DELIVERY OF HYDROPHILIC DRUGS USING SUGAR GLASS MICRO/NANO-PARTICLES

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Declaration

I declare that this written submission represents my ideas in my own words, and where others' ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be a cause for disciplinary action by the Institute and can also evoke penal action from the sources that have thus not been properly cited, or from whom proper permission has not been taken when needed.

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Approval Sheet

This thesis entitled "Delivery of hydrophilic drug by using Sugar glass micro/nano particles" by Nitin Kumar Talreja is approved for the degree of Master of Technology.

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List of Figures

LIST OF TABLES

ABSTRACT

Most of the techniques followed in novel drug delivery systems for encapsulating hydrophilic drugs in nanoparticles/Microparticles have some drawbacks such as burst release and low encapsulation of drug in polymer particles because of rapid partitioning of water soluble drug into the aqueous phase. Double emulsion is one of those techniques which has a major limitation of burst release. We hypothesised that applying double emulsion technique with Sugar glass nanoparticles could result in better encapsulation yield of hydrophilic drug in nano or micro sized particles. Here sugar glass nanoparticles are prepared by flash freezing the inverse micellar suspension of drug along with other excipeints followed by drying off of the solvents leaving behind the surfactant coated nanoparticles which were then encapsulated in the polymer. As a model drug we used 5-FU and metformin as a Hydrophilic drug. The new system formulated called sugar glass nanoparticle when encapsulated in polymer particles by double emulsion technique gave better encapsulation of the drug and the burst release from the polymer system unlike conventional technique was also minimised. We have also evaluated both the conventional and modified water in oil in oil, double emulsion method using sugar glass nano particles as an encapsulant. There was a initial burst release of 20-35 % and then it followed sustained release similar to bimodal release pattern, whereas in conventional methods, 90 % of burst release was observed. Also the size of particles was in range of 300-900 nm. Encapsulation of the drug increased to 64.33 % when using sugar glass system unlike W/O/W double emulsion method where the encapsulation was 4.06 %. It is concluded that this new modified double emulsion technique incorporating sugar glass particles of drug is better and advantageous than conventional double emulsion technique in terms of size, shape, release profile and % encapsulation.

Contents

Chapter 1

INTRODUCTION

1.1 CANCER

CANCER, One of the leading widespread disease in the world. Patients of cancer are huge in number. There are numerous ways in which the disease can be treated but every therapy has a drawback. Use of chemotherapy is one of the most commonly used treatments of Cancer. Chemotherapy can be defined as the use of drugs or medications to treat a disease. 1 out of every 7 deaths in the United States is due to Cancer [cancer.org]. Cancer is a disease of body cells. The cells which are then called cancerous cells become abnormal and keep on dividing and forming more cells without control. Normally the cells are expected to divide and reproduce in an orderly and controlled manner [63]. The abnormal division and forming of these cells begin to form a clump, which is also known as a tumor. These Cancer cells are disorganized because they do not differentiate into the tissue of the organ and they never fulfill the function of the organ. It is likely that the Cancer cells can affect the other tissues even further if the cells break away from the tumor. When these cells separate themselves from the origin, they can be transferred around the body through arteries and veins or through the lymphatic system. When these cells accumulate in another organ or tissue, it is more than likely that it will start growing there and multiply to form another tumor. Cancer cells also produce proteolytic enzymes that attach the membrane and allow them to invade the tissue [66]

TYPES OF CANCER- There are some categories of cancers that begin in specific types of cells and few of them illustrated below.

Carcinoma

Carcinoma is the most common type of cancer. It is formed by epithelial cells; these cells are cover the inside and outside surfaces of the body and often have a structure like column. Carcinomas are found in different epithelial cell types have specific names,

Adenocarcinoma

Cancer which forms in epithelial cells that produce fluids or mucus. Tissues with this type of epithelial cell are sometimes known as glandular tissues. Most cancers of the breast, prostate and colon are Adenocarcinoma.

Basal cell carcinoma is a cancer which begins in the lower last layer; also called as basal layer of the epidermis(outer layer of skin).

Squamous cell carcinoma is a cancer which that originate in cells that are epithelial in origin and lie just below the outer surface of the skin. Squamous cells form lining for many other organs, such as the stomach, bladder, lungs, intestines and kidneys. Squamous cells look flat, like fish scales, when viewed under a microscope. Squamous cell carcinomas are sometimes known as epidermoid carcinomas.

Transitional cell carcinoma is a cancer which forms in a type of epithelial tissue which is called transitional epithelium, or urothelium. This tissue is made up of many layers of epithelial cells that can get bigger and smaller, is found in the linings of the bladder, ureters, and part of the kidneys (renal pelvis), and other organs. Some cancers of the bladder, kidneys and ureters are transitional cell carcinomas.

Sarcoma:

Sarcoma is a cancer which is formed in bone and soft tissues, including muscle, blood vessels, fat, lymph vessels, and fibrous tissue.

Osteosarcoma is the most common type of cancer. It is the cancer of bone. The most common types of soft tissue sarcoma are Kaposi sarcoma, malignant fibrous histiocytoma, leiomyosarcoma, liposarcoma and dermatofibrosarcoma protuberans.

Leukemia:

Cancers which are begun in the blood-forming tissue of the bone marrow are known as leukemias. Solid tumors are not formed in this cancer type. Instead, large numbers of abnormal white blood cells (leukemia cells and leukemic blast cells) build up in the blood and bone marrow, crowding out normal blood cells. The low level of normal blood cells lead to lower oxygen level in the body and problems arises in controlling bleeding and fight.

Lymphoma:

Lymphoma begins in lymphocytes. These are disease-fighting white blood cells which are part of the immune system. In lymphoma, mutated lymphocytes accumulate in lymph nodes and lymph vessels, as well as in other organs.

Multiple Myeloma:

Multiple myeloma is a cancer that starts from the plasma cells; these are kind of immune cells. The abnormal plasma cells, known as myeloma cells, build up in the bone marrow and form tumor like masses in bones. Multiple myeloma is also known as plasma cell myeloma and Kahler disease.

CAUSES OF CANCER- Cancer is a tangled group of diseases with as many possible causes. These are lifestyle factors such as diet, and physical activity; tobacco use; Few types of infections; epigenetic factors and exposure of body to different types of chemicals, carcinogens and radiation.

Chemical carcinogens

It may be defined as something (chemical, radiation, etc) which can harm or damage a cell and make it to turn into a cancerous cell. The general rule says, the more the exposure to a carcinogen, the greater the risk. Some of the Examples are:

- Tobacco smoking leads to cancer of the lung, mouth, oesophagus, throat, pancreas and bladder. A prevelant notion is that smoking causes about 1 in 5 of common cancers. About 1 in 8 death occurs because of tobbaco smoking induced lung cancer. The more you smoke, the greater you become prone to Cancer.
- Chemicals in the laboratories such as asbestos, mercury, benzene, formaldehyde, etc. If you have worked with these without protective measures you have an increased risk of developing certain cancers.

Age

The older you become, the chances are that you will develop a cancer. This is probably due to the persistence of damage to cells over time. Also, the body's defences and resistance against abnormal cells may become less good as we grow old. For example, the capability to repair damaged cells, and the immune system which destroys abnormal cells, may becomes negligent with age. So, eventually one damaged cell may manage to survive and multiply out of control into a cancer. Most cancers develop in aged people. [64]

Lifestyle factors

We are what we eat. Diet and other lifestyle factors can increase or decrease the risk of developing cancer. For example:

- If we eat fruits and vegetables you have a reduced risk of developing these cancers. The specefic way in which they protect us against cancer is not fully derived. These foods are rich in vitamins and minerals, and also contain chemicals called antioxidants. They may protect against carcinogens that get into the body.

- Eating too much fatty food possibly increases the risk of developing certain cancers.
- The risk of developing certain cancers is increased by obesity, lack of regular exercise, drinking too much alcohol and eating a lot of red meat. [65]

Some examples of studies looking at lifestyle factors and cancer:

A large review of data (Parkin et al) found that about 4 in 10 cancers diagnosed in the UK each year - over 130,000 in total - are caused by avoidable lifestyle factors and choices including smoking, alcohol, weight and diet. Quote from lead author Professor Parkin ... "Many people believe cancer is down to fate or 'in the genes' and that it is the luck of the draw whether they get it. Looking at all the evidence, it's true that around 55% of all cancers are caused by things we mostly have the power to change." [66]

One more research study (Kirkegaard et al) followed up over 56,000 people for 10 years. It looked at lifestyle factors and rates of cancer. The study concluded that by following those suggestions on keeping physically active, keeping body weight in check, not smoking, drinking alcohol in moderation and having a healthy diet, the risk of developing bowel cancer could be reduced by as much as 24%. But, the study found that even improving in some of these lifestyle factors had some reduction in risk. [66]

A study which followed up 363,978 people found that about 15 in 100 of all cancers in men and about 5 in 100 in women in Europe are caused by consuming alcohol. Most cancer cases were observed with people who consumed higher than the recommended toxic limits. But the survey also found that even drinking more than three units a day for men and more than one unit a day for women increased the risk of developing certain cancers.[66]

A study that followed up over 10,00,000 people in the USA suggests that cutting the amount of red meat in most people's diets to 42 g per day (equal to about one large steak a week) could significantly reduce the incidence of certain cancers. [66]

Radiation

Radiation is a carcinogen. if we are exposed to radioactive materials and nuclear rays, this can increase the risk of myeloma and other cancers. Too much sun exposure and sunburn /UV rays increase your risk of developing skin cancer. The more the intensity of radiation, the greater the risk of developing cancer. But the risk from small doses, such as from a single X-ray test, is very small. [68]

Infection

Some germs (viruses and bacteria) are linked to certain cancers. For example, people with persistent infection with the hepatitis B virus or the hepatitis C virus have an increased risk of developing cancer of the liver. Another instance is the relation between the human papilloma virus (HPV) and cervical cancer. Most women who develop cervical cancer have been infected with a strain of HPV at some point in their life. Another link is between the H.pylori, a germ (bacterium) to stomach cancer.

One research study estimated that about one in five cancers - 2.5 million a year globally - are caused by treatable or curable infections. They estimated that four infections - HPV, H. pylori, and hepatitis B and C viruses - accounted for 2 million cases of cervical, stomach and liver cancers in 2007-8. Most of these were in the developing countries. Initiatives such as immunization against HPV and hepatitis B are helping to combat these infections. But, most viruses and viral infections are not linked to cancer. [66]

Immune system

People with a poor immune system have an increased risk of developing certain cancers. For example people on immunosuppressive therapy. [67]

Genetic make-up

Some cancers have a strong genetic link. For example, in certain childhood cancers the abnormal gene or genes which may trigger a cell to become abnormal and cancerous are inherited. Other types of cancer may have some genetic factor which is less clear-cut. It may be that in some people their genetic make-up means that they are less resistant to the effect of carcinogens or other factors such as diet.

Most cancers are probably due to a multitude of factors. Not everyone who has contact with a potential cancer-causing substance (carcinogen) or has an unhealthy lifestyle will develop cancer. For example, not all smokers develop cancer of the lung. In fact, we are all probably exposed to low doses of carcinogens a lot of the time.

The body has certain mechanisms which may protect us from developing cancer. For example, it is thought that many cells which are damaged by carcinogens can repair themselves. Also, the body's immune system may be able to destroy some types of abnormal cells before they multiply into a tumor. Perhaps one carcinogen may only damage one gene, and two or more genes may need to be damaged or altered to trigger the cells to multiply out of control. In many cases it is likely that there is a combination of factors (such as genetic makeup, exposure to a carcinogen, age, diet, the state of your immune system, etc) which turns oncogenes to active state. These may play an active part in initiating the signal to a cell to become abnormal, and allowing it to multiply uncontrollably into a cancer. [American society of cancer]

There are more than 100 types of cancer, including, skin cancer, lung cancer, colon cancer, prostate cancer, breast cancer and lymphoma. Symptoms vary depending on the type. Oral chemotherapy is usually taken in the form of a tablet or capsule. Now these pills have many side effects which generally are categorized as adverse effects. These ill effects include

Fatigue, pain, (Headache, Muscle pain, stomach pain, pain from damage of nerve), burning sensation, hairfall, mouth and throat sores, mucositis. Diarrohea, constipation, irregular bowel syndrome an some nervous system effects are common side effects of cancer drugs. These drugs also cause trouble thinking clearly and concentrating after chemotherapy. This is often referred

as chemo brain by cancer survivors. Appetite loss is also observed in almost all the patients of cancer chemotherapy.

