

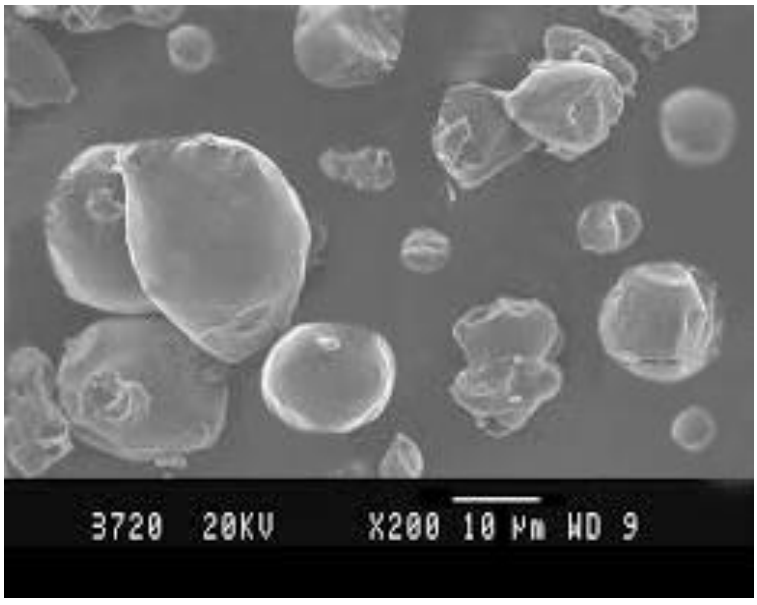

Figure 6.28 Scanning electron image of proniosomal formulation LS604

6.1.5.8 *In vitro* drug release study

The dialysis method was used to investigate the *in vitro* release of the letrazole from proniosome. The results are shown in following tables and figures

Table 6.6 *In vitro* release study of LS201

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.051	1.277781769	0.006388909	0.638890884	25.55563537	0	1.407486683	1.871832	1
2	0.086	2.122548667	0.010612743	1.074052151	42.96208606	0.30103	1.633085361	1.756164	1.41421356
3	0.132	3.232813735	0.016164069	1.650410172	66.01640687	0.47712125	1.819651883	1.531269	1.73205081
4	0.160	3.908627254	0.019543136	2.020645069	80.82580274	0.60205999	1.907550027	1.282717	2
5	0.195	4.753394153	0.023766971	2.482114791	99.28459162	0.69897	1.996881854	-0.14545	2.23606798

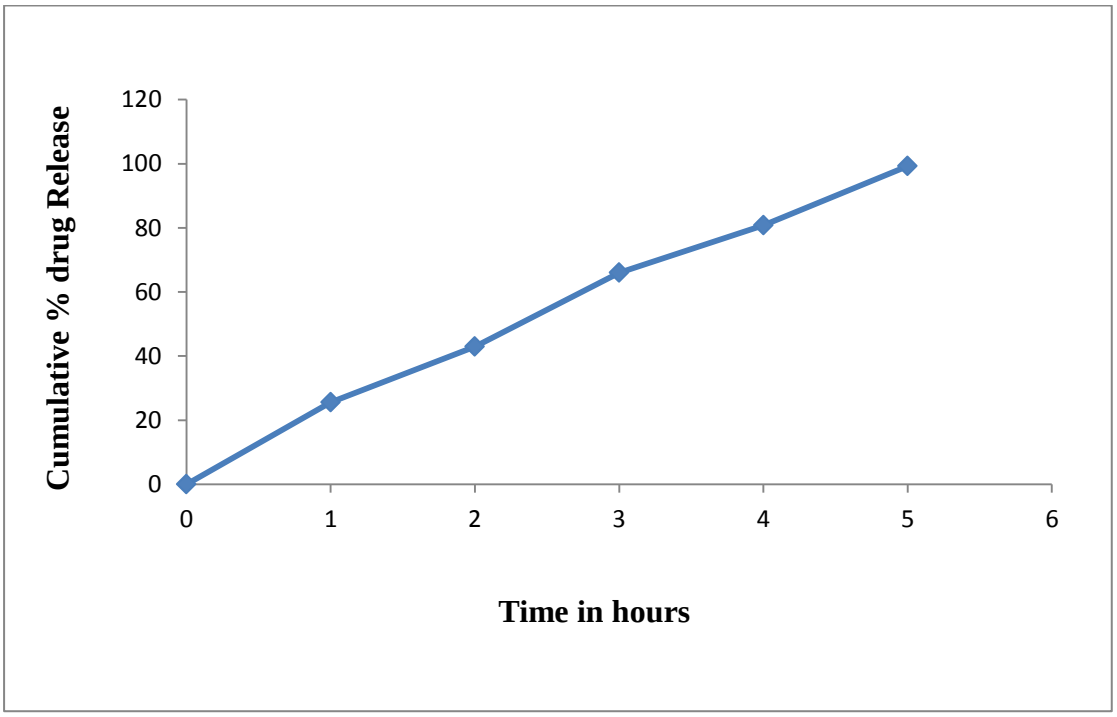

Figure 6.29 *In Vitro* drug release of formulation LS201

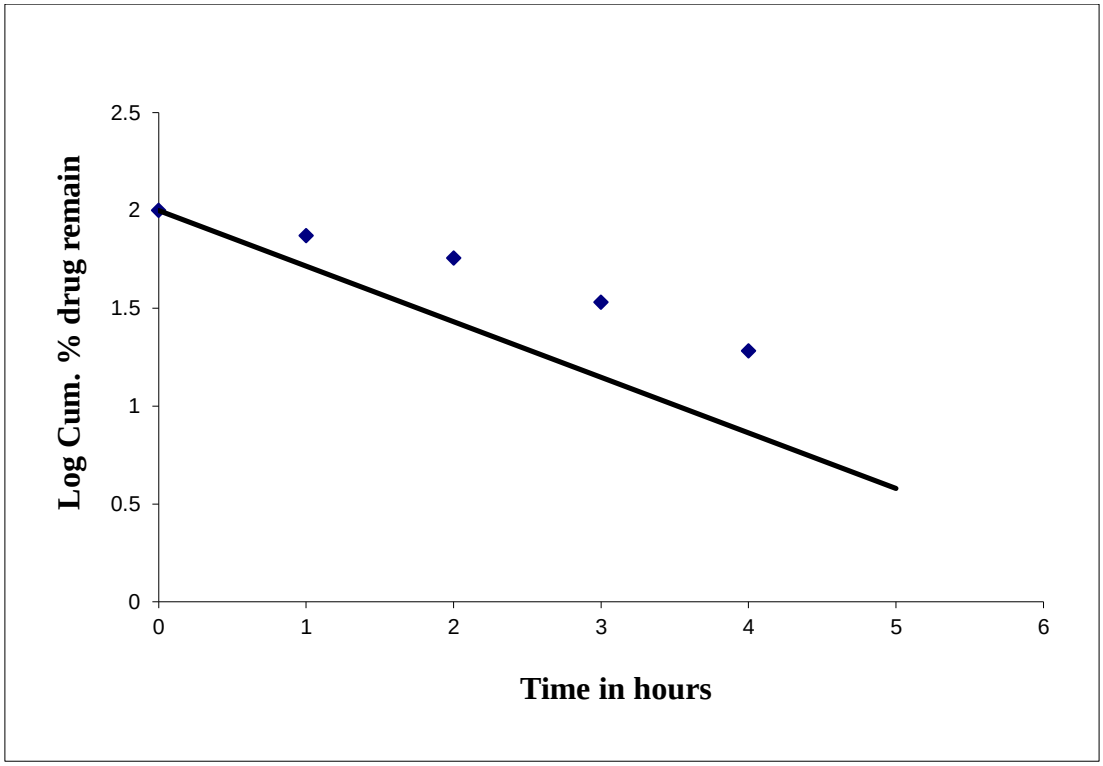

Figure 6.30 First order kinetics of formulation LS201

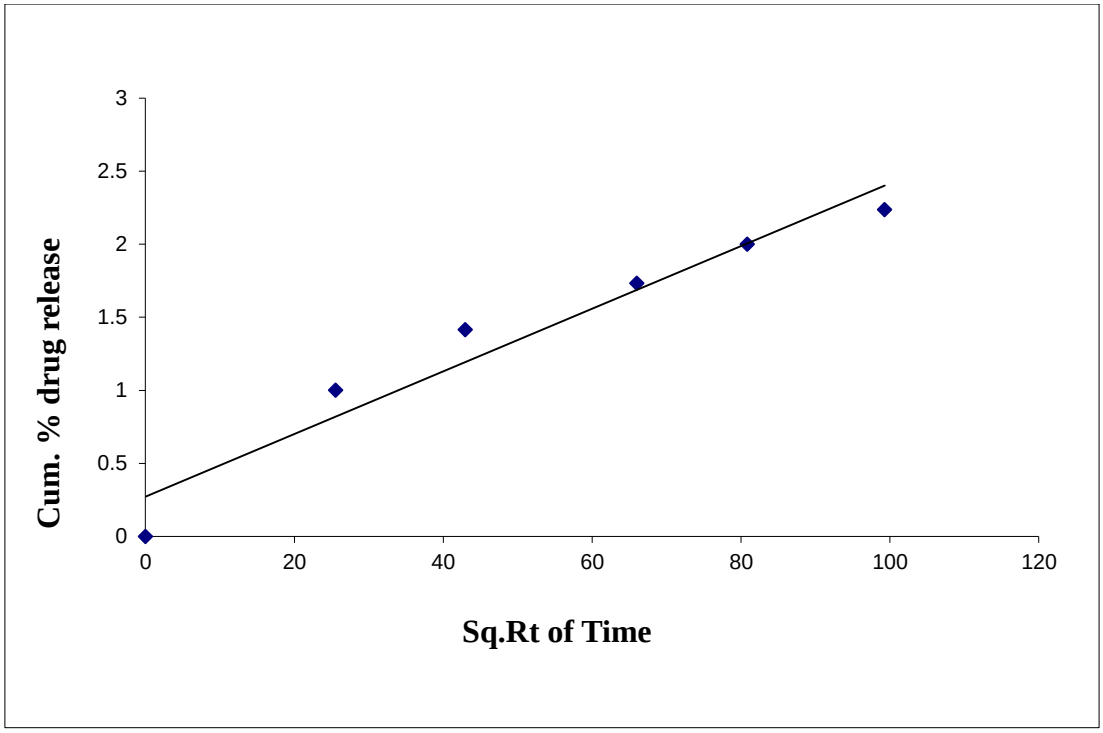

Figure 6.31 Higuchi's plot for formulation LS201

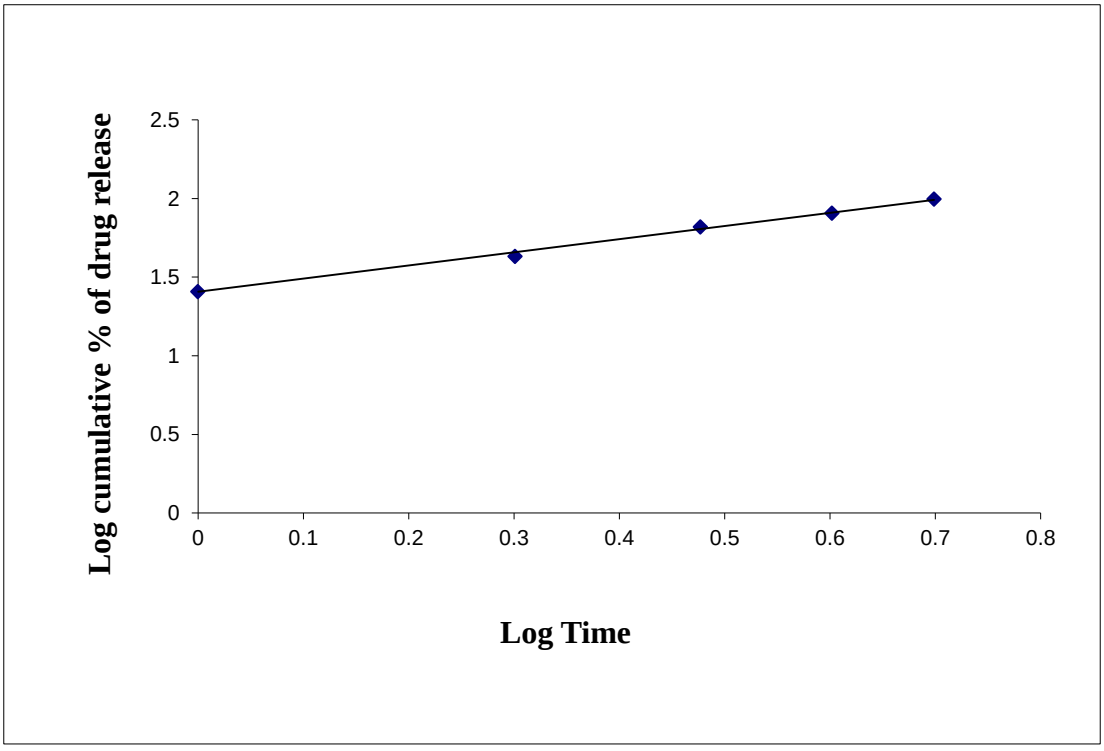

Figure 6.32 Korsmeyer & peppas plot for formulation LS201

Table 6.7 *In vitro* release study of LS202

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.042	1.060555995	0.00530278	0.530277997	21.21111989	0	1.326563599	1.896465	1
2	0.072	1.784641908	0.00892321	0.902926514	36.11706056	0.30103	1.557712397	1.805385	1.41421356
3	0.119	2.919043172	0.014595216	1.487973565	59.5189426	0.47712125	1.774655207	1.607252	1.73205081
4	0.140	3.425903312	0.017129517	1.770594067	70.82376266	0.60205999	1.850178996	1.465029	2
5	0.165	4.029308239	0.020146541	2.106555564	84.26222254	0.69897	1.92563291	1.196943	2.23606798
6	0.193	4.705121758	0.023525609	2.484755406	99.39021622	0.77815125	1.997343635	-0.21482	2.44948974

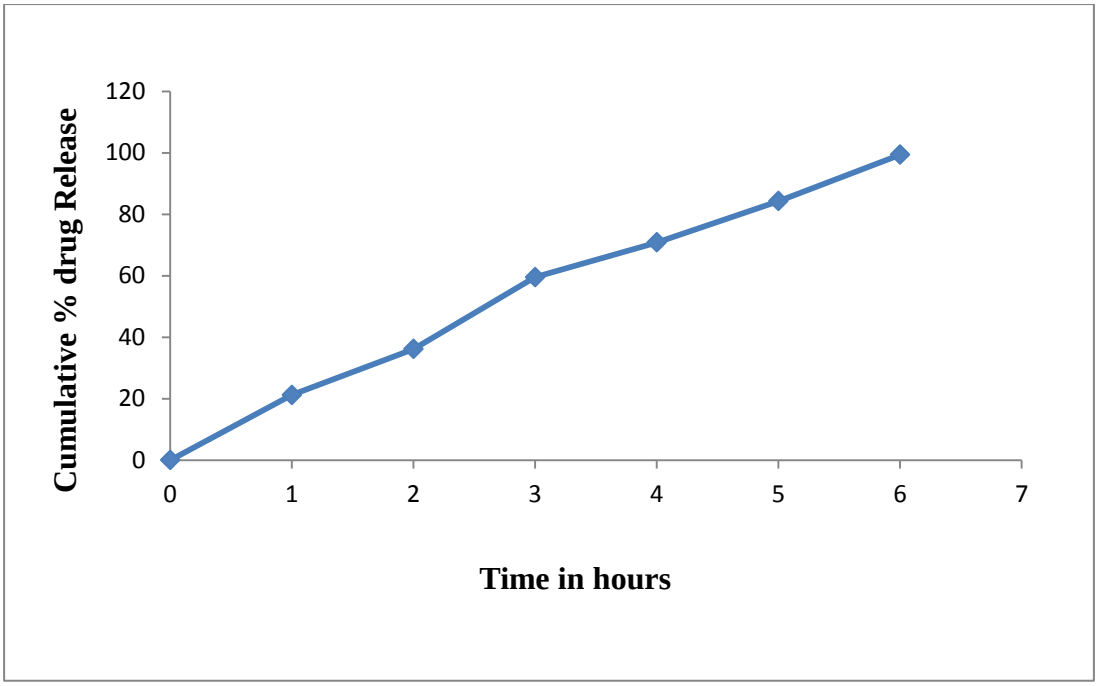

Figure 6.33 *In vitro* drug release of formulation LS202

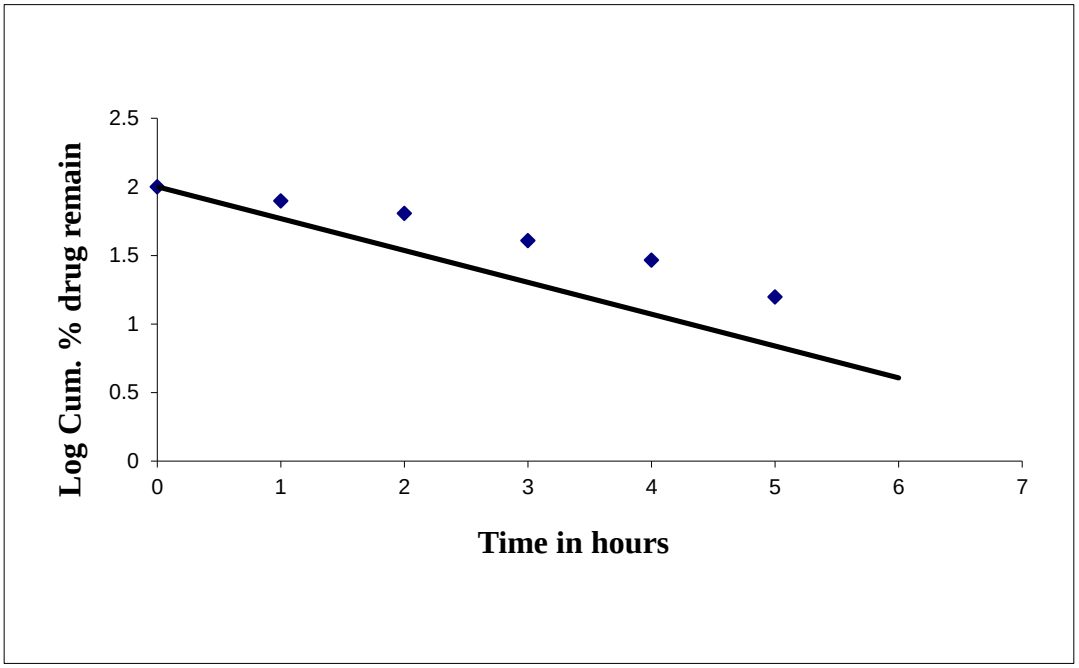

Figure 6.34 First order kinetics of formulation LS202

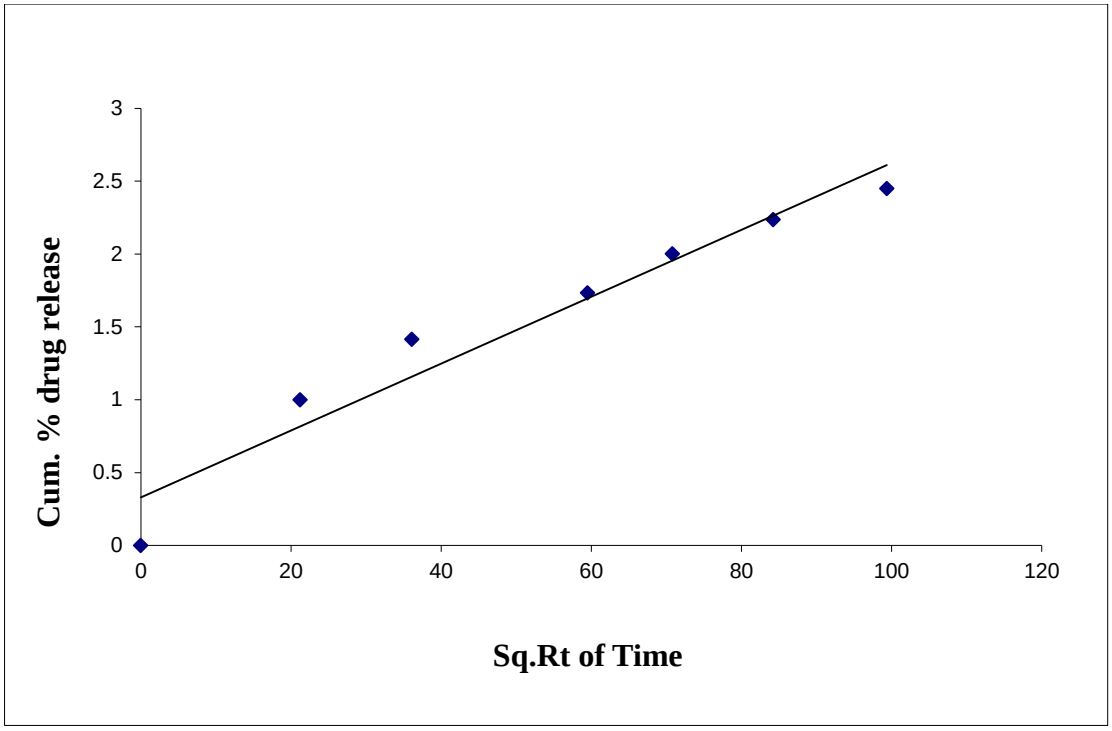

Figure 6.35 Higuchi's plot for formulation LS202

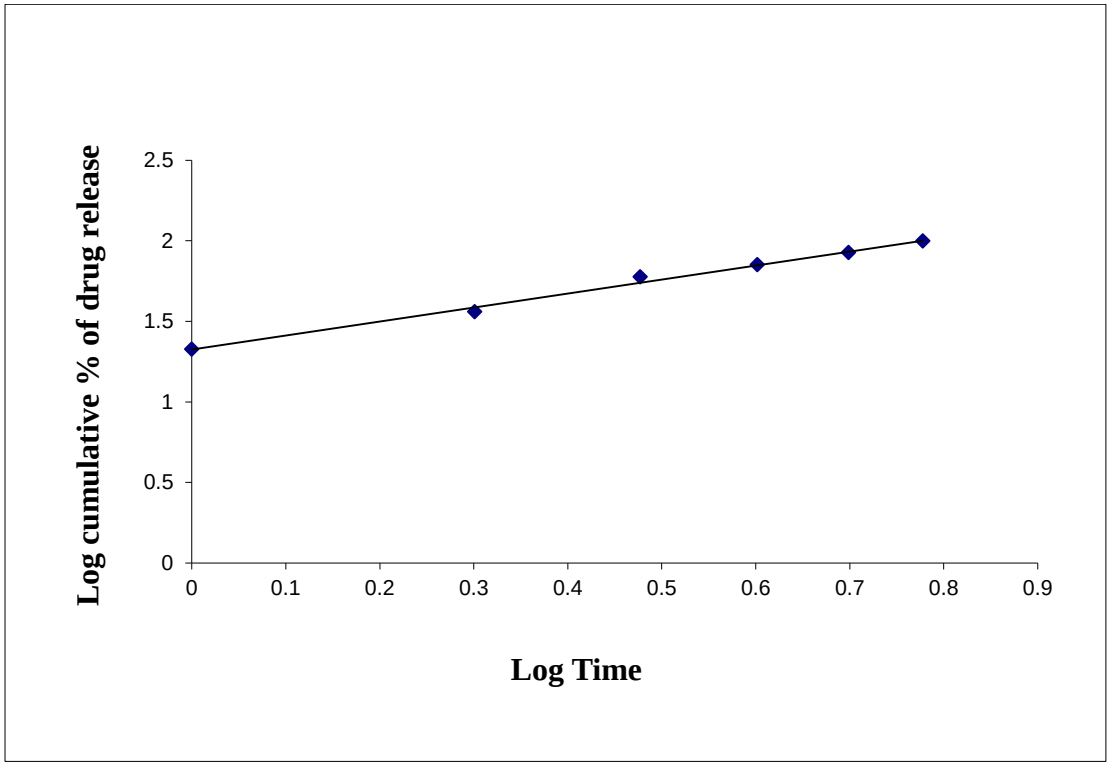

Figure 6.36 Korsmeyer & peppas plot for formulation LS202

Table 6.8 *In vitro* release study of LS203

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.037	0.939875009	0.004699375	0.469937504	18.79750018	0	1.274100098	1.909569	1
2	0.061	1.51914374	0.007595719	0.76897062	30.7588248	0.30103	1.487969738	1.840364	1.41421356
3	0.084	2.074276273	0.010371381	1.061728324	42.46913296	0.47712125	1.628073395	1.759901	1.73205081
4	0.106	2.60527261	0.013026363	1.347969255	53.9187702	0.60205999	1.731739978	1.663524	2
6	0.147	3.594856691	0.017974283	1.868814022	74.75256088	0.77815125	1.873626075	1.402217	2.44948974
8	0.194	4.729257956	0.02364629	2.471963221	98.87852884	0.90308999	1.995101996	0.049788	2.82842712

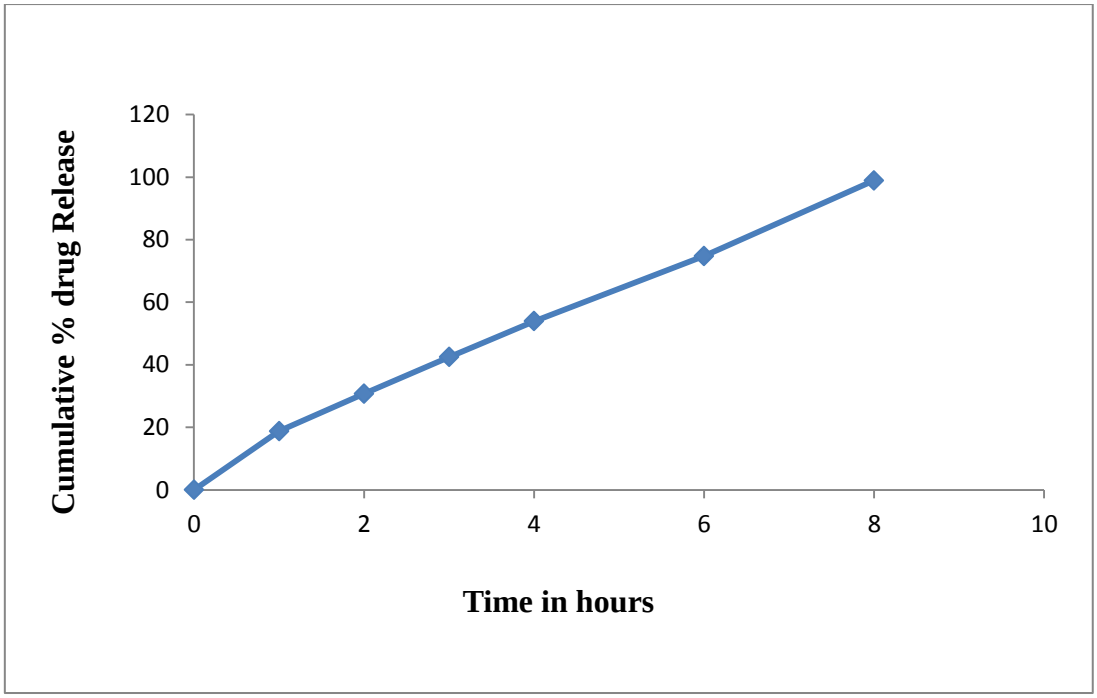

Figure 6.37 *In vitro* drug release of formulation LS203

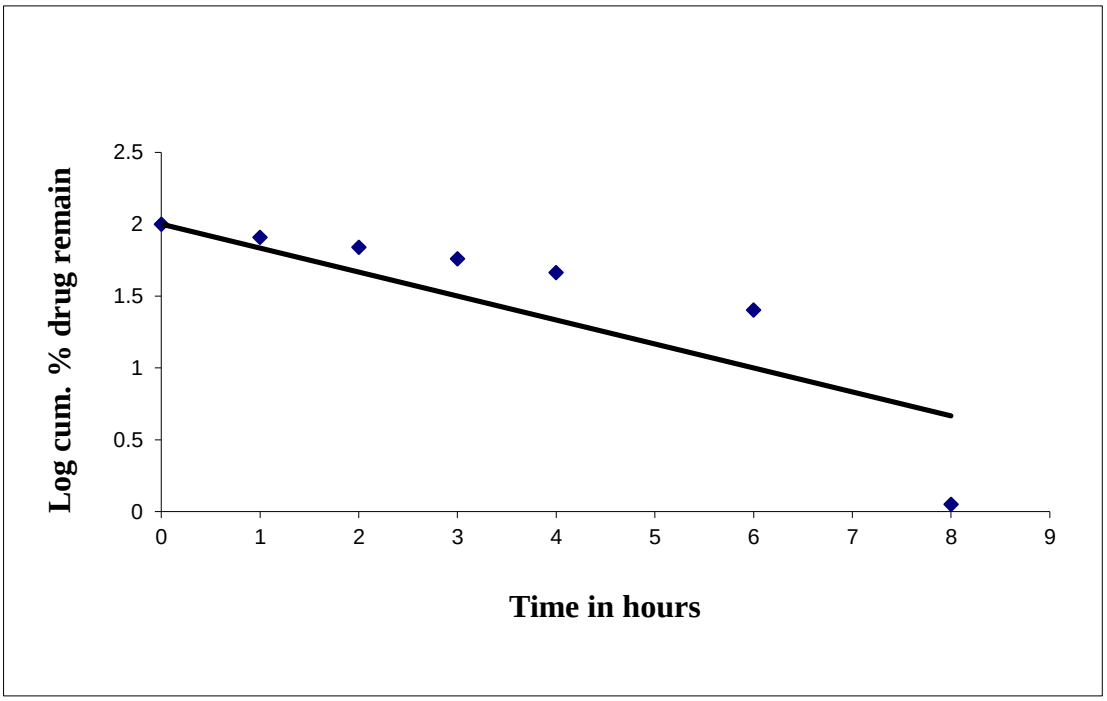

Figure 6.38 First order kinetics of formulation LS203

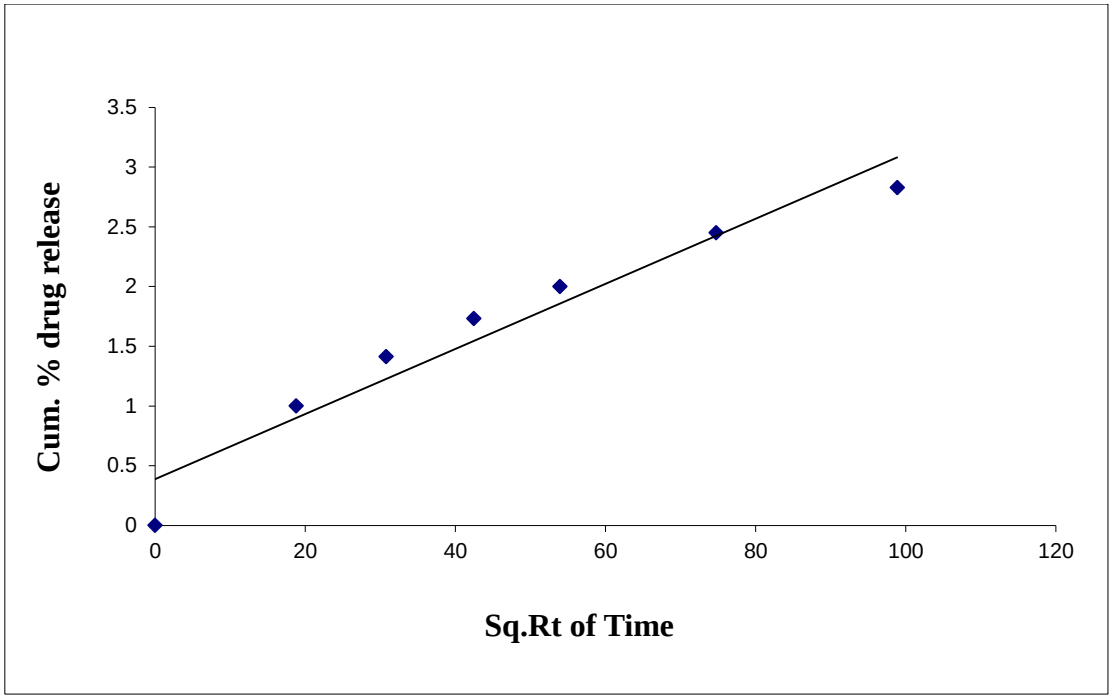

Figure 6.39 Higuchi's plot for formulation LS203

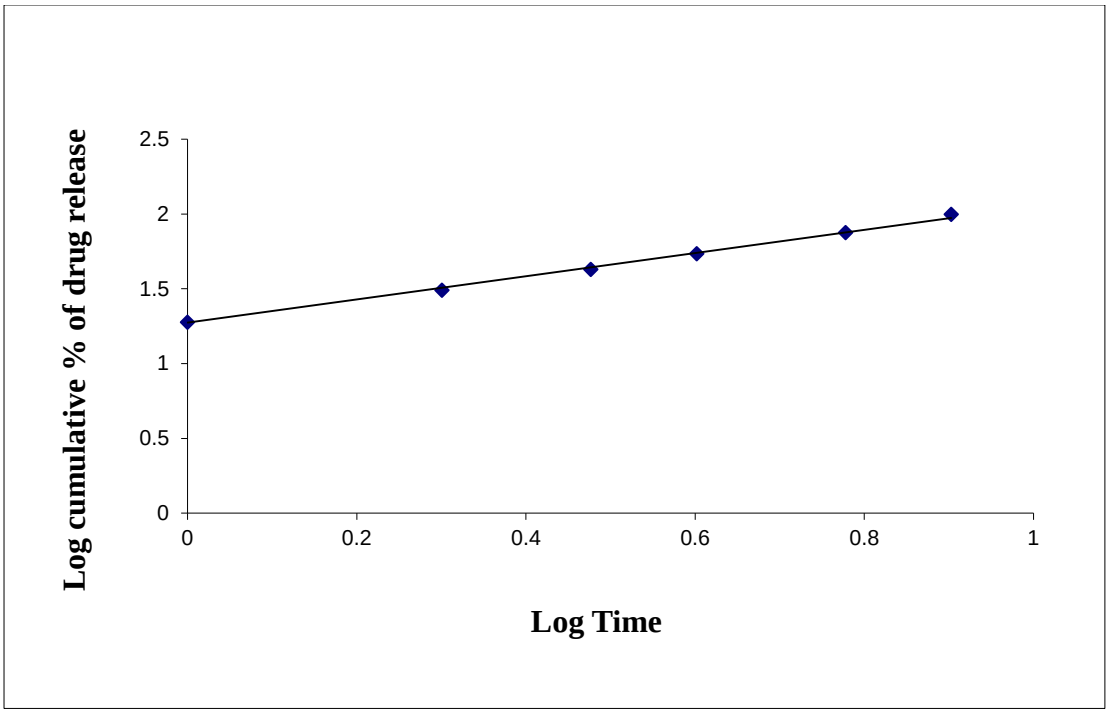

Figure 6.40 Korsmeyer & peppas plot for formulation LS203

Table 6.9 *In vitro* release study of LS204

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.032	0.819194023	0.00409597	0.409597012	16.38388047	0	1.214416771	1.92229	1
2	0.056	1.398462754	0.006992314	0.707423317	28.29693269	0.30103	1.451739362	1.855538	1.41421356
4	0.088	2.170821062	0.010854105	1.107587099	44.30348395	0.60205999	1.64643788	1.745828	2
6	0.128	3.136268946	0.015681345	1.612019251	64.48077006	0.77815125	1.809430215	1.550464	2.44948974
8	0.159	3.884491057	0.019422455	2.017492996	80.69971985	0.90308999	1.906872027	1.285564	2.82842712
10	0.194	4.729257956	0.02364629	2.478721356	99.14885425	1	1.9962877	-0.07	3.16227766

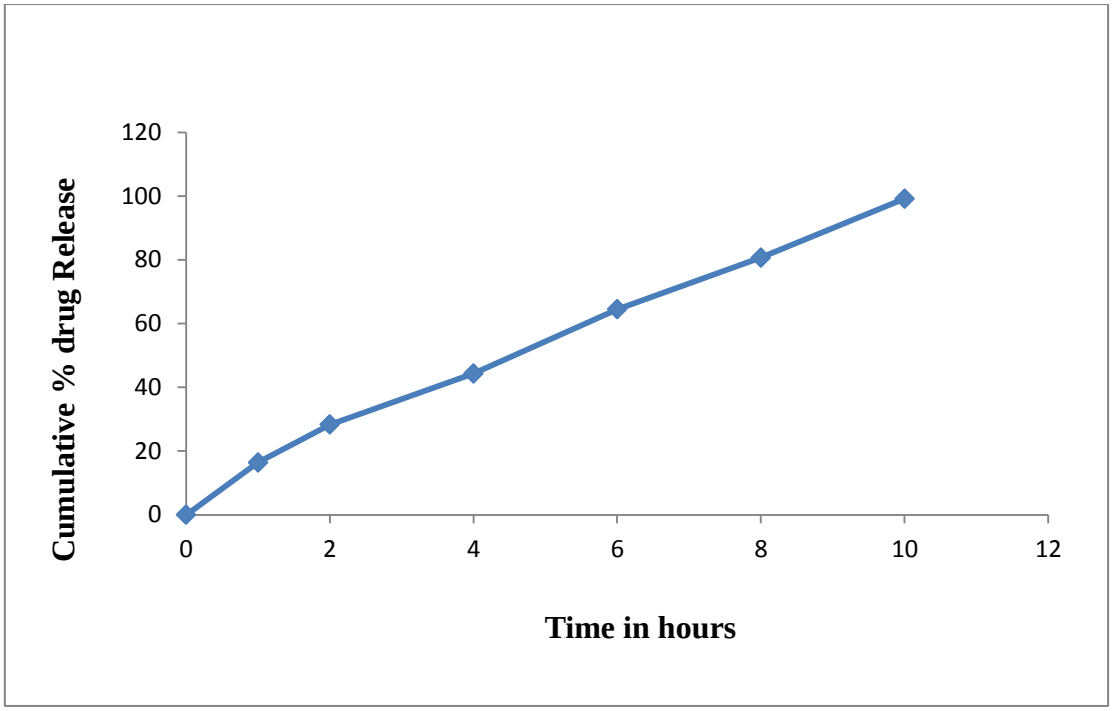

Figure 6.41 *In vitro* drug release of formulation LS204

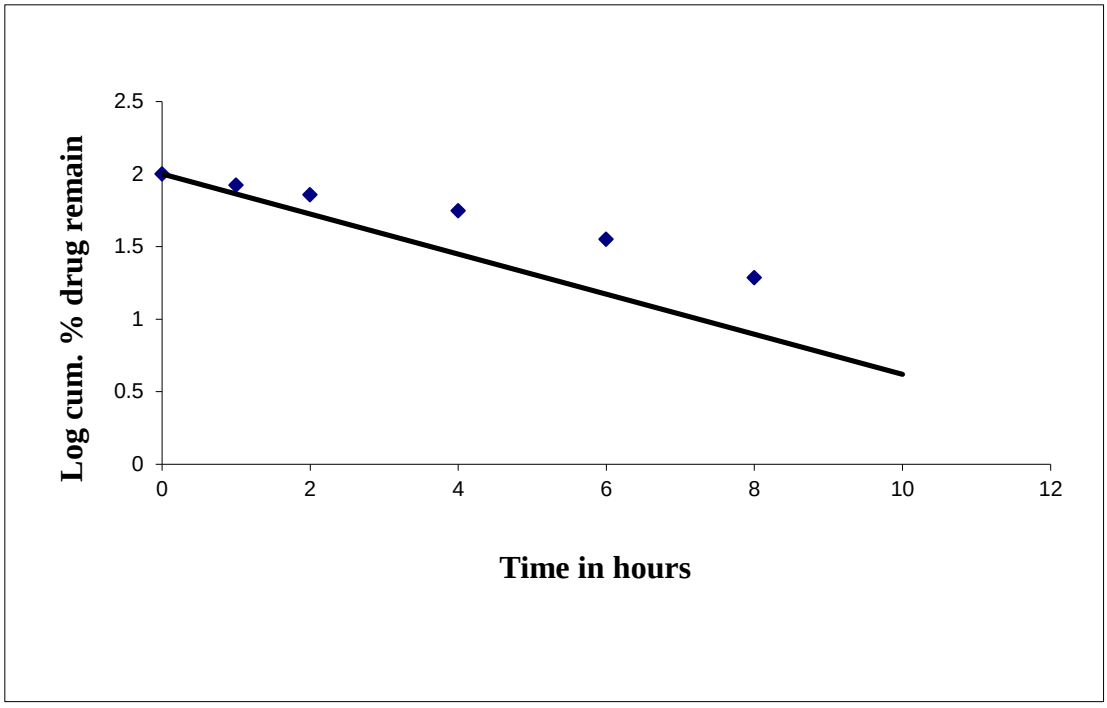

Figure 6.42 First order kinetics of formulation LS204

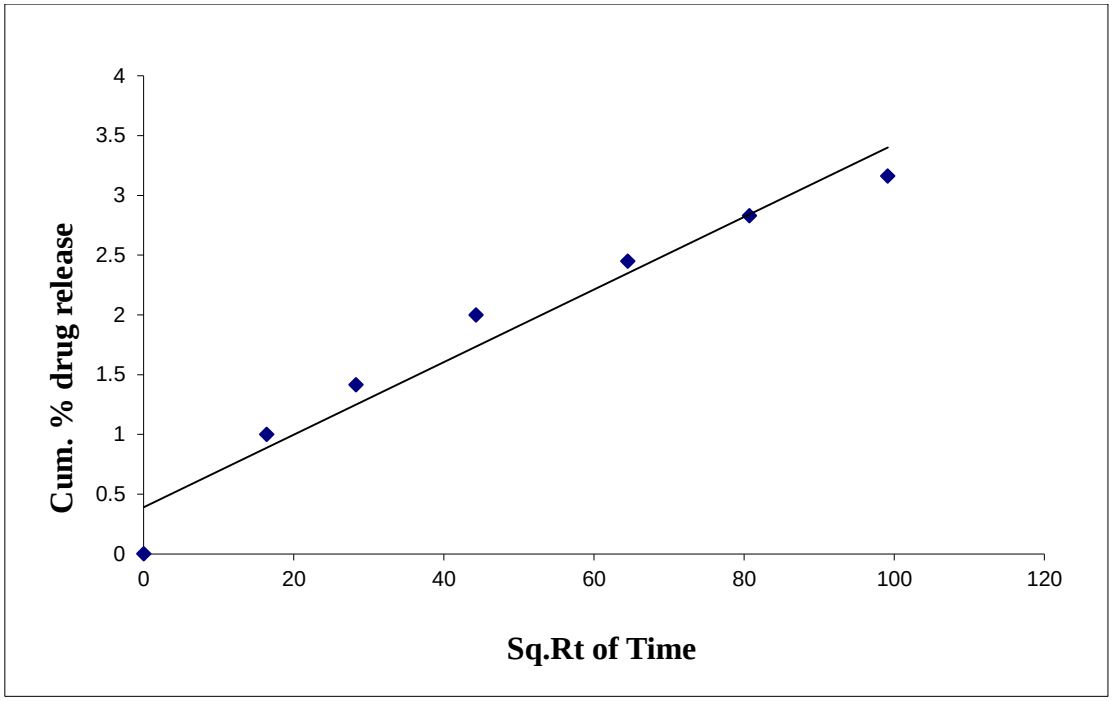

Figure 6.43 Higuchi's plot for formulation LS204

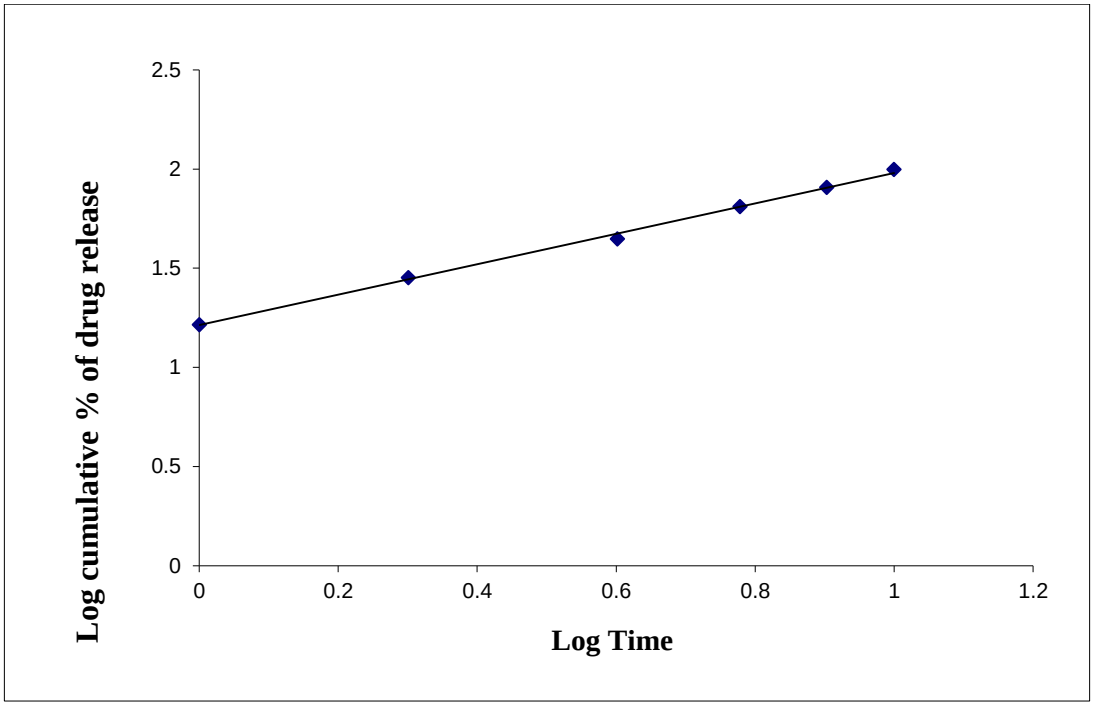

Figure 6.44 Korsmeyer & peppas plot for formulation LS204

Table 6.10 *In vitro* release study of LS205

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.025	0.650240644	0.003251203	0.325120322	13.00481287	0	1.114104107	1.939495	1
2	0.048	1.205373177	0.006026866	0.609188995	24.3675598	0.30103	1.386812041	1.878708	1.41421356
4	0.074	1.832914302	0.009164572	0.935013289	37.40053157	0.60205999	1.572877775	1.796571	2
6	0.110	2.701817398	0.013509087	1.38779398	55.51175921	0.77815125	1.744384991	1.648245	2.44948974
8	0.137	3.35349472	0.016767474	1.740650815	69.62603261	0.90308999	1.842771649	1.482502	2.82842712
10	0.163	3.981035845	0.019905179	2.087956325	83.518253	1	1.921781401	1.217003	3.16227766
12	0.192	4.680985561	0.023404928	2.477741542	99.10966166	1.07918125	1.996115994	-0.05044	3.46410162

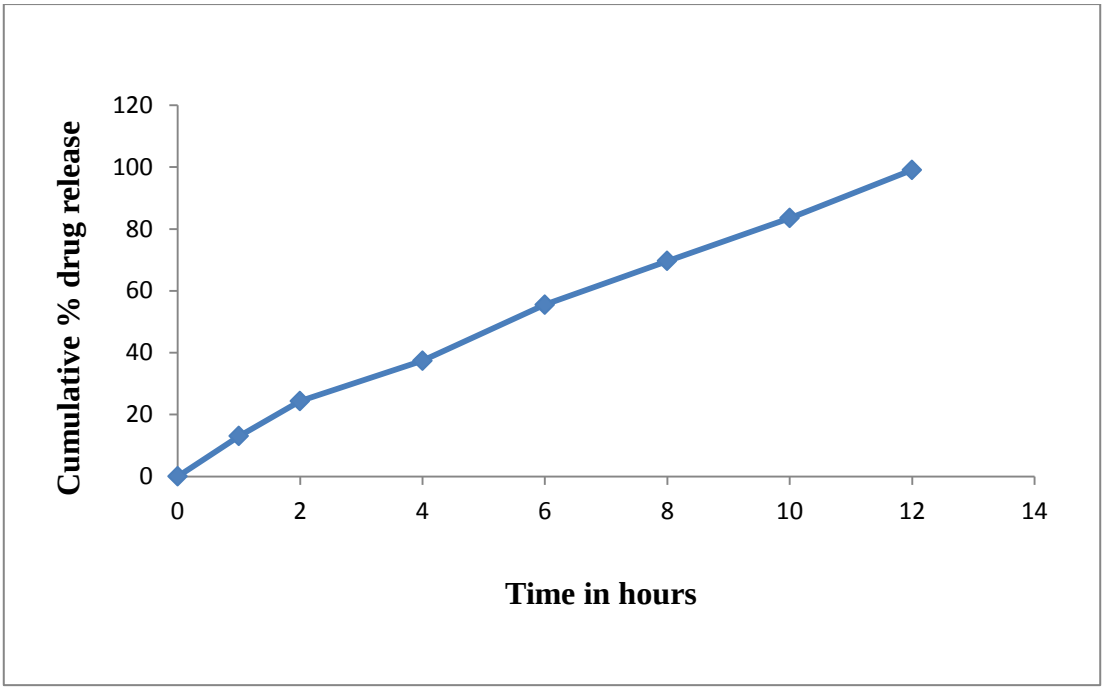

Figure 6.45 *In vitro* drug release of formulation LS205

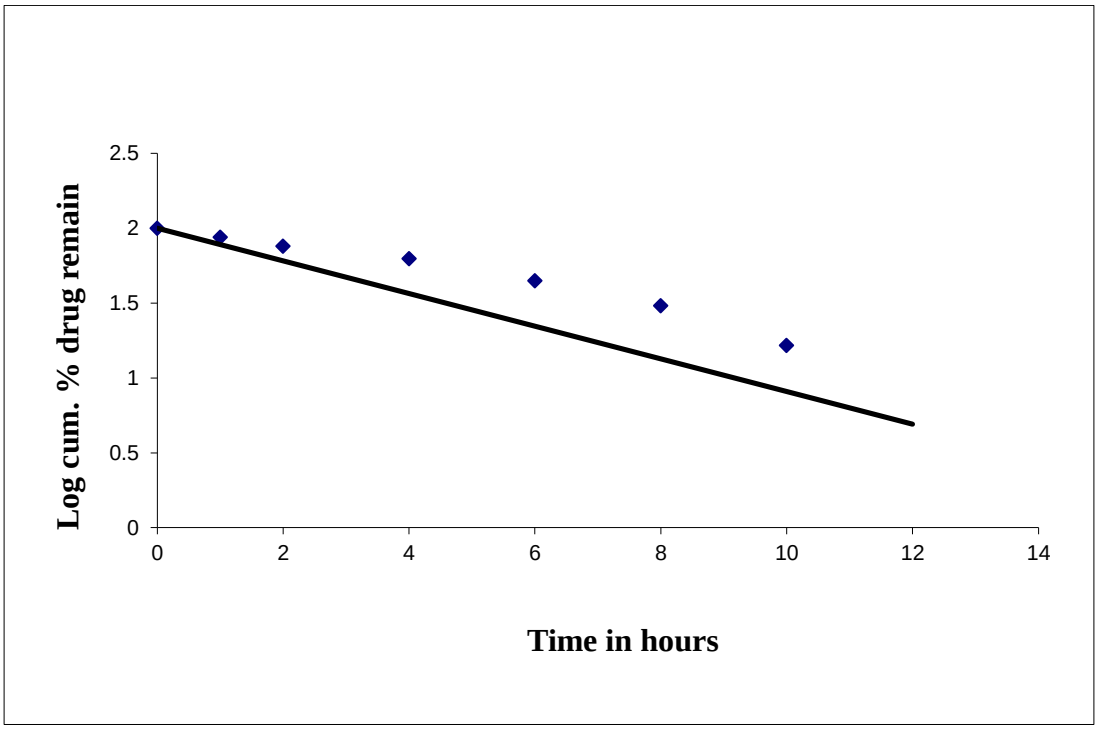

Figure 6.46 First order kinetics of formulation LS205

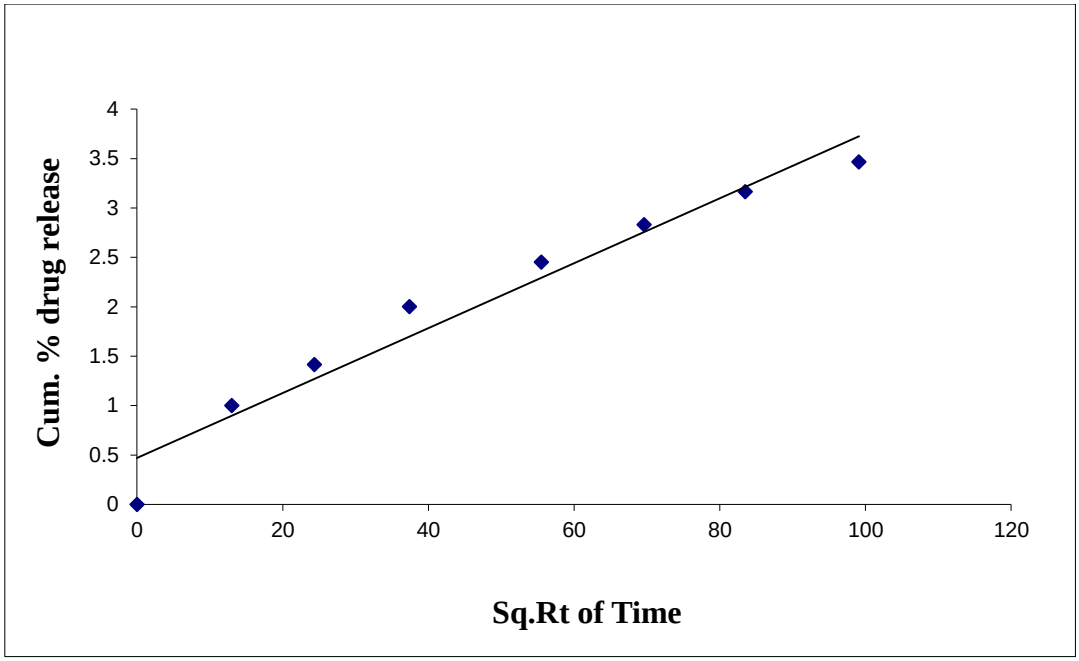

Figure 6.47 Higuchi's plot for formulation LS205

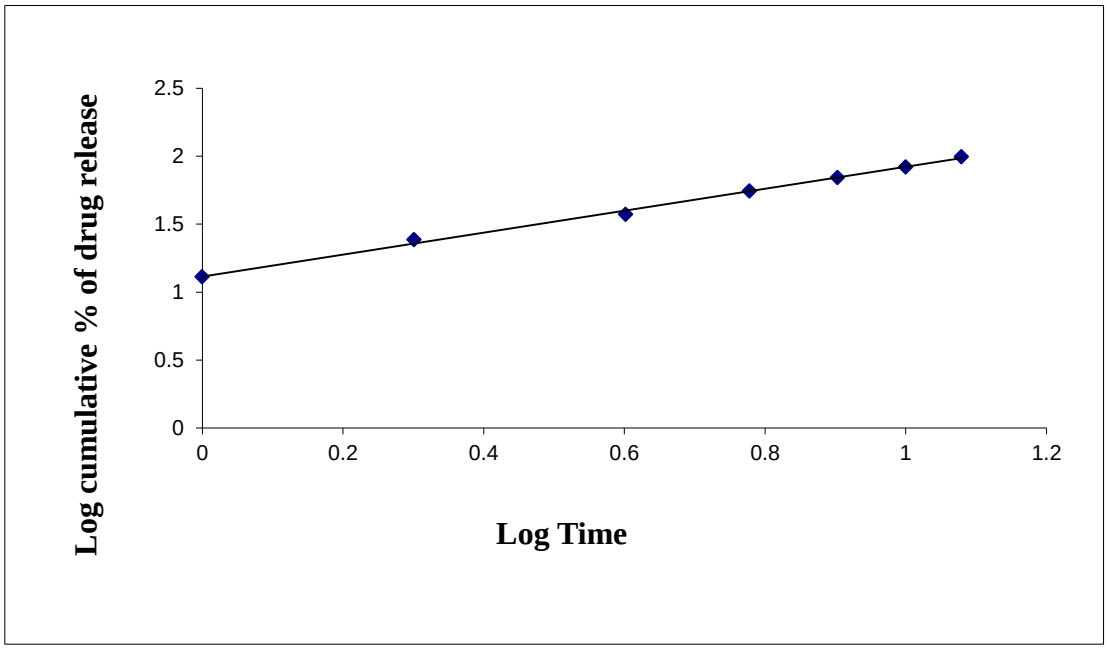

Figure 6.48 Korsmeyer & peppas plot for formulation LS205

Table 6.11 *In vitro* release study of LS206

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.020	0.529559658	0.002647798	0.264779829	10.59119316	0	1.024944889	1.95138	1
2	0.036	0.915738812	0.004578694	0.463165003	18.5266001	0.30103	1.267795727	1.911016	1.41421356
4	0.058	1.446735148	0.007233676	0.737820559	29.51282235	0.60205999	1.470010744	1.84811	2
6	0.083	2.050140076	0.0102507	1.053990374	42.15961497	0.77815125	1.624896636	1.762231	2.44948974
8	0.118	2.894906975	0.014474535	1.496875224	59.87500898	0.90308999	1.777245592	1.603415	2.82842712
10	0.141	3.450039509	0.017250198	1.803390561	72.13562244	1	1.858149784	1.445049	3.16227766
12	0.169	4.125853028	0.020629265	2.175797716	87.03190863	1.07918125	1.939678508	1.112876	3.46410162
14	0.191	4.656849364	0.023284247	2.482554414	99.30217657	1.14612804	1.996958768	-0.15625	3.74165739

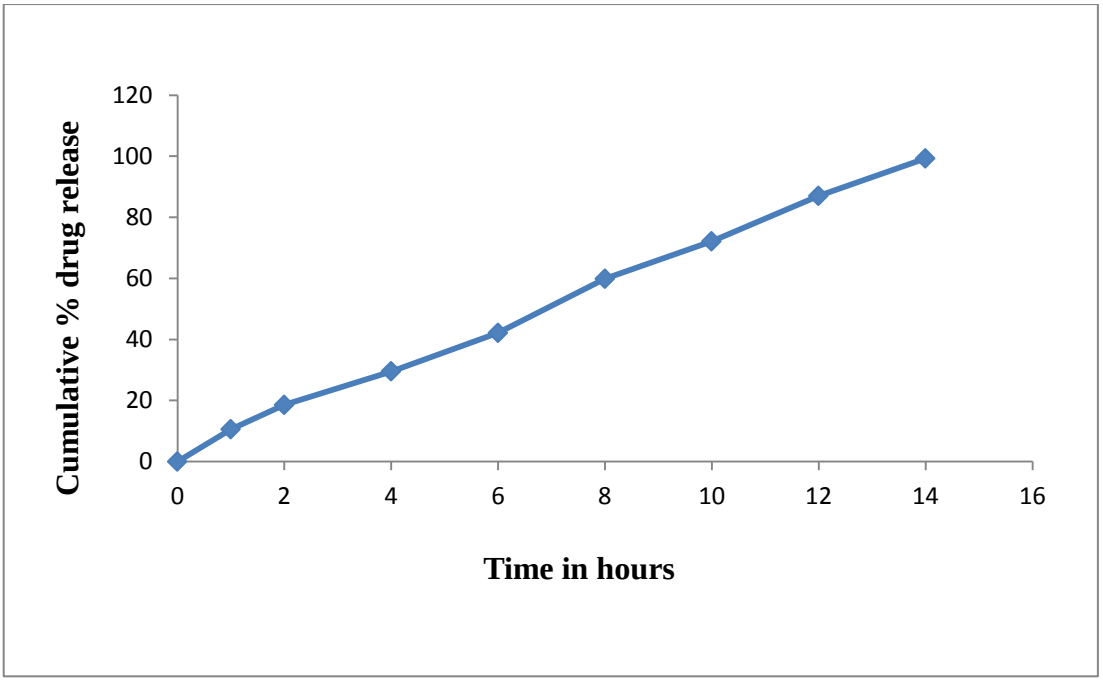

Figure 6.49 *In vitro* drug release of formulation LS206

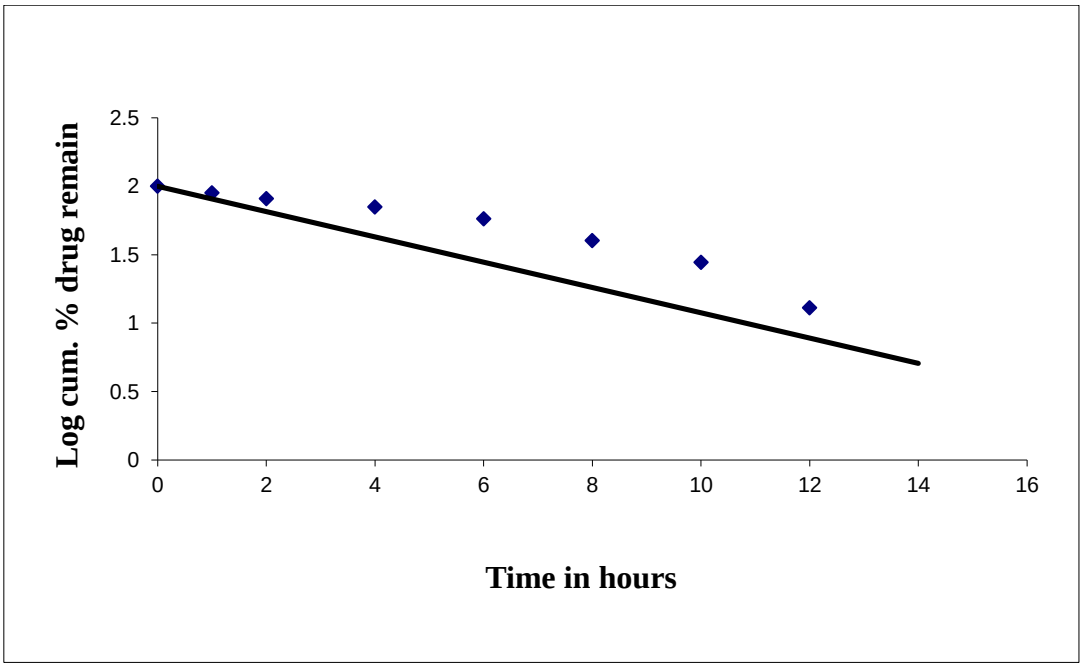

Figure 6.50 First order kinetics of formulation LS206

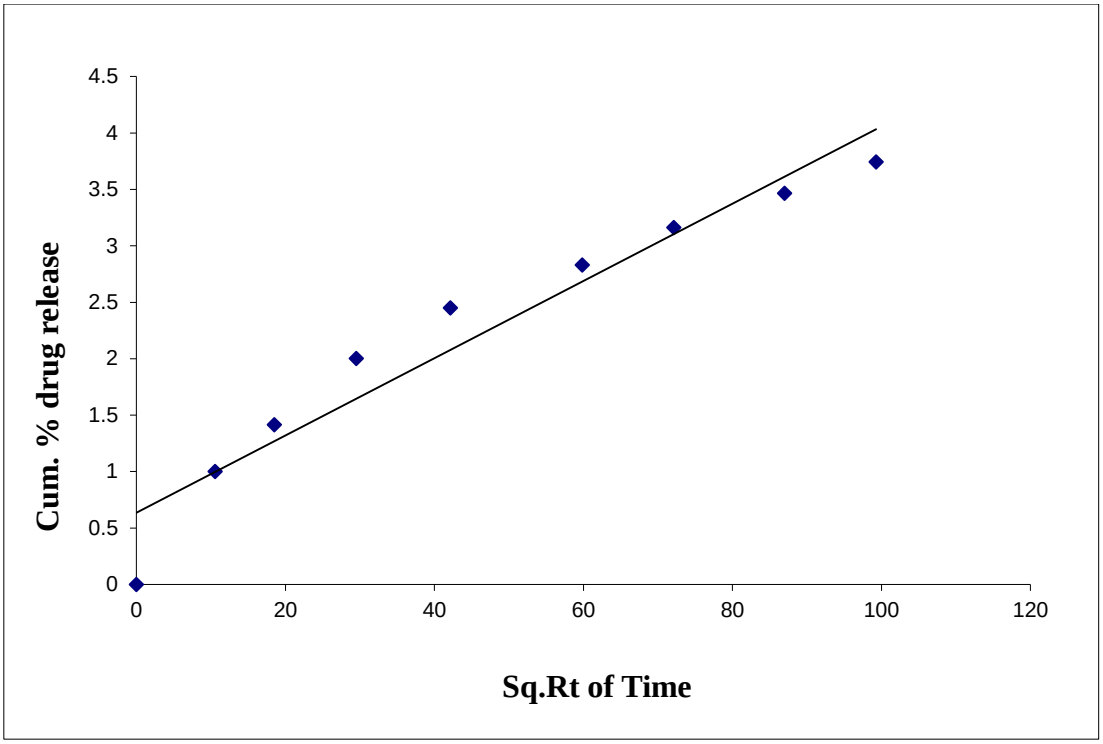

Figure 6.51 Higuchi's plot for formulation LS206

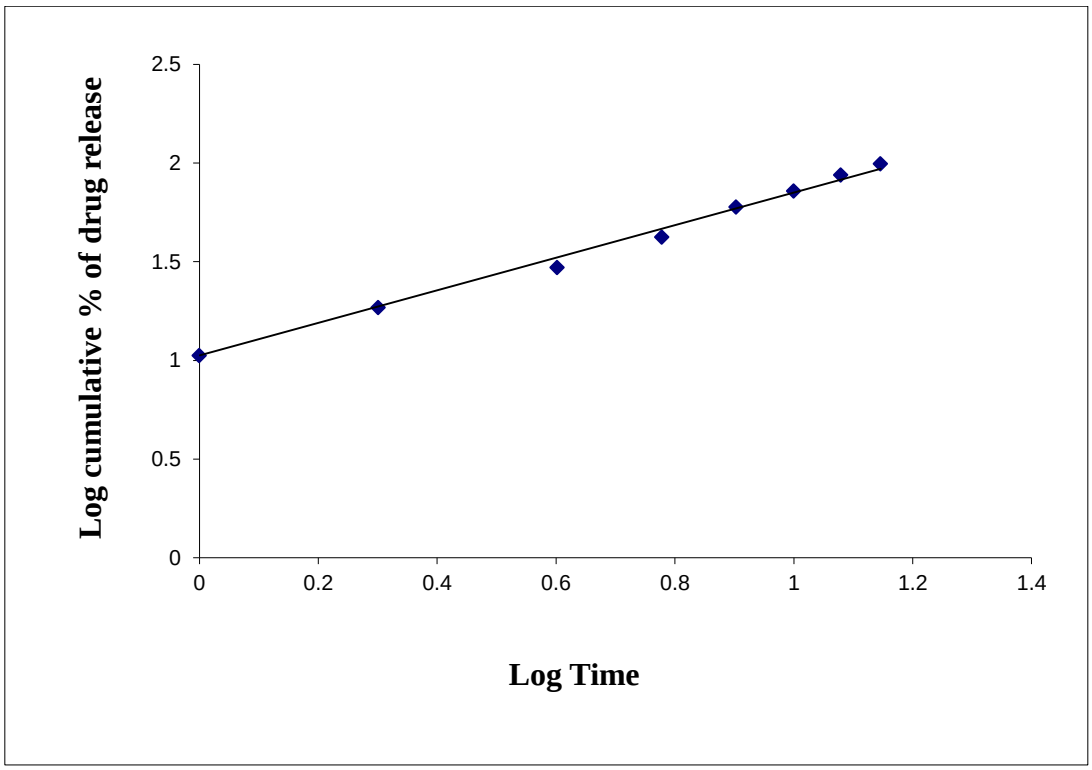

Figure 6.52 Korsmeyer & peppas plot for formulation LS206

Table 6.12 *In vitro* release study of LS207

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.013	0.360606278	0.001803031	0.180303139	7.212125566	0	0.858063279	1.967491	1
2	0.028	0.722649235	0.003613246	0.36493068	14.59722721	0.30103	1.164270368	1.931472	1.41421356
4	0.049	1.229509374	0.006147547	0.625587242	25.02348969	0.60205999	1.398347875	1.874925	2
6	0.071	1.760505711	0.008802529	0.903380504	36.13522017	0.77815125	1.557930705	1.805261	2.44948974
8	0.098	2.412183033	0.012060915	1.246824222	49.8729689	0.90308999	1.697865222	1.700072	2.82842712
10	0.123	3.015587961	0.01507794	1.572648517	62.90594067	1	1.798691661	1.569304	3.16227766
12	0.140	3.425903312	0.017129517	1.807962072	72.31848287	1.07918125	1.859249307	1.44219	3.46410162
14	0.155	3.787946268	0.018939731	2.023242583	80.92970333	1.14612804	1.908107948	1.280357	3.74165739
18	0.190	4.632713167	0.023163566	2.483505495	99.34021981	1.25527251	1.997125117	-0.1806	4.24264069

