Formulation and Evaluation of Doxorubicin Nanosponges

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In partial fulfilment of the requirements for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

Submitted by

MUMTAJ.J

(Reg No.: 261710759)

Under the guidance of

Dr. Barish M.Pharm., Ph.D,FICS,FAGE

Professor & Head

Department of Pharmaceutics

RVS College of Pharmaceutical sciences

Coimbatore-641402

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DEPARTMENT OF PHARMACEUTICS

RVS COLLEGE OF PHARMACEUTICAL SCIENCES

SULUR, COIMBATORE–641 402.

DECLARATION

I hereby declare that this dissertation work entitled **"FORMULATION AND EVALUATION OF DOXORUBICIN NANOSPONGES"** submitted by me, in partial fulfilment of the rerequirements for the degree **of MASTER OF PHARMACY IN PHARMACEUTICS** to The Tamil Nadu Dr.M.G.R Medical university, Chennai is the result of my original and independent research work carried out under the institutional guidance of **Dr. Barish,** M.Pharm, Ph.D, FICS, FAGE, Professor, and HOD of DEPARTMENT OF PHARMACEUTICS, RVS College of Pharmaceutical Sciences, during the academic year 2018 – 2019

Date:

Place: Sulur **J.MUMTAJ**

(Reg .261710759)

CERTIFICATE

This is to certify that this dissertation entitled **"FORMULATION AND EVALUATION OF DOXORUBICIN NANOSPONGES"** submitted by **J.MUMTAJ (Reg:261710759)** to The Tamil Nadu Dr.M.G.R Medical University, Chennai in partial fulfilment for the degree of **MASTER OF PHARMACY IN PHARMACEUTICS** under the institutional guide of **Dr.Barish,** M.Pharm, Ph.D, FICS, FAGE, Professor, and HOD of DEPARTMENT OF PHARMACEUTICS, RVS College of Pharmaceutical Sciences, during the academic year 2018 – 2019

Dr. Barish M.Pharm,Ph.D, FICS,FAGE
Professor and HOD,
Department of Pharmaceutics,
RVS College of Pharmaceutical Sciences,
Sulur, Coimbatore- 641 402

Place: Sulur

Date:

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I have fully satisfied with her performance and work. I have forward this dissertation work for evaluation.

Dr. R. Venkatanarayan, M.Pharm, Ph.D
Principal
RVS College of Pharmaceutical Sciences
Sulur, Coimbatore.

Place: Sulur
Date:

CERTIFICATE

This is to certify that the dissertation work entitled **"FORMULATION AND EVALUATION OF DOXORUBICIN NANOSPONGES"** is a bonafied work done by **Mrs. J. MUMTAJ (Reg.No:261710759)** for the partial fulfillment of the University rules and regulations for the award of degree of **MASTER OF PHARMACY IN PHARMACEUTICS** under the supervision and guidance during the academic year 2018 -2019.

Dr . Barish M.Pharm, Ph.D, FICS, FAGE
Professor and HOD,
Department of Pharmaceutics,
RVS College of Pharmaceutical Sciences,
Sulur, Coimbatore – 641 402.

Dr. R. Venkatanarayan, M.Pharm, Ph.D
Principal
RVS College of Pharmaceutical Sciences
Sulur, Coimbatore-641 402.

EVALUATION CERTIFICATE

Dissertation title : Formulation and Evaluation of Doxorubicin Nanosponges

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Mrs. J.Mumtaj
(Reg No: 261710759)

ABSTRACT

The pharmaceutical and health care industry has been creating and using nano-scale materials for resolving many physical, biological and chemical problems related with the treatment of disease. The hydrophobic nature of most of the drugs presents a challenge for effective in vivo delivery. Shrinking materials to nano size has profoundly enhanced the efficacy of such drugs. An ideal drug therapy attains effective drug concentration at the target site for a specified period of time and minimizes general and local side effects. To obtain a desirable therapeutic response, the correct amount of drug should be transported and delivered to the site of action with subsequent control of drug input rate. Nanosponges are made of microscopic particles with few nanometers wide cavities, in which a large variety of substances can be encapsulated. These particles possess the ability to carry both lipophilic and hydrophilic substances and thereby improving the solubility of poorly water soluble molecules. The studies conducted in this field proves that the tiny mesh-like structures called nanosponges may revolutionise the treatment of many diseases and early trials suggest this technology is up to five times more effective at delivering drugs for breast cancer than conventional methods.

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INTRODUCTION

The pharmaceutical and health care industry has been creating and using nano-scale materials for resolving many physical, biological and chemical problems related with the treatment of disease. The hydrophobic nature of most of the drugs presents a challenge for effective in vivo delivery. Shrinking materials to nano size has profoundly enhanced the efficacy of such drugs. A number of polymers have been studied and used for formulating Novel drug delivery systems (NDDS).

An ideal drug therapy attains effective drug concentration at the target site for a specified period of time and minimizes general and local side effects. To obtain a desirable therapeutic response, the correct amount of drug should be transported and delivered to the site of action with subsequent control of drug input rate. The distribution of drug to other tissues therefore seems unnecessary,wasteful and a potential cause of toxicity. Targeted drug delivery is the delivery of drug to receptor, organ or any part of the body to which one wishes to deliver the drug exclusively. Targeting drug delivery has long been a problem for medical researchers i.e., how to get them to the right place in the body and how to control the release of the drug to prevent overdoses. The development of new and complex molecule called Nanosponges has the potential to solve this problem. Nanosponges are made of microscopic particles with few nanometers wide cavities, in which a large variety of substances can be encapsulated. These particles possess the ability to carry both lipophilic and hydrophilic substances and thereby improving the solubility of poorly water soluble molecules. The studies conducted in this field proves that the tiny mesh-like structures called nanosponges may revolutionise the treatment of many diseases and early trials suggest this technology is up to five times more effective at delivering drugs for breast cancer than conventional methods. The nanosponge is about the size of a virus with a ‘backbone’ (a scaffold structure) of naturally degradable polyester. They ‘cross link’ segments of the polyester to form a spherical shape that has many pockets (or cavities) where drugs can be encapsulated. The polyester is biodegradable, which means that when it breaks down in the body, the drug can be released on a known schedule.

The nanosponges are encapsulating type of nanoparticles which encapsulates the drug molecules within its core. Based on the method of associating with drugs, the nanoparticles are classified into encapsulating nanoparticles, conjugating nanoparticles and complexing

nanoparticles. The encapsulating nanoparticle is represented by nanosponges and nanocapsules. Nanosponges such as alginate nanosponge, which are sponge like nanoparticles contains many holes that carry the drug molecules. The second category is conjugating nanoparticle, which links to drugs through covalent bonds. The third type is complexing nanoparticle, which attracts the molecules by electrostatic charges. The nanosponges are solid in nature and can be formulated as oral, parenteral, topical or inhalational dosage forms. For oral administration, these may be dispersed in a matrix of excipients, diluents, lubricants and anticaking agents which is suitable for the preparation of tablets or capsules. For parenteral administration, these can be simply mixed with sterile water, saline or other aqueous solutions. For topical administration, they can be effectively incorporated into topical hydrogl. When compared to the other nanoparticles, they are insoluble both in water and organic solvents, porous, non-toxic and stable at high temperatures up to 300°C. They are capable of capturing, transporting and selectively releasing a huge variety of substances because of their specific 3D structure containing cavities of nanometric size and tunable polarity. Furthermore, nanosponges show a notable advantage in comparison with the common nanoparticles that is they can be easily regenerated by different treatments, such as washing with eco-compatible solvents, mild heating, stripping with moderately inert hot gases or changing ionic strength.

The simple chemistry of polymers and cross linkers poses no problems in the preparation and this technology can be easily ramped up to commercial production levels. They can be mixed with water and used as a transport fluid.

They are also used to mask unpleasant flavours, to convert liquid substances to solids. The chemical linkers allow the nanosponges to bind preferentially to the target site. The nanosponges could be either in crystalline or in paracrystalline form. The loading capacity of nanosponges depends mainly on the degree of crystallisation. Paracrystalline nanosponges show different loading capacities. The nanosponges can be formulated to be of specific size and to release drugs over time by varying the proportion of cross linker to polymer. These nanosponges can be magnetized when they are synthesised in the presence of compounds having magnetic properties. The tiny shape of nanosponges enables the pulmonary and venous delivery of drug in a controlled manner.

Targeting Sites by Nanosponges

"Tagging" drug-loaded nanosponges ensures desired pharmacological response by targeting only disease affected cells and leaving the healthy ones unharmed. Drugs encapsulated within the nanosponge pores are shielded from premature destruction and stability of drug is enhanced. This tiny sponge circulates around the tumour cell until they encounter the surface to release their drug cargo in a sustained manner. Nanosponge is three to five times more effective at decreasing tumour growth than direct injection. The targeted delivery systems of nanosponge have several basic advantages like, the drug is released at the tumour instead of circulating widely through the body, and it is more effective for a given dosage. The nanosponges have basic features such as fewer harmful side effects as smaller amounts of the drug will come into contact with healthy tissue.

Difference between Nanoparticles and Nanosponges

The thin line of distinction among nanoparticles and nanosponges is the difference in porosity and size. Nanoparticles have size in nanometer whereas nanosponges have pores in nanometers while their overall size can extend up to micrometers, and are usually smaller than 5μm. Many times nanosponges have been reported as nanoporous nanoparticles / microparticles. Nanosponges show diverse domains in their structure, since they have both hydrophobic and hydrophilic groups.

ADVANTAGES OF NANOSPONGES

1) Being amphiphilic in nature, nanosponges can carry both hydrophobic molecules in the hydrophobic cavity and hydrophilic molecules in the spaces between the hydrophobic moieties simultaneously. Hydrophobic drugs can be loaded into the nanosponge structure to consequently increase their solubility
2) The superior properties of nanosponges have been attributed to 'tunability', that is the ability to control the structure of particles and control the nature and size of aperture.
3) Nanosponges have the ability to produce predictable/controlled drug release.
4) Nanosponges can be tagged with specific linkers to target diseased cells hence achieving greater efficacy while reducing side-effects, decreasing dose and dosing frequency and in turn increasing patient compliance.
5) Nanosponges can significantly reduce the irritation of drugs without reducing their efficacy.
6) Biodegradable in nature and easy scale up for commercial production.