DRUGS FOR CANCER- Serendipity led to the use of nitrogen mustard to kill unwanted cells and began the era for development of cytotoxic drugs to kill cancer cells. Depending on the type, location and severity of cancer, chemotherapy for a specefic individual after translational research can be modified. Chemotherapy includes vast number of drugs and there use depends on the type of cancer and best suited mechanism to stop reproduction of cell. To further define different mechanisms of drug, let us look at some examples.

Methotrexate and vinca alkaloids are called anti metabolites. They kill dividing cells only during a specific part or parts of the cell cycle which is known as S phase and M phase. These drug inhibit the spindle formation and alignment of chromosomes. [69,71]

Alkylating agents and platinum derivatives are toxic to both tumor and normal cells whether they are in the proliferating or resting phase. They have a linear dose–response curve; that is, the greater the dose of the drug, the greater the fractional cell kill. And thus this is leads to death of normal cells as well. [70]

Oxaliplatin belongs to a new class of platinum agent. It contains a platinum atom complexed with oxalate. It forms reactive platinum complexes that are believed to inhibit DNA synthesis by forming interstrand and intrastrand cross-linking of DNA molecules [72]

Histone deacetylase (HDAC) inhibitors- These are a relatively new class of anti-cancer agents that play potential roles in epigenetic or non-epigenetic regulation, inducing death, apoptosis, and cell cycle arrest in cancer cells. [63]

Pyrimidine analogues- These drugs resemble pyrimidine molecules and work by either inhibiting the synthesis of nucleic acids (e.g. fluorouracil, inhibiting enzymes involved in DNA synthesis

(e.g. cytarabine, which inhibits DNA polymerase) or by becoming incorporated into DNA (e.g. gemcitabine), interfering with DNA synthesis and resulting in cell death.

1.2. 5-Fluorouracil

Instigated as a rationally synthesised anticancer agent 40 years ago, continues to be used in the prophylaxis of various common malignancies including cancer of the rectum, colon, skin and breast. This drug is an analogue of uracil; pyrimidine ring analogue, and is metabolised by the same metabolic pathways as that of uracil. Although several potential sites of antitumour activity have been discovered. The picture still remains blurred as, the meticulous mechanism of action and the extent to which each of these sites contributes to tumour or host cell toxicity is very unclear.

Various assay methods are available to quantify 5-fluorouracil in serum, plasma and other biological fluids. Unfortunately, there is no substantial proof that plasma drug concentrations can predict antitumour effect or host cell toxicity. The recent establishment of clinically useful pharmacodynamic assays provides a far better alternative to plasma drug concentrations, since these assays allow the detection of active metabolites of 5-fluorouracil in biopsied tumour or normal tissue.

5-Fluorouracil has a poor absorption after oral administration, with unpredictable bioavailability. The parenteral preparation is the major dosage form, used intravenously (bolus or continuous infusion). A liitle while back, studies have demonstrated the pharmacokinetic rationale and clinical feasibility of hepatic arterial infusion and intraperitoneal administration of 5-fluorouracil. In addition, 5-fluorouracil continues to be used in topical preparations for the treatment of malignant skin cancers.

Following parenteral administration of 5-fluorouracil, there is speedy distribution of the drug and rapid elimination with an apparent half-life of 8 to 20 minutes approx. The rapid elimination is because of the swift catabolism of 5-fluorouracil in the liver.

As in case of all drugs, precautions should be taken in administering 5-fluorouracil in various neoplastic states. In general, however, there are no set recommendations for dose adjustment in the presence of renal or hepatic dysfunction. Drug interactions continue to be described with other antineoplastic drugs, as well as with other classes of agents.[74]

As we can clearly conclude from the illustrated examples, that most of the anticancer drugs are harmful for normal cells of the body. As a preventive measure to this side effect, we can reduce the dose of the drug, but that will lead to inadequate pharmacological effect because drug won't reach to the desired concentration. There should be a solution that though lesser amount of drug is ingested, it exerts equal therapeutic effect. and this is possible by making the drug reach specefic site where cancer cells are present. Imagine a situation where there are minimal side effects, now we can think of what are the ways we can minimize these side effects. These are directly proportional to amount of drug being ingested in our body. If we can treat a particular disease at a low dose then it is beneficial in both the ways, i.e. it reduces the side effects, lowers the incidence of drug interaction and thereby reducing the cost of the medicine. Unfortunately due to lack of specification and solubility of drug molecules, patients have to take high doses of the drug to achieve the expected therapeutic effects for the prophylaxis of diseases. The problems in conventional dosage form are not limited to as stated above, we do observe repeated ingestion of drugs which is not patient friendly. To solve/ overcome these problems, We need a novel drug delivery system.

1.3 Novel Drug Delivery System

The process by which a drug is delivered can have a significant effect on its therapeutic effect. Some drugs have an optimum therapeutic window within which maximum benefit is derived, and concentrations above or below this range can induce adverse effects or produce no benefits at all. Furthermore, the very slow progress in the prophylaxis of the treatment of chronic diseases, has suggested a growing need for a multidisciplinary approach to the delivery of

therapeutics to specefic sites. And this led to innovative ideas on regulating the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and pharmacology of drugs were generated. These new strategies, were called drug delivery systems (DDS) and are based on multidisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology [75]. There are various drug carriers and formulations present in the pharmaceuticals, which are used for the delivery of therapeutic agents to the targeted site, at the same time maintaining the adjunct dose levels by behaving as controlled release and sustained release systems etc. [1]. One such emerging technique is the use of nanoparticles to deliver drugs at target site. These nanoparticles are used as widely in the field of diagnosis, target drug delivery, bio-sensing, cellular uptake, etc., in the bio-medical field. There has been a lot of research on the delivery of drugs using different carriers like nanocapsules, nanoparticles and microsphere. These nanoparticles were evaluated to deliver the drugs and maintain the concentration in a controlled manner. Controlled drug delivery systems (DDS) have several advantages compared to the conventional forms of drugs.[] A drug reaches only to the place of action, unlike conventional forms where it is distributed in whole body, hence its influence on vital tissues and undesirable side effects can be minimized. Stockpiling of therapeutic agents in the target site increases as a result of which, the required doses of drugs are lower. This modern form of therapy is especially important when there is a confusion between the dose or the concentration of a drug and its therapeutic results or toxic effects. Cell-specific delivery can be achieved by attaching/incorporating drugs in specially designed carriers. Various nanostructures, including liposomes, polymers, dendrimers, complex structures of silicon and carbon, and magnetic nanoparticles, have been tested as carriers in drug delivery systems.[2]

For over 30 years, scientists have appreciated the prospective benefits of nanomedicine and technology in providing huge improvements in drug delivery and drug targeting. Improving delivery techniques that has negligible toxicity and improved therapeutic efficacy offers great

potential advantages to patients, and opens up opportunities for new markets in pharmaceutical industry. Other approaches to drug delivery are focused on crossing blood brain barrier; a physical barrier to most of the foreign agents in the body, in order to better target the drug and improve its effectiveness; or on finding alternative and better routes for the delivery of drugs other than via the gastro-intestinal tract, which generally leads to first pass metabolism.

Chapter 2

LITERATURE REVIEW

The literature review covers all the information obtained which is relevant to my project. It covers the Types of nanoparticle Systems, methods to prepare nanoparticles, examples of system encapsulating hydrophobic and hydrophilic drugs and relevant procedures to characterize nanoparticles.

2.1 TYPES OF NANOPARTICLE SYSTEM

Nanoparticles can be made from either metals or polymers and also from other synthetic compounds. In general, nanoparticles used in the field of biotechnology ranges in between 20 and 520 nm, seldom exceeding 800 nm. The nano size of these particles facilitates all communications with biomolecules on the surface of cell and within the cells in way that results in and can be designated to various physiochemical and biochemical properties of these cells [7]. Similarly, its huge application in drug delivery system and noninvasive bioimaging has discovered various advantages over conventional pharmaceutical agents [7]

Metallic Nanoparticles-:

As the name suggests, these are made of metals. Metallic nanoparticles have captivated researchers for over a century and are now are widely utilized in biomedical sciences and engineering. They are a focus of interest because of their huge potential in nanotechnology. Today these materials can be synthesized and modified with various chemical functional groups which allow them to be conjugated with antibodies, ligands, and drugs of interest and thus

opening a wide range of potential applications in biotechnology, magnetic separation, and preconcentration of target analytes, targeted drug delivery, and vehicles for gene and drug delivery and more importantly diagnostic imaging.

Polymeric Nanoparticles-:

Polymer nanoparticles have attracted the interest of many research groups and have been utilized in an increasing number of fields during the last decades. Generally, two main strategies are employed for their preparation: the dispersion of preformed polymers and the polymerization of monomers. [4] Many polymers now have been discovered which are biodegradable, and hence the gradual degradation results in the controlled release delivery of drugs, also the toxicity associated with the external material is minimised as compared to Metallic nanoparticles which are generally toxic [5]

The below stated picture represents the toxicity of nanoparticles and also illustrates the possible mechanism of toxicities associated with these metallic nanoparticles.

This is the reason polymeric nanoparticles have gained much of attention in drug delivery. Also another property to be stated is that of biocompatibility of polymers like PLGA.

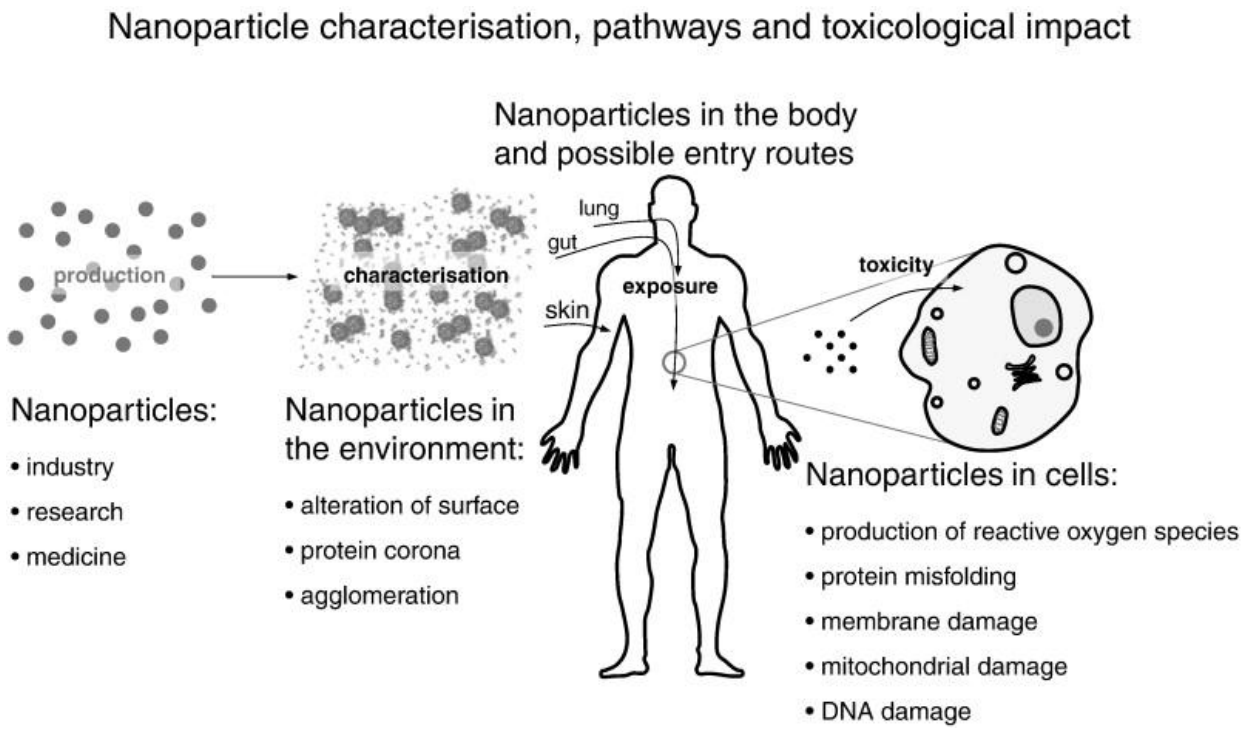

Figure 2.1.1: Nanoparticles: Overview

PLGA or poly(lactic-co-glycolic acid) is a copolymer which is used in a host of Food and Drug Administration (FDA) approved therapeutic devices, owing to its biodegradability and biocompatibility. (Wiki). Poly(lactic-co-glycolic acid) (PLGA) is one of the most successfully developed biodegradable polymers. Among the different polymers developed to formulate polymeric nanoparticles, PLGA has attracted considerable attention due to its attractive properties:

- Biodegradability and biocompatibility.
- FDA and European Medicine Agency approval in drug delivery systems for parenteral administration,
- Well described formulations and methods of production adapted to various types of drugs e.g. hydrophilic or hydrophobic small molecules or macromolecules,
- Protection of drug from degradation,

- Possibility of sustained release,
- Possibility to modify surface properties to provide stealthness and/or better interaction with biological materials.
- Possibility to target nanoparticles to specific organs or cells.[6]

Polymers can also be used to coat other types of nanoparticles. Polyethylene glycol (PEG) is a hydrophilic polymer that has been used to coat the surface of nanoparticles, which allows them to avoid clearance by the reticuloendothelial system [8]. The degradation time can vary from several months to several years, depending on the molecular weight and copolymer ratio [9] and [10] The forms of PLGA are usually identified by the monomers ratio used. For example, PLGA 50:50 identifies a copolymer whose composition is 50% lactic acid and 50% glycolic acid. Poly(lactic acid) (PLA) has also been used to a lesser extent than PLGA due to the lower degradation rate.