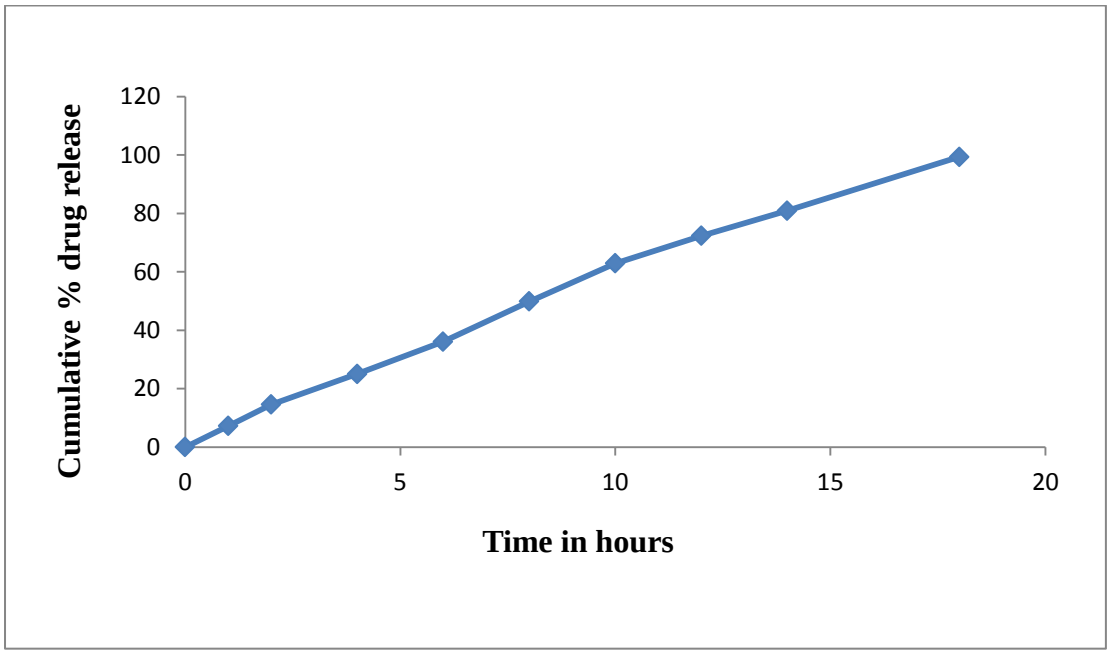

Figure 6.53 *In vitro* drug release of formulation LS207

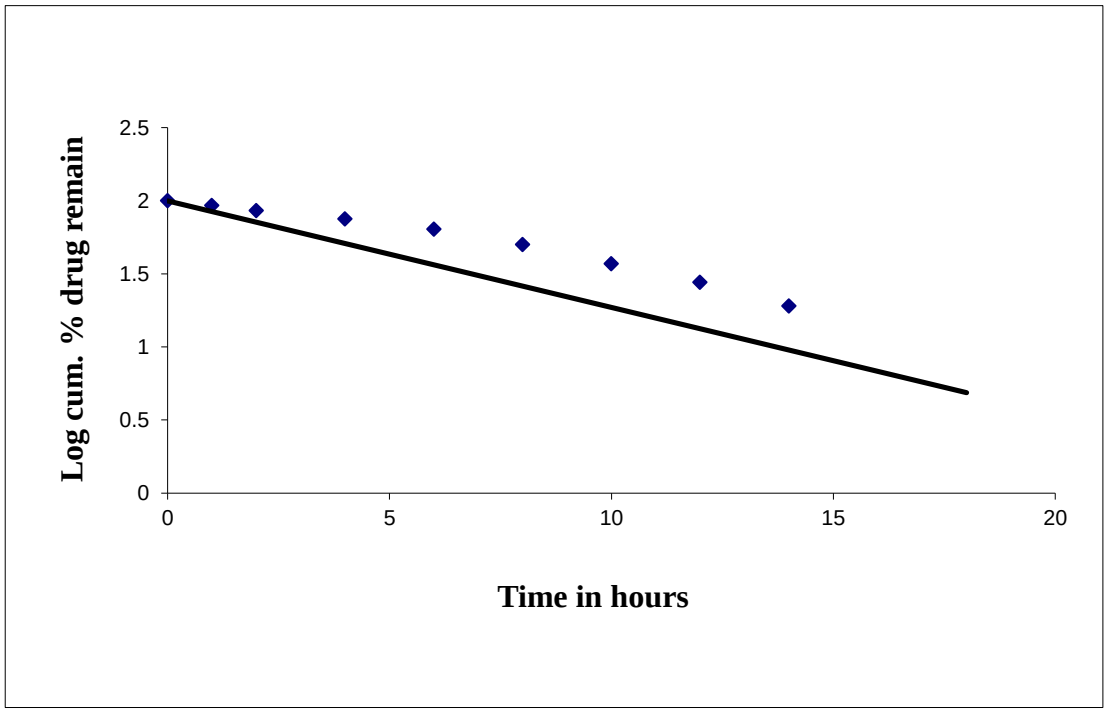

Figure 6.54 First order kinetics of formulation LS207

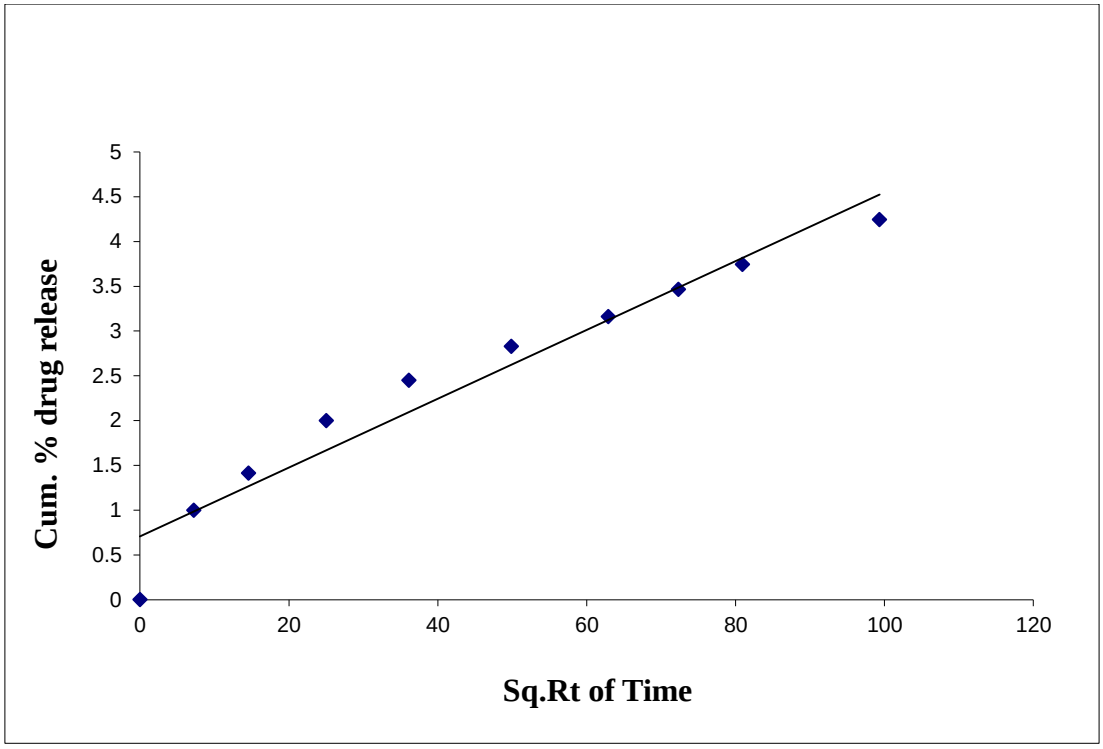

Figure 6.55 Higuchi's plot for formulation LS207

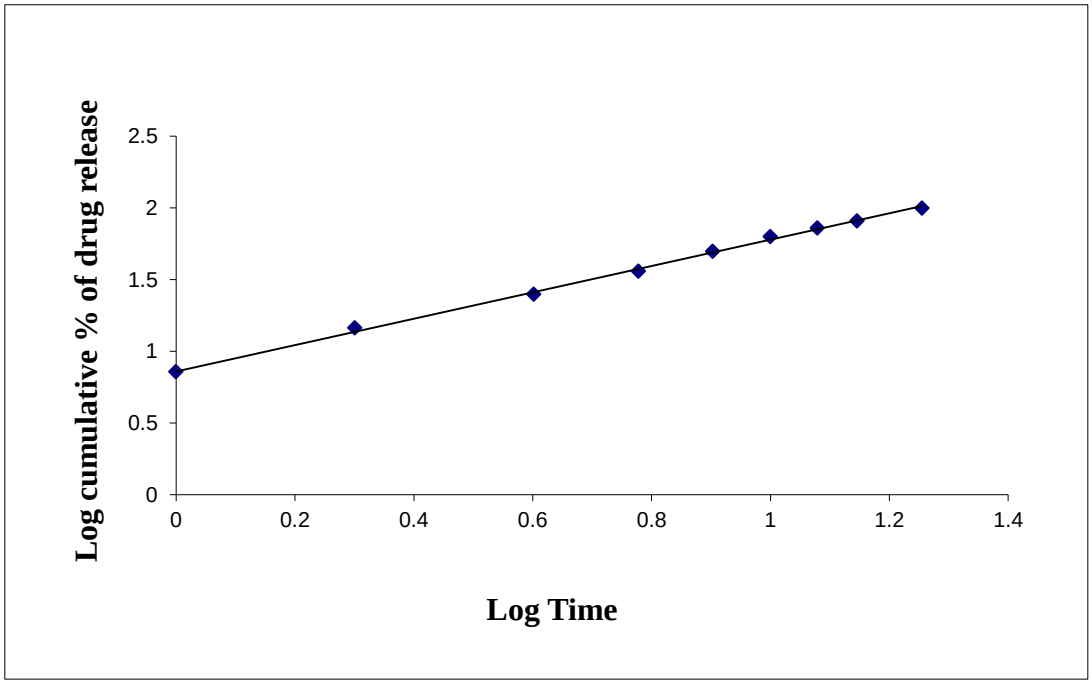

Figure 6.56 Korsmeyer & peppas plot for formulation LS207

Tablet 6.13 *In vitro* release study of LS208

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.012	0.336470081	0.00168235	0.168235041	6.729401623	0	0.827976449	1.969745	1
2	0.026	0.674376841	0.003371884	0.340553121	13.62212485	0.30103	1.134244856	1.936403	1.41421356
4	0.049	1.229509374	0.006147547	0.624863156	24.99452626	0.60205999	1.39784491	1.875093	2
6	0.064	1.591552331	0.007957762	0.818179728	32.72718914	0.77815125	1.514908706	1.82784	2.44948974
8	0.089	2.194957259	0.010974786	1.135797716	45.43190863	0.90308999	1.657360982	1.736939	2.82842712
10	0.133	3.256949932	0.01628475	1.688743625	67.54974499	1	1.829623714	1.511218	3.16227766
12	0.149	3.643129086	0.018215645	1.914402701	76.57610804	1.07918125	1.88409329	1.369659	3.46410162
14	0.168	4.101716831	0.020508584	2.180127864	87.20511458	1.14612804	1.940541957	1.107036	3.74165739
18	0.191	4.656849364	0.023284247	2.498711299	99.94845198	1.25527251	1.999776072	-1.28779	4.24264069

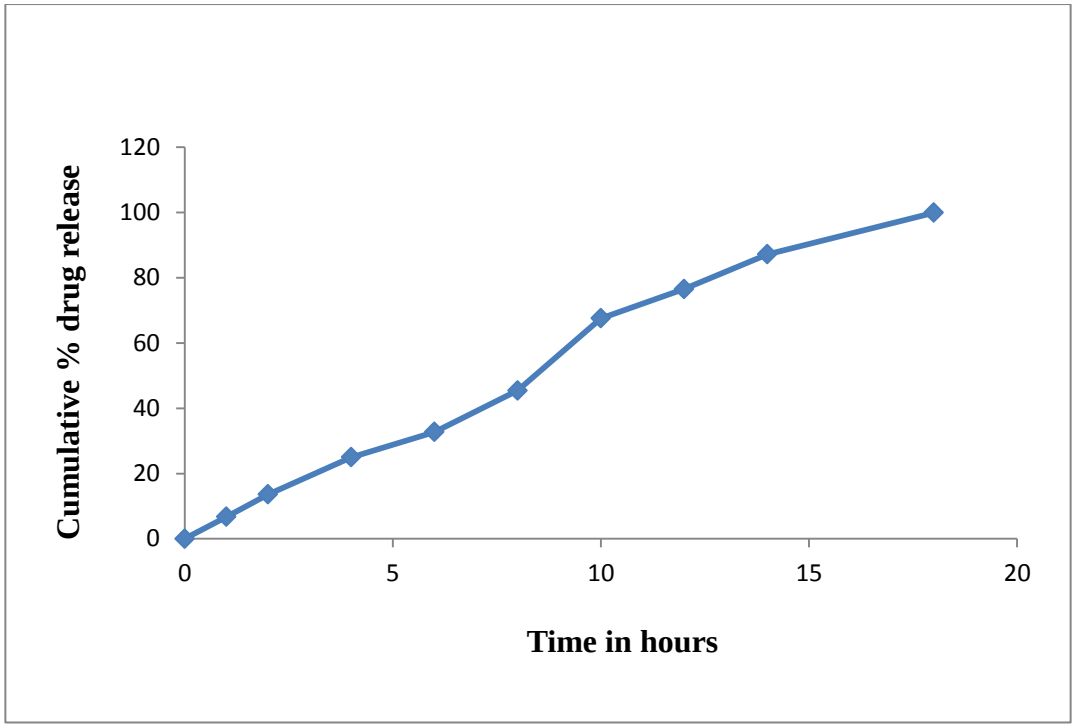

Figure 6.57 *In vitro* drug release of formulation LS208

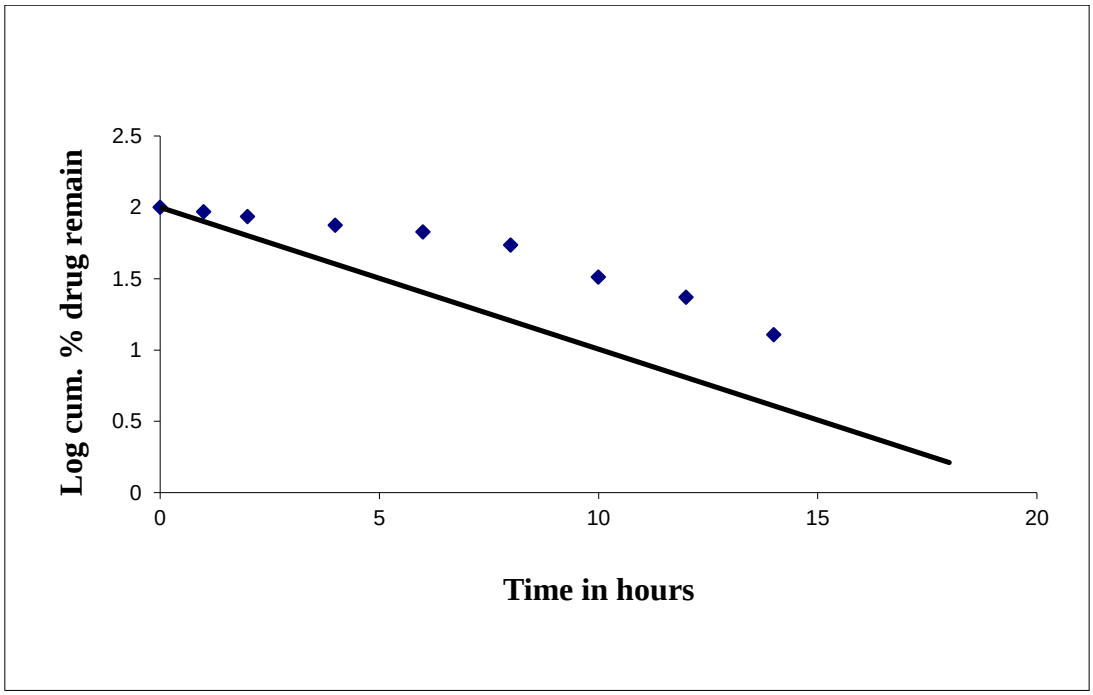

Figure 6.58 First order kinetics of formulation LS208

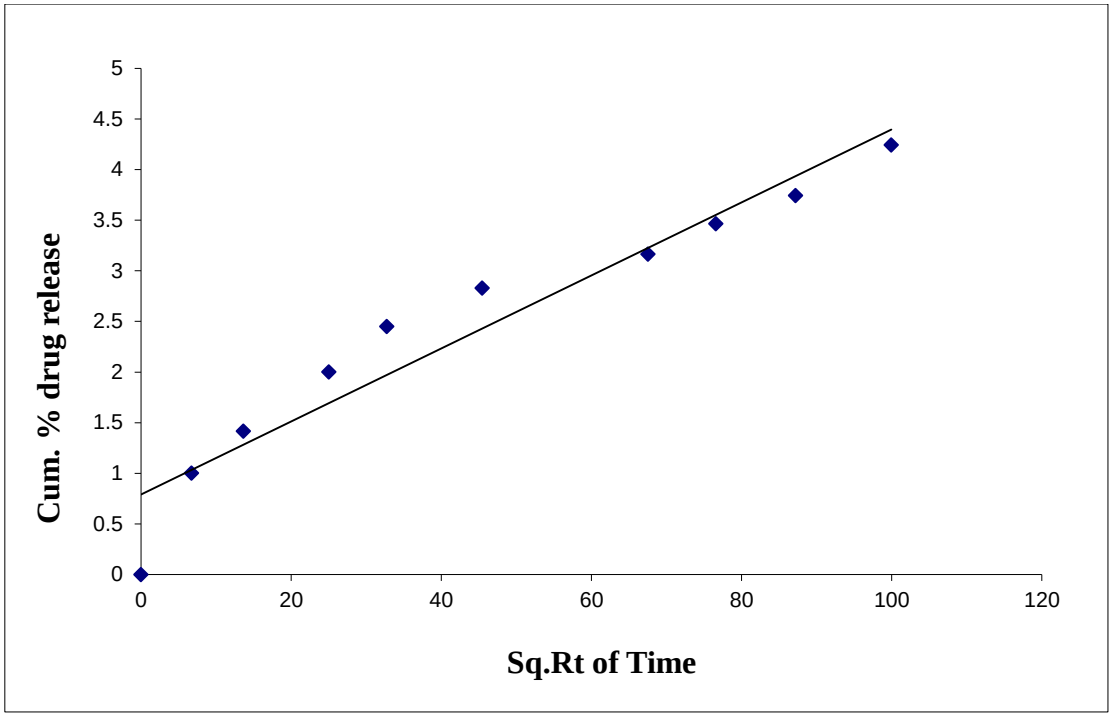

Figure 6.59 Higuchi's plot for formulation LS208

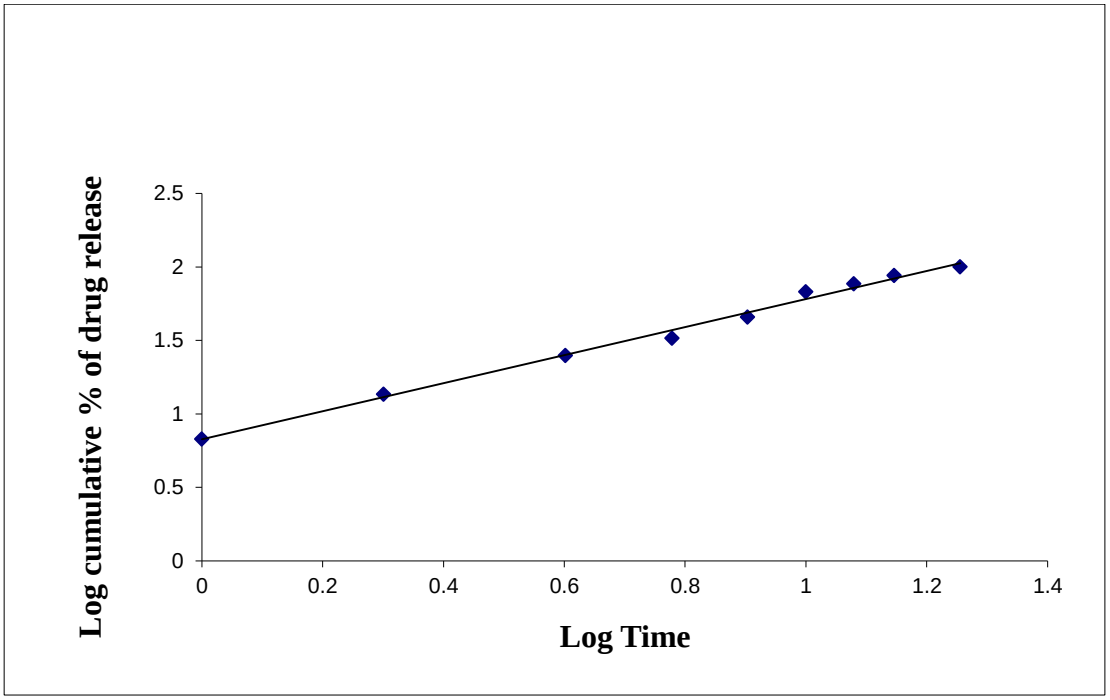

Figure 6.60 Korsmeyer & peppas plot for formulation LS208

Table 6.14 *In vitro* release study of LS209

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.012	0.336470081	0.00168235	0.168235041	6.729401623	0	0.827976449	1.969745	1
2	0.023	0.601968249	0.003009841	0.304348826	12.17395302	0.30103	1.085431621	1.943623	1.41421356
4	0.038	0.964011206	0.004820056	0.491389986	19.65559945	0.60205999	1.293486293	1.904956	2
6	0.058	1.446735148	0.007233676	0.74239207	29.69568278	0.77815125	1.472693315	1.846982	2.44948974
8	0.094	2.315638244	0.011578191	1.191310969	47.65243876	0.90308999	1.678085132	1.718896	2.82842712
10	0.138	3.377630917	0.016888155	1.745463688	69.81854752	1	1.84397081	1.47974	3.16227766
12	0.156	3.812082465	0.019060412	1.996465771	79.85863085	1.07918125	1.90232186	1.304089	3.46410162
14	0.170	4.149989225	0.020749946	2.203539976	88.14159902	1.14612804	1.945180925	1.074026	3.74165739
18	0.190	4.632713167	0.023163566	2.486401839	99.45607356	1.25527251	1.99763131	-0.26446	4.24264069

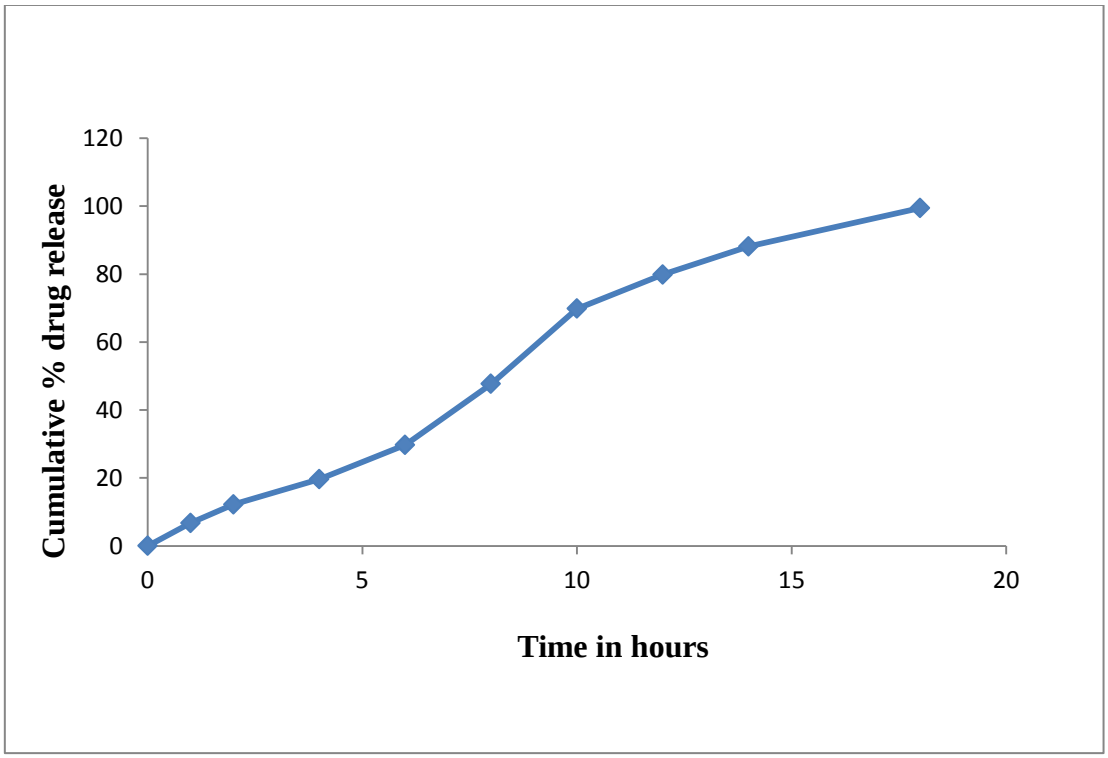

Figure 6.61 *In vitro* drug release of formulation LS209

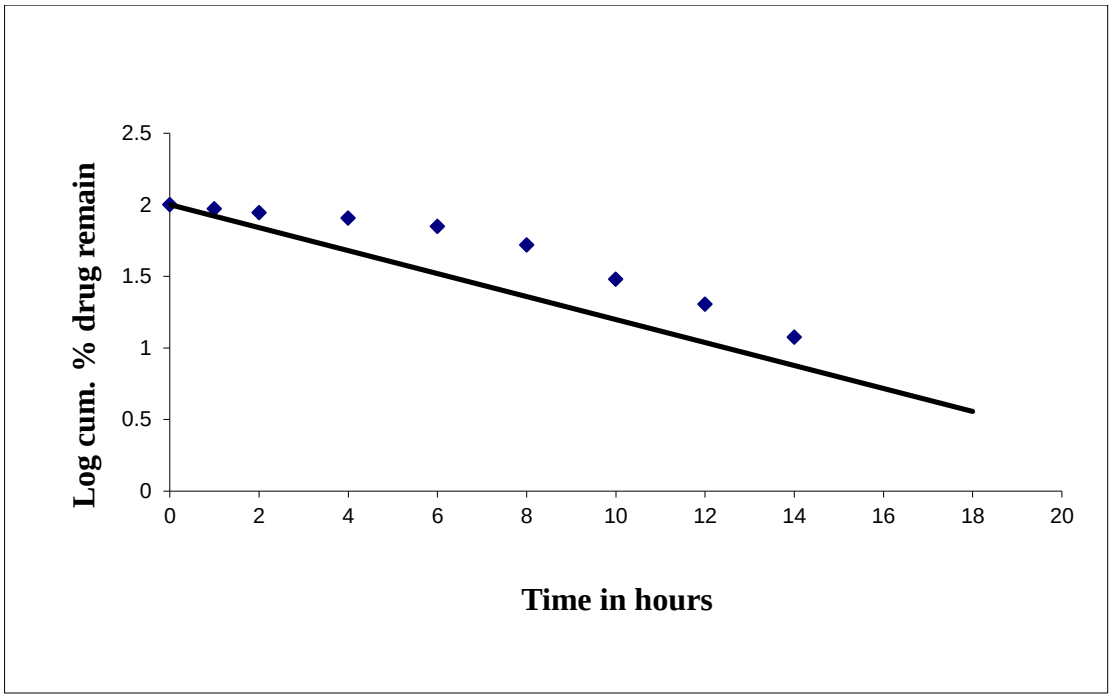

Figure 6.62 First order kinetics of formulation LS209

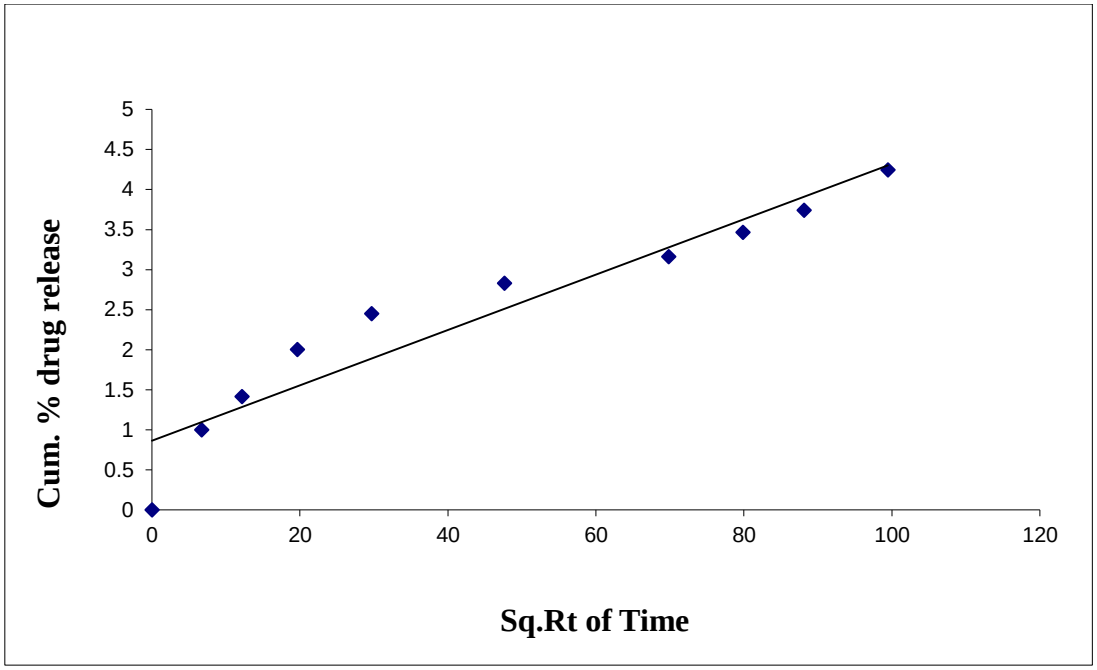

Figure 6.63 Higuchi's plot for formulation LS209

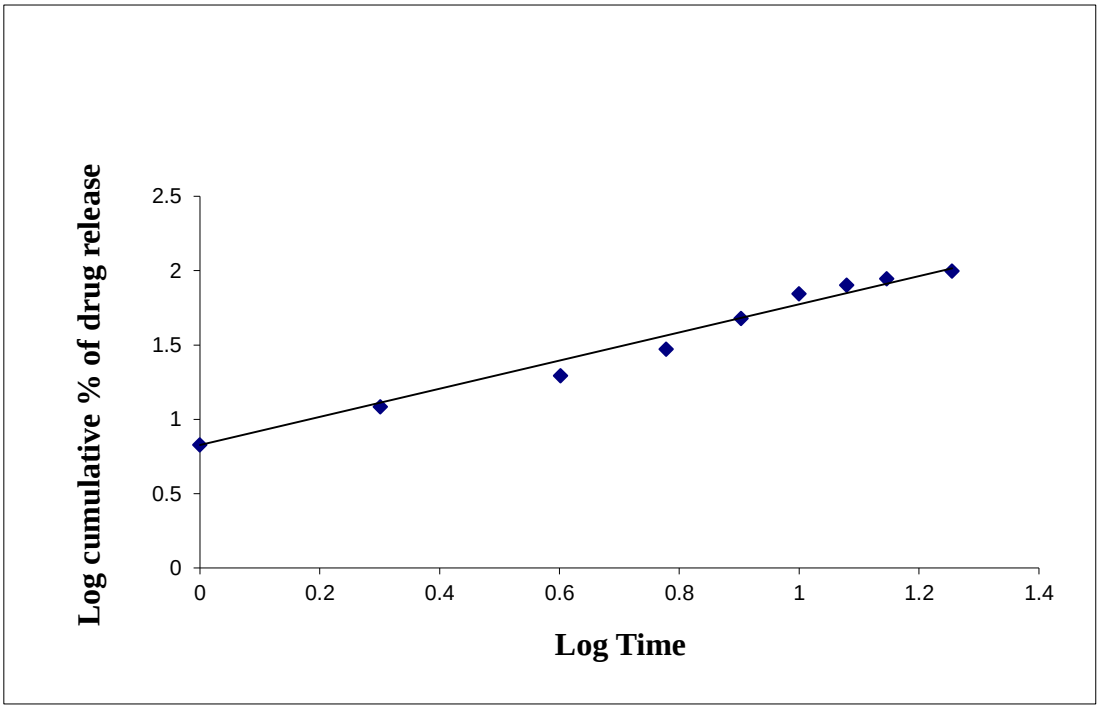

Figure 6.64 Korsmeyer & peppas plot for formulation LS209

Table 6.15 *In vitro* release study of LS210

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.008	0.239925293	0.001199626	0.119962646	4.798505854	0	0.681106029	1.978644	1
2	0.021	0.553695855	0.002768479	0.279247181	11.16988722	0.30103	1.048048788	1.94856	1.41421356
4	0.034	0.867466418	0.004337332	0.44166942	17.66677681	0.60205999	1.247157323	1.915575	2
6	0.055	1.374326557	0.006871633	0.703774154	28.15096617	0.77815125	1.449493305	1.856421	2.44948974
8	0.096	2.363910639	0.011819553	1.212309461	48.49237842	0.90308999	1.685673486	1.711871	2.82842712
10	0.141	3.450039509	0.017250198	1.779013002	71.16052008	1	1.852239113	1.459987	3.16227766
12	0.158	3.86035486	0.019301774	2.018671072	80.7468429	1.07918125	1.907125551	1.284502	3.46410162
14	0.173	4.222397816	0.021111989	2.238296099	89.53184398	1.14612804	1.951977529	1.01987	3.74165739
18	0.190	4.632713167	0.023163566	2.485677753	99.42711012	1.25527251	1.997504817	-0.24193	4.24264069

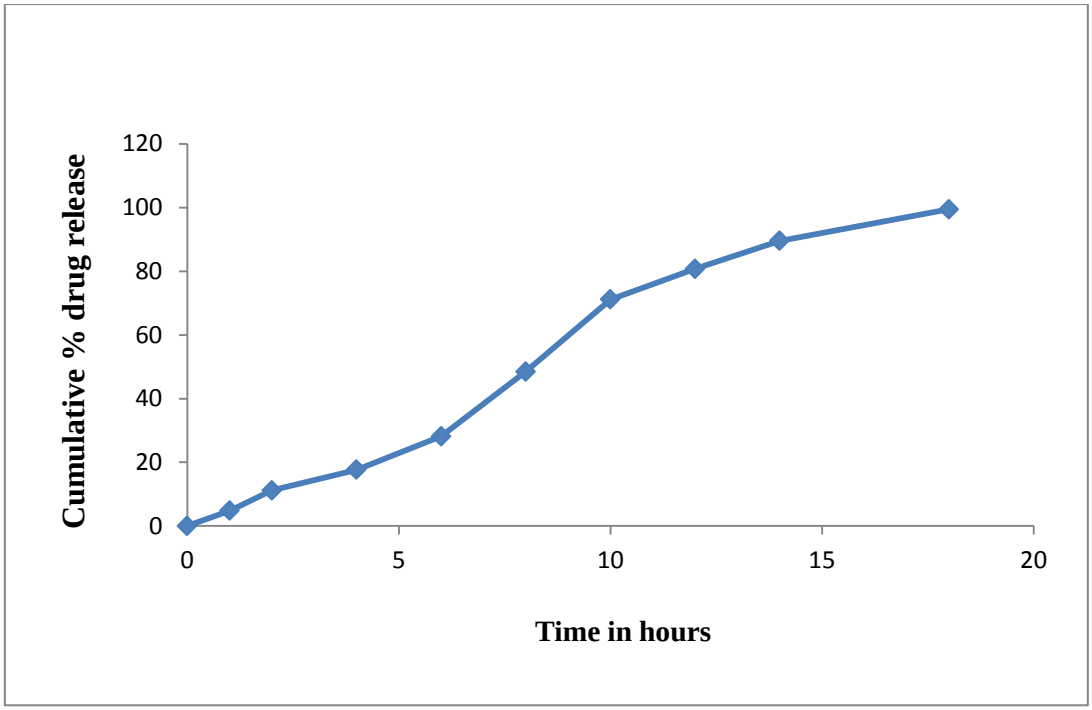

Figure 6.65 *In vitro* drug release of formulation LS210

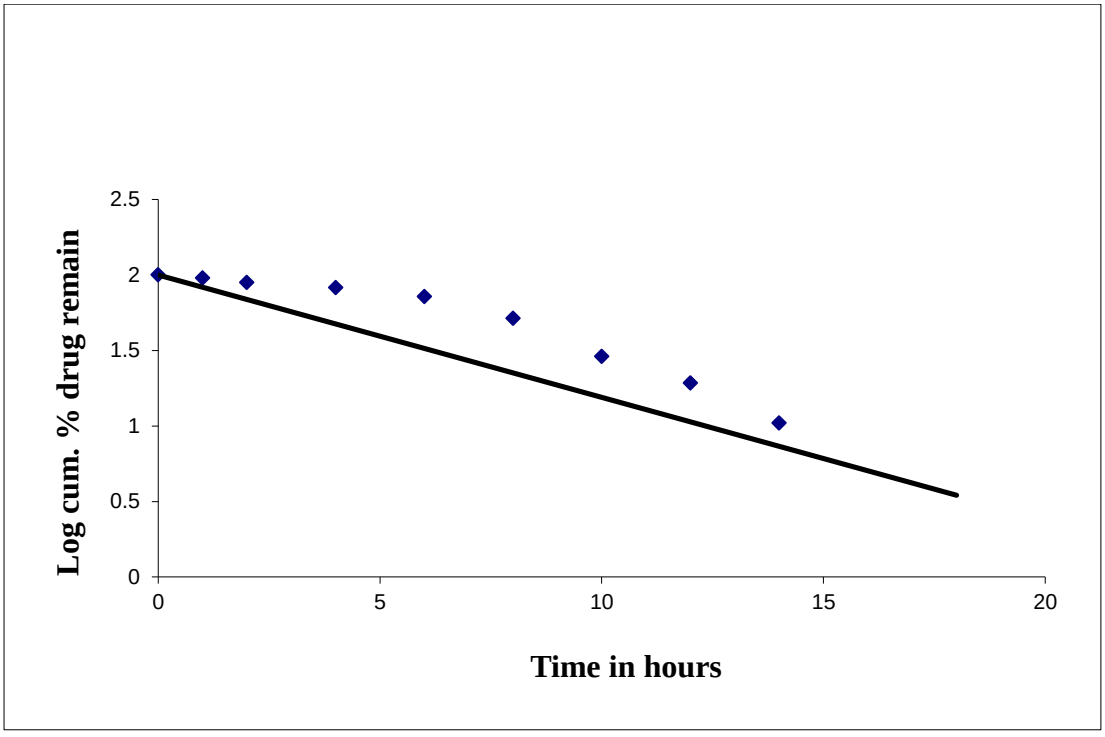

Figure 6.66 First order kinetics of formulation LS210

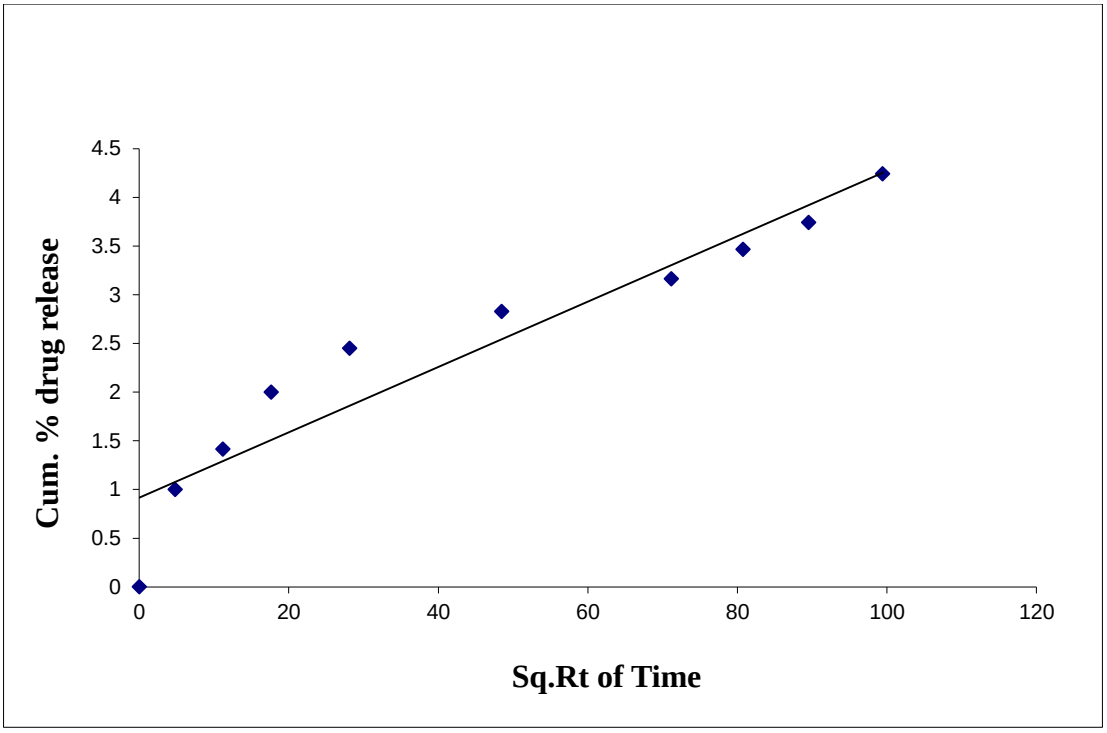

Figure 6.67 Higuchi's Plot for formulation LS210

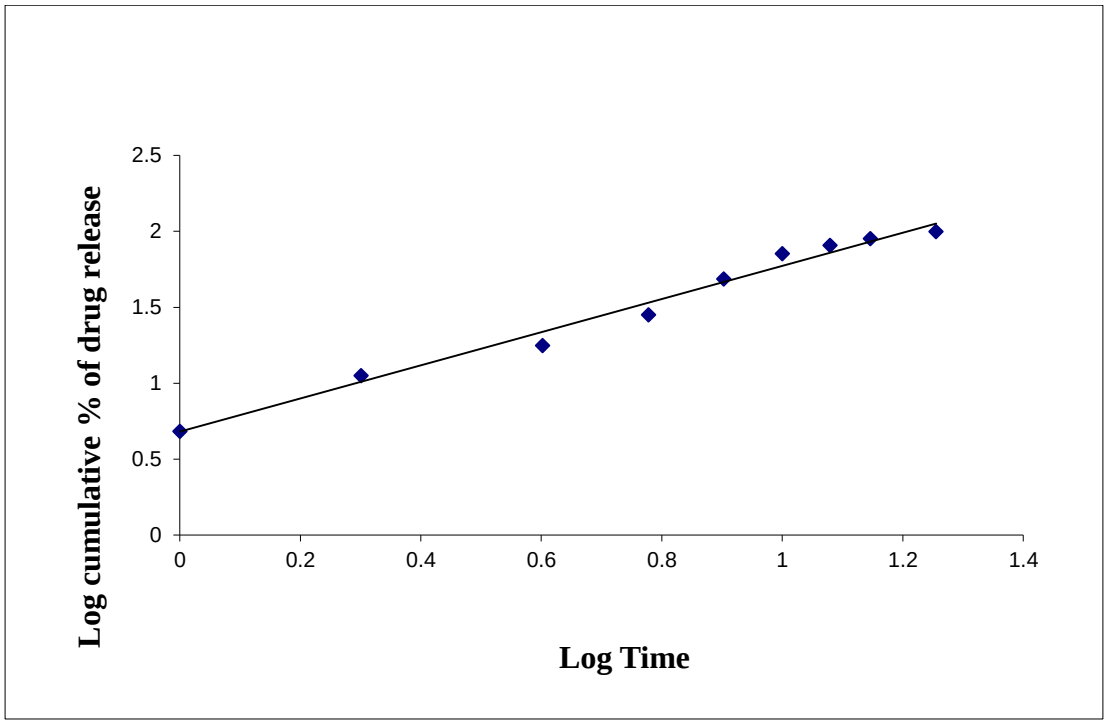

Figure 6.68 Korsmeyer & peppas plot for formulation LS210

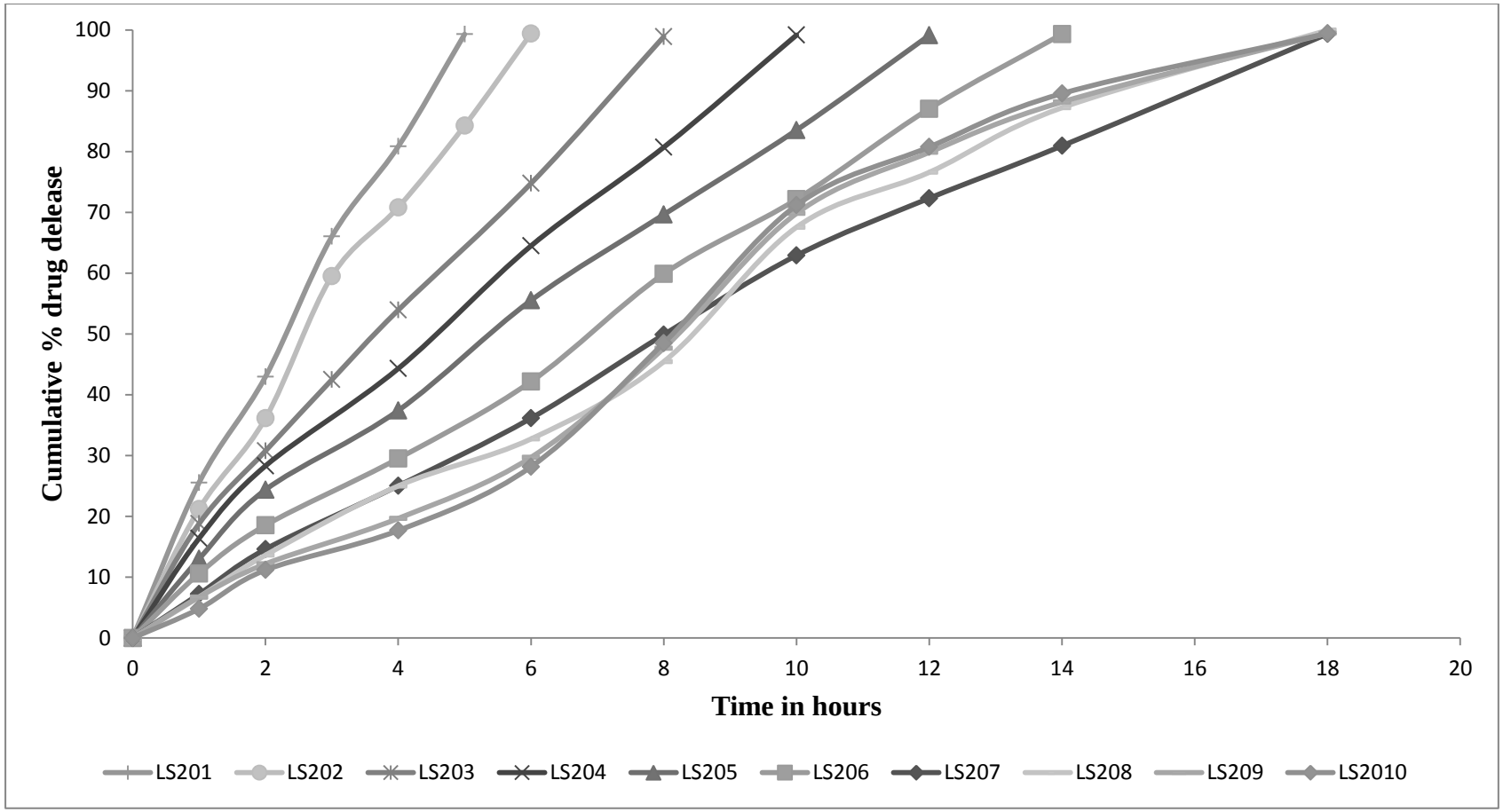

Figure 6.69 Comparative dissolution profile of LS 201- LS 210

6.1.5.9 Kinetic values obtained from different plots of formulations (LS201-210) of letrozole

Table 6.16 Drug release mechanism (LS 201- LS 210)

Formula Code	Zero order		First Order		Higuchis model		Koresmayer & Peppas	
	K_0	R^2	K_1	R^2	K_H	R^2	*n*	R^2
LS201	18.5322	0.9982	0.8369	-0.8597	44.0017	0.9716	0.8512	0.9982
LS202	15.6182	0.9951	0.6896	-0.8510	41.1225	0.9715	0.8736	0.9967
LS203	11.3129	0.9997	0.4872	-0.8831	34.5283	0.9700	0.7956	0.9988
LS204	9.0747	0.9993	0.3869	-0.8699	31.4629	0.9775	0.7718	0.9987
LS205	7.6944	0.9989	0.3067	-0.8642	29.0634	0.9769	0.8026	0.9986
LS206	6.8913	0.9991	0.2669	-0.8497	27.2939	0.9661	0.8513	0.9970
LS207	5.5161	0.9972	0.2142	-0.8574	24.5723	0.9718	0.9109	0.9991
LS208	5.8394	0.9908	0.3090	-0.8115	25.6385	0.9616	0.9548	0.9966
LS209	6.0510	0.9843	0.2378	-0.8908	26.2650	0.9529	0.9942	0.9910
LS210	6.2262	0.9809	0.2406	-0.9009	26.8055	0.9482	1.1017	0.9916

Table 6.17 *In vitro* release study of LS 601

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.033	0.843330221	0.004216651	0.42166511	16.86660441	0	1.227027659	1.919776	1
2	0.058	1.446735148	0.007233676	0.731800876	29.27203505	0.30103	1.466452917	1.849591	1.41421356
4	0.083	2.050140076	0.0102507	1.047970692	41.91882767	0.60205999	1.622409128	1.764035	2
6	0.121	2.967315566	0.014836578	1.527059838	61.08239351	0.77815125	1.785916046	1.590146	2.44948974
8	0.148	3.618992888	0.018094964	1.882571654	75.30286617	0.90308999	1.876811507	1.392647	2.82842712
10	0.171	4.174125422	0.020870627	2.19632785	87.853114	1	1.94375716	1.084465	3.16227766
12	0.192	4.680985561	0.023404928	2.491499174	99.65996696	1.07918125	1.998520739	-0.46848	3.46410162

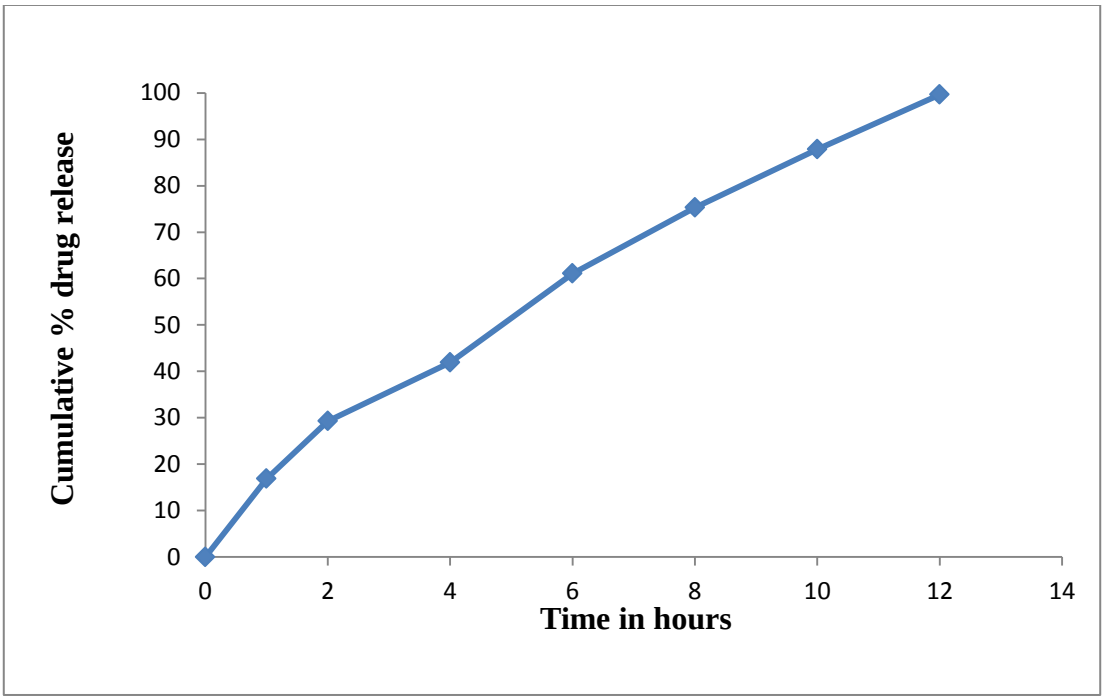

Figure 6.70 *In vitro* drug release of formulation LS601

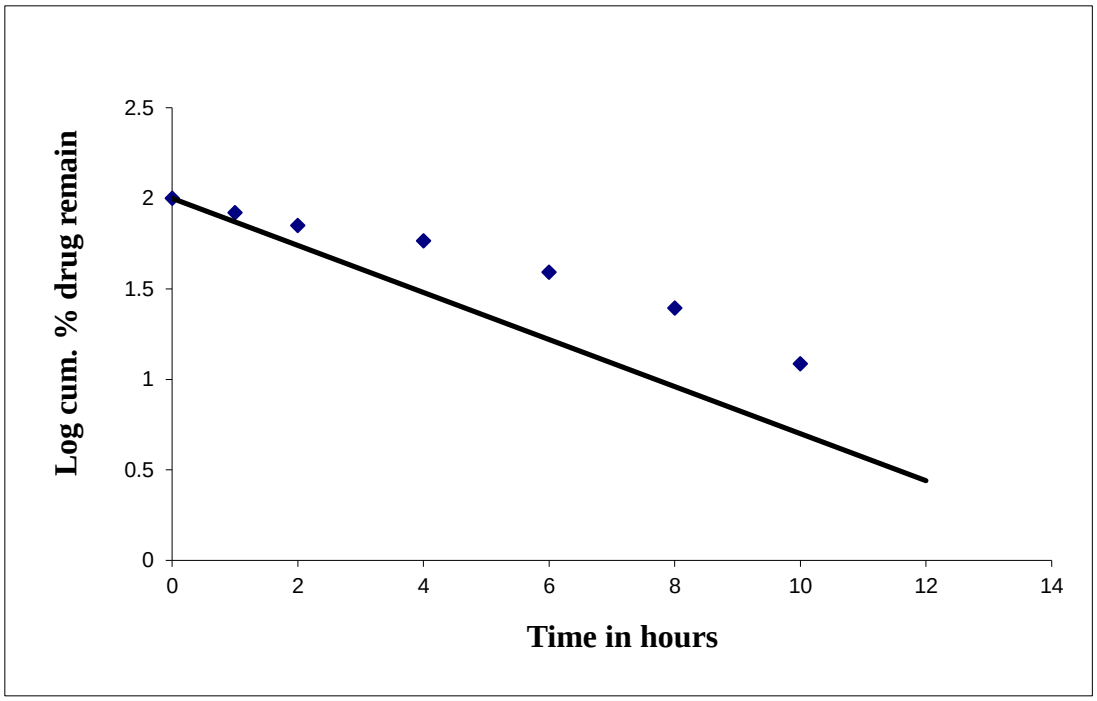

Figure 6.71 First order kinetics of formulation LS601

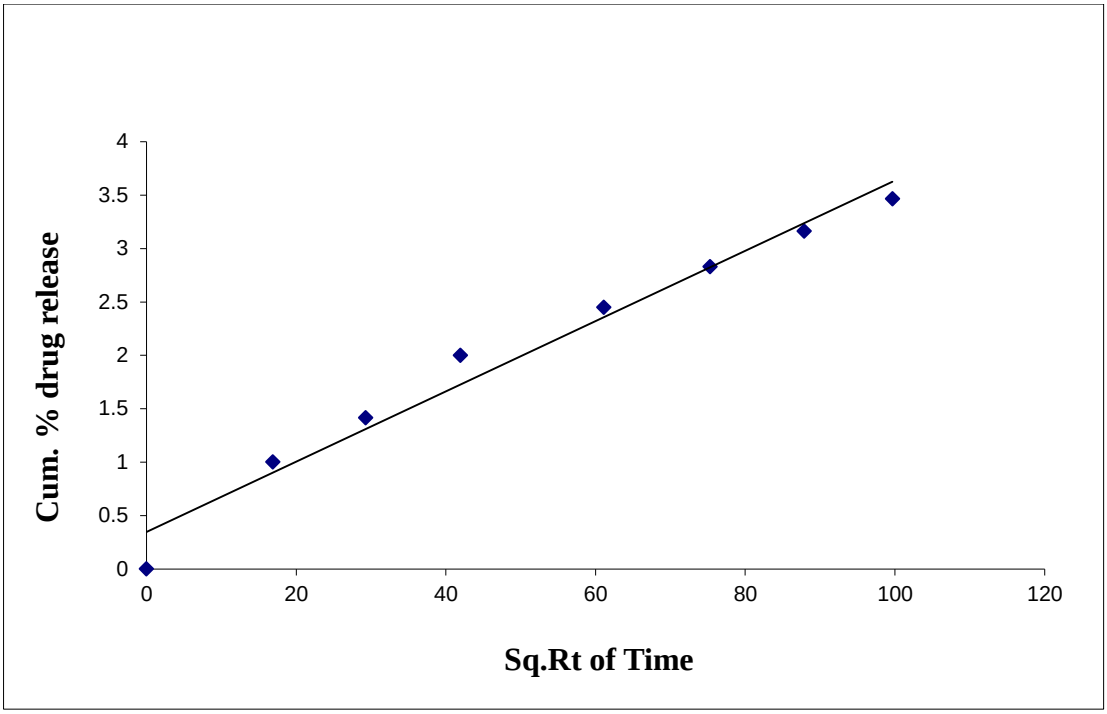

Figure 6.72 Higuchi's plot for formulation LS601

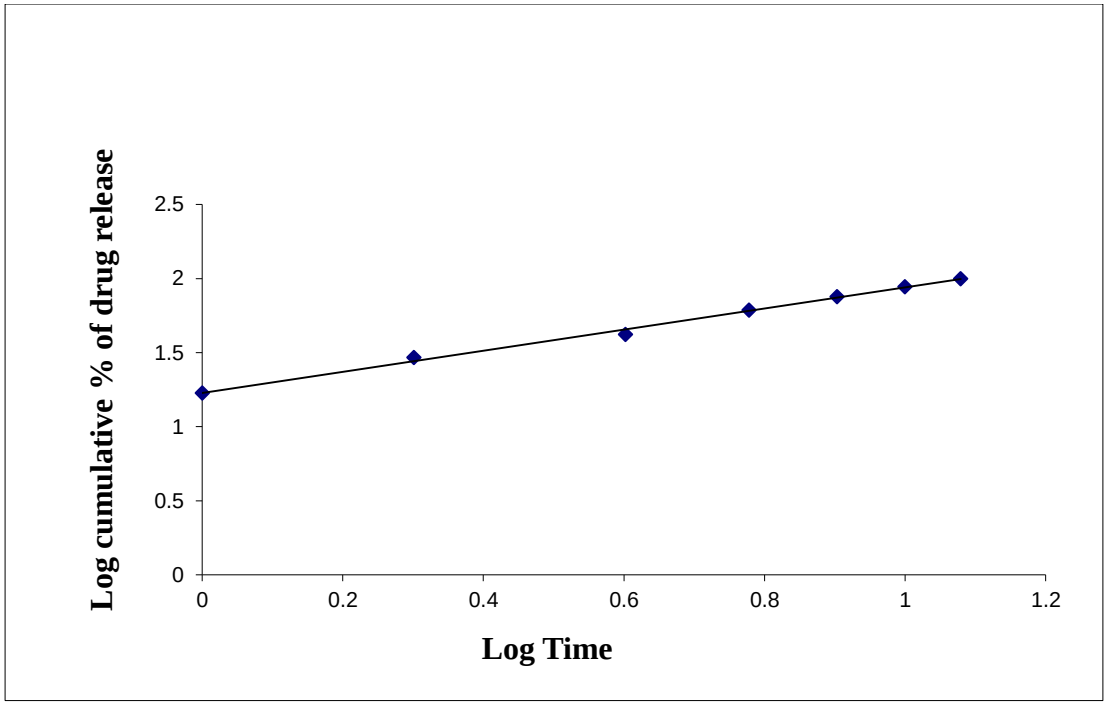

Figure 6.73 Korsmeyer & peppas plot for LS601

Table 6.18 *In vitro* release study of LS 602

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.029	0.746785432	0.003733927	0.373392716	14.93570864	0	1.174225833	1.929747	1
2	0.051	1.277781769	0.006388909	0.646358739	25.85434954	0.30103	1.412533616	1.870086	1.41421356
4	0.070	1.736369514	0.008681848	0.888430429	35.53721715	0.60205999	1.550683416	1.809309	2
6	0.096	2.363910639	0.011819553	1.219564686	48.78258746	0.77815125	1.688264832	1.709418	2.44948974
8	0.119	2.919043172	0.014595216	1.52077006	60.83080238	0.90308999	1.784123545	1.592945	2.82842712
10	0.134	3.281086129	0.016405431	1.73098197	69.23927879	1	1.840352536	1.487997	3.16227766
12	0.153	3.739673874	0.018698369	1.993086704	79.72346814	1.07918125	1.901586183	1.306994	3.46410162
14	0.170	4.149989225	0.020749946	2.235641118	89.42564471	1.14612804	1.95146208	1.024254	3.74165739
16	0.188	4.584440773	0.022922204	2.494366784	99.77467136	1.20411998	1.999020306	-0.64718	4

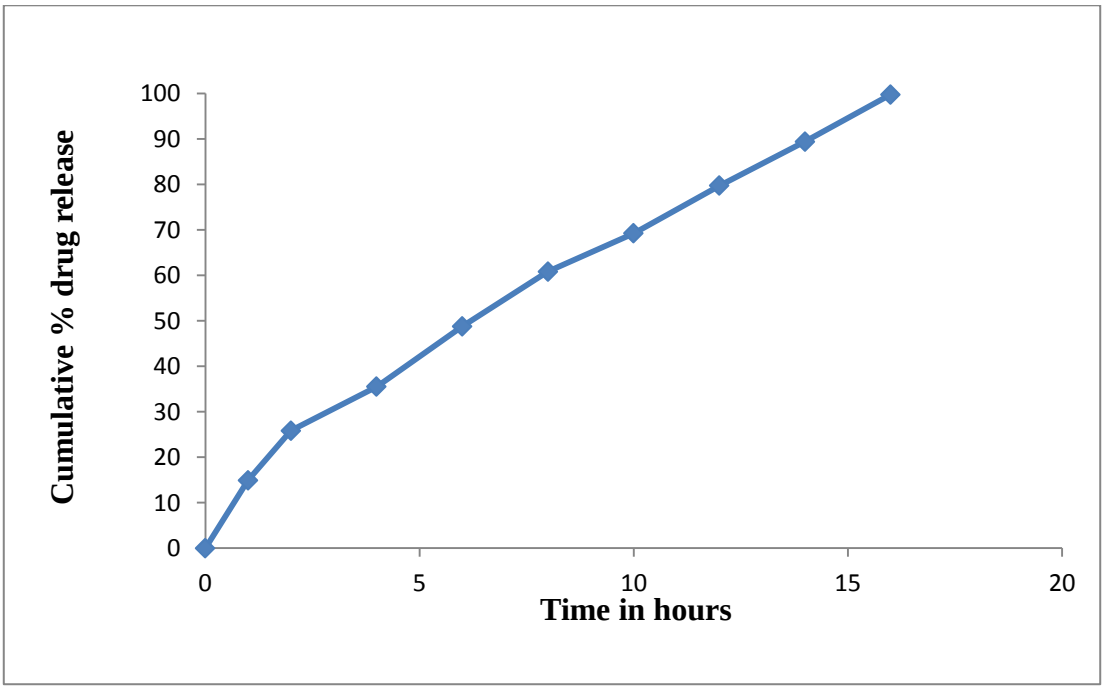

Figure6.74 *In vitro* drug release of formulation LS602

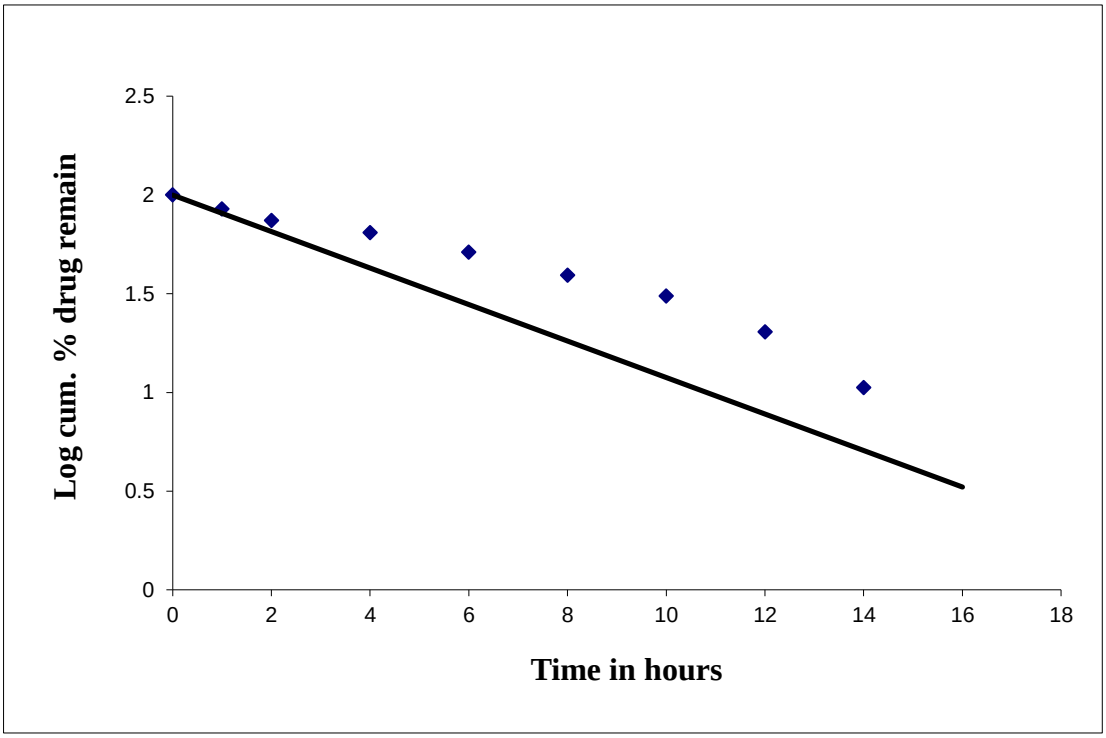

Figure6.75 First order kinetics of formulation LS602

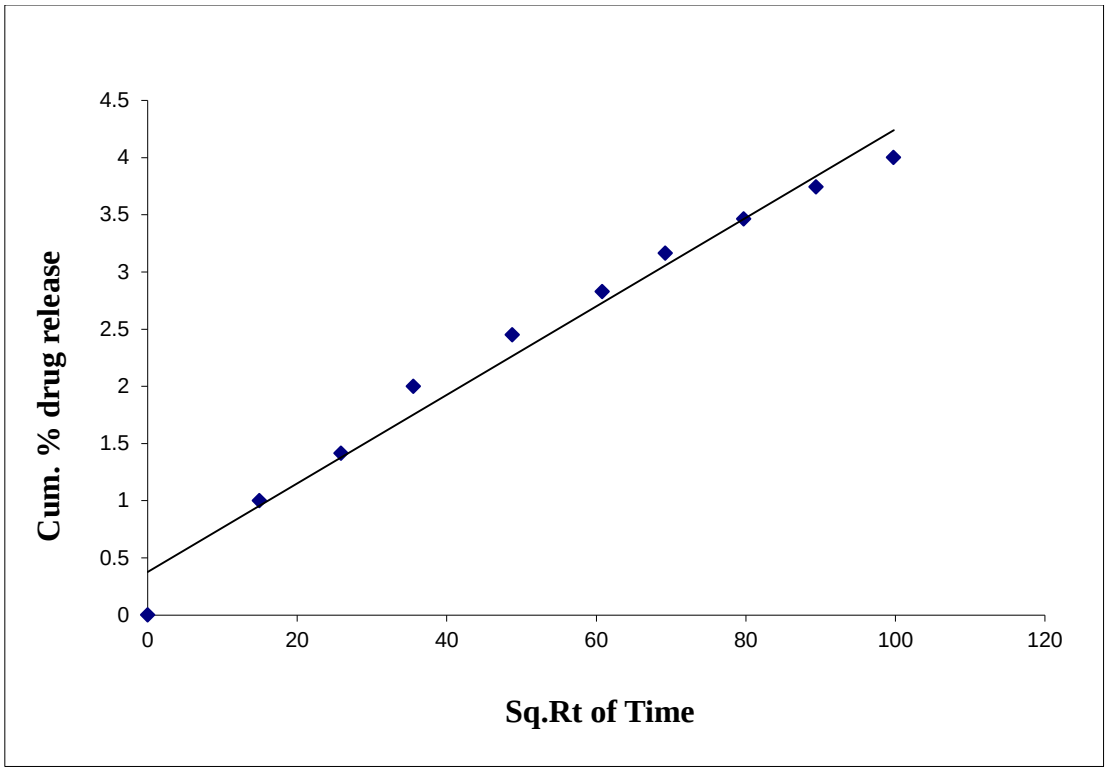

Figure6.76 Higuchi's plot for formulation LS602

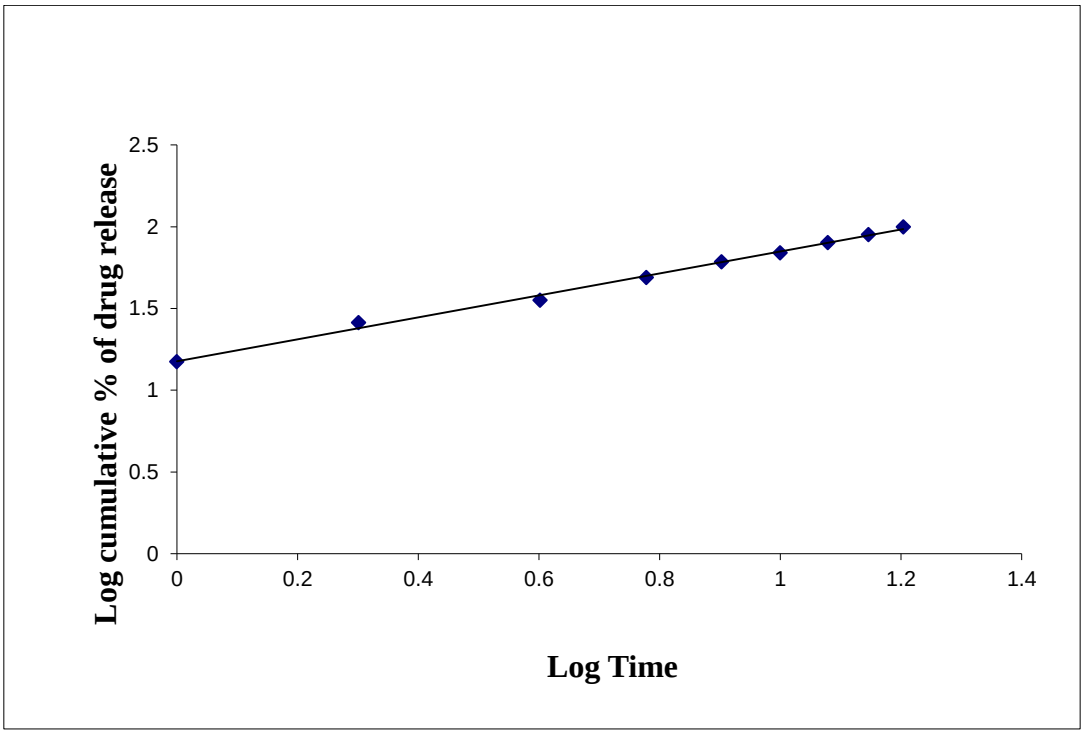

Figure6.77 Korsmeyer & peppas plot for formulation LS602

Table 6.19 *In vitro* release study of LS 603

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.029	0.749199052	0.003745995	0.374599526	14.98398104	0	1.175627215	1.929501	1
2	0.036	0.915738812	0.004578694	0.465361396	18.61445586	0.30103	1.269850345	1.910547	1.41421356
4	0.063	1.567416134	0.007837081	0.800357446	32.01429782	0.60205999	1.505343981	1.832418	2
6	0.084	2.074276273	0.010371381	1.069461677	42.77846706	0.77815125	1.631225218	1.757559	2.44948974
8	0.097	2.388046836	0.011940234	1.247089721	49.88358882	0.90308999	1.697957691	1.69998	2.82842712
10	0.115	2.822498384	0.014112492	1.488195963	59.52783852	1	1.774720113	1.607156	3.16227766
12	0.130	3.18454134	0.015922707	1.697442425	67.897697	1.07918125	1.831855044	1.506536	3.46410162
14	0.144	3.5224481	0.01761224	1.898241218	75.92964873	1.14612804	1.880411391	1.381482	3.74165739
16	0.162	3.956899648	0.019784498	2.150691473	86.02765893	1.20411998	1.934638105	1.145269	4
18	0.176	4.294806408	0.021474032	2.319644853	92.78579412	1.25527251	1.967481489	0.858189	4.24264069
20	0.186	4.536168379	0.022680842	2.483273903	99.33095611	1.30103	1.997084616	-0.17455	4.47213595