7) They mix with water and are used as a transport fluid. They can be used to mask unpleasant flavours.

DISADVANTAGE

The only disadvantage of this nanosponges is their ability to include only small molecules.

CHARACTERISTIC FEATURES OF NANOSPONGES

- Nanosponges of specific size can be synthesized by changing the crosslinker to polymer ratio.
- They are nontoxic, porous particles, insoluble in most organic solvents and stable up to 300^0C. They are stable at the pH range of 1-11.
- They form clear and opalescent suspension in water.
- They can be reproduced by simple thermal desorption, extraction with solvents, by using microwaves and ultrasounds.
- Their three-dimensional structure allows capture, transportation and selective release of a variety of substances.
- Chemical linkers permit nanosponges to bind preferably to the target site.
- By complexing with different drugs nanosponges can form inclusion and non-inclusion complexes.
- By adding magnetic particles into the reaction mixture, magnetic properties can also be imparted to nanosponges.

POLYMERS USED IN NANOSPONGES PREPARATION

There are various polymers and cross linkers are used in the preparation of nanosponges.

Polymers: Hyper cross linked Polystyrenes, Cyclodextrines and its derivatives like Alkyloxycarbonyl Cyclodextrins, Methyl β-Cyclodextrin, Hydroxy Propyl β-Cyclodextrins.

Copolymers: Poly(valerolactoneallylvalerolactone), Poly(valerolactoneallylvalerolactone oxepanedione), Ethyl Cellulose, Poly vinyl alcohol.

Cross linker: Carbonyl diimidazoles, Carboxylic acid dianhydrides, Diarylcarbonates, Dichloromethane, Diisocyanates, Diphenyl Carbonate, Epichloridine, Gluteraldehyde, Pyromellitic anhydride, 2,2-bis (acrylamido)Acetic acid.

PREPARATION OF NANOSPONGES

Nanosponges are prepared mainly on the criteria of delivery system,polymer and nature of drug and solvents

1) Nanosponges prepared from hyper-cross linked β-cyclodextrins:

β-cyclodextrin nanosponges were prepared by placing 100ml of dimethyl formamide (DMF) in a round bottomed flask and 17.42g of anhydrous β-CD was added and shaken to achieve complete dissolution. Then 9.96g of carbonyl diimidazole (61.42m mol) was added and the solution was allowed to react for 4hrs at 1000°C. Once condensation polymerization was complete, the block of hyper cross linked cyclodextrin was roughly ground and an excess of deionised water was added to remove DMF. Finally residual by-products or unreacted reagents were completely removed by soxhlet extraction with ethanol.The white powder thus obtained was dried overnight in an oven at 600°C. The fine powder obtained was dispersed in water. The colloidal part that remained suspended in water was recovered and lyophilized. The obtained nanosponges are sub-micron in dimension and with a spherical shape.

2) Emulsion solvent diffusion method:

Nanosponges can be prepared by using different proportion of ethyl cellulose and polyvinyl alcohol. The dispersed phase containing ethyl cellulose and drug was dissolved in 20ml dichloromethane and slowly added to a definite amount of polyvinyl alcohol in 150ml of aqueous continuous phase. The reaction mixture was stirred at 1000 rpm for 2hrs in a magnetic stirrer. The nanosponges formed were collected by filtration and dried in oven at 40°C for 24hrs. The dried nanosponges were stored in vaccum desiccators to ensure the removal of residual solvents.

3) Quasi-emulsion solvent diffusion:

The inner phase is prepared using eudragit RS 100 and added to a suitable solvent. Drug to be incorporated is made into a solution and dissolved under ultrasonication at 35ºC. This inner phase added into external phase containing polyvinyl alcohol which acts as emulsifying agent. The mixture is then stirred at 1000-2000 rpm for 3hr at room temperature and dried in an hot air oven at 40ºC for 12hr [7].

4) Ultrasound- Assisted Synthesis:

In this method, polymers react with cross- linkers in absence of solvent and under sonication. Here, the polymer and cross- linker are mixed in a flask. Place the flasks in an ultrasound bath filled with water and heated to 90°C and then sonicate for 5 hrs. It was then allowed to cool and washed with water to remove the unreacted polymer. Dry the product under vacuum and store at 250°C [8].

LOADING OF DRUG INTO NANOSPONGES

Suspend the prepared nanosponges in water and sonicate to avoid the presence of aggregates and then centrifuge the suspension to collect the colloidal fraction.Separate the supernatant and then dry the sample by freeze drying. The aqueous suspension of nanosponges was prepared and dispersed the amount of the drug to be loaded in it. Maintain the suspension under constant stirring for specific time required for complexation. After complexation, separate the uncomplexed (undissolved) drug from complexed drug by centrifugation. Then obtain the solid crystals of nanosponges by solvent evaporation or by freeze drying [7].

FACTORS AFFECTING DRUG RELEASE FROM NANOSPONGES

1. Physical and chemical properties of entrapped active pharmaceutical ingredients.
2. Physical properties of sponge system such as pore diameter, pore volume, and resiliency.
3. Properties of vehicle, in which the sponges are finally dispersed.
4. Particle size, pore characteristics, and compositions can be considered as vital parameters
5. External triggers such as pressure, temperature, and solubility of actives.
6. Temperature: Some entrapped actives can be too viscous at room temperature to flow spontaneously from sponges onto the skin but increased skin or environment temperature can result in increased flow rate and ultimately drug release.

CHARACTERIZATION OF NANOSPONGES

1. Particle size determination

The sizes of particles are maintained during polymerization for the formation of free-following powders having fine aesthetic appearance. Particle size analysis of loaded and unloaded nanosponges can be carried out by laser light diffractometry or Malvern zeta sizer.

2. Determination of loading efficiency

The loading efficiency of prepared nanosponge is determined by subtracting the un-entrapped drug from the total amount of drug. The un-entrapped drug must be estimated by any suitable method of analysis.The method used for separation of un-entrapped drug by gel filtration, dialysis and ultra centrifugation. The loading efficiency is calculated as:

$$Loading\ efficiency = \frac{Actual\ drug\ content\ in\ nanosponge}{Theoretical\ drug\ content} \times 100$$

3. Compatibility Studies

The drug should be compatible with the polymers which are used for the preparation of nanosponges. The compatibility of drug with adjuvants can be determined by Thin Layer Chromatography (TLC) and Fourier Transform Infrared Spectroscopy (FT-IR). Crystalline characteristics can be studied by powder Xray diffraction (XRD) and Differential Scanning Colorimetry (DSC).

4. Zeta Potential

Zeta potential is a measure of surface charge. The surface charge of nanosponge can be determined by using Zeta sizer.

5. Solubility studies

The most widely used approach to study inclusion complexation is the phase solubility method described by Higuchi and Connors, which examines the effect of a nanosponge, on the solubility of drug. Phase solubility diagrams indicate the degree of complexation.

6. In vitro release studies

In vitro release kinetics experiments are carried out using a multi compartment rotating cell. An aqueous dispersion of nanosponges (1ml) containing the drug is placed in the donor compartment, while the receptor compartment separated by a hydrophilic dialysis membrane is filled with phosphate buffer of requires pH. The experiment is carried out for 24hr. At fixed time intervals, the receptor buffer is completely withdrawn and replaced with fresh buffer. The amount of drug in the medium is determined by the suitable analytical method and drug release is calculated to determine the release pattern.

7. Microscopy studies

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) can be used to study the microscopic aspects of the nanosponges. The difference in crystallization state of the raw materials and the product seen under electron microscope indicates the formation of the inclusion complexes.

MARKETED FORMULATIONS

Table 1: Marketed formulations of nanosponges

Drug	Administration Route	Trade Name	Dosage Form
Dexamethasone	Dermal	Glymesason	Tablet
Iodine	Topical	Mena- gargle	Solution
Alprostadil	I.V	Prostavastin	Injection
Piroxicam	Oral	Brexin	Capsule

APPLICATIONS

Nanosponges have many applications in the pharmaceutical field due to their biocompatibility and versatility. Some of them are as follows

a) Nanosponges in Solubility Enhancement:

Presence of crosslinking agent and cavities in the nanosponge structure helps interaction with active molecules. These features allow several substances to be included and get solubilized in the thus formed cavities. The hydrophilic hydroxyl groups on the external surface remain exposed to the environment, while the hydrophobic functionality of the complex hides in the interior cavity of the cyclodextrin the net effect is that a water soluble complex is formed.

b) Nanosponges in Drug Delivery:

Nanosponges have spherical shape and nanometric in size making them ideal in preparing various dosage forms like topical, parenteral, aerosol, tablets and capsules. It is found that highest solubility and in vitro drug release is observed in inclusion complex.

c) Nanosponges for Protein Delivery:

The major obstacle in protein formulation development is the maintenance of the native protein structure both during the formulation process and upon the long term storage. The nanosponges were found to be stable at 300°C and high protein complexation capacity was also observed.

d) Nanosponges in Enzyme Immobilization:

The enzyme immobilization is particularly relevant for lipases, as itimproves their stability and modifies properties like enantioselectivity as well as the reaction rates. As a consequence, the demand for new solid supports, suitable for family of enzymes is constantly growing.

e) Nanosponges as a Carrier for Delivery of Gases:

Hypoxia (deficiency of adequate oxygen supply) is related to various pathologies, from inflammation to cancer. Sometimes it can be difficult to deliver oxygen in appropriate form and doses in clinical practice. Nanosponge formulations were developed as oxygen delivery systems for topical application which were having the ability to store and to release oxygen slowly over time.

f) Nanosponges as Protective Agent -against Photo Degradation:

Nanosponges were prepared by encapsulating gamma-oryzanol showing a good protection from photodegradation. Gamma-oryzanol (a ferulic acid ester mixture), an anti-oxidant and usually employed to stabilize food and pharmaceutical raw materials, moreover, used as a sunscreen in the cosmetics industry. Its applications are limited due to its high instability and photodegradation. With the gammaoryzanol loaded nanosponges a gel and an O/W emulsion are formulated.

g) Modulating Drug Release:

A drug loaded into the nanosponge is retained and released slowly over time. Hydrophilic nanosponges are employed to enhance the drug absorption across biological barriers, to modify the drug release rate and as a potent drug carrier in immediate release formulations. Hydrophobic nanosponges are utilized as sustained release carriers for water soluble drugs, including peptide and protein drugs and they protect the drug during its passage through the stomach. This drug is released very slowly at pH 1.1, whereas release is faster if pH is raised to 7.4.

h) Effective Delivery Carriers:

Antitumor drugs such as paclitaxel, camptothecin and tamoxifen shows bioavailability problem (because of poor aqueous solubility) hence nanosponges can be used as vehicles in order to improve their solubility as well as bioavailability. Complexes showed high effect than that of

the drug alone. After loading the drug in nanosponges the mean absolute bioavailability of paclitaxel was increased and found to be 2.5 fold higher than the plain drug.