METABOLISM OF PLGA

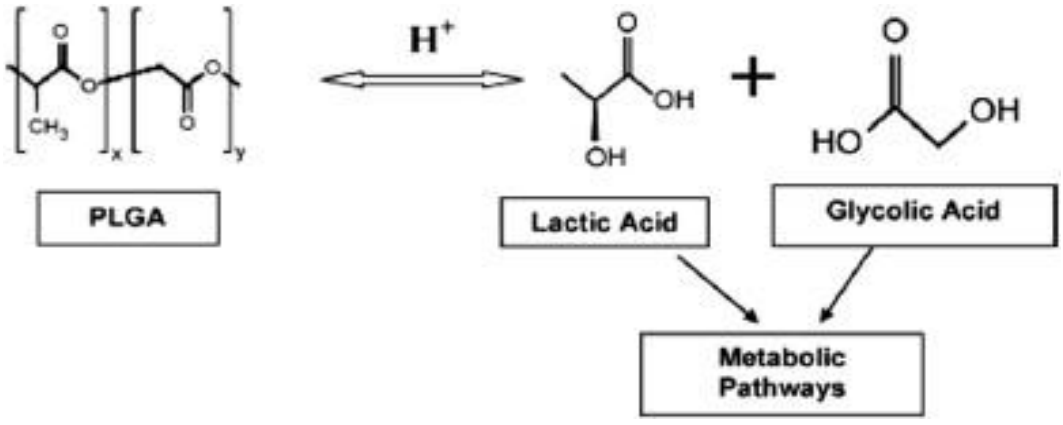

Figure 2.1.2: Metabolism of PLGA

2.2 METHODS TO PREPARE NANOPARTICLES

There are several methods to prepare nanoparticles. Depending on the method of preparation, the structural organization may differ.

The drug is either entrapped inside the core of a "nanocapsule" as well as entrapped in or adsorbed on the surface of a matrix "nanosphere".

The most common technique used for the preparation of PLGA nanoparticles is the emulsification-solvent evaporation technique. This technique allows the encapsulation of hydrophobic drugs and consists in dissolving the polymer and the compound in an organic solvent (e.g., dichloromethane). The emulsion oil (O) in water (W) i.e. O/W is prepared by adding water and a surfactant (e.g., polysorbate-80, poloxamer-188) to the polymer solution. The nanosized droplets are induced by sonication or homogenization. The solvent is then evaporated or extracted and the nanoparticles collected after centrifugation [1] and [16].

A modification of this technique, the double emulsion W/O/W, was used to encapsulate hydrophilic drugs, such as peptides, proteins and nucleic acids. Nanoparticles can also be prepared by the nanoprecipitation method which is also known as the interfacial deposition method [17]. Briefly, the drug and the polymer are dissolved in an organic solvent (such as acetone) and added drop wise to water. The organic solvent is allowed to evaporate and the pellets formed are collected after centrifugation. Other techniques exist such as the spray-drying method [1]. Drug loading into nanoparticles is achieved by two methods: (i) the incorporation of the drug during the nanoparticles production and (ii) the adsorption of the drug on nanoparticles after their production.

Solvent evaporation was the first method developed to prepare PNPs from a preformed polymer [25]. Although originally proposed by polymer chemists, These proccess are followed in pharmaceutical technology where biodegradable polymers have been applied in the production of drugs [26]. In this method, polymer solutions are prepared in volatile solvents and emulsions are formulated. In the past, Organic solvents like dichloromethane and chloroform were widely used,

but are now replaced with ethyl acetate which is less toxic. The emulsion thus formed is converted into a nanoparticle suspension after the evaporation of solvent, and this solvent actually diffuses through the continuous phase of the emulsion [27] and [28].

Nanoprecipitation-- The nanoprecipitation method was developed by Fessi et al. [50] for the preparation of PNP. It is also called as solvent displacement method. The basic principle of this technique is based on the interfacial deposition of a polymer after displacement of a semipolar solvent, miscible with water, from a lipophilic solution. Rapid diffusion of the solvent into non-solvent phase results in the decrease of interfacial tension between the two phases, which increases the surface area and leads to the formation of small droplets of organic solvent [40] and [41]. Nanoprecipitation system consists of three basic components: the polymer (synthetic, semi synthetic or natural), the polymer solvent and the non-solvent of the polymer. Organic solvent (i.e., ethanol, acetone, hexane, methylene chloride or dioxane) which is miscible in water and easy to remove by evaporation is chosen as polymer solvent. Due to this reason, acetone is the most frequently employed polymer solvent in this method [50], [42] and [43].

Sometimes, it consists of binary solvent blends, acetone with small amount of water [44], blends of acetone with ethanol [45], [46] and [47] and methanol [48]. On the other hand, the non-solvent phase consisting of a non-solvent or a mixture of non-solvents is supplemented with one or more naturally occurring or synthetic surfactants

2.3 EXAMPLES OF SYSTEM ENCAPSULATING HYDROPHOBIC DRUGS

PLGA nanoparticles loaded with hydrophobic, poorly soluble drugs are most commonly formulated by nanoprecipitation. Drug release and effective response of PLGA nanoparticles are influenced by

(i) the surface modification, (ii) the method of preparation,

(iii) the particle size,

(iv) the molecular weight of the encapsulated drug and

(v) the ratio of lactide to glycolide moieties [3].

PLGA nanoparticles are described in the literature as effective nanocarriers for the encapsulation of various anti-cancer agents such as paclitaxel [19], 9-nitrocamptothecin [20], cisplatin [21], etc. but also for the encapsulation of various drugs such as haloperidol [22], estradiol [23], etc. Other systems include the microspheres and Nanodots.

2.4 EXAMPLES OF SYSTEM ENCAPSULATING HYDROPHILIC DRUGS

1. Valproic acid- Hydrogel nanoparticles [14]

2. Isoniazid- Inhalable chitosan nanoparticles [30]

3. Allopurinol- polycyanoacrylate nanoparticles [31]

4. lamivudine- Formulation and evaluation of ethosomes for transdermal delivery

2.5 CHALLENGES TO THE ENCAPSULATION AND DELIVERY OF HYDROPHILIC DRUGS

The results of the encapsulation efficiency analysis demonstrated that more lipophilic drugs, such as cyclosporin and indomethacin, do not suffer from the problems of drug leakage to the external medium, resulting in improved drug content in the nanoparticles.

In spite of the fact that valproic acid is a liquid that is very sparingly soluble in water, very low encapsulation efficiency was obtained. Ketoprofen, a drug sparingly soluble in water, demonstrated intermediate values of encapsulation that were well correlated with its

intermediate lipophilicity. More hydrophilic drugs, such as vancomycin and phenobarbital, were poorly encapsulated in PLGA nanoparticles. [11] Hydrophilic drug encapsulation/delivery is complex when the procedure called double emulsion technique is followed, the reason to the complexity of latter can be in short explained as the drug inside the polymer suspension has high affinity to external phase solvent, i.e. is the aqueous phase and as a result the encapsulation efficiency is less. The major problems are

1. Low encapsulation efficiency

2. Burst release

3. Loading capacity

4. Drug loss due to inefficient solvent system

2.6 INITIATIVES TAKEN TO SOLVE SUCH PROBLEMS

Hydrophilic drug delivery still remains a challenge; this either being attributed to the fragility and poor cellular penetration of macromolecules, or to the unsuitable pharmacokinetics and toxicity of small drugs, for instance anticancer agents. By offering more favorable pharmacokinetics and protection of the drug, encapsulation in polymer nanoparticles constitutes an attractive possibility to overcome these problems. [12] Refer table 2.6.1

Table 2.6.1 Strategies to overcome problems in Hydrophilic drug delivery

DRUG	PREPARATION METHOD	STRATEGY	RESULT	REFERENCE
5-FU	Monomer emulsion polymerisation	pH increase	Enhancement of the polymerisation rate	[32]
Vasopressin PLA	Emulsion-solvent evaporation	Reverse micelles	Enhancement of drug solubility in the polymer network	[33]
doxorubicin hydrochloride (CHITOSAN)	Chitosan ionic gelation	Polyanion (dextran sulphate)	2-fold increase of EE	[34]
Gemcitabine	Redox radical emulsion polymerisation	Chitosan/Ion pair formation	Ionic interaction between SiRNA and chitosan and increase of nanoparticle loading	[36]

Aqueous-core nanocapsules-:

Nanocapsules with an aqueous core are a result of relatively recent technology developed for encapsulating hydrophilic molecules. Aqueous-core nanocapsules (ACN) consist of nanoparticles exhibiting a core shell structure, the core being composed of liquid water, generally surrounded by a thin polymer shell. The advantages of such a structure for encapsulating hydrophilic

molecules lie firstly in the high drug encapsulation efficiency due to the optimised drug solubility in the nanoparticle core and low polymer content compared to polymer nanospheres. Secondly, for entrapment in polymer nanoparticles; since the drug is 'protected' within the nanocapsules, tissue irritation at the administration site as well as the burst effect are reduced, and the drug remains protected against degradation [38]

2.7. SUGAR GLASS NANOPARTICLES

This was one of the best initiated research for the delivery of Hydrophilic molecules, either protein or drugs; and had a good encapsulation efficiency along with a controlled release profile, better storage possibilities of the formulation i.e., the storage activity loss was less. The author here tried it for the delivery of protiens and also followed the procedure of double emulsion, and achieved a very high encapsulation efficiency for protiens.

RESULTS IN SGNP TECHNIQUE

The post encapsulation recovery of proteins activity after incubation into polycaprolactone (PCL) + chloroform solution compared to a common approach where HRP was added from buffer was very high. Results for the protective performance of the SGnPs with respect to process related stresses shows that the SGnP system provide excellent protection from array of non-aqueous solvents, showing excellent sustained release profiles (30% during 30 days) without significant burst release (only 8 to 11 % release on day 1) from nanofiber or gas-foamed scaffolds made of PCL and induced bone formation within 30 days compared to scaffold with BMP2 encapsulated by conventional technique. This system also offers excellent encapsulation efficiency ($\approx$ 100%) of the protein incorporated into the polymer scaffold, with only minor activity loss ($<$ 5%) at the encapsulation step [35]

Reviewing the literature we can conclude that there are 2 specific problems to be solved with respect to hydrophilic drug delivery and thus we aim at a developing a system with minimal burst release and higher encapsulation efficiency, and this can be summarized in 3 objectives given below

1. Optimization of process parameters and selection of the most preferred technique.

2. Development of sugar glass nanoparticles and encapsulation of latter into the polymer so as to achieve higher encapsulation and lower burst release.

3. Physical and biological characterization/evaluation of the system.

2.8 Encapsulation of Hydrophilic drugs-

It is not possible with single emulsion solvent evaporation method. So a modified technique called double emulsion is to be followed; A coin has 2 sides so do all techniques have pros and cons. The main aim is to achieve higher encapsulation efficiency and a controlled release profile. Talking about the latter, the release is attributed to the rate of degradation of Plga polymer. Even if polymer release is achieved, there are still chances of burst release in the formulation.

Now for the encapsulation; the drug incorporated should not release from the polymer system into the external solvent and the possibility for such release is very high if we use a aqueous solvent; but using a less aqueous solvent can solve this problem; another option left is to use some different techniques to make these Nanoparticles for example if we follow a method called Oil in Oil emulsion technique which excludes the use of water. However this technique has not been tested on plga.