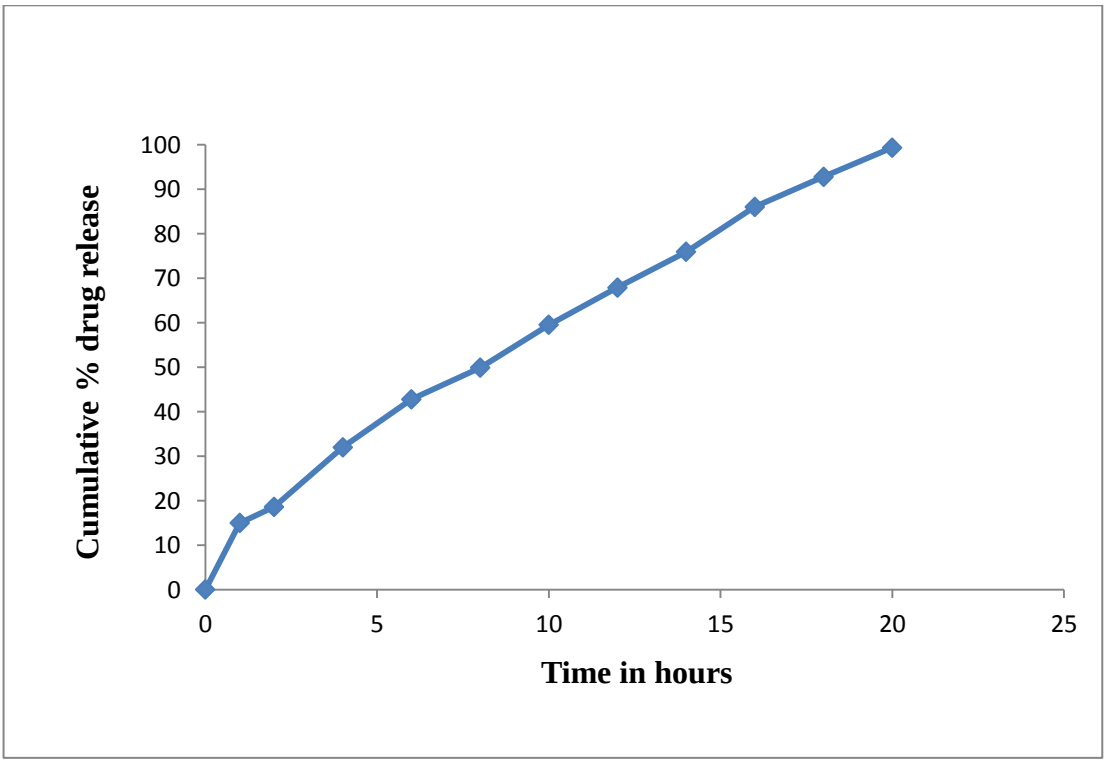

Figure 6.78 *In vitro* drug release of formulation LS603

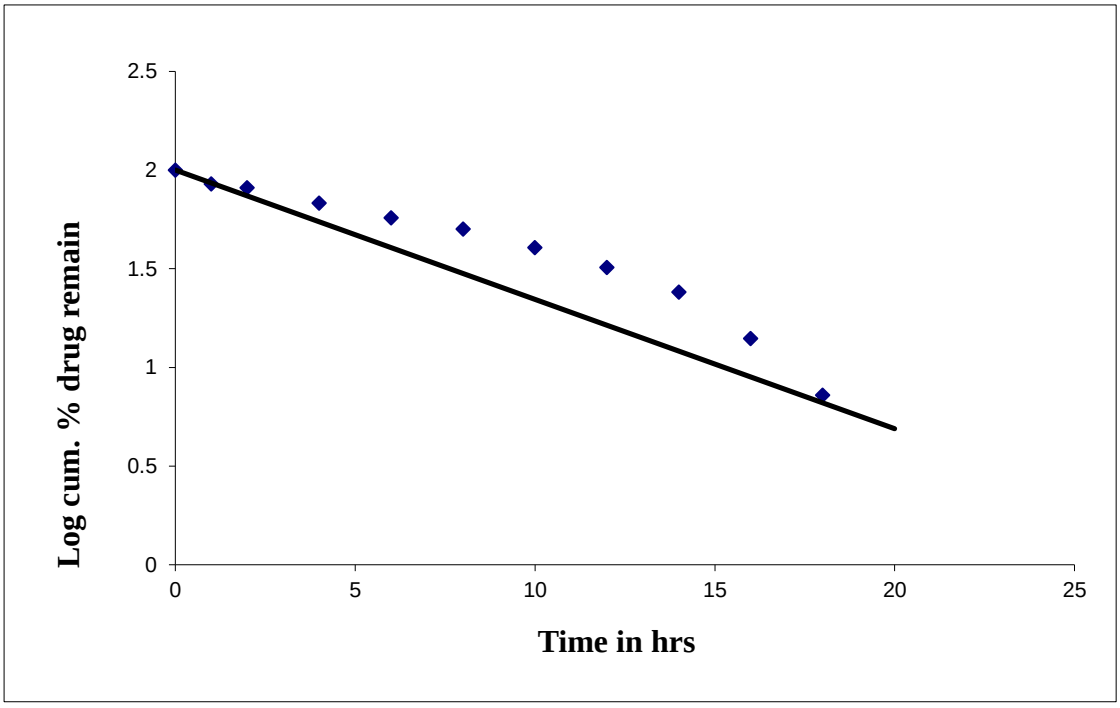

Figure 6.79 First order kinetics of formulation LS603

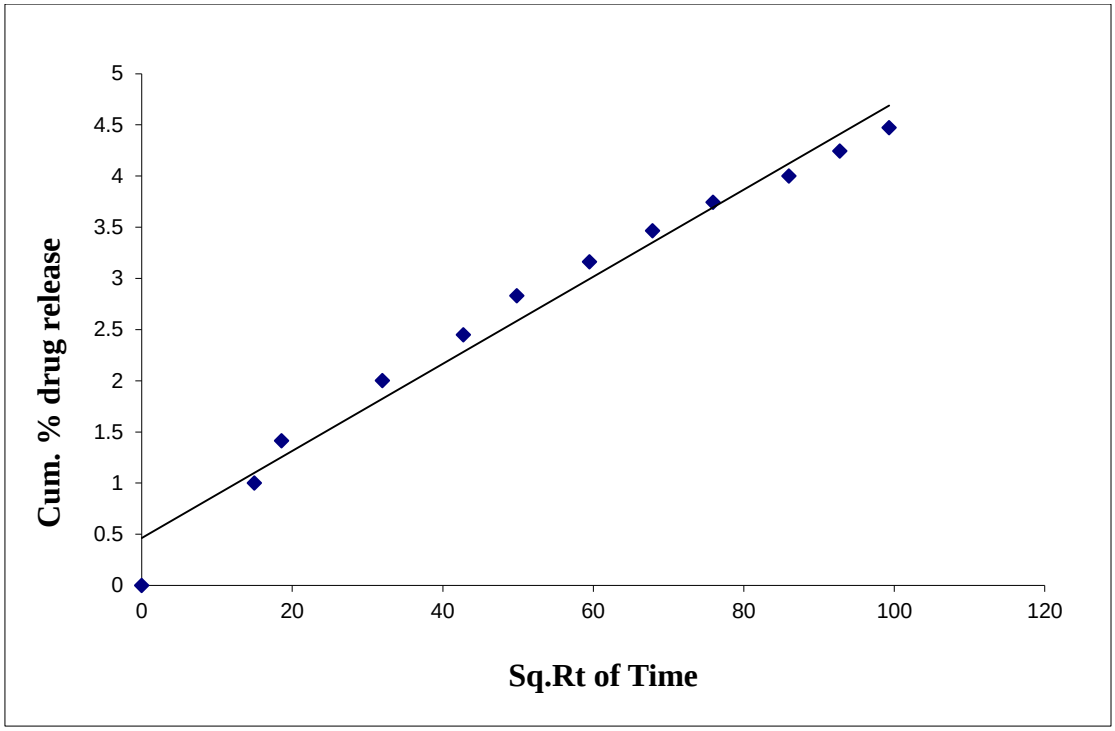

Figure 6.80 Higuchi's plot for formulation LS603

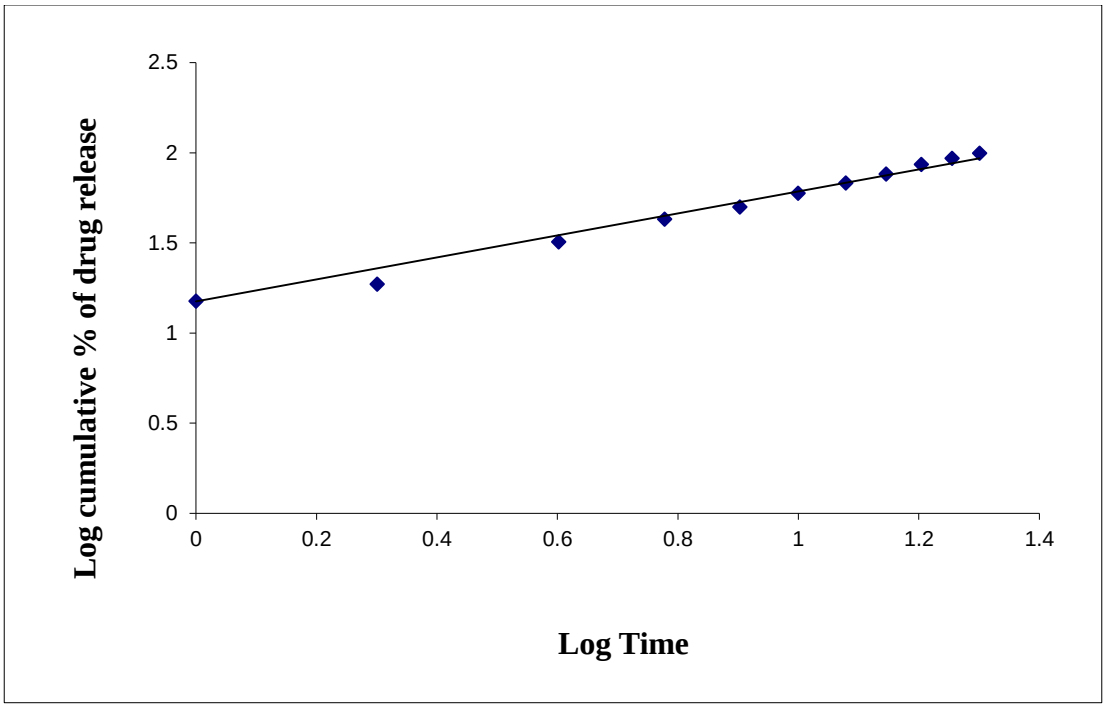

Figure 6.81 Korsmeyer & peppas plot for formulation LS603

Table 6.20 *In vitro* release study of LS 604

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.021	0.553695855	0.002768479	0.276847928	11.0739171	0	1.0443013	1.94903	1
2	0.033	0.843330221	0.004216651	0.427202069	17.08808275	0.30103	1.2326933	1.91862	1.414214
4	0.049	1.229509374	0.006147547	0.628724948	25.14899792	0.60206	1.4005207	1.8742	2
6	0.068	1.688097119	0.008440486	0.870313914	34.81255657	0.778151	1.5417359	1.81416	2.44949
8	0.083	2.050140076	0.0102507	1.068216364	42.72865455	0.90309	1.6307192	1.75794	2.828427
10	0.098	2.412183033	0.012060915	1.269739243	50.78956971	1	1.7057745	1.69206	3.162278
12	0.114	2.798362187	0.013991811	1.48695065	59.478026	1.079181	1.7743565	1.60769	3.464102
14	0.126	3.087996552	0.015439983	1.659751455	66.39005819	1.146128	1.822103	1.52647	3.741657
16	0.138	3.377630917	0.016888155	1.835448603	73.41794411	1.20412	1.8658022	1.42459	4
18	0.149	3.643129086	0.018215645	1.968197687	78.72790748	1.255273	1.8961287	1.32781	4.242641
20	0.162	3.956899648	0.019784498	2.161514259	86.46057036	1.30103	1.9368181	1.1316	4.472136
22	0.174	4.246534013	0.02123267	2.345900438	93.83601753	1.342423	1.9723696	0.78986	4.690416
24	0.183	4.463759787	0.022318799	2.496978665	99.87914661	1.380211	1.9994748	-0.9177	4.898979

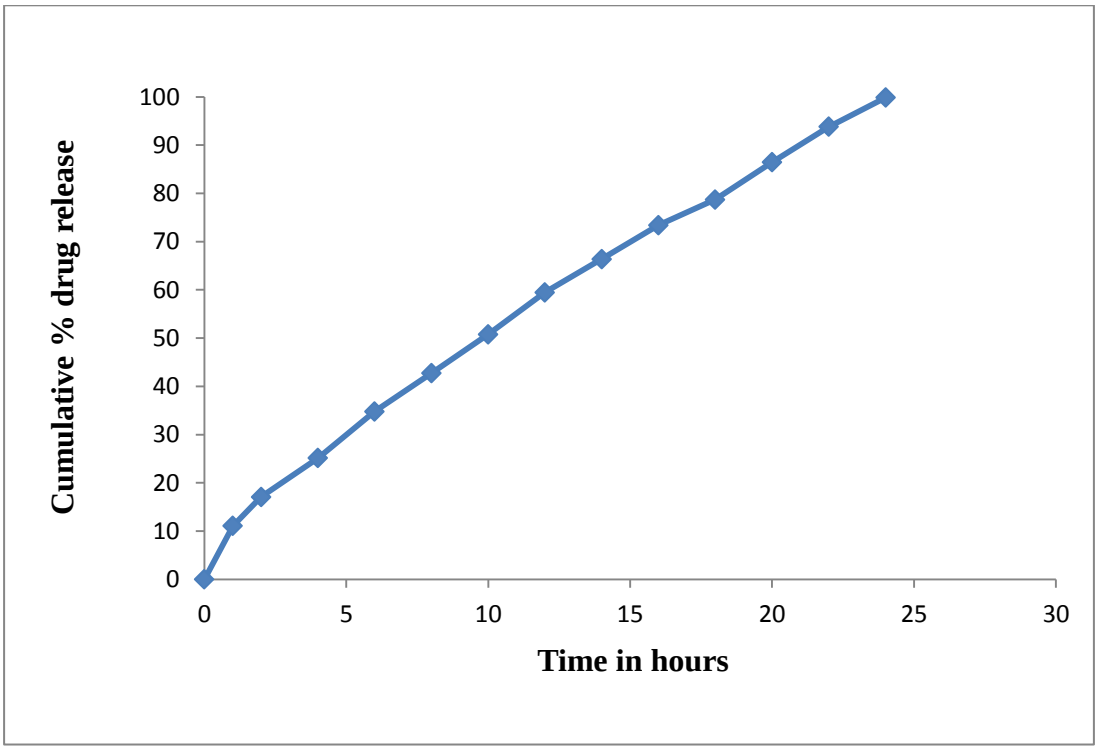

Figure 6.82 *In vitro* drug release of formulation LS604

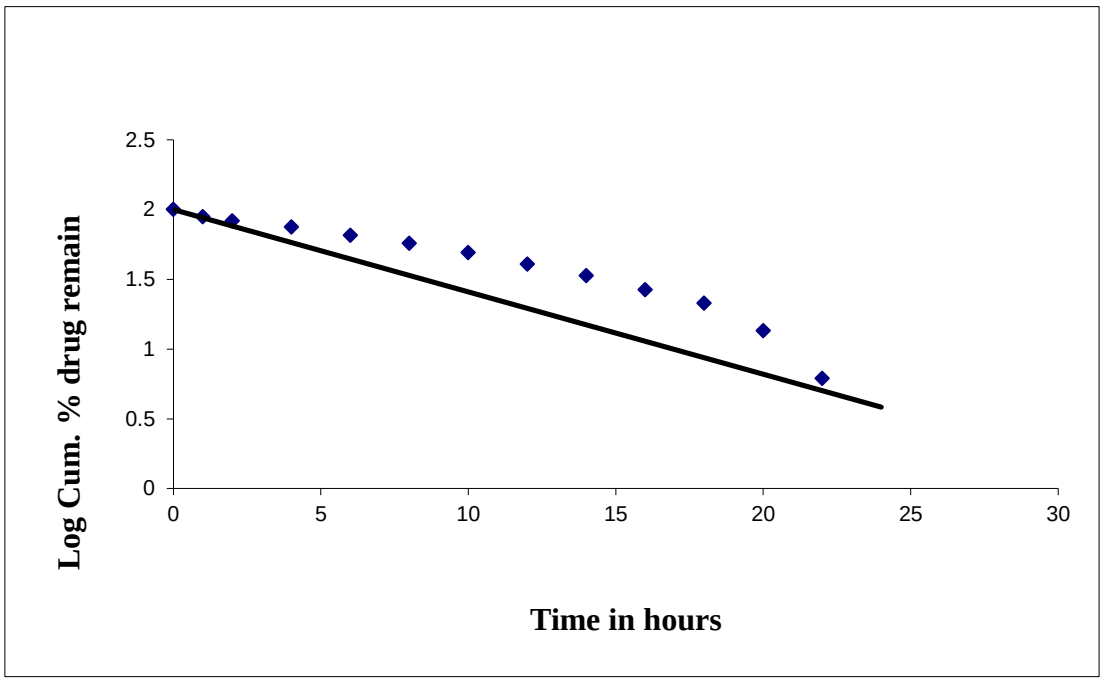

Figure 6.83 First order kinetics of formulation LS604

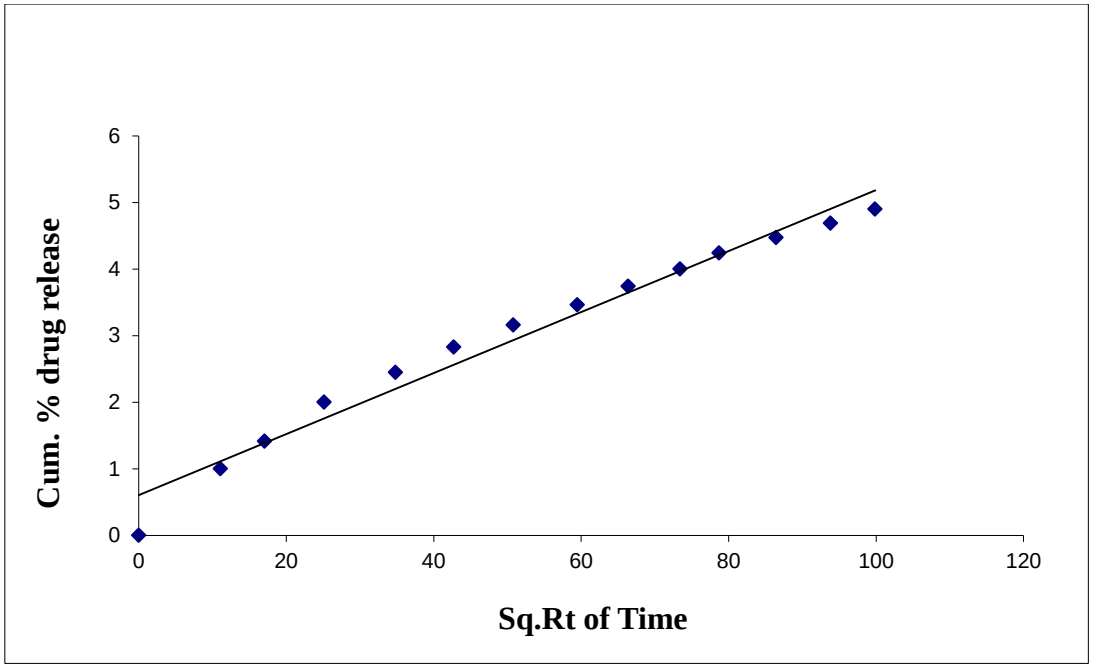

Figure 6.84 Higuchi's plot for formulation LS604

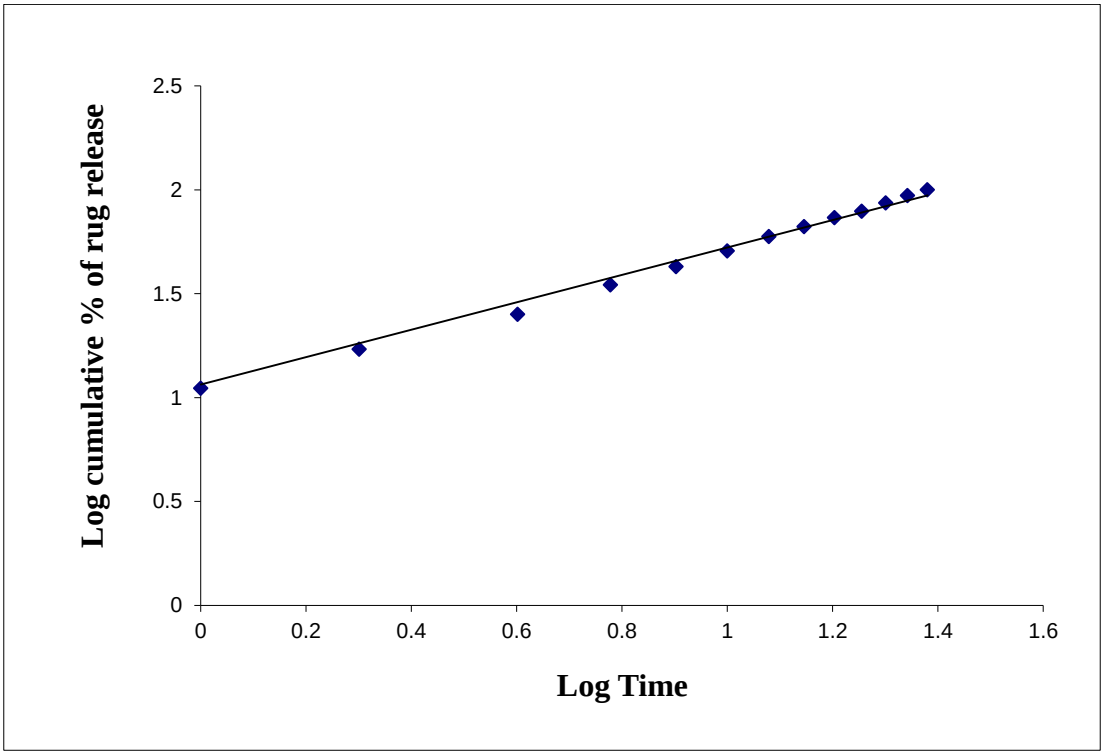

Figure 6.85 Korsmeyer & peppas plot for formulation LS604

Table 6.21 *In vitro* release study of LS 605

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.026	0.674376841	0.003371884	0.33718842	13.48753681	0	1.129932643	1.937079	1
2	0.042	1.060555995	0.00530278	0.537021766	21.48087063	0.30103	1.332051879	1.894975	1.41421356
4	0.059	1.470871345	0.007354357	0.752785001	30.11140004	0.60205999	1.478730949	1.844406	2
6	0.078	1.929459091	0.009647295	0.996787587	39.87150348	0.77815125	1.600662612	1.77908	2.44948974
8	0.088	2.170821062	0.010854105	1.136763164	45.47052654	0.90308999	1.657729983	1.736631	2.82842712
10	0.106	2.60527261	0.013026363	1.375697148	55.02788593	1	1.740582828	1.652943	3.16227766
12	0.119	2.919043172	0.014595216	1.558635156	62.34540622	1.07918125	1.794804459	1.575818	3.46410162
14	0.131	3.208677538	0.016043388	1.73264277	69.3057108	1.14612804	1.840769022	1.487058	3.74165739
16	0.144	3.5224481	0.01761224	1.921614827	76.86459306	1.20411998	1.885726332	1.364277	4
18	0.156	3.812082465	0.019060412	2.066432009	82.65728037	1.25527251	1.917281112	1.239117	4.24264069
20	0.169	4.125853028	0.020629265	2.261438115	90.4575246	1.30103	1.956444699	0.979661	4.47213595
22	0.184	4.487895984	0.02243948	2.483718124	99.34872495	1.34242268	1.997162298	-0.18624	4.69041576

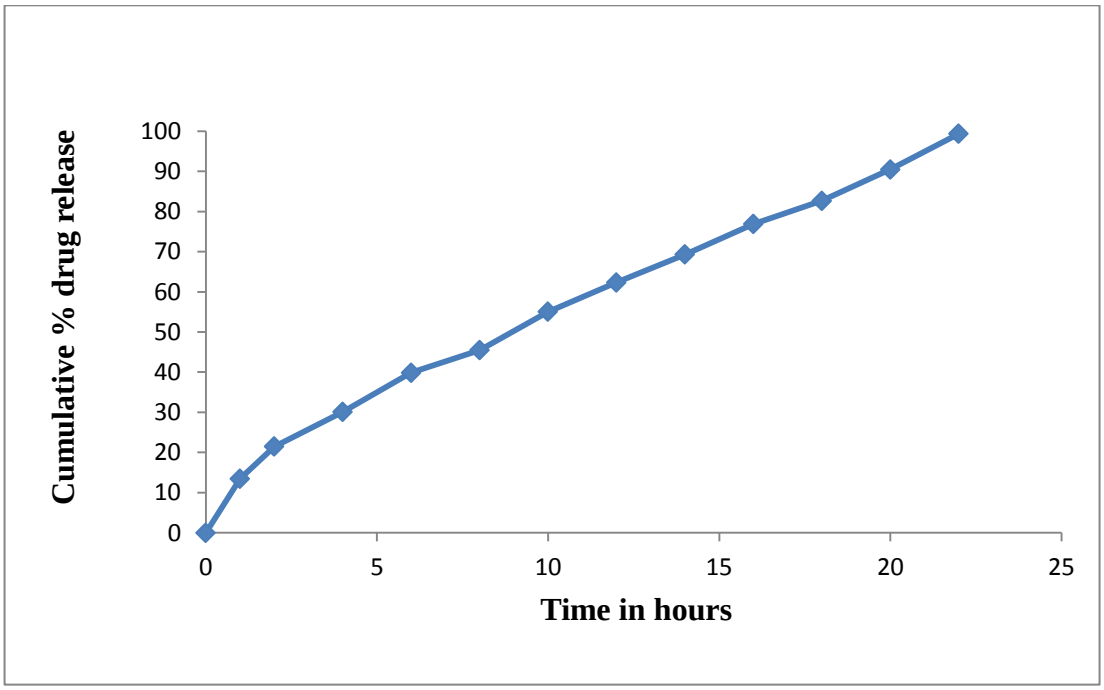

Figure 6.86 *In vitro* drug release of formulation LS605

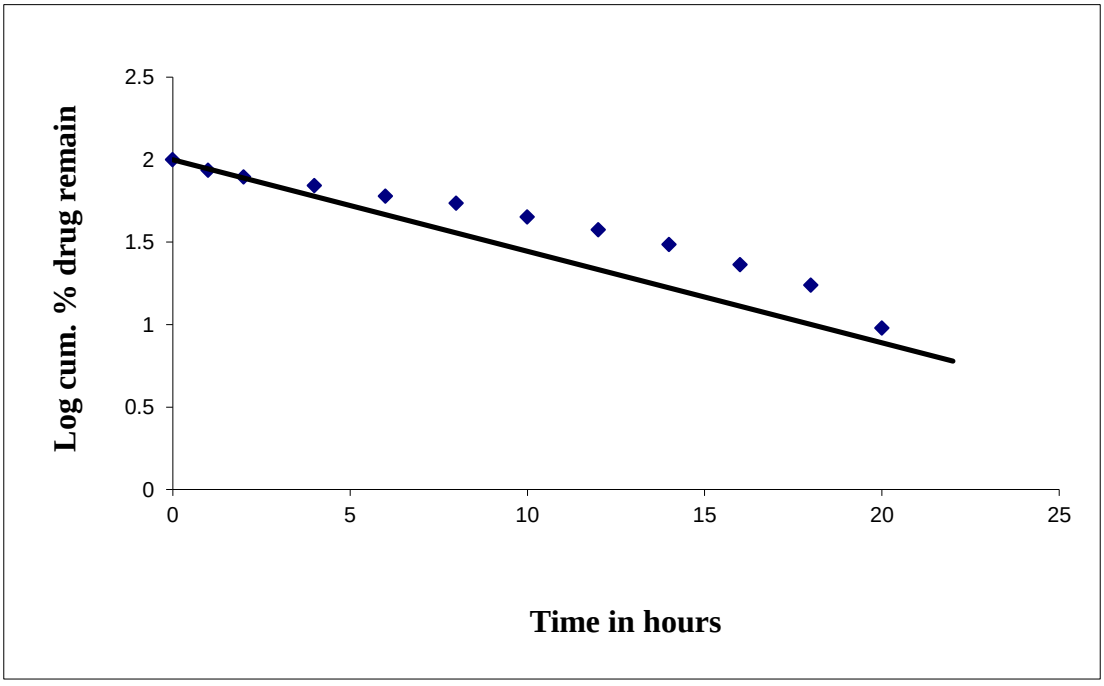

Figure 6.87 First order kinetics of formulation LS605

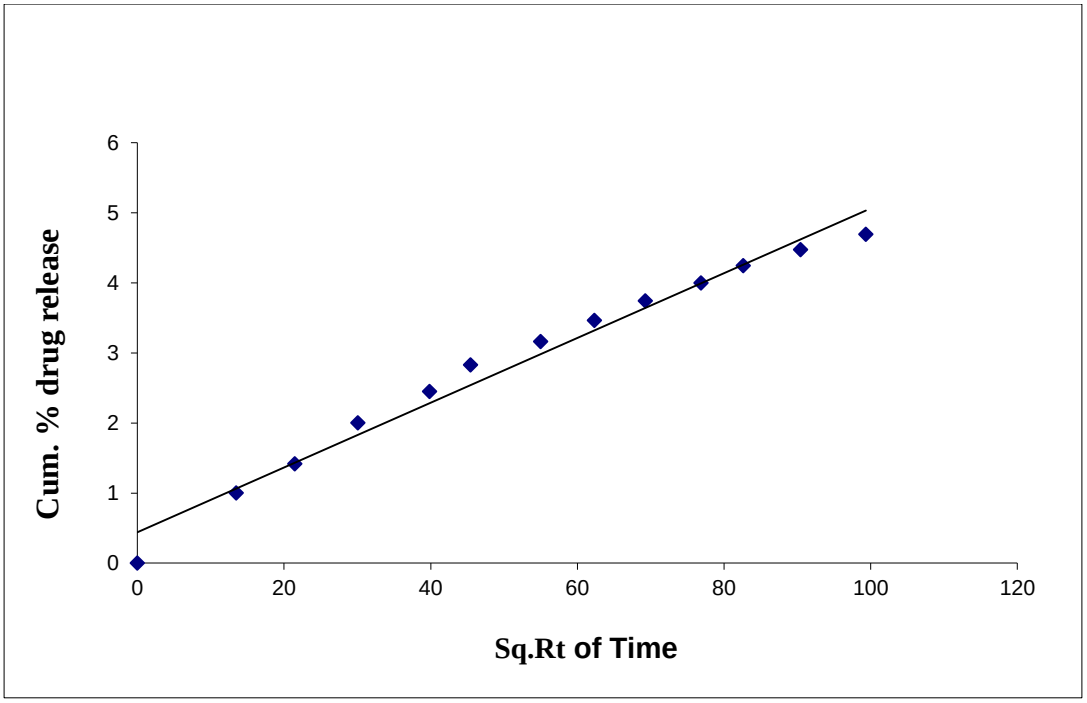

Figure 6.88 Higuchi's Plot for formulation LS605

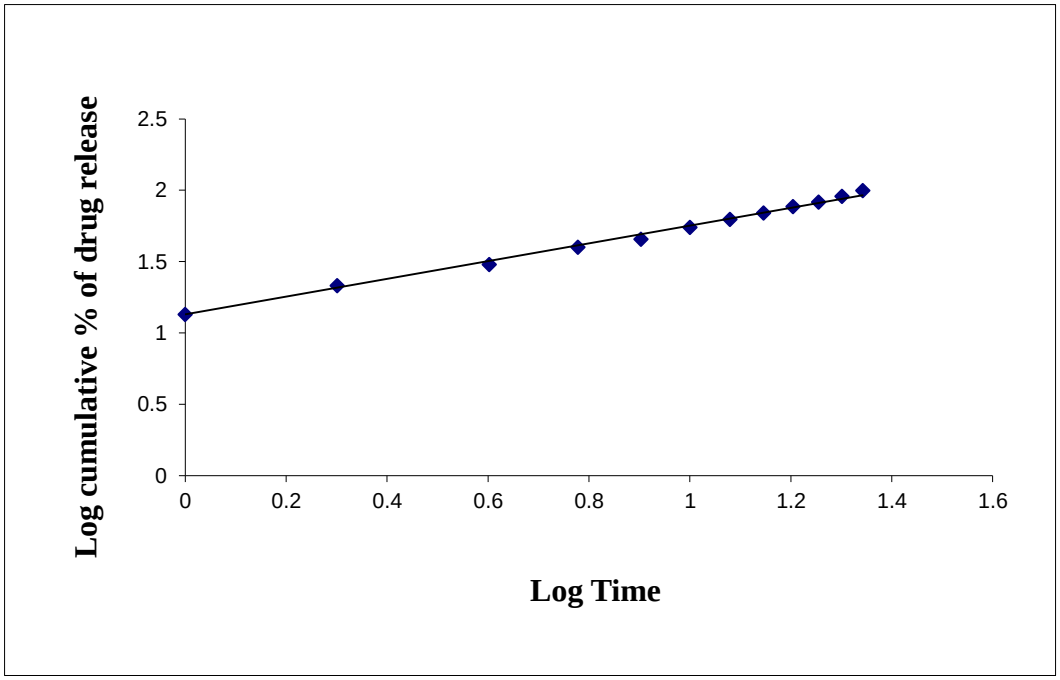

Figure 6.89 Korsmeyer & peppas plot for formulation LS605

Table 6.22 *In vitro* release study of LS 606

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.030	0.770921629	0.003854608	0.385460815	15.41843258	0	1.1880402	1.92728	1
2	0.041	1.036419797	0.005182099	0.525919115	21.0367646	0.30103	1.3229789	1.89742	1.414214
4	0.049	1.229509374	0.006147547	0.632828101	25.31312406	0.60206	1.4033457	1.87324	2
6	0.064	1.591552331	0.007957762	0.826144674	33.04578694	0.778151	1.5191161	1.82578	2.44949
8	0.093	2.291502047	0.01145751	1.192035055	47.6814022	0.90309	1.678349	1.71866	2.828427
10	0.108	2.653545004	0.013267725	1.395971554	55.83886215	1	1.7469366	1.64504	3.162278
12	0.126	3.087996552	0.015439983	1.639732778	65.58931111	1.079181	1.8168331	1.53669	3.464102
14	0.140	3.425903312	0.017129517	1.839566123	73.58264492	1.146128	1.8667754	1.42189	3.741657
16	0.153	3.739673874	0.018698369	2.030710437	81.2284175	1.20412	1.909708	1.2735	4
18	0.171	4.174125422	0.020870627	2.247936211	89.91744846	1.255273	1.953844	1.00357	4.242641
20	0.186	4.536168379	0.022680842	2.470698944	98.82795776	1.30103	1.9948798	0.06894	4.472136

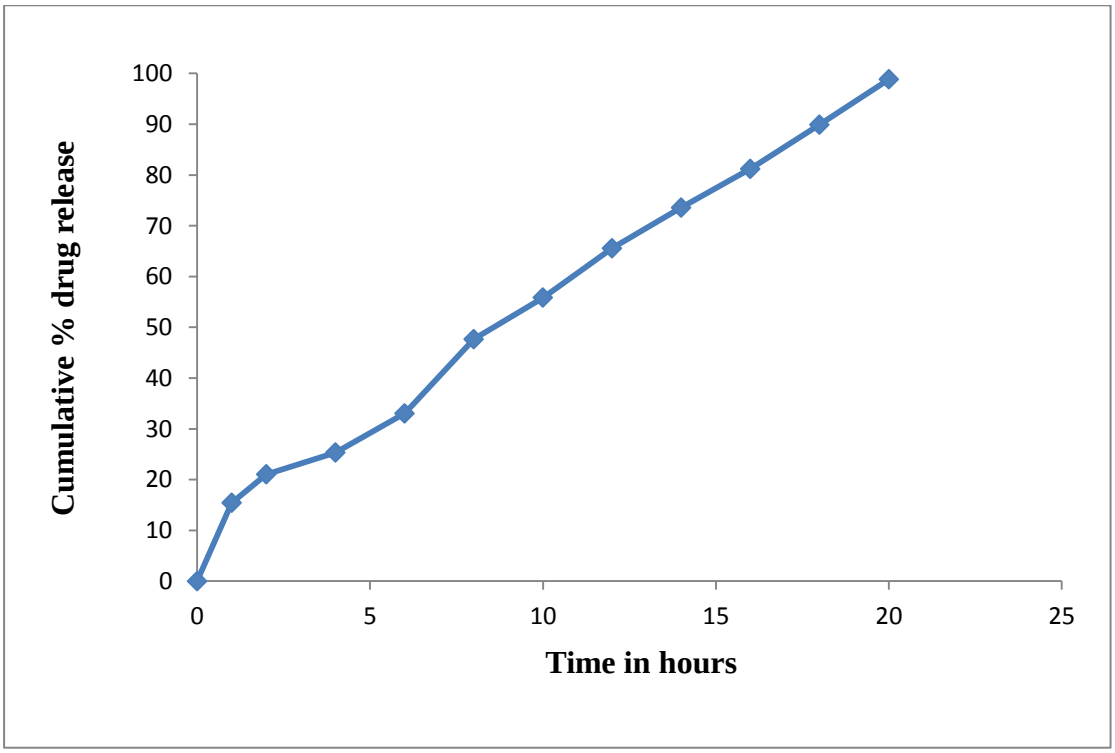

Figure 6.90 *In vitro* drug release of formulation LS606

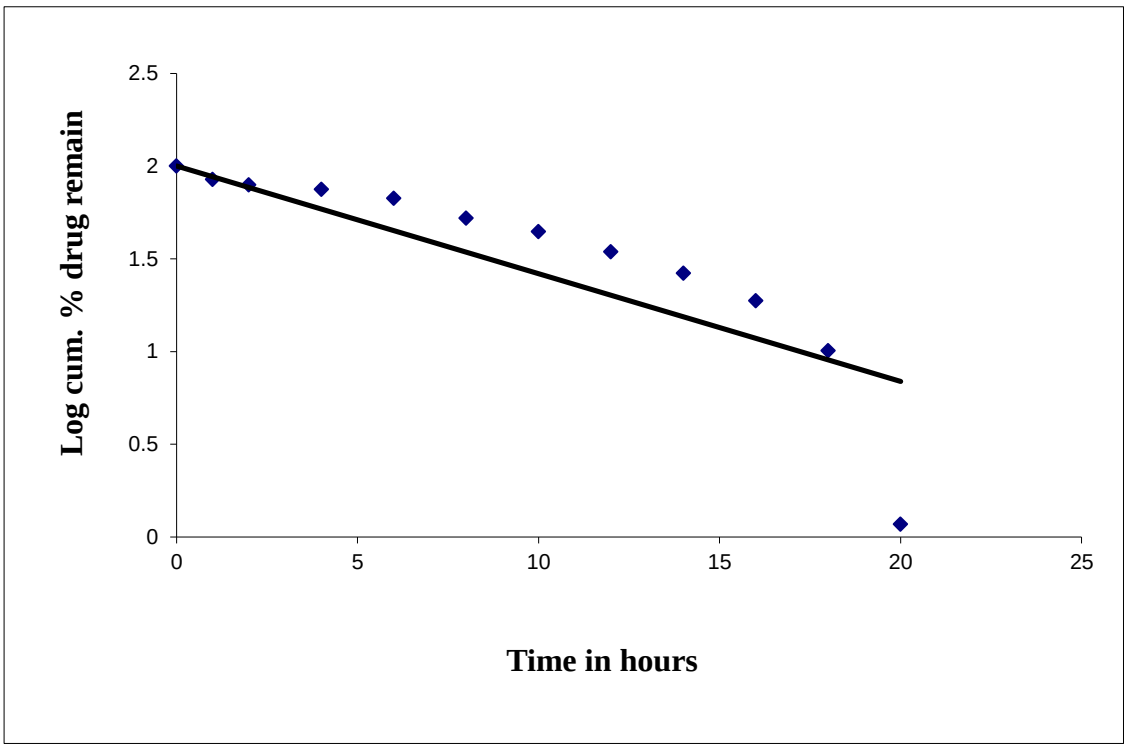

Figure 6.91 First order kinetics of formulation LS606

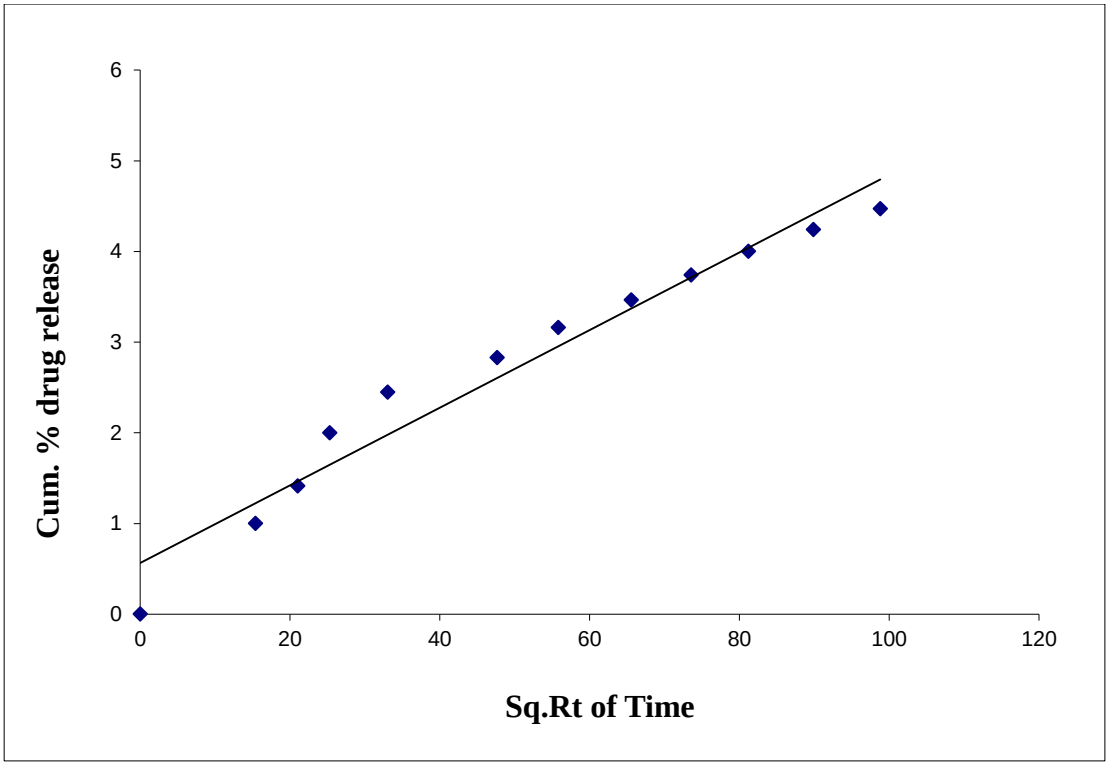

Figure 6.92 Higuchi's plot for formulation LS606

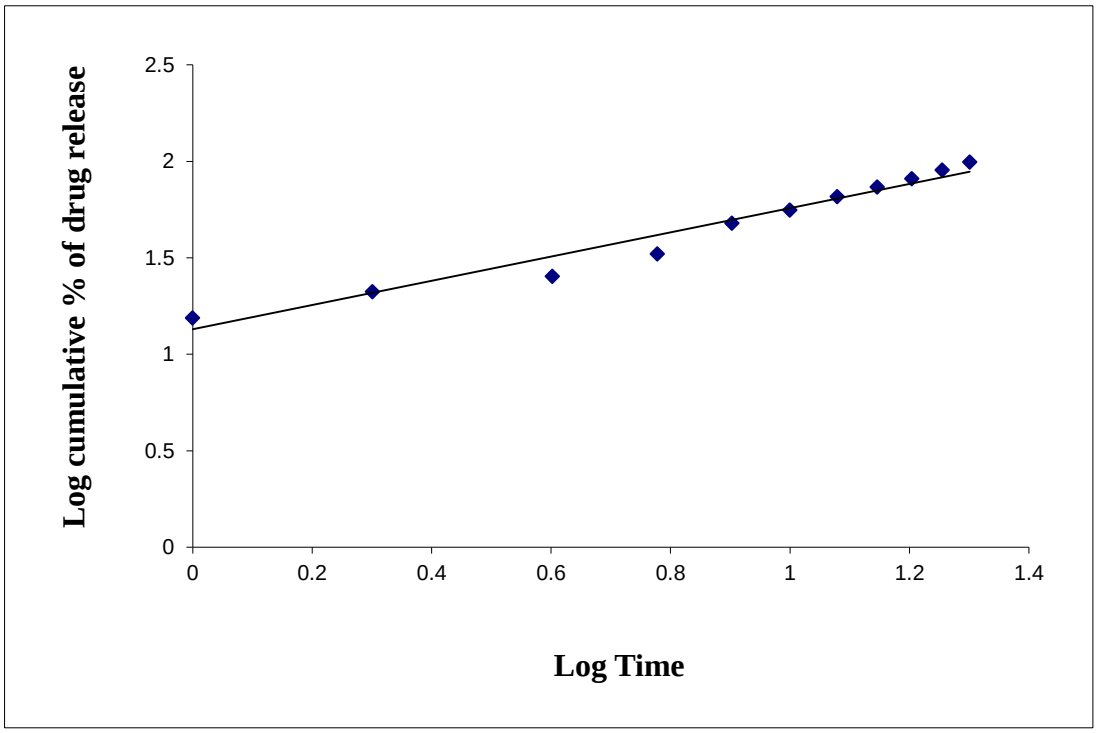

Figure 6.93 Korsmeyer & peppas plot for formulation LS606

Table 6.23 *In vitro* release study of LS 607

Time in hrs	absorbance	concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.013	0.360606278	0.001803031	0.180303139	7.212125566	0	0.858063279	1.967491	1
2	0.028	0.722649235	0.003613246	0.36493068	14.59722721	0.30103	1.164270368	1.931472	1.41421356
4	0.049	1.229509374	0.006147547	0.625587242	25.02348969	0.60205999	1.398347875	1.874925	2
6	0.069	1.712233317	0.008561167	0.879244307	35.16977229	0.77815125	1.546169556	1.811778	2.44948974
8	0.101	2.484591624	0.012422958	1.282545794	51.30183176	0.90308999	1.710132872	1.687513	2.82842712
10	0.128	3.136268946	0.015681345	1.633230371	65.32921486	1	1.815107439	1.539964	3.16227766
12	0.140	3.425903312	0.017129517	1.809410244	72.37640974	1.07918125	1.859597036	1.44128	3.46410162
14	0.152	3.715537677	0.018577688	1.988486459	79.53945837	1.14612804	1.900582629	1.310917	3.74165739
18	0.190	4.632713167	0.023163566	2.484229581	99.36918325	1.25527251	1.99725172	-0.2001	4.24264069

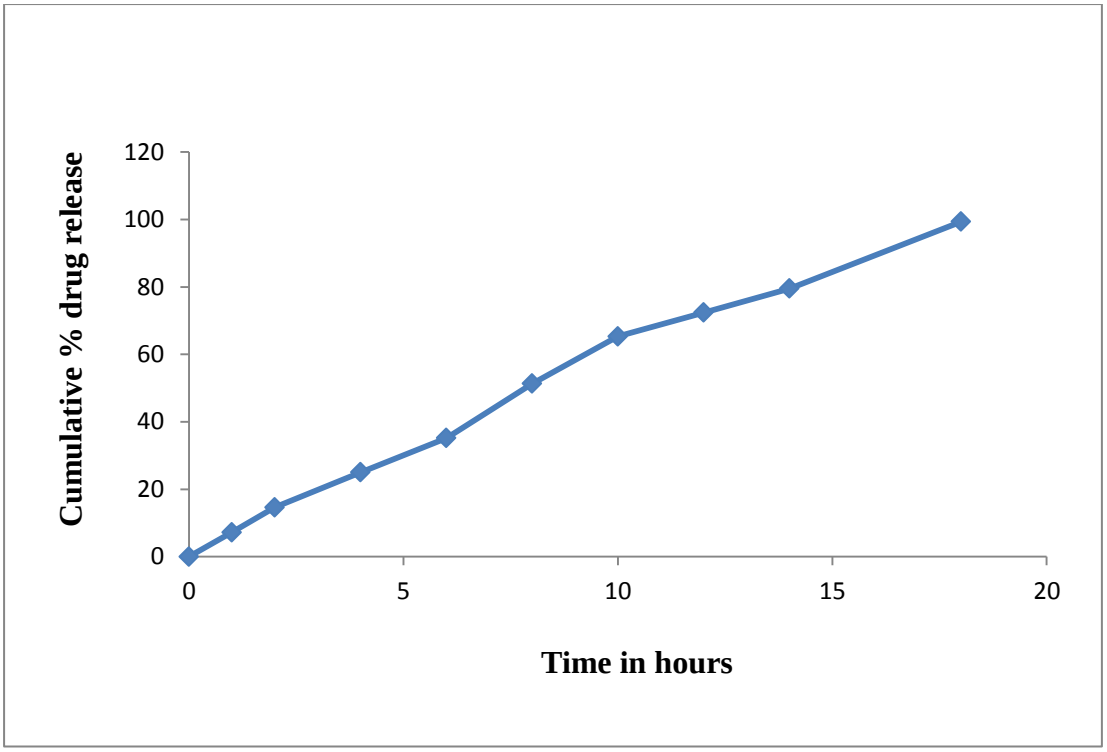

Figure 6.94 *In vitro* drug release of formulation LS607

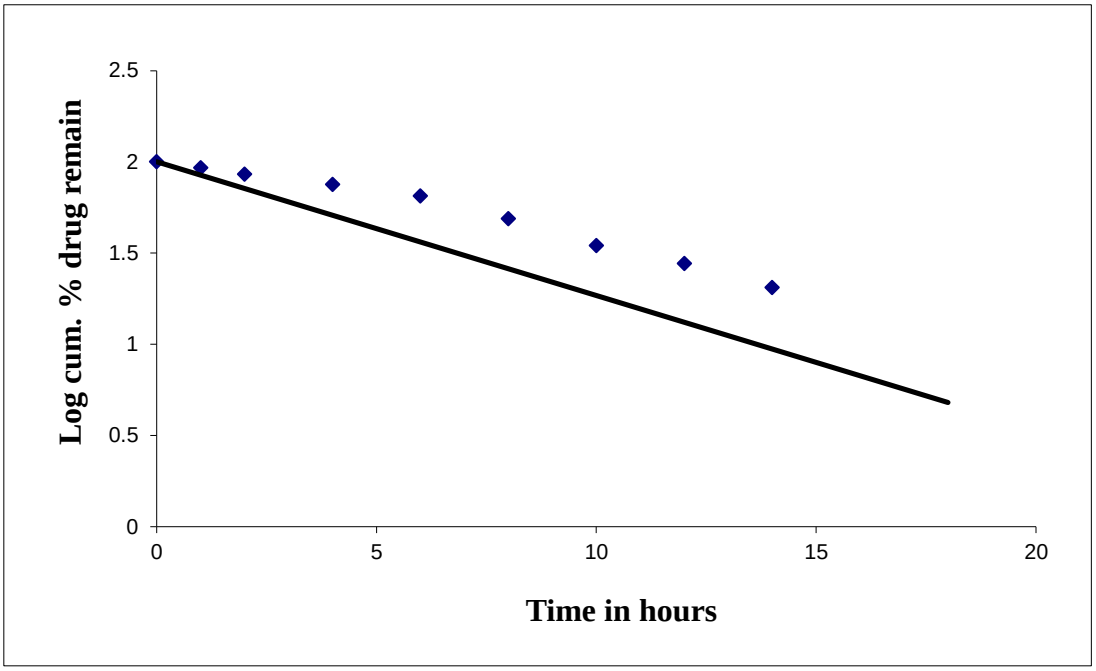

Figure 6.95 First order kinetics of formulation LS607

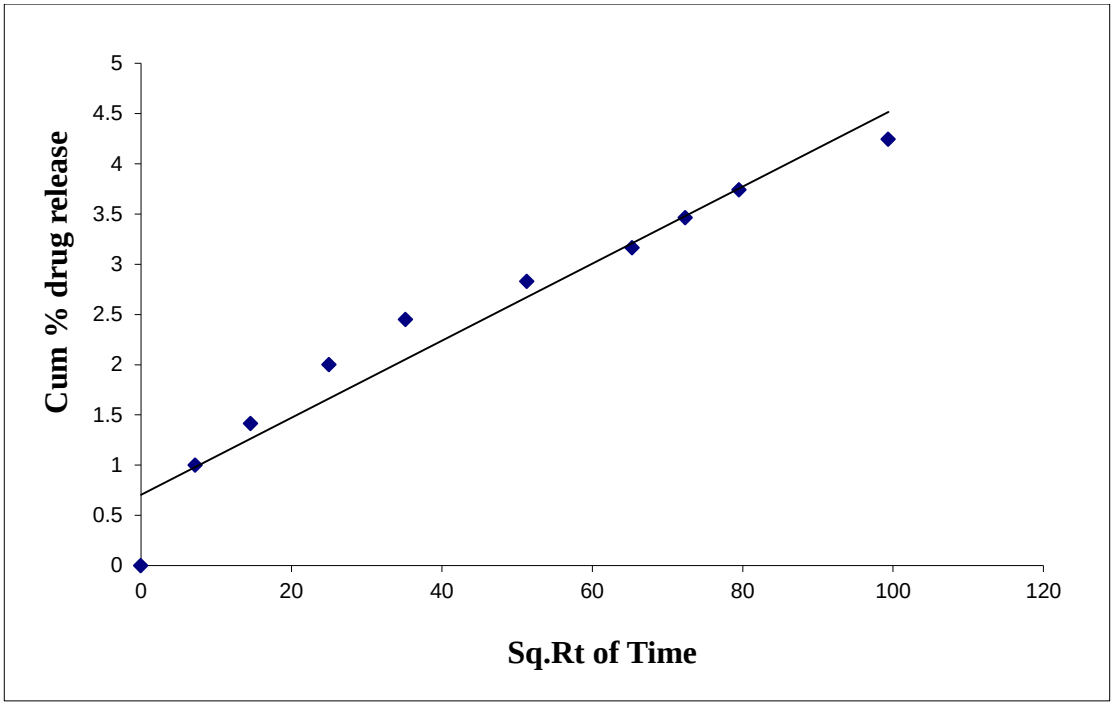

Figure 6.96 Higuchi's plot for formulation LS607

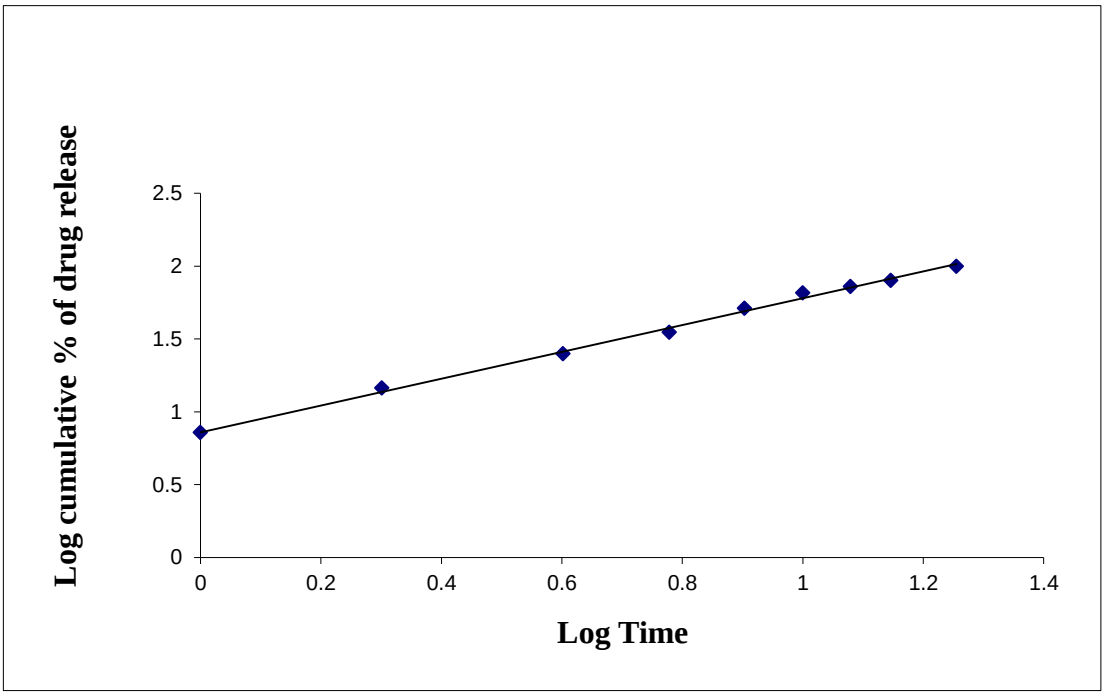

Figure 6.97 Korsmeyer & peppas plot for formulation LS607

Table 6.24 *In vitro* release study of LS 608

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.018	0.481287264	0.002406436	0.240643632	9.625745277	0	0.9834344	1.95604	1
2	0.032	0.819194023	0.00409597	0.414409884	16.57639537	0.30103	1.2194901	1.92129	1.414214
4	0.054	1.35019036	0.006750952	0.688099993	27.52399971	0.60206	1.4397115	1.86019	2
6	0.069	1.712233317	0.008561167	0.882623375	35.30493499	0.778151	1.5478354	1.81087	2.44949
8	0.089	2.194957259	0.010974786	1.141107679	45.64430716	0.90309	1.6593866	1.73525	2.828427
10	0.128	3.136268946	0.015681345	1.633713095	65.34852381	1	1.8152358	1.53972	3.162278
12	0.146	3.570720494	0.017853602	1.882301559	75.29206235	1.079181	1.8767492	1.39284	3.464102
14	0.159	3.884491057	0.019422455	2.074894045	82.9957618	1.146128	1.9190559	1.23056	3.741657
18	0.190	4.632713167	0.023163566	2.487850011	99.51400043	1.255273	1.9978842	-0.3134	4.242641

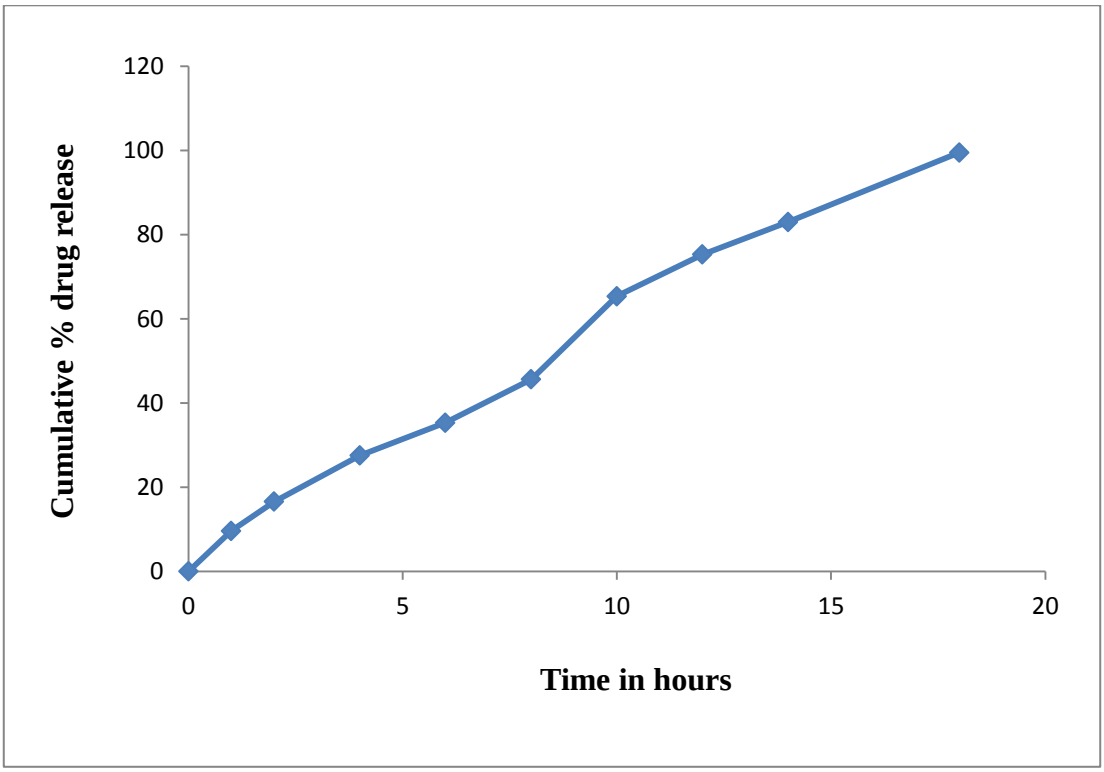

Figure 6.98 *In vitro* drug release of formulation LS608

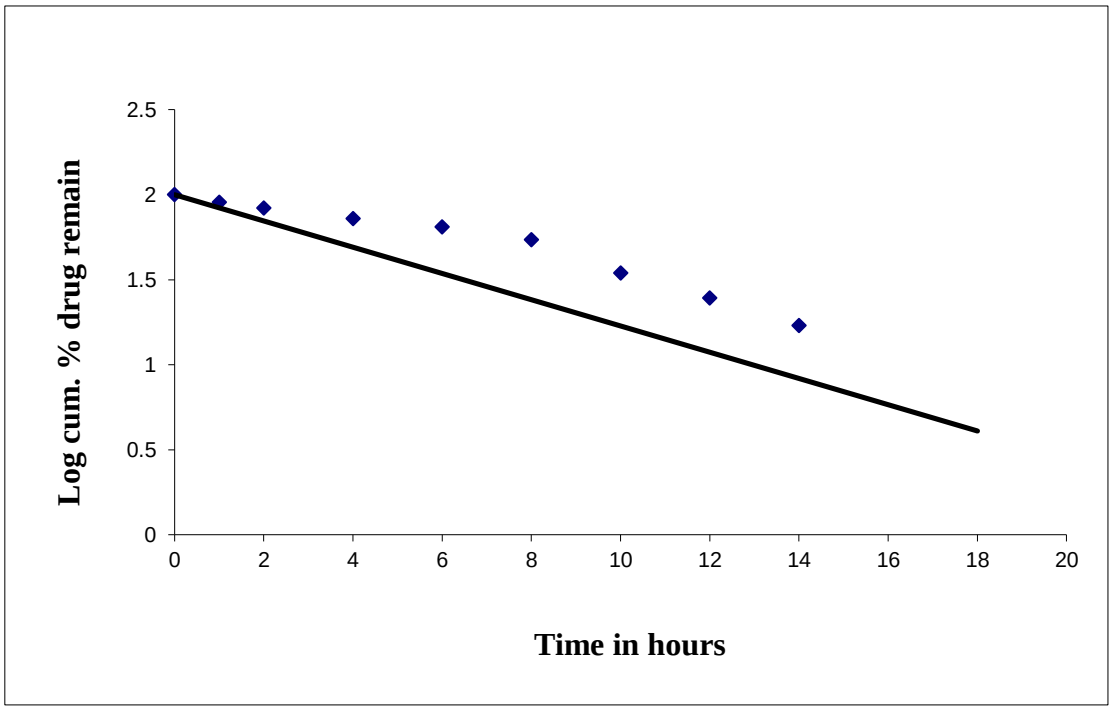

Figure 6.99 First order kinetics of formulation LS608

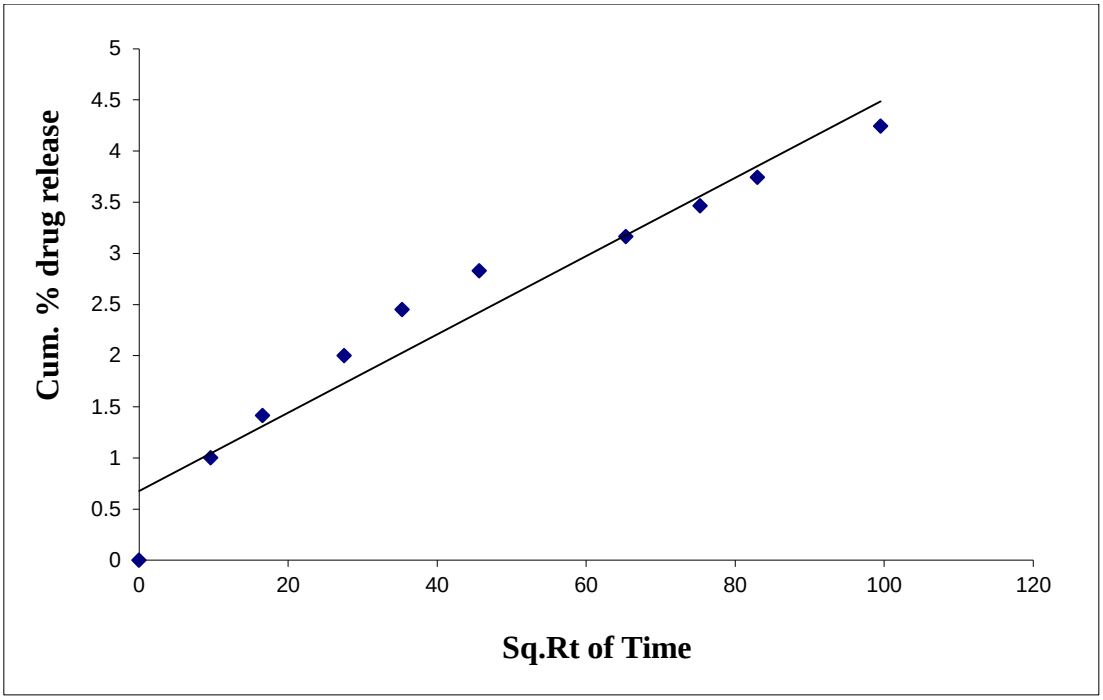

Figure 6.100 Higuchi's plot for formulation LS608

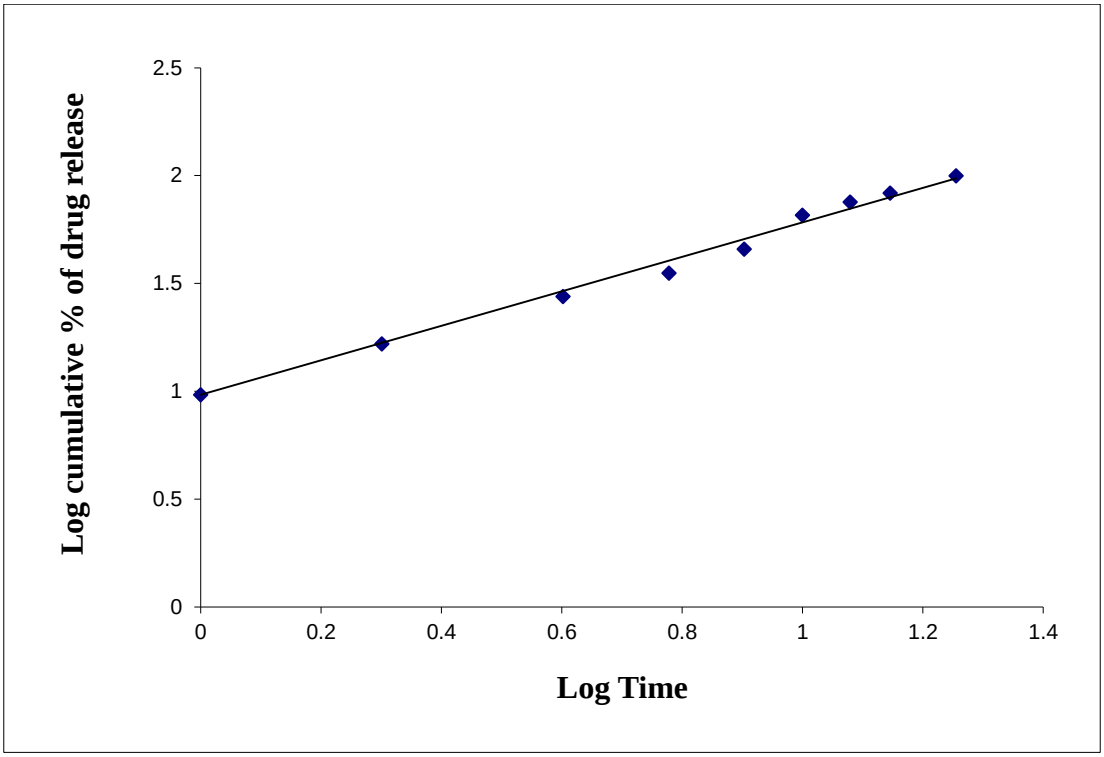

Figure 6.101 Korsmeyer & peppas plot for formulation LS608

Table 6.25 *In vitro* release study of LS 609

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.012	0.336470081	0.00168235	0.168235041	6.729401623	0	0.827976449	1.969745	1
2	0.028	0.722649235	0.003613246	0.364689318	14.58757273	0.30103	1.163983034	1.931521	1.41421356
4	0.043	1.084692192	0.005423461	0.552937289	22.11749156	0.60205999	1.34473587	1.89144	2
6	0.076	1.881186696	0.009405933	0.962031463	38.48125853	0.77815125	1.585249267	1.789007	2.44948974
8	0.094	2.315638244	0.011578191	1.198069104	47.92276417	0.90308999	1.68054186	1.716648	2.82842712
10	0.130	3.18454134	0.015922707	1.655677035	66.22708139	1	1.821035616	1.528569	3.16227766
12	0.151	3.69140148	0.018457007	1.940952518	77.63810071	1.07918125	1.890074903	1.349509	3.46410162
14	0.168	4.101716831	0.020508584	2.183024208	87.32096832	1.14612804	1.941118543	1.103086	3.74165739
18	0.190	4.632713167	0.023163566	2.489539545	99.58158178	1.25527251	1.99817902	-0.37839	4.24264069

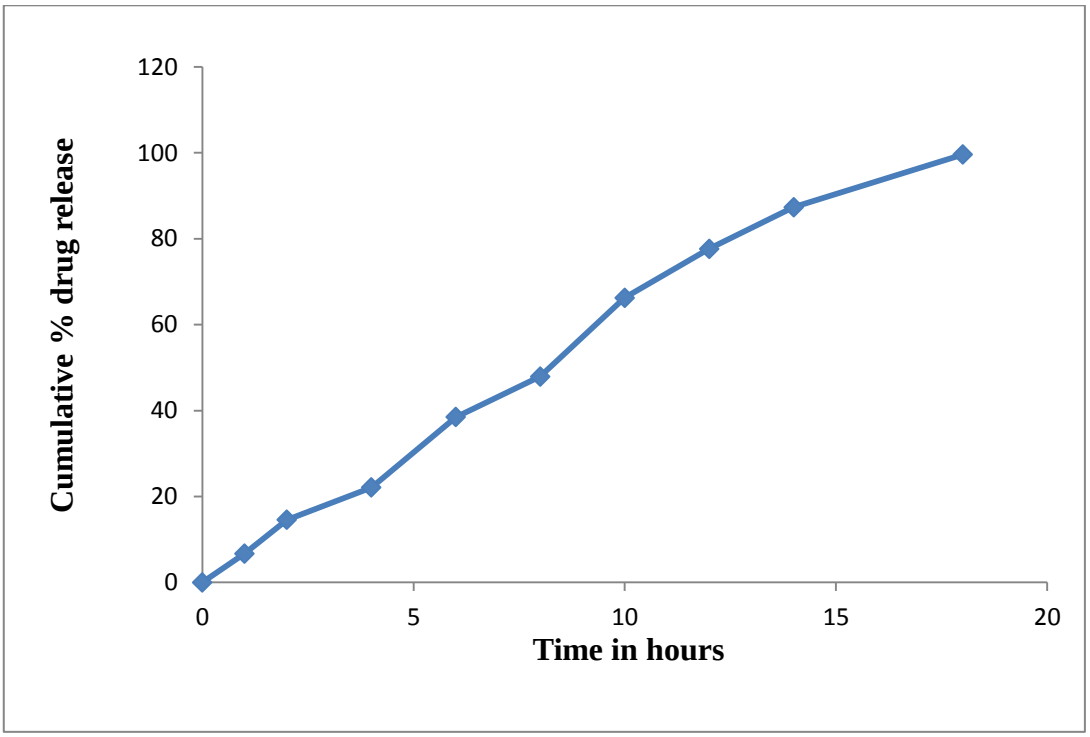

Figure 6.102 *In vitro* drug release of formulation LS609

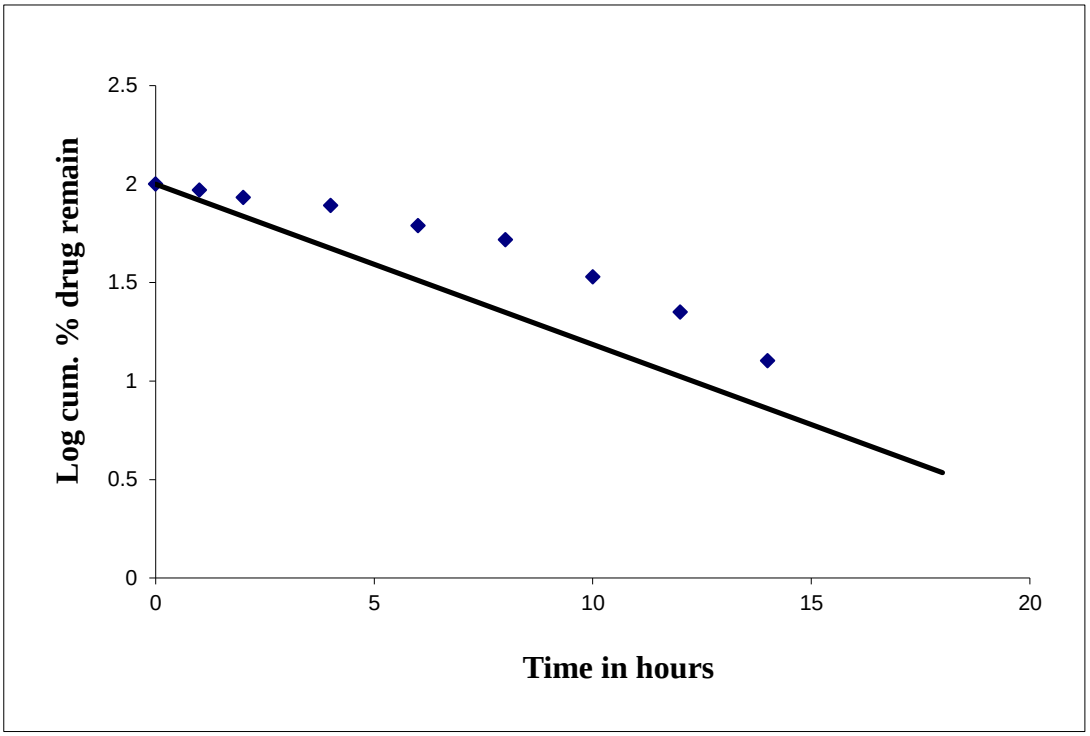

Figure 6.103 First order kinetics of formulation LS609

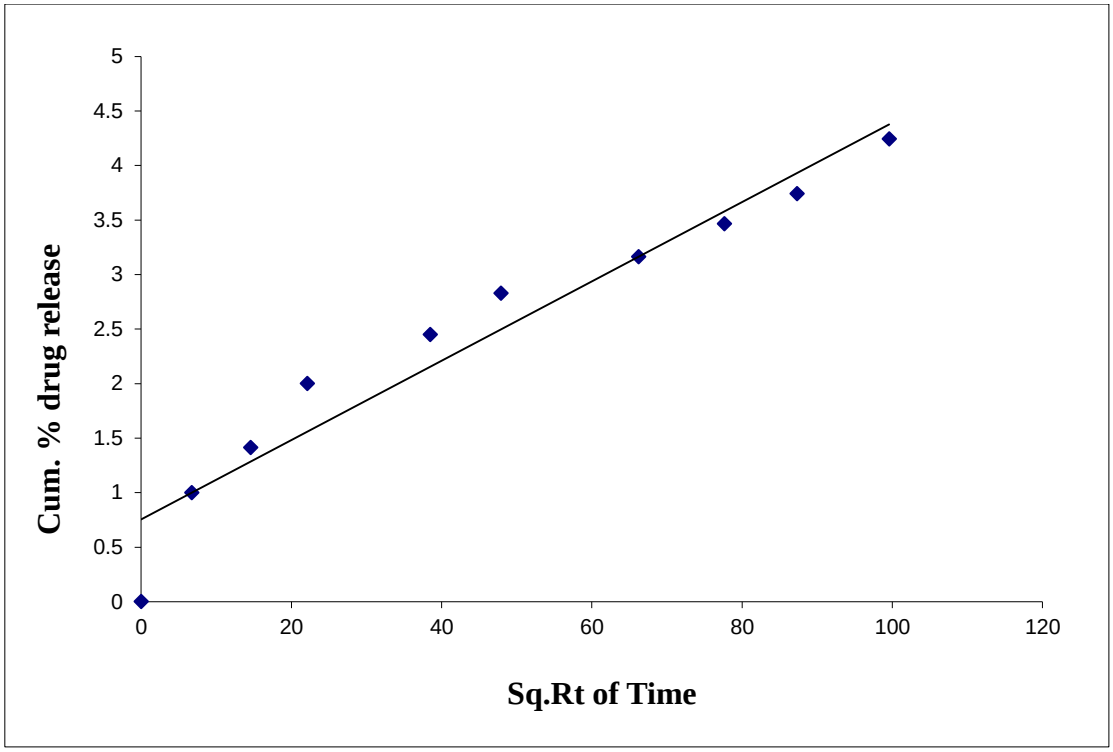

Figure 6.104 Higuchi's plot for formulation LS209

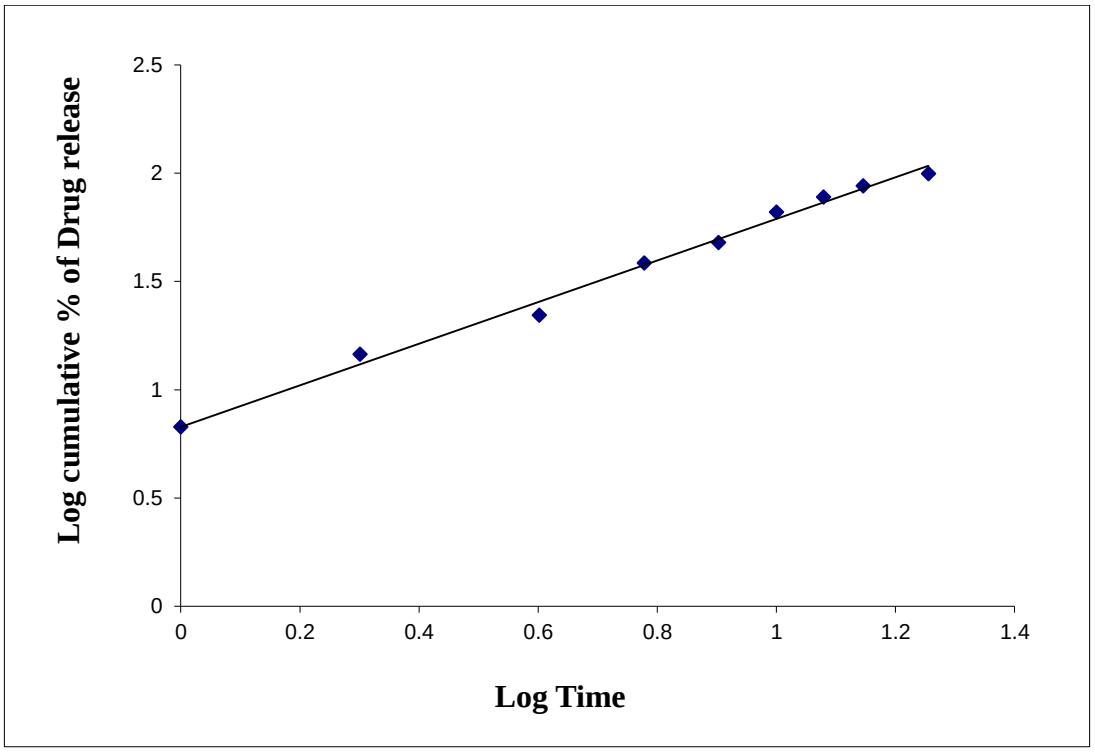

Figure 6.105 Korsmeyer & peppas plot for formulation LS609

Table 6.26 *In vitro* release study of LS 610

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.020	0.529559658	0.002647798	0.264779829	10.59119316	0	1.024944889	1.95138	1
2	0.034	0.867466418	0.004337332	0.439028805	17.56115222	0.30103	1.244553007	1.916132	1.41421356
4	0.058	1.446735148	0.007233676	0.737337835	29.4935134	0.60205999	1.469726511	1.848229	2
6	0.094	2.315638244	0.011578191	1.186256734	47.45026938	0.77815125	1.676238682	1.72057	2.44948974
8	0.126	3.087996552	0.015439983	1.595592271	63.82369083	0.90308999	1.804981915	1.558424	2.82842712
10	0.154	3.763810071	0.01881905	1.964378996	78.57515983	1	1.895285273	1.330918	3.16227766
12	0.172	4.198261619	0.020991308	2.21924287	88.76971482	1.07918125	1.948264825	1.050391	3.46410162
14	0.190	4.632713167	0.023163566	2.478451261	99.13805043	1.14612804	1.996240374	-0.06452	3.74165739