Future Prospects

Nanosponges are effective carriers for targeted delivery of drugs to lungs, liver and spleen. A simple approach for formulating Palladium/Silver and Palladium/Silver/Aluminium nanosponges, which contain network of nanowires has been reported. This strategy establishes the first time preparation of alloy nanosponges with network nanowires via self-regulated reduction of sodium dodecylsulfate (SDS) and adding the second or third metal salt during synthesis without additional reducing agent. Further studies on kinetics and biochemical interactions of nanosponges within organisms are vital. These studies must mainly focus on research on nanosponges translocation pathways, its accumulation in the targeted area, short and long-term toxicity, their interactions with cells, the receptors and signalling pathways involved, cytotoxicity, and their surface functionalization for an effective phagocytosis. There is only a sparse knowledge about the effects of nanosponges exposure on the lymphatic and immune systems, as well as various organs. In order to clarify the possible role of nanosponges in diseases recently associated with them (such as Crohn's disease, neurodegenerative diseases, autoimmune diseases, and cancer), nanoscale characterization techniques should be used to a larger extent to identify nanosponges at disease sites in affected organs or tissues, and to establish relevant interaction mechanisms. The cytotoxicity of the nanoparticles or their degradation product remains a major problem and also the improvement in the biocompatibility obviously is a serious concern for the future.

LITERATURE REVIEW

- *Priyanka et al (2018)* formulated ibuprofen loaded nanosponges for topical application. Emulsion solvent diffusion method was selected to prepare ibuprofen loaded nanosponges using different rations of drug: polymer. The obtained nanosponges have been evaluated for physiochemical characteristics and in vitro release studies. The shape and morphology of drug loaded nanosponges were investigated and confirmed by SEM. FTIR results were in agreement with standard spectral studies and moreover it was identified that there was no intraction between drug and polymer. Entrapment efficiency of the NS was found to be around 70.41%. The production yield and in vitro release studies was also good. Overall this study resulted in porous nature of nanosponges which provides a channel for the release of the drug and the method is quick and reproducible.
- *Sornsuvit et al (2018)* aimed to determine the pharmacokinetic parameters and bioavailability of silymarin 140mg self micro-emulsifying drug delivery system (SMEDDS) formulation. An open label, single-dose pharmacokinetic study was conducted. Twelve healthy volunteers were included in the study. After the volunteers had fasted overnight for 10 h, a single-dose generic silymarin 140mg SMEDDS soft capsule was administered. Then 10ml blood sample were taken at 0.0, 0.25, 0.50, 0.75, 1.0, 1.33, 1.67, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0, and 12.0 h. The plasma silymarin concentration were analysed using validated LC-MS/MS. The pharmacokinetic parameters were analysed and calculated.
- *Gangadharappa et al (2017*) proposed the study to improve the solubility of celecoxib using beta-cyclodextrin and NN-methylene bisacrylamide nanosponge hydrogel formulation. Celecoxib is an extremely lipophilic and poorly water soluble NSAID which has gastrointestinal side effects when used chronically. Solubility of celecoxib is 40% when administered as a capsule dosage form. Nanosponges of celecoxib were prepared and characterized by differential scanning colorimetry, X-ray diffractometry. FT-IR analysis and evaluated by zeta-potential and polydispersity index and drug entrapment efficiency. Further, celecoxib nanosponges were dispersed in 1% Carbopol 934 hydrogel and the gel was evaluated for its viscosity, pH, spread ability, in vitro diffusion. In vivo, pharmacokinetic and skin irritation studies conducted using rats. These results confirm that nanosponges formulation is ideal for increasing the solubility and bioavailability of poorly water soluble drug like celecoxib.

- *Shilpa et al (2017)* developed and characterized silybin loaded solid lipid nanoparticle gel for irritant contact dermatitis (ICD). sICD is associated with reduced skin water content, emerging in dry skin condition relapsing eczema. In this study, the silybin loaded nanoparticles was prepared by the ultrasonicprobe sonication method and further evaluated for particle size and entrapment efficiency. Results of optimized batch showed mean particle size 139+_0.35 nm and entrapment efficiency 90.97+_0.91%. Optimized batch was freeze dried and characterized by field emission scanning electron microscopy (FE-SEM),it shows particles are in nano range, with spherical morphology and smooth surface. Ex vivo study of gel exhibited prolonged drug relese, where as the skin irritation study shows no irritancy.
- *Cristian Radu et al (2017)* aimed to address one of the major challenges of the actual era of nanomedicine namely, the bioavailability of poorly water soluble drugs The design of these 100 nm sized carrier nanoparticles was based on natural polymers and their biological properties such as cellular uptake potential, cytotoxicity and 3D penetrability were tested using a colon cancer cell line as the in vitro culture model. Comparative scanning electron microscopy (SEM) and atomic force microscopy (AFM) measurement demonstrated that the silymarin loaded Poly(3-HydroxyButyrate-co-3-HydroxyValerate) (PHBHV) nano carries significantly decreased HT-29 cells viability after 6 and 24 h of treatment.
- *El-nahas et al. (2017)* formulated and characterized silymarin-loaded Eudragit nanoparticles (SNPs) and evaluated their hepatoprotective and cytotoxic effects after oral administration. SNPs were prepared by nanoprecipitation technique and were evaluated for particle size, entrapment efficiency, TEM, solid-state characterization, and in vivo drug release. The hepatoprotective activity was evaluated after oral administration of selected SNPs in carbon tetrachloride-intoxicated rats. Potential in vivo acute cytotoxicity study was also assessed. Morphology of the selected SNPs revealed spherical and uniformly distributed nanoparticles. DSC and FT-IR studies suggested the presence of silymarin in an amorphous state and absence of chemical interaction. The hepatoprotective evaluation of the selected SNPs in CCL4 intoxicated rats revealed significant improvement in the activities of different biochemical parameters compared to the marketed product. The histopathological and toxicity studies were also carried out. The obtained results suggested that the selected SNPs were safe and potentially offered enhancement in the pharmacological hepatoprotective properties of silymarin.

- *Ahmad et al. (2017)* developed and optimized nanoemulsion of silymarin. Nanoemulsion was made by aqueous titration method. Sefsol218, Kolliphor RH40 and polyethylene glycol 400 were used as oil phase, surfactant and co-surfactant while distilled water acted as an aqueous phase. Nanoemulsion was characterized on the basis of particle size, viscosity, electrical conductivity and refractive intex. Further, in vitro release, in vivo pharmacokinetic study, stability study and cancer cell line studies were also performed. The stability study also showed considerably stable formulations at refrigerator temperature as compared with room temperature. The cancer cell line studies also confirmed that silymarin nanoemulsion may be an efficient carrier for oral delivery of silymarin against human hepatocellular carcinoma without damaging normal cells.
- *Hsuan et al (2017)* assembled flavonoid silymarin and zein into spherical silymarin-zein nanoparticles that could be effectively adsorbed on to bacterial cellulose nanofibers. Silymarin-zein nanoparticles greatly changed the wettability and swelling property of bacterial cellulose films due to the formation of nanoparticles/nanofibers nanocomposites. Silymarin-zein nanoparticles enhanced the release of sparingly soluble silymarin from the nanocomposite films. The active films showed more effective antioxidant and antibacterial activities as compared with pure films and lipid oxidation. These findings suggest that the nanoparticle/nanofiber composites may offer a suitable platform for modification of bacterial cellulose films with improved drug release properties and biological activities
- *Younis et al. (2016)* developed nano-formulations of silymarin (SM), a drug commonly used for liver diseases, to overcome it's poor solubility and poor bioavailability; antifibrotic effect of these formulations has not been tested yet. This study was aimed to formulate and evaluate silymarin-loaded eudragit RS 100 nanoparticles (SMnps) and to test the capability of SMnps to reverse an established fibrosis model. SMnps were prepared by solvent evaporation and nano-precipitation techniques. The influence of drug entrapment efficiency (EE)% and in vitro drug releases were investigated. Formulations of SMnps represent a step forward in the field of anti-fibrotic drug development.
- *Bhagyashree Subhash P. and Dr. Mohite S. K (2016)* aimed to produce controlled release artesunate nanosponges for topical and oral delivery. Nanosponges using three different polymers ethyl cellulose, Poly (methyl methacrylate) and Pluronic F-68 (poloxamer 188) were prepared successfully using PVA as surfactant by emulsion solvent evaporation

method. The effects of different drug: polymer rations, surfactant concentration, stirring speeds and time, sonication time on the physical characteistics of the nanosponges as well as the drug entrapment efficiency of the nanosponges were investigated. Particle size analysis and surface morphology of nanosponges were performed. The scanning electrone microscopy of nanosponges showed that they were spherical in shape and spongy in nature. These small sponges can circulate around the drug in a controlled and predictable manner which is more effective for a particular given dosage.