Another method of interest includes the non aqueous encapsulation of drug substance, illustrated in the paper; Non-aqueous encapsulation of excipient-stabilized spray-freeze dried BSA into poly(lactide-co-glycolide) microspheres results in release of native protein. [76]

Modified nanoprecipitation method-

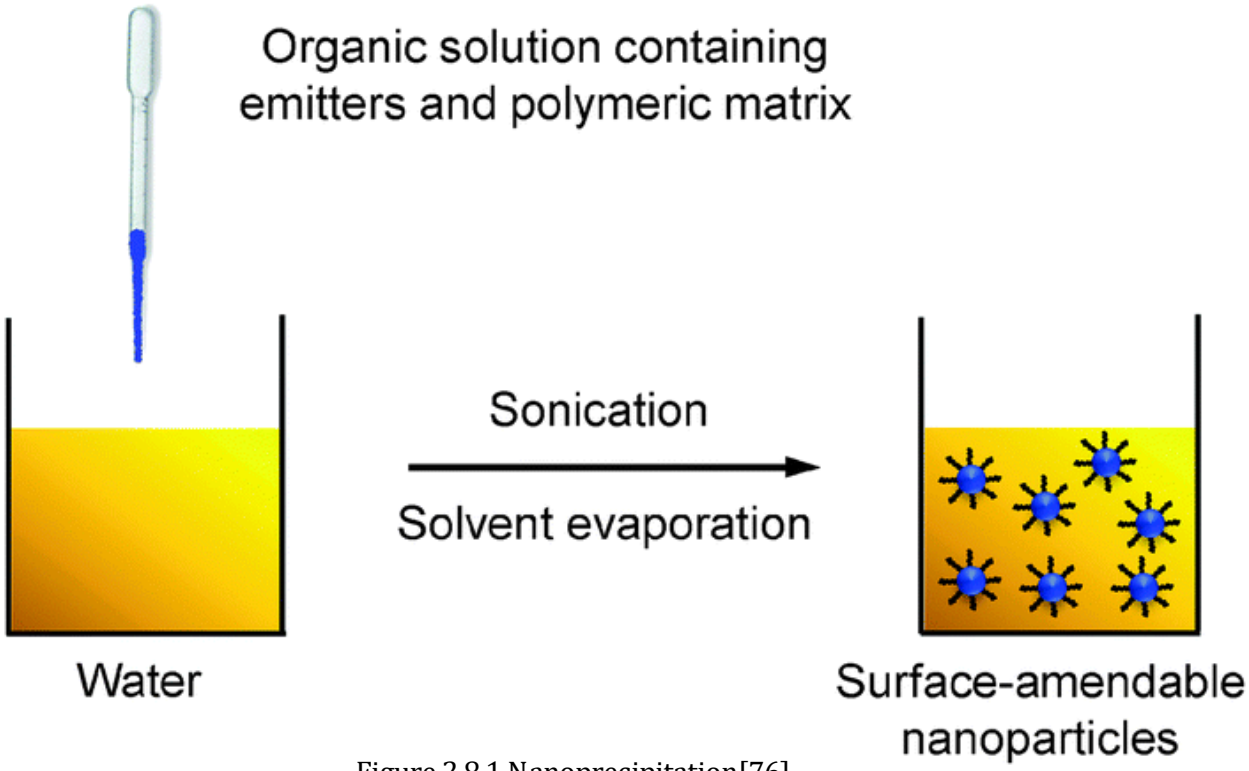

Figure 2.8.1 Nanoprecipitation[76]

In summary, we aim to create a robust and biocompatible nanoparticle platform that can selectively deliver a chemotherapeutic agent to the tumor site. Nanoparticle drug delivery is advantageous when compared to direct delivery of the drug in that it can prolong the timeframe of the effectiveness of the drug and can be directed to the tumor site via the EPR effect and selective targeting. PLGA nanoparticles are advantageous in that they are biocompatible and nontoxic.

Chapter 3

MATERIALS AND METHOD

3.1 MATERIALS

PLGA (50:50), DCM, 5 FU (hychem), PVA; high molecular wieght, Trehalose dihydrate, metformin (gift sample), liquid nitrogen (bought from Sangareddy), (Isoctane, AOT, tween 20, span 80, paraffin oil, n hexane),General solvents were of analytical grade and were procured from Hychem laboratories

3.2 EXPERIMENTATION

This project has been carried out in steps for which the methods are described.

1. Optimization of process parameters for water in oil in water emulsion method.

2. Preparation of polymer nano particles of 5 FU (different loading), by O/O and W/O method.

3. Characterization of nano particles for the particle size, EE and release, surface morphology.

4. Preparation of sugar glass nano particles by flash freezing.

5. Encapsulation of prepared SGnp's into polymer and further characterization of the same as above.

3.2.1 STANDARD PLOT FOR DRUGS

The standard plot for drugs was made by using UV Spectrophotometer. The standard solution was prepared by dissolving 10 mg of drug in 100 mL pbs (ph 7.4). Further dilutions were done to make a note of readings at different concentrations. The resulting graph was extrapolated.

3.2. PREPARATION OF PLGA NANOPARTICLES

PLGA nanoparticles have been mostly prepared by double emulsion–solvent diffusion [46], solvent emulsion–evaporation [36], interfacial deposition [42] and nano precipitation method [47] Generally in emulsification – diffusion method, the polymer to be used is dissolved in organic solvents (PC, BA, EtAc, MEK, etc.), poured and homogenised in aqueous phase having stabilizer and subsequently emulsified by homogenizer. In solvent evaporation method, the polymers are dissolved in volatile organic solvent (CHCl3, EtAc, acetone, DCM, etc.) and injected into aqueous phase on stirring (addition of emulsifier/stabilizer is optional) and sonicated. Interfacial deposition methods have been used for the formation of both nanocapsule and nanospheres. The nanoparticles are synthesized in the interfacial layer of water and organic solvent (water Immiscible). At last the nanoparticles are recovered by centrifugation.

BY W/O/W METHOD

The double emulsion solvent evaporation method was employed for the formation of drug-encapsulated PLGA Nanoparticles, similar to previously described. Briefly, fluorouracil (or 5-FU) was dissolved in water. Polymer was likewise dissolved in DCM and mixed with the drug solution and homogenised at 12 k for 1 minute to form a Primary emulsion. NPs were formed by adding the drug–polymer solution/ primary emulsion to PVA (Self emulsifying agent; Here acting as an external phase for the secondary emulsion) and homogenizing at 18 k for 5 mins. The resulting NP emulsion was allowed to stir uncovered for 3h at room temperature for the evaporation of organic solvent. The emulsion is converted into a nanoparticle suspension on evaporation of the solvent for the polymer, which is allowed to diffuse through the continuous phase of the emulsion. Nanoparticles were recovered by centrifugation (12 k rpm for 10 mins). The PLGA–NPs were resuspended, washed with water and collected. Further proccess includes the resuspension of formed NPs in sufficient amount of water, freezing at -800 C. The freezed

Nps were lyophilised in (Instrumrent) and then were characterised for aforementioned paramenters.

BY WATER IN OIL IN OIL EMULSION METHOD

As this is also a double emulsion method, Two emulsions were prepared. The primary emulsion was prepared by homogenizing the aqueous phase containg drug with the polymer solution (polymer dissolved in dcm). The homogenization was done for 1 min at 20 k and then this primary emulsion was injected drop wise into liquid parrafinn to prepare the final emulsion. This is further homogenised for 5 minutes to form a stable emulsion. The is now called secondary emulsion. The secondary emulsion is kept on stirring for the solvent evaporation for 3 hrs. n hexane was added at regular intervals for particle hardening. After stirring the emulsion is centrifuged at 12 k rpm for 5 minutes to recover the particles. These particles were washed with n hexane and then were resuspended in the same. The n hexane was allowed to evaporate and particles were collected.

3.3. PREPARATION OF SUGAR GLASS NANOPARTICLES

Sgnp were prepared by inverse micelles of AOT in isooctane in a 50 mL centrifuge tube. Aqueous phase of 0.4 mL to 0.6 mL containing the drug and other excipients (trehalose and tween 20) was then added. This mixture was vortexed for 3 mins to obtain a clear suspension. The pH of aqueous phase was adjusted to give maximum drug solubility and stability at room temperature. The inverse micelle suspension was flash frozen by injecting it into a vial containing liquid nitrogen. The vial with the frozen particles and iso ocatne was lyopjilised to evaporate water and iso octane. After this process the particles were recovered by centrifugation and then were washed with iso osctane. The washing was repeated for 3 times to remove the remaining AOT. Particles were resuspended in Iso octane and stored at -800 C for future use.

ENCAPSULATION OF SGNPS by BOTH THE METHODS

The prepared sgnps were analysed for the drug content and accordingly specefied amount of sgnps were mixed with the polymer solution and vortexed for 1 min to get a stable emulsion. As this method involves the mixing of solid particles in the polymer solution, this is also called as S/O/O or S/O/W depending upon the final phase of the double emulsion. The procedure followed is same as previously described.

3.4. CHARACTERIZATION

1. SIZE ANALYSES-

i) BY MICROSCOPY- Slide was prepared by suspending particles in water and then was viewed under microscope. The morphology was observed and needful calculations were done to measure the size of particles.

ii) BY DLS- The mean particle size, size distribution and polydispersity index were determined by dynamic light scattering (BIC 90 plus, Brookhaven Instruments Corp.). The analyses were performed at a scattering angle of 90° and a temperature of 25 °C. For each sample, the mean particle diameter, polydispersity and standard deviation for ten determinations were calculated.

The sample was prepared by suspending 5 mg of polymer particles in water and sonicating at for 1 min.

2. ENCAPSULATION EFFICIENCY-

3 replicates of the prepared sample were prepared by dissolving 5 mg of polymer particles in 1 mL of DCM and then 3-4 mL of pbs was added. These replicates were incubated for 24 hrs in the incubator shaker at 37^0 C and then the supernatant was withdrawn. The concentration of drug was measured in the supernatant by measuring the absorbance in UV Spectrophotometer

at 269 nm for fluorouracil and 234 nm for metformin. This absorbance gave the corresponding concentration and the total amount was calculated.

Encapsulation efficiency of the 5-FU microsphere delivery system. The encapsulation efficiency (EE) was calculated using the formula as below:

$$\text{drug in}\frac{\text{Nps}}{\text{total}} \times 100\% = \text{EE}$$

3. RELEASE PROFILE

In vitro release- The release tests were performed at 37 ° C. Microspheres (5 mg) weighed were re-suspended in 10 ml PBS (pH 7.4) release medium. Then the samples were incubated in a simulating biological fluid incubator at 37 ° C under a shaking rate of 150±10 rpm. The release medium was taken out, and then the supernatant of the samples was drawn (around 1 ml) for each day, and another 1 ml of fresh release medium was then placed. The concentration of the 5-FU and Metformin was measured using UV Spectrophotometer. Average and standard deviation of the data were obtained from three replicates of the same sample.

4. SHAPE AND MORPHOLOGY

Analysis for shape and morphology of all samples of nanoparticles was done with SEM (Scanning Electron Microscopy).

Chapter 4

RESULTS AND DISCUSSION

4.1. Development of Standard plots

EXPERIMENTAL CONDITIONS- According to the solubility characteristics of drugs, phosphate buffer pH 7.4 was selected as solvent for analysis. From the UV scanning of both the drugs, 234nm and 269nm were the wavelengths selected for estimation of metformin and 5 Fluorouracil respectively.

PREPARATION OF STOCK SOLUTIONS-

5 FU and Metformin 10 mg each were accurately weighed and dissolved separately in phosphate buffer pH 7.4. Final volume was adjusted to 100ml with phosphate buffer pH 7.4 to get a concentration of 100µg/ml. 1ml of each from the above prepared solutions were further separately diluted to 10ml to get a concentration of 10µg/ml of each 5 FU and Metformin. These were used as stock solutions.

WAVELENGTH SELECTION- The above prepared stock solutions of 5 FU and Metformin were scanned in the range of 200-400nm to determine the wavelength of maximum absorption for both the drugs. 5 FU showed absorption maxima at 269nm whereas Metformin showed at 234 nm.

The standard curves for both the drugs are depicted below.