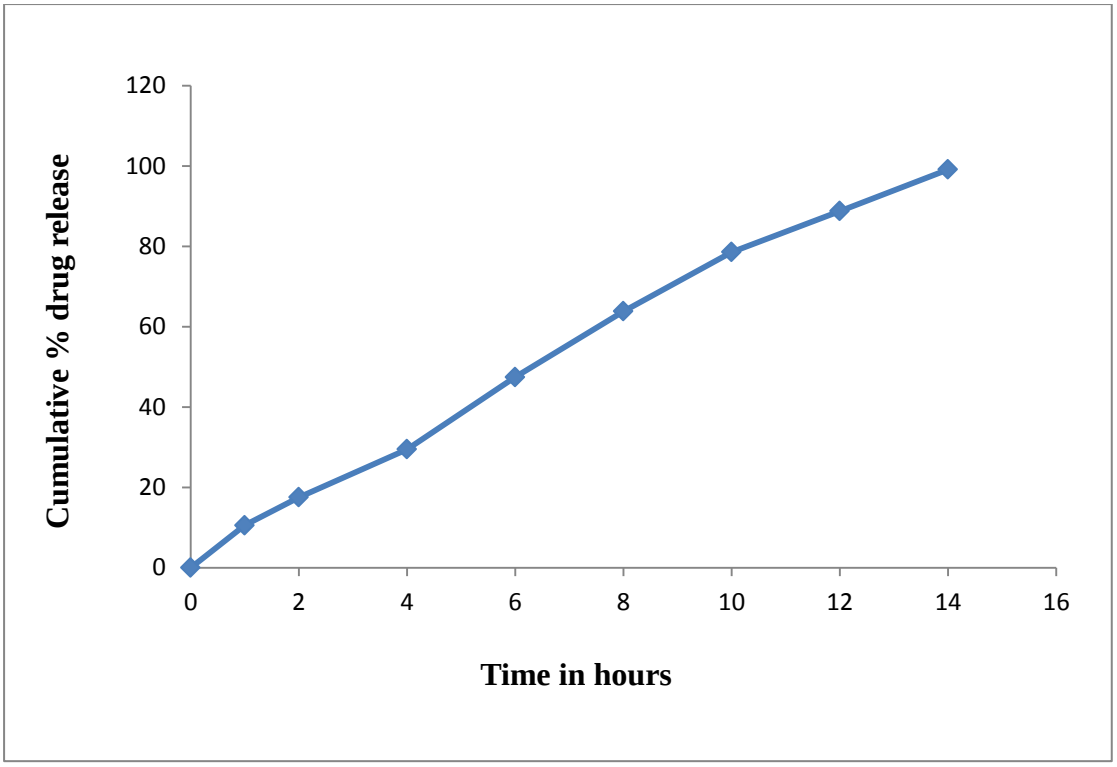

Figure 6.106 *In vitro* drug release of formulation LS610

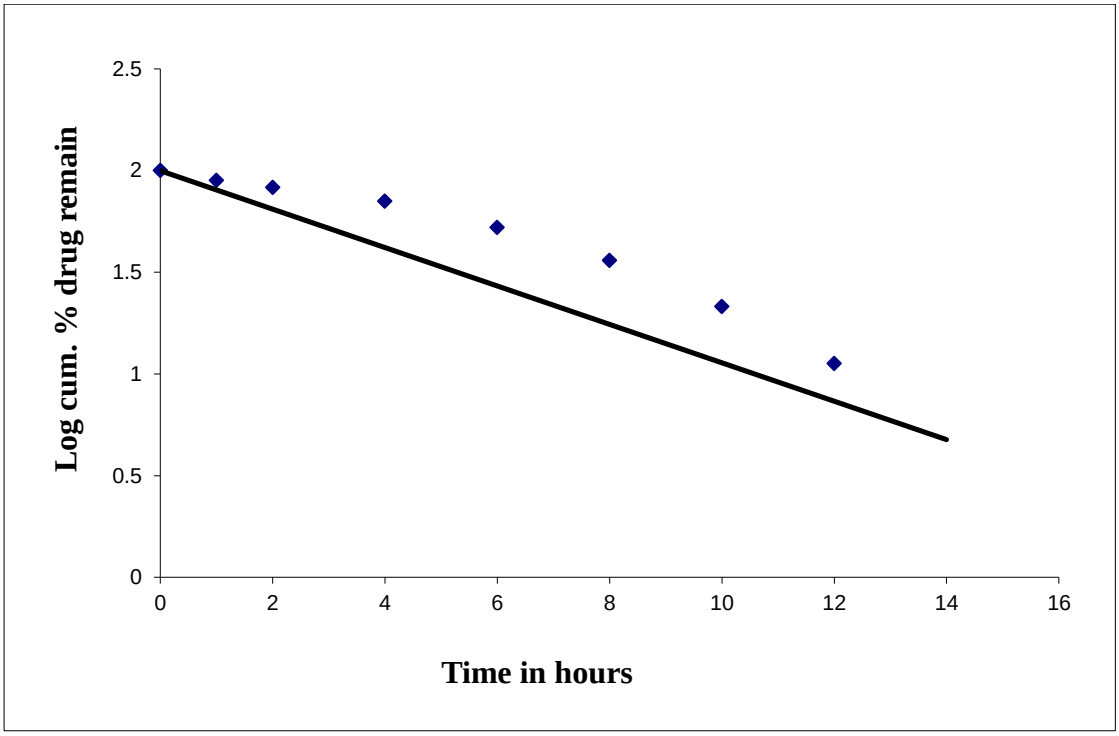

Figure 6.107 First order kinetics of formulation LS610

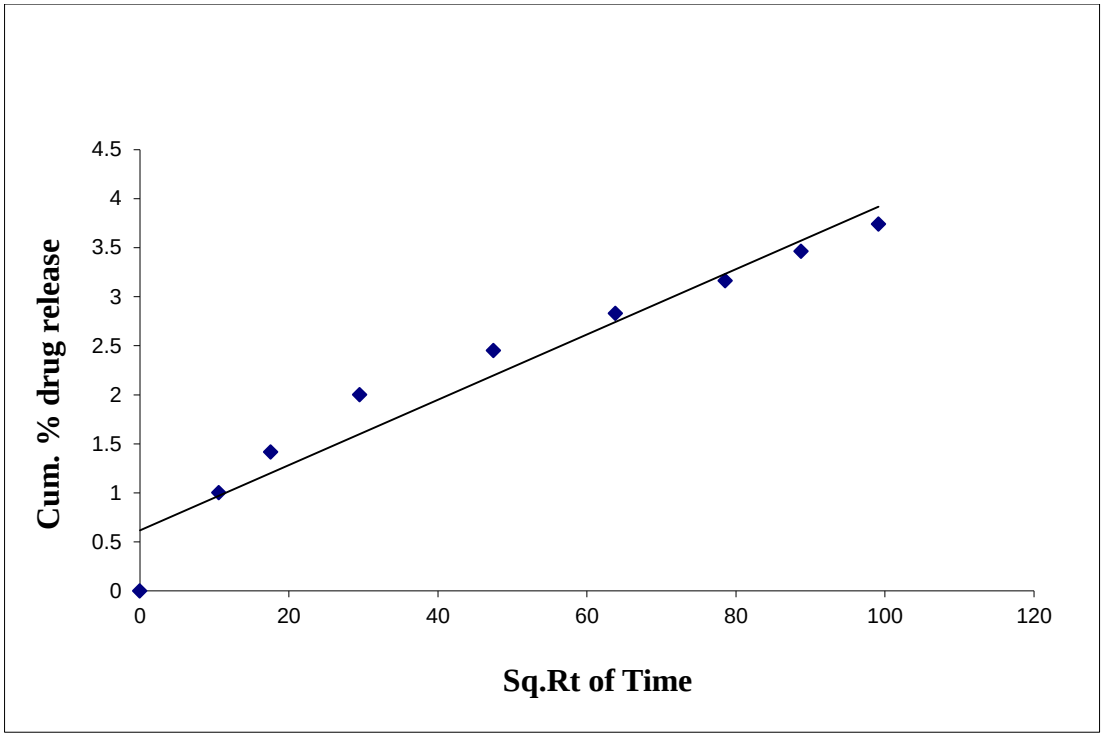

Figure 6.108 Higuchi's plot for formulation LS610

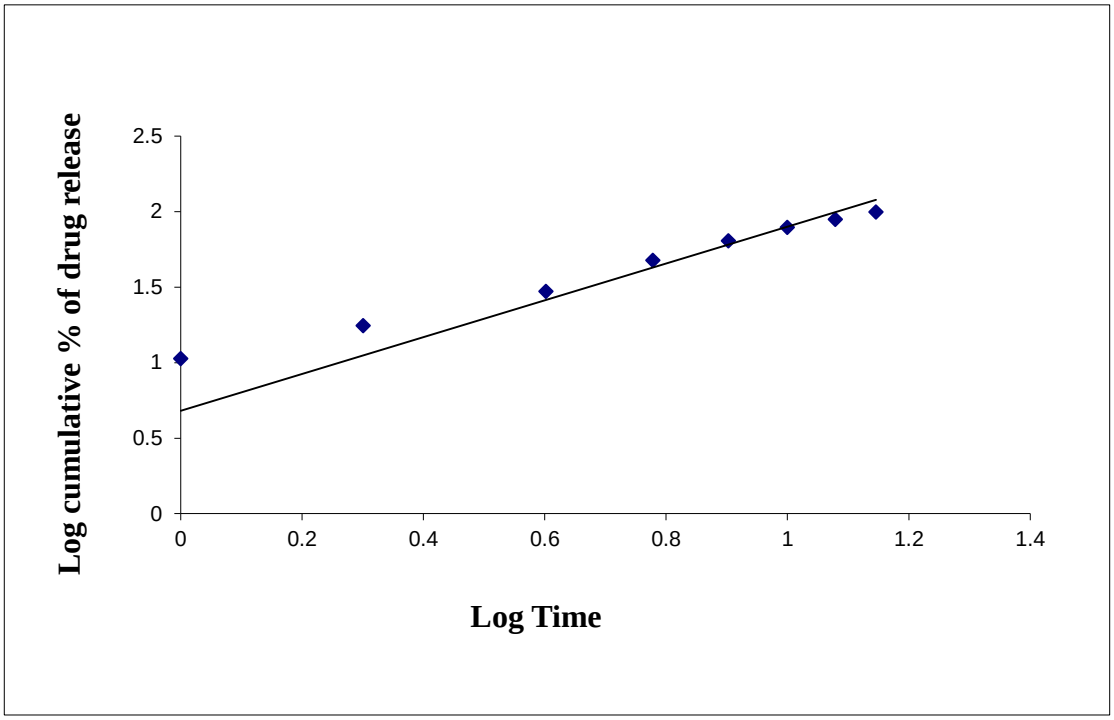

Figure 6.109 Korsmeyer & peppas plot for formulation LS610

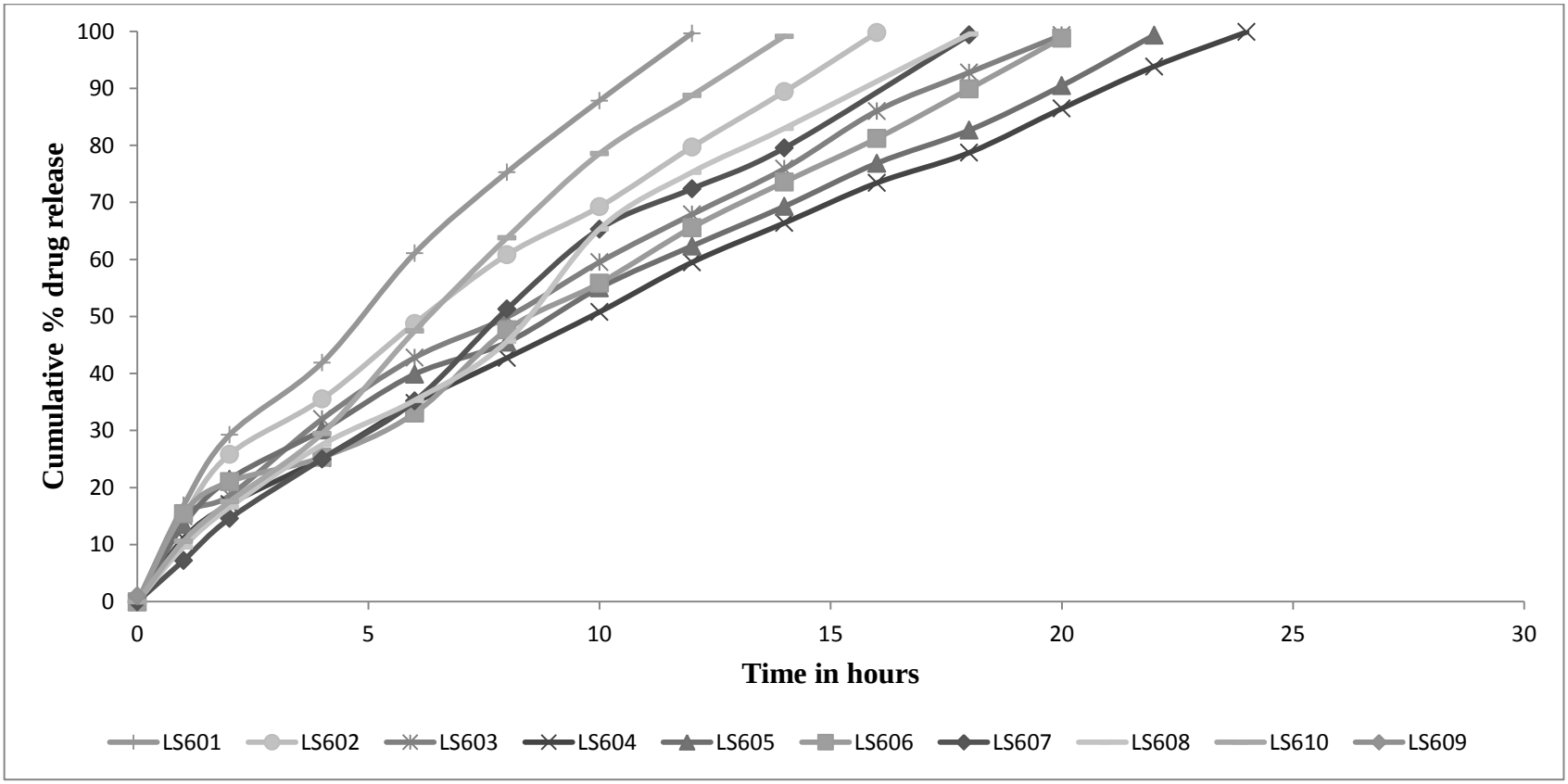

Figure 6.110 Comparative dissolution profile of LS 601-LS610

6.1.5.9 Kinetic values obtained from different plots of formulations (LS601-610) of letrozole

Table 6.27 Drug release mechanism (LS601-610)

Formula Code	Zero order		First Order		Higuchis model		Koresmayer & Peppas	
	K_0	R^2	K_1	R^2	K_H	R^2	*n*	R^2
LS601	7.4944	0.9963	0.3656	-0.8573	29.5931	0.9867	0.7098	0.9981
LS602	5.4894	0.9971	0.2623	-0.8178	25.2760	0.9891	0.6695	0.9980
LS603	4.4817	0.9973	0.1812	-0.8744	22.9652	0.9883	0.6626	0.9955
LS604	3.8235	0.9978	0.1716	-0.7934	21.2384	0.9862	0.7030	0.9983
LS605	3.9083	0.9977	0.1523	-0.8444	21.1806	0.9892	0.6339	0.9978
LS606	4.4636	0.9978	0.1601	-0.8736	22.3383	0.9775	0.6459	0.9810
LS607	5.5101	0.9949	0.2149	-0.8545	24.6108	0.9717	0.9127	0.9983
LS608	5.4801	0.9944	0.2272	-0.8554	24.5502	0.9692	0.8210	0.9959
LS609	5.8044	0.9917	0.2412	-0.8724	25.6998	0.9667	0.9526	0.9963
LS610	7.0744	0.9968	0.2686	-0.8849	28.2896	0.9708	0.8781	0.9972

Table 6.28 Stability study of optimized formulation LS 604

Temperature	Values obtained at zero time			Values obtained at 1st month			Values obtained at 2st month			Values obtained at 3rd month		
	Vesicle size in nm	Drug remain in %	*In vitro* drug release in %	Vesicle size in nm	Drug remain in %	*In vitro* drug release in %	Vesicle size in nm	Drug remain in %	*In vitro* drug release%	Vesicle size in nm	Drug remain in %	*In vitro* drug release%
5±3˚C	650	83.64	99.87	655	83.32	99.58	657	83.08	99.16	660	82.92	98.85
25±2˚C	650	83.64	99.87	660	82.25	98.25	665	81.35	97.35	670	80.12	96.86
40±2˚C	650	83.64	99.87	665	80.25	97.35	670	76.25	94.45	680	72.28	92.92

	Values obtained at zero time	Values obtained at 1st month	Values obtained at 2st month	Values obtained at 3rd month
Colour	Yellow	No changes	No changes	No changes
Appearance	Cyrstalline	No changes	No changes	No changes

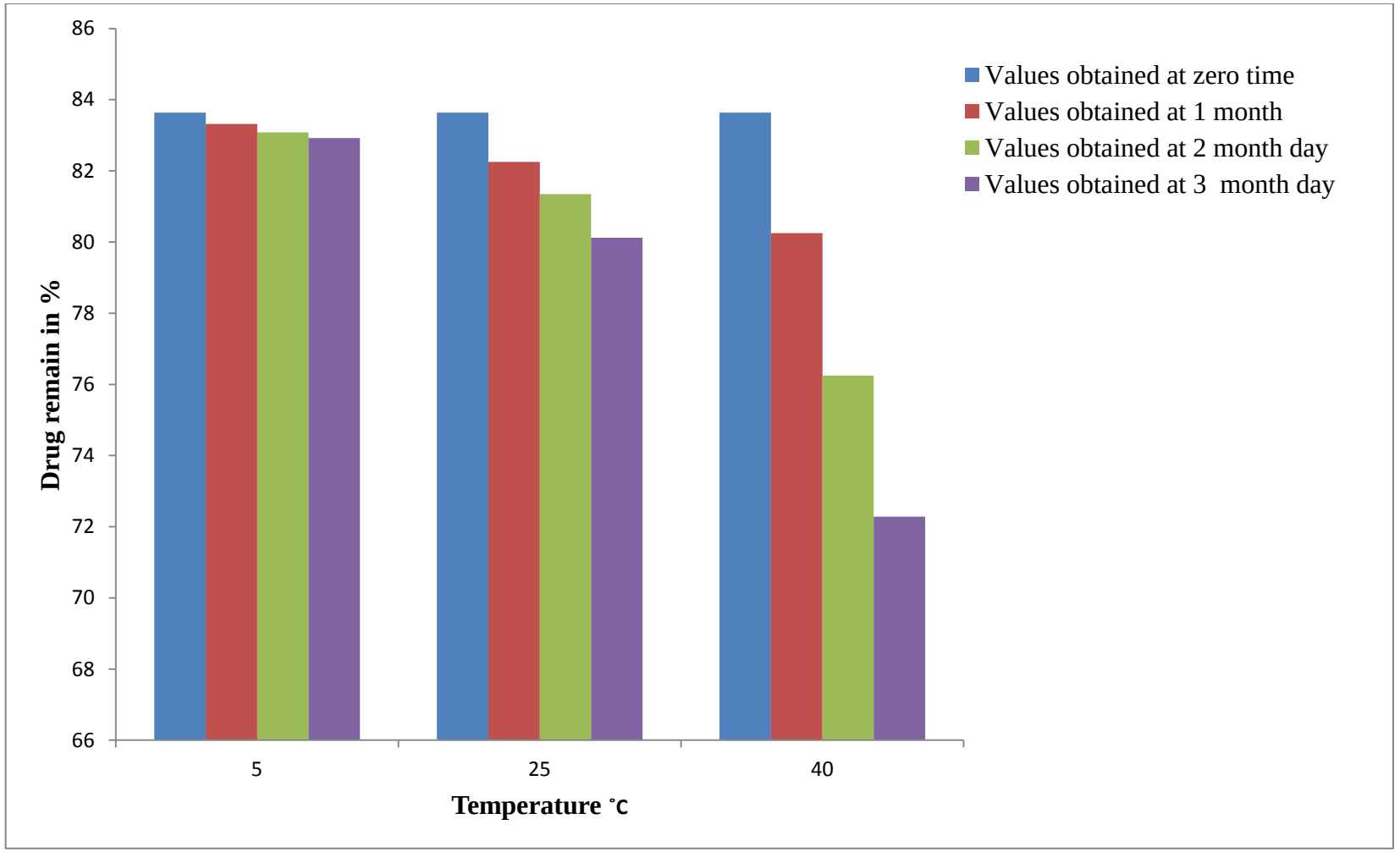

Figure 6.111 Entrapment efficiency in stability studies of optimized formulation LS 604

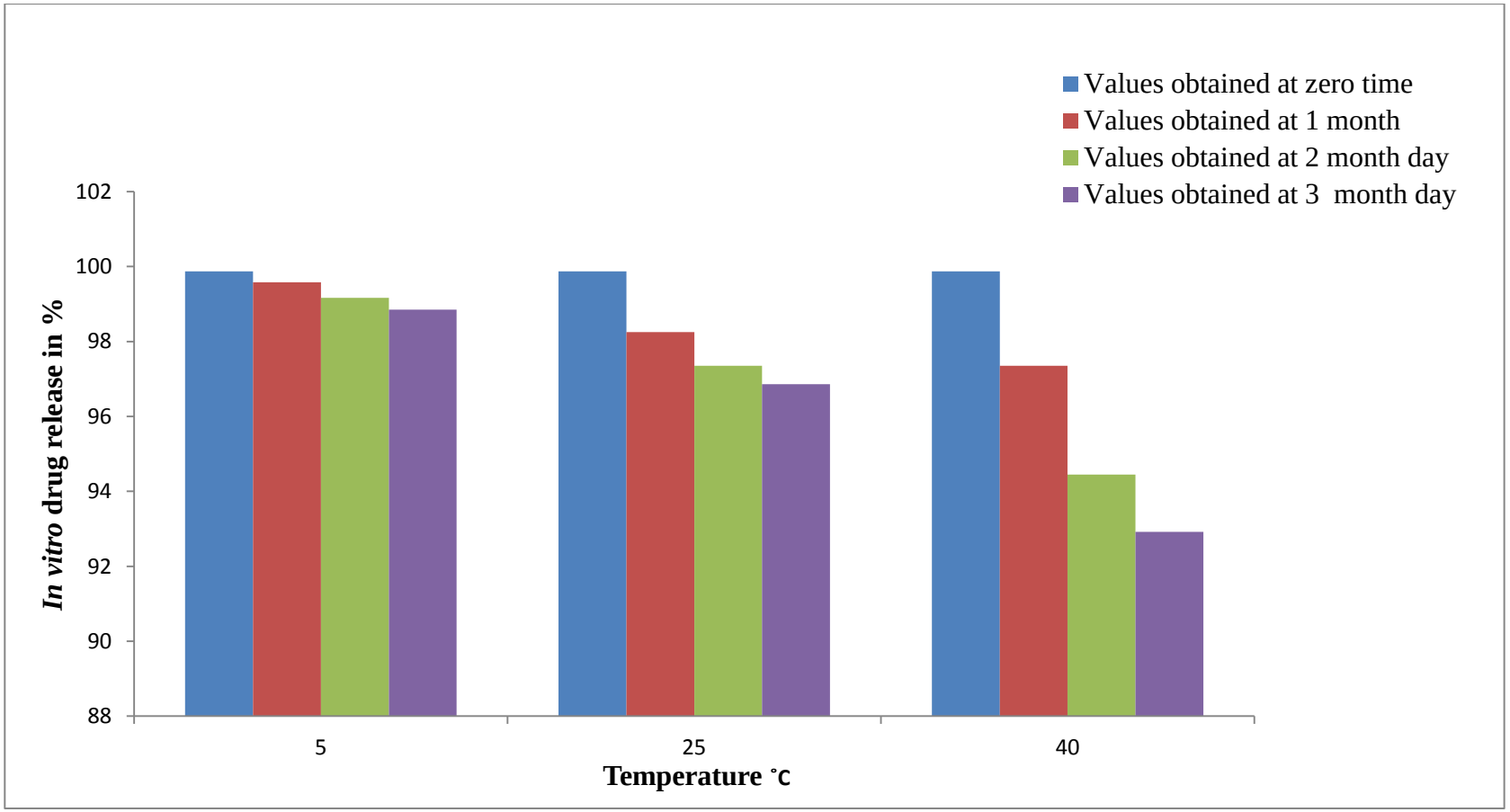

Figure6.112 a. *In vitro* drug release during stability studies of optimized formulation LS 604

6.1.5.10 *In vivo* pharmacokinetic study of optimized proniosome in animal model

Pharmacokinetic parameters	Pure letrazole	LS604
Cmax (mcg/ml)	42.57±0.3214	29.17±0.058
T max (h)	12.00±0.0000	18.00±0.00
ElimRateConst (h^{-1})	0.064±0.0003	0.0202±0.001
Half life (h)	10.825±0.0517	34.39±0.169
AUC0_t (mcg.h/ml)	1045.87±10.852	1402.67±3.7167
AUC0_inf (mcg.h/ml)	1065.13±9.8921	1824.5±10.07
AUMC0_t (mcg.h/ml)	22659.47±195.61	49428.4±175.97
AUMC0_inf (mcg.h/ml)	2444.83±62.165	53131.2±967.72

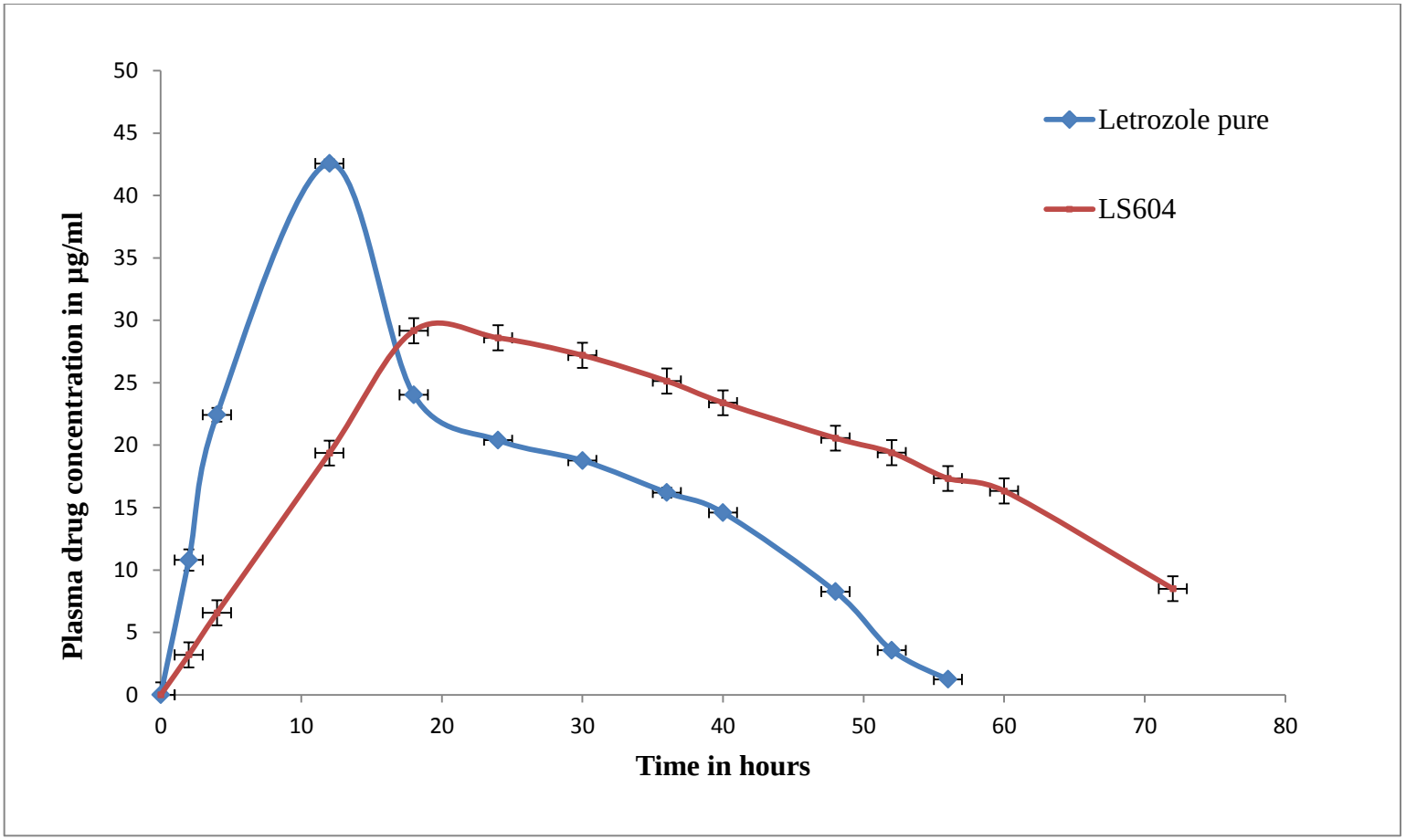

Figure 6.112 b. Comparison of mean plasma concentration of pure letrozole and proniosomal formulation LS604

RALOXIFENE

6.2 Preformulation

Preformulation is the science of the physicochemical characterization of the solid and solution properties of compounds. Before starting prototype formulation of Raloxifine proniosome preformulation studies for raloxifine and pure drug was performed. Following were the results of preformulation studies carried out for Raloxifine pure drug, which were carried out as per methods, explained in the methodology section.

6.2.1.1 Identification of raloxifene

Drug :Raloxifene
Colour :Dark yellow
Odour :Odourless
Appearance :Crystalline Powder

6.2.1.2 Solubility study

Raloxifene was soluble in ethanol,DMSO ,dimethyl formamide and insoluble in water.

6.2.3 Melting point

The procedure for determination is same as section 5.1.3. The melting point of Raloxifene was found to be 146-147 °C. This matches with the standard melting temperature range 143-147 °C indicating the identity of Raloxifene .

6.1.2.4 Angle of repose

- Raloxifene - 31°38'±0.64
- Maltodextrin - 30°06'±0.14

6.2.2 Drug -excipient compatability studies

6.2.2.1 FT IR spectroscopy of raloxifene

Compatibility studies were performed using FT IR spectrophotometer. The FT IR spectrum of pure drug and physical mixture of drug and formulation were studied by making a KBr Pellets.

The spectral details for all types of formulations are shown as follows

Table 6.29 FTIR spectrum peaks in raloxifene

S. No.	Wave Number in cm^{-1}		Functional group
	Range	Observed	
1.	3650-3600 cm^{-1}	3643.10 cm^{-1}	O-H
2.	1250-970cm-1	1234.67 cm^{-1}	C-O Phenolic
3.	1600-1475 cm-1	1519.82 cm^{-1}	C=C Aromatic
4.	1730-1705 cm-1	1730.36 cm^{-1}	C=O Keto
5.	1300-1000 cm-1	1051.39 cm^{-1}	C-O Ether
6.	1350-1200 cm-1	1234.67 cm^{-1}	C-N

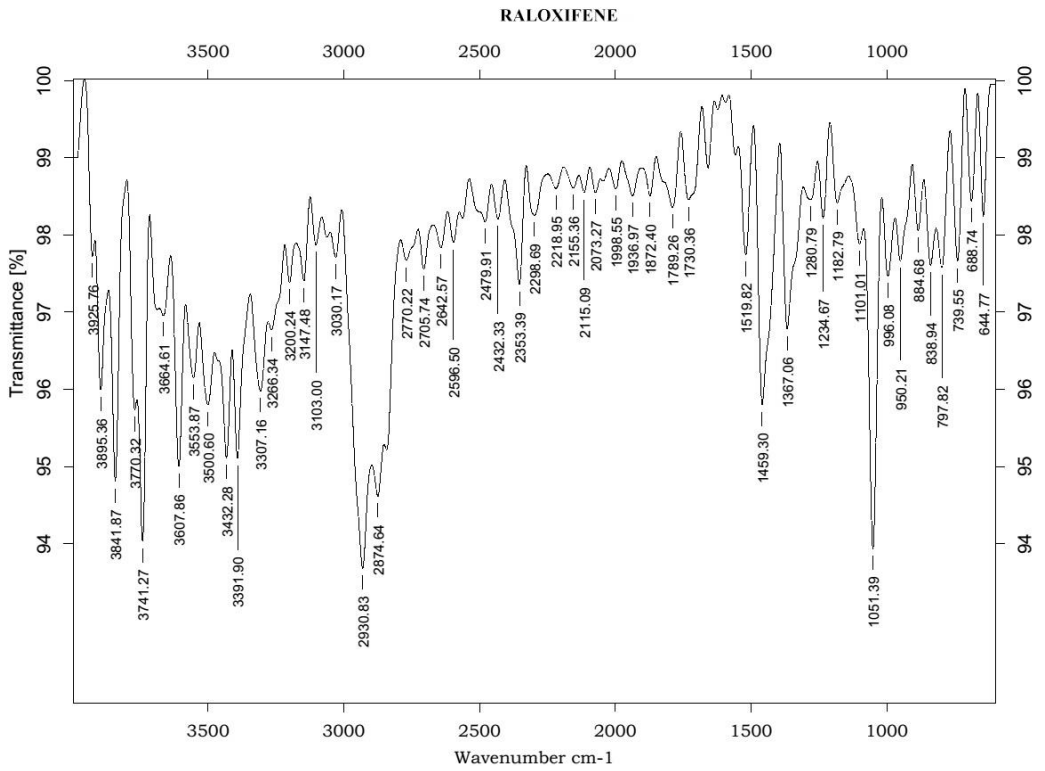

Figure 6.113 FT IR spectrum of raloxifene

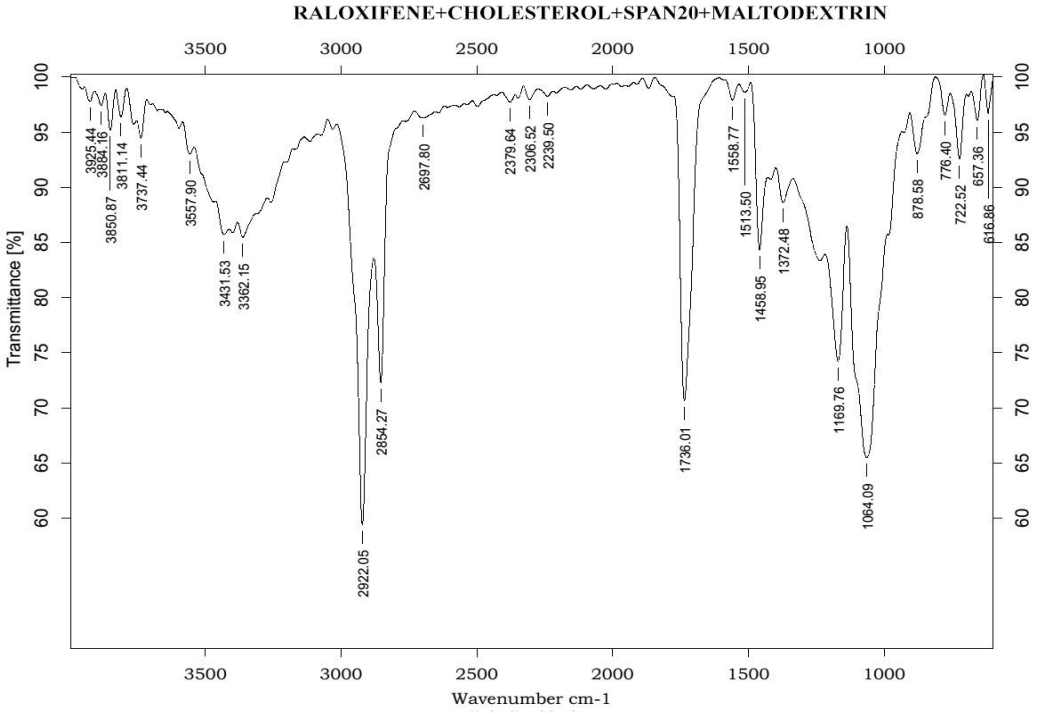

Figure 6.114 FT IR spectrum of physical mixture of raloxifene+cholesterol+span20+maltodextrin

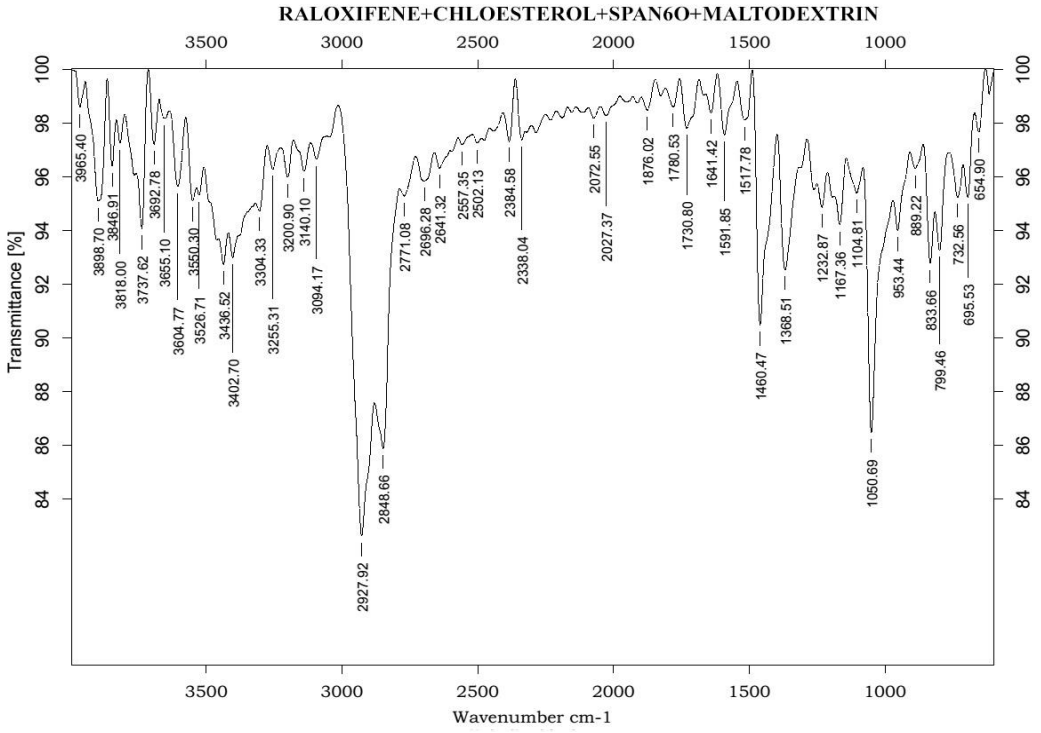

Figure 6.115 FT IR spectrum of physical mixture of

Raloxifene+cholesterol+span 60+maltodextrin

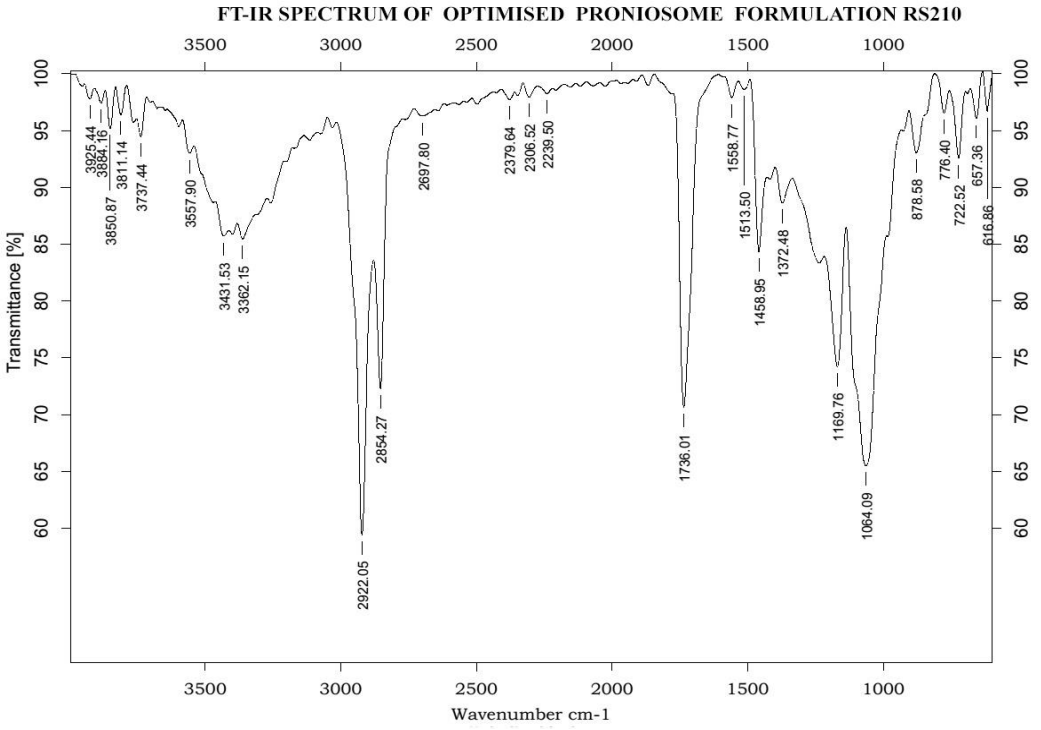

Figure 6.116 FT IR spectrum of optimised proniosome formulation RS210

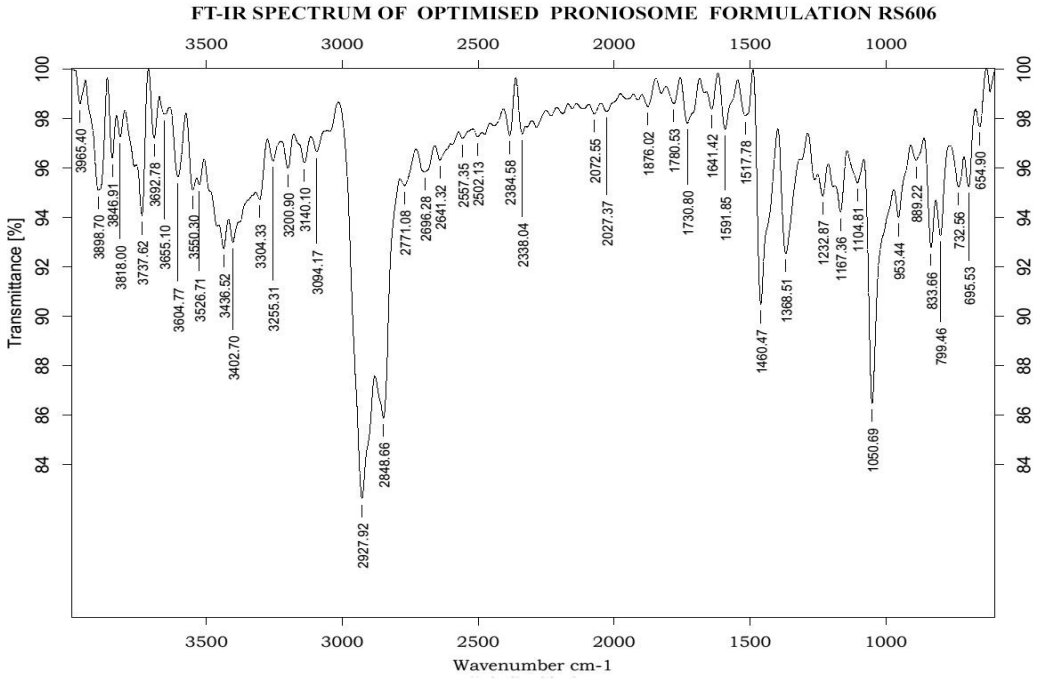

Figure 6.117 FT-IR spectrum of optimised proniosome formulation RS606

Table 6.30 Characteristic absorption peaks in FT IR spectrum

(KBr Disc) peaks at					Indications
Pure drug Raloxifene	**Physical Mixture**		**Formulations**		
	Raloxifene+Cholesterol+ Span 60+Maltodextrin	**Raloxifene+Cholesterol+ Span 60+Maltodextrin**	**RS210**	**RS606**	
3643.10 cm^{-1}	3557.10 cm^{-1}	3857.10 cm^{-1}	3657.10 cm^{-1}	3857.10 cm^{-1}	O-H
1234.67 cm^{-1}	1232.87 cm^{-1}	1432.87 cm^{-1}	1332.87 cm^{-1}	1432.87 cm^{-1}	C-O Phenolic
1519.82 cm^{-1}	1517.78 cm^{-1}	1617.78 cm^{-1}	1617.78 cm^{-1}	1617.78 cm^{-1}	C=C Aromatic
1730.36 cm^{-1}	1730.80 cm^{-1}	1830.80 cm^{-1}	1830.80 cm^{-1}	1830.80 cm^{-1}	C=O Keto
1051.39 cm^{-1}	1050.69 cm^{-1}	1070.69 cm^{-1}	1050.69 cm^{-1}	1070.69 cm^{-1}	C-O Ether
1234.67 cm^{-1}	1232.87 cm^{-1}	1332.87 cm^{-1}	1132.87 cm^{-1}	1332.87 cm^{-1}	C-N
3643.10 cm^{-1}	3604.77 cm^{-1}	3804.77 cm^{-1}	3304.77 cm^{-1}	3804.77 cm^{-1}	O-H

6.2.2.2DSC study

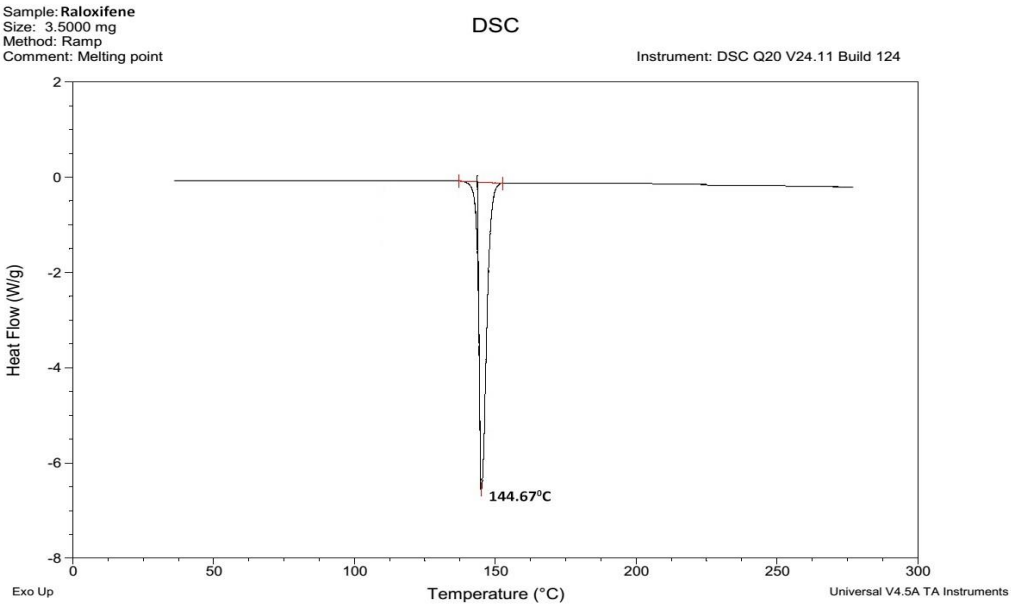

Figure 6.118 DSC Thermogram of raloxifene

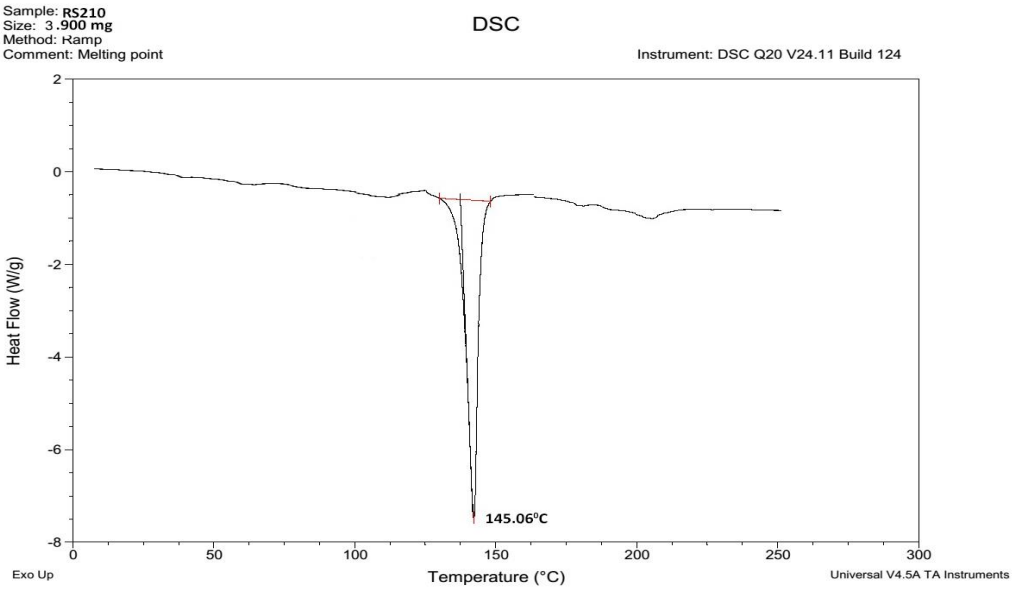

Figure 6.119 DSC Thermogram of RS210

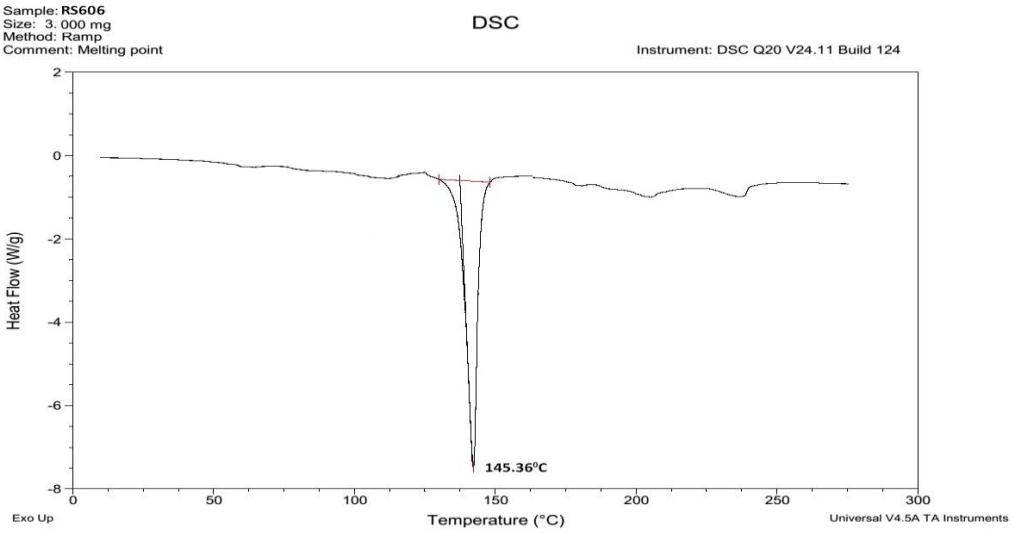

Figure 6.120 DSC Thermogram of RS606

6.2.3 Construction of calibration curve

6.2.3.1 Calibration curve of raloxifene

6.2.3.1.1 Construction of raloxifene calibration curve by UV spectroscopy

Calibration curve of raloxifene was constructed by ploting absorbance (nm) vs Concentration (µg/ml). The results were optained as followes

Table 6.31 Calibration curve of raloxifene in P^H 7.4 phosphate buffer at 289nm

Concentration in µg/ml /ml	Absorbance Trial 1	Absorbance Trial 2	Absorbance Trial 3	Mean Absorbance
0	0	0	0	0
10	0.123	0.125	0.121	0.1230±0020
20	0.236	0.231	0.232	0.2330±0026
30	0.376	0.378	0.378	0.3773±0012
40	0.501	0.497	0.499	0.4990±0020
50	0.589	0.592	0.59	0.5903±0015
60	0.72	0.706	0.703	0.7097±0091

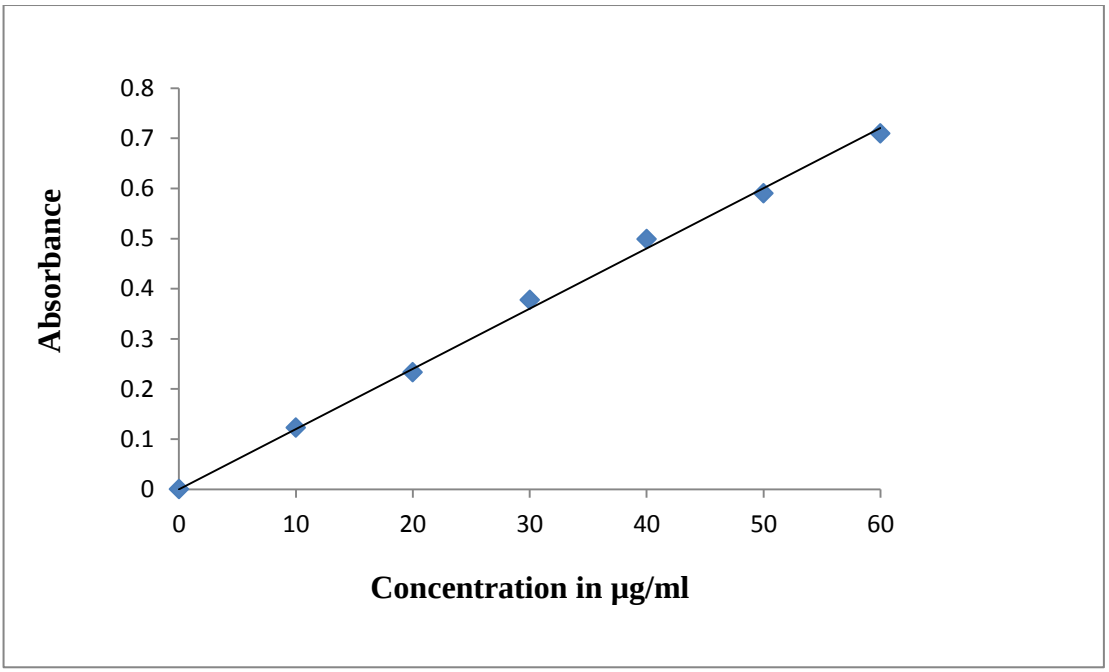

Figure 6.121 Calibration curve of raloxifene by UV spectroscopy

Slope	0.0119
Intecept	0.0050
Regression	0.9989

6.2.3.1.2 Construction of raloxifene calibration curve by HPLC

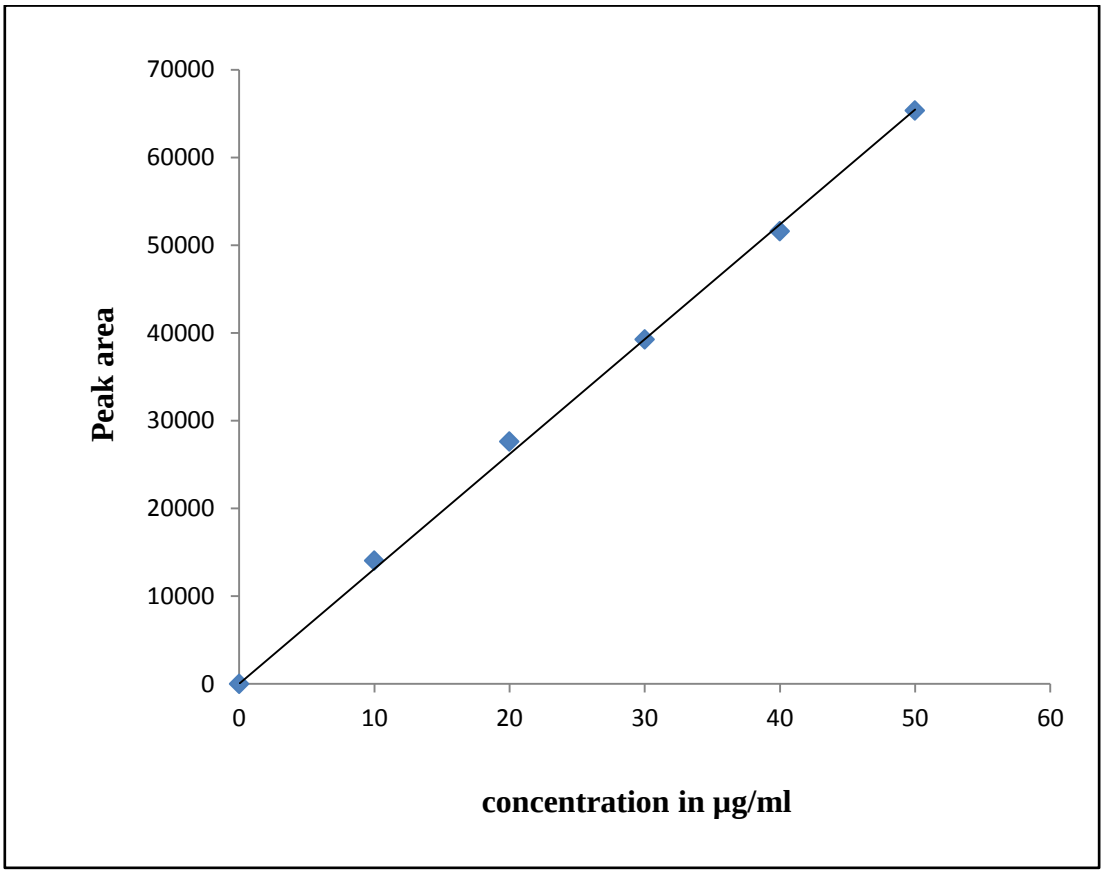

Figure 6.122 Calibration curve of raloxifene by HPLC

Slope	1288.135
Intercept	768.8413
Regression	0.999568

6.2.4 Formulation of proniosome

6.2.4.1 Formulation of raloxifene loaded proniosomes

According to the procedure given in the methodology proniosomes where formulated using sapn 20 and span 60, stored in a sealed container and used it for evaluation.

6.2.5 Evaluation of proniosomes

6.2.5.1 Morphological evaluation

After hydrsating the proniosome using phosphate buffer, proniosome derived neosomes were absorbed under microscope and the photomicrograph given as follows

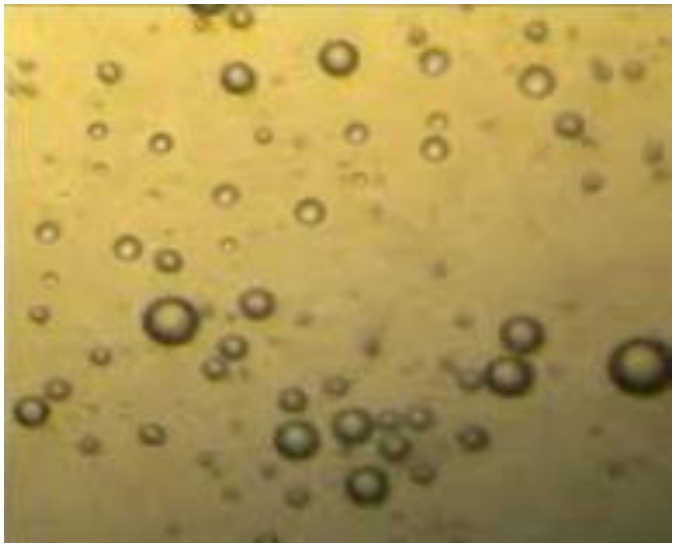

Figure 6.123 Photomicrograph of pronisome RS 210

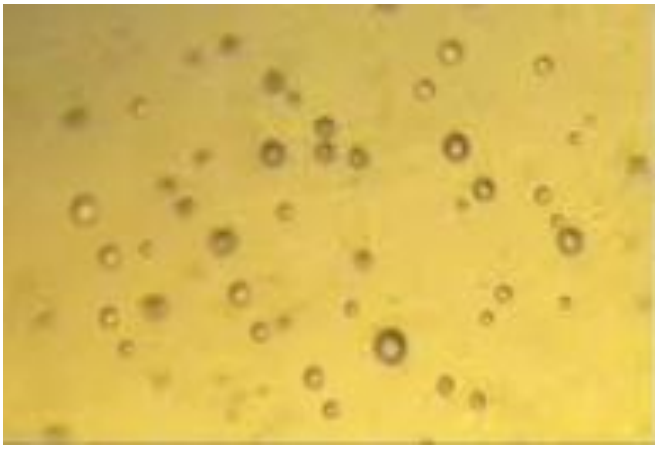

Figure 6.124 Photomicrograph of pronisome RS606

6.2.5.2 Characteristics of raloxifine loaded proniosome

Angle of Repose, Vesicle Size, Zeta Potential, Entrapment Efficiency, Number of Vesicles Per Cubic mm were determined as perprocedure given in methodology and the results were summarised as follows

Table 6.32 Characteristics of formulation RS201-RS210

Formulation	*Angle of Repose in Θ ± STD	Vesicle size in nm	Zeta potential in *mv*	*Entrapment Efficiency in % ± STD	Number of vesicle/ mm^3X10^5
RS201	31°15'±0.99	120	+14	40.41 ±0.6	3.2
RS202	32°14'±0.96	150	+16	44.42 ±0.60	2.8
RS203	31°03'±0.41	220	+17	50.50 ±0.85	2.6
RS204	31°20'±0.63	280	+16	54.04 ±0.77	2.5
RS205	32°03'±0.61	340	+17	57.24 ±0.70	2.4
RS206	32°29'±0.87	420	+18	60.94 ±0.38	2.2
RS207	31°41'±0.85	460	+16	63.86 ±0.56	2
RS208	31°17'±0.46	490	+19	69.44 ±0.65	1.9
RS209	30°65'±0.48	510	+20	72.16 ±0.80	1.6
RS210	29°87'±0.48	530	+24	76.10 ±0.36	1.4

Table 6.33 Characteristics of formulation RS601-RS610

Formulation	*Angle of Repose in Θ ± STD	Vesicle size in nm	Zeta potential in *mv*	*Entrapment Efficiency in % ± STD	Number of vesicle/ mm^3X10^5
RS601	31°19'±0.55	240	+14	57.52 ±0.54	3.2
RS602	30°72'±0.30	360	+14	63.16 ±0.58	2.3
RS603	31°54'±0.45	440	+16	68.78 ±0.85	2.1
RS604	31°01'±0.89	580	+19	72.98 ±0.44	2.1
RS605	30°39'±0.46	640	+20	77.74 ±0.45	1.8
RS606	29°22'±0.17	690	+22	82.44 ±0.44	1.4
RS607	30°32'±0.82	660	+21	74.96 ±0.77	1.8
RS608	31°38'±0.20	640	+20	70.74 ±0.59	2
RS609	31°18'±0.46	640	+20	64.96 ±0.40	2.3
RS610	31°22'±0.97	610	+19	61.76 ±0.47	2.8

6.2.5.3 Vesicle size

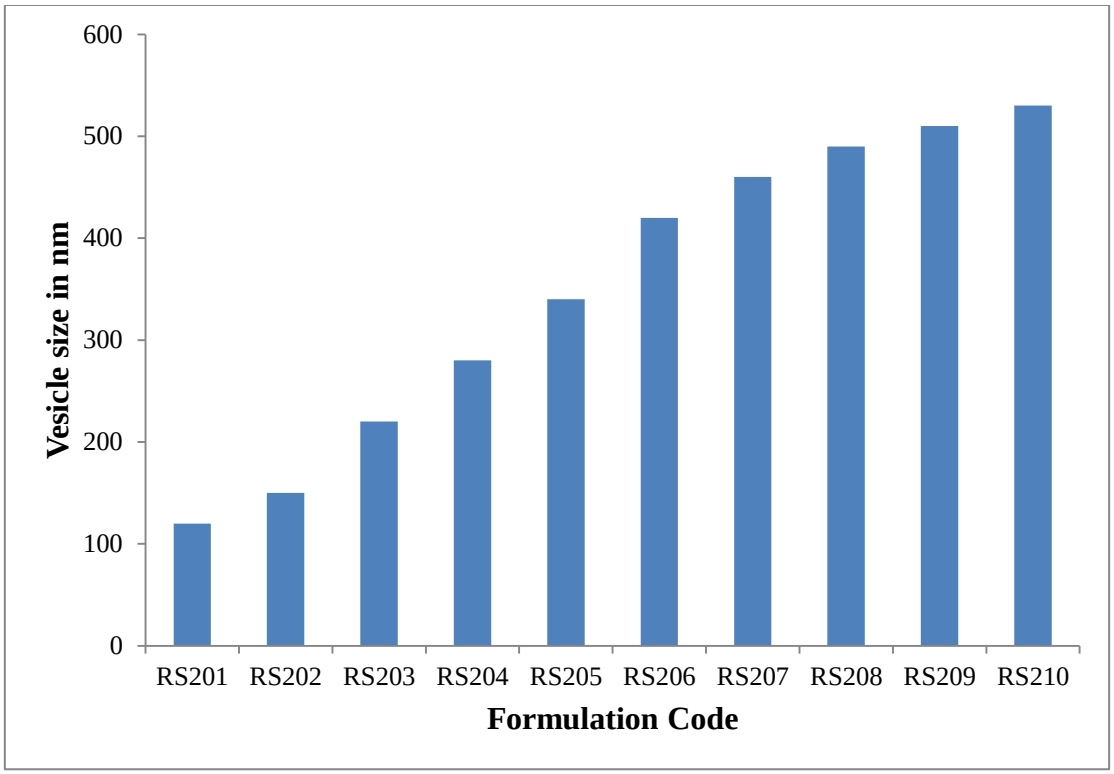

Figure 6.125 Vesicle size of formulation RS201- RS210

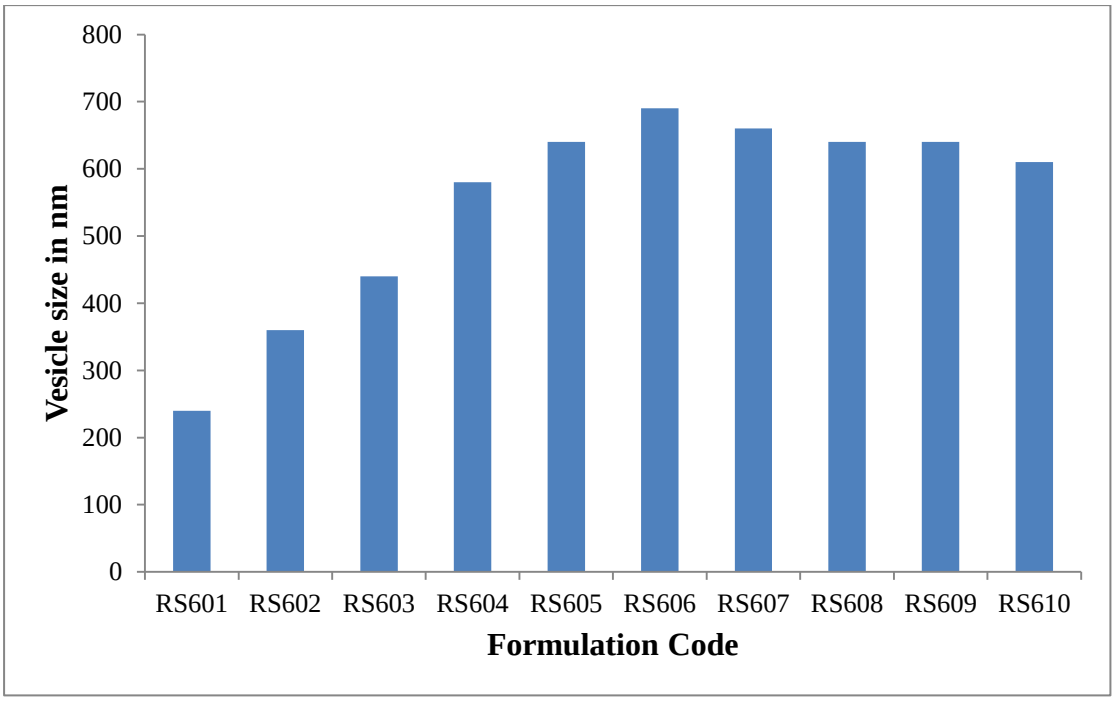

Figure 6.126 Vesicle size of formulation RS 601- RS 610

RS210		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 110	**Peak 1:**	110	100.00	143.80
PdI: 0.67	**Peak 2:**	0.000	0.0	0.000
Intercept: 0.747	**Peak 3:**	0.000	0.0	0.000

Result quality : **Refer to quality report**

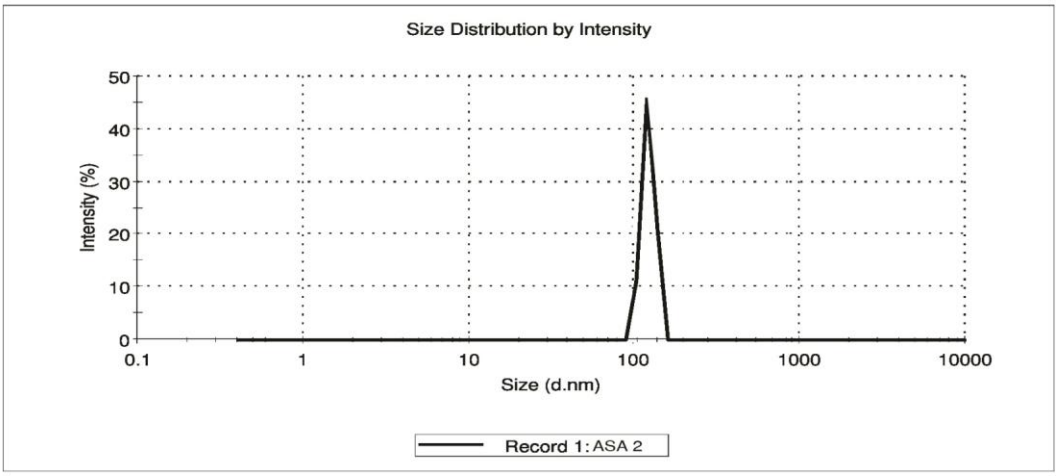

Figure 6.127 Size distribution by intensity of formulation RS210

Results

RS606		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 604	**Peak 1:**	604	100.00	798.61
PdI: 0.67	**Peak 2:**	0.000	0.0	0.000
Intercept: 0.747	**Peak 3:**	0.000	0.0	0.000