- *Amiri et al. (2016)* prepared nanoniosomal silybin and evaluate its cytotoxicity in the T-47D breast cancer cell line. Silibinin is a chemotherapeutic agent active against cancer. Niosomes are biodegradable, biocompatible, safe and effective carriers for drug delivery. Niosomes were prepared by reverse phase evaporation of a mixture span 20, silibinin, PEG-2000 and cholesterol in chloroform and methanol solvent (1:2 v/v). The solvent phase was evaporated using a rotary evaporator and the remaining gel phase was hydrated in phosphate buffer saline. Mean size, size distribution and zeta potential of niosomes were measured with a zetasizer instrument and then nanoparticles underwent scanning electron microscopy. The drug releasing pattern was evaluated by dialysis and the cytotoxicity of nanoniosomes in T-47D cells was assessed by MTT assay. The amount of encapsulated drug and the level of drug loading were determined 98.6+_2.7% and 22.3+_ 1.8%, respectively; released drug was estimated about 18.6+_2.5% after 37 hours. The cytotoxic effects of nanoniosome were significantly increased when compared with the free drug. This study finding suggests that silibinin nanoniosomes could serve as new drug formulation for breast cancer therapy.
- *Zhang et al. (2016)* carried out a study with the objective to prepare silyblin nanoparticles (NPs) and optimize the prepared nanoparticles using central composite rotatable design-response surface methodology. Hepatocellular carcinoma (HCC) is a most common liver malignancy. HCC was induced in rats by supplementing 100 mg/L of diethylnitrosamine (DENA) in drinking water for 8 weeks. Saline, silybin 30mg/kg body weight and nanoformulation of silybin equivalent to silybin dose were administered orally to 3 groups of 6 animals each. Anticancer activity was evaluated by counting the live nodules. The results showed that silybin NPs under optimized conditions gave rise to the entrapment efficiency (EE) of 88% drug loading of 15%, mean diameter of 216 nm of the NPs prepared and zeta potential value of -15mV. In rats treated with silybin NPs, the number of neoplastic nodules was significantly lower, the animals did not exhibit

decrease in mean body weight, the number of liver nodules was reduced by >93% with significantly high localization in the liver. It was concluded that orally administered silybin NPs showed improved efficacy and safety compared to silybin for the treatment of HCC in rats.

- *Li J. et al. (2016)* determined the expression status and role of AMPK in esophageal squamous cell carcinoma (ESCC) and investigated whether silibinin, a nontoxic natural product, could activate AMPK to inhibit ESCC development. Emerging evidence suggested that activation of adenosine monophosphate-activated protein kinase (AMPK) may suppress cancer growth. We found that silibinin induced apoptosis, and inhibited ESCC cell proliferation in vitro and tumorigenicity in vivo without any adverse effects. Silibinin also markedly suppressed the invasive potential of ESCC cells in vitro and their ability to form lung metastasis in nude mice. This preclinical study supported that AMPK is a valid therapeutic target and suggested that silibinin may be a potentially used as a therapeutic agent and chemo sensitizer for oesophageal cancer.
- *Viswanad V. and Jilsha G. (2015)* formulated cephalexin into nanosponges loaded hydrogel as it can enhance skin permeation. Nanosponges of cephalexin were prepared using hydroxyl ethyl cellulose and poly vinyl alcohol by emulsion solvent evaporation method. The particle size and entrapment efficiency was found in the range of 200-400 nm and 88.5%-95.6% respectively. Based on the characterization, nanosponges with high entrapment efficiency and least particle size were selected for hydrogel formulation. Five different formulations of hydrogels were prepared by using Carbopol 934 with varying concentration of penetration enhancer (propylene glycol) and various evaluation studies were carried out. The in vitro release studies revealed that the formulation with higher concentration of penetration enhancer (15% propylene glycol) showed greater drug release. From the kinetic study, the best linearity was found with first order and Higuchi's equation.
- *Aldawsari et al. (2015)* carried out a research aiming to formulate lemongrass-loaded ethyl cellulose nanosponges with a topical hydrogel with an enhanced antifungal effect and decreased irritation. Lemongrass-loaded ethyl cellulose nanosponges were formulated. The emulsion solvent evaporation technique was used for the preparation of nanosponges. The prepared formulations were evaluated for particle size, citral content, and in vivo release. The selected hydrogel formulation,F9, was subjected to surface morphological investigations, using scanning and transmission electron microscopy,

where results showed that the nanosponges possess a spherical uniform shape with a spongy structure, the integrity of which was not affected by integration into the hydrogel.

- *Srinivas P. and Jahnavi Reddy A. (2015)* formulated controlled release isoniazid (INH) nanosponges for topical delivery. Nanosponges using ethyl cellulose polymer were prepared successfully using PVA as surfactant by emulsion solvent evaporation method. Particle size analysis and surface morphology of nanosponges were performed. The scanning electron microscopy of nanosponges showed that they were spherical in shape and spongy in nature. The particle size of the formulations was found to be in the range of 47.18% to 74.86%. The optimized nanosponge formulation was selected for formulating nanogels using various gelling agents like Carbopol 934, Carbopol 940, HPMC K4M and studied for pH, viscosity and in vitro drug release. Of the various formulations prepared, F2 was found to show the maximum sustained drug release of 74.26% in 10 hours.
- *Seema et al. (2015)* developed curcumin loaded nanosponges which increased the bioavailability and retention time at the colon. Clinical importance of curcumin in colon disease like inflammatory bowel diseases (Chron's disease and ulcerative colitis) irritable bowel syndrome and colon cancer has been reported. Due to small size and porous nature of the noval delivery system they can easily bind to poorly-soluble drugs within their matrix and prevent drug from rapid metabolism and excretion, improve their bioavailability. The nanosponge of curcumin is prepared by solvent evaporation method. Further the formulated nanosponges are evaluated for its phase solubility, particle size determination, zeta potential, SEM,TEM analysis, loading efficiency, and production yield and in vitro release study.
- *Lockhart et al. (2015)* reported the synthesis and encapsulation of polyester nanosponge particles (NPs) co-loaded with tamoxifen (TAM) and quercetin (QT) to investigate the loading, release and in vitro metabolism of a dual drug formulation. The NPs were made in two variations, 4% and 8% crosslinking densities, to evaluate the effects on metabolism and release kinetics. The stability of the formulation was established in simulated gastrointestinal fluids, and the metabolism of TAM was shown to be reduced 2-fold and 3-fold for NP-4%s and NP-8%s, respectively, while QT metabolism was reduced 3 and 4-fold. This work demonstrates the suitability of the nanosponges not only as a dual release drug delivery system but also enabling a regulated metabolism through the capacity of the nano network. The variation in crosslinking enables a dual release

with tailored release kinetics and suggests improved bioavailability aided by a reduced metabolism.

- *Wang Y et al. (2014*) aimed to identify the CXCR4 antagonists that could reduce and/or inhibit tumour metastasis from natural products. C-X-C chemokine receptor type 4 (CXCR4) signalling has been demonstrated to be involved in cancer invasion and migration; therefore, CXCR4 antagonist can serve as an anti-cancer drug by pre-venting tumor metastasis. According to the molecular docking screening, we reported here silibinin as a novel CXCR4 antagonist. Biochemical characterization showed that silibinin blocked chemokine ligand 12(CXCL12)-induced CXCR4 internalization by competitive binding to CXCR4, therefore inhibiting down-stream intracellular signalling.
- *Jana et al. (2014)* carried out a study to improve the solubility and bioavailability of nebivolol. The present study attempts to overcome these issues through nanoparticulate delivery system using widely used carrier EudragitRS100. The solvent evaporation (single emulsion) technique was used for developing nanoparticles. The physiochemical characterization confirmed the particle size in nano range with smooth and spherical morphology. Further, Fourier transforms infrared (FTIR) spectroscopy and differential scanning colorimetry (DSC) studies confirm compatibility of drug-polymer combination. The in vitro drug release study of the prepared nanoparticles showed prolongation of drug release with reduced burst release in comparison with pure drug powder.
- *Sadiq and Abdul Rassol (2014)* aimed to develop and evaluate silibinin (SIL) loaded solid lipid nanoparticals (SLN) in an attempt to increase its oral bioavailability and targeting the lower part of GI tract. Solvent emulsification evaporation method with slight modification was used to prepare the SLNs and glyceryl monostearate (GMS), trimyristin (TM), tripalmitin (TP) and tristearin (TS) were investigated as solid lipid matrix. Tween 20 (T20), tween 80 (T80), polyvinyl alcohol (PVA), poloxamer 188 (P188), sodium cholate (SC) and sodium deoxycholate (SDC) were investigated as emulsifiers. The formulations were evaluated for entrapment efficiency (EE), particle size distribution and in-vitro release profile. Furthermore, the optimized formula (F2) was further investigated by TEM, FTIR and DSC studies. All the prepared SLNs are within submicromal range and acceptable polydispersity index (PI). It can be concluded that SIL could easily incorporated into SLN containing TS and P188 for oral use.

- *Pooja et al. (2014)* tried to develop silibinin loaded chitosan nanoparticles so as to improve its bioavailability. This study presents fabrication and characterization of chitosan-tripolyphosphate based encapsulation of silibinin. Various preparations of silibinin encapsulated chitosan-tripolyphosphate nanoparticles were studied for particle size, morphology, zeta-potential, and encapsulation efficiencies. Preparations were also evaluated for cytotoxic activities in vitro. No chemical interactions between silibinin and chitosan were observed in FTIR analysis. Powder x-ray diffraction analysis revealed transformed physical state of silibinin after encapsulation. Surface morphology and thermal behaviour were determined using TEM and DSC analysis. Encapsulated silibinin displayed increased dissolution and better cytotoxicity against human prostate cancer cell (DU145) than silibinin alone.
- *Ramteke et al. (2014*) proposed a detailed study about the various mathematical models of drug dissolution. When a new solid dosage from is developed, it is very important to study drug release or dissolution. The quantitative analysis of values obtained in dissolution or release rates is easier when mathematical formulae are used to describe the process. The mathematical modelling helps to optimize the design of a therapeutic device to yield information on the efficacy of various release models. In this paper review of the different mathematical models used to determine the kinetics of drug release from drug delivery system such as, zero order, first order, Hixson-Crowell,Higuchi , Weibull, Korsemeyer-Peppas, Hopfenberg, Baker-Lonsdale and gompertz model were carried out.
- *Raja et al. (2013*) formulated nanosponge loaded with ciprofloxacin antibiotic and resulted in sustained release. The drug is acid labile and hence it is entrapped with ethyl cellulose for its sustained release. As the drug is made into nanoparticle the density was found to be increased. Among all the formulated batches starting from F1 through F5 the final batch (F5) is considered as the best entrapped (90.80%) nanosponge with greater percentage drug release (99.4%). The characterization by SEM concluded the appearance as a nanosponge.