OPTIMUM PARAMETERS

Table 4.1.1 optimum parameters for standard plot

PARAMETERS	5 FLUOROURACIL	METFORMIN
ʎ max (nm)	269 nm	234 nm
Min conc (ug/mL)	2	2
Max conc (ug/mL)	10	12
Equation	y = 0.0476x + 0.0238	y = 0.0798x + 0.0209
Corelation cofficient	R2 = 0.983	R2 = 0.9991

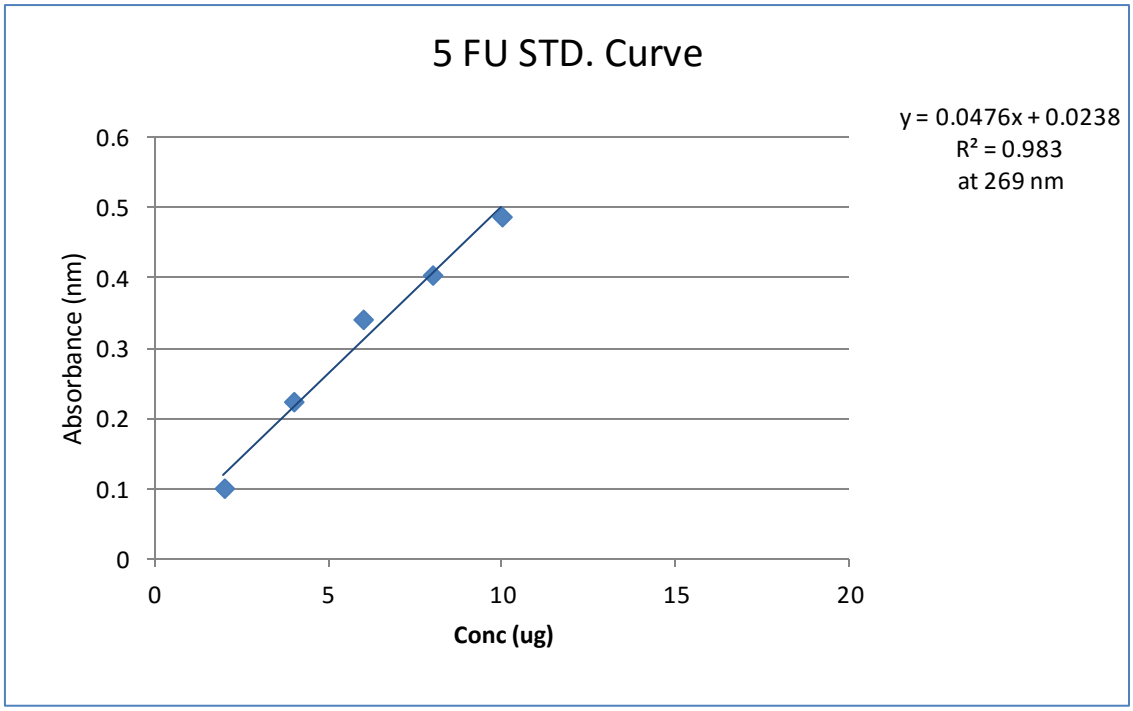

Figure 4.1.2. Standard curve for 5 FU

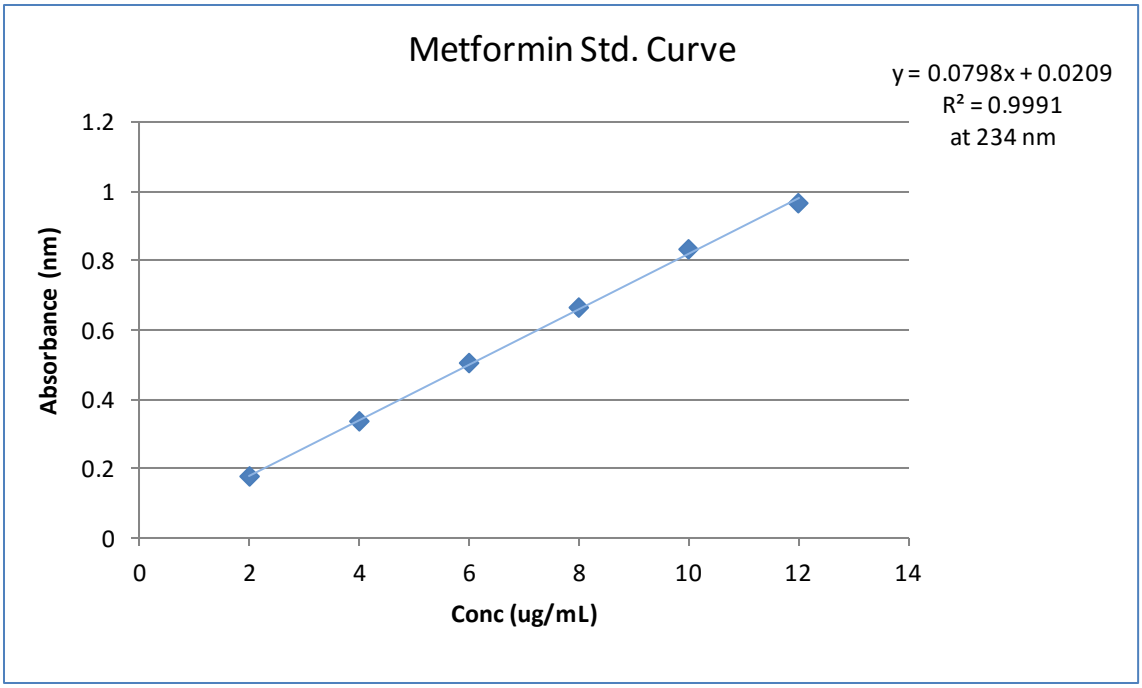

Figure 4.1.1 Standard curve for Metformin

4.2. OPTIMIZATION OF PROCESS PARAMETERS

FOR W/O/W DOUBLE EMULSION TECHNIQUE

The polymer concentration and the pva concentration along with other formulation variables were optimised by running trials with alternate parameters and it was found that the best results were derived when 2.5 % polymer concentration and 2.5 % of PVA was used. The ratio of drug is to polymer also plays a crucial role in the encapsulation of drug in the polymer particles. Size, on the other hand can be tuned by homogenizing or sonicating the emulsion at primary or secondary stage. It was observed that the speed of homogenization can affect the size of particles to a great extent. And then the final parameters were decided by which final batches of particles were prepared. Details of formulation variables are summarized in the table 4.2.1.

Table 4.2.1 Optimization of process parameters

SN	Primary Emulsion			Secondary Emulsion		Lyophilized	Avg. Particle Size (um)	Encapsulation(%)
	DRUG Con. (w/v)%	PLGA Con. (w/v)%	Homogenization Speed (RPM) & Time	PVA Con. (w/v)%	Homogenization Speed (RPM) and Time	Y/N		
1	2.5	2.5	12500, 20 Sec.	0.25	8000, 10 Sec.	N	1.0-2.5	
2	2.5	2.5	12000, 10 Sec	0.185	12000, 45 Sec	N	1.0-2.0	
3	2.5	2.5	12000, 20 Sec	0.25	12000, 5 min.	Y	1.0-2.0	
4	2.5	2.5	12000, 20 Sec	0.25	12000, 5 min.	N	1.5-2.0	
5	2.5	2.5	12000, 20 Sec.	0.25	20000, 5 Min.	N	0.5-2.0	
6	2.5	2.5	12000, 20 Sec.	0.25	30000, 5 Min.	Y	1.0-1.5	
7	2.5	2.5	12000, 20 Sec.	0.25	30000, 5 Min.	N	0.5-1.5	
8	5	2.5	Son. - 10 Sec.* 3	0.25	Son.- 10Sec.*3	N	1.0-2.0	17.71
9	10	2.5	Son. - 10 Sec.* 3	2.5	Son. - 10 Sec.* 3	N	1.0-2.0	2.97
10	2.5	2.5	20000, 1 Min.	2.5	18000, 5 min.	N	0.5-1.5	8.01
11	2.5	2.5	20000, 1 Min.	2.5	12000, 5 Min.	Y	1.0-1.5	25.88

4.3. PARTICLE SIZE ANALYSIS

BY MICROSCOPY

Particle size analysis was done by DLS and microscopy where slides of different batches were prepared by suspending the nanoparticles in water and then smearing on the slide. Respective slides were observed and the size measurement was done on Carl Ziess fluorescent microscope. fig 4.3.1 shows the size distribution of batches prepared by W/O/W and fig 4.3.0 shows the size distribution of batches prepared by W/O/O method. The size of particles lie in the range of 0.5 um to 2.5 um and average size for W/O/O was 900 nm and 765 nm for W/O/O. fig. shows the images acquired from the microscope.

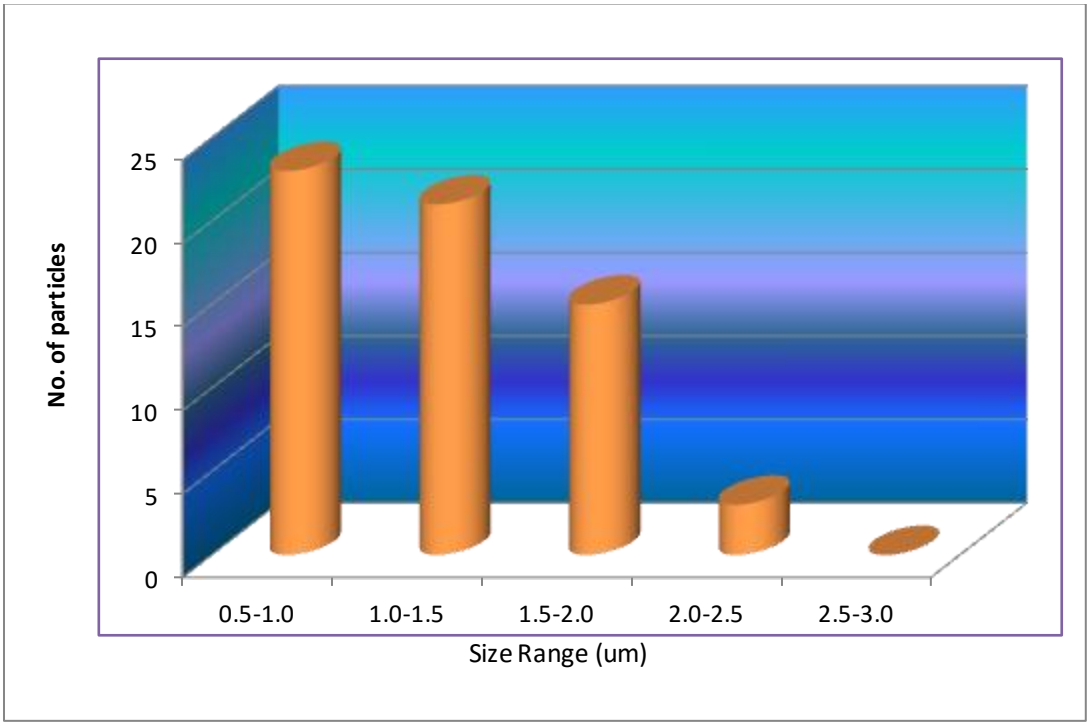

figure 4.3.0: Particle size distribution of metformin nanoparticles prepared by w/o/o

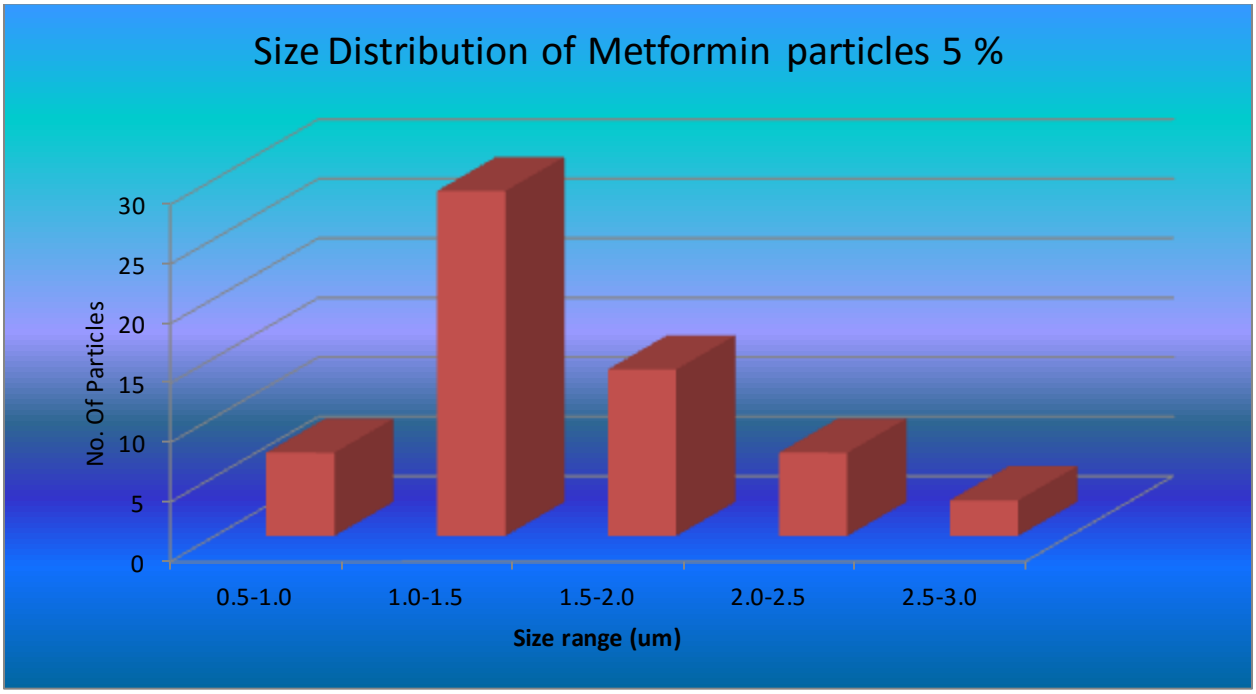

figure 4.3.1: Particle size distribution of metformin nanoparticles prepared by w/o/w

BY DLS-

The polydispersity index and average size of particles were determined by dynamic light scattering and the results are summarized in the table 4.3.1

Table 4.3.1: Data obtained from DLS

Technique	% Loading	Polydispersity Index	Average Diameter (nm)
W/0/W	1	0.337	847
W/O/O	1	0.264	634
W/O/W With SGnps	1	0.453	1858

and fig (4.3.2, 4.3.3, 4.3.4) depicts the details obtained by DLS for W/o/o, W/o/W and Sgnp respectively.