Result quality : **Refer to quality report**

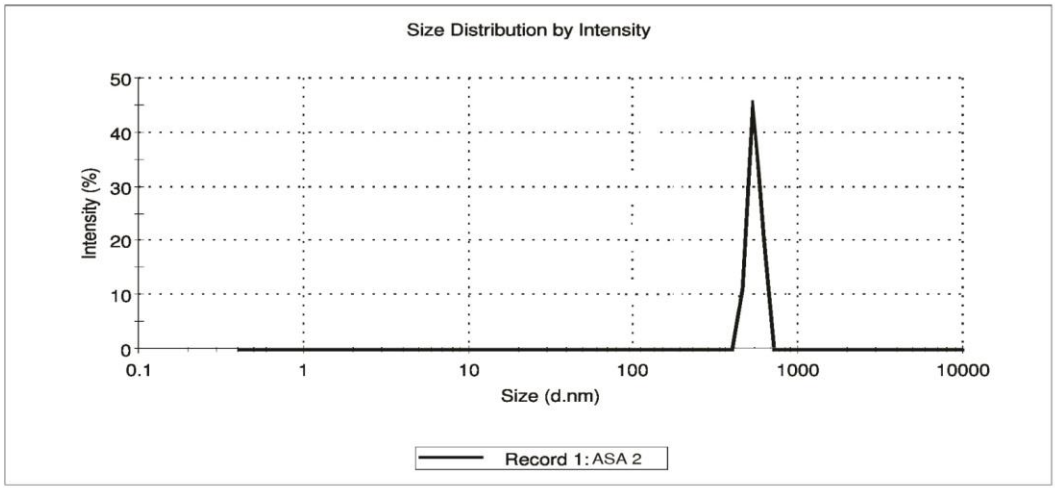

Figure 6.128 Size distribution by intensity of formulation RS606

6.2.5.4 Zeta potential

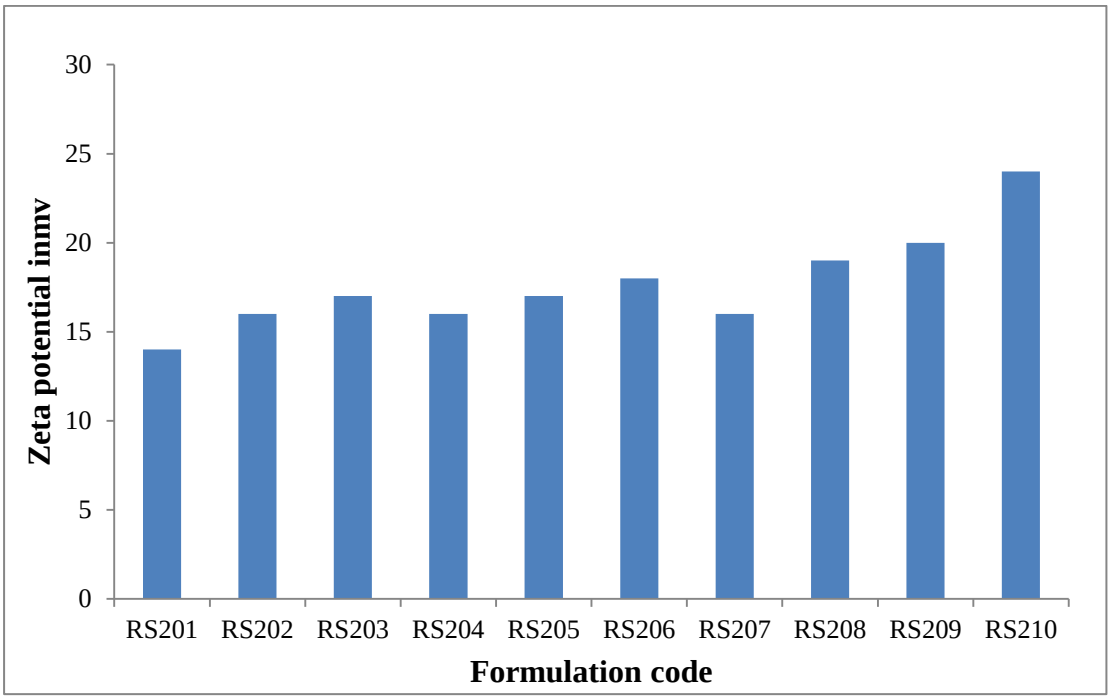

Figure 6.129 Zeta potential of formulation RS201- RS 210

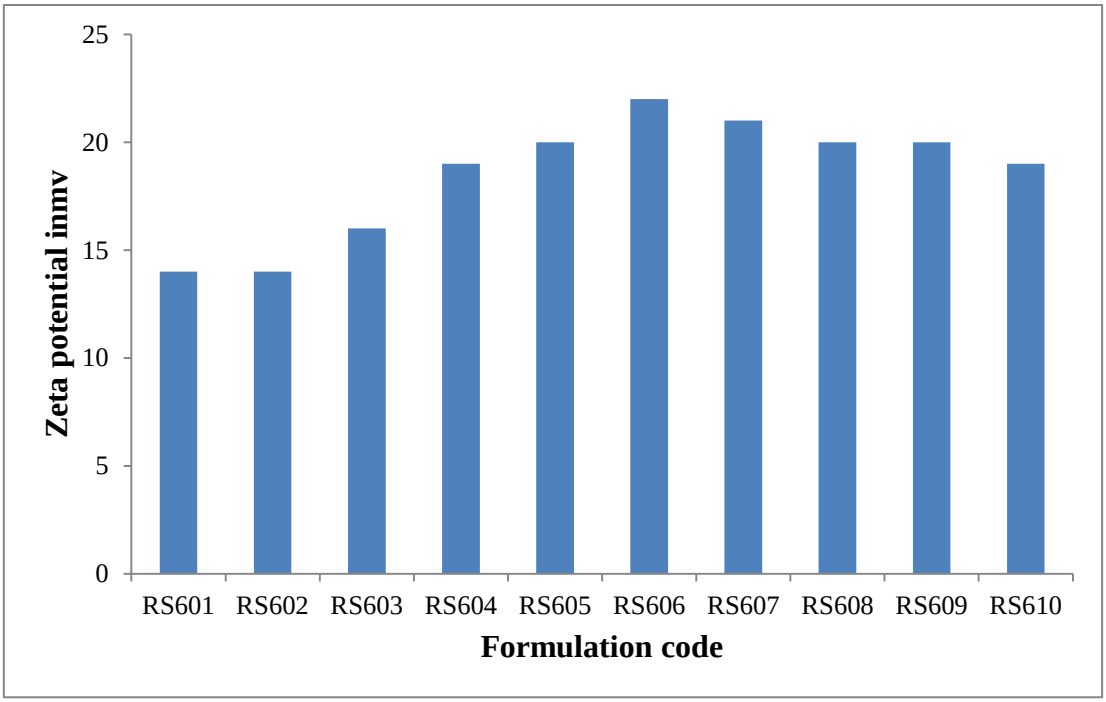

Figure 6.130 Zeta potential of formulation RS 601- RS 610

6.2.5.5 Entrapment efficiency

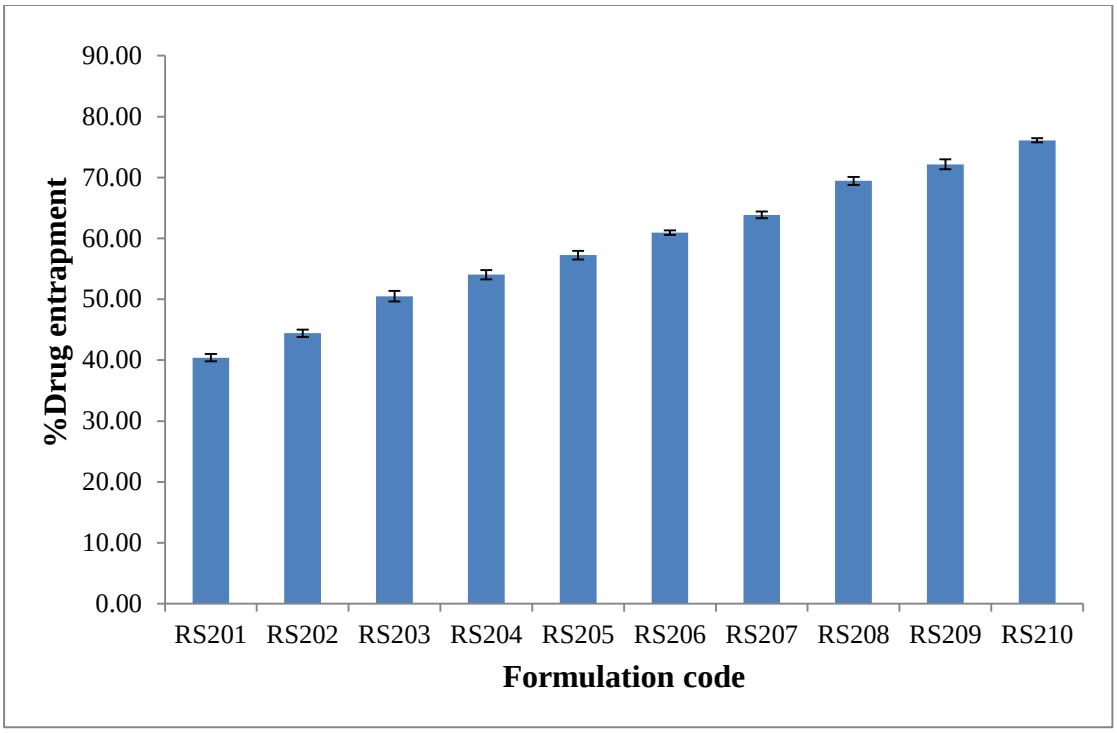

Figure 6.131 Entrapment efficiencyof formulation RS201- RS 210

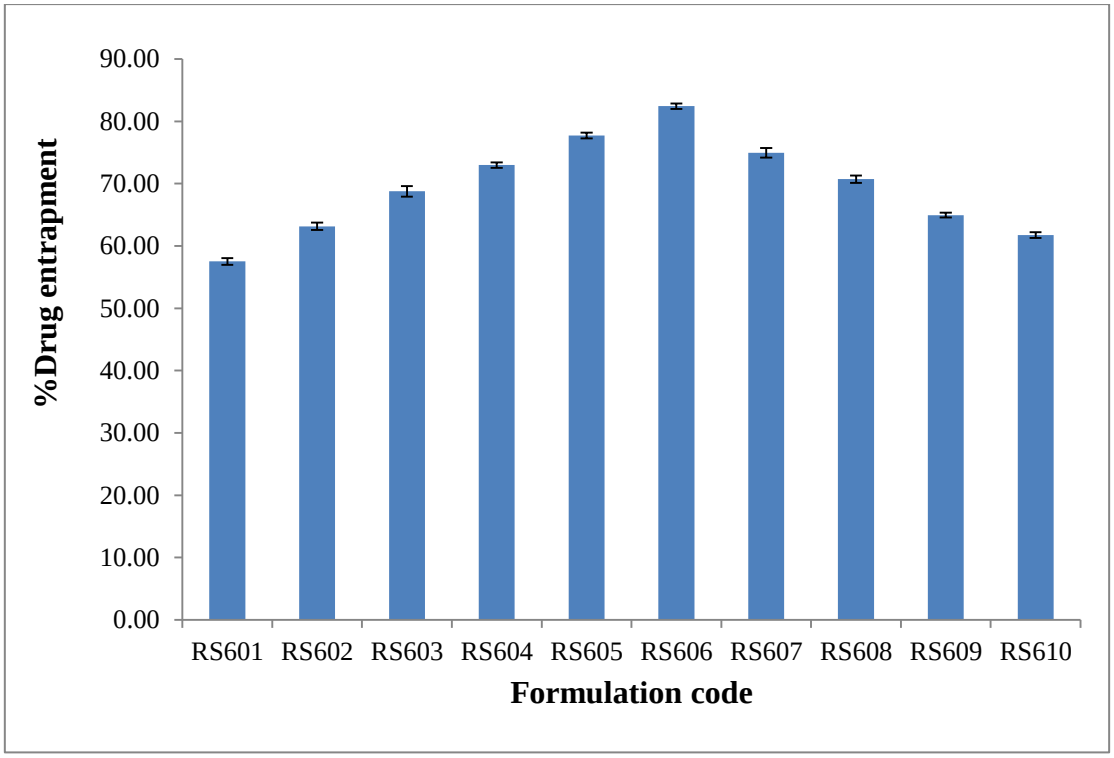

Figure 6.132 Entrapment efficiencyof formulation RS 601-RS 610

6.2.5.6 Number of vesicles per mm^3

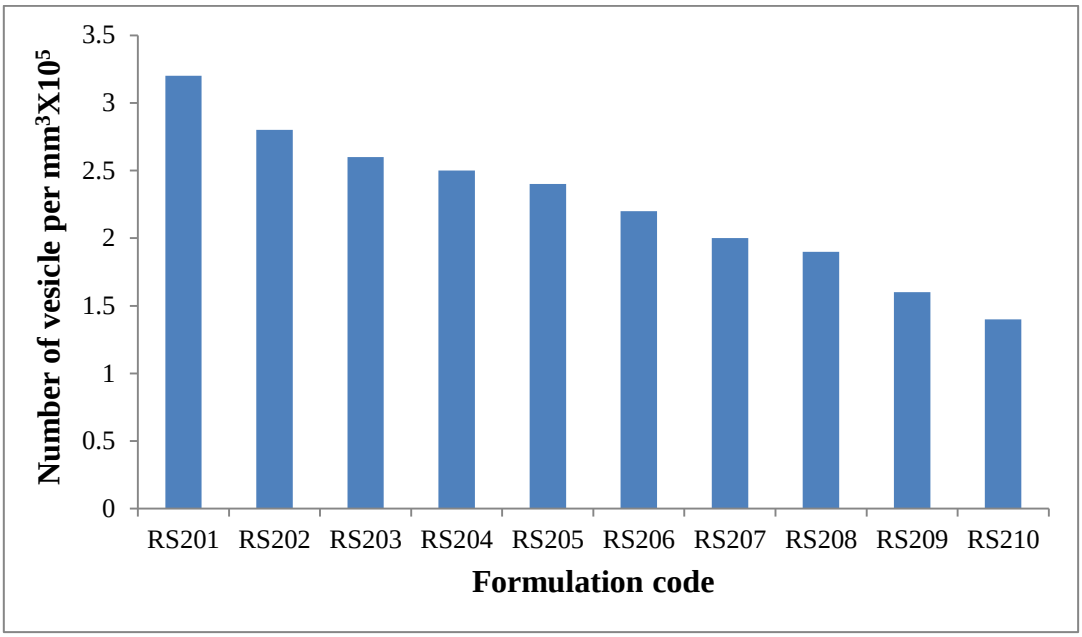

Figure 6.133 Number of vesicles per cubic mm of formulation RS201-RS210

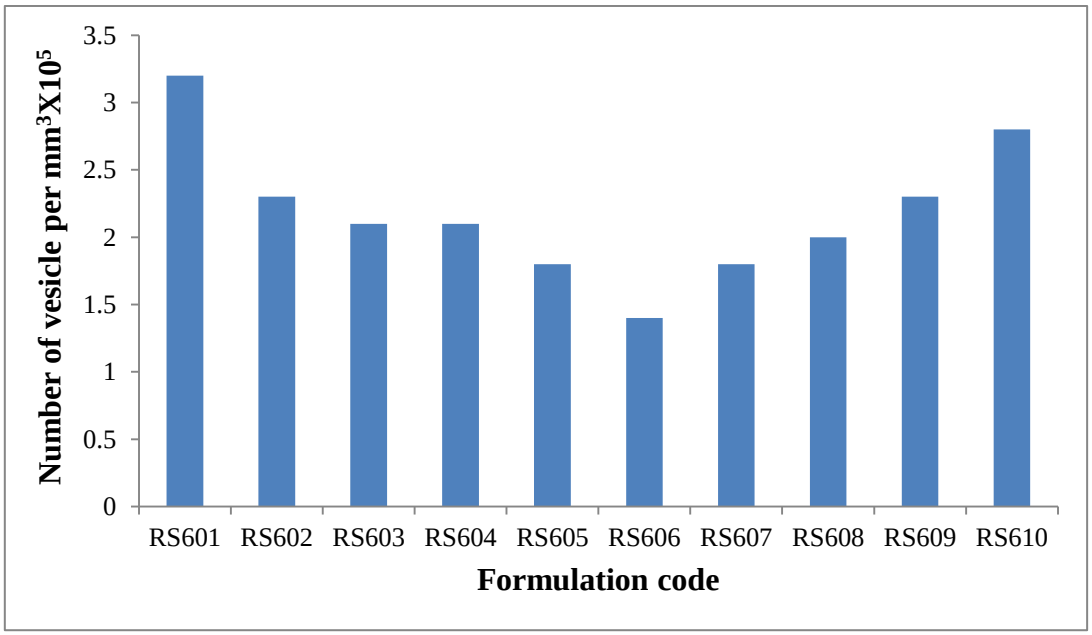

Figure 6.134 Number of vesicles per cubic mm of formulation RS601-RS610

6.2.5.7 Scanning Electron Microscopy (SEM)

Shape and surface charesterstics of proniosome examined by scanning electron microscopy shown in figures

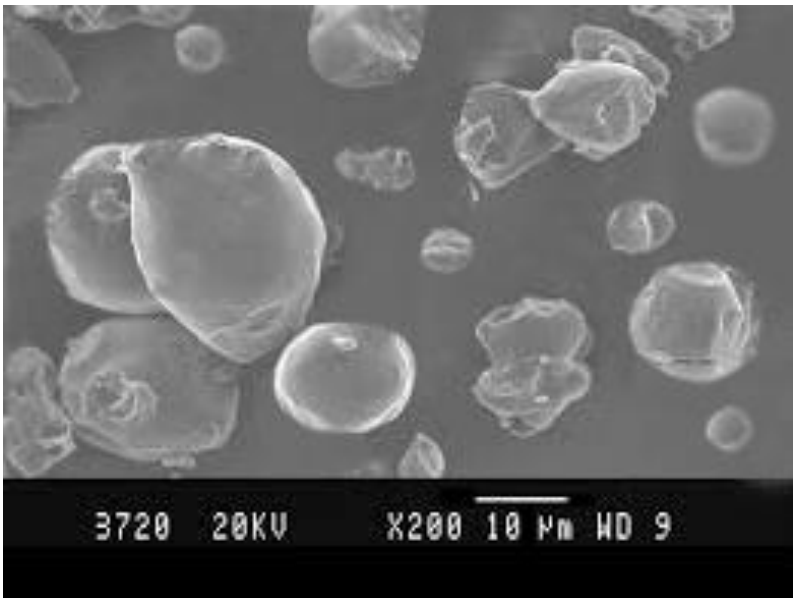

Figure 6.135 Scanning electron image of proniosomal formulation RS204

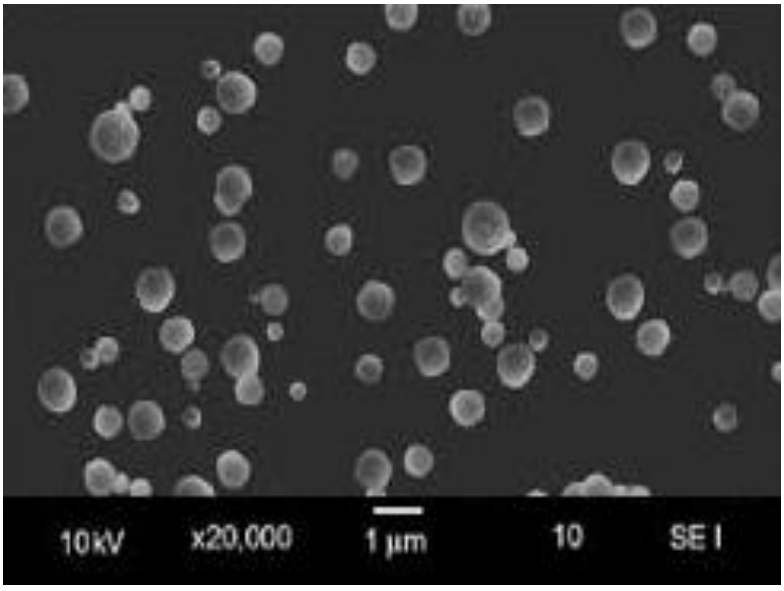

Figure 6.136 Scanning electron image of proniosomal formulation RS606

6.2.5.8 *In vitro* drug release study

The dialysis method was used to investigate the *in vitro* release of the Raloxifene from proniosome results are shown in following tables and figures

Table 6.34 *In vitro* release study of RS201

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.148	24.0484533	0.120242266	12.02422665	20.04037775	0	1.301905903	1.902871	1
2	0.258	42.54880368	0.212744018	21.51488638	35.85814396	0.30103	1.554587806	1.807142	1.41421356
4	0.364	60.37641406	0.30188207	30.8541796	51.42363266	0.60205999	1.711162753	1.686425	2
6	0.543	90.48152968	0.452407648	46.51050155	77.51750259	0.77815125	1.889399772	1.351845	2.44948974
8	0.689	115.0365402	0.575182701	59.6928221	99.48803684	0.90308999	1.997770861	-0.29076	2.82842712

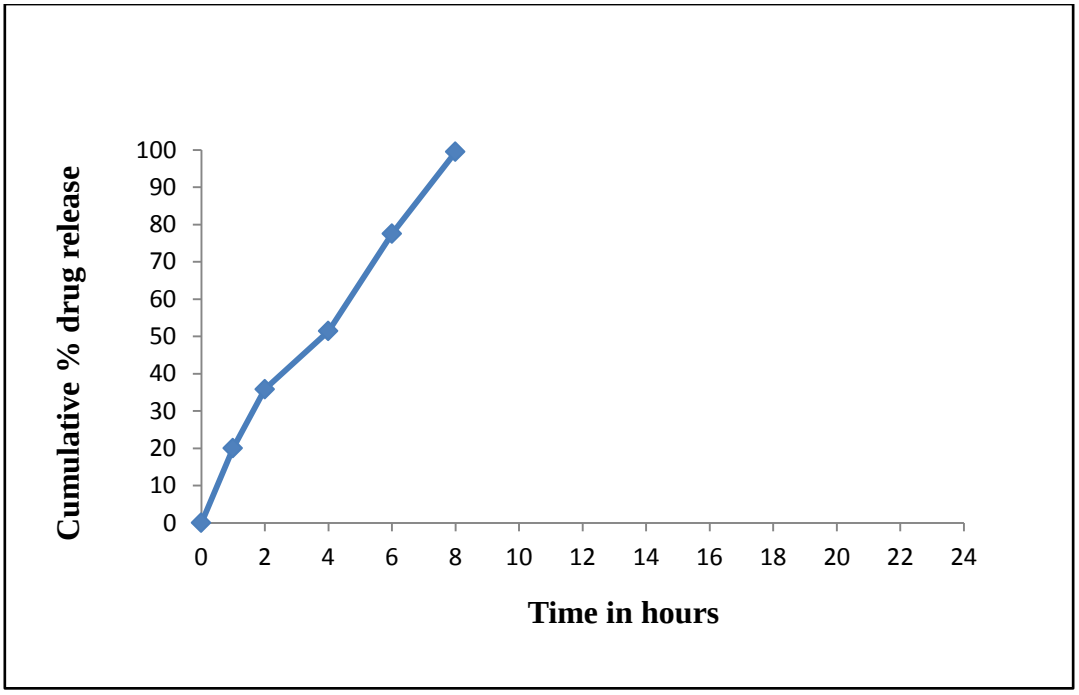

Figure 6.137 *In vitro* drug release of formulation RS201

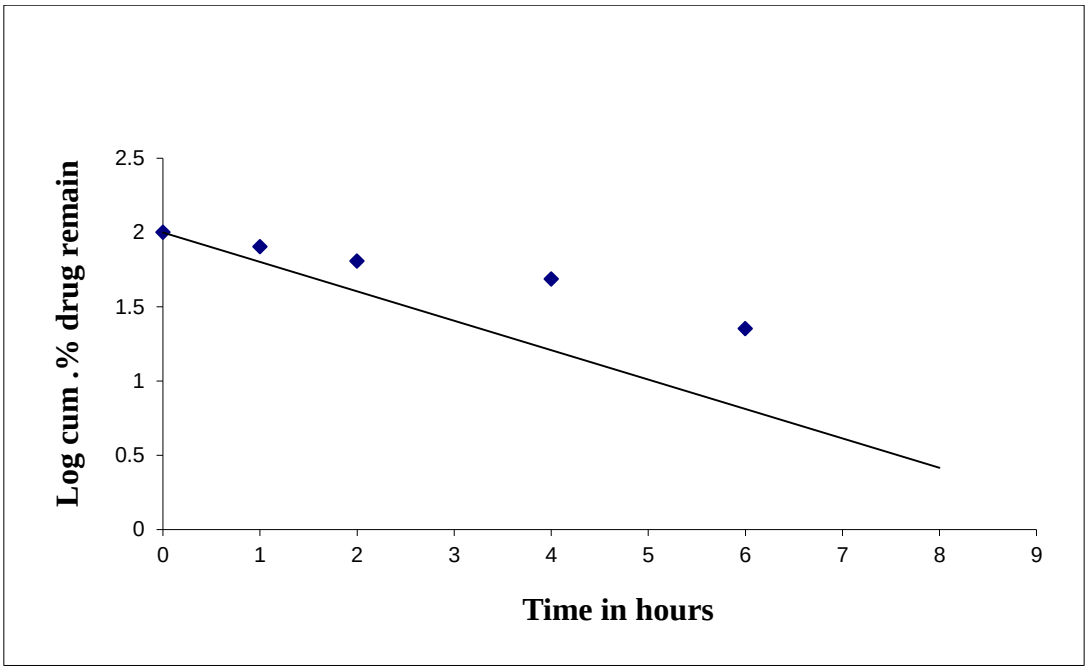

Figure 6.138 First order kinetics of formulation RS201

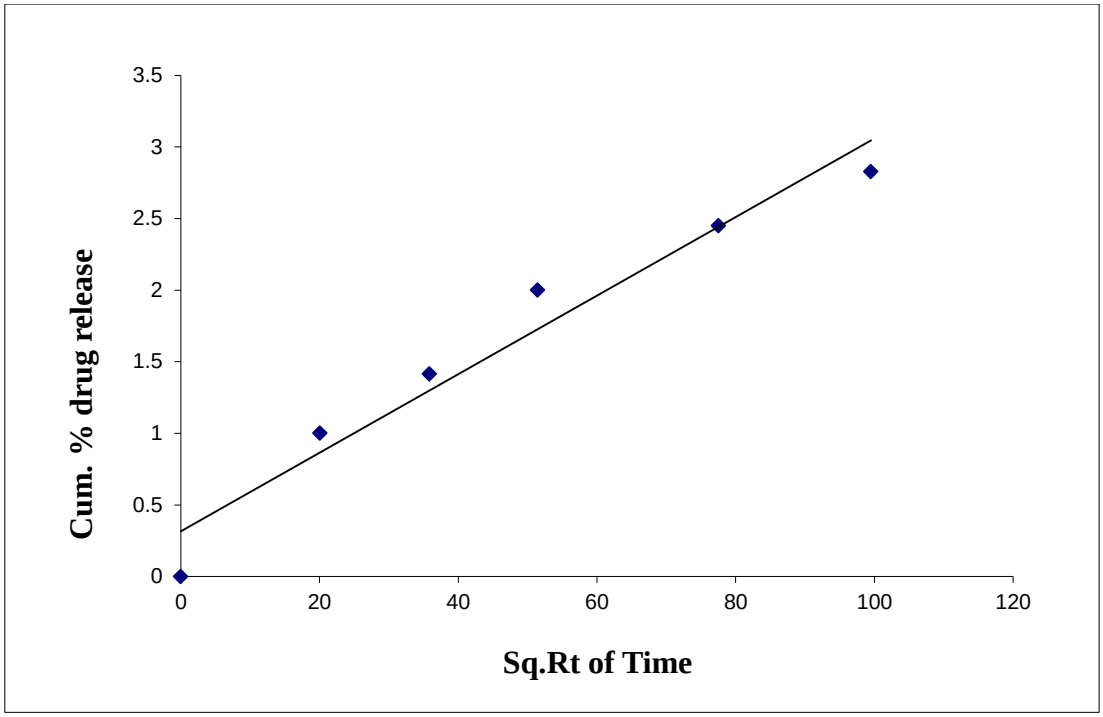

Figure 6.139 Higuchi's plot for formulation RS201

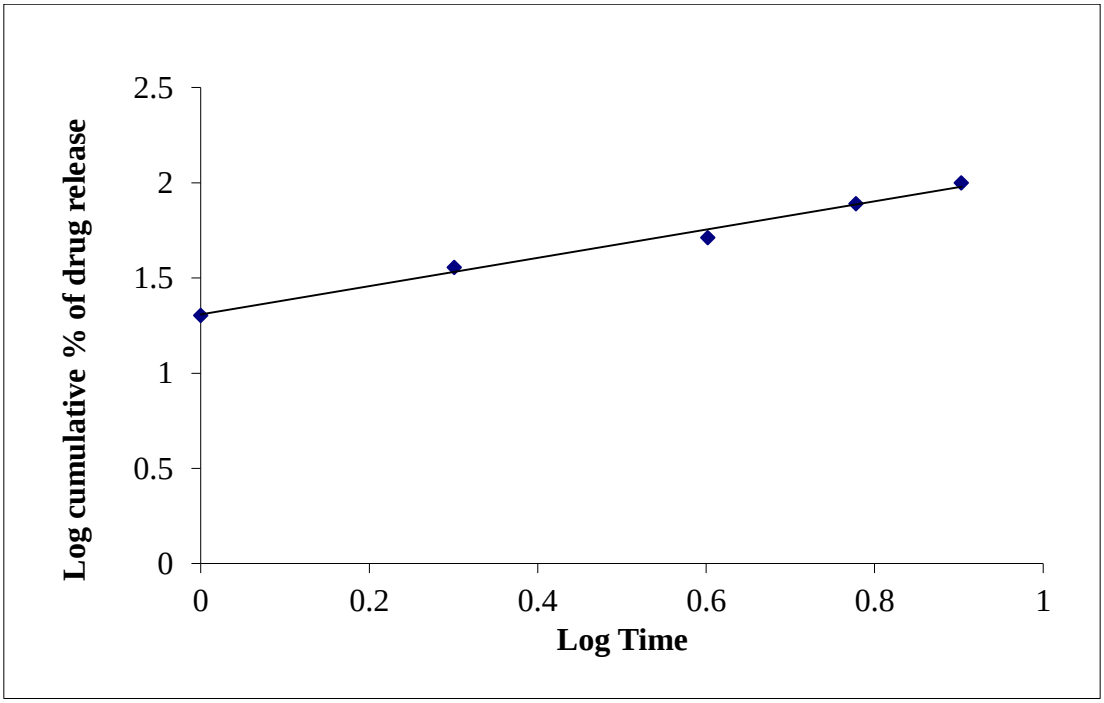

Figure6.140 Korsmeyer & peppas plot for formulation RS201

Table 6.35 *In vitro* release study of RS202

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.132	21.35749324	0.106787466	10.67874662	17.79791104	0	1.250369032	1.914883	1
2	0.216	35.48503354	0.177425168	17.9560917	29.9268195	0.30103	1.476060564	1.845552	1.41421356
4	0.304	50.28531385	0.251426569	25.71108219	42.85180365	0.60205999	1.631969106	1.757003	2
6	0.412	68.44929422	0.342246471	35.29592552	58.82654253	0.77815125	1.769573324	1.614617	2.44948974
8	0.537	89.47241966	0.447362098	46.49198118	77.4866353	0.90308999	1.889226803	1.35244	2.82842712
10	0.681	113.6910602	0.568455301	59.49602563	99.16004271	1	1.996336705	-0.07574	3.16227766

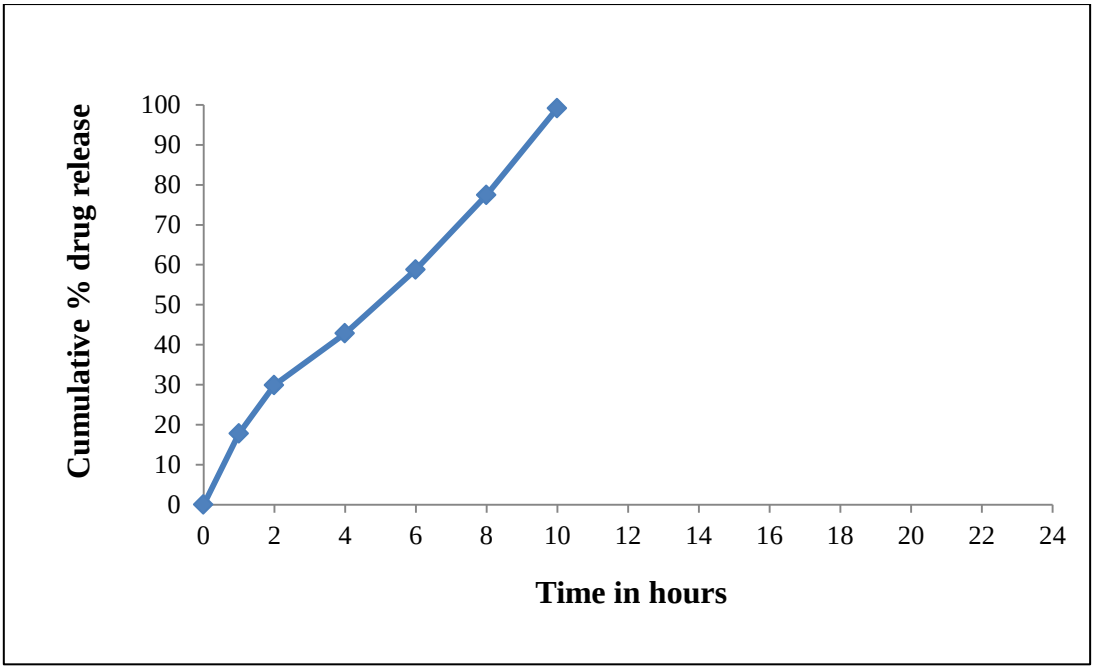

Figure 6.141 *In vitro* drug release of formulation RS202

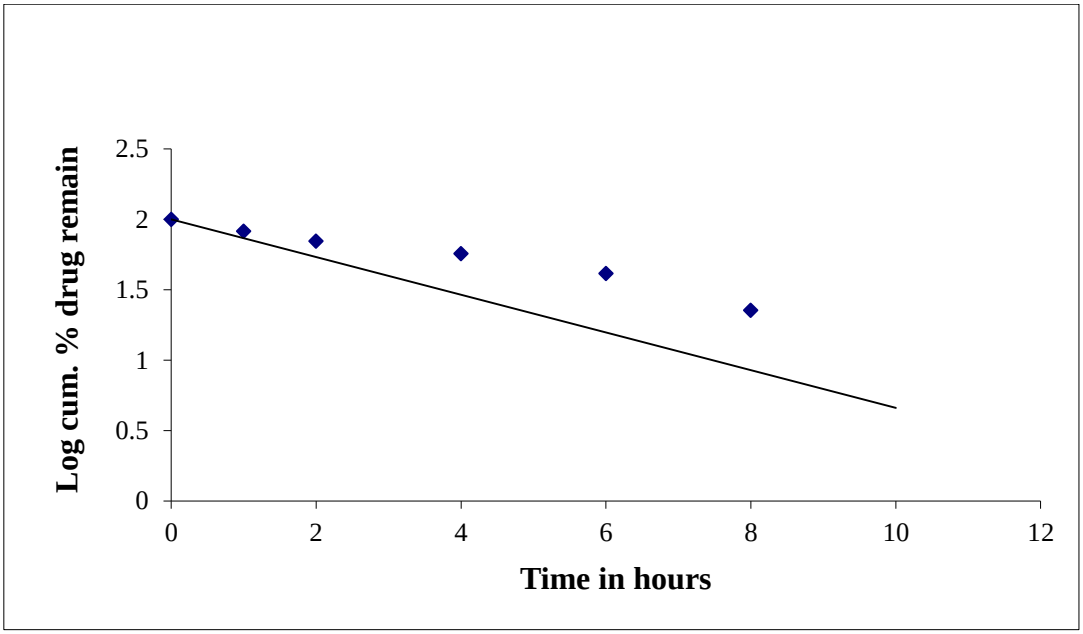

Figure 6.142 First order kinetics of formulation RS202

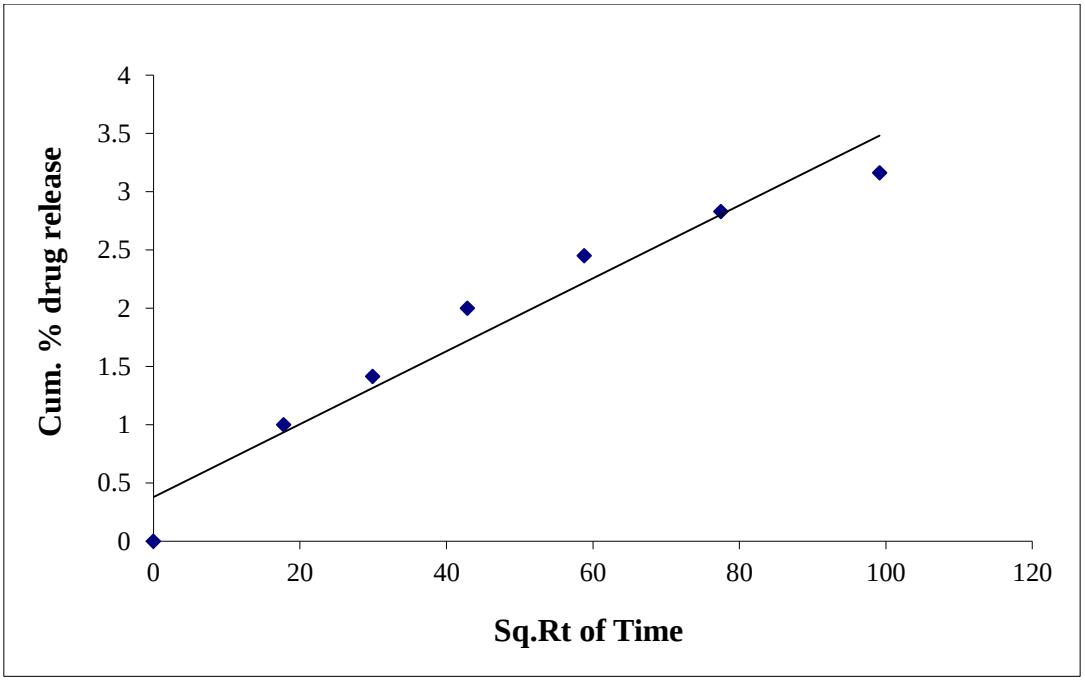

Figure6.143 Higuchi's Plot for formulation RS202

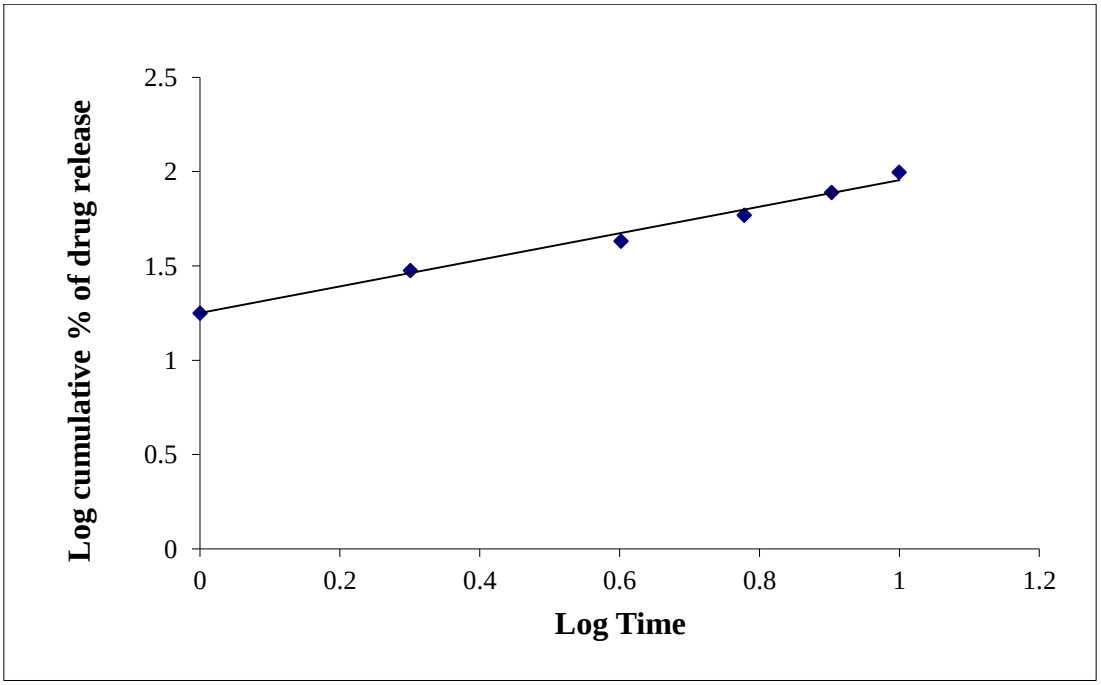

Figure6.144 Korsmeyer & peppas plot for formulation R202

Table 6.36 *In vitro* release study of RS203

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.118	19.00290319	0.095014516	9.501451597	15.83575266	0	1.19963871	1.925128	1
2	0.192	31.44859345	0.157242967	15.91432576	26.52387626	0.30103	1.423636993	1.866146	1.41421356
4	0.269	44.39883872	0.221994194	22.70393433	37.83989055	0.60205999	1.577949872	1.793512	2
6	0.364	60.37641406	0.30188207	31.13671038	51.8945173	0.77815125	1.715121477	1.682195	2.44948974
8	0.446	74.16758434	0.370837922	38.63605967	64.39343278	0.90308999	1.808841578	1.55153	2.82842712
10	0.542	90.31334468	0.451566723	47.45061568	79.08435946	1	1.898090601	1.320471	3.16227766
12	0.675	112.6819501	0.563409751	59.53805186	99.23008643	1.07918125	1.99664337	-0.11356	3.46410162

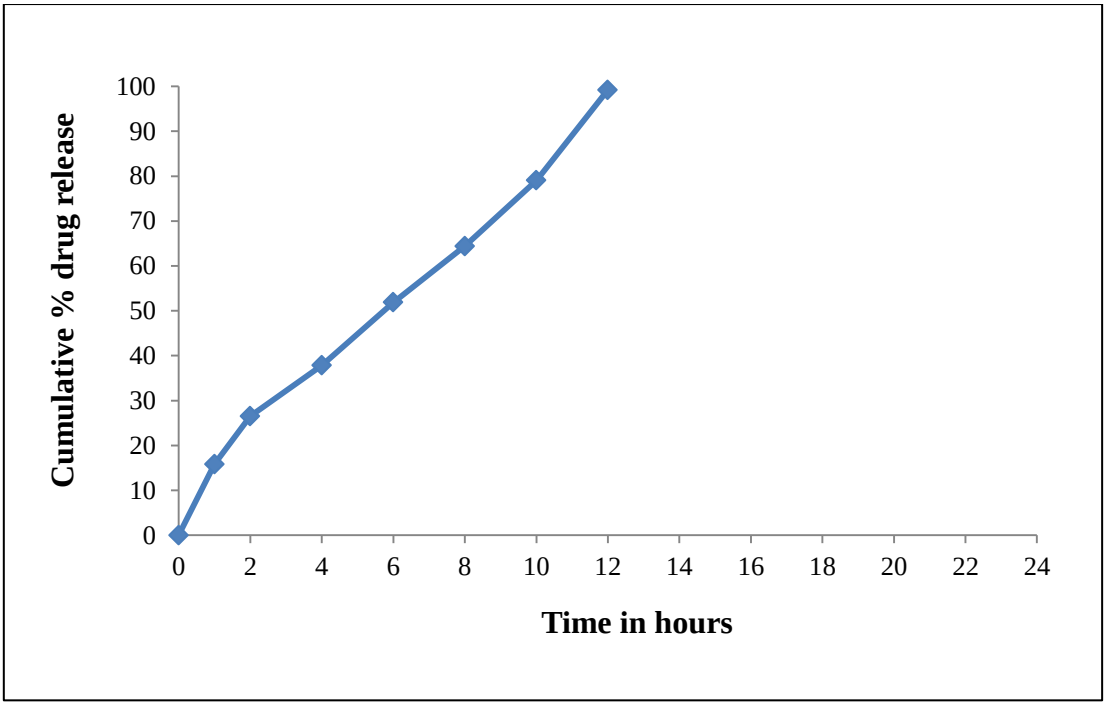

Figure 6.145 *In vitro* drug release of formulation RS203

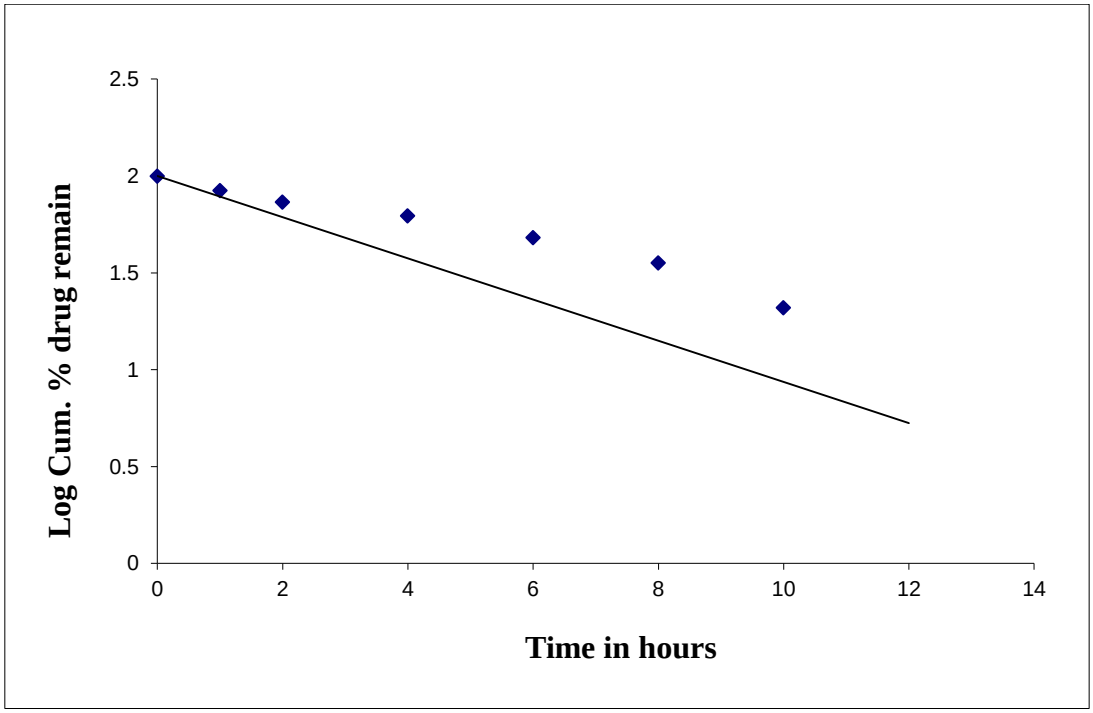

Figure 6.146 First order kinetics of formulation RS203

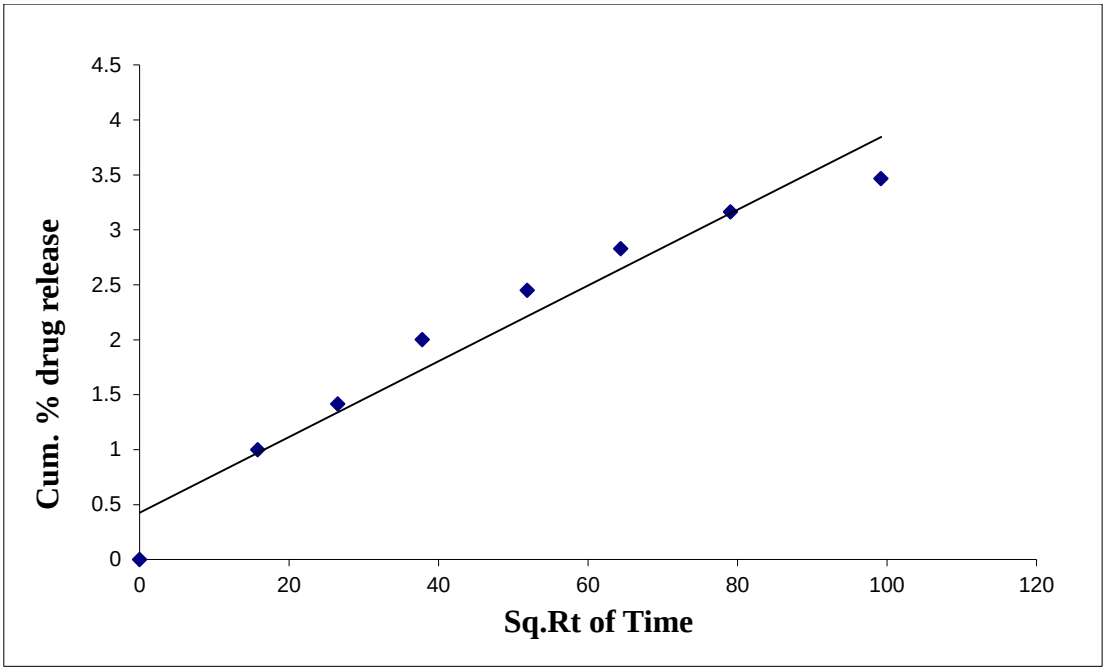

Figure6.147 Higuchi's plot for formulation RS203

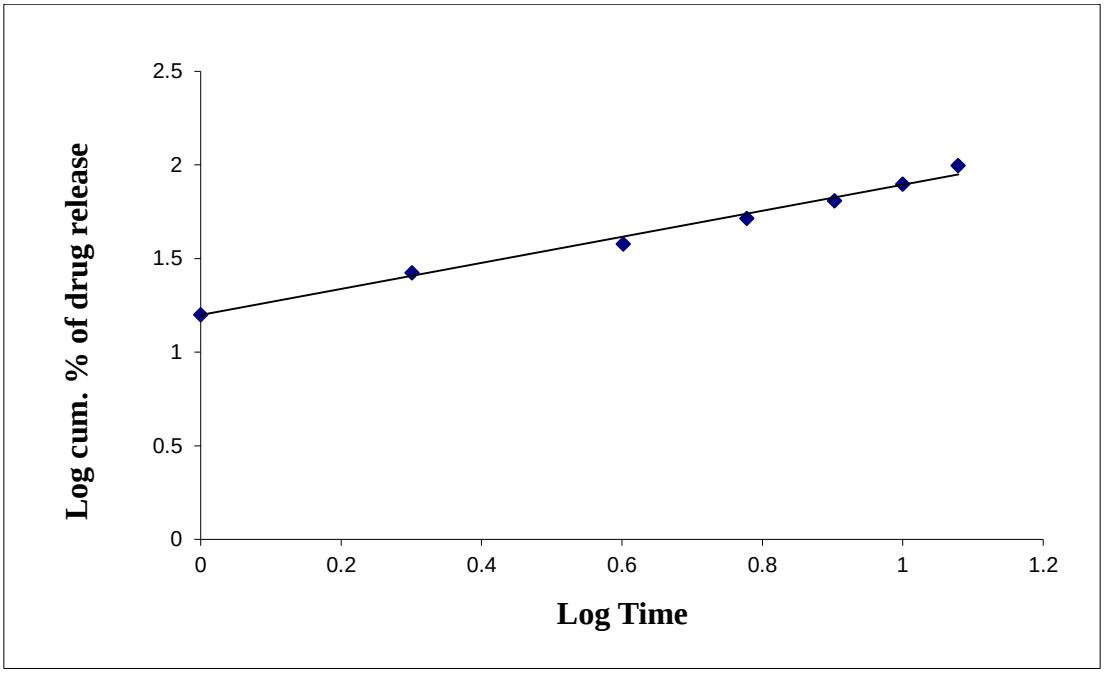

Figure 6.148 Korsmeyer & peppas plot for formulation RS203

Table 6.37 *In vitro* release study of RS204

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.098	15.63920312	0.078196016	7.819601562	13.03266927	0	1.115033374	1.939356	1
2	0.156	25.39393333	0.126969667	12.85335869	21.42226449	0.30103	1.330865377	1.8953	1.41421356
4	0.223	36.66232856	0.183311643	18.74149565	31.23582608	0.60205999	1.494652996	1.837362	2
6	0.306	50.62168385	0.253108419	26.08779658	43.47966096	0.77815125	1.638286149	1.752205	2.44948974
8	0.388	64.41285414	0.322064271	33.48959856	55.8159976	0.90308999	1.746758691	1.645265	2.82842712
10	0.467	77.69946942	0.388497347	40.77703474	67.96172456	1	1.832264391	1.505669	3.16227766
12	0.555	92.49974972	0.462498749	48.95416959	81.59028264	1.07918125	1.911638438	1.265047	3.46410162
14	0.669	111.6728401	0.558364201	59.46571228	99.10952047	1.14612804	1.996115375	-0.05038	3.74165739

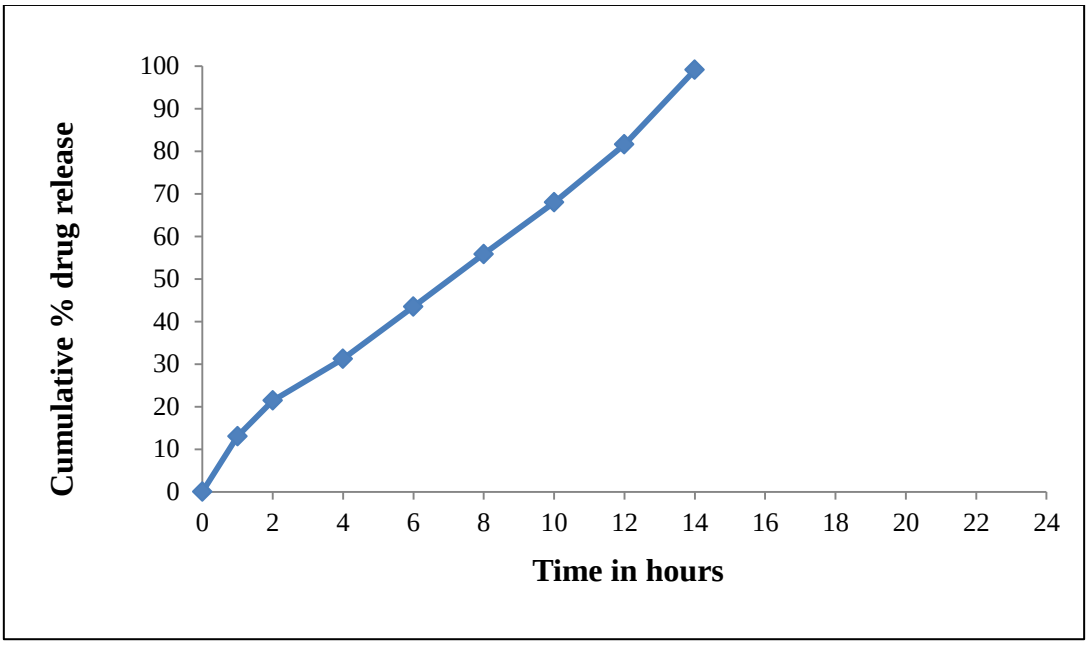

Figure 6.149 *In vitro* drug release of formulation RS204

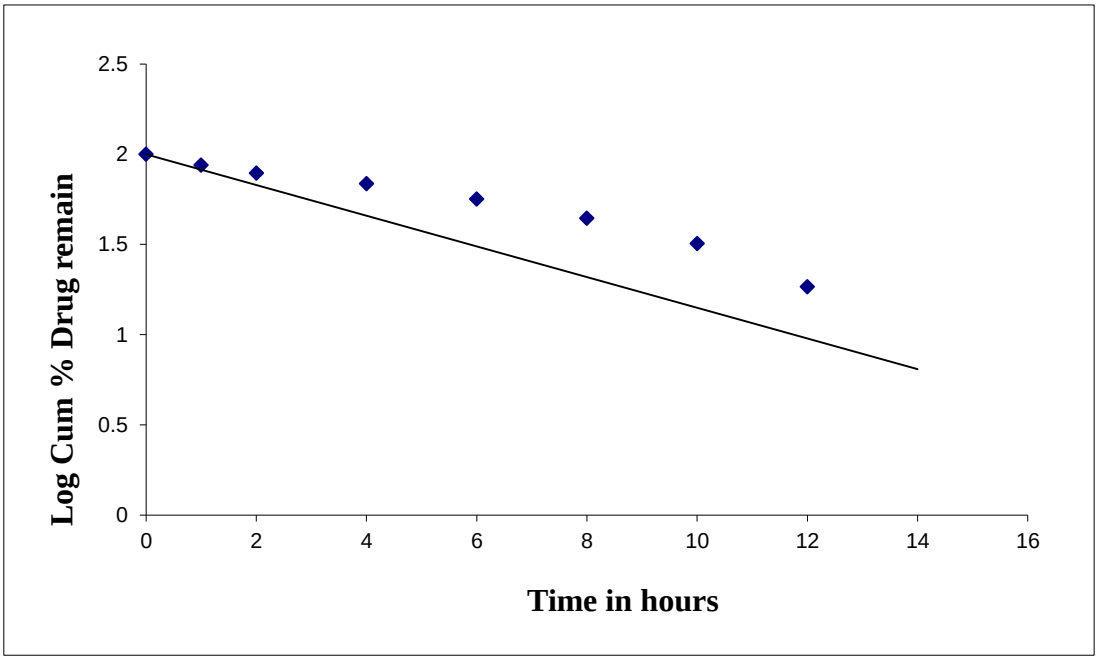

Figure 6.150 First order kinetics of formulation RS204

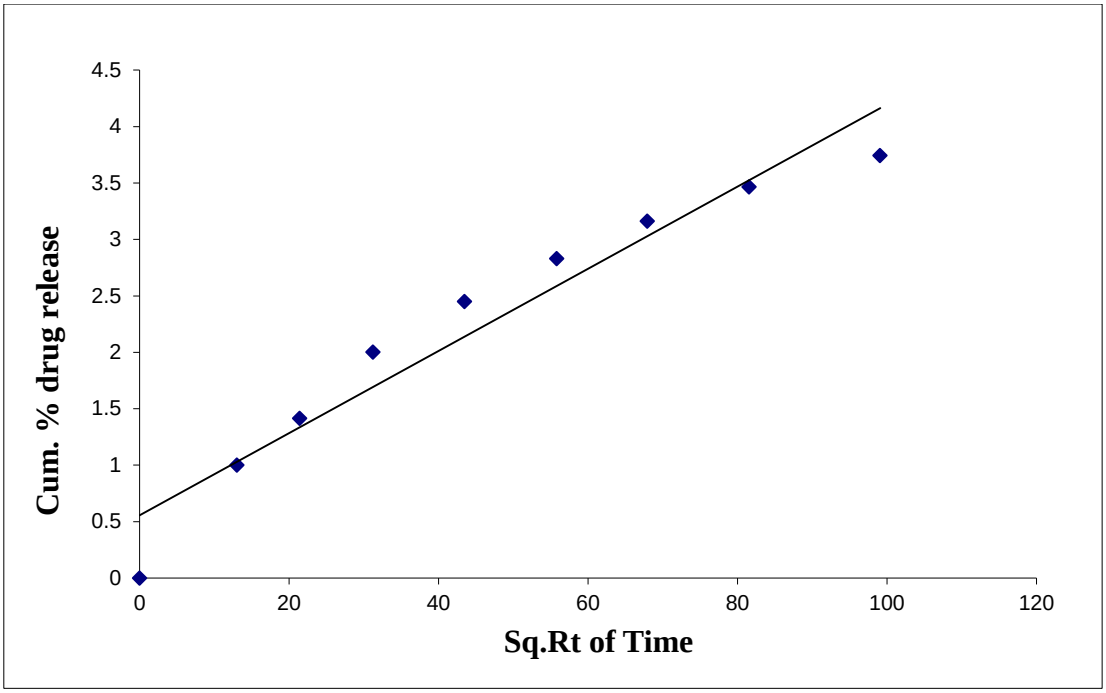

Figure 6.151 Higuchi's plot for formulation RS204

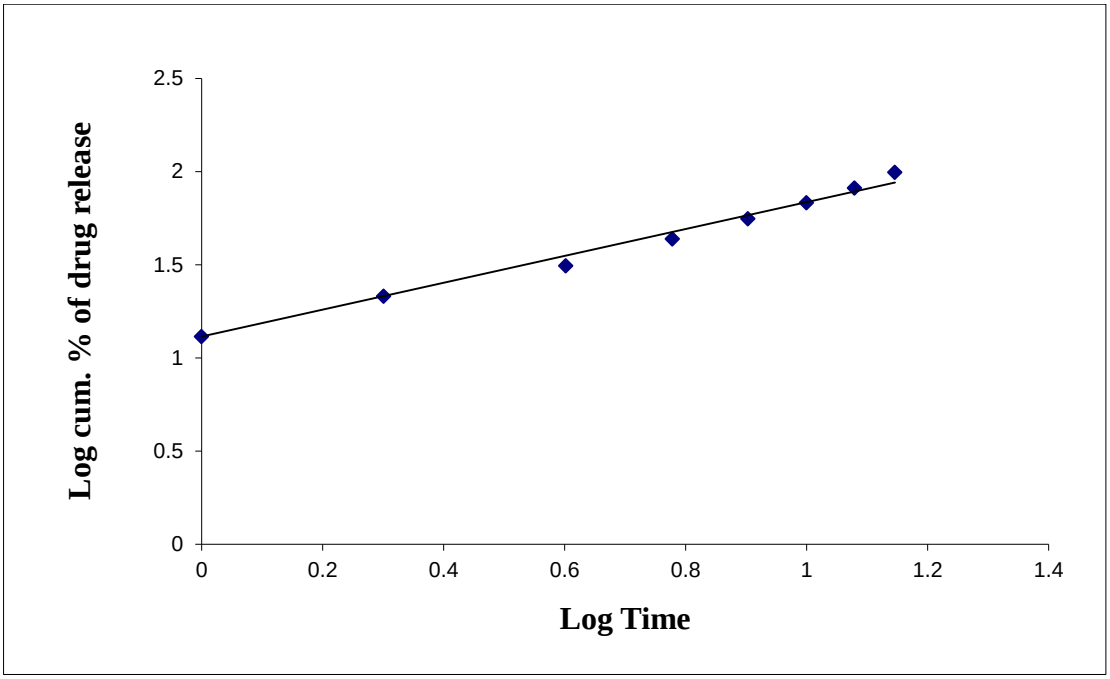

Figure 6.152 Korsmeyer & peppas plot for formulation RS204

Table 6.38 *In vitro* release study of RS205

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.086	13.62098308	0.068104915	6.810491541	11.35081923	0	1.055027207	1.947675	1
2	0.142	23.03934328	0.115196716	11.65588147	19.42646912	0.30103	1.288393872	1.906192	1.41421356
4	0.201	32.96225848	0.164811292	16.84773251	28.07955418	0.60205999	1.448390208	1.856852	2
6	0.278	45.91250375	0.229562519	23.65247773	39.42079621	0.77815125	1.595725392	1.782324	2.44948974
8	0.361	59.87185904	0.299359295	31.09128041	51.81880068	0.90308999	1.714487357	1.682878	2.82842712
10	0.436	72.48573431	0.362428672	37.99693663	63.32822772	1	1.801597334	1.564332	3.16227766
12	0.482	80.22224447	0.401111222	42.59004905	70.98341509	1.07918125	1.85115689	1.462646	3.46410162
14	0.548	91.3224547	0.456612274	48.94237661	81.57062769	1.14612804	1.911533804	1.265511	3.74165739
16	0.551	91.82700971	0.459135049	59.69673641	99.49456068	1.20411998	1.997799339	-0.29633	4

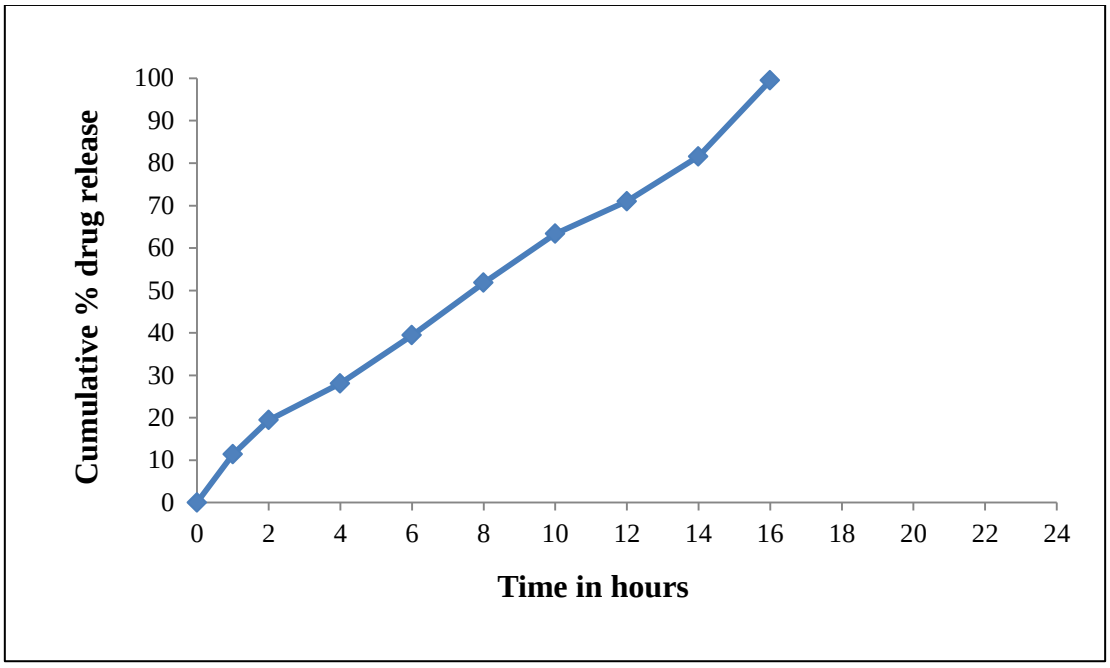

Figure 6.153 *In vitro* Drug Release of formulation RS205

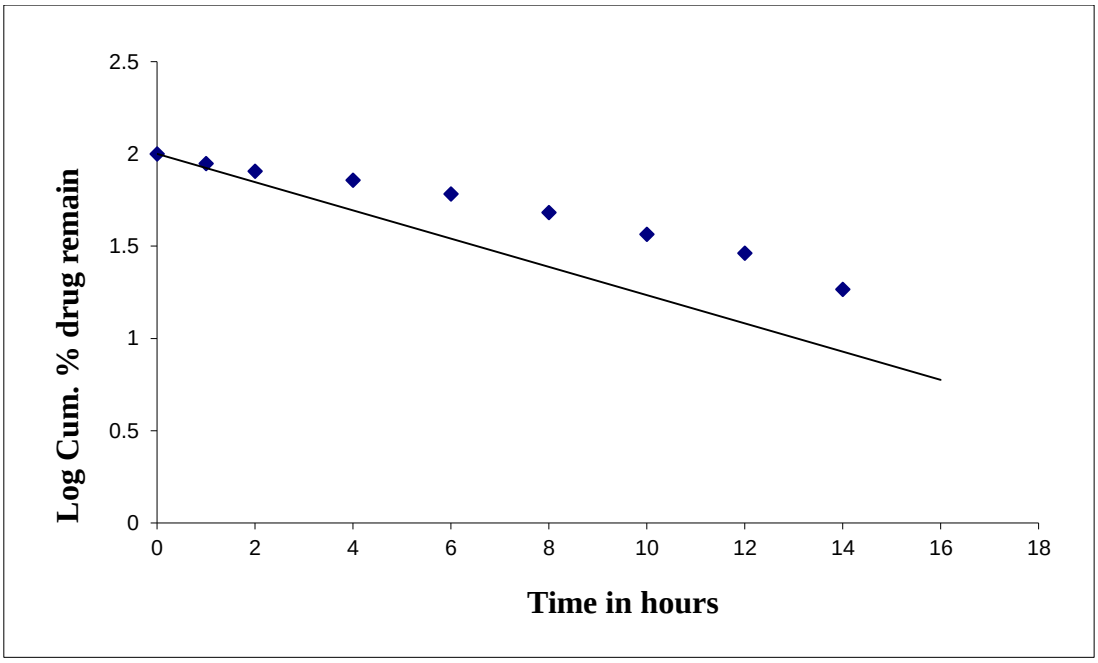

Figure 6.154 First order kinetics of formulation RS205

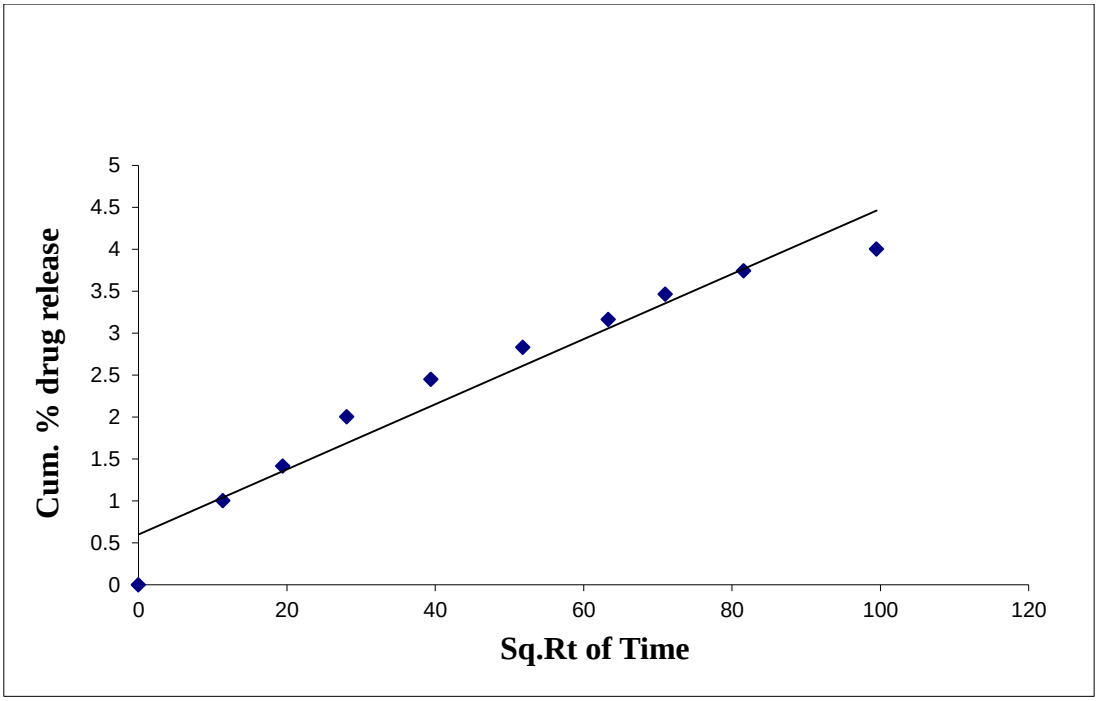

Figure 6.155 Higuchi's plot for formulation RS205

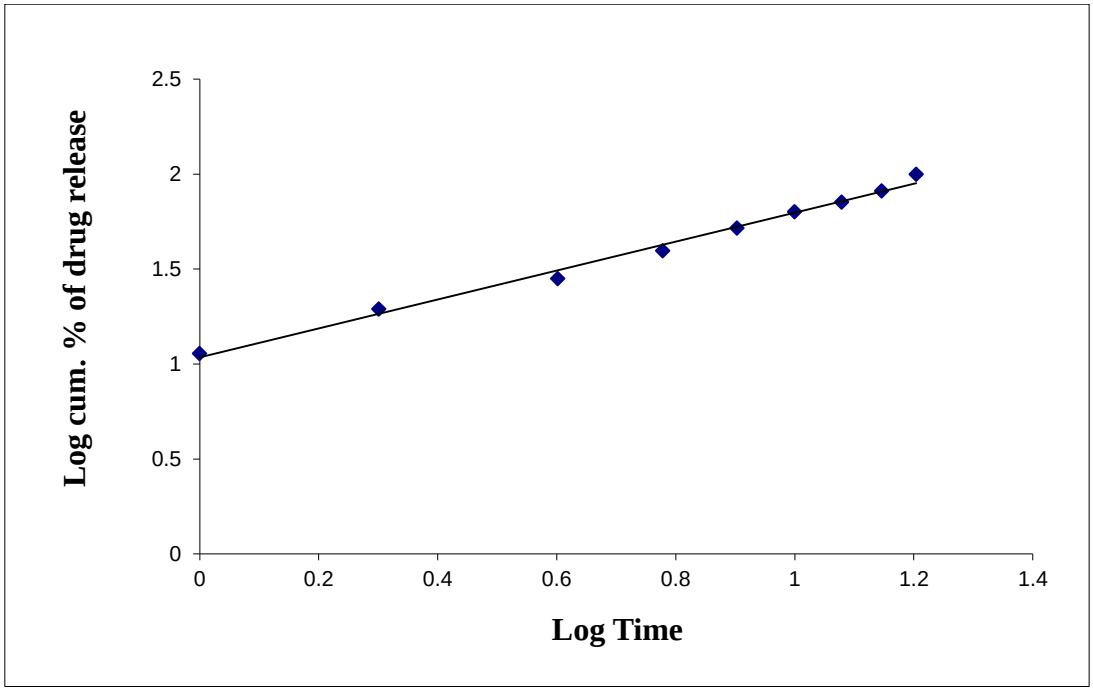

Figure 6.156 Korsmeyer & peppas plot for formulation RS205

Table 6.39 *In vitro* release study of RS206

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.072	11.26639303	0.056331965	5.633196516	9.38866086	0	0.972603652	1.957183	1
2	0.126	20.34838322	0.101741916	10.28685554	17.14475924	0.30103	1.234131391	1.91832	1.41421356
4	0.183	29.93492842	0.149674642	15.28361197	25.47268662	0.60205999	1.406074753	1.872315	2
6	0.249	41.03513865	0.205175693	21.13306637	35.22177729	0.77815125	1.546811267	1.811429	2.44948974
8	0.333	55.16267895	0.275813395	28.60718791	47.67864651	0.90308999	1.678323918	1.718679	2.82842712
10	0.396	65.75833417	0.328791671	34.45664231	57.42773718	1	1.759121704	1.629127	3.16227766
12	0.442	73.49484433	0.367474222	38.98248073	64.97080121	1.07918125	1.812718222	1.54443	3.46410162
14	0.489	81.39953949	0.406997697	43.66977675	72.78296126	1.14612804	1.862029721	1.434841	3.74165739
16	0.492	81.9040945	0.409520473	53.2830013	88.80500217	1.20411998	1.948437429	1.049024	4
18	0.561	93.50885975	0.467544299	59.90442487	99.84070811	1.25527251	1.999307653	-0.79781	4.24264069