AIM AND OBJECTIVE

- To formulate doxorubicin nanosponges so as to target cancer cells particularly breast cancer, colorectal cancer using different polymers.
- To increase the bioavailability of doxorubicin by developing doxorubicin nanosponges.
- To reduce the dose, dosing frequency and to reduce dose dependent side effects of doxorubicin.
- To facilitate drug targeting or selective uptake of drug.
- Deliver the drug at specified site in a controlled manner without showing any burst effect.

To achieve the objective the following steps were carried out:

- The preformulation studies.
- Formulation of doxorubicin nanosponges by general full factorial design.
- Characterization of prepared doxorubicin nanosponges.
- In-vitro evaluation of doxorubicin nanosponges.

DRUG PROFILE

DRUG NAME: Doxorubicin

SYNONYM(S): ADR,**1** Adria,**2** Dox,**2** hydroxyl daunorubicin,**2** NSC-123127**2**

COMMON TRADE NAME(S): generic available,**3** ADRIAMYCIN®,**4** RUBEX®**5** (USA)

CLASSIFICATION: anthracycline antineoplastic antibiotic**6**

Special pediatric considerations are noted when applicable, otherwise adult provisions apply.

MECHANISM OF ACTION:

Doxorubicin binds directly to DNA via intercalation between base pairs on the DNA helix.2 Doxorubicin also inhibits DNA repair by inhibiting topoisomerase II. These actions result in the blockade of DNA and RNA synthesis and fragmentation of DNA.4 Doxoubicin is also a powerful iron-chelator. The iron-doxorubicin complex can bind DNA and cell membranes producing free radicals that immediately cleave DNA and cell membranes. Although maximally cytotoxic in S phase, doxorubicin is not cell cycle-specific.2

PHARMACOKINETICS:

Interpatient variability	clearance reduced in obese patients (i.e., >130% ideal body weight
Oral Absorption	not stable in gastric acids; not absorbed from GI tract
Distribution	widely distributed in plasma and in tissues cross blood brain barrier? no volume of distribution4 25 L/kg plasma protein binding4 70%
Metabolism	in the liver and other tissues by an aldo-keto reductase enzyme active metabolite doxorubicinol inactive metabolite(s) doxorubicinone, aglycones and conjugates
Excretion	predominantly in bile

	urine4 3-10% as metabolites feces4 40-50% as unchanged drug terminal half life8 20-48 h clearance9 27.5-59.6 L/h/m2
Gender	terminal half life4: male 54 h; female 35 h clearance4: male 113 L/h; female 44 L/h
Children	increased risk for delayed cardiotoxicity4

USES: ***Primary uses:*** ***Other uses:***

*Bladder carcinoma
*Breast cancer
Endocrine carcinoma10
Ewing's sarcoma12,13
*Gastric cancer
*Gynecological carcinoma
*Head and neck cancer
*Hepatic carcinoma
Hepatoma14
Kaposi's sarcoma15
*Leukemia, acute lymphoblastic
*Leukemia, acute myeloblastic
*Lung cancer
*Lymphoma, Hodgkin's
*Lymphoma, non-Hodgkin's
*Neuroblastomas
Osteosarcoma16,17
Pancreatic cancer18
*Sarcoma, soft tissue
*Testicular carcinoma
*Thyroid carcinoma
Urothelial carcinoma19
*Wilm's tumour

Multiple myeloma6
Prostate cancer6
Thymoma11

SPECIAL PRECAUTIONS:

Contraindicated in patients with the following conditions:

- hypersensitivity to doxorubicin, anthracyclines (e.g., epirubicin, daunorubicin), or anthracenediones (e.g., mitoxantrone, mitomycin)
- previous therapy with high cumulative doses of anthracyclines (e.g., doxorubicin, daunorubicin, epirubicin, idarubicin) or some anthracenediones (e.g., mitoxantrone)
- severe hepatic impairment
- severe myocardial insufficiency or recent myocardial infarction, severe arrhythmias, or history of severe cardiac disease

Cardiac toxicity is a risk of doxorubicin therapy that may be manifested by early (acute) or late (delayed) effects.20 Cardiac function should be assessed at baseline and continue during treatment. Risk factors for developing doxorubicin-induced cardiotoxicity include:3,7,20-23

- high cumulative dose, previous therapy with other anthracyclines or anthracenediones, prior or concomitant radiotherapy to the mediastinal/pericardial area
- pre-existing heart disease
- extremes of age
- liver disease
- concomitant chemotherapy, especially bevacizumab, cyclophosphamide, PACLitaxel, and trastuzumab
- concomitant use of drugs that can suppress cardiac contraction
- whole body hyperthermia
- female gender (mainly in children)

Carcinogenicity: Doxorubicin is carcinogenic in animals and is potentially carcinogenic in humans

Mutagenicity: Mutagenic in the Ames test.3 Doxorubicin is clastogenic in mammalian *in vitro* and *in vivo* chromosome tests.7

Fertility: Treatment with doxorubicin may produce gonadal suppression, resulting in amenorrhea or azoospermia.11

Pregnancy: Doxorubicin is classed as FDA Pregnancy Category D.24 There is positive evidence of human fetal risk, but the benefits from use in pregnant women may be acceptable despite the risk. Chemotherapy protocols including doxorubicin have been administered during

pregnancy to treat breast cancer.25 For more information, refer to BC Cancer's Cancer Management Guidelines *Breast Cancer in Pregnancy*.

Breastfeeding should not occur while a mother is undergoing chemotherapy with doxorubicin because Doxorubicin is secreted into breast milk.3,4

SIDE EFFECTS:

The table includes adverse events that presented during drug treatment but may not necessarily have a causal relationship with the drug. Because clinical trials are conducted under very specific conditions, the adverse event rates observed may not reflect the rates observed in clinical practice. Adverse events are generallyincluded if they were reported in more than 1% of patients in the product monograph or pivotal trials, and/or determined to be clinically important.

Hyperuricemia may result from cell lysis by doxorubicin and may lead to electrolyte disturbances or acute renal failure.34 It is most likely with highly proliferative tumours of massive burden, such as leukemias, high-grade lymphomas, and myeloproliferative diseases. The risk may be increased in patients with preexisting renal dysfunction, especially ureteral obstruction. Suggested prophylactic treatment for high-risk patients35:

- aggressive hydration: 3 L/m²/24 hr with target urine output >100 ml/h
- if possible, discontinue drugs that cause hyperuricemia (e.g., thiazide diuretics) or acidic urine (e.g., salicylates)
- monitor electrolytes, calcium, phosphate, renal function, LDH, and uric acid q6h x 24-48 hours
- replace electrolytes as required
- allopurinol 600 mg po initially, then 300 mg po q6h x6 doses, then 300 mg po daily x 5-7 days

Urine should be alkalinized only if the uric acid level is elevated, using sodium bicarbonate IV or PO titrated to maintain urine pH>7. Rasburicase (FASTURTEC®) is a novel uricolytic agent that catalyzes the oxidation of uric acid to a water-soluble metabolite, removing the need for alkalinization of the urine.36 It may be used for treatment or prophylaxis of hyperuricemia; however, its place in therapy has not yet been established. Aluminum hydroxide (AMPHOGEL®) may be added orally if phosphate becomes elevated. If aluminum hydroxide has been added, discontinue sodium bicarbonate.37

Cardiotoxicity is thought to be due to free radical damage as myocardial tissue is susceptible to these highly reactive species.38 Anthracycline cardiotoxicity may present with early or late effects.39,40 The following information applies to all anthracyclines, anthracenediones and mitoxantrone.

Early cardiotoxic effects are not dose-related and may present from mild ECG changes to life-threatening arrhythmias.20,38,39 These events may occur during or immediately after a single dose of anthracycline treatment,20,38 but do not predict subsequent development of delayed cardiotoxicity and are not considered indications for suspension of therapy.

Late cardiotoxic effects, which are dose-related and clinically the most important type of cardiotoxic effect, present as reduced LVEF or symptomatic CHF, and typically occur weeks to years after completion of treatment. Abnormalities in LVEF are associated with all the anthracyclines and their derivatives. LVEF changes are related to the total cumulative dose, are irreversible and refractory to medical therapy.

Prevention and treatment: Cardiac assessment should occur at baseline and throughout therapy. Monitor for symptomatic congestive heart failure (CHF) or reduced left ventricular ejection fraction (LVEF). Sensitive, non-invasive methods to measure LVEF include radionucleotide angiography (RNA), MUGA, or echocardiogram.40 Late cardiotoxic effects may be prevented by stopping treatment with the associated anthracycline once patients have reached the suggested maximum cumulative dose.23,38 Management of anthracycline cardiotoxicity includesdiscontinuation of the drug and initiating standard treatment of CHF.

Cardiotoxicity risk can be reduced but not eliminated with the use of alternative anthracyclines (i.e., epirubicin or liposomal doxorubicin) or by altering the frequency of administration (once a week vs. once every 3 weeks, or continuous infusion).40 Cardioprotectant therapy with dexrazoxane may be considered for patients with cumulative doxorubicin-equivalent doses greater than 300 mg/m2.

Local effects: Extravasation of doxorubicin can occur with or without accompanying stinging or burning sensation, and even if blood returns well on aspiration of the infusion needle.3 Extravasation of doxorubicin will result in severe ulceration and soft tissue necrosis.4 To minimize the risk of thrombosis or perivenous extravasation, the dose should be infused over

3 to 10 minutes, depending on the vein size and the dose.7 For more information on prevention and treatment of extravasation with doxorubicin, refer to BC Cancer Policy III-20 *Prevention and Management of Extravasation of Chemotherapy*. Also, monitor for local erythematous streaking along vein and/or facial flushing which may indicate a too rapid infusion rate.4 This has traditionally been called the "doxorubicin flare."