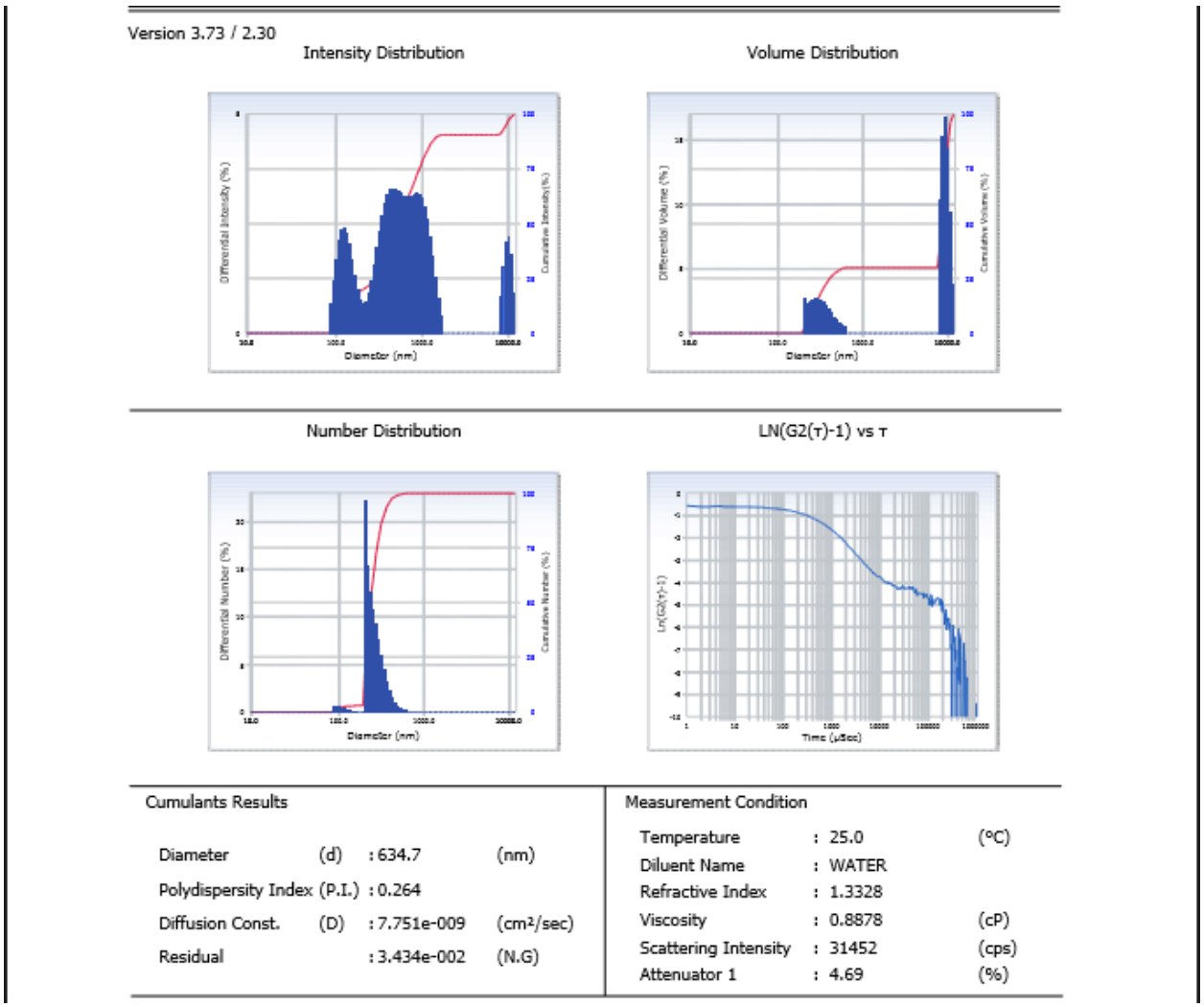

Figure 4.3.2: DLS data of W/O/O Fluorouracil nanoparticles

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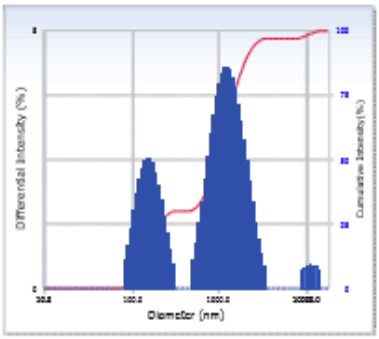
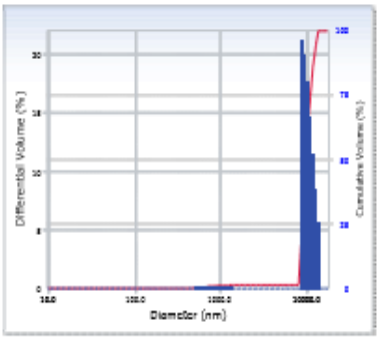
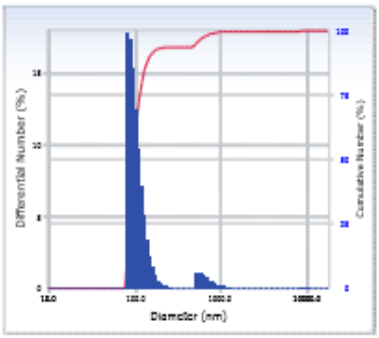
Intensity Distribution



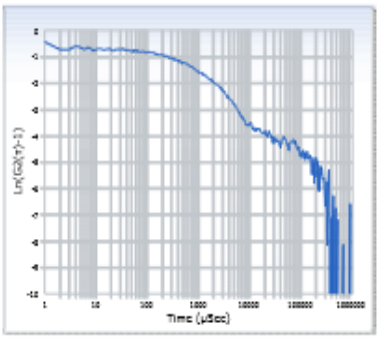
Volume Distribution



Number Distribution



LN(G2(τ)-1) vs τ



Cumulants Results

Diameter	(d)	: 847.0	(nm)
Polydispersity Index	(P.I.)	: 0.337	
Diffusion Const.	(D)	: 5.808e-009	(cm²/sec)
Residual		: 4.128e-002	(N.G)

Measurement Condition

Temperature	: 25.0	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3328	
Viscosity	: 0.8878	(cP)
Scattering Intensity	: 4620	(cps)
Attenuator 1	: 0.9	(%)

Figure 4.3.3: DLS data of W/O/W Fluorouracil nanoparticles

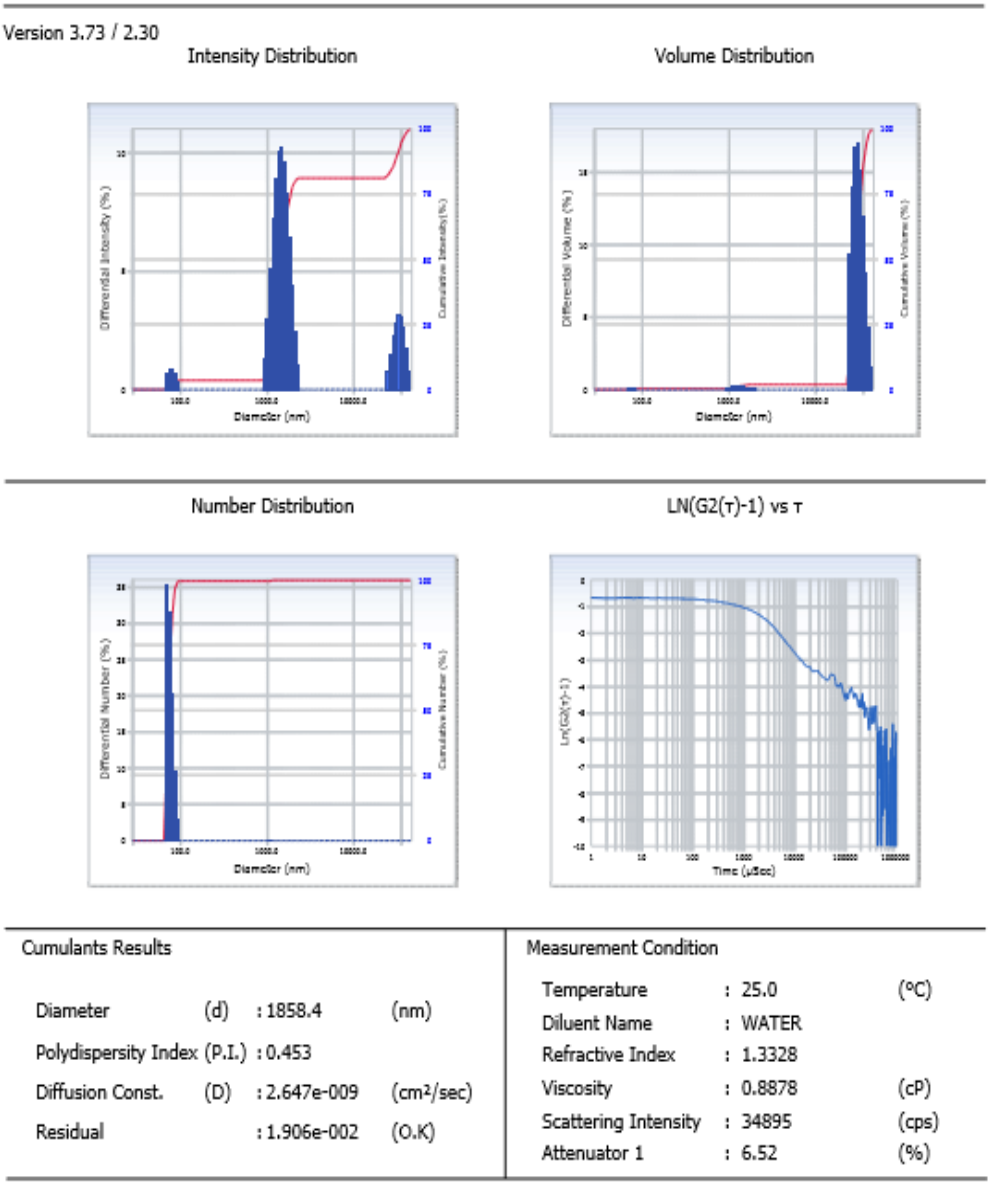


Figure 4.3.4: DLS data of W/O/W with Fluorouracil SgNp nanoparticles

The polydispersity index as we can see is 0.337 and 0.453 which indicates a optimum distribution of particles and the extent of homogenity in the size of particles.

SEM IMAGES OF MICROPARTICLES

Sem image of microparticles were acquired at different magnifications and 12.00 kv to see the porosity and morphology of particles. The particles prepared by W/o/W are not porous and are little bigger in size as sgnp's are incorporated in them. One of such image is given below.