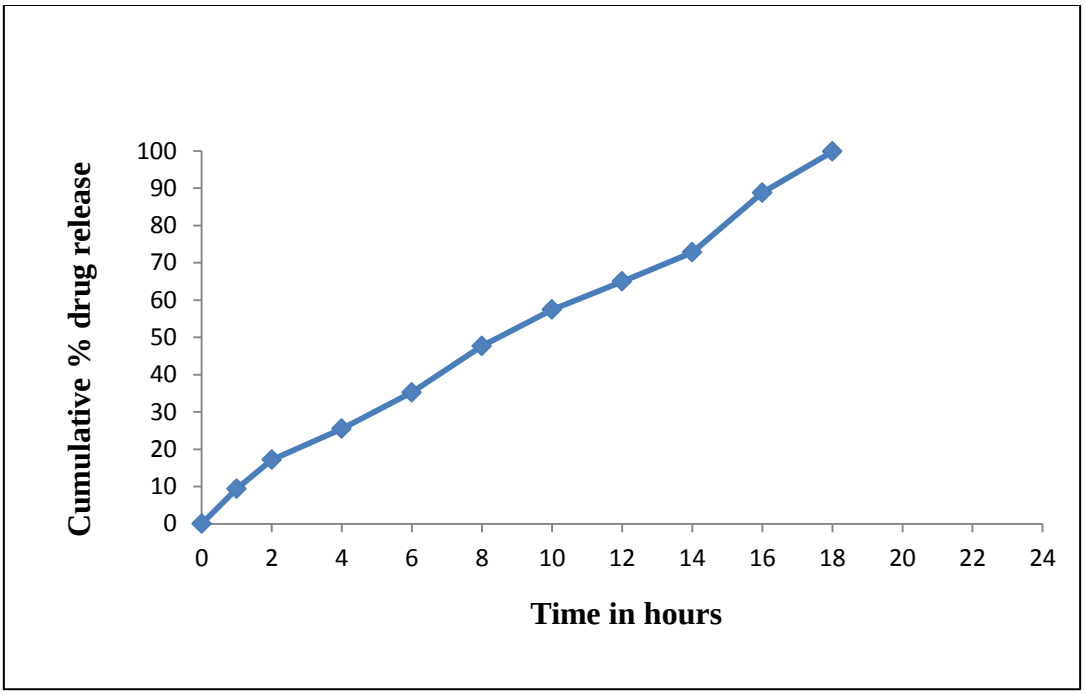

Figure 6.157 *In vitro* drug release of formulation RS206

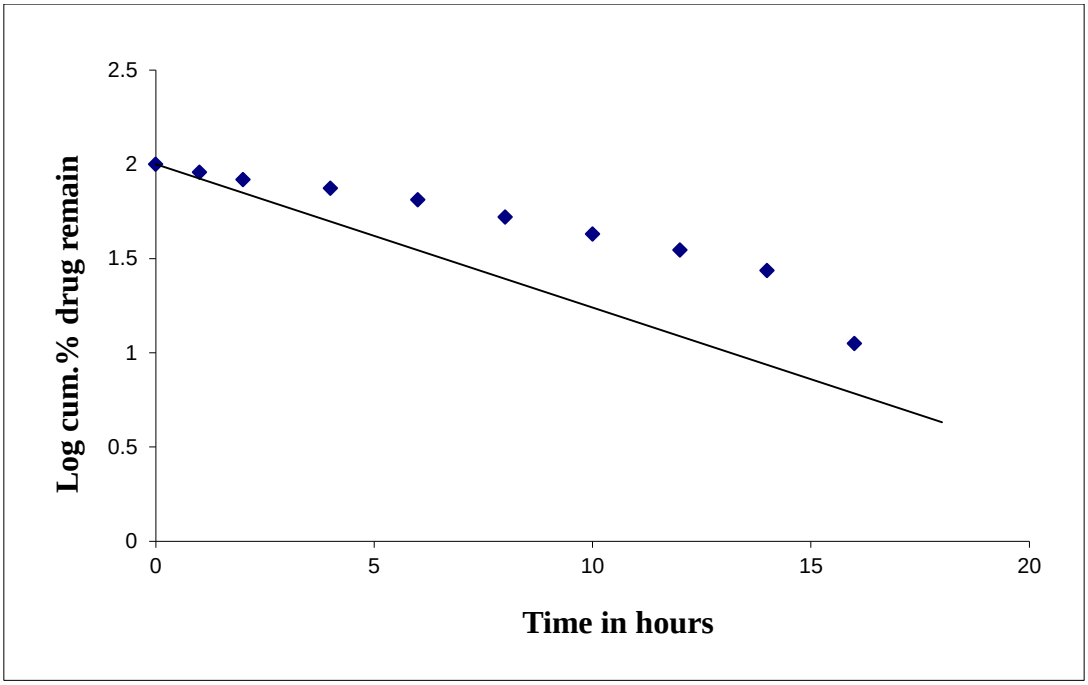

Figure 6.158 First order kinetics of formulation RS206

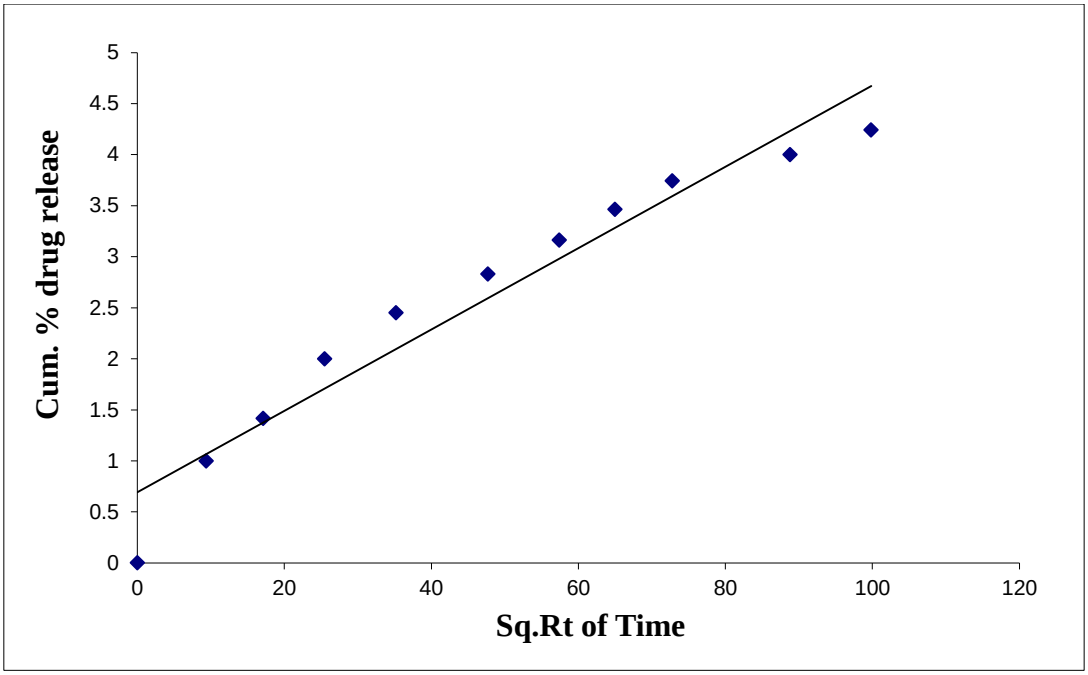

Figure 6.159 Higuchi's plot for formulation RS206

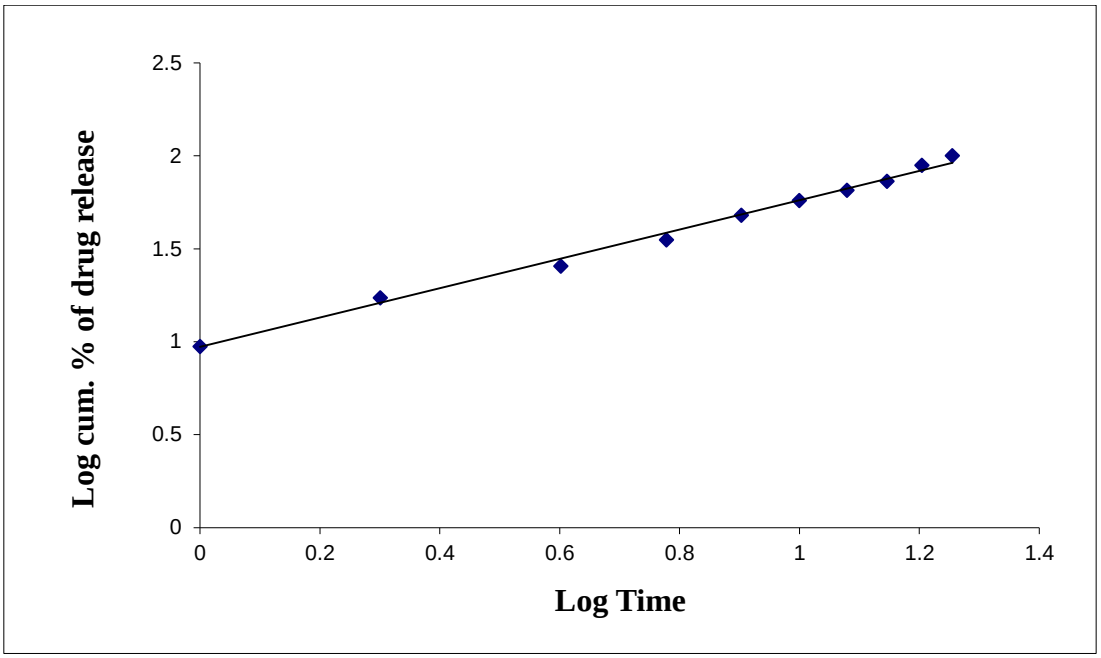

Figure 6.160 Korsmeyer & peppas plot for formulation RS206

Table 6.40 *In vitro* release study of RS207

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.066	10.25728301	0.051286415	5.128641506	8.547735843	0	0.931851092	1.961194	1
2	0.104	16.64831314	0.083241566	8.426729402	14.044549	0.30103	1.147507798	1.934273	1.41421356
4	0.172	28.08489338	0.140424467	14.31150265	23.85250442	0.60205999	1.377533985	1.881656	2
6	0.234	38.5123636	0.192561818	19.8060867	33.01014449	0.77815125	1.518647425	1.826009	2.44948974
8	0.321	53.1444589	0.265722295	27.50725798	45.84542997	0.90308999	1.66129605	1.733635	2.82842712
10	0.374	62.05826409	0.31029132	32.49560517	54.15934194	1	1.733673379	1.661251	3.16227766
12	0.426	70.80388427	0.354019421	37.4889979	62.48166316	1.07918125	1.795752581	1.574244	3.46410162
14	0.466	77.53128441	0.387656422	41.56073681	69.26789468	1.14612804	1.840531988	1.487592	3.74165739
16	0.468	77.86765442	0.389338272	50.64501952	84.40836587	1.20411998	1.926385492	1.192892	4
18	0.522	86.94964461	0.434748223	55.96469116	93.27448527	1.25527251	1.969762861	0.827726	4.24264069
20	0.558	93.00430474	0.465021524	59.86151767	99.76919612	1.30103	1.998996473	-0.63676	4.47213595

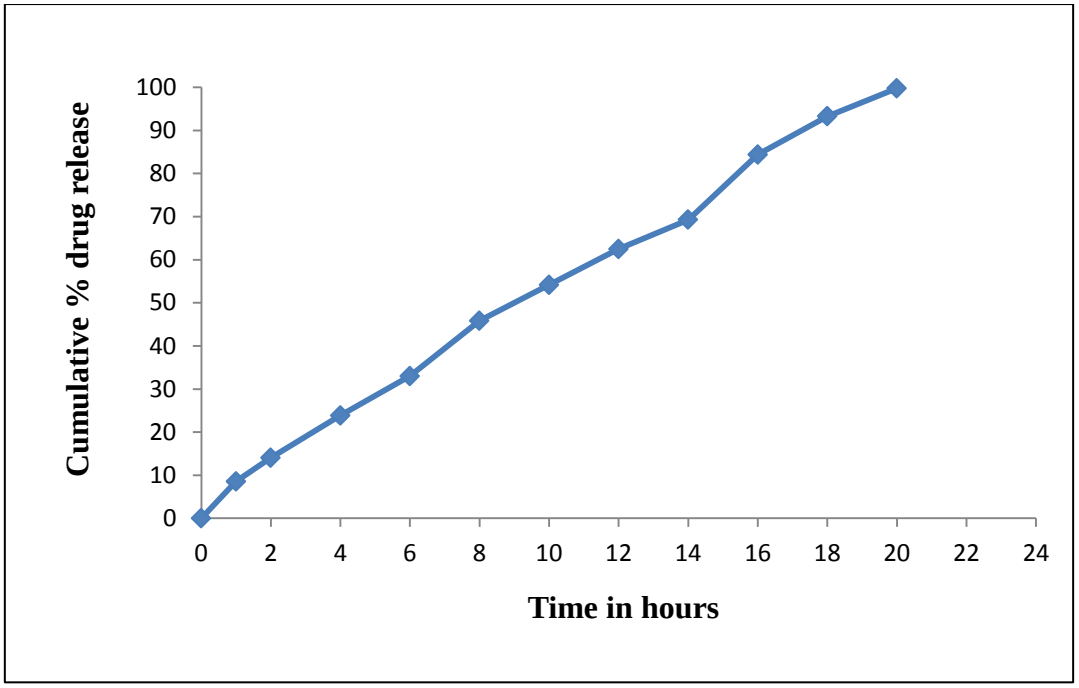

Figure 6.161 *In vitro* drug release of formulation RS207

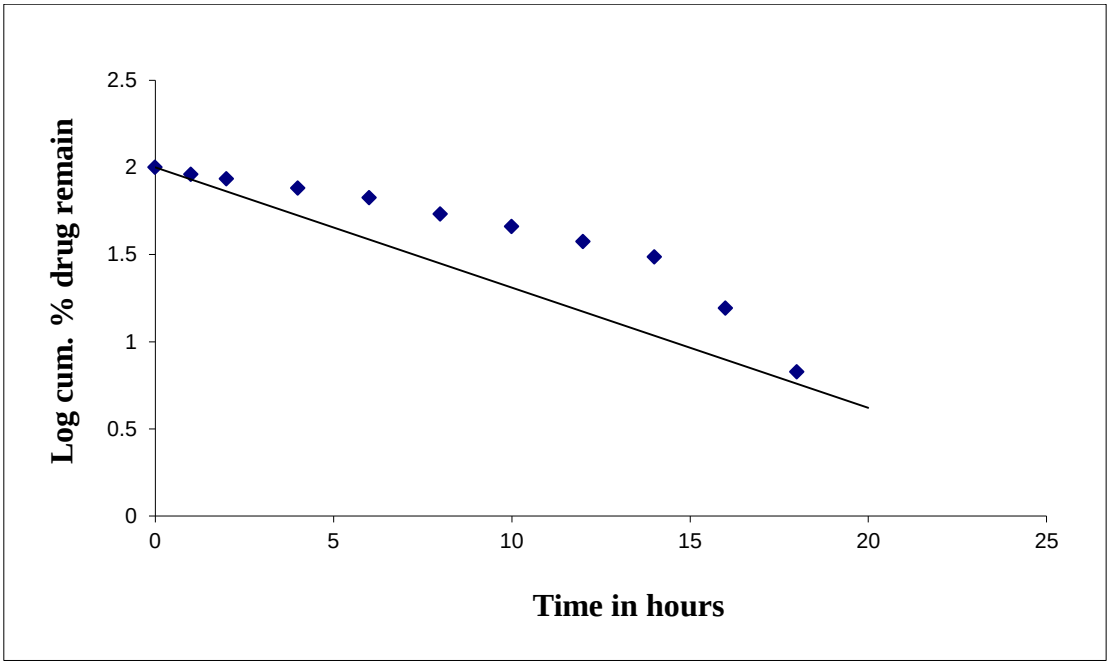

Figure 6.162 First order kinetics of formulation RS207

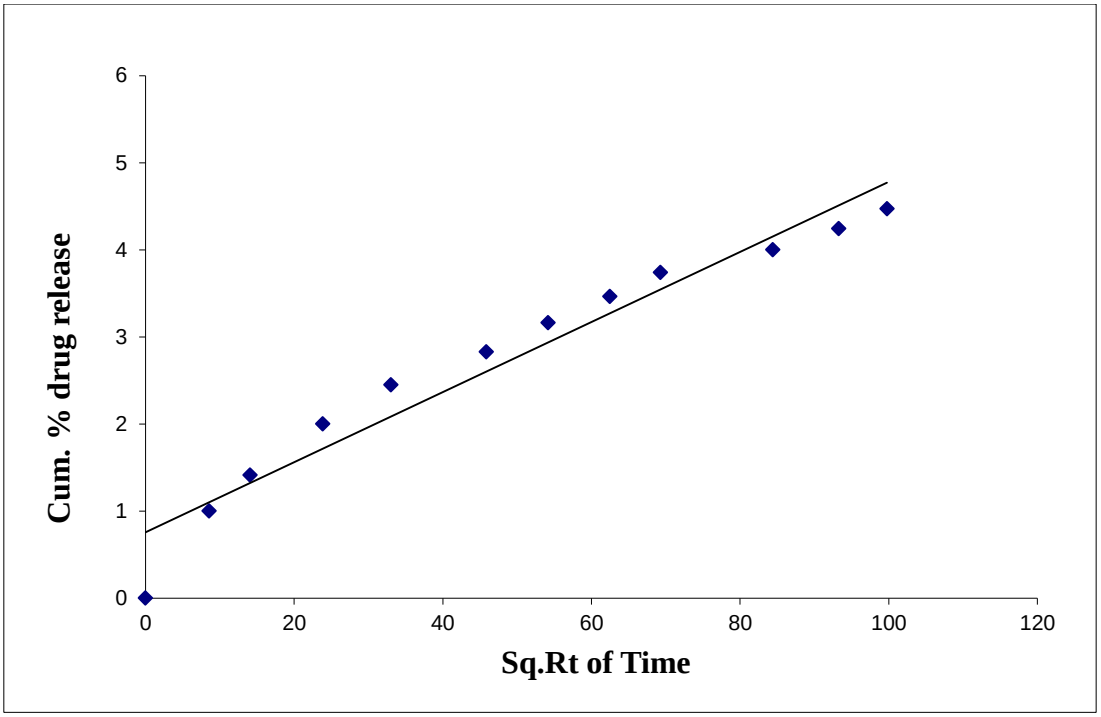

Figure 6.163 Higuchi's plot for formulation RS207

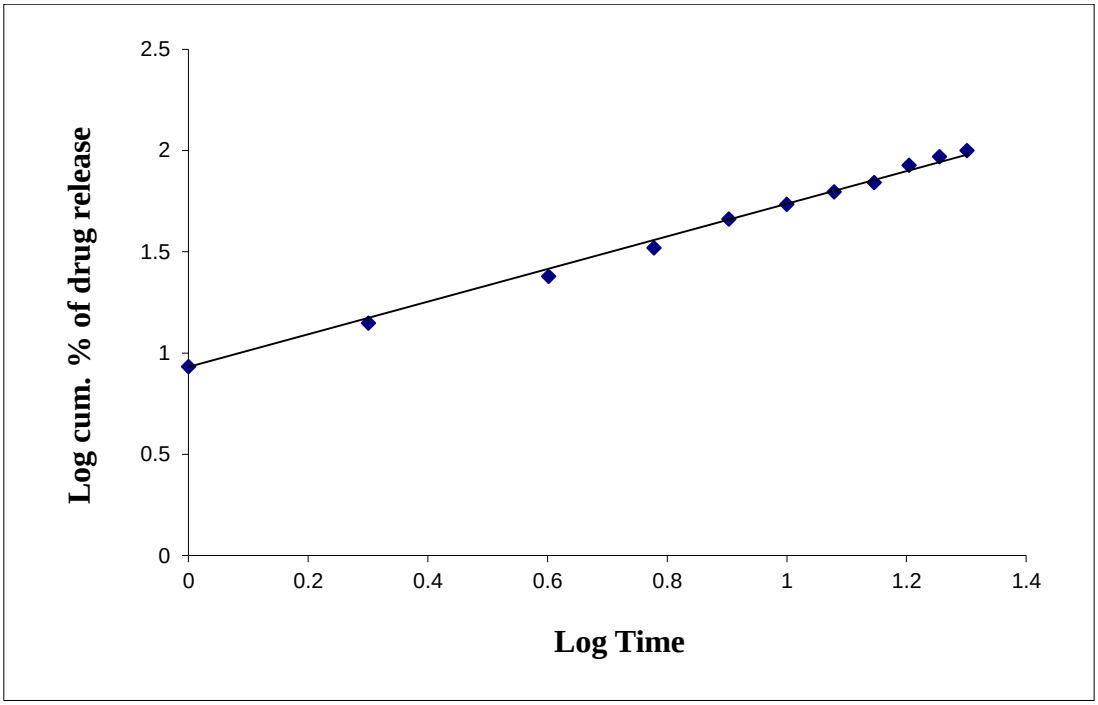

Figure 6.164 Korsmeyer & peppas plot for formulation RS207

Table 6.41 *In vitro* release study of RS208

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.054	8.239062969	0.041195315	4.119531485	6.865885808	0	0.836696576	1.969109	1
2	0.093	14.79827811	0.073991391	7.481529683	12.46921614	0.30103	1.095839153	1.942161	1.41421356
4	0.160	26.06667334	0.130333367	13.26371008	22.10618347	0.60205999	1.34451377	1.891503	2
6	0.219	35.98958855	0.179947943	18.48583442	30.80972403	0.77815125	1.488687808	1.840045	2.44948974
8	0.302	49.94894384	0.249744719	25.82540795	43.04234658	0.90308999	1.63389594	1.755552	2.82842712
10	0.357	59.19911903	0.295995595	30.94998498	51.58330831	1	1.712509192	1.684995	3.16227766
12	0.408	67.77655421	0.338882771	35.83069376	59.71782294	1.07918125	1.776103967	1.605113	3.46410162
14	0.444	73.83121434	0.369156072	39.53578937	65.89298228	1.14612804	1.818839164	1.532844	3.74165739
16	0.451	75.00850936	0.375042547	48.61502653	81.02504422	1.20411998	1.908619277	1.278181	4
18	0.487	81.06316949	0.405315847	52.39244169	87.32073614	1.25527251	1.941117388	1.103094	4.24264069
20	0.526	87.62238462	0.438111923	56.48268095	94.13780158	1.30103	1.973764052	0.768061	4.47213595
22	0.552	91.99519471	0.459975974	59.54530984	99.24218307	1.34242268	1.996696309	-0.12044	4.69041576

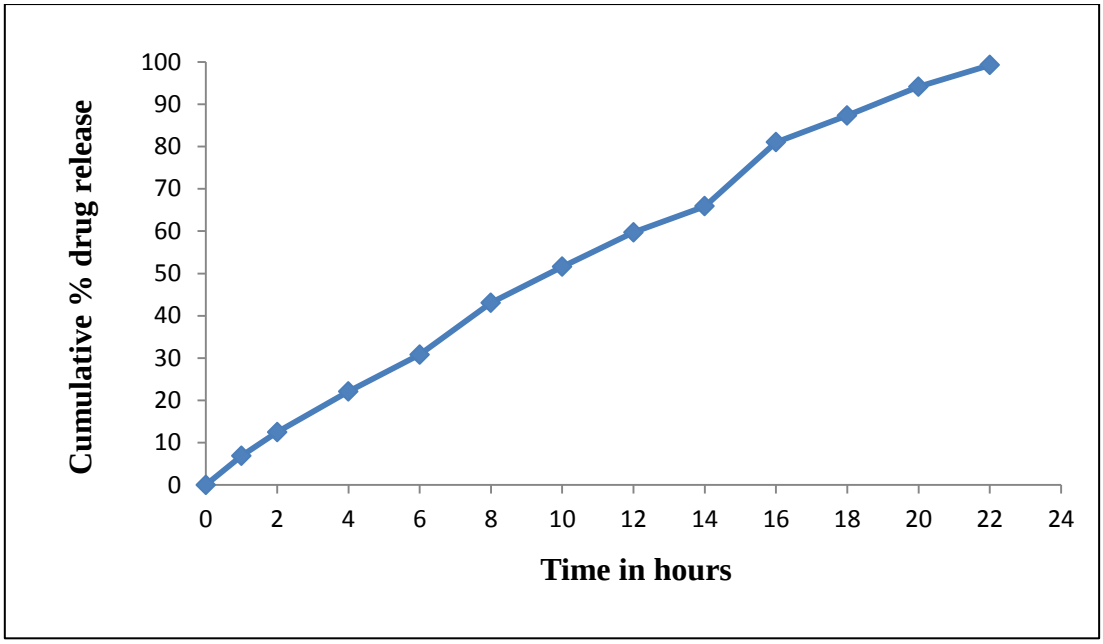

Figure 6.165 *In vitro* drug release of formulation RS208

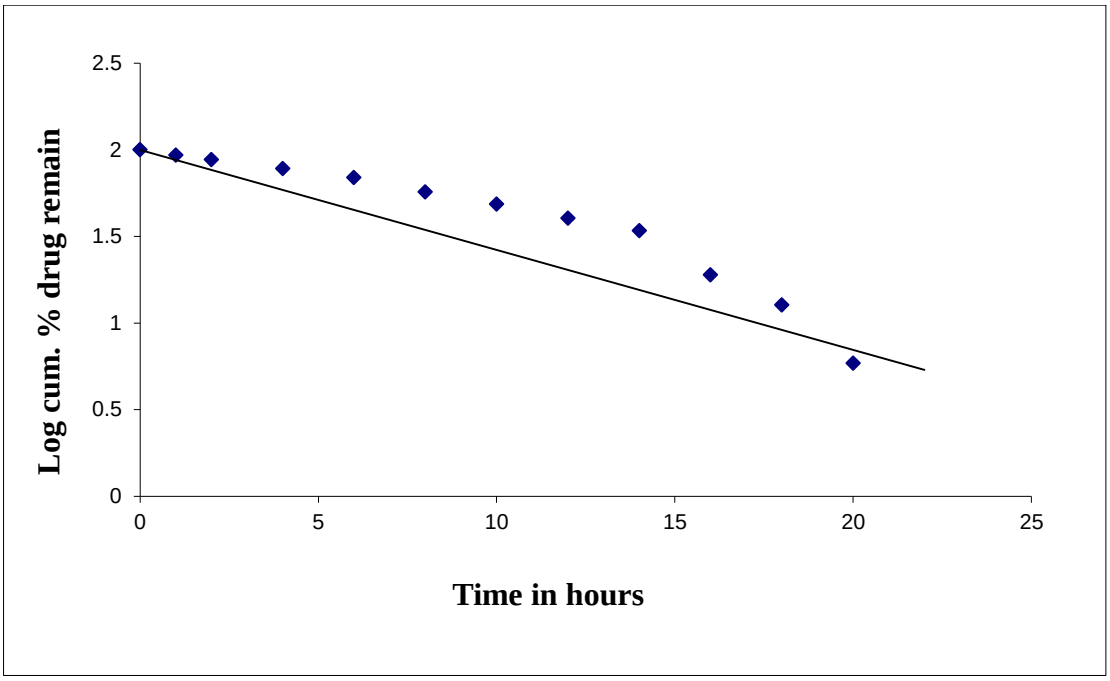

Figure 6.166 First order kinetics of formulation RS208

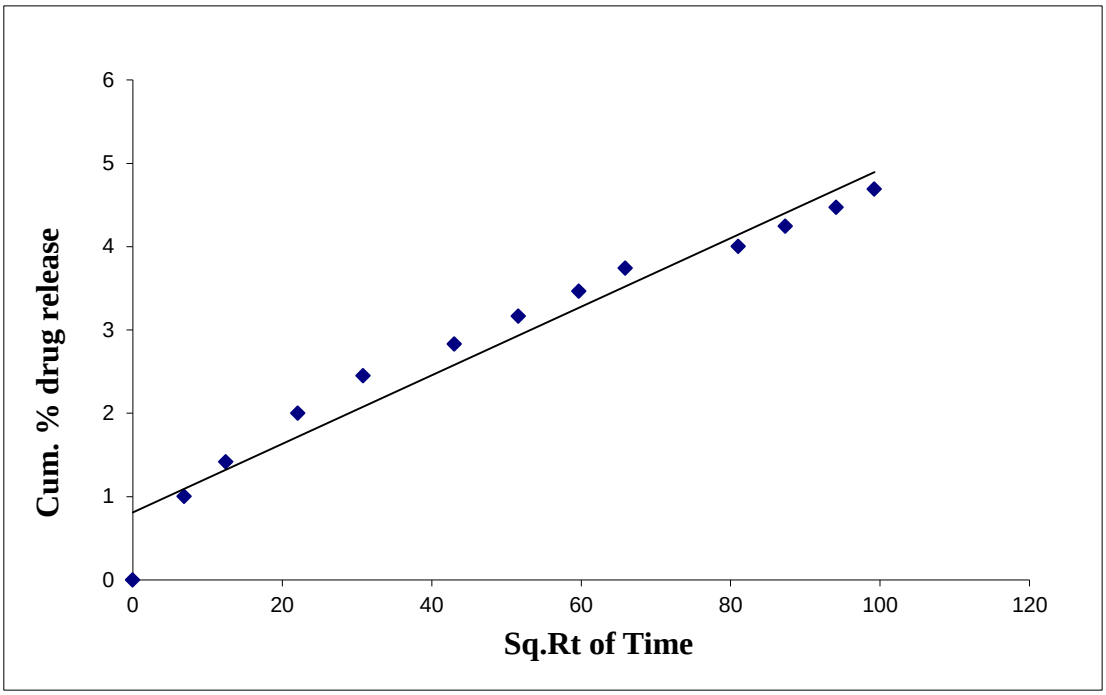

Figure 6.167 Higuchi's plot for formulation RS208

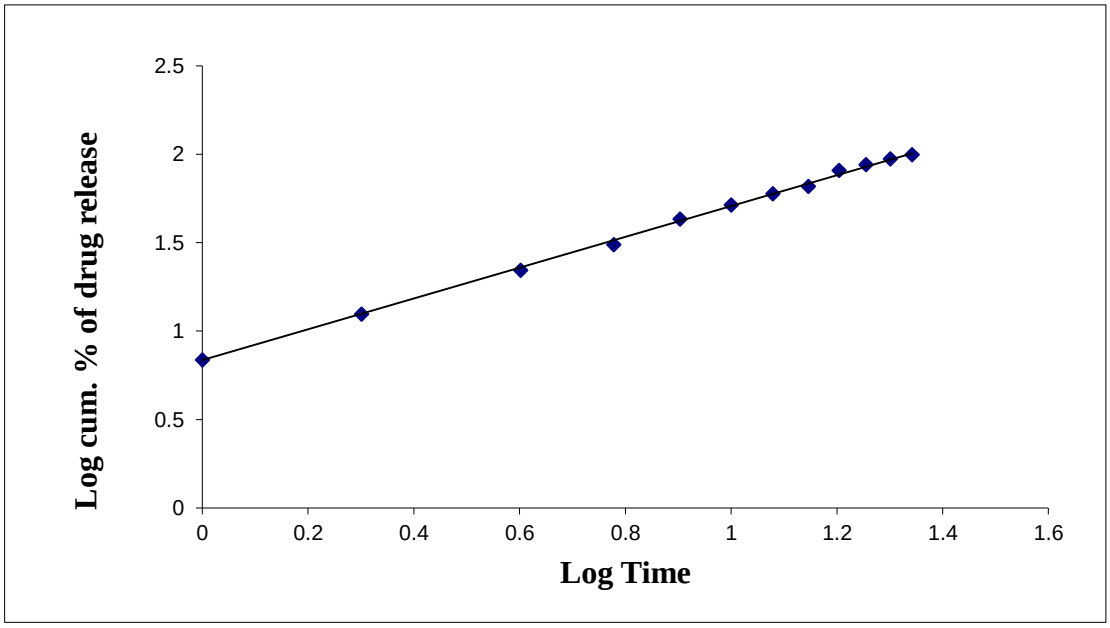

Figure 6.168 Korsmeyer & peppas plot for formulation RS208

Table 6.42 *In vitro* release study of RS209

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.048	7.229952948	0.036149765	3.614976474	6.02496079	0	0.779954225	1.973013	1
2	0.078	12.27550305	0.061377515	6.210051056	10.35008509	0.30103	1.01494392	1.95255	1.41421356
4	0.142	23.03934328	0.115196716	11.7147262	19.52454366	0.60205999	1.290580892	1.905663	2
6	0.201	32.96225848	0.164811292	16.90657723	28.17762872	0.77815125	1.449904442	1.85626	2.44948974
8	0.254	41.87606367	0.209380318	21.69310241	36.15517069	0.90308999	1.558170416	1.805126	2.82842712
10	0.321	53.1444589	0.265722295	27.74606067	46.24343444	1	1.665050081	1.730432	3.16227766
12	0.376	62.3946341	0.31197317	32.90259285	54.83765475	1.07918125	1.739078873	1.654776	3.46410162
14	0.416	69.12203424	0.345610171	36.89023926	61.48373211	1.14612804	1.788760222	1.585644	3.74165739
16	0.431	71.64480929	0.358224046	46.10066073	76.83443454	1.20411998	1.885555899	1.364843	4
18	0.476	79.21313445	0.396065672	50.6012714	84.33545233	1.25527251	1.926010179	1.194918	4.24264069
20	0.511	85.09960957	0.425498048	54.3366403	90.56106717	1.30103	1.956941532	0.974923	4.47213595
22	0.563	93.84522975	0.469226149	59.56044649	99.26741082	1.34242268	1.996806694	-0.13514	4.69041576

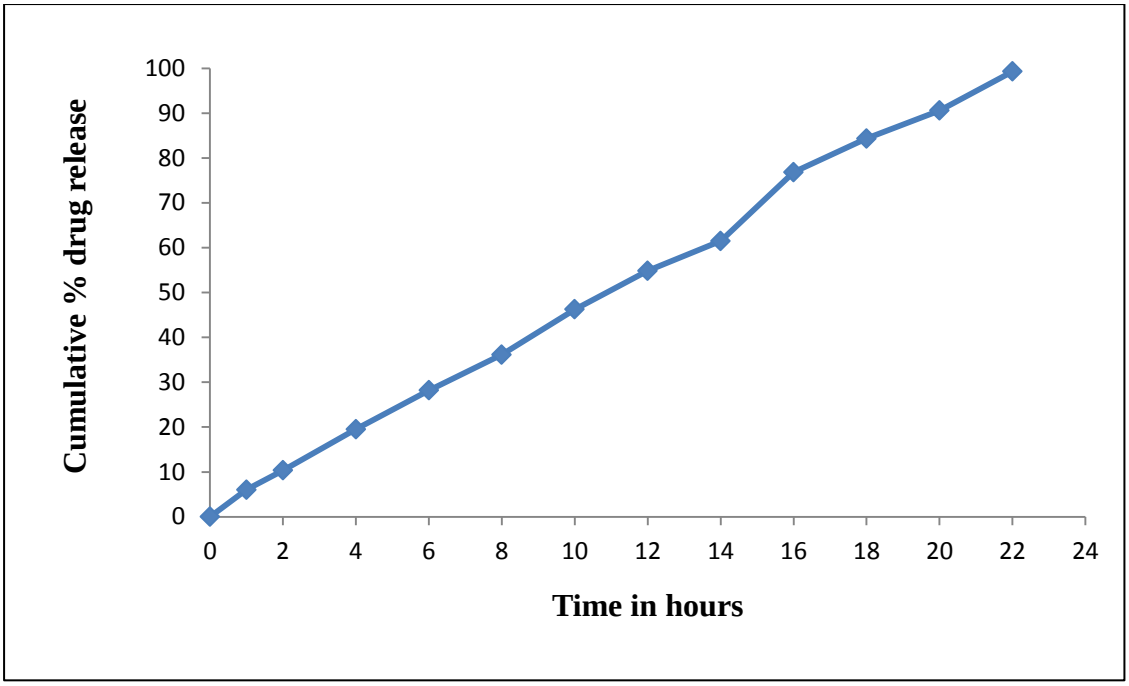

Figure 6.169 *In vitro* drug release of formulation RS209

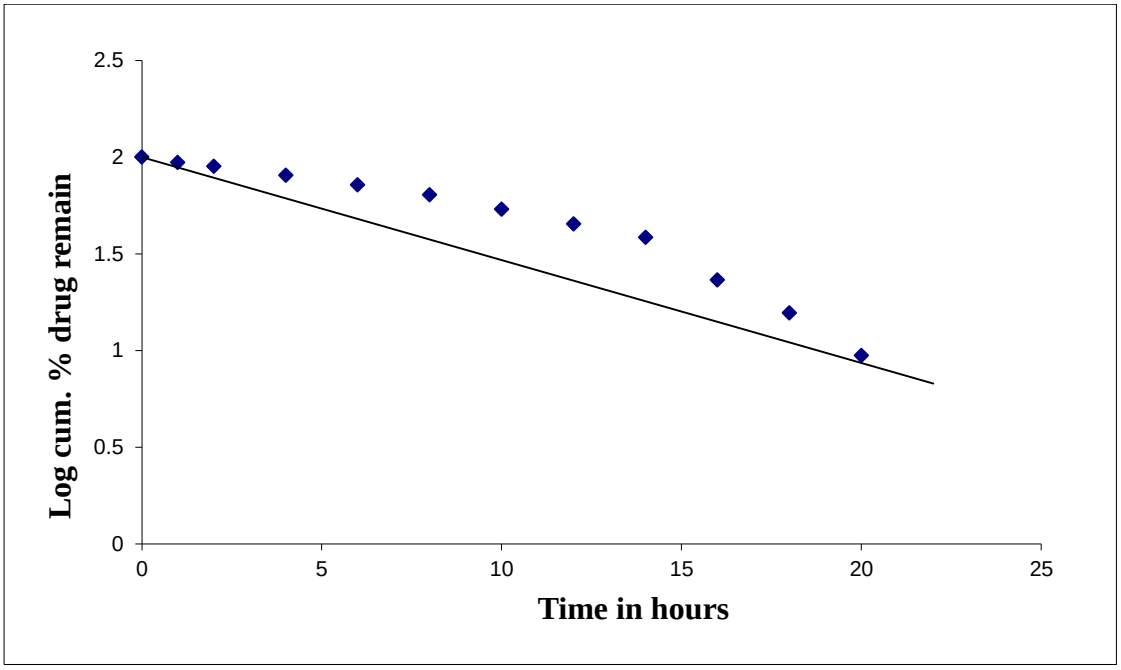

Figure 6.170 First order kinetics of formulation RS209

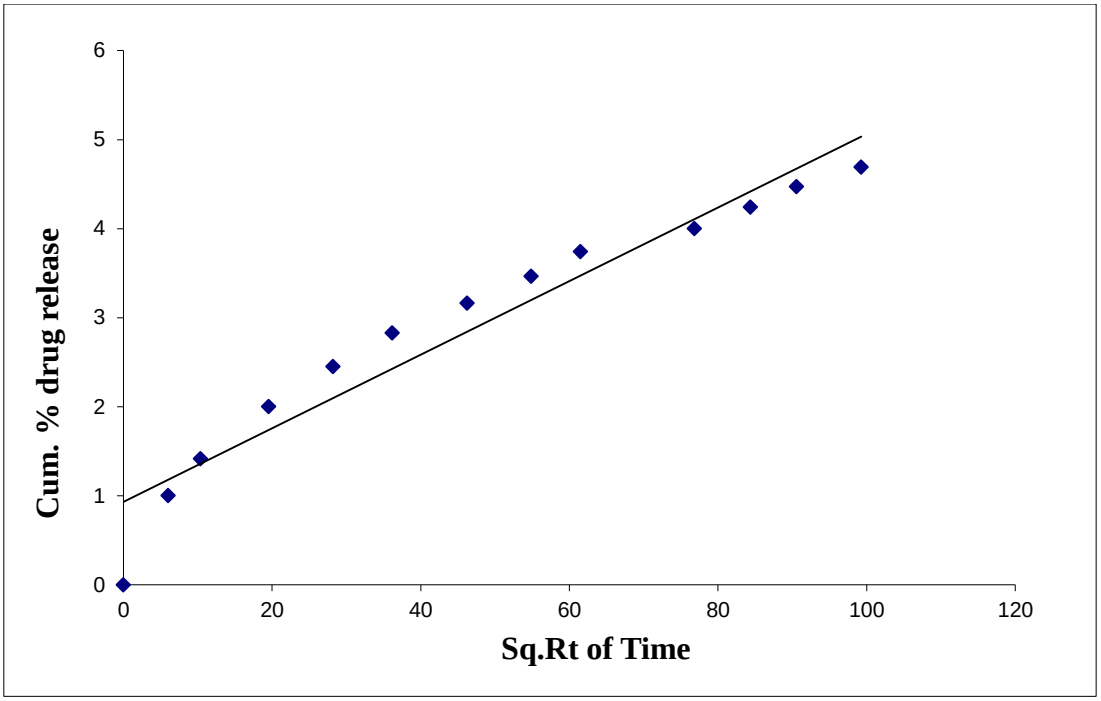

Figure 6.171 Higuchi's plot for formulation RS209

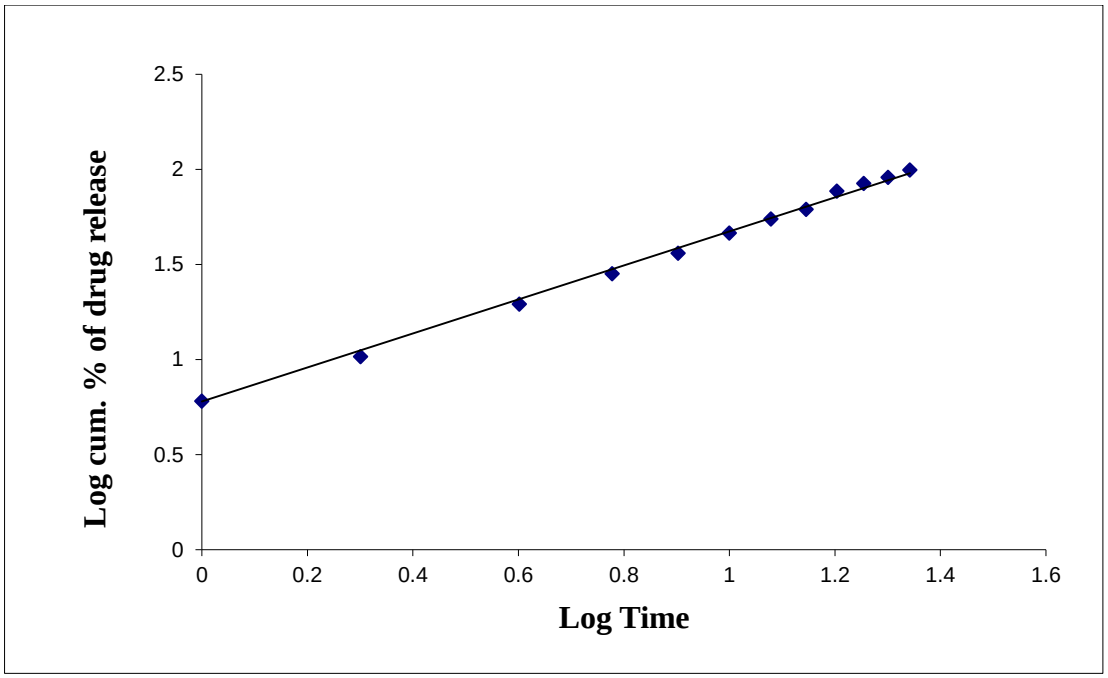

Figure 6.172 Korsmeyer & peppas plot for formulation RS209

Table 6.43 *In vitro* release study of RS210

Time in hrs	Absorbance	Concentration In µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.039	5.716287917	0.02858144	2.858143958	4.763573264	0	0.677932849	1.978803	1
2	0.066	10.25728301	0.051286415	5.185804385	8.643007308	0.30103	0.93666488	1.960742	1.41421356
4	0.121	19.5074582	0.097537291	9.913464811	16.52244135	0.60205999	1.218074219	1.92157	2
6	0.182	29.76674342	0.148833717	15.238182	25.39697	0.77815125	1.404781906	1.872756	2.44948974
8	0.234	38.5123636	0.192561818	19.90865953	33.18109921	0.90308999	1.520890769	1.824899	2.82842712
10	0.298	49.27620382	0.246381019	25.67570327	42.79283879	1	1.631371098	1.75745	3.16227766
12	0.346	57.34908399	0.28674542	30.2049054	50.34150899	1.07918125	1.701926229	1.695994	3.46410162
14	0.389	64.58103914	0.322905196	34.39437381	57.32395635	1.14612804	1.758336157	1.630184	3.74165739
16	0.399	66.26288918	0.331314446	42.66211833	71.10353055	1.20411998	1.851891166	1.460845	4
18	0.436	72.48573431	0.362428672	46.43616979	77.39361631	1.25527251	1.88870514	1.354231	4.24264069
20	0.484	80.55861448	0.402793072	51.19746721	85.32911202	1.30103	1.931097226	1.166456	4.47213595
22	0.524	87.28601462	0.436430073	55.36675343	92.27792238	1.34242268	1.965097808	0.887734	4.69041576
24	0.563	93.84522975	0.469226149	59.51922114	99.19870191	1.38021124	1.996505989	-0.09621	4.89897949

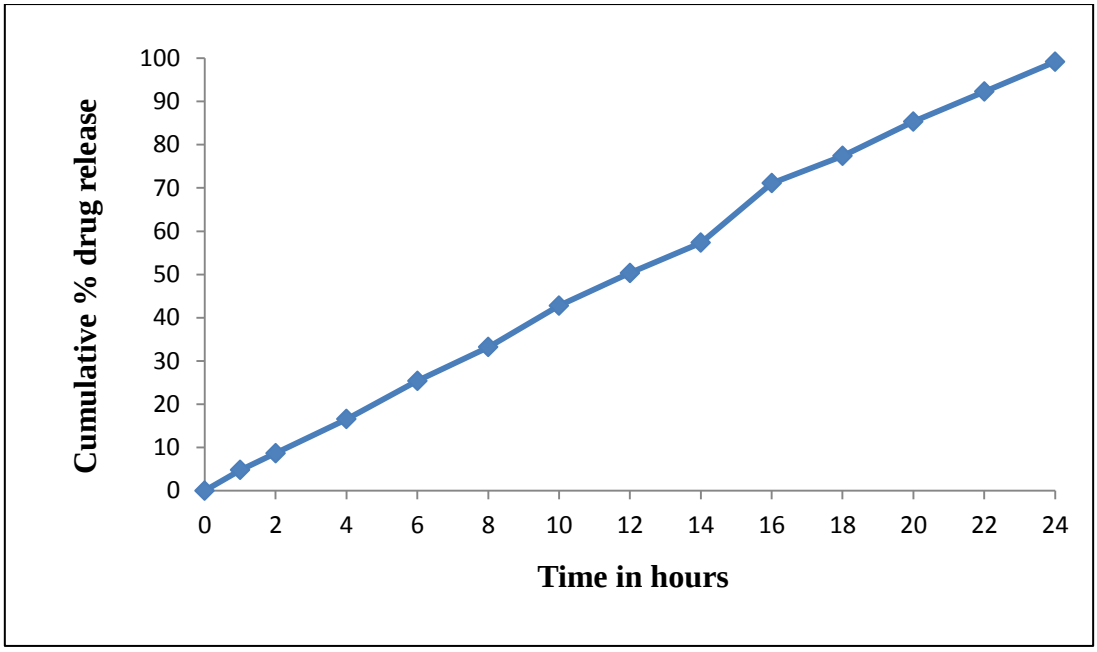

Figure6.173 *In vitro* drug release of formulation RS210

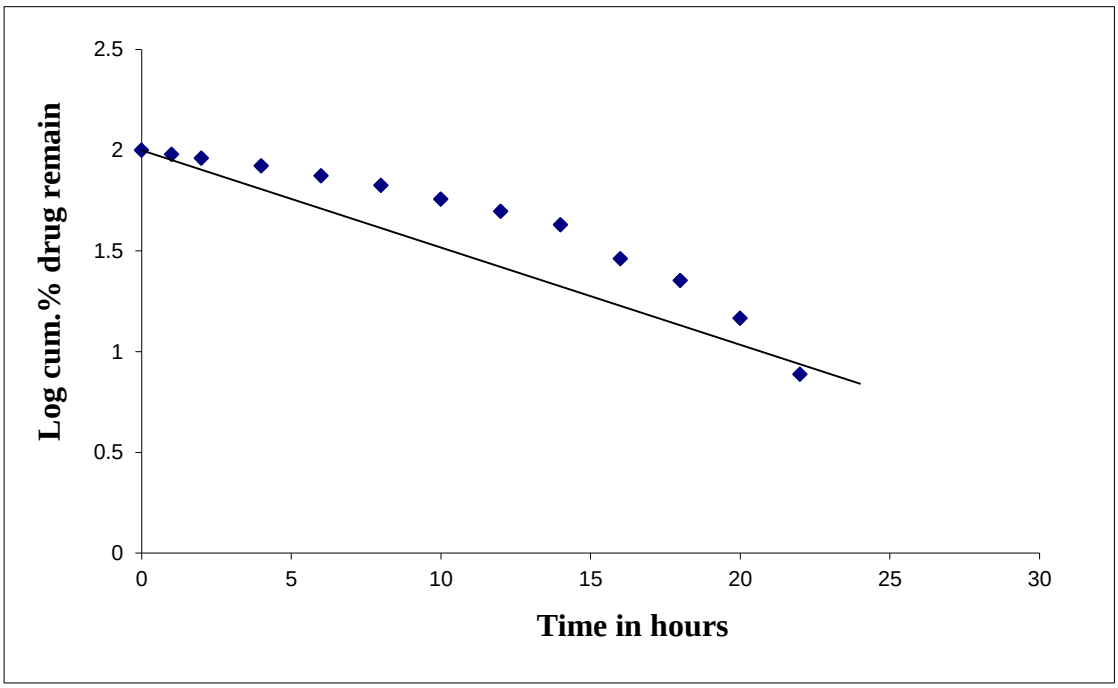

Figure 6.174 First order kinetics of formulation RS210

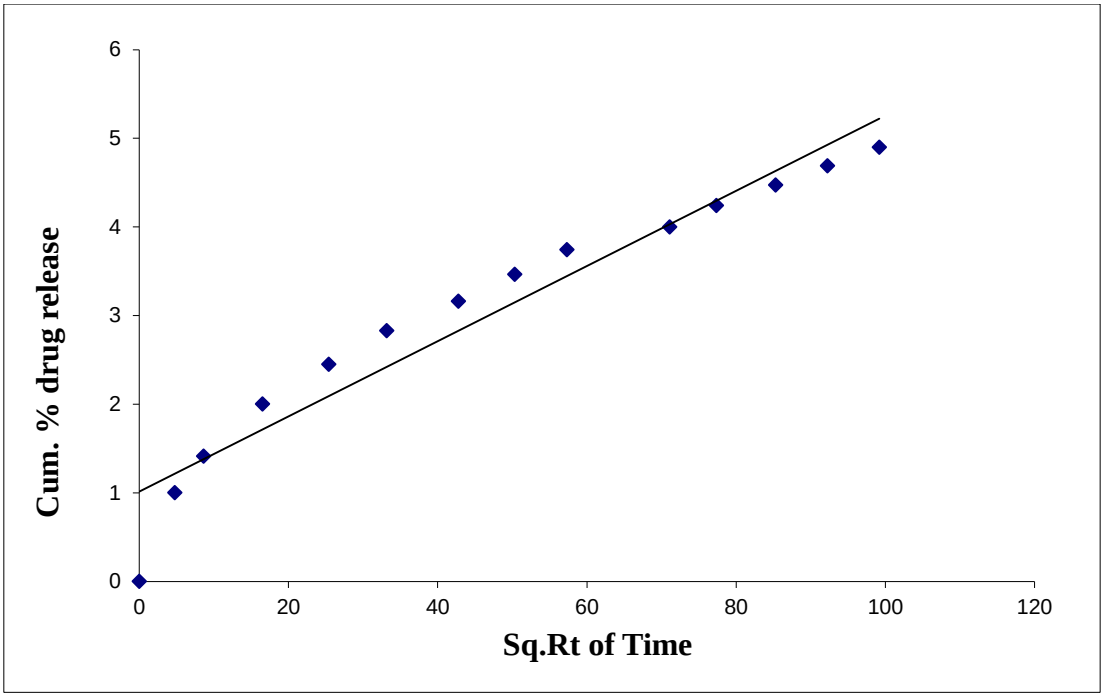

Figure 6.175 Higuchi's plot for formulation RS210

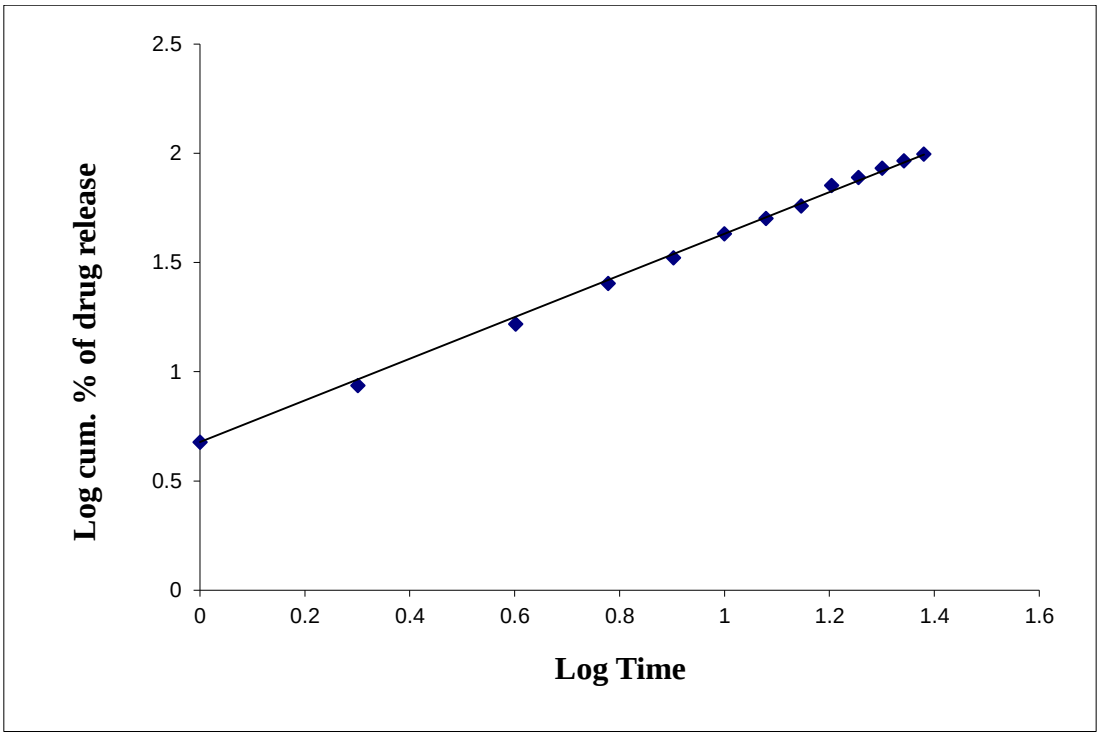

Figure 6.176 Korsmeyer & peppas plot for formulation RS210

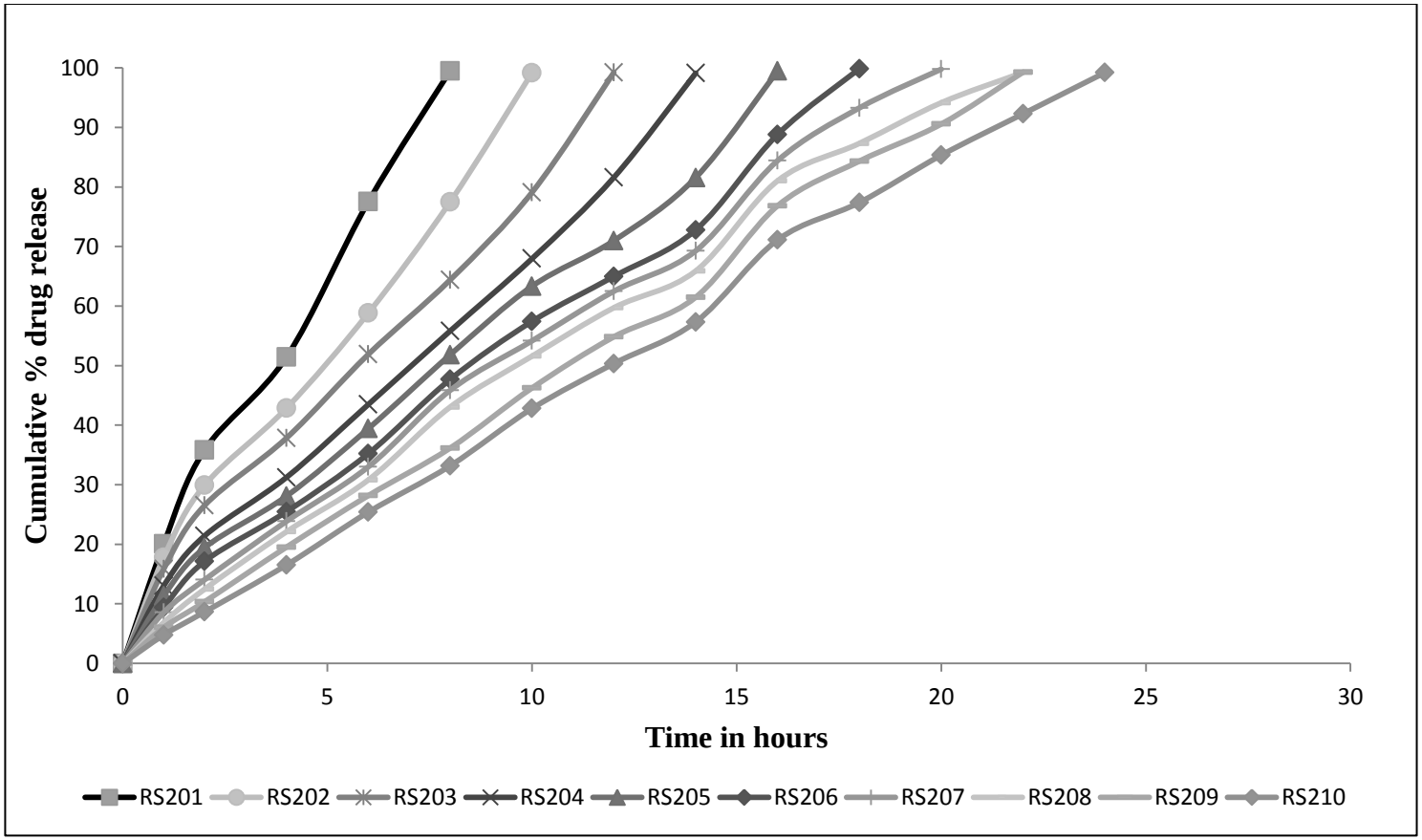

Figure 6.177 Comparative dissolution profiles of RS 201-210

6.2.5.9 Kinetic values obtained from different plots of formulations (RS201-210) of raloxifene

Table 6.44 Drug release mechanism (RS201-210)

Formula Code	Zero order		First Order		Higuchis model		Koresmayer & Peppas	
	K_0	R^2	K_1	R^2	K_H	R^2	*n*	R^2
RS201	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
RS202	9.1929	0.9941	0.3772	-0.8479	30.2800	0.9732	0.7154	0.9941
RS203	7.1984	0.9969	0.3003	-0.8278	27.5475	0.9742	0.7063	0.9948
RS204	6.3743	0.9978	0.2424	-0.8259	25.7636	0.9684	0.7478	0.9945
RS205	5.5898	0.9975	0.2208	-0.7943	24.3214	0.9716	0.7616	0.9960
RS206	5.1414	0.9978	0.2270	-0.7670	23.5911	0.9699	0.7990	0.9970
RS207	4.8531	0.9985	0.2042	-0.8157	23.5201	0.9724	0.8346	0.9983
RS208	4.5157	0.9972	0.1659	-0.8841	23.0996	0.9753	0.8787	0.9993
RS209	4.5088	0.9988	0.1557	-0.8503	22.5385	0.9653	0.9231	0.9989
RS210	4.2081	0.9990	0.1417	-0.8619	21.9883	0.9657	0.9765	0.9994

Table 6.45 *In vitro* release study of RS601

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.164	26.73941335	0.133697067	13.36970668	22.28284446	0	1.347970629	1.890517	1
2	0.203	33.34908399	0.16674542	16.94193613	28.23656022	0.30103	1.45081179	1.855903	1.41421356
4	0.282	46.58524377	0.232926219	23.89350686	39.82251143	0.60205999	1.600128646	1.779434	2
6	0.384	63.74011413	0.318700571	32.93679447	54.89465746	0.77815125	1.739530079	1.654228	2.44948974
8	0.498	82.91320452	0.414566023	43.16074081	71.93456802	0.90308999	1.85693764	1.448172	2.82842712
10	0.574	95.69526479	0.478476324	50.38090299	83.96817166	1	1.924114697	1.204983	3.16227766
12	0.673	112.3455801	0.561727901	59.66301331	99.43835552	1.07918125	1.997553933	-0.25054	3.46410162

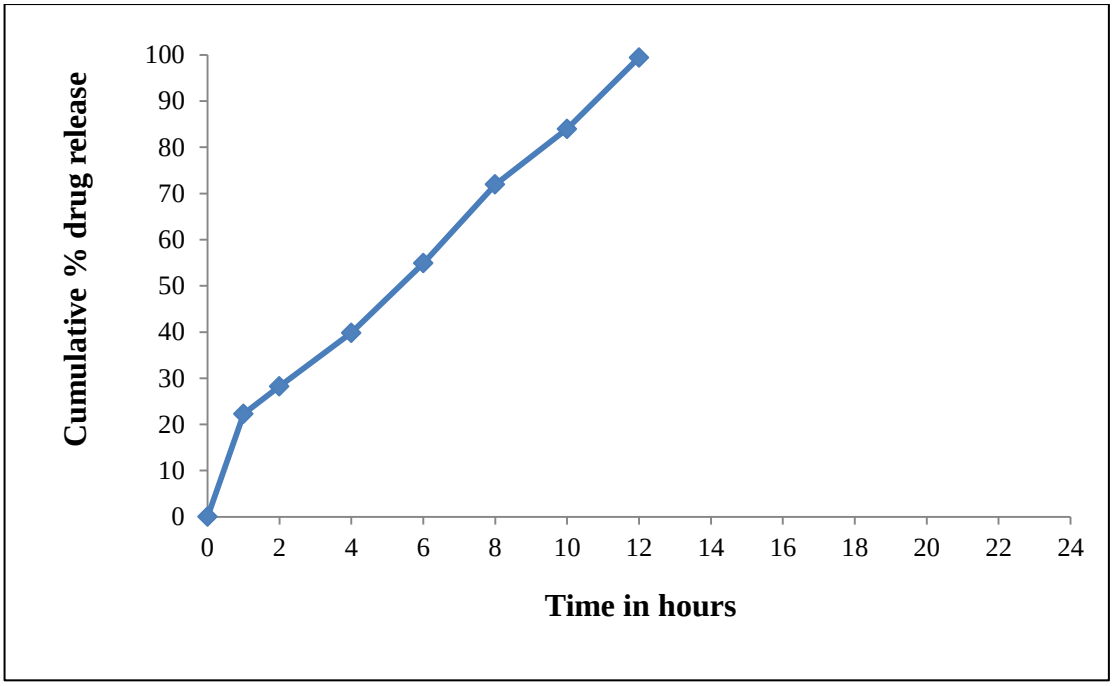

Figure 6.178 *In vitro* drug release of formulation RS601

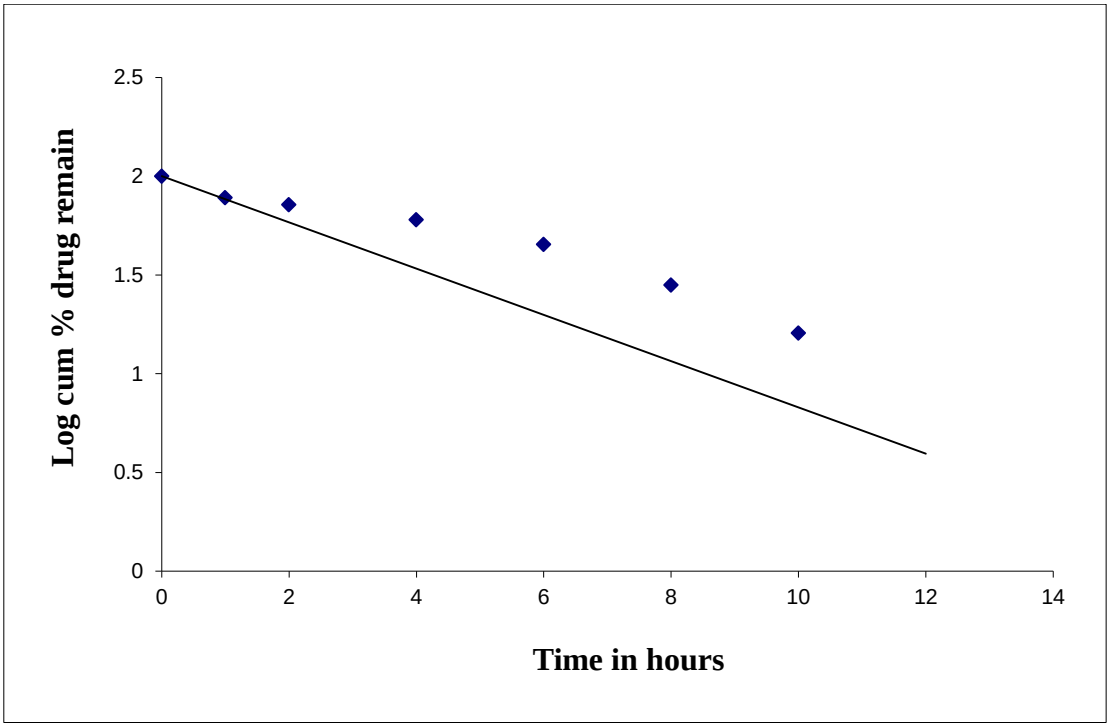

Figure 6.179 First order kinetics of formulation RS601

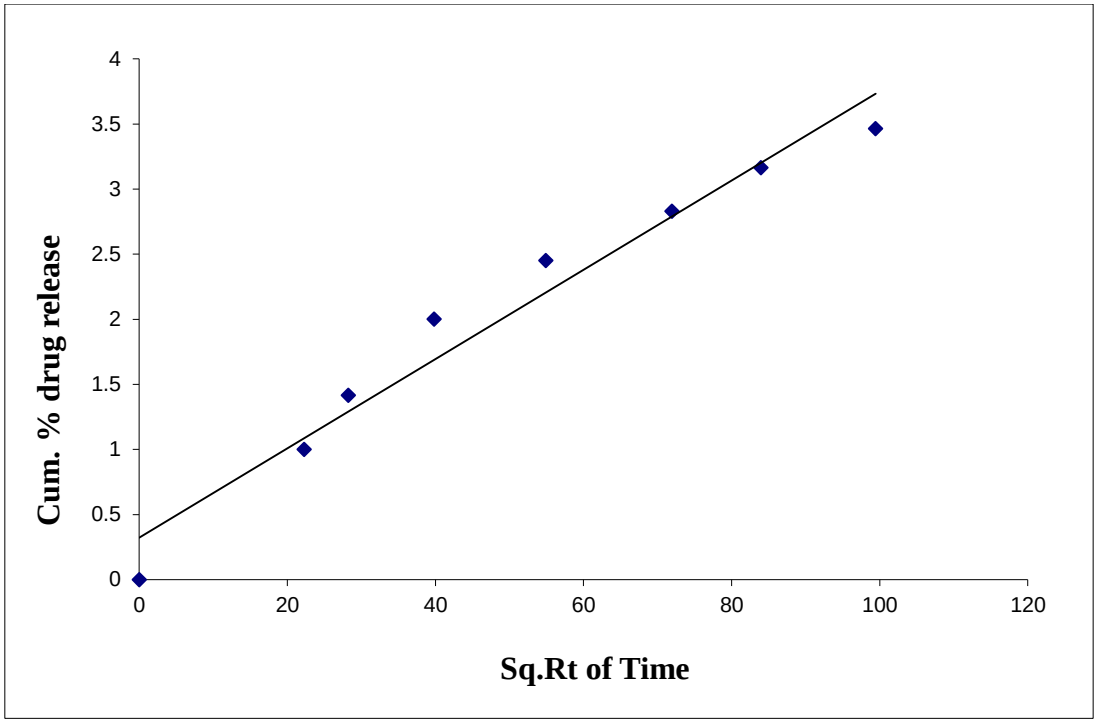

Figure 6.180 Higuchi's plot for formulation RS601

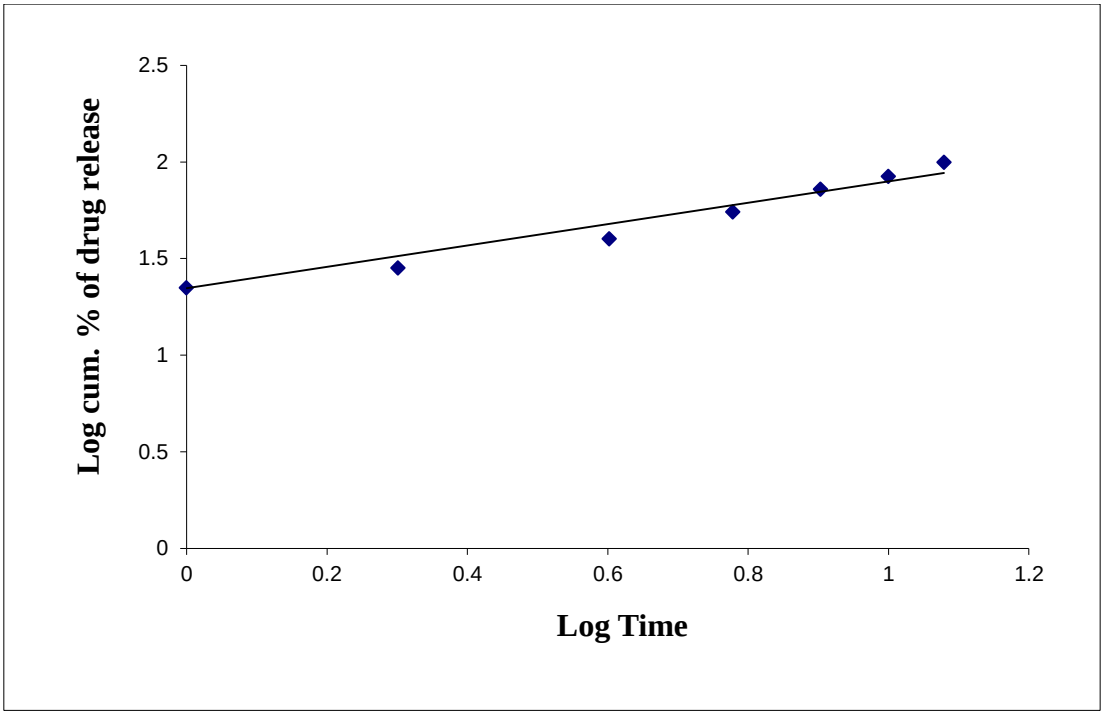

Figure 6.181 Korsmeyer & peppas plot for formulation RS601

Table 6.46 *In vitro* release study of RS602

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.144	23.37571328	0.116878566	11.68785664	19.47976107	0	1.289583626	1.905905	1
2	0.186	30.43948343	0.152197417	15.45349885	25.75583141	0.30103	1.410875574	1.870662	1.41421356
4	0.248	40.86695365	0.204334768	20.97162879	34.95271465	0.60205999	1.543480911	1.813229	2
6	0.321	53.1444589	0.265722295	27.51905096	45.86508493	0.77815125	1.661482202	1.733477	2.44948974
8	0.384	63.74011413	0.318700571	33.34832316	55.58053859	0.90308999	1.744922751	1.647573	2.82842712
10	0.466	77.53128441	0.387656422	40.88130944	68.13551573	1	1.833373548	1.503307	3.16227766
12	0.573	95.52707979	0.477635399	50.65451997	84.42419995	1.07918125	1.926466954	1.19245	3.46410162
14	0.670	111.8410251	0.559205126	59.76676344	99.6112724	1.14612804	1.998308488	-0.41035	3.74165739