INTERACTIONS:

AGENT	EFFECT	MECHANISM	MANAGEMENT
barbiturates56 (e.g., phenobarbital)	delayed, moderate possible; decreased pharmacological effects of doxorubicin	doxorubicin metabolism increased by barbiturates via CYP3A4 induction	monitor therapy
calcium channel blockers7 (e.g., verapamil)4	doxorubicin-induced cardiotoxicity may be increased	additive toxicity	monitor cardiac function throughout treatment
cyclosporine57	increased pharmacological effects of doxorubicin	doxorubicin metabolism decreased by cyclosporine either by competition for CYP3A4 or p-glycoprotein inhibition	consider therapy modification

AGENT	EFFECT	MECHANISM	MANAGEMENT
digoxin tablets58	delayed, moderate, suspected; decreased pharmacological effects of digoxin	digoxin absorption decreased by antineoplastic agents due to alteration of intestinal mucosa	monitor for decreased effect of digoxin
paclitaxel59	increased doxorubicin pharmacological effects4	doxorubicin clearance decreased either by competition for CYP3A4 or p-glycoprotein	monitor for increased cardiotoxicity (e.g., congestive heart failure) or consider using docetaxel instead of paclitaxel59
quinolones56 (e.g., ciprofloxacin)	delayed, moderate, possible; the antimicrobial effect of quinolones may be decreased	quinolone absorption decreased by antineoplastic agents due to alteration of intestinal mucosa	monitor therapy
stavudine60	decreased pharmacological effects of stavudine	stavudine metabolism to active drug is decreased by doxorubicin due to inhibition of phosphorylation	avoid concomitant use
trastuzumab61	increased cardiotoxicity	unknown	consider therapy modification

SUPPLY AND STORAGE:

Injection3: Mayne Pharma supplies doxorubicin in single-dose vials of sterile, preservative-free, lyophilized red powder of 10 mg, 50 mg and 150 mg sizes.3 The formulation contains lactose.41 Store vials between 15-20ºC and protect from light (keep intact vials in their carton until use).

Novopharm supplies doxorubicin in single-dose vials of sterile, isotonic, preservative-free solution of 10 mg/5 mL, 50 mg/25 mL and 200 mg/100 mL sizes.41 The formulation contains hydrochloric acid for pH adjustment. Refrigerate vials and protect from light (keep intact vials in their carton until use).

DOSAGE GUIDELINES:

Refer to protocol by which patient is being treated. Numerous dosing schedules exist and depend on disease, response and concomitant therapy. Guidelines for dosing also include consideration of absolute neutrophil count (ANC). Dosage may be reduced, delayed or discontinued in patients with bone marrow depression due to cytotoxic/radiation therapy or with other toxicities.

Adults:

BC Cancer usual dose noted in *bold, italics*

		Cycle Length:
Intravenous:	1 week72:	25 mg/m2 IV for one dose on day 1 (total dose per cycle 25 mg/m2)
		15-20 mg IV for one dose on day 1
2		*(total dose per cycle 15-20 mg)*
		60 mg/m2 IV for one dose on day 1
2 weeks73:		*(total dose per cycle 60 mg/m2)*
		40-75 mg/m2 IV for one dose on day 1
3 weeks10,16-18,73-94:		*(total dose per cycle 40-75 mg/m2)*
		50 mg/m2 IV for one dose on day 1
4 weeks95:		*(total dose per cycle 50 mg/m2)*
		30 mg/m2 IV for one dose on day 2
4 weeks96:		*(total dose per cycle 30 mg/m2)*

	30 mg/m2 IV for one dose on days 1 and 8
4 weeks97:	*(total dose per cycle 60 mg/m2)*
	25-30 mg/m2 IV for one dose on days 1 and
4 weeks98,99:	*15*
	(total dose per cycle 50-60 mg/m2)
	75 mg/m2 IV for one dose on day 1
6 weeks12,13:	*(total dose per cycle 75 mg/m2)*
	50 mg/m2 IV for one dose on day 1
8 weeks91:	*(total dose per cycle 50 mg/m2)*
Suggested maximum cumulative dose48:	3 week cycle: 550 mg/m2
	1 week cycle: 700 mg/m2
	If risk factors are present:
	3 week cycle: 400-450 mg/m2
	1 week cycle: 550 mg/m2

Children:		Cycle Length:
Intravenous1:	N/A	45-90 mg/m2 IV continuous infusion (24-96 h)
	N/A	30-45 mg/m2 IV daily x 3 or weekly
	1 week:	20-30 mg/m2 IV for one dose on day 1 (total dose per cycle 20-30 mg/m2)
	3 weeks:	40-75 mg/m2 IV for one dose on day 1 (total dose per cycle 40-75 mg/m2)

EXCIPIENTS PROFILE

EUDRAGIT® E 100

1. **Commercial form**

EUDRAGIT® E 100

Solid substance

EUDRAGIT® E 100 is described in the monographs quoted above.

EUDRAGIT® E PO

Solid substance obtained from EUDRAGIT® E 100.

EUDRAGIT® E PO is described in the Ph. Eur. and JPE monographs quoted above.

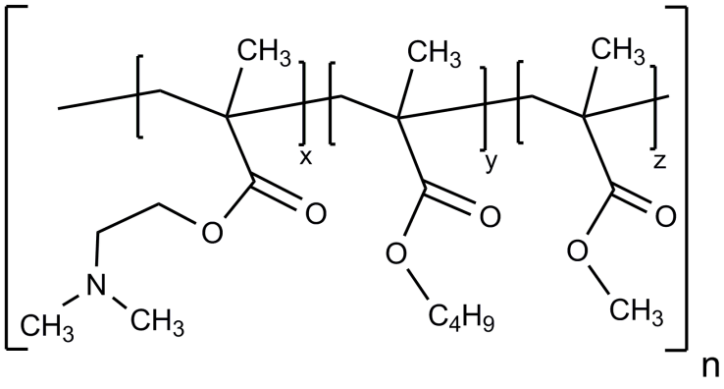
The polymer conforms to the USP/NF monograph quoted above.

EUDRAGIT® E 12,5

Solution of EUDRAGIT® E 100 with 12.5 % (w/w) dry substance in a mixture of 60 % (w/w) Isopropyl Alcohol Ph. Eur. / USP and 40 % (w/w) Acetone Ph. Eur. / NF.

2. Chemical structure

EUDRAGIT® E 100 is a cationic copolymer based on dimethylaminoethyl methacrylate, butyl methacrylate, and methyl methacrylate with a ratio of 2:1:1.



The monomers are randomly distributed along the copolymer chain. Based on SEC method the weight average molar mass (Mw) of EUDRAGIT® E 100; EUDRAGIT® E PO and EUDRAGIT® E 12,5 is approximately 47,000 g/mol.

3. Characters

Description

EUDRAGIT® E 100: colourless to yellow tinged granules with a characteristic amine-like odour.

EUDRAGIT® E PO: white powder with a characteristic amine-like odour.

EUDRAGIT® E 12,5: light yellow liquid of low viscosity, clear to slightly cloudy with a characteristic odour of the solvents.

Solubility

1 g of EUDRAGIT® E 100 or EUDRAGIT® E PO dissolves in 7 g methanol, ethanol, isopropyl alcohol, acetone, ethyl acetate, methylene chloride or 1 N hydrochloric acid to give clear to slightly cloudy solutions. EUDRAGIT® E 12,5 is mixable with these solvents and with petroleum ether in a ratio of 1:1.

The solid substance is practically insoluble in petroleum ether and water. The polymer is precipitated from EUDRAGIT® E 12,5 when mixed with water in a ratio of 1:1.

4. Tests

Test solution

Either EUDRAGIT® E 12,5 is used for the Test solution, or a corresponding solution of EUDRAGIT® E 100 or EUDRAGIT® E PO: 12.5 % (w/w) dry substance is dissolved in a mixture of 60 % (w/w) isopropyl alcohol and 40 % (w/w) acetone.

Particle size

EUDRAGIT® E PO: Dv50 < 50 µm

The particle size is determined by laser light diffraction according to Ph. Eur. 2.9.31 / light diffraction measurement USP <429>.

Film formation

When the Test solution is poured onto a glass plate, a clear film forms upon evaporation of the solvents.

Dry substance / Residue on evaporation

EUDRAGIT® E 100 / EUDRAGIT® E PO: not less than 98.0 %

The test is performed according to Ph. Eur. 2.2.32 d.

1 g is dried in an oven for 3 hrs at 110°C.

EUDRAGIT® E 12,5: 11.9 - 13.1 %

The test is performed according to Ph. Eur. 2.2.32 d. 20 g quartz sand are mixed with 1 g of the solution and dried in an oven for 5 hrs at 110°C.

Loss on drying

EUDRAGIT® E 100 / EUDRAGIT® E PO: max. 2.0 % according to "Dry substance / Residue on evaporation".

Assay

Dimethylaminoethyl (DMAE) groups on dry substance (DS): 20.8 - 25.5 %

Alkali value: 162 – 198 mg KOH per g DS

The assay is performed according to Ph. Eur. 2.2.20 "Potentiometric titration" or USP <541>. 0.2 g EUDRAGIT® E 100 / EUDRAGIT® E PO or 1.6 g EUDRAGIT® E 12,5 are dissolved in 96 ml glacial acetic acid and 4 ml water. 0.1 N perchloric acid is used as the titrant. 1 mL of 0.1 N perchloric acid is equivalent to 7.21 mg of dimethylaminoethyl groups.

The alkali value (AV) states how many mg KOH are equivalent to the basic groups contained in 1 g dry substance (DS).

$$\text{AV (mg KOH / g DS)} = \frac{\text{ml 0.1 N } HClO_4 \cdot 561}{\text{sample weight (g)} \cdot \text{DS (\%)}}$$

DMAE groups (%) = AV (mg KOH / g DS) . 0.1286

JPE: EUDRAGIT® E 100 / EUDRAGIT® E PO: 4.0 - 6.0 % Nitrogen on dry substance

The test is performed according to JP method “Nitrogen determination”.

Colour

Absorbance (A): max. 0.300

The test is performed according to Ph. Eur. 2.2.25 or USP monograph. The yellow colour of the test solution is determined against water at 420 nm in a 1 cm cuvette.

Viscosity / Apparent viscosity

3 - 6 mPa . s

The viscosity of the Test solution is determined by means of a Brookfield viscometer (UL adapter / 30 rpm / 20°C).

The test is performed according to Ph. Eur. 2.2.10 or USP <912> method II.

Viscosity / Kinematic viscosity

JPE: EUDRAGIT® E 100 / EUDRAGIT® E PO: 2.5 - 5.5 mm2 / s

The test is performed according to the JPE monograph.

Refractive index

nD20: 1.380 - 1.385

The refractive index of the Test solution is determined according to Ph. Eur. 2.2.6.