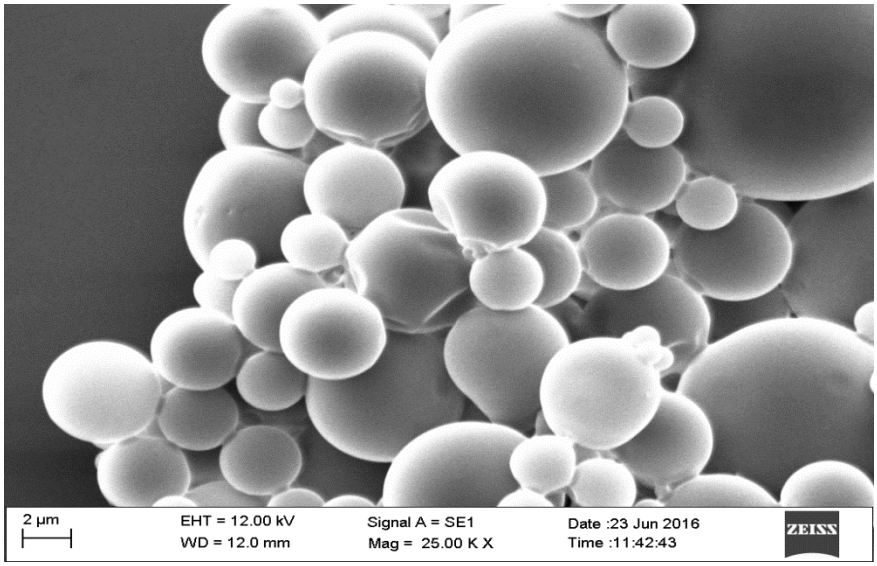


Figure 4.3.5 Sem Image of 5 FU Sgnp at 25.00 kx

4.4 COMPARISON OF ENCAPSULATION EFFICIENCY

Table 4.4.1, 4.4.2 and 4.4.3 shows the comparison between encapsulation efficiency of conventional method and method in which sgnp's are incorporated.

Table 4.4.1: Comparison of EE of 1 % loading with different loading

TECHNIQUE	% LOADING	% EE	STD DEV
W/O/W	1	4.06	1.2
SG+ W/O/W	1	64.33	3.5
W/O/O	1	88	3
SG+ W/O/O	1	60	3

As we can clearly see that sugar glass nanoparticles have higher encapsulation efficiency, no matter which technique is followed. As in 1 % loading, the EE of particles by conventional w/o/w technique is 4.06 and that of sugar glass is 64.33 %.

Table 4.4.2 ENCAPSULATION EFFICIENCY OF 5 % LOADING

TECHNIQUE	% LOADING	% EE	STD DEV
W/O/W	5	2.01	0.215
SG+ W/O/W	5	55.33	3.5
W/O/O	5	48.66	3.7
SG+ W/O/O	5	50.66	3

Table: 4.4.3: ENCAPSULATION EFFICIENCY OF 1 % LOADING of METFORMIN

TECHNIQUE	% LOADING	% EE	STD DEV
W/O/W	1	2.36	0.37
SG+ W/O/W	1	33	0.5
W/O/O	1	44.33	3.05
SG+ W/O/O	1	56	1.7

4.5 RELEASE PROFILE STUDY

Figure 4.5.1 shows the release profile of fluorouracil particles prepared by w/o/w, as we can see there is a 76 % of burst release in 1 hour which is very high. Contrary to this, the release in 1 hour for same loading with sugar glass nanoparticles of fluorouracil is only 22 %, shown in fig. 4.5.2.

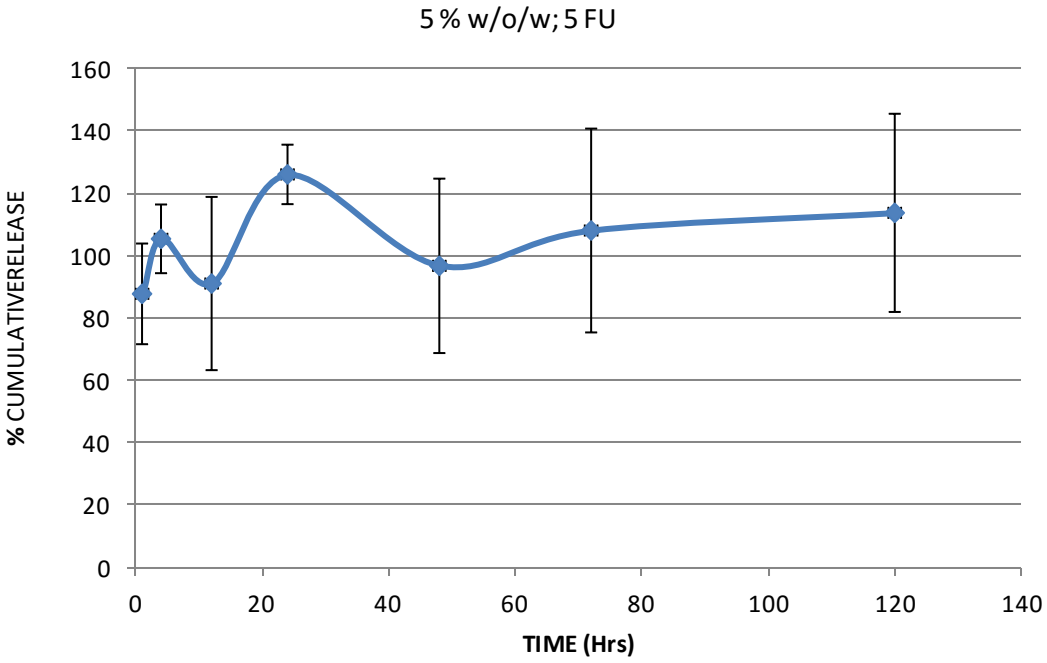


Figure 4.5.1 Release profile of 5 FU NP prepared by W/O/W

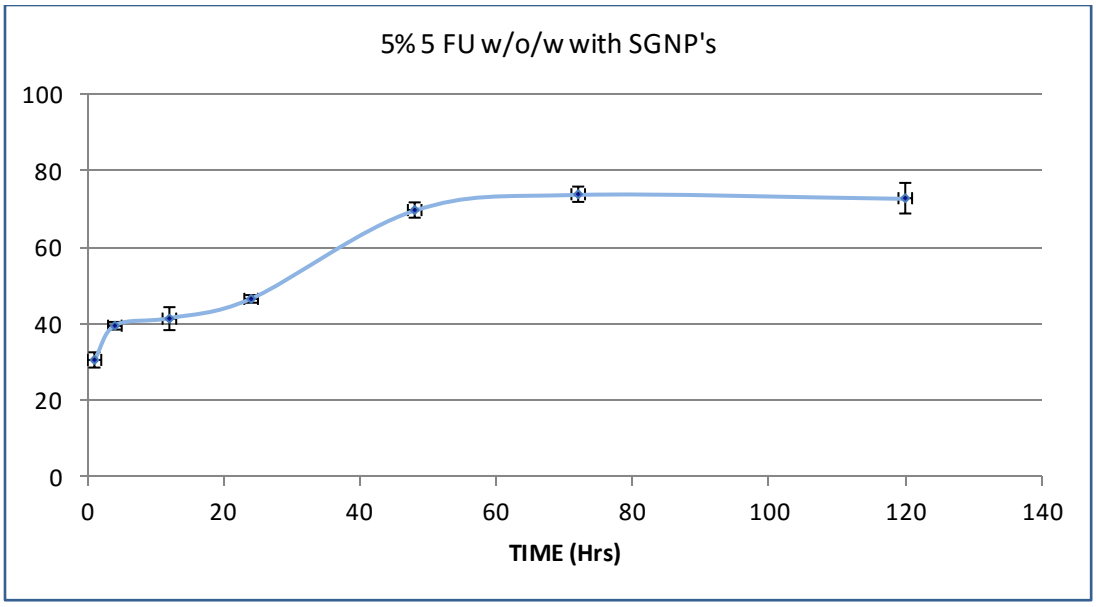


Figure 4.5.2 Release profile of 5 FU SgNp prepared by W/O/W

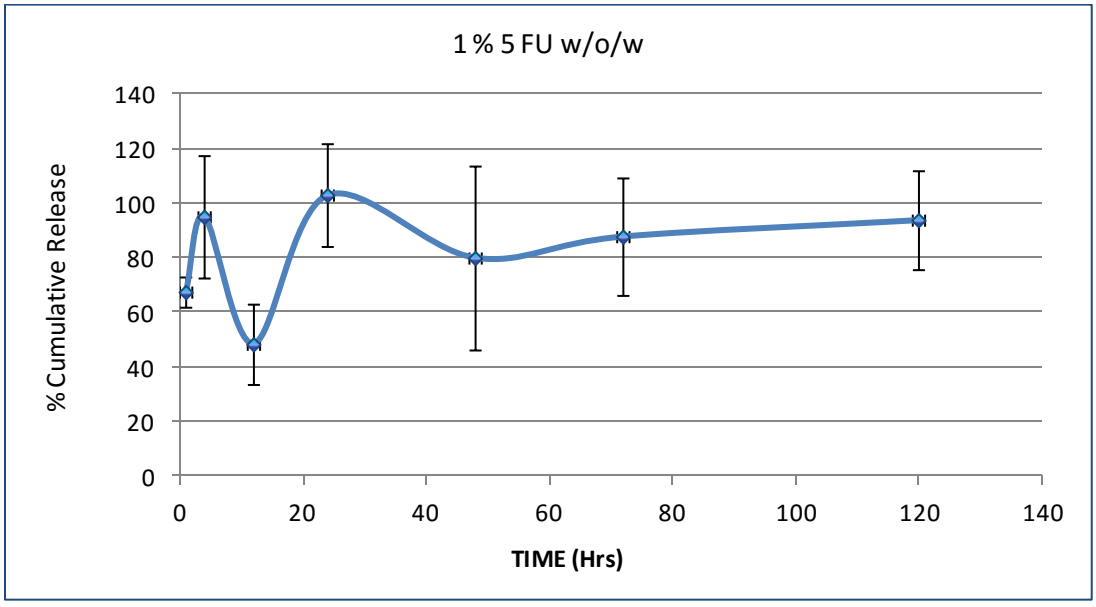


Figure 4.5.3: Release profile of 5 FU NP 1 % prepared by W/O/W

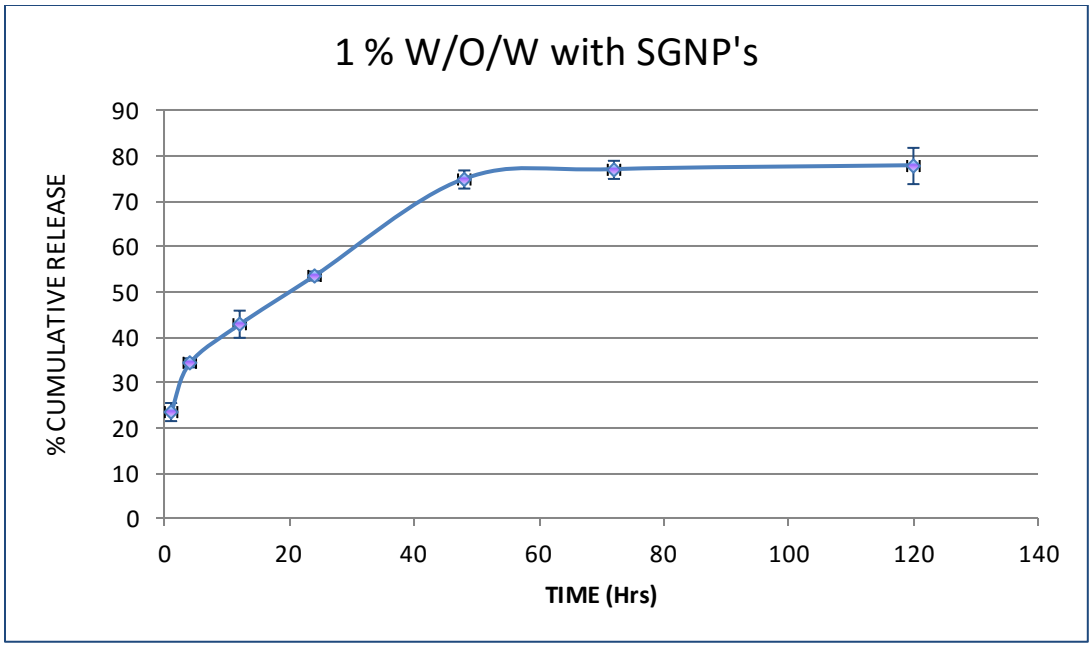


Figure 4.5.4: Release profile of 5 FU SgNP 1 % prepared by W/O/W

The above depicted figures (4.5.3 and 4.5.4) shows the release profile of 1 % loading of 5 FU by conventional method and by incorporation of sgnps in the same technique. There was burst release of 67 % in conventional method, while only 22 % was released in 1 hour from sgnp system.

Similar comparisons are done between respective loadings and the graphs of release profile has been plotted. (fig 4.5.5 and 4.5.6) shows the comparison between release profiles of sgnp and np prepared by conventional technique for 5 % and 1 % loading respectively.