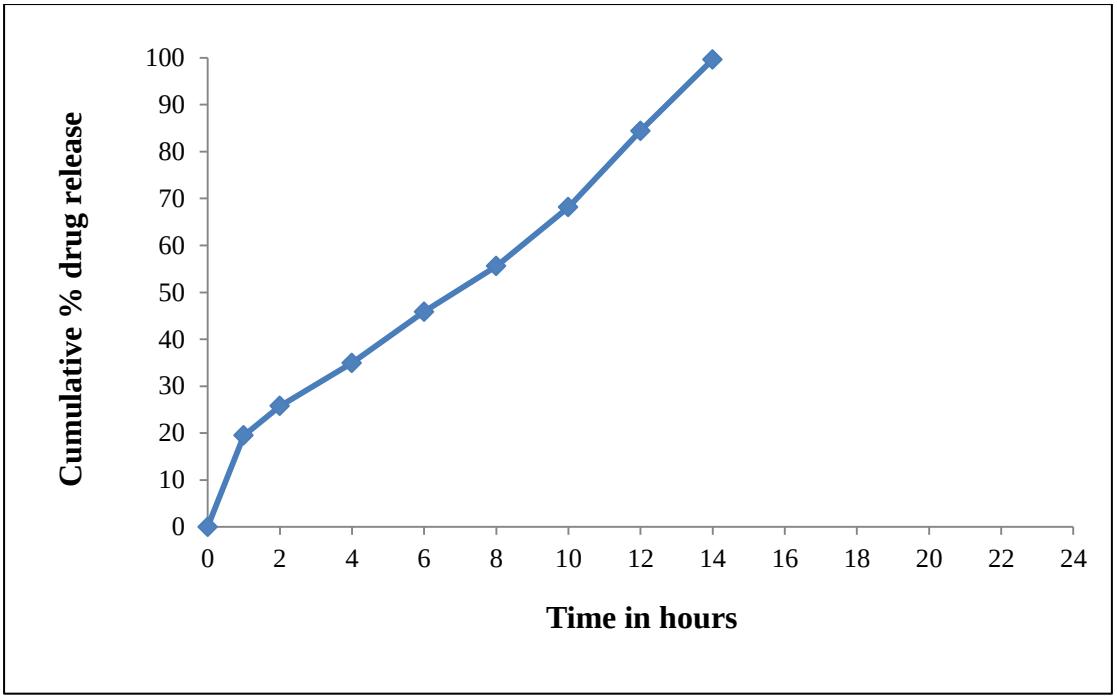

Figure 6.182 *In vitro* drug release of formulation RS602

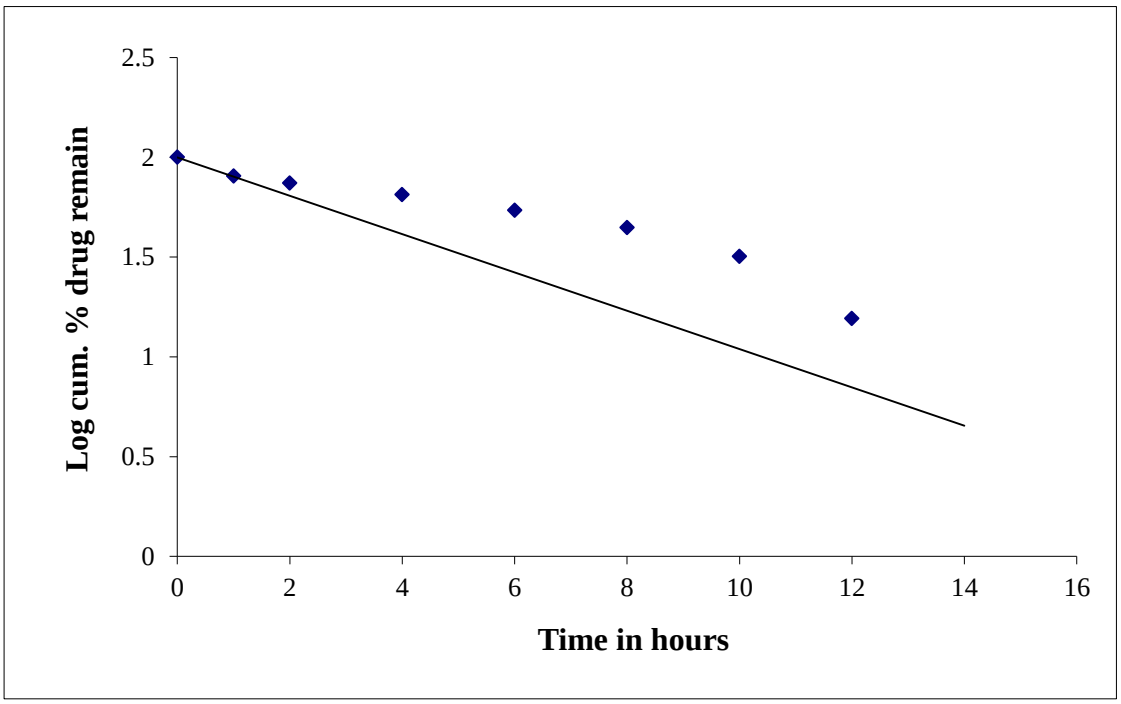

Figure 6.183 First order kinetics of formulation RS602

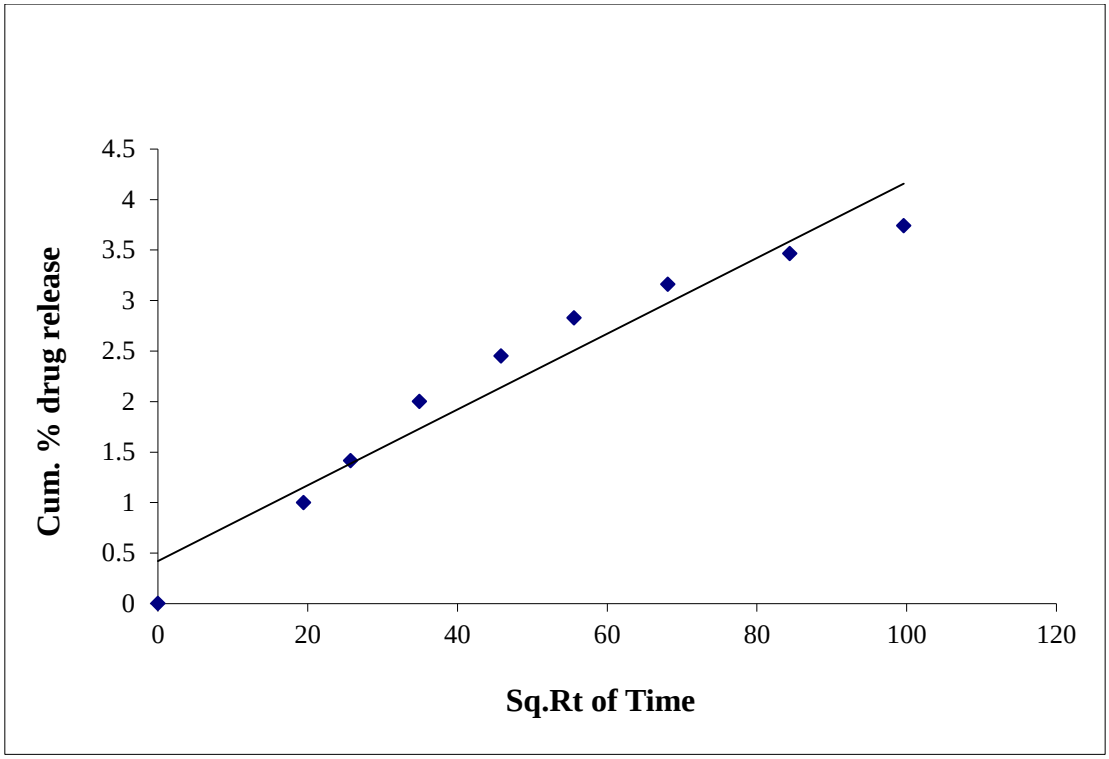

Figure 6.184 Higuchi's Plot for formulation RS602

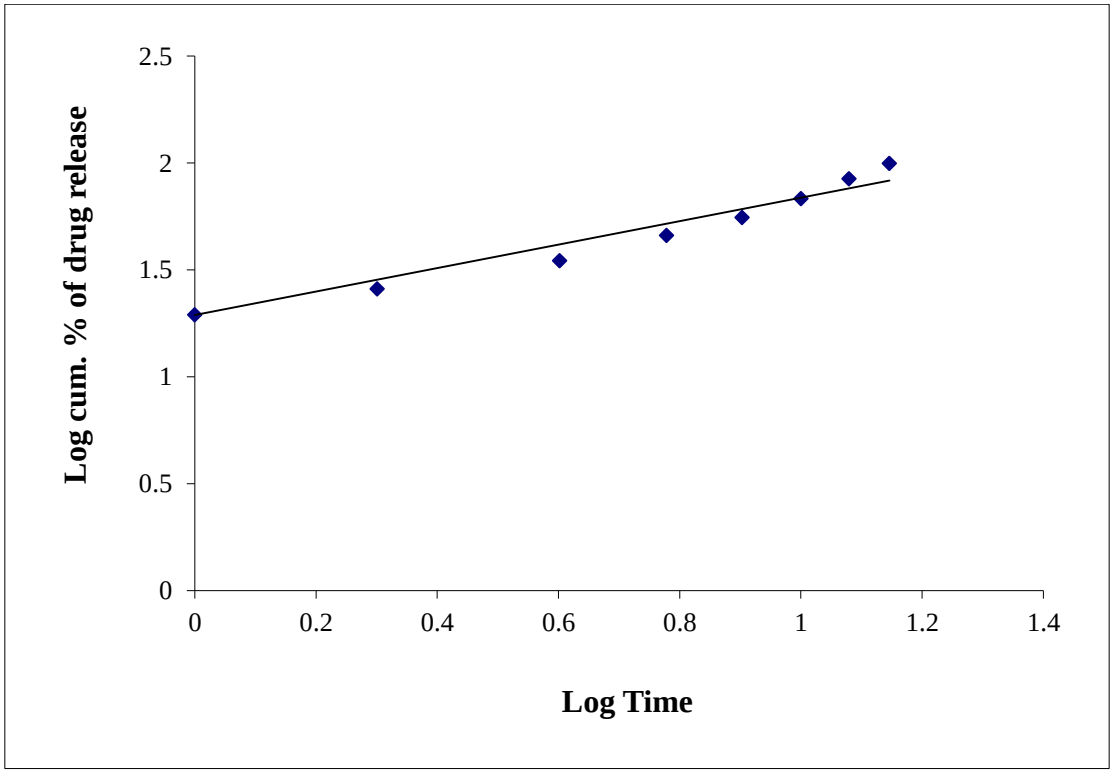

Figure 6.185 Korsmeyer & peppas plot for formulation RS602

Table 6.47 *In vitro* release study of RS603

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.129	20.85293823	0.104264691	10.42646912	17.37744853	0	1.239986011	1.917099	1
2	0.173	28.25307839	0.141265392	14.33506858	23.89178096	0.30103	1.378248524	1.881432	1.41421356
4	0.230	37.83962359	0.189198118	19.41087196	32.35145327	0.60205999	1.509893794	1.830258	2
6	0.288	47.59435379	0.237971769	24.6666333	41.11105549	0.77815125	1.613958627	1.770034	2.44948974
8	0.346	57.34908399	0.28674542	30.01994194	50.03323656	0.90308999	1.699258598	1.698681	2.82842712
10	0.409	67.94473921	0.339723696	35.89126039	59.81876731	1	1.776837459	1.604023	3.16227766
12	0.468	77.86765442	0.389338272	41.53216538	69.22027564	1.07918125	1.840233324	1.488265	3.46410162
14	0.538	89.64060467	0.448203023	48.19731705	80.32886175	1.14612804	1.904871613	1.293829	3.74165739
16	0.551	91.82700971	0.459135049	59.59918911	99.33198185	1.20411998	1.9970891	-0.17521	4

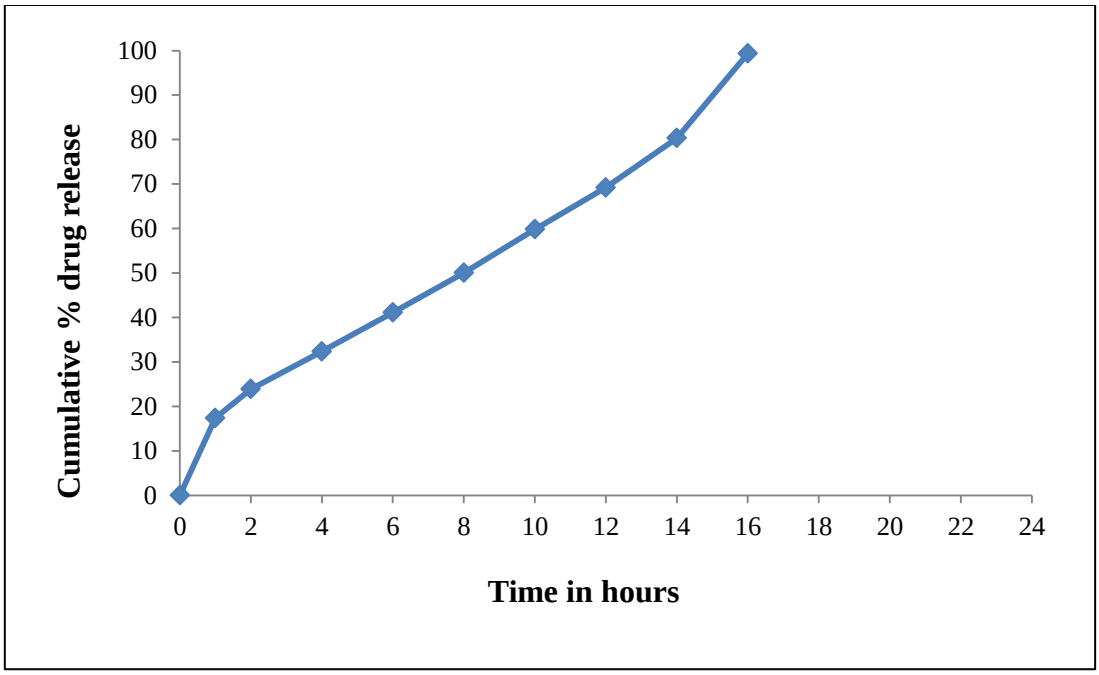

Figure 6.186 *In vitro* drug release of formulation RS603

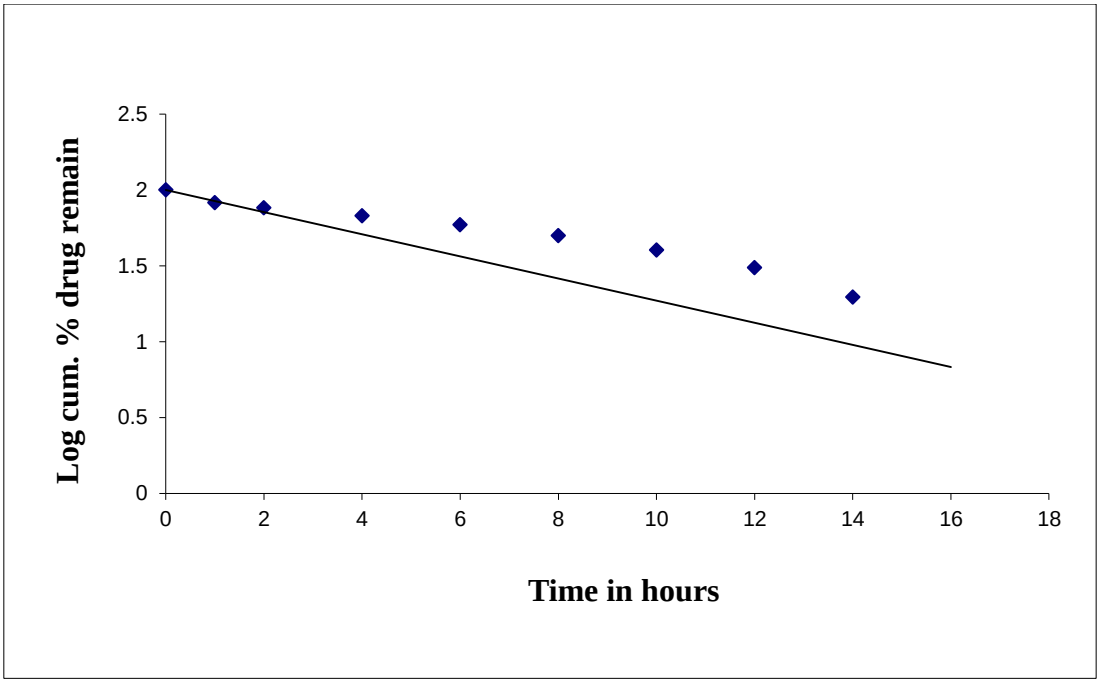

Figure 6.187 First order kinetics of formulation RS603

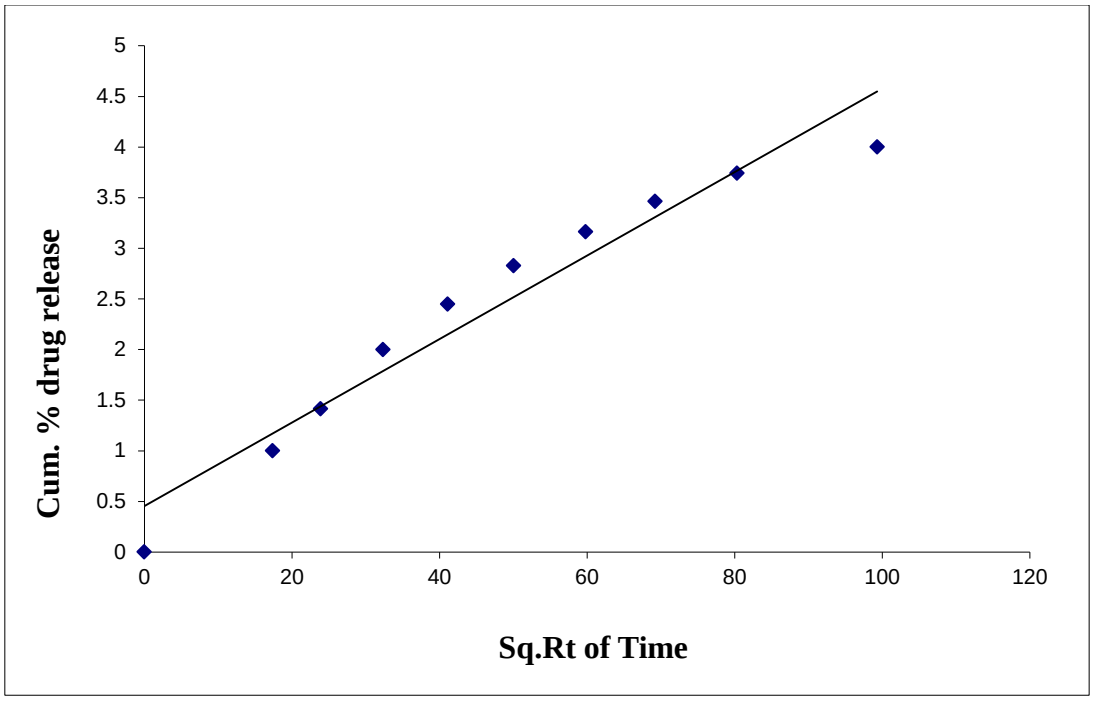

Figure 6.188 Higuchi's plot for formulation RS603

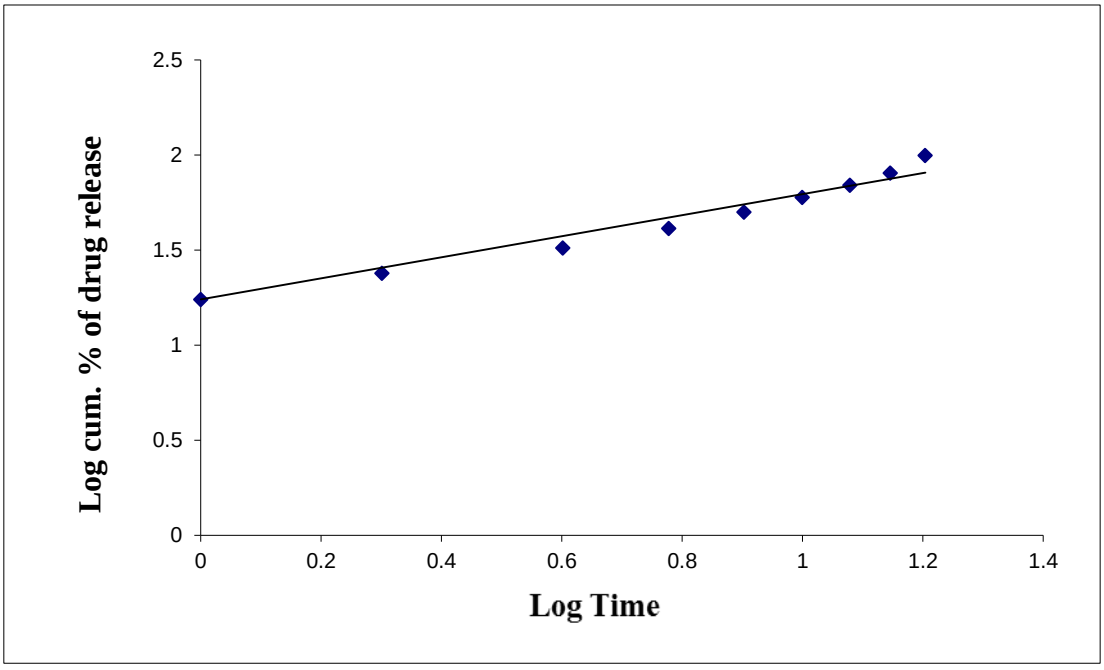

Figure 6.189 Korsmeyer & peppas plot for formulation RS603

Table 6.48 *In vitro* release study of RS604

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.106	16.98468315	0.084923416	8.492341576	14.15390263	0	1.150876203	1.933721	1
2	0.148	24.0484533	0.120242266	12.19407348	20.3234558	0.30103	1.307997557	1.90133	1.41421356
4	0.217	35.65321854	0.178266093	18.23694063	30.39490106	0.60205999	1.482800734	1.842641	2
6	0.268	44.23065372	0.221153269	22.88219041	38.13698402	0.77815125	1.581346345	1.791431	2.44948974
8	0.321	53.1444589	0.265722295	27.78139954	46.30233257	0.90308999	1.66560287	1.729955	2.82842712
10	0.366	60.71278406	0.30356392	32.09700671	53.49501118	1	1.728313283	1.6675	3.16227766
12	0.418	69.45840424	0.347292021	37.07694464	61.79490773	1.07918125	1.790952688	1.582121	3.46410162
14	0.467	77.69946942	0.388497347	41.89206127	69.82010211	1.14612804	1.84398048	1.479718	3.74165739
16	0.478	79.54950445	0.397747522	51.75251777	86.25419628	1.20411998	1.935780233	1.13817	4
18	0.562	93.67704475	0.468385224	59.61178296	99.3529716	1.25527251	1.997180861	-0.18908	4.24264069

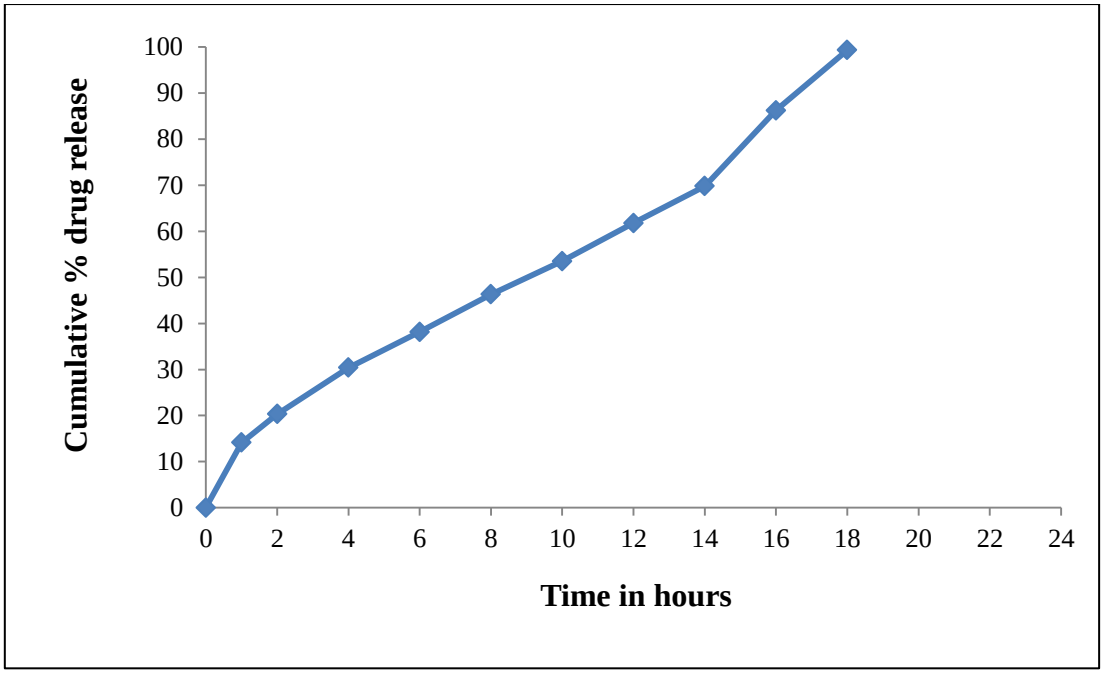

Figure 6.190 *In vitro* drug release of formulation RS604

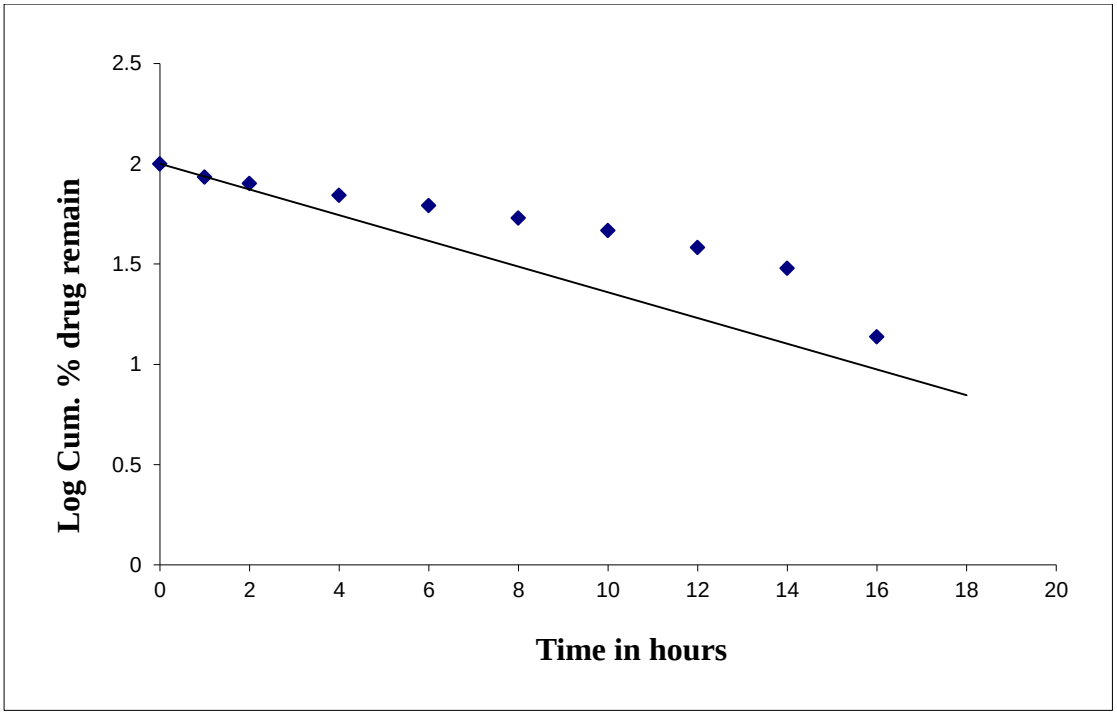

Figure 6.191 First order kinetics of formulation RS604

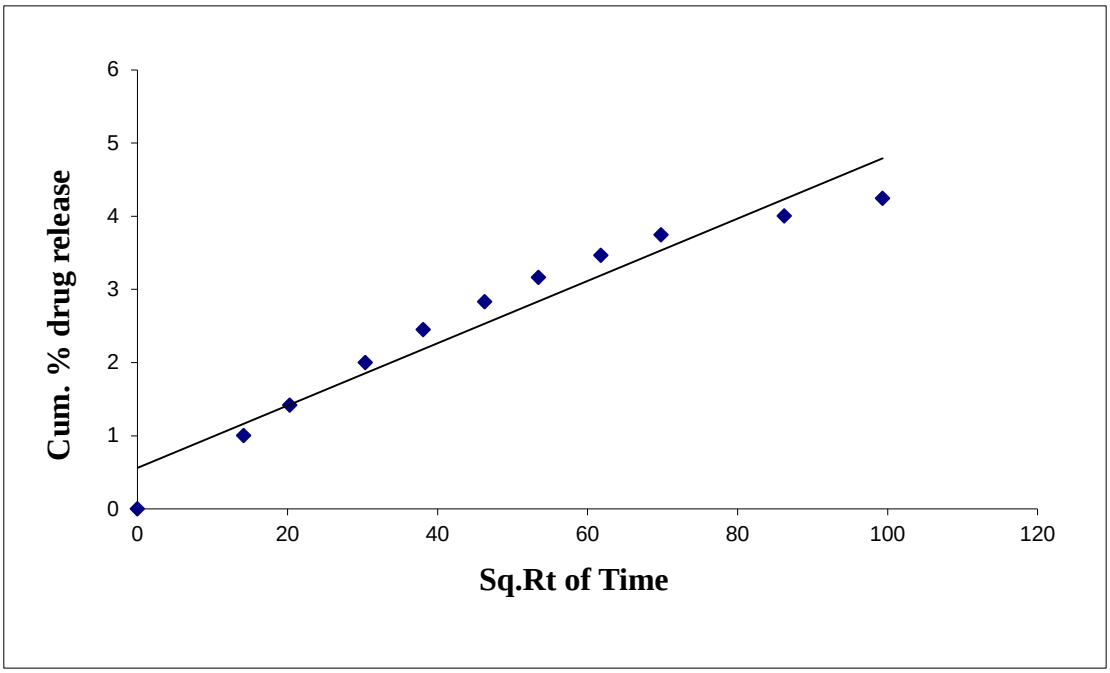

Figure 6.192 Higuchi's plot for formulation RS604

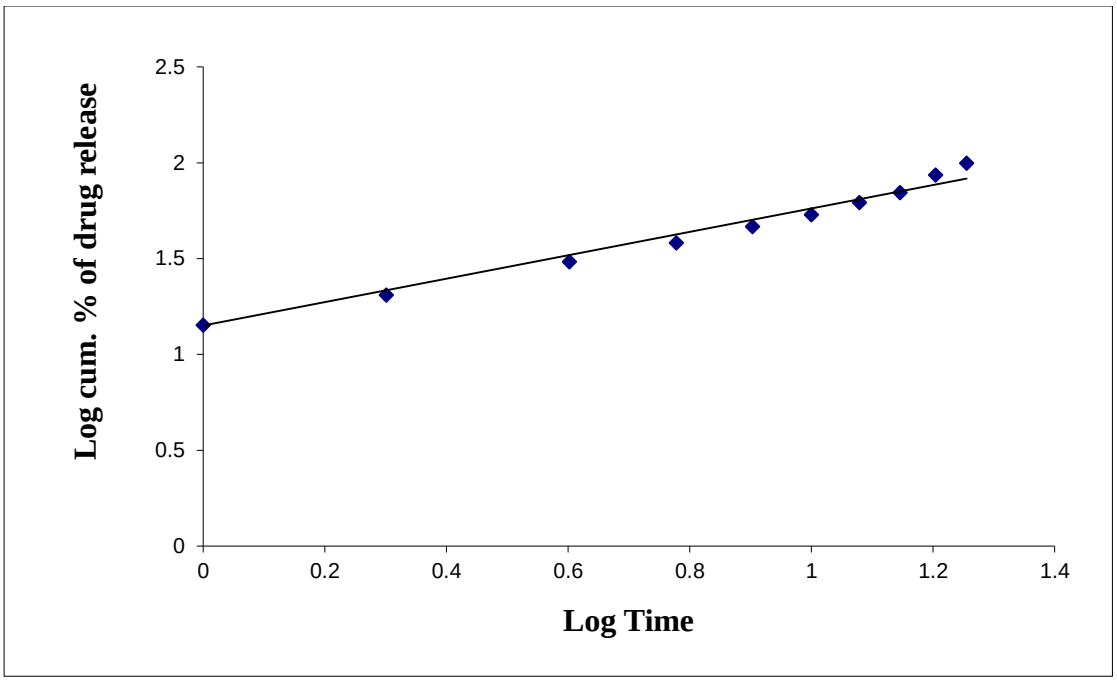

Figure 6.193 Korsmeyer & peppas plot for formulation RS604

Table 6.49 *In vitro* release study of RS605

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.098	15.63920312	0.078196016	7.819601562	13.03266927	0	1.115033374	1.939356	1
2	0.135	21.86204825	0.109310241	11.08741616	18.47902693	0.30103	1.266679098	1.911269	1.41421356
4	0.206	33.8031835	0.169015918	17.27660426	28.79434044	0.60205999	1.459307135	1.852515	2
6	0.256	42.21243368	0.211062168	21.81926119	36.36543531	0.77815125	1.560688791	1.803693	2.44948974
8	0.294	48.60346381	0.243017319	25.43690059	42.39483432	0.90308999	1.627312942	1.760461	2.82842712
10	0.343	56.84452898	0.284222645	30.04346781	50.07244636	1	1.69959881	1.69834	3.16227766
12	0.402	66.76744419	0.333837221	35.57337071	59.28895118	1.07918125	1.772973768	1.609712	3.46410162
14	0.434	72.1493643	0.360746822	38.93200521	64.88667534	1.14612804	1.812155522	1.545472	3.74165739
16	0.438	72.82210431	0.364110522	47.56555211	79.27592018	1.20411998	1.899141292	1.316475	4
18	0.484	80.55861448	0.402793072	52.16202823	86.93671372	1.25527251	1.939203219	1.116052	4.24264069
20	0.561	93.50885975	0.467544299	59.44273701	99.07122835	1.30103	1.995947548	-0.03209	4.47213595

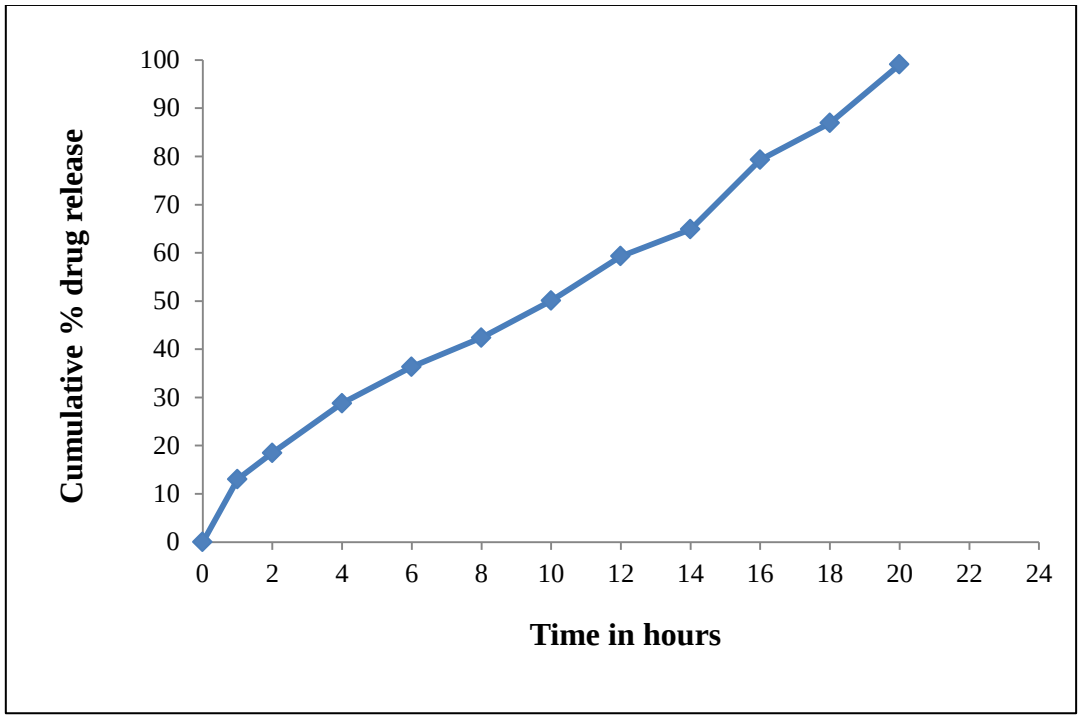

Figure 6.194 *In vitro* drug release of formulation RS605

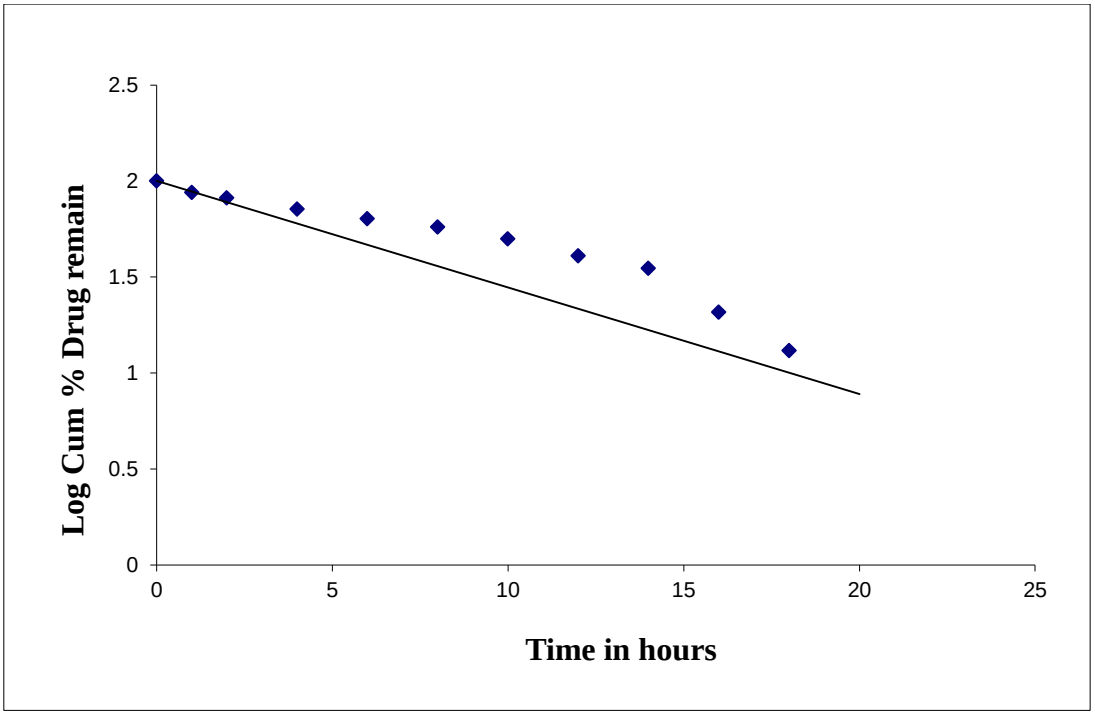

Figure 6.195 First order kinetics of formulation RS605

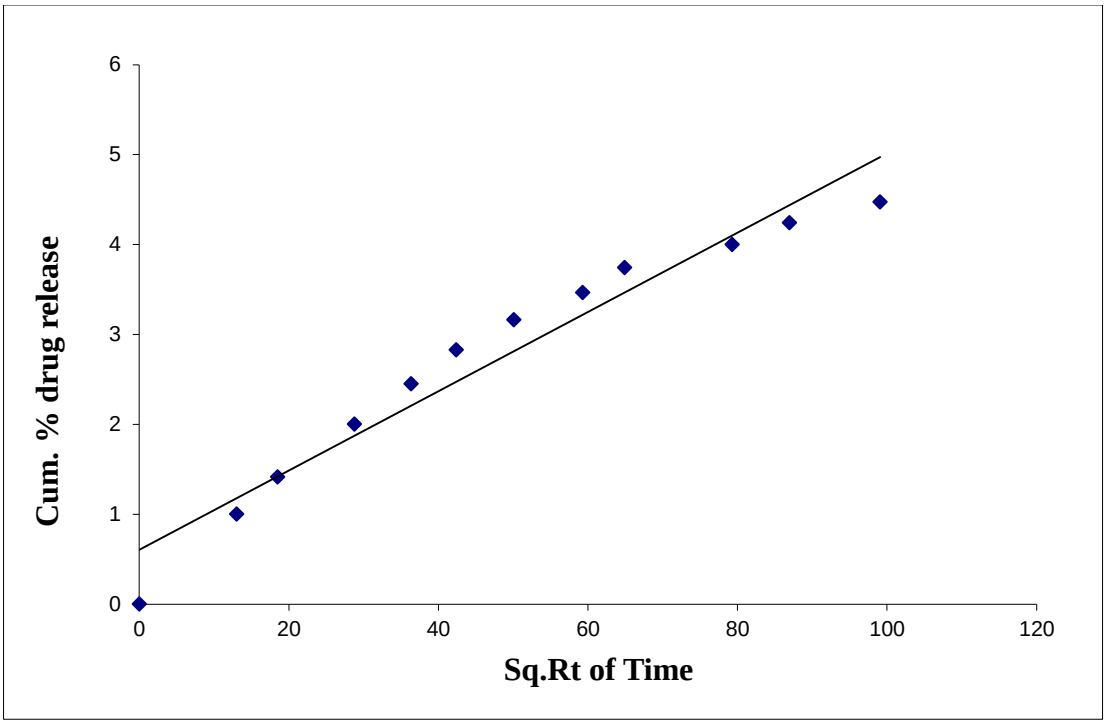

Figure 6.196 Higuchi's plot for formulation RS605

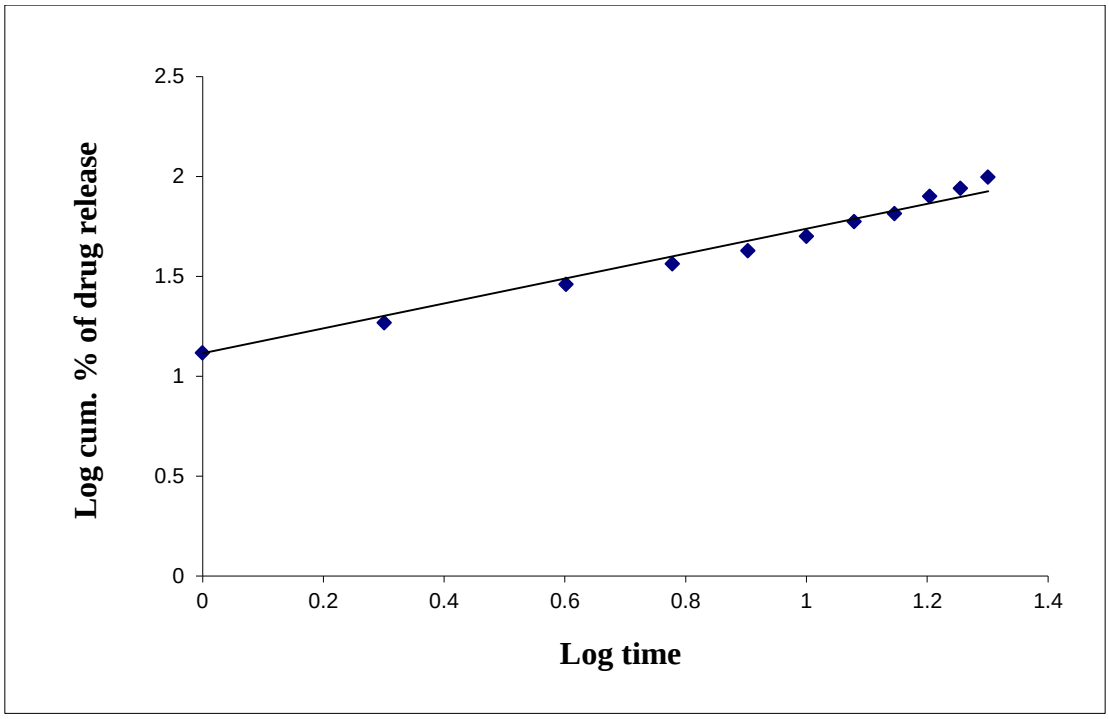

Figure 6.197 Korsmeyer & peppas plot for formulation RS605

Table 6.50 *In vitro* release study of RS606

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.064	9.920913004	0.049604565	4.960456502	8.267427504	0	0.917370395	1.962524	1
2	0.112	17.99379317	0.089968966	9.096105716	15.16017619	0.30103	1.180704249	1.9286	1.41421356
4	0.178	29.0940034	0.145470017	14.82614876	24.71024794	0.60205999	1.392877103	1.876736	2
6	0.231	38.04144559	0.190207228	19.59080989	32.65134982	0.77815125	1.51390114	1.828329	2.44948974
8	0.270	44.56702373	0.222835119	23.23401341	38.72335569	0.90308999	1.587972985	1.787295	2.82842712
10	0.317	52.47171889	0.262358594	27.63203123	46.05338539	1	1.663261561	1.731964	3.16227766
12	0.369	61.21733907	0.306086695	32.52955851	54.21593086	1.07918125	1.734126919	1.660714	3.46410162
14	0.398	66.09470417	0.330473521	35.58041446	59.30069076	1.14612804	1.773059752	1.609587	3.74165739
16	0.402	66.76744419	0.333837221	43.51767544	72.52945907	1.20411998	1.860514438	1.438867	4
18	0.415	68.95384923	0.344769246	45.27855241	75.46425401	1.25527251	1.877741283	1.389799	4.24264069
20	0.476	79.21313445	0.396065672	51.09773351	85.16288918	1.30103	1.930250387	1.171349	4.47213595
22	0.521	86.78145961	0.433907298	55.67402743	92.79004572	1.34242268	1.967501389	0.857933	4.69041576
24	0.558	93.00430474	0.465021524	59.65326459	99.42210765	1.38021124	1.997482966	-0.23815	4.89897949

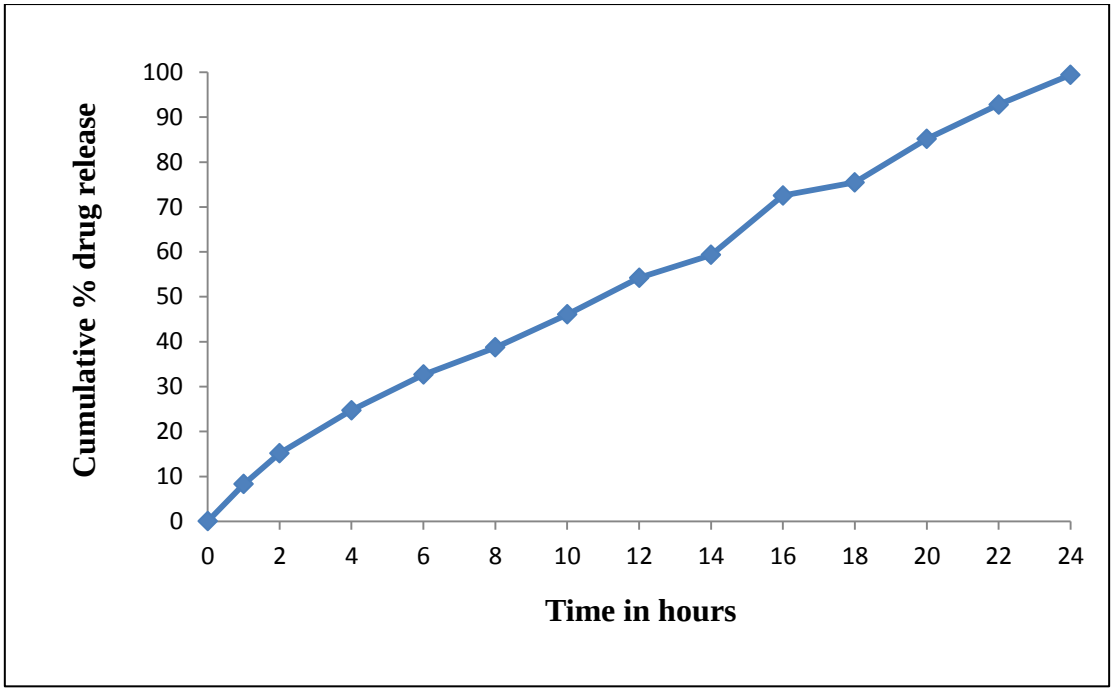

Figure 6.198 *In vitro* drug release of formulation RS606

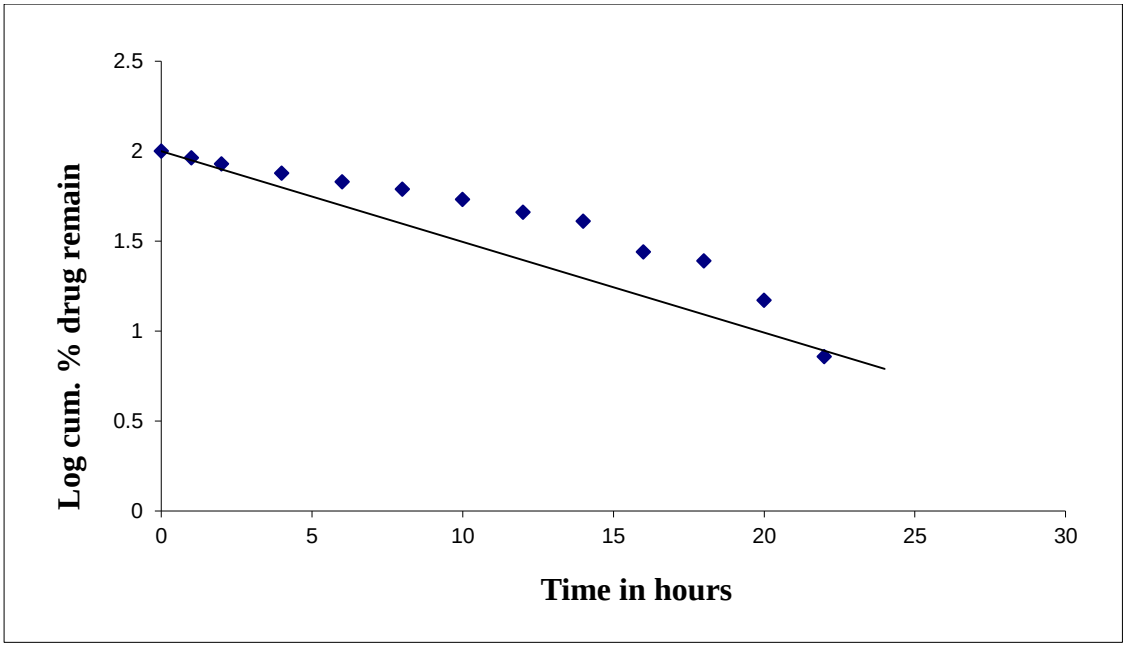

Figure 6.199 First order kinetics of formulation RS606

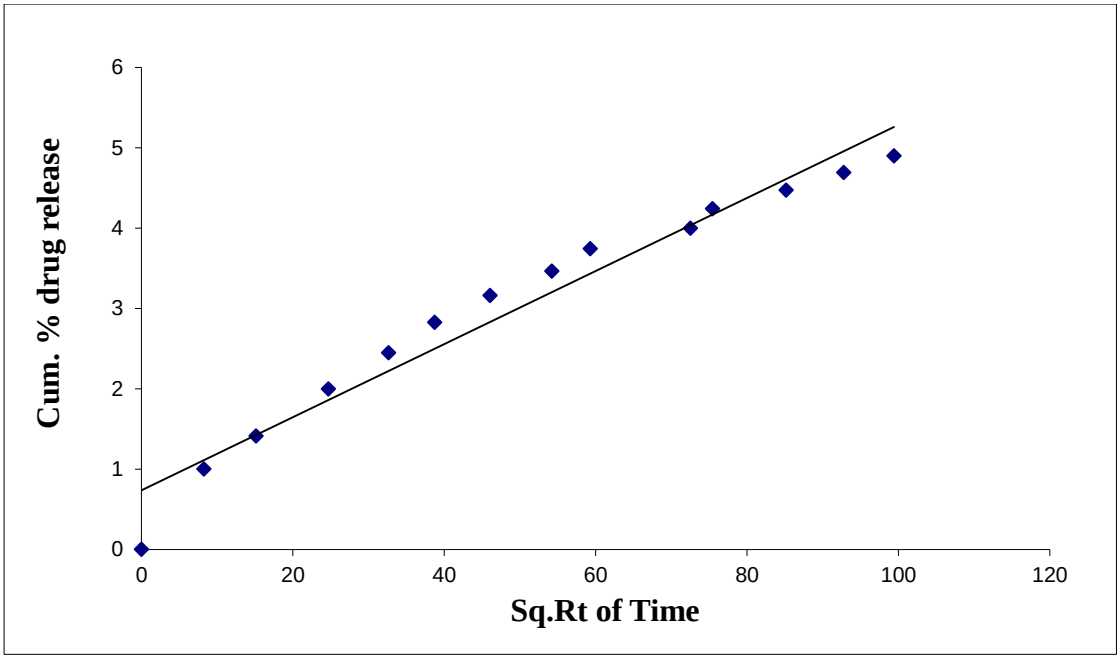

Figure 6.200 Higuchi's plot for formulation RS606

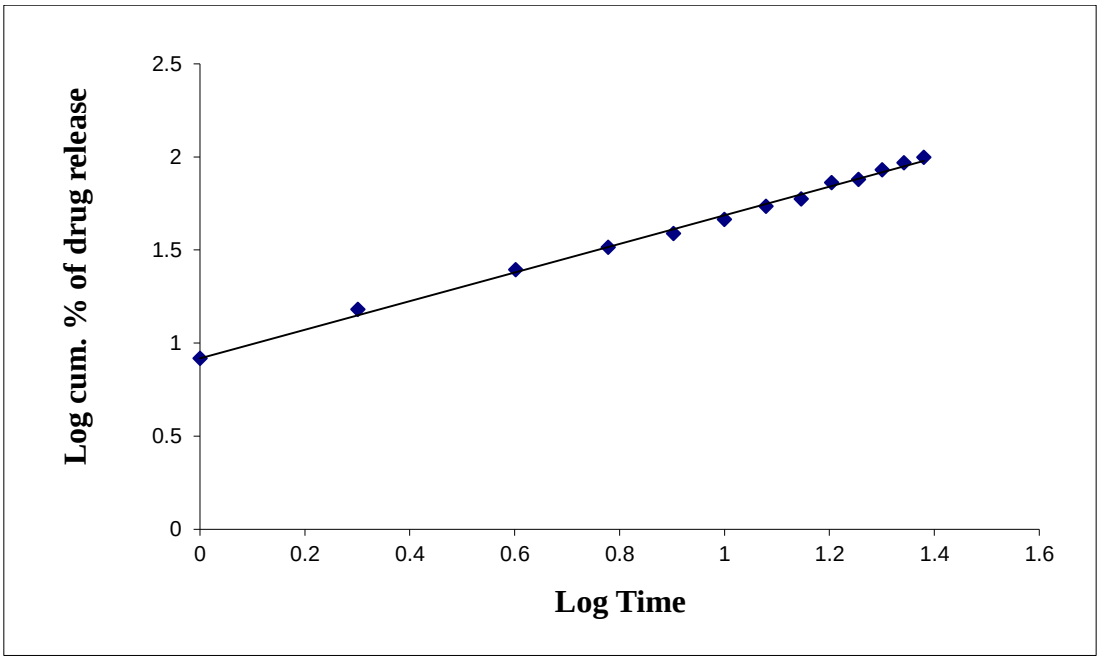

Figure 6.201 Korsmeyer & peppas plot for formulation RS606

Table 6.51 *In vitro* release study of RS607

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.044	6.557212934	0.032786065	3.278606467	5.464344112	0	0.737538041	1.975596	1
2	0.098	15.63920312	0.078196016	7.885173691	13.14195615	0.30103	1.118660014	1.93881	1.41421356
4	0.162	26.40304335	0.132015217	13.42348583	22.37247639	0.60205999	1.349714058	1.890016	2
6	0.211	34.64410852	0.173220543	17.80804885	29.68008142	0.77815125	1.472465088	1.847078	2.44948974
8	0.258	42.54880368	0.212744018	22.10683752	36.8447292	0.90308999	1.566375369	1.80041	2.82842712
10	0.302	49.94894384	0.249744719	26.23239564	43.72065939	1	1.640686703	1.750349	3.16227766
12	0.344	57.01271399	0.28506357	30.26377015	50.43961691	1.07918125	1.702771779	1.695135	3.46410162
14	0.389	64.58103914	0.322905196	34.61805987	57.69676644	1.14612804	1.761151474	1.626374	3.74165739
16	0.427	70.97206928	0.354860346	45.24039443	75.40065739	1.20411998	1.877375132	1.390924	4
18	0.483	80.39042947	0.401952147	50.65929522	84.43215871	1.25527251	1.926507893	1.192228	4.24264069
20	0.532	88.63149464	0.443157473	55.58373211	92.63955351	1.30103	1.966796453	0.866904	4.47213595
22	0.567	94.51796977	0.472589849	59.41328461	99.02214102	1.34242268	1.995732312	-0.00972	4.69041576

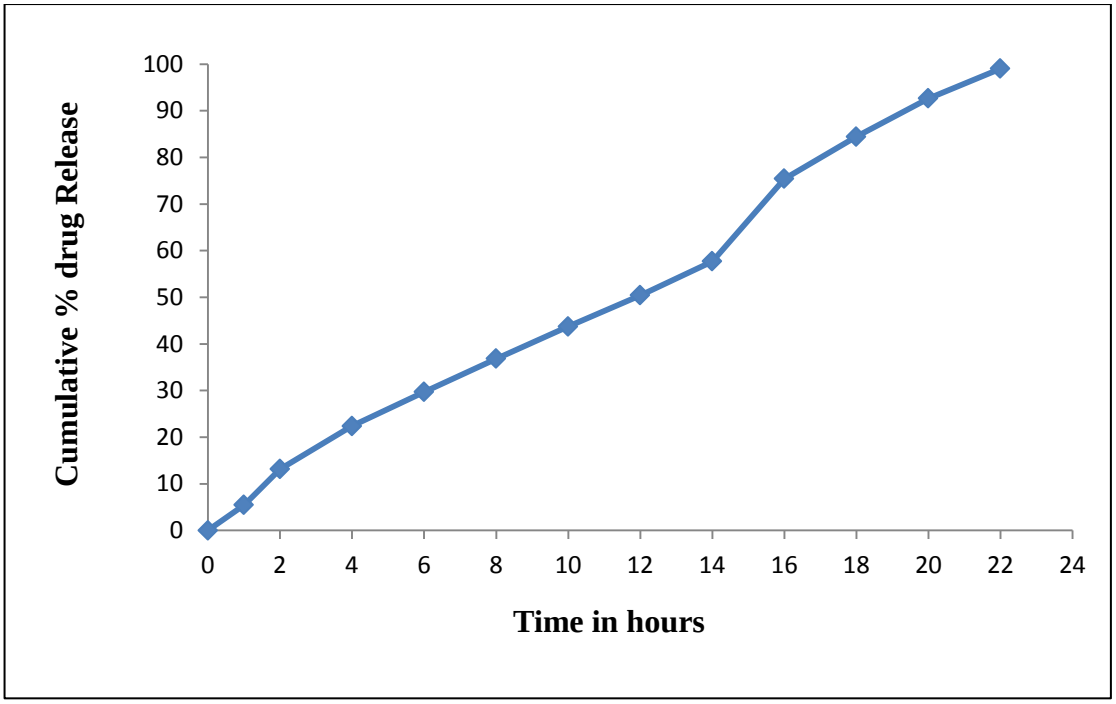

Figure 6.202 *In vitro* drug release of formulation RS607

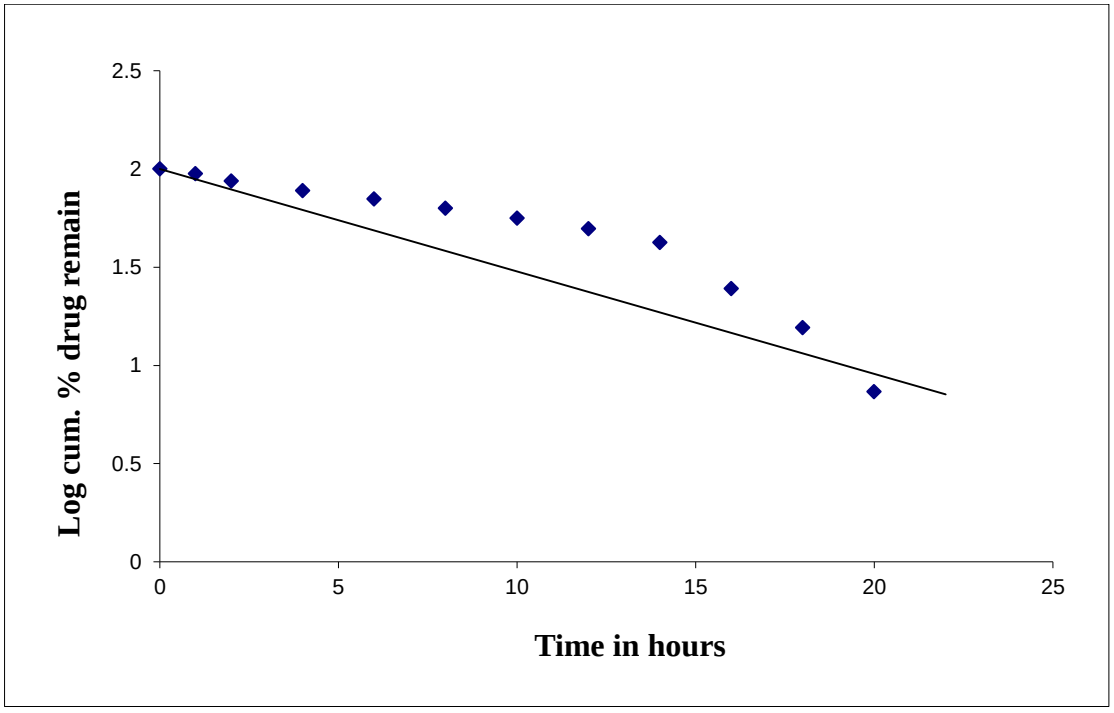

Figure 6.203 First order kinetics of formulation RS607

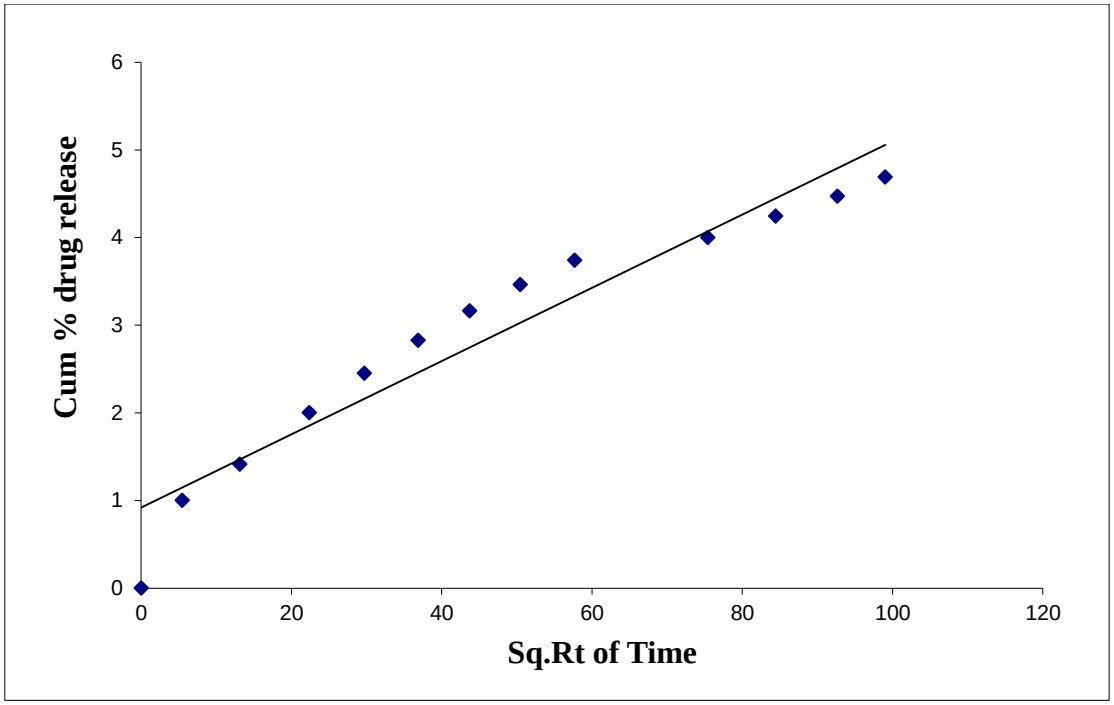

Figure 6.204 Higuchi's plot for formulation RS607

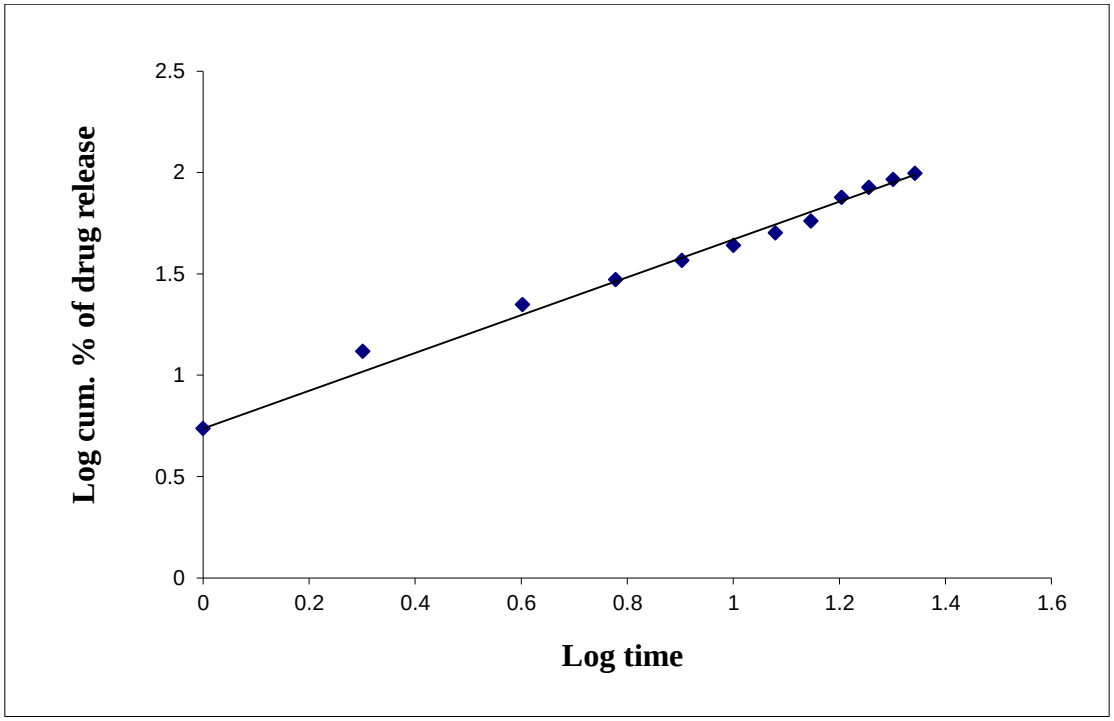

Figure 6.205 Korsmeyer & peppas plot for formulation RS607

Table 6.52 *In vitro* release study of RS608

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.034	4.875362899	0.024376814	2.43768145	4.062802416	0	0.608825702	1.981987	1
2	0.081	12.78005806	0.06390029	6.438782661	10.73130443	0.30103	1.030652515	1.950699	1.41421356
4	0.146	23.71208329	0.118560416	12.03259586	20.05432643	0.60205999	1.30220808	1.902795	2
6	0.203	33.29862849	0.166493142	17.06298929	28.43831548	0.77815125	1.453903868	1.854681	2.44948974
8	0.246	40.53058364	0.202652918	21.01195315	35.01992191	0.90308999	1.544315173	1.81278	2.82842712
10	0.312	51.63079387	0.258153969	26.9673641	44.94560683	1	1.652687248	1.740792	3.16227766
12	0.364	60.37641406	0.30188207	31.85648213	53.09413688	1.07918125	1.725046565	1.671227	3.46410162
14	0.411	68.28110922	0.341405546	36.41259385	60.68765642	1.14612804	1.783100367	1.594529	3.74165739
16	0.432	71.81299429	0.359064971	46.03086395	76.71810658	1.20411998	1.884897876	1.367018	4
18	0.502	83.58594454	0.417929723	52.63546902	87.72578169	1.25527251	1.943127247	1.088994	4.24264069
20	0.576	96.0316348	0.480158174	59.69417359	99.49028932	1.30103	1.997780694	-0.29268	4.47213595