Relative density

d: 0.811 - 0.821 20 20

The relative density of the Test solution is determined according to Ph. Eur. 2.2.5

5 Purity

Sulphated ash / Residue on ignition

Max. 0.1 %

The test is performed according to Ph. Eur. 2.4.14 or USP <281>.

1 g EUDRAGIT® E 100, EUDRAGIT® E PO or EUDRAGIT® E 12,5 is used for the test.

Heavy metals

Max. 20 ppm

The test is performed according to Ph. Eur. 2.4.8 method C or USP <231> method II.

1 g EUDRAGIT® E 100, EUDRAGIT® E PO or EUDRAGIT® E 12,5 is used for the test.

Arsenic:

JPE: EUDRAGIT® E 100 / EUDRAGIT® E PO: max. 2 ppm

The test is performed according to JP Method 3.

g EUDRAGIT® E 100 or EUDRAGIT® E PO is used for the test

Monomers

EUDRAGIT® E 100 / EUDRAGIT® E PO total of monomers: < 2500 ppm

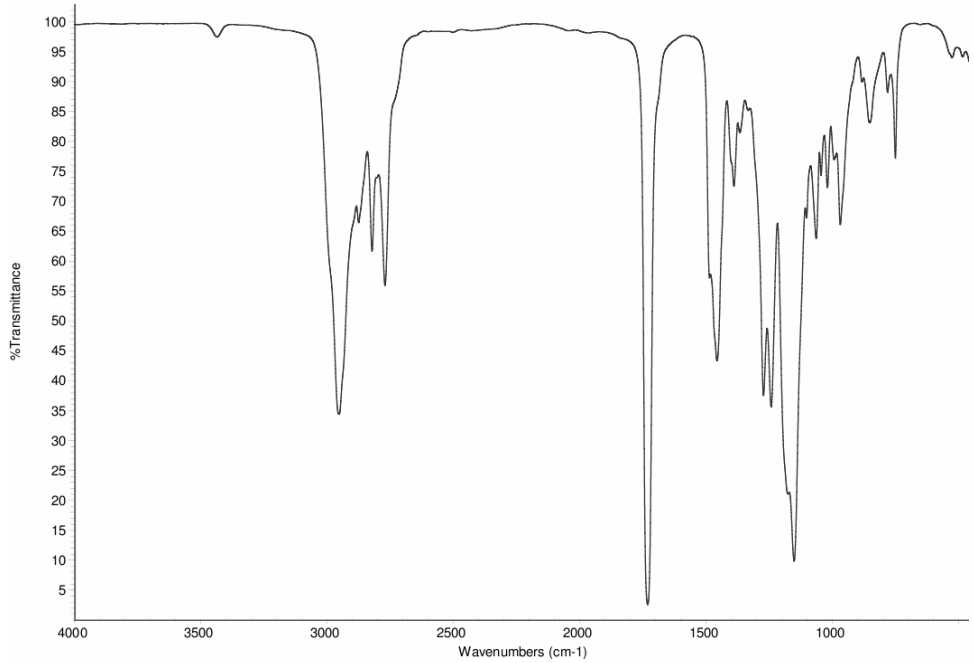
Butyl methacrylate: < 1000 ppm

Methyl methacrylate: < 500 ppm

Dimethylaminoethyl methacrylate: < 1000 ppm

EUDRAGIT® E 12,5: total of monomers max. 0.04 %

The test is performed according to the Ph. Eur., USP/NF or JPE monograph on 1 g EUDRAGIT® E 100 / EUDRAGIT® E PO or 8 g EUDRAGIT® E 12,5.



7. Detection in dosage forms

The dosage forms are extracted using the solvents listed under "Solubility," if necessary after crushing. Insoluble substances are isolated by filtration or centrifugation. The clear filtrate is boiled down and the residue identified by IR spectroscopy.

8. Storage

EUDRAGIT® E 100: Protect from warm temperatures (USP, General Notices). Protect from moisture. Any storage between 8°C and 25°C fulfils this requirement. EUDRAGIT® E 100 tends to form lumps at warm temperatures (≥ 30°C). This has no influence on the quality. The lumps are easily broken up again.

EUDRAGIT® E PO: Store at temperatures up to 25°C. Protect from moisture. Any storage between 8°C and 25°C fulfils this requirement. Temperatures above 25°C will cause caking of EUDRAGIT® E PO.

EUDRAGIT® E 12,5: Protect from warm temperatures (USP, General Notices). Store in tightly closed containers.

9. Stability

Minimum stability dates are given on the product labels and batch-related Certificates of Analysis. Storage Stability data are available upon request.

ETHYL CELLULOSE

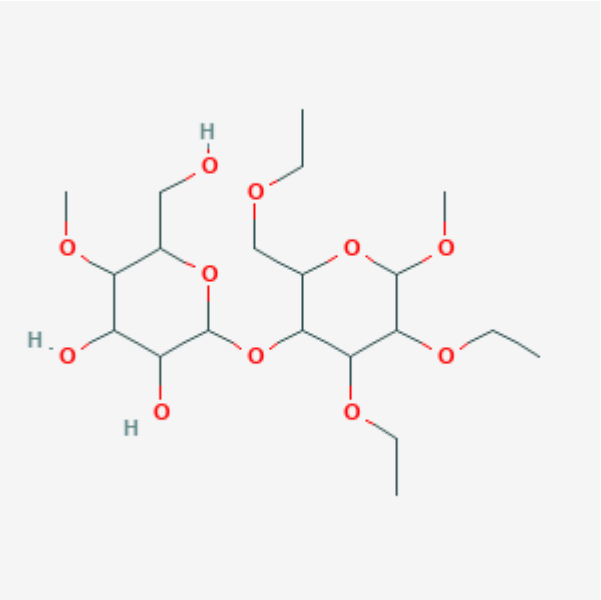
	Revised specification prepared at the 76th JECFA (2012), published in FAO JECFA Monographs 13 (2012) superseding specifications prepared at the 26th JECFA (1982), published in FNP 25 (1982) and FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' was established at the 35th JECFA (1989).
SYNONYMS	INS No. 462
DEFINITION	Ethyl ether of cellulose, prepared from wood pulp or cotton by treatment with alkali and ethylation of the alkali cellulose with ethyl chloride. The article of commerce can be specified further by viscosity. Antioxidants permitted for use in food may be added for stabilizing purposes.
Chemical names	Cellulose ethyl ether, ethyl ether of cellulose
C.A.S. number	9004-57-3
Assay	Not less than 44% and not more than 50% of ethoxyl groups (-OC2H5) on the dried basis (equivalent to not more than 2.6 ethoxyl groups per anhydroglucose unit).
DESCRIPTION	Free-flowing, white to light tan powder
FUNCTIONAL USES	Tableting aid, binder, filler, diluent of colour and other food additives

CHARACTERISTICS

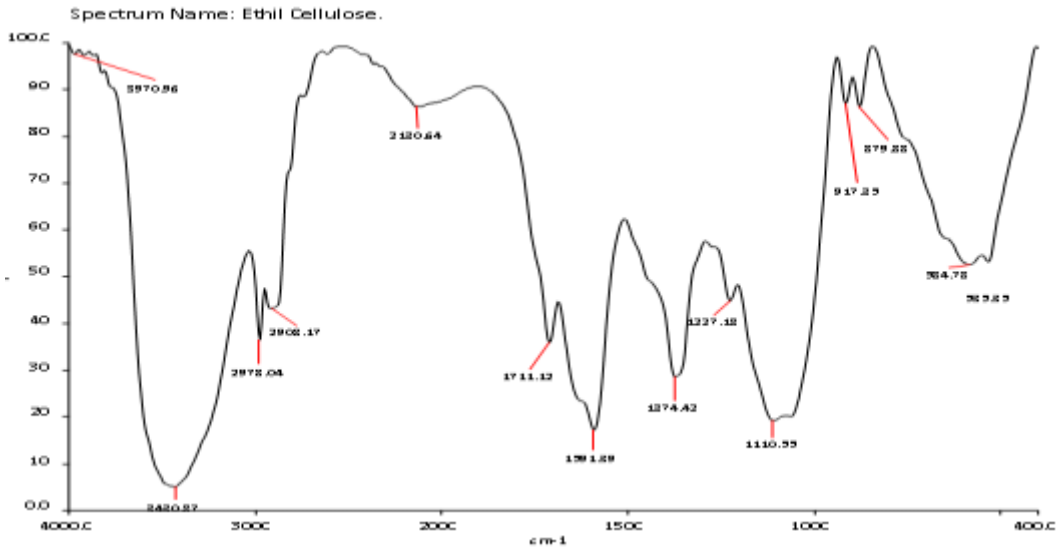
IDENTIFICATION	
Solubility (Vol. 4)	Practically insoluble in water, in glycerol, and in propane-1,2-diol, but soluble in varying proportions in certain organic solvents, depending upon the ethoxyl content. Ethyl cellulose containing less than 46-48% of ethoxyl groups is freely soluble in tetrahydrofuran, methyl acetate and aromatic hydrocarbon ethanol mixtures. Ethyl cellulose containing 46-48% or more of ethoxyl groups is freely soluble in ethanol, methanol, toluene and ethyl acetate.
Film forming test	Dissolve 5 g of the sample in 95 g of an 80:20 (w/w) mixture of toluene-ethanol. A clear, stable, slightly yellow solution is formed. Pour a few ml of the solution onto a glass plate, and allow the solvent to evaporate. A thick, tough continuous, clear film remains. The film is flammable.

pH (Vol. 4)	Neutral to litmus (1 in 20 suspension)
PURITY	
Loss on drying (Vol. 4)	Not more than 3% (105o, 2 h)
Sulfated ash	Not more than 0.4% Test 1 g of the sample
Lead	Not more than 2 mg/kg Determine using an AAS (Electrothermal atomization technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").
METHOD OF ASSAY	Determine the ethoxyl content as directed under *Ethoxyl and Methoxyl Group Determination* (see Volume 4).

STRUCTURE:



IR OF ETHYL CELLULOSE:



PLAN OF WORK

Preformulation Studies:

- Construction of calibration curve
- Solubility studies
- FT-IR Compatibility studies

Formulation of doxorubicin nanosponges:

Preparation of nanosponges of doxorubicin using 2 different polymers (ethyl cellulose, eudragit) using emulsion solvent diffusion method.