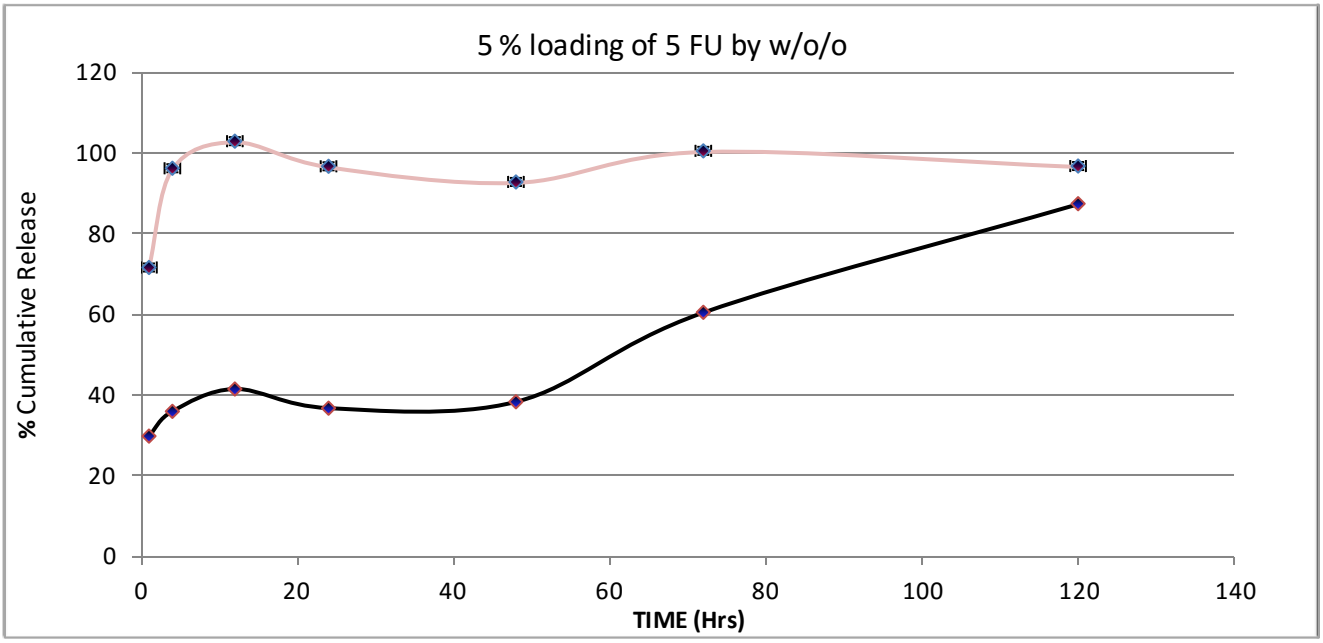


Figure 4.5.5 Release profile of 5 FU SgNp and np made by W/O/O technique.

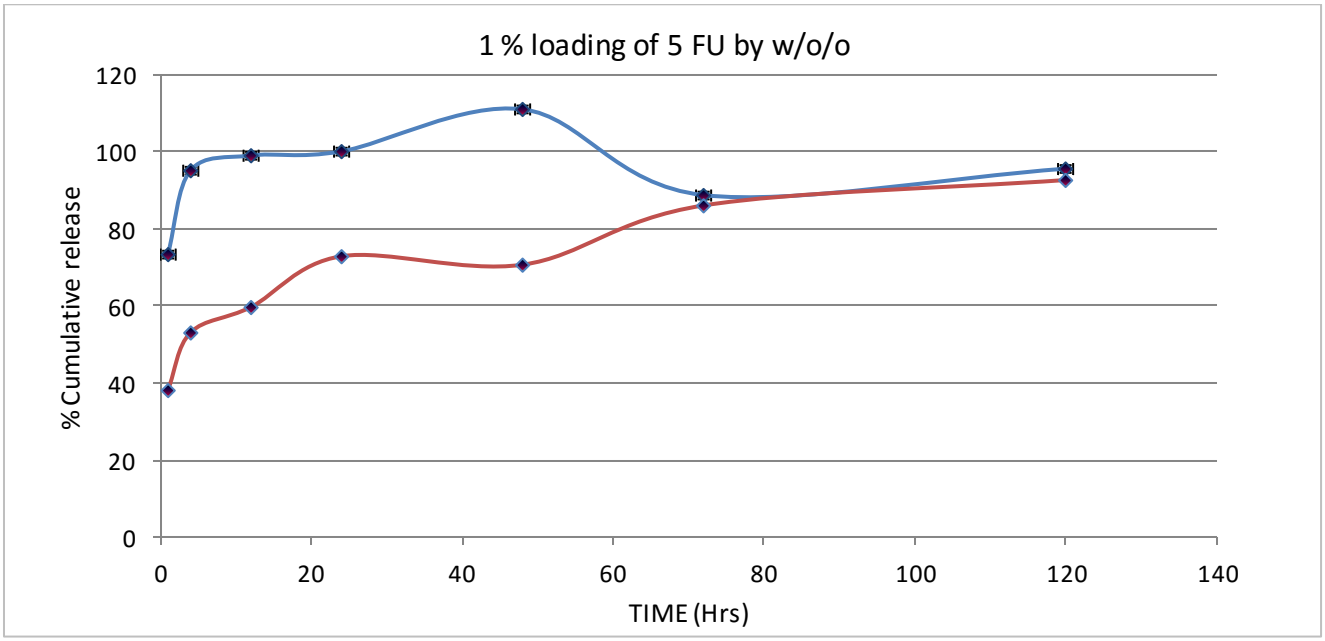
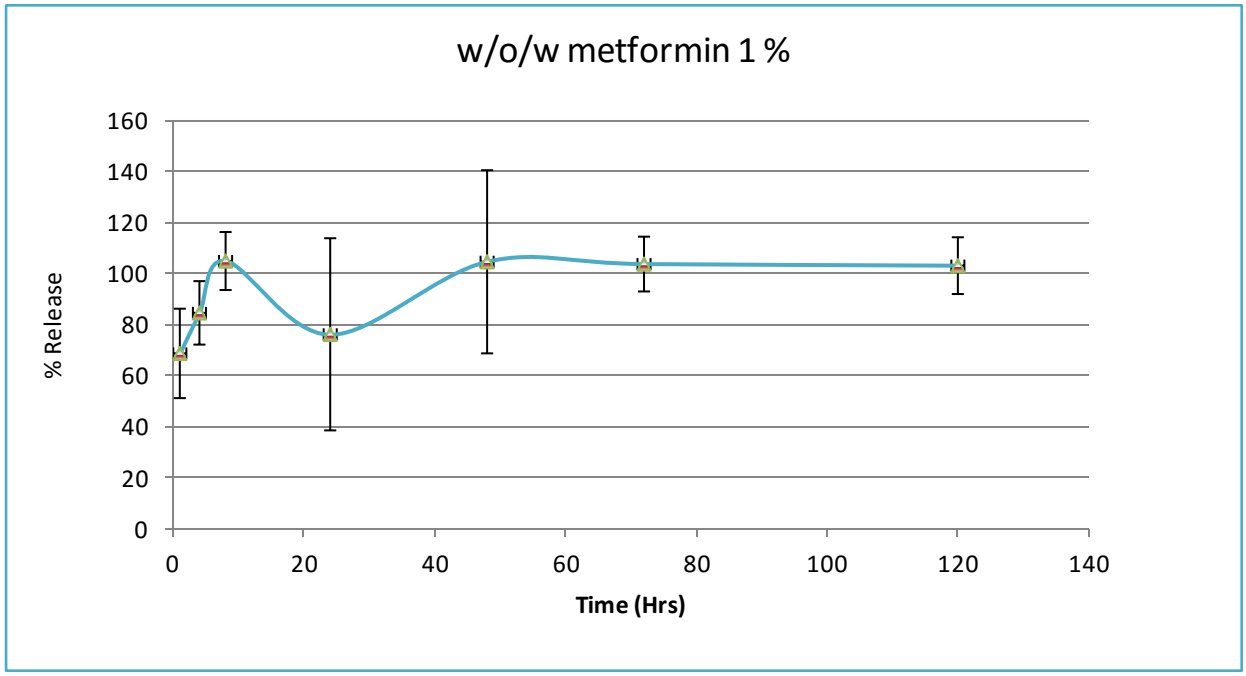


Figure 4.5.6 Release profile of 5 FU SgNp and np made by W/O/O technique.

Figure 4.5.7 and 4.5.8 represents the graph for release profile evaluated for metformin nanoparticles and sgnp of metformin with 1 % loading and prepared by w/o/w method

Figure 4.5.7: Release profile of Metformin Np 1 % prepared by W/O/W



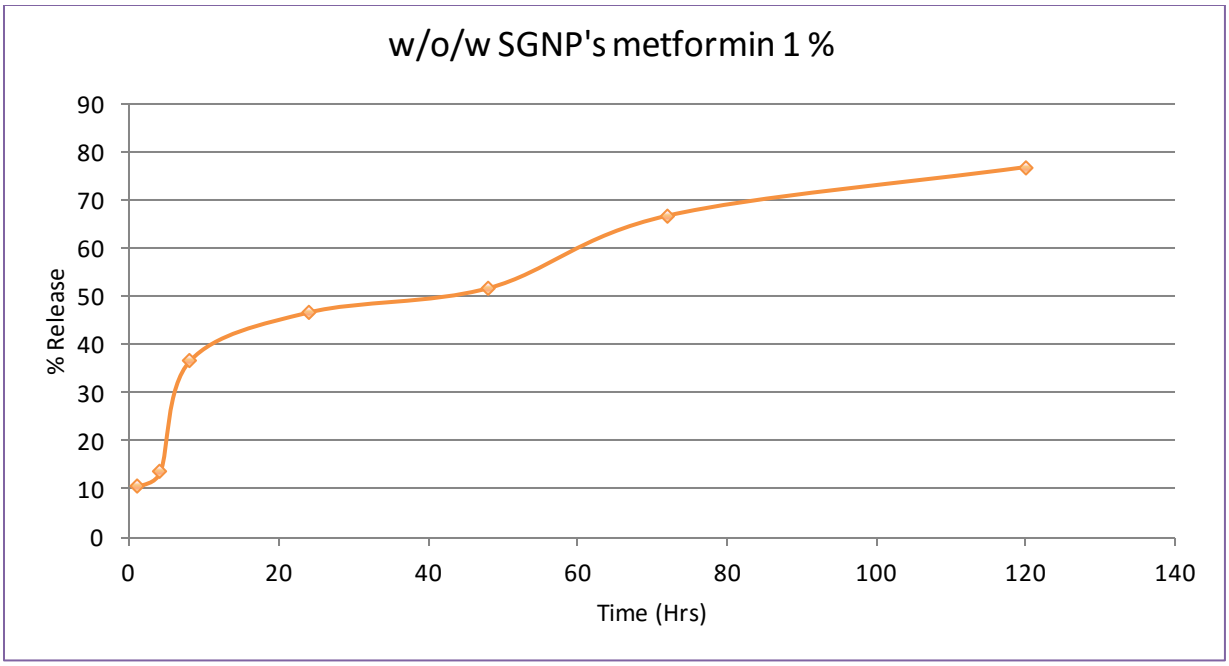


Figure 4.5.8: Release profile of Metformin SgNP 1 % prepared by W/O/W

It can be clearly concluded that the release profile for sgnp is controlled and has shown an acceptable minimal burst release of 20 %- 30 %. A good encapsulation of hydrophilic is demonstrated and thus this technique can be used for future delivery systems for hydrophilic drugs. The details of the experiments carried out for demonstration of release and encapsulation efficiency can be found in previous chapters.

SUMMARY

The novel approach used here gave better encapsulation efficiency unlike conventional methods. This method can also be called as solid in oil in oil or solid in oil in water. The absence of burst release may be due to the longer path that the drug has to travel in polymer particles and also the system has shown controlled release pattern which is attributed to the degradability of polymer. We have also evaluated both the conventional and modified water in oil in oil, double emulsion method using sugar glass nano particles as an encapsulant. There was a initial burst release of 20-35 % and then it followed sustained release similar to bimodal release pattern unlike other where it was around 90 %. Also the size of particles was in range of 300-900 nm. Encapsulation of the drug increased to 64.33 % when using sugar glass system unlike W/O/W double emulsion method where the encapsulation was around 4 %. It is concluded that this new modified double emulsion technique incorporating sugar glass particles of drug is better and advantageous than conventional double emulsion technique in terms of size, shape, release profile and encapsulation efficiency. This novel approach can be very useful in solving the problems associated with delivery of hydrophilic drug.

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