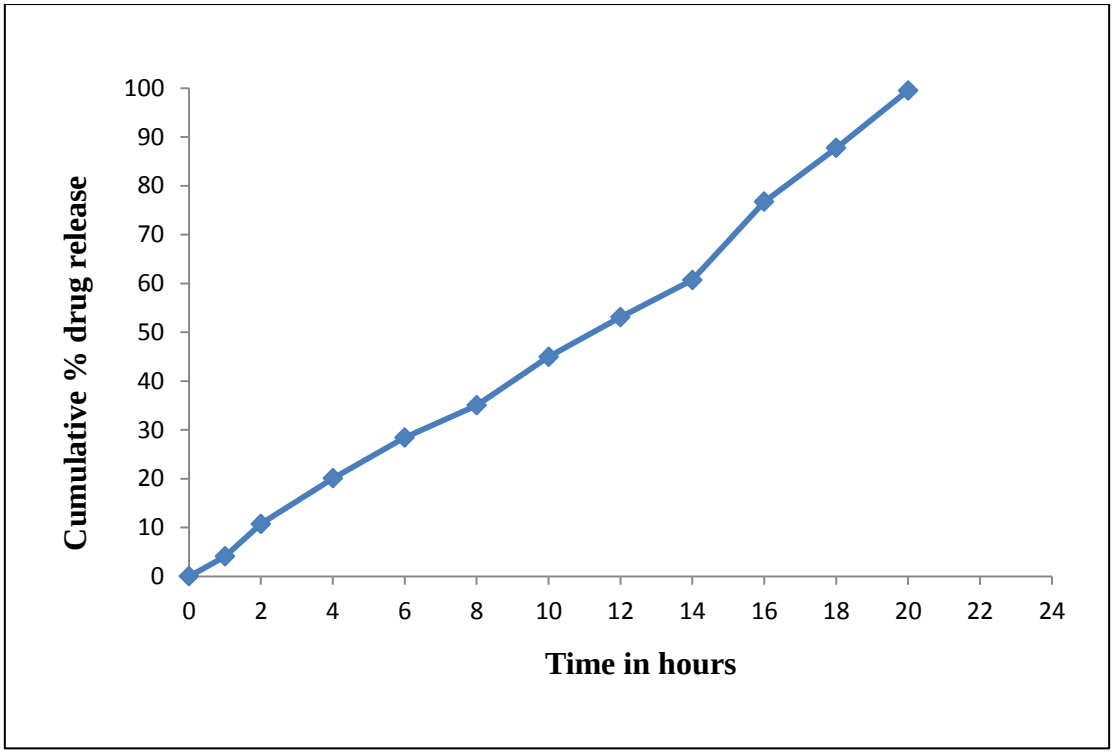

Figure 6.206 *In vitro* drug release of formulation RS608

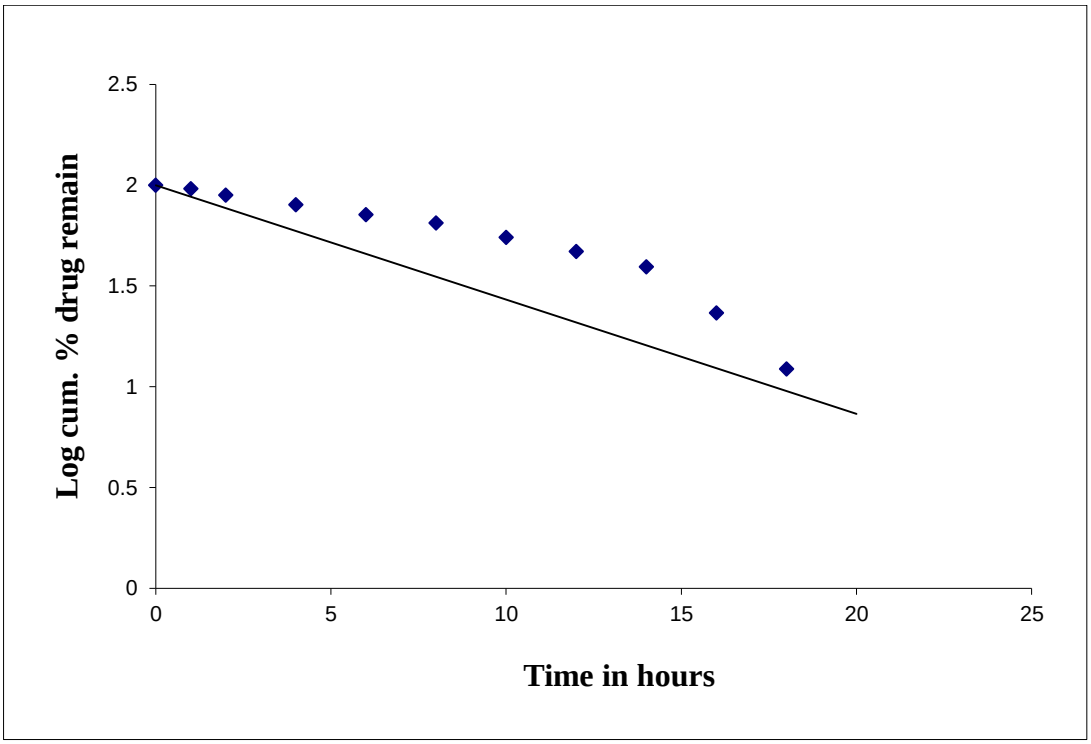

Figure 6.207 First order kinetics of formulation RS608

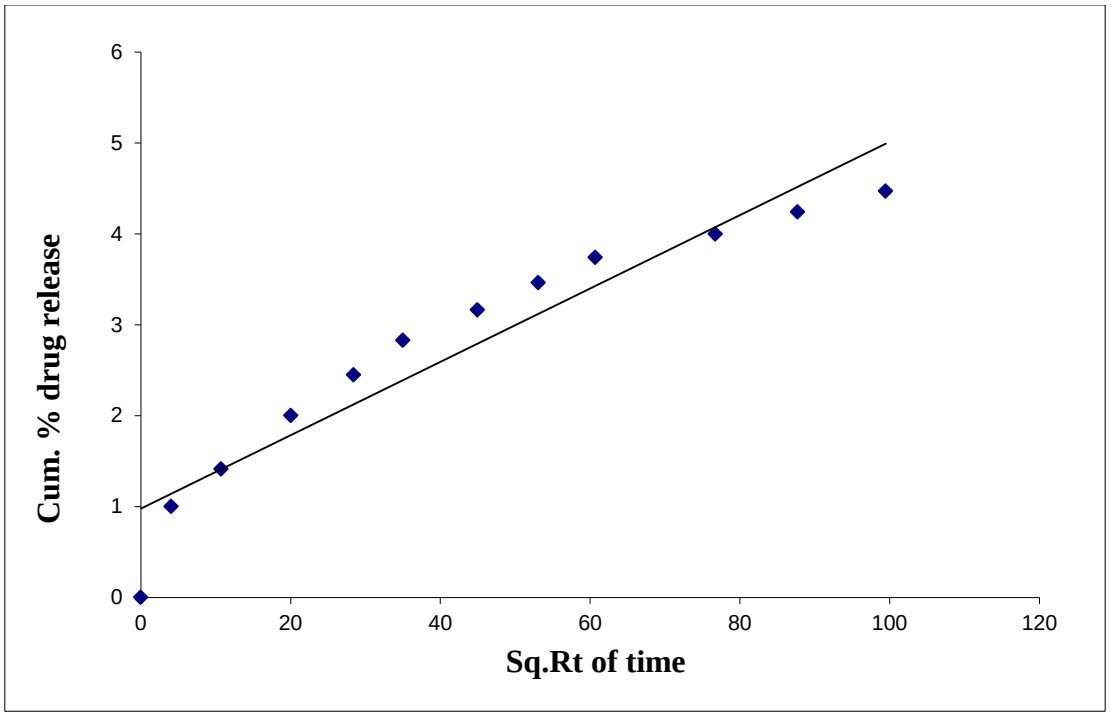

Figure 6.208 Higuchi's plot for formulation RS608

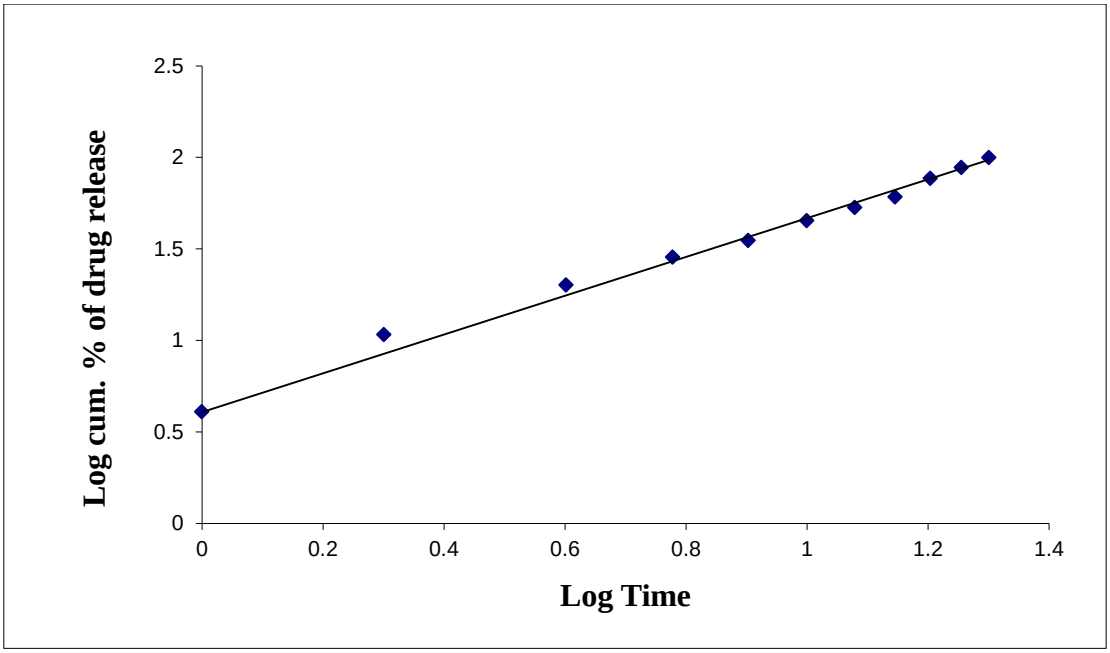

Figure 6.209 Korsmeyer & peppas plot for formulation RS608

Table 6.53 *In vitro* release study of RS609

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.028	3.866252878	0.019331264	1.933126439	3.221877398	0	0.50810901	1.985777	1
2	0.064	9.920913004	0.049604565	4.999119031	8.331865052	0.30103	0.920742227	1.962218	1.41421356
4	0.128	20.68475323	0.103423766	10.48024827	17.46708046	0.60205999	1.242220321	1.916627	2
6	0.191	31.28040845	0.156402042	15.98492342	26.64153903	0.77815125	1.42555931	1.86545	2.44948974
8	0.238	39.18510361	0.195925518	20.25007508	33.75012514	0.90308999	1.528275387	1.821185	2.82842712
10	0.302	49.94894384	0.249744719	26.02384623	43.37307705	1	1.637220234	1.753023	3.16227766
12	0.397	65.92651917	0.329632596	34.51212334	57.52020556	1.07918125	1.759820429	1.628182	3.46410162
14	0.462	76.8585444	0.384292722	40.63740114	67.7290019	1.14612804	1.830774676	1.508812	3.74165739
16	0.482	80.22224447	0.401111222	51.15798378	85.2633063	1.20411998	1.93076217	1.1684	4
18	0.576	96.0316348	0.480158174	59.86490139	99.77483565	1.25527251	1.999021021	-0.6475	4.24264069

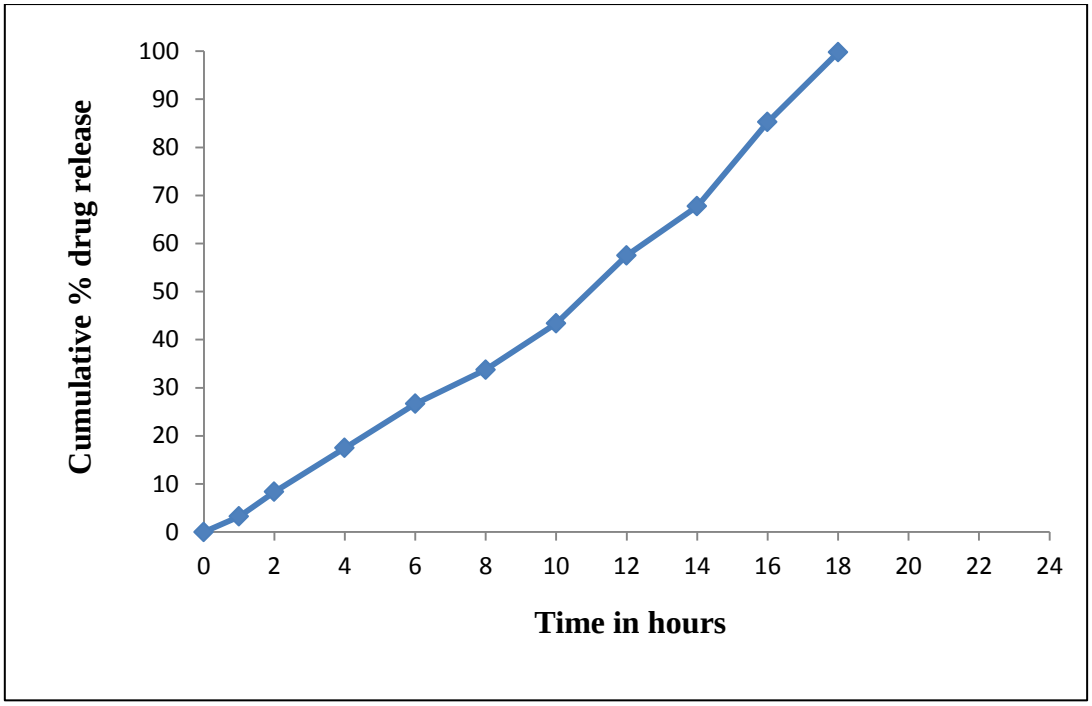

Figure 6.210 *In vitro* drug release of formulation RS609

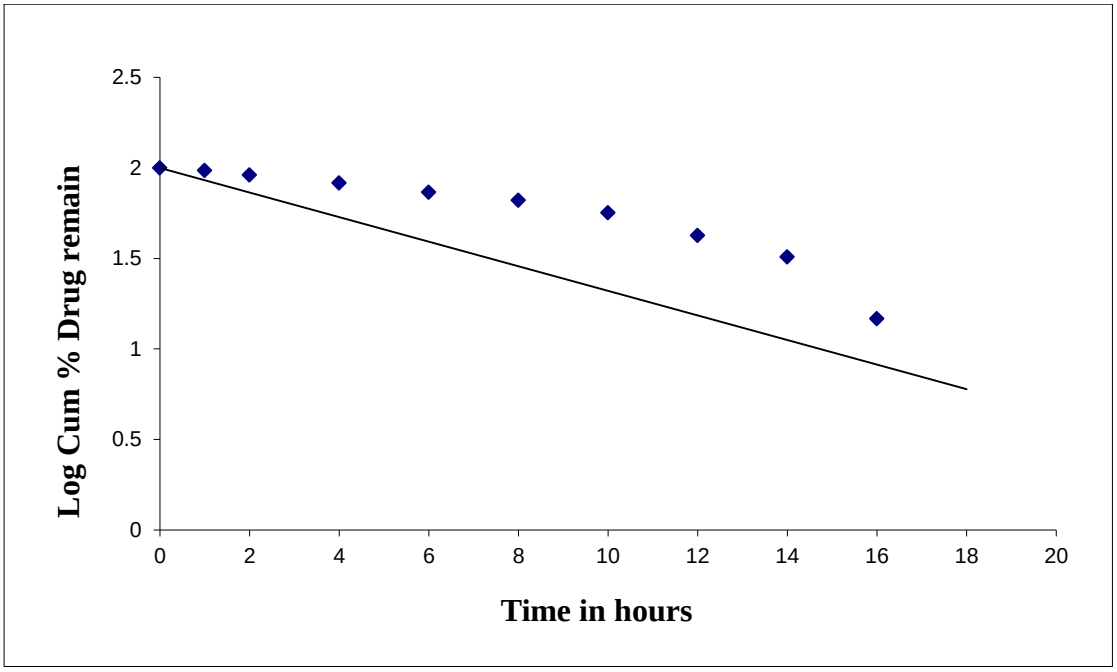

Figure 6.211First order kinetics of formulation RS609

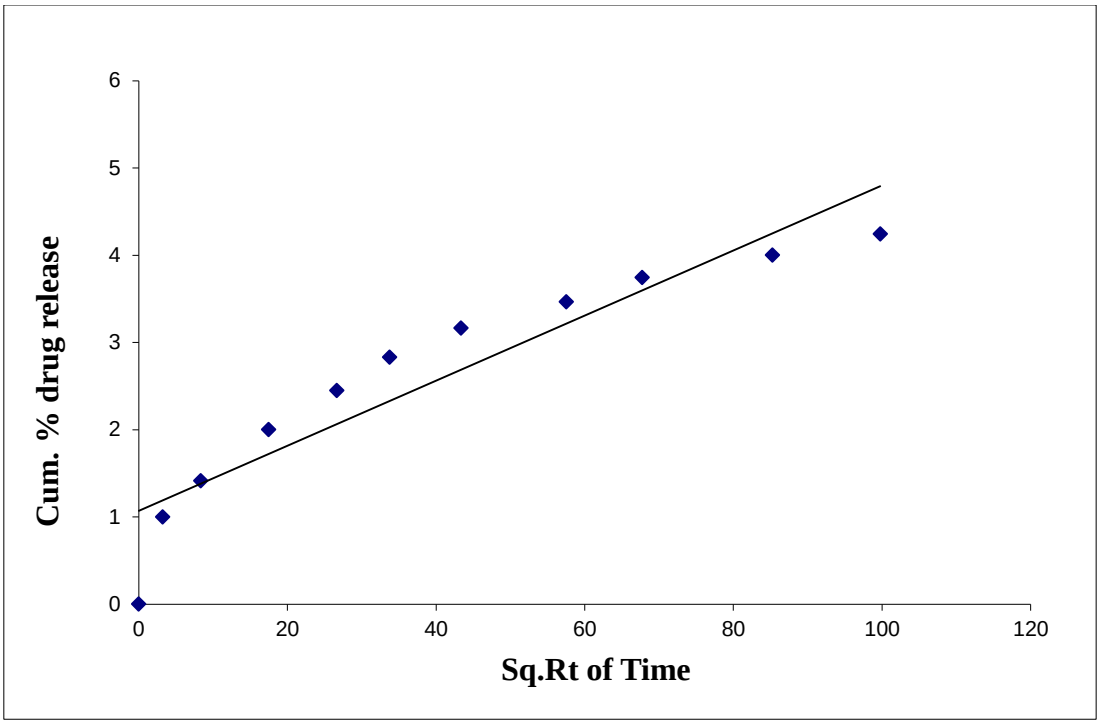

Figure 6.212 Higuchi's plot for formulation RS609

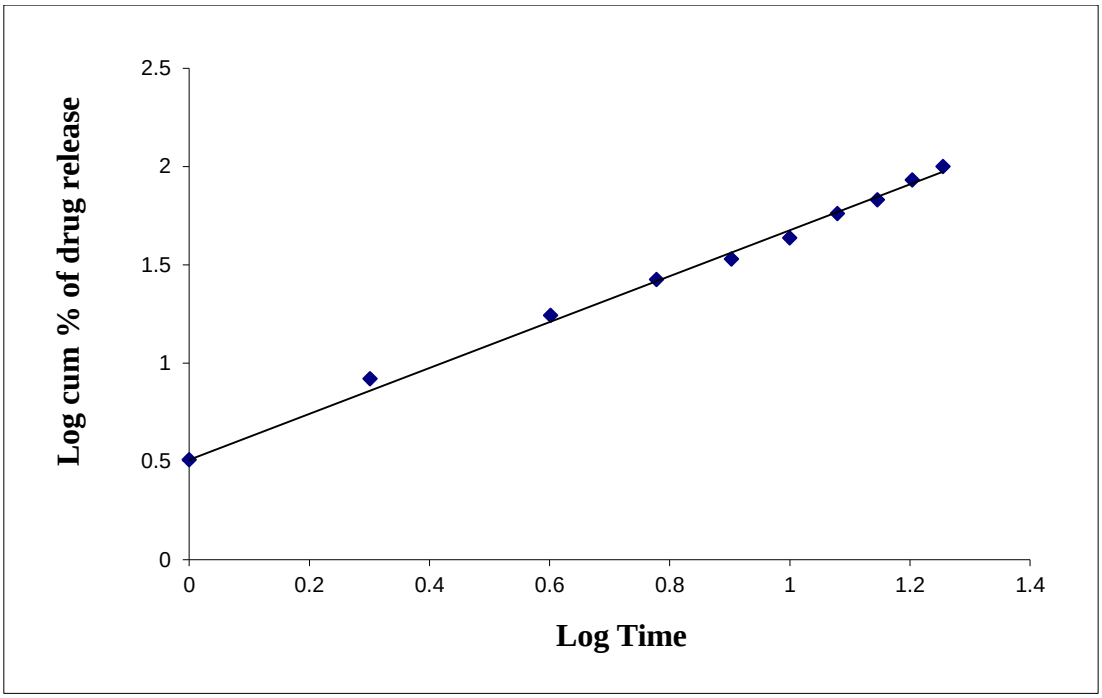

Figure 6.213 Korsmeyer & peppas plot for formulation RS609

Table 6.54 *In vitro* release study of RS610

Time in hrs	Absorbance	Concentration in µg/ml /ml	Amount present in mg	Cumulative amount present in 100 ml	Cumulative % of drug release	Log Time	Log Cum % Drug Release	Log cum % remain	Sq.Rt.Time
0	0.000	0.0000	0	0	0	0	0	2	0
1	0.023	3.025327861	0.015126639	1.51266393	2.521106551	0	0.401591201	1.988911	1
2	0.058	8.911802983	0.044559015	4.48615477	7.476924617	0.30103	0.873723002	1.96625	1.41421356
4	0.119	19.1710882	0.095855441	9.704915407	16.17485901	0.60205999	1.208840504	1.923374	2
6	0.186	30.43948343	0.152197417	15.53082391	25.88470651	0.77815125	1.413043245	1.869908	2.44948974
8	0.249	41.03513865	0.205175693	21.13304635	35.22174392	0.90308999	1.546810855	1.811429	2.82842712
10	0.346	57.34908399	0.28674542	29.70037041	49.50061735	1	1.694610615	1.703286	3.16227766
12	0.398	66.09470417	0.330473521	34.64667134	57.74445223	1.07918125	1.761510266	1.625884	3.46410162
14	0.432	71.81299429	0.359064971	38.16676344	63.6112724	1.14612804	1.803534083	1.560967	3.74165739
16	0.475	79.04494944	0.395224747	50.04123536	83.40205893	1.20411998	1.921176772	1.220054	4
18	0.577	96.1998198	0.480999099	59.40912003	99.01520005	1.25527251	1.995701869	-0.00665	4.24264069

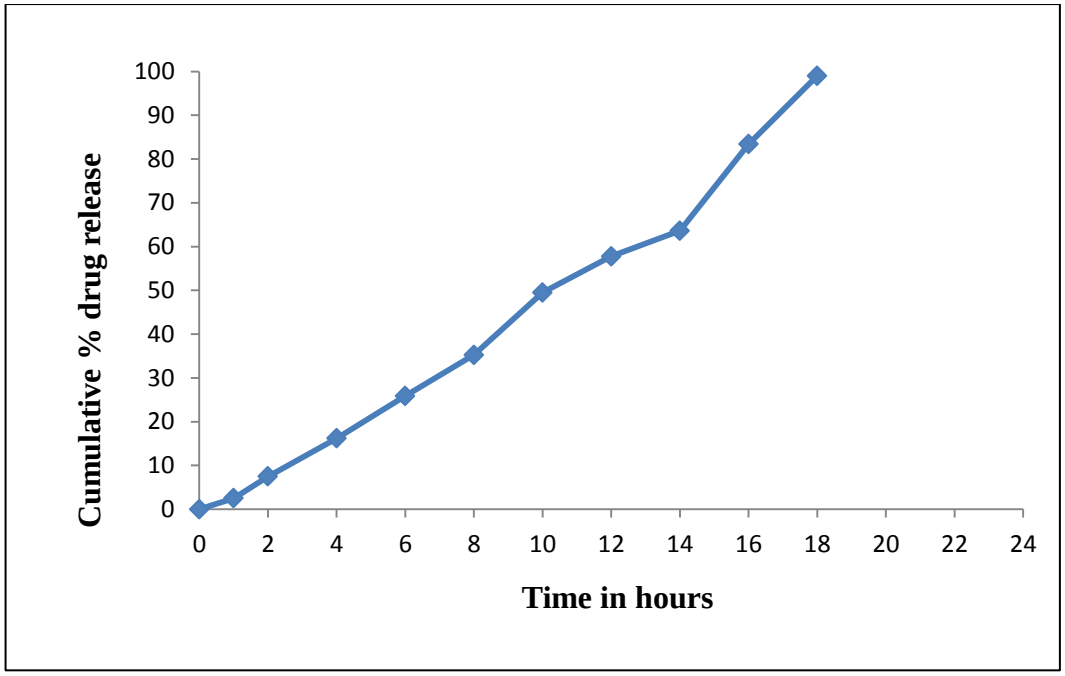

Figure 6.214 *In vitro* drug release of formulation RS610

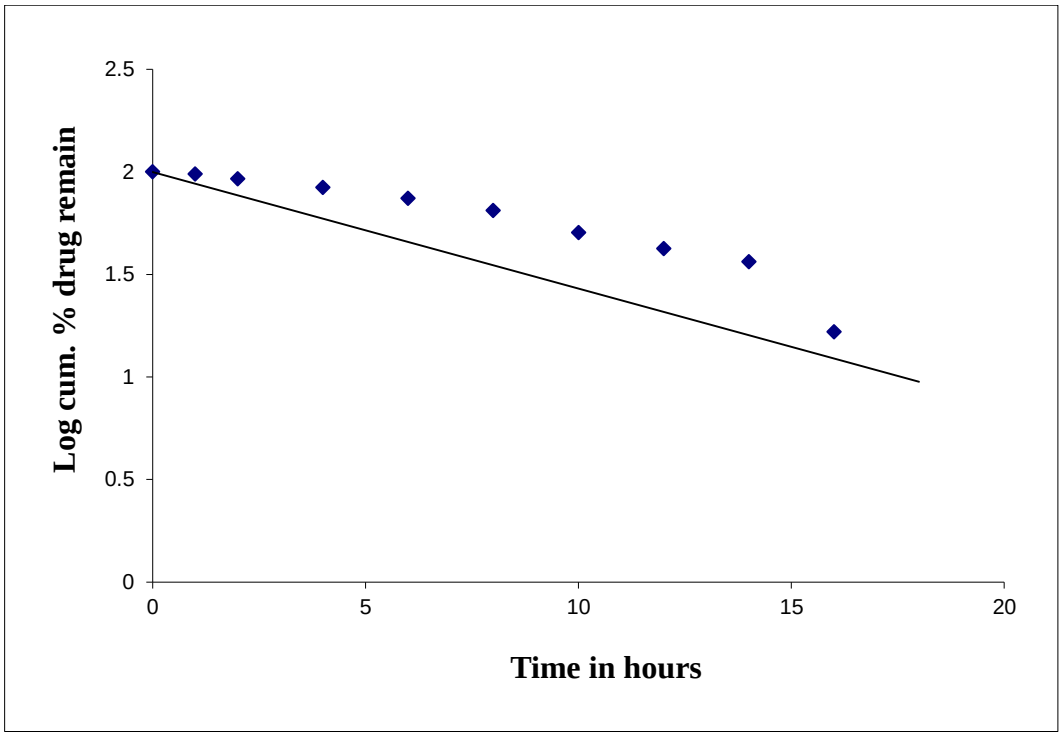

Figure 6.215 First order kinetics of formulation RS610

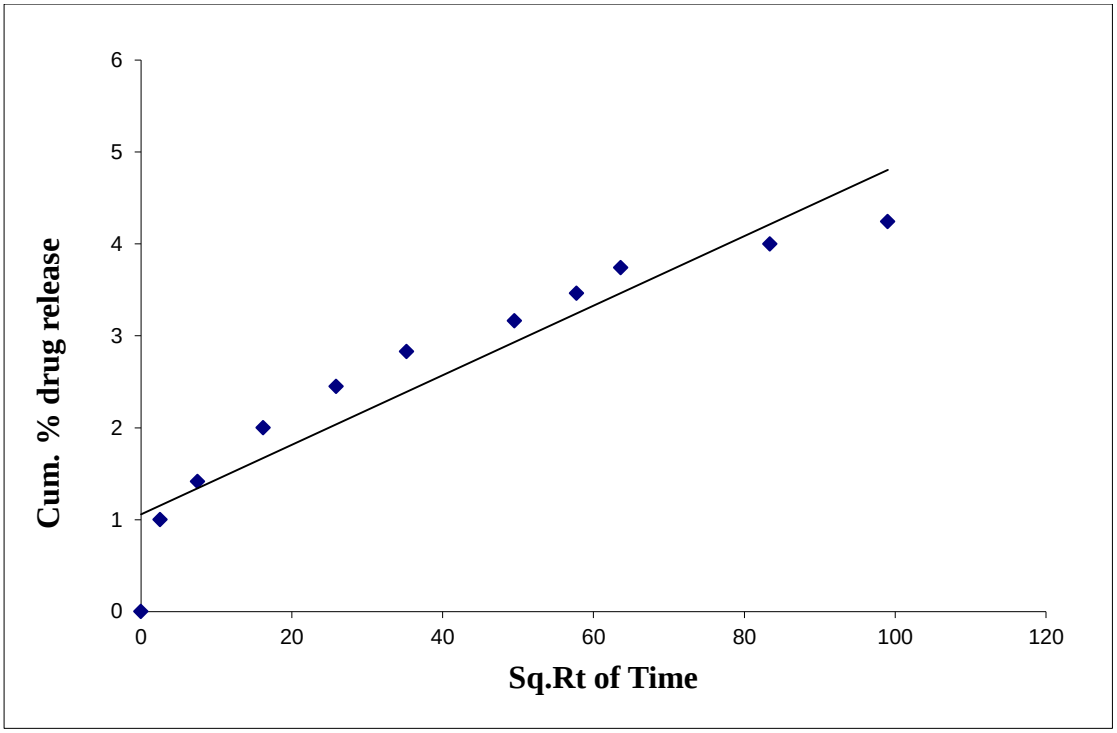

Figure 6.216 Higuchi's plot for formulation RS610

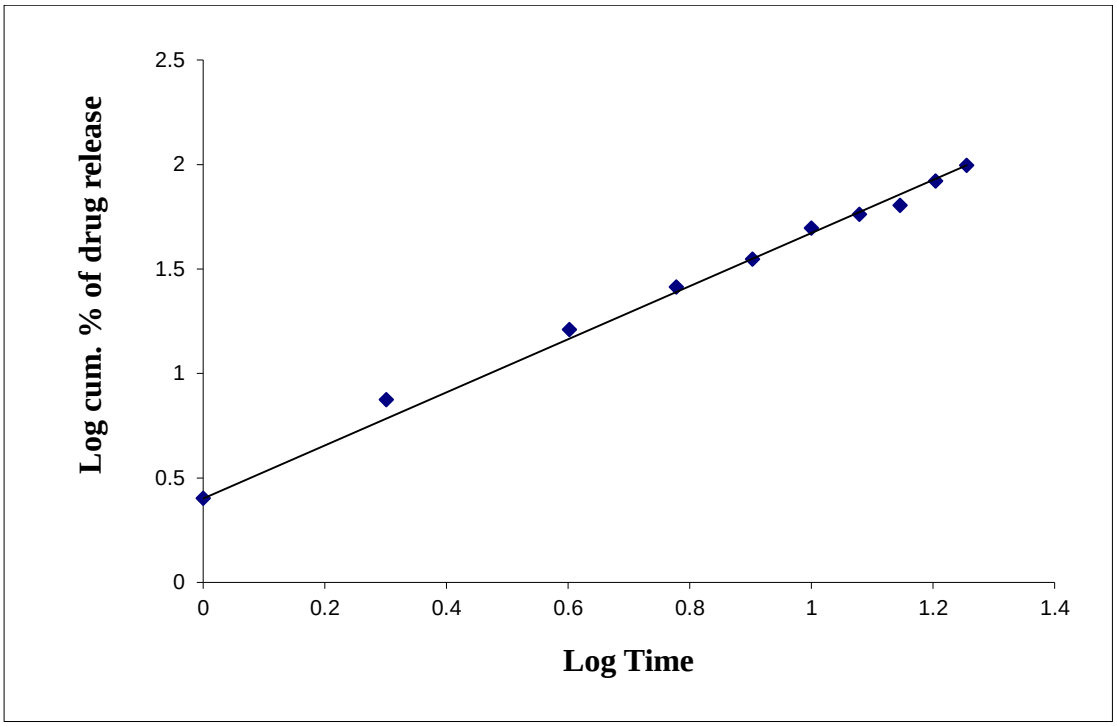

Figure 6.217 Korsmeyer & peppas plot for formulation RS610

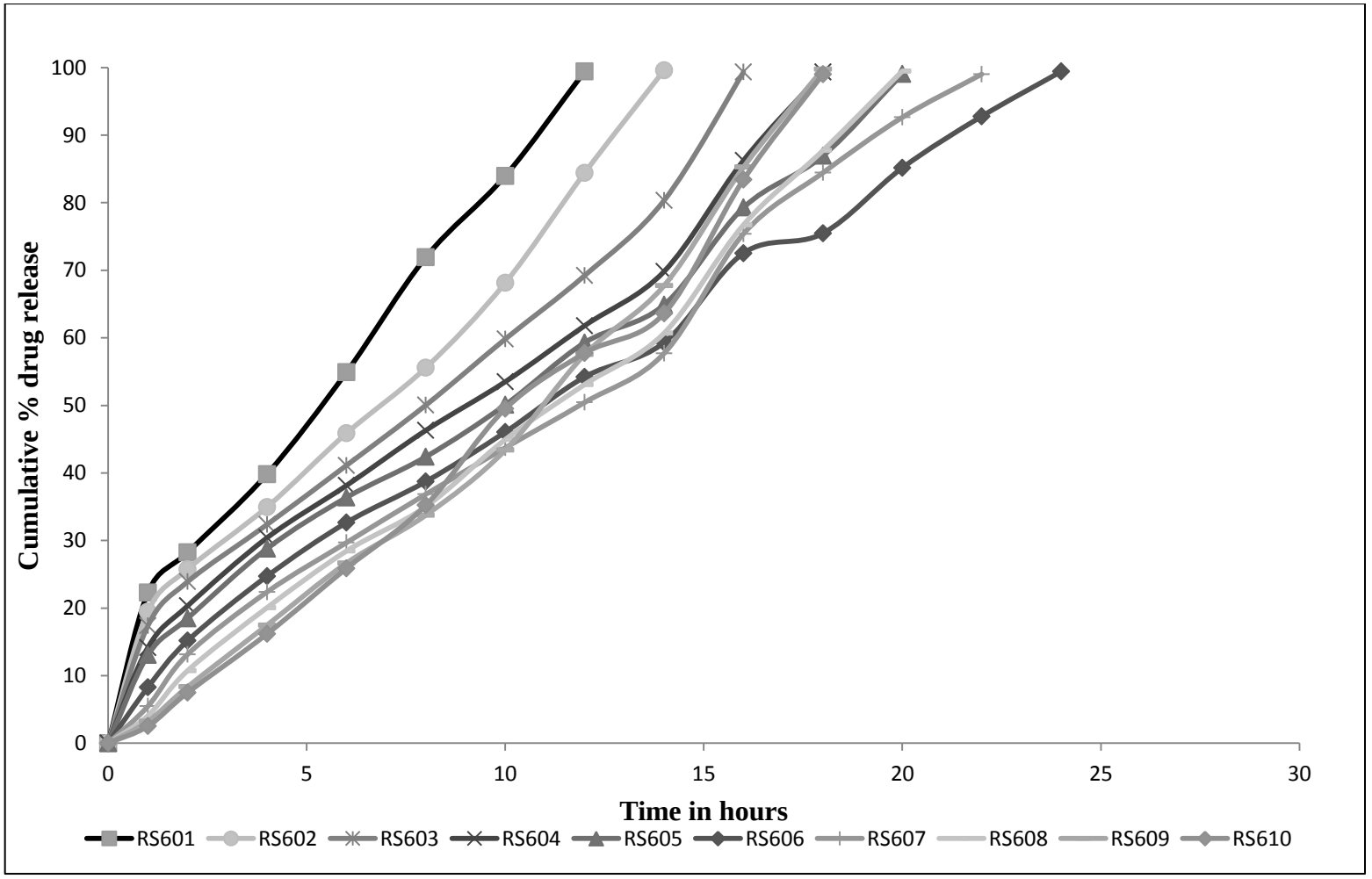

Figure 6.218 Comparative dissolution profile of RS 601-RS610

6.2.5.9 Kinetic values obtained from different plots of formulations (RS601-RS610) of raloxifene

Table 6.55 Drug release mechanism (RS601-RS610)

Formula Code	Zero order		First Order		Higuchis model		Koresmayer & Peppas	
	K_0	R^2	K_1	R^2	K_H	R^2	*n*	R^2
RS601	7.0906	0.9988	0.3266	-0.8457	28.0726	0.9813	0.6130	0.9850
RS602	6.3610	0.9910	0.2749	-0.7991	25.1534	0.9713	0.6071	0.9816
RS603	5.0866	0.9935	0.2053	-0.7876	22.8817	0.9713	0.6034	0.9849
RS604	4.6844	0.9933	0.1824	-0.7942	22.0630	0.9693	0.6507	0.9904
RS605	4.3222	0.9966	0.1558	-0.8254	21.4565	0.9727	0.6665	0.9922
RS606	3.8677	0.9983	0.1440	-0.8427	21.0283	0.9783	0.7648	0.9983
RS607	4.4135	0.9953	0.1522	-0.8604	22.0840	0.9607	0.8926	0.9953
RS608	4.8278	0.9956	0.1710	-0.7913	22.4491	0.9517	1.0088	0.9966
RS609	5.4969	0.9922	0.2121	-0.7476	23.3715	0.9337	1.1424	0.9981
RS610	5.4504	0.9935	0.1721	-0.7976	23.2822	0.9387	1.2196	0.9980

6.2.5.10 Stability study of optimized formulation

Table 6.56 Stability study of optimized raloxifene formulation RS 606

Temperature	Values obtained at zero time			Values obtained at 1st month			Values obtained at 2st month			Values obtained at 3rd month		
	Vesicle size in nm	Drug remain in %	*In vitro* drug release in %	Vesicle size in nm	Drug remain in %	*In vitro* drug release in %	Vesicle size in nm	Drug remain in %	*In vitro* drug release%	Vesicle size in nm	Drug remain in %	*In vitro* drug release%
5±3˚C	690	82.44	99.42	694	82	99.16	696	81.86	98.95	700	81.36	98.35
25±2˚C	690	82.44	99.42	695	80.25	98.65	700	80	97.25	710	79.82	96.54
40±2˚C	690	82.44	99.42	698	81.25	96.35	710	75.35	95.45	720	72.58	93.92

	Values obtained at zero time	Values obtained at 1st month	Values obtained at 2st month	Values obtained at 3rd month
Colour	Dark Yellow	No changes	No changes	No changes
Appearance	Crystalline	No changes	No changes	No changes

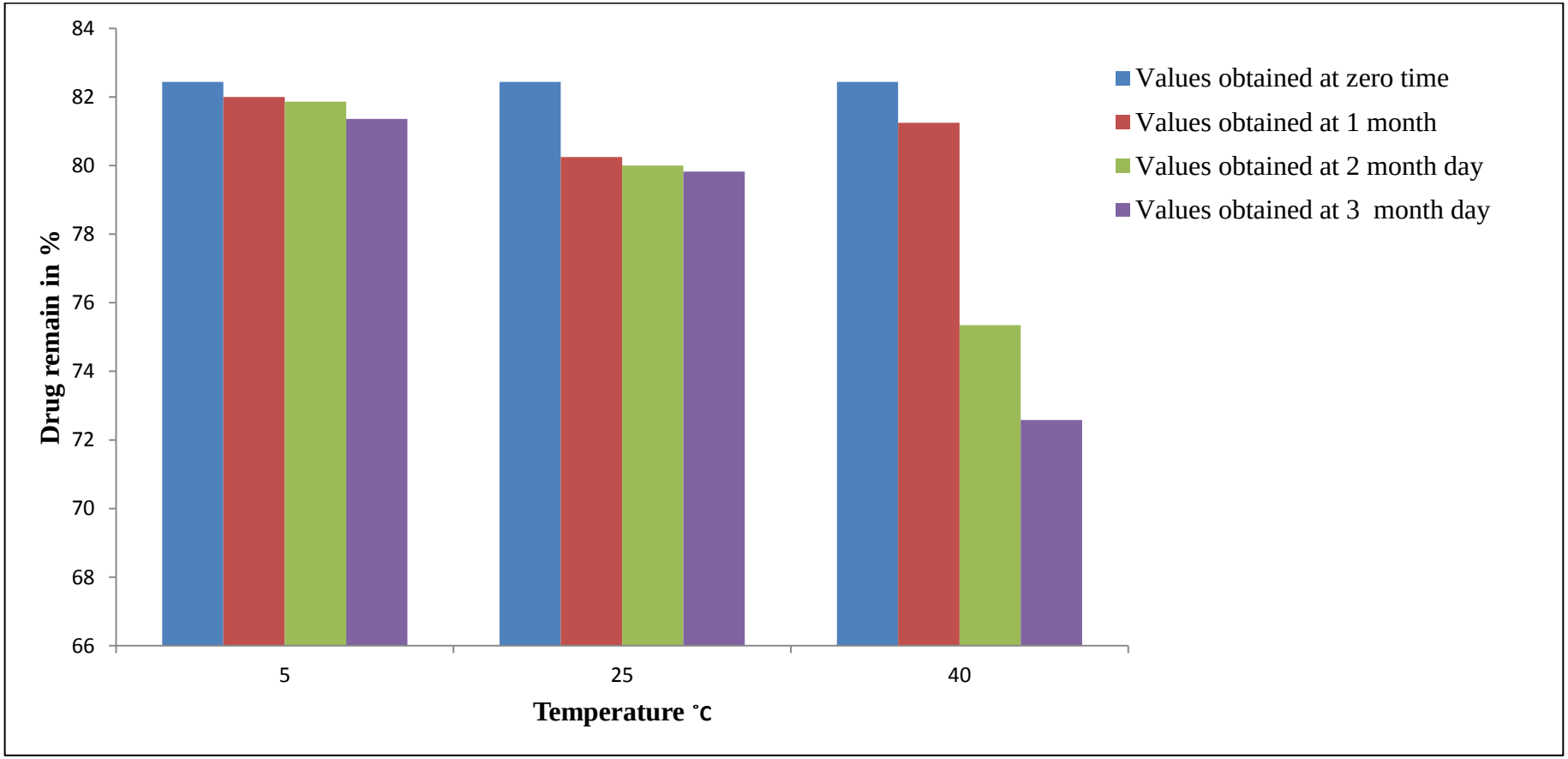

Figure 6.219 Entrapment efficiency in stability studies of optimized formulation RS 606

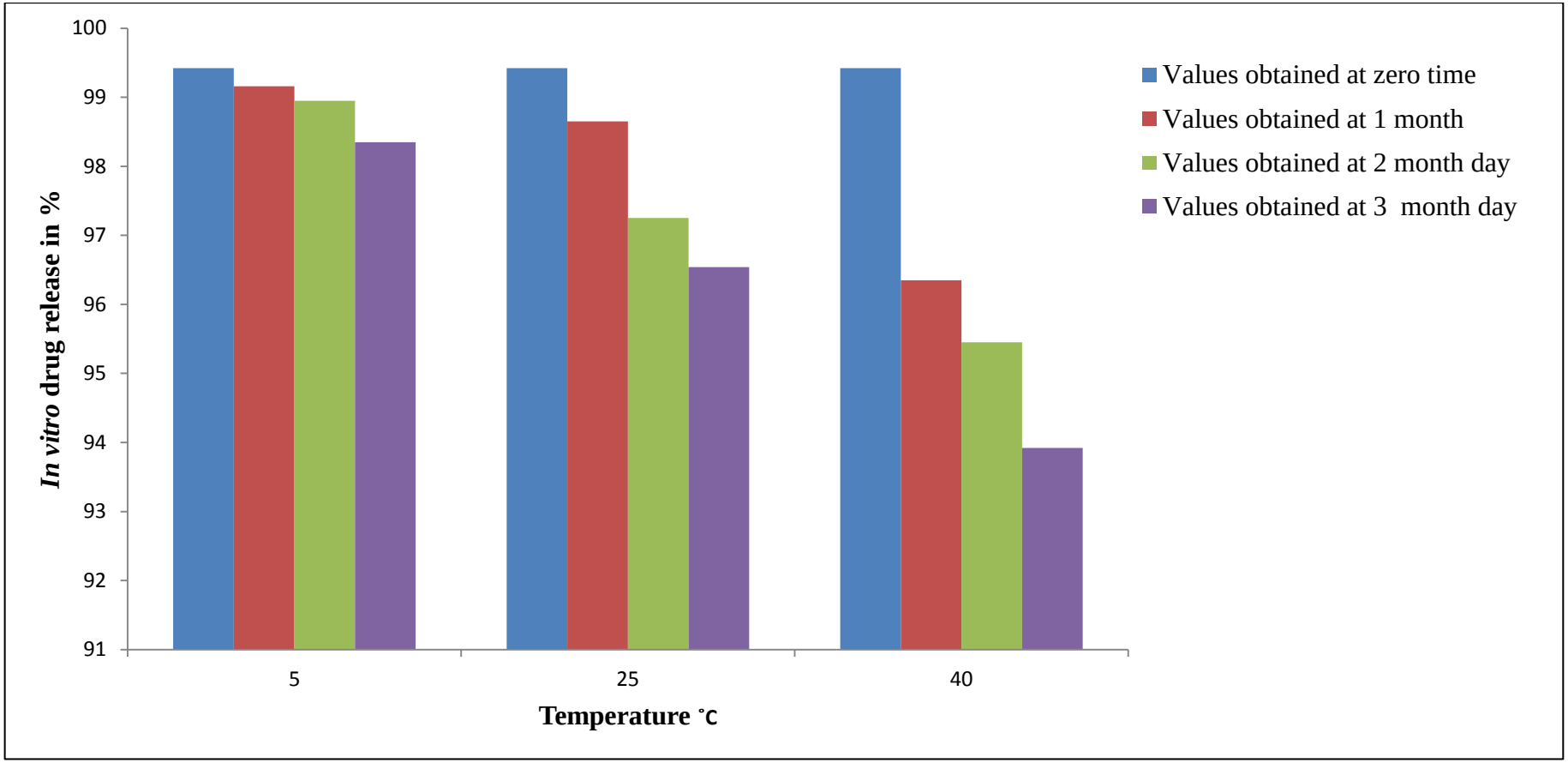

Figure 6.220 *In vitro* drug release during stability studies of optimized formulation RS 606

6.2.5.11 *In vivo* pharmacokinetic study of optimized proniosome in animal model

Pharmacokinetic parameters	Pure raloxifene	RS606
Cmax (mcg/ml)	69.17±0.21	45.2±0.10
T max (h)	4.0±0.00	18±0.00
ElimRateConst (h^{-1})	0.03±0.007	0.018±0.006
Half life (h)	23.08±0.06	37.95±0.16
AUC0_t (mcg.h/ml)	1605.67±1.06	1994.97±4.53
AUC0_inf (mcg.h/ml)	2420.32±4.35	2898.46±12.47
AUMC0_t (mcg.h/ml)	28168.8±45.74	70895.87±199.71
AUMC0_inf (mcg.h/ml)	62131.62±354.52	117423.99±1382.69

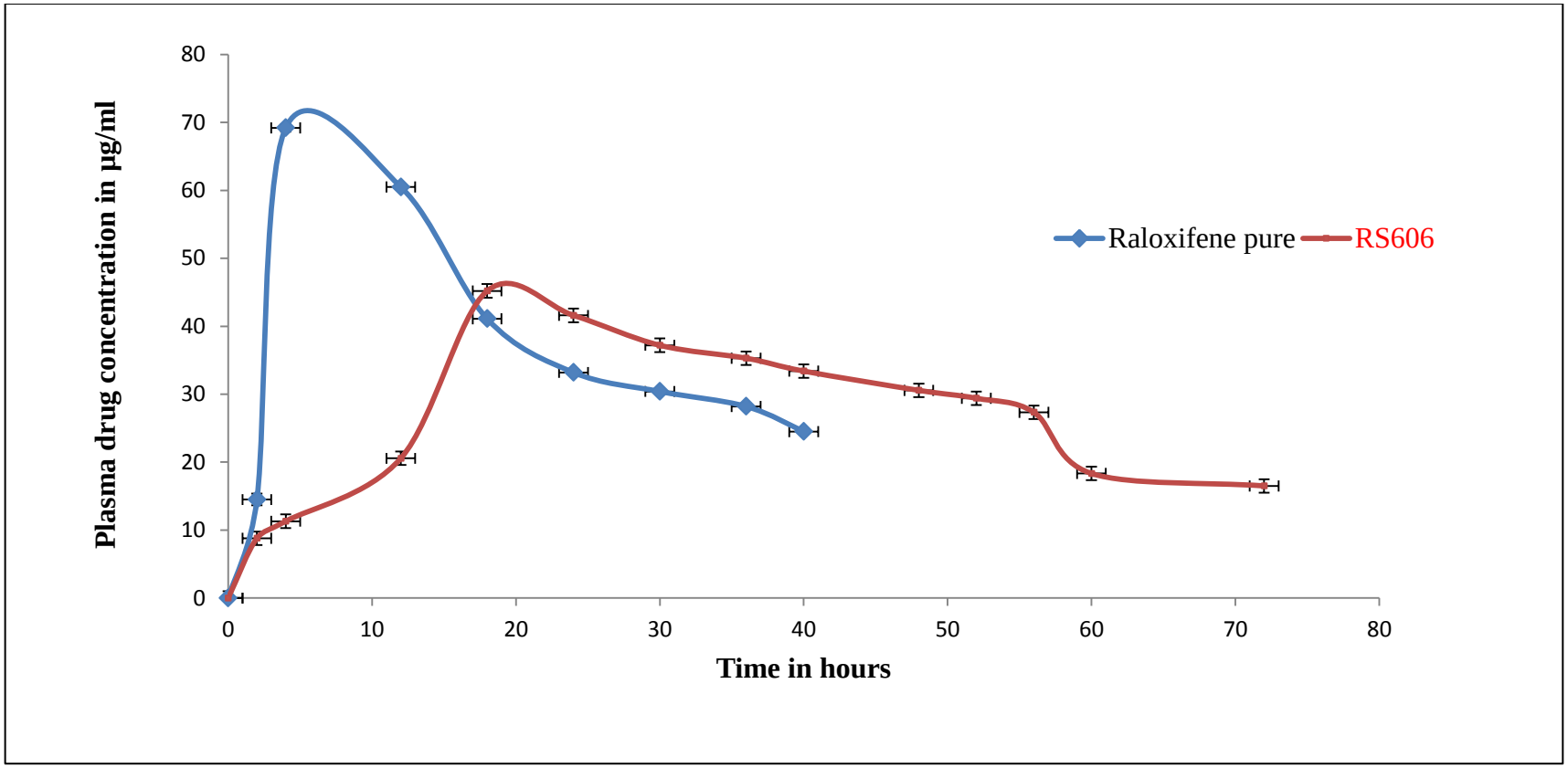

Figure 6.221 Comparison of mean plasma concentration of pure raloxifene and proniosomal formulation RS606

7. DISCUSSIONS

Letrozole loaded proniosome formulation

Preformulation studies were carried out to confirm and identify the purity of drug and the results obtained were matched with standard specifications indicated that the selected drug and excipients were suitable for formulations.

The slurry method was found to be simple and suitable for laboratory scale preparation of letrozole proniosomes which were prepared using different proportions of non-ionic surfactant span 20, span 60 and cholesterol as membrane stabilizer along with maltodextrin carrier. The objective in developing proniosomes was to devise a method of producing a nonionic surfactant based dosage at the point of use to avoid problems of physical and chemical stability found in storage of some surfactant-based dosage forms. By creating a dry formulation, issues related to hydrolysis of the active ingredient or surfactants are avoided; by forming the suspension as needed, precipitation and aggregation are avoided. Although the sorbitol-based proniosomes accomplished these objectives, the effect of the carrier on entrapment efficiency remained problematic.[35] However, making proniosomes with a reduced amount of sorbitol was a tedious process and began to compromise the advantages of proniosomes related to minimizing film thickness. The use of maltodextrin as the carrier in the proniosome preparation permitted flexibility in the amounts of surfactant and other components, which greatly enhances the potential application of proniosomes in a scaled-up production environment.

Although maltodextrin is a polysaccharide which has minimal solubility in organic solvents. Thus, it was possible to coat the maltodextrin particles by simply adding surfactant in organic solvent to dry maltodextrin and evaporating the solvent.

Cholesterol, which has a property to abolish the gel to liquid transition of niosomes, this found to prevent the leakage of drug from the niosomal formulation. The slower release of drug from vesicles may be attributed to the fact that vesicles consist of several concentric sphere of bilayer separated by aqueous compartment.

Compatibility studies were performed using FT-IR spectrophotometer. The IR spectrum of pure drug and physical mixture of drug and excipients were studied by making a KBr pellet. FT-IR Spectra of letrozole, span 20, span 60, maltodextrin, cholesterol, physical mixture of drug and carrier and LS207,LS604 formulations were recorded and given under results and analysis. The letrozole present in the formulation LS207 and LS604 was confirmed by FT-IR spectra. The pure letrazole (Figure No 6.1)exhibits characteristic peaks at 2378.07 cm^{-1} (C≡N Stretching), 1170.64 cm^{-1} (C=N Stretching), 1058.35 cm^{-1} (C-N amine), 1460.18 cm^{-1} (C=C Aromatic). All these peaks have appeared in physical mixture, LS207 and LS604 formulations indicated no chemical interaction between letrozole, cholesterol, maltodextrin, span 20 or span 60 and also the drug is compatible with the excipients. It also confirmed that the stability of drug during formulation.

The thermotropic behaviour and the physical state of the drug in proniosome powder were ascertained from the DSC thermograms. DSC analysis was carried out for Letrozole and letrozole –loaded proniosomes (LS207,LS604). It was evident from Figure 6.10 that the DSC thermogram of letrozole showed sharp endothermic peak at 184.21°C which corresponds to the melting of the drug which possessed crystalline behaviour .On the other hand, the absence of conspicuous peak over the range of 184 to 185.5°C in letrozole loaded proniosome formulation.The DSC thermograms showed that there were no physical or chemical interaction in between drug and excipient.

Construction of standard curve was obtained by plotting the standard concentration (2µg to 12µg) against absorbance which was measured at λ_{max} of 240nm.The linear regression analysis was done on absorbance data points. A straight line generated to facilitate the calculation of amount of drug by using Y=mx+c equation where Y=absorbance,m=slope,x=concentration.The standard curve showed good linearity with R^2 value of 0.9997,which suggested that it obeys Beer-Lamberts law.

The powder flow properties were assessed from Angle of repose. Angle of repose of maltodextrin powder compared with proniosome formulation by fixed funnel method. Results of measurements of the angle of repose of proniosome powder and pure maltodextrin are summarized in table 6.4 and table 6.5and the values were within the range of 30°16'±0.73 to 33°27'±0.41 and 29°11'±0.31to32°58'±0.64 for LS201 to LS210 and LS601 to LS610 respectively. It was indicated that the fluidity of proniosome dry powder is equal to or better than that of maltodextrin powder and proniosome formulations have appreciable flow property.

Niosomes were spherical and few being elongated shape while observed by optical microscope and given in figure 6.15 and 6.16. The smaller size may result from efficient hydration of a uniform and thin film of surfactant mixture at low surfactant loading.

Vesicle size and size distribution is an important parameter for the vesicular systems.[163] The mean size of the vesicles was in the range of 110-690 nm in case of letrozole proniosomes using span20 and 490-650nm in case of letrozole proniosome using span 60. Larger vesicle size was also a reason for higher entrapment of drug.

Shape and surface characteristics of proniosome examined by Scanning Electron Microscopy analysis, is shown in the figure 6.27 and 6.28. Letrozole loaded maltodextrin proniosome (LS207 and LS604 formulation) are evaluated for surface morphology. Surface morphology confirmed the coating of surfactant in carrier. Due to the porous surface of maltodextrin particles, which provides more surface area for the coating of the surfactant mixture makes it as a effective carrier. Surfactant coating was confirmed from surface morphology and the average particle size was in the range of 690±0.42nm and 650±0.28nm in case of LS207 and LS604 respectively.

The zeta potential analysis was performed to get information about the surface properties of the niosome derived from proniosomes. Zeta potential is an important parameter to maintain stability of niosomes. Zeta potential was determined using malvern zeta sizer and the values lied in between +16mv to +36mv in case of letrozole loaded proniosomes and zeta potential was determined as +32mv for

formulation LS207 and +36mv for LS604. Colloidal particle with zeta potential around ± 30mv is physically stable. Zeta potential is a measure of net charge of niosome. When the charge was higher on the surface of vesicles leads to repulsive force. Due to repulsive forces between vesicles could avoid agglomeration and prevent faster settling. So evenly distributed suspension was obtained indicated that prepared proniosome formulation LS207 and LS604 would yield better stable suspension.

One of the important parameter to evaluate the proniosome powder is the number of vesicles formed after hydration. The maximum benefit from the proniosome formulations can be speculated when abundant numbers of vesicles are formed after hydration in the gastrointestinal tract. Number of vesicles formed after hydration is appeared between 0.9 to 3.8 mm^3x10^5 for letrozole loaded proniosomes has given in Figure 6.25 and 6.26. Among all the formulations, the proniosome formulation containing span 60 and cholesterol at a ratio of 0.7:0.3(LS604) has exhibited 0.9 mm^3x10^5 number of vesicles which is in well correlation with the size and entrapment efficiency results.

The Entrapment efficiency of all proniosomal formulations are reported in figure 6.23 and 6.24.The drug entrapped in proniosomes were found in the range of 42.26±0.77 to76.32±0.93% and 54.18 ±0.36 to83.64±0.42% in case of letrozole proniosomes using span20 and letrozole proniosomes using span 60 has given in table 6.4 and 6.5 respectively. The entrapment efficiency was found to be the highest with the formulation LS207 and LS604 among the formulation of letrozole proniosomes using span20 and letrozole proniosomes using span 60 respectively. Higher entrapment efficiency was observed for optimal concentration of surfactant which might be due to the high fluidity of the vesicles.Entrapment efficiency was also affected by incresaed cholesterol content. From the table, increase in cholesterol, one of the common additives for preparing stable proniosomes, is seen to increase entyrapment efficiency of letrozole. An increase in cholesterol content has also been found to result in increase in microviscosity of the membrane leading to more rigidity of the bilayers. [164] Cholesterol seems to have an ability to cement the leaking space in a bilayer membrane.[165] All span-type surfactants have the same head group with different alkyl

chains. Increase in the alkyl chain length has been found to lead to a higher Entrapment efficiency.[49] Span 60 has a longer saturated alkyl chain (C16) compared to that of span 20 (C10). A larger alkyl chain lowers the HLB value of a surfactant and this tends to increase entrapment of the drug.[166] It was also observed that very high cholesterol content (LS609,LS610) had a lowering effect on drug entrapment to the vesicles (59.32 ±0.80 %,54.18 ±0.36 %).This could be due to the fact that cholesterol beyond a certain level starts disrupting the regular bi-layered structure leading to loss of drug entrapment. It is clear that the LS604 formula, which was composed of Span 60 and cholesterol in a 0.7:0.3 ratio, seems to be the most suitable for an efficient encapsulation of letrozole as it exhibits the highest entrapment efficiency (83.64±0.42%). Larger vesicle size was also a reason for higher entrapment of drug.

The dialysis method was used to investigate the *in vitro* letrozole release from niosomes. Results are shown in tables & figures under results and analysis. For all 10 prepared proniosome formulations, release study was carried out and comparison dissolution profile was given in figure 6.69. Linear release was found in most of the formulations and almost 90% release of drug was observed within 18hrs. Formulations (LS206 to LS210) which had high cholesterol ratio shown controlled release. A cumulative release of 99.34% was given by the best formulation LS207 at the end of 18 hr.Formulations which have higher cholesterol content(LS206 to LS210) are seen to have less drug release over a period of 18 hr. Among these formulations which were prepared using span20 and cholesterol. LS207 has higher entrapment and better percentage of cumulative drug release. Hence, increase in cholesterol ratio seems to result in a more intact bilayer and consequent reduction in permeability. The release study was conducted for all the ten formulations using span 60 as comparison dissolution profile was shown in the figure 6.110. Most of the formulations were found to have a linear release and the formulations were found to provide approximately 90% release within a period of 24 hours. The formulation which have optimum ratio of span 60 and cholesterol(LS604)was found to control the drug release (99.87%) than other formulations. When compared the formulations with span20 and span 60, formulations with span 60 shown better entrapment and extending the release for nearly 24 hours. Among all formulations LS604 was

selected as best formulation because of its highest entrapment efficiency and consistent release profile of Letrozole.

Mathematical models are used to predict the drug release mechanism and compared the release profile. For all the formulations (LS201 to LS210 and LS601 to LS610), the cumulative per cent drug release vs time (zero order), the cumulative per cent drug release vs square root of time (Higuchi plot), and log cumulative per cent drug remaining vs time (first order) were plotted separately .In each case, R^2 value was calculated from the graph and reported in table 6.16 and 6.27. The formulations shown linearity and high correlation values for zero order. From this it was assumed that drug release from all the formulations followed either near zero or zero order kinetics. Highuchi's plot correlation values which indicated the mechanism of release of drug from formulation was diffusion. The release exponent value of korsmeyer-peppas plot revealed the fact that the drug release follows super case II transport diffusion.

Physical stability of optimized proniosomal formulations were studied for a period of three months. The colour appearance,vesicle size, remaining drug content and release study was estimated at the interval of one month over a period of three months. From the results it was monitored that there was not much variation in the colour and appearance and also the drug leakage from the vesicles and cumulative percentage drug release was less at 5±3°C when compared with 25±2°C and 40±2°. Approximately more than 90% of letrozole was retained in optimized proniosomal formulations after the three-month period. Thus, Span 60 proniosomes of letrozole seemed to exhibit good stability at low temperature. At high temperature entrapment efficiency and release rate was reduced to a particular rate. This might be, upon storing leakage from vesicles occurred at high temperature due to phase transition of surfactant and lipid. The results of this study assumed that the stability of proniosomes could be maintained at 5±3°C followed by 25±2°C .

Pharmacokinetic parameters such as T_{max} , C_{max}, AUC_{0-t}, $t_{½}$ were determined by means of PK solver MS excel add in function for both pure drug and optimized formulation (LS604). All results were expressed in the mean ± standard deviation (SD). The data obtained were shown under the results 6.1.5.10 and figure 6.112b. The elimination half life was extended from 10.825±0.0517 to 34.39±0.169 hours for pure

drug and optimized formulation respectively.The increased values were observed for T_{max} , AUC_{0-t}, $AUMC^{0-t}$ and $t_{½}$ in the optimized formulation compartitive to a pure drug. The *in vivo* study also explains more appropriateness of the formulation of LS604 as proniosomal formulation

Raloxifene loaded proniosome formulation

Preformulation studies were carried out to confirm and identify the purity of drug and the results obtained were matched with standard specifications. So the selected drug and excipients were suitable for formulations.

The slurry method was found to be simple and suitable for laboratory scale preparation of raloxifene proniosomes which were prepared using different proportions of non-ionic surfactant span 20, span 60 and cholesterol as membrane stabilizer along with maltodextrin carrier. Maltodextrin based proniosomes could be used for efficient oral delivery of lipophilic or amphiphilic drugs. Further, during formulation, the amount of carrier could be easily adjusted with the surfactant enabling the preparation of proniosomes with high surfactant:carrier mass ratio, as a result of high surface area and porous structure of maltodextrin. Raloxifene loaded maltodextrin based proniosomes with non-ionic surfactant i.e., span 60 and span20 were prepared by slurry method. While formulating proniosomes, stability issues raised by aqueous niosomes could be resolved. For achieving maximum therapeutic benefit from the proniosome formulations, the stability of the vesicles formed after hydration with gastric fluids may also equally be important for achieving maximum therapeutic benefit from the proniosome formulations. Therefore, several methods were employed to improve the stability of vesicular systems.

The stability and morphology of the niosomes would greatly vary depending on the non-ionic surfactant concentration and cholesterol leading to disruption of vesicles, [167] leading to leakage of free drug before drug diffusion and fusion of vesicle with gastrointestinal membrane following any alteration in their composition. [168] Highest entrapment could be observed with an increase in phase transition temperature of span which has same polar head groups with varied alkyl chain. The phase transition temperatures for span 20, 40 and 60 are 16, 42 and 53°C, respectively whereas span 80 having the lowest phase transition temperature (12°C). Span 60 was selected as a

surfactant because of its high phase transition temperature and to facilitate stable vesicle formation and to improve the oral delivery of raloxifene from proniosomes.[53]

Cholesterol, which has a property to abolish the gel to liquid transition of niosomes, this found to prevent the leakage of drug from the niosomal formulation. The slower release of drug from multilamellar vesicles may be attributed to the fact that multilamellar vesicles consist of several concentric sphere of bilayer separated by aqueous compartment.

Compatibility studies were performed using FTIR spectrophotometer. The IR spectrum of pure drug and physical mixture of drug and polymer were studied by making a KBr pellet. FTIR Spectra of raloxifene, span20,span 60, maltodextrin,cholesterol, physical mixture of drug and carrier and RS210,RS604 formulations were recorded and given under results and analysis. The Raloxifene present in the formulation RS210 and RS606 was confirmed by FT-IR spectra. The pure Raloxifene (Figure 6.113) exhibited characteristic peaks at 3643.10 cm^{-1} (O-H Stretching), 1234.67 cm^{-1} (C-O Phenolic), 1519.82 cm^{-1} (C=C Aromatic), 1730.36 cm^{-1} (C=O Keto), 1051.39 cm^{-1} (C-O Ether), 1234.67 cm^{-1}, (C-N amine). All these peaks have appeared in physical mixture and RS210, RS606 formulations indicated no chemical interaction between raloxifene, cholesterol, span 20 or span 60.FT-IR study results revealed that all characteristic peaks of raloxifene were appeared in the proniosomal formulation spectra, which indicated there was no phenomenal change in the position of peaks after successful method of preparation and also the drug is compatible with the components excipients. It also confirmed that the stability of drug during formulation.

The thermotropic behaviour and the physical state of the drug in proniosome powder were ascertained from the DSC thermograms. DSC analysis was carried out for raloxifene and raloxifene loaded proniosomes (RS210, RS606). It was evident from figure 6.118, that the DSC thermogram of raloxifene showed sharp endothermic peak at 144.67°C which corresponds to the melting of the drug which possessed crystalline behaviour .On the other hand, the absence of conspicuous peak over the range of 145°C to 145.36°C in raloxifene loaded proniosome formulation.

The DSC thermograms showed there were no physical or chemical interaction between drug and excipient.

Construction of standard curve was obtained by plotting the standard concentration (10µg to 60µg) against absorbance which was measured at λ_{max} of 289nm.The linear regression analysis was done on absorbance data points. A straight line generated to facilitate the calculation of amount of drug by using Y=mx+c equation where Y=absorbance,m=slope,x=concentration.The standard curve showed good linearity with R2 value of o.9989,which suggested that it obeys Beer Lamberts law.

Angle of repose of maltodextrin powder compared with proniosome formulation by fixed funnel method. Results of measurements of the angle of repose of proniosome powder and pure maltodextrin were summarized in table 6.32 and 6.33 and the readings were within the range of 29°87'±0.48 to 32°29'±0.63 and 29°22'±0.17 to 31°54'±0.45 for RS201 to RS210 and RS601 to RS610 respectively. A small angle of repose (<30) is an indicative of good flow properties of proniosome powder formulations. While comparing angle of repose of maltodextrin powder with proniosome formulation, angle of repose of proniosome powder was nearly similar to that of maltodextrin powder.

Niosomes were spherical and some are elongated while observed by optical microscope and given in figure 6.123 and 6.124. The smaller size may result from efficient hydration of a uniform and thin film of surfactant mixture at low surfactant loading.

Vesicle size and size distribution is an important parameter for the vesicular systems.[167] The mean size of the vesicles was in the range of 240-690 nm in case of Raloxifene proniosomes using span20 and 490-650nm in case of raloxifene proniosome using span 60. Larger vesicle size was also a reason for higher entrapment of drug.

Shape and surface characteristics of proniosome examined by Scanning Electron Microscopy analysis, is shown in the figure 6.135 and 6.136. raloxifene loaded maltodextrin Proniosome (RS210 and RS606 formulation) are evaluated for surface morphology. Surface morphology confirms the coating of surfactant in carrier. Due to

the porous surface of maltodextrin particles, which provides more surface area for the coating of the surfactant mixture makes it as a effective carrier. Surfactant coating was confirmed from surface morphology and the average particle size was in the range of 530±0.42nm and 690±0.38nm in case of RS210 and RS606 respectively.

The zeta potential analysis was performed to get information about the surface properties of the niosome derived from proniosomes. Zetapotential is an important parameter to maintain stability of niosomes. Zeta potential was determined using Malvern zeta sizer and the values came in between +14mv to+24mv in case of raloxifene loaded proniosomes and zeta potential was determined as +24mv for formulation RS210 and +22mv for RS606. Colloidal particle with zeta potential around ± 30mv is physically stable. Zeta potential is a measure of net charge of niosome. When the charge was higher on the surface of vesicles leads to repulsive force. Due to repulsive forces between vesicles could avoid agglomeration and prevent faster settling. So evenly distributed suspension was obtained indicated that prepared proniosome formulation RS210and RS606 would yield better stable suspension.

One of the important parameter to evaluate the proniosome powder is the number of vesicles formed after hydration. The maximum benefit from the proniosome formulations can be speculated when abundant numbers of vesicles are formed after hydration in the gastrointestinal tract. Number of vesicles formed after hydration is appeared between $1.4mm^3x10^5$ to $3.2mm^3x10^5$ in case of raloxifene loaded proniosomes. Among all the formulations, the proniosome formulation containing span 60 and cholesterol at a ratio of 0.5:0.5(RS606) has exhibited $1.4mm^3x10^5$ which is in well correlation with the size and entrapment efficiency results.

The drug entrapped in proniosomes were found in the range of 40.41±0.6 to 76.10±0.36% and 57.52 ±0.36 to82.44±0.44% in case of raloxifene proniosomes using span20 and raloxifene proniosomes using span 60 has given in Table 6.32 and 6.33 respectively. Entrapment efficiency was studied for all the 10 formulations to find the best, in terms of entrapment efficiency. Higher entrapment efficiency of the vesicles of span 60 is predictable because of its higher alkyl chain length. The entrapment efficiency was found to be higher with the formulation RS606 (82.44%),

which may have an optimum surfactant and cholesterol ratio to provide a high entrapment of raloxifene and might be due to the high fluidity of the vesicles. Very low cholesterol content was also found to cause low entrapment efficiency , which might be because of leakage of the vesicles. The higher entrapment may be explained by high cholesterol content (50% of the total lipid). There are reports that entrapment efficiency was increased, with increasing cholesterol content and by the usage of span-60 which has higher phase transition temperature. It was also observed that very high cholesterol content (RS609, RS610) had a lowering effect on drug entrapment to the vesicles (64.96,61.76%) when compared with other formulations.. This could be due to the fact that cholesterol beyond a certain level starts disrupting the regular bi-layered structure leading to loss of drug entrapment. All span-type surfactants have the same head group with different alkyl chains. Increase in the alkyl chain length has been found to lead to a higher entrapment efficiency. Span 60 has a longer saturated alkyl chain (C16) compared to that of span 20 (C10). A larger alkyl chain lowers the HLB value of a surfactant and this tends to increase entrapment efficiency of the drug.It is clear that the RS606 formula, which was composed of span 60, cholesterol in a 5:5 ratio, seems to be the most suitable for an efficient encapsulation of raloxifene as it exhibits the highest entrapment efficiency (82.44±0.44%). The larger vesicle size may also contribute to the higher entrapment efficiency.

The dialysis method was used to investigate the *in vitro* raloxifene release from niosomes. Results are shown in tables & figures under results and analysis. For all prepared proniosome formulations, release study was carried out and comparison of dissolution profiles were given in figure 6.174 and 6.213. Linear release was found in most of the formulations and almost 90% release of drug was observed within 18-24hours. Formulations (RS206 to RS210) which had high cholesterol ratio shown controlled release. A cumulative release of 99.19% was given by the best formulation RS210 at the end of 24 hour. Formulations which have higher cholesterol content (RS206 to RS210) were seen to have less drug release over a period of 20-24 hr. Among these formulations which were prepared using span20 and cholesterol,RS210 has higher entrapment and better percentage of cumulative drug release. Hence, increase in cholesterol ratio seems to result in a more intact bilayer and consequent reduction in permeability. Most of the formulations prepared using span 60 were found to have a linear release and the formulations were found to provide

approximately 90% release within a period of 24 hours. The formulation which have optimum ratio of span 60 and cholesterol in RS606 was found to control the drug release(99.42%) than other formulations. When compared the formulations with span 20 and span 60,formulations with span 60 shown better entrapment and extending the release for nearly 24 hours. Among all formulations RS606 was selected as best formulation because of its highest entrapment efficiency and consistent release profile of raloxifene.

Mathematical models are used to predict the release mechanism and compare release profile. For all the formulations (RS201 to RS210 and RS601 to RS610), the cumulative per cent drug release vs time (zero order), the cumulative per cent drug release vs square root of time (Higuchi plot), and log cumulative per cent drug remaining vs time (first order) were plotted separately In each case, R^2 value was calculated from the graph and reported in Table 6.44 and 6.55. The formulations shown linearity and high correlation values., for zero order. From this it was assumed that from all the formulations drug release followed either near zero or zero order kinetics. Highuchi's plot correlation values indicated the mechanism of release of drug from formulation was diffusion. The release exponent value of korsmeyer-peppas plot revealed the fact that the drug release follows non fickian diffusion.

Physical stability of optimized proniosomal formulations were studied for a period of three months.The colour appearance,vesicle size, remaining drug content and release study was estimated at the interval of one month over a period of three months. From the results it was monitored that there is not much variation in the colour and appearance and also the drug leakage from the vesicles and cumulative percentage of drug release was less at 5±3°C when compared with 25±2°C and 40±2°. Approximately more than 90% of raloxifene was retained in optimized proniosomal formulations after the three-month period. Thus, Span 60 proniosomes of raloxifene seemed to exhibit good stability at low temperature. At high temperature entrapment efficiency and release rate was reduced to a particular rate. This might be, upon storing leakage from vesicles occurred at high temperature due to phase transition of surfactant and lipid.The results indicated that the stability of proniosomes could be maintained at 5±3°C followed by 25±2°C .

Pharmacokinetic parameters such as T_{max} ,C_{max}, AUC_{0-t}, $t_{½}$ were determined by means of PK solver MS excel add in function for both pure drug and optimized formulation (RS606). All results were expressed in the mean ± standard deviation (SD). The data obtained were shown under the results 6.2.5.11 and figure 6.218. The elimination half life was extended from 23.08±0.06to 37.95±0.16hours for pure drug and optimized formulation respectively.The increased values were observed for T_{max} , AUC_{0-t}, $AUMC^{0-t}$ and $t_{½}$ in the optimized formulation compartitive to a pure drug. The *in vivo* study also explains more appropriateness of the formulation of RS606 as proniosomal formulation

SUMMARY AND CONCLUSION

Currently proniosomes have been studied by researcher as a choice of oral drug delivery system for anticancer drugs to provide a better oral bioavailability considering, high penetration property of the niosome encapsulated agents through biological membrane and the stability of them.

Cancer is a leading cause of death worldwide. The four most common cancers occurring worldwide are lung, female breast, bowel and prostate cancer. Breast cancer is the most frequently diagnosed cancer and the primary cause of cancer-related death in women worldwide. Aromatase inhibitors and selective oestrogen receptor modulator are used for the treatment of breast cancer in postmenopausal women. The most important goal of cancer chemotherapy is to minimize the exposure of normal tissues to drugs while maintaining their therapeutic concentration in tumors.

Proniosomes proved to be the potential carriers for efficient oral delivery of lipophilic or amphiphilic drugs. Henceforth an attempt was made to improve the oral delivery of letrozole and raloxifene by loading into maltodextrin based proniosome powders separately.

Letrozole loaded maltodextrin based proniosomes and raloxifene loaded maltodextrin based proniosomes were prepared by slurry method with different ratio of span 20, span 60 and cholesterol and evaluated for flow properties and the results indicated acceptable flow properties.

The formation of niosomes and surface morphology of optimized proniosome formulations were studied by optical and scanning electron microscopy, respectively which has showed smooth surface of proniosome. FT IR, differential scanning calorimetry studies performed to understand the solid state properties of the drug revealed the absence of chemical interaction.Further evaluated for entrapment efficiency, *in vitro* release, kinetic data analysis, stability study and pharmacokinetic analysis. The formulation LS604, RS606 which showed higher entrapment efficiency (83.64%, 82.44%) and *in vitro* releases of 99.87% and 99.42% respectively at the end of 24 hours was found to be best among all formulations. The drug release was explained by zero order kinetics. The stability study results showed that the prepared

proniosome formulations were stable. The pharmacokinetic data obtained from *in vivo* study shows better bioavailability when compared with pure letrozole and raloxifene.

In conclusion, we can state that, besides providing the controlled systemic delivery of letrozole and raloxifene, an attempt was made to prepare proniosomal drug delivery system and evaluate its performance. Proniosome provides an effective means of delivering the drug through the oral route. The stable proniosome formulation was prepared and it is highly successful in enhancing oral bioavailability of the drug. Thus a dry free flowing product like proniosomes will be a promising industrial product.

IMPACT OF THE STUDY

- The ultimate goal of drug delivery research is to help patients by developing clinically useful formulation.
- The above research proposal is expected to give a newer and safer anticancer proniosome formulation to solve the problem associated with the currently available formulations.
- Formulating the chemotherapeutic agents in proniosome, maximize the efficacy of the drug by targeting the drug at the site.
- Due to its site specific delivery it may reduce systemic side effects of chemotherapeutic agents.
- When compared to conventional drug delivery systems we can minimize the quantity of drug to the patient thereby we can improve patient convenience.
- Effective nature of proniosome drug delivery may minimize duration of hospitalization and reduce the health care cost.
- Based on the above reasons such drug delivery system is currently needed for effective drug delivery in chronic conditions.

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