Characterisation of doxorubicin nanosponges:

- ❖ Particle size determination
- ❖ SEM
- ❖ Zeta potential
- ❖ Entrapment efficiency

Evaluation of doxorubicin nanosponges:

- ❖ In vitro release study

EXPERIMENTAL METHODS

I. PREFORMULATION STUDIES:

Physical characteristics:

By visual examination the drug was tested for its physical characters like colour, odour and texture.

Solubility test:

Doxorubicin powder (about 1mg) was taken in a test tube and solubility in ethanol, water, dichloromethane and chloroform was tested.

Preparation of stock solution

The standard stock solution of doxorubicin was prepared by transferring accurately weighed quantity (10 mg) of doxorubicin raw material in 100 ml of volumetric flask. The drug was dissolved in few ml of ethanol and the volume was made up to get a stock solution of 100 µg/ml.

Selection of Wavelength

The standard stock solution was scanned in the range of 200 to 400 nm in UV spectrophotometer using phosphate buffer pH 7.4 as blank. The absorbance maximum was found at 288 nm.

Construction of calibration curve of doxorubicin:

From the standard stock solution of doxorubicin 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 ml were withdrawn to 10ml of volumetric flask and then made up volume with phosphate buffer pH 7.4 to get a concentration range of 5-40 µg/ml. The absorbance of these solutions was measured at 288 nm using JASCO V-530 UV 1600 UV – visible spectrophotometer. Phosphate buffer pH 7.4 was used as blank. The calibration curve was plotted between concentration and absorbance.

PREPARATION OF BUFFER SOLUTION

Phosphate buffer pH 7.4:

An accurately weighed quantity of 20.209 gm of disodium hydrogen phosphate and 8 gm of potassium hydrogen phosphate was dissolved in sufficient water to produce 1000ml.

Drug Excipient Compatibility Studies

T-IR spectrum pf drug was recorded using FT-IR Spectro photometer (Shimadzu JASCO 4100). The diffuse reflectance technique was utilised in the mid IR 4000-400 cm spectral region. The procedure consist of dispersing the sample in KBr(100mg) using a mortar, triturating the materials into a fine powder bed into the holder using compression gauge. The pressure was around 5 tons for 5 minutes. The pellet was placed in the light path and the spectrum was recorded. The characteristic peaks of the functional groups were interpreted.

The FTIR spectrum of doxorubicin, polymers ethyl cellulose and eudragit were recorded. The spectrum of physical mixture of doxorubicin, polymer and co-polymer were also documented to check for their compatibility.

II. FORMULATION OF DOXORUBICIN NANOSPONGES BY EMULSION SOLVENT DIFFUSION METHOD:

Emulsion solvent diffusion method was used to formulate doxorubicin loaded nanosponges by using a suitable polymer. Dispersed phase consist of specified amount of drug and polymer which was dissolved in 20 ml of an organic solvent dichloromethane. Aqueous phase consist of specified amount of poly vinyl alcohol dissolved in 100 ml distilled water. Disperse phase was added drop by drop into aqueous phase by stirring on magnetic stirrer at 1000 rpm for about 2 hours. The nanosponges formed were collected by filtration and dried in oven at 40°c for about 24 hours. They were then kept in the vacuum desiccators to remove the residual solvent. The doxorubicin nanosponges were formulated using polymers ethyl cellulose and eudragit.

III. CHARACTERIZATION OF NANOSPONGES

FTIR Spectroscopy of nanosponges

Before formulating a drug substance into dosage form, it is essential that it should be chemically and physically compatible. Compatibility studies give information needed to define the drug substance and provide a frame work for the drug combination with pharmaceutical excipients in the fabrication dosage form. This study was carried out by using infrared

spectrophotometer to find if there is any possible chemical intraction between the doxorubicin and polymers.

A few mg of sample (doxorubicin nanosponges) was weighed and mixed with 100 mg of potassium bromide (dried at 40-50°c). The mixture was taken and compressed under 10- ton pressure in hydraulic press to form a pellet was scanned from 4000-400 cm-1 in IR spectrophotometer.

Determination of percentage yield

Doxorubicin loaded nanosponges were weighed after drying. Percentage yield was calculated by

$$\% \text{ yield} = \frac{\text{practical weight of nanosponges obtained}}{\text{Theoretical weight (drug + polymers)}} \times 100$$

Scanning electron microscopy (SEM)

SEM analysis was performed to determine their microscopic characters (shape & morphology) of prepared doxorubicin nanosponges. Nanosponges were prepared and dried well to remove the moisture content and images were taken using scanning electron microscopy (Hitachi X650, Tokyo, Japan) in different magnifications. Samples were placed on glass slide kept under vacuum and then by using sputter coater unit, samples were coated with a thin gold layer, operated at 15kv acceleration voltage.

Particle size determination

The average mean diameter and size distribution of loaded nanosponges is found by Dynamic Light Scattering method using Malvern zeta sizer at 25°c. The dried nanosponges were dispersed in water to obtain proper light scattering intensity for doxorubicin nanosponges.

Determination of Zeta potential

Zeta potential is a measure of surface charge. The surface charge (electrophoretic mobility) of nanosponge can be determined by using Zeta sizer (Malvern Instrument) having zeta cells, polycarbonate cell with gold plated electrodes and using water as medium for sample preparation. It is essential for the characterisation of stability of the nanosponges.

Determination of Entrapment Efficiency

The entrapment efficiency of nanosponges were determined by adding 10 ml of phosphate buffer of pH 7.4 and sonicated in a bath sonicator and filtered. 1ml of filtrate is made up to 10 ml with phosphate buffer and was assayed spectrophotometrically at 288 nm (UV visible spectrophotometer, model UV-1601 PC, Shimadzu). The amount of entrapped drug was calculated from the equation.

$$\text{Entrapment efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

In vitro release studies

Drug release was determined by dialysis method; two ml of each formulation (test and control) were poured into dialysis bags and put into 25 ml phosphate buffer (pH 7.4) and stirred (100 rpm, room temperature). At predetermined time intervals, 2 ml of phosphate buffer was taken and then substituted by fresh phosphate buffer. Finally, the amounts of released doxorubicin in phosphate buffer were measured by spectrophotometer at 288 nm. Aliquots withdrawn were assayed at each time interval for the drug released at λmax of 288 nm using UV-Visible spectrophotometer by keeping phosphate buffer pH 7.4 as blank and the amount of released drug was estimated by the standard curve.

RESULTS AND DISCUSSION

I. PREFORMULATION STUDIES

Physical Characteristics

Doxorubicin was checked for its colour, odour and texture. Doxorubicin is red coloured powder in appearance, odourless and amorphous in nature.

Solubility

Solubility test for Doxorubicin was carried out in different solvents such as ethanol, water, dichloromethane and chloroform and results are given in Table 1.

Table 1: Solubility test for Doxorubicin in different solvents

Sl. No	Solvent	Soluble	Sparingly Soluble	Insoluble
1.	Ethanol	✓	-	-
2.	Dichloromethane	✓	-	-
3.	Chloroform	-	✓	-
4.	Water	✓	-	-

Selection of Wavelength

The Doxorubicin stock solution of concentration 100µg/mL was scanned in the range of 200-400nm for $\lambda_{max.}$ using double beam UV Spectrophotometer. The absorption peak obtained is shown in Figure 1.

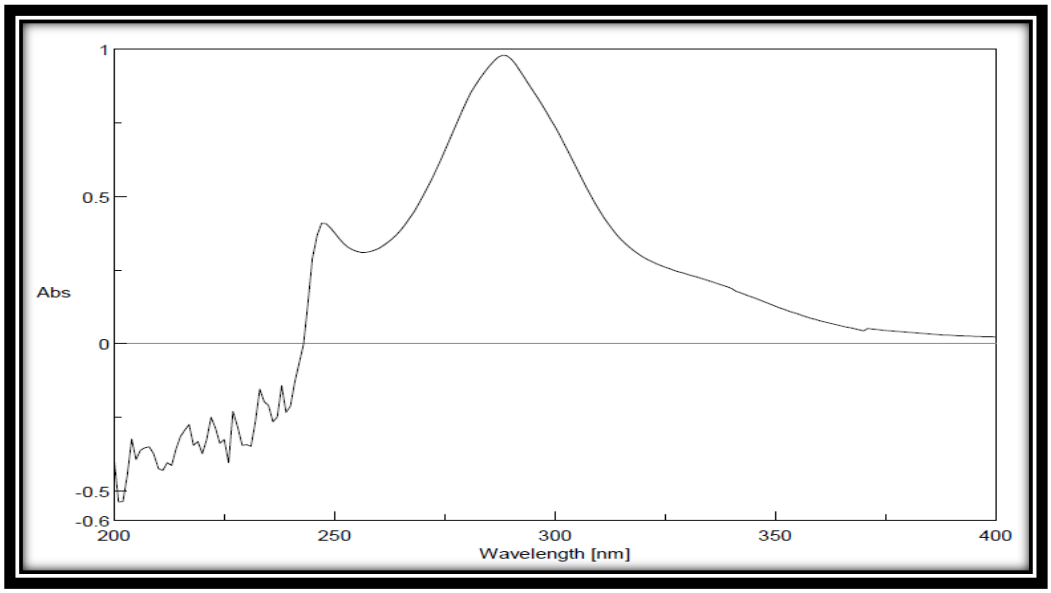


Figure 1: UV spectra of Doxorubicin

The maximum absorption of Doxorubicin was found to be at 232nm and hence it is selected as the wavelength for further studies.

Construction of calibration curve of Doxorubicin

In the calibration curve, linearity was obtained between 5-40 µg/ml concentration of Doxorubicin and the regression value was found to be $r^2 = 0.9996$. Hence we can conclude that Doxorubicin obeys Beer Lambert's Law at the concentration between 5-40 µg/ml. The results are shown in Table 2 and Figure 5.

Table 2: Concentration and absorbance values for estimation of Doxorubicin

Sl.No	Concentration (µg/ml)	Absorbance (AU) at 232nm
1.	5	0.1686
2.	10	0.3624
3.	15	0.5357
4.	20	0.6963
5.	25	0.8770
6.	30	1.0693
7.	35	1.2700
8.	40	1.4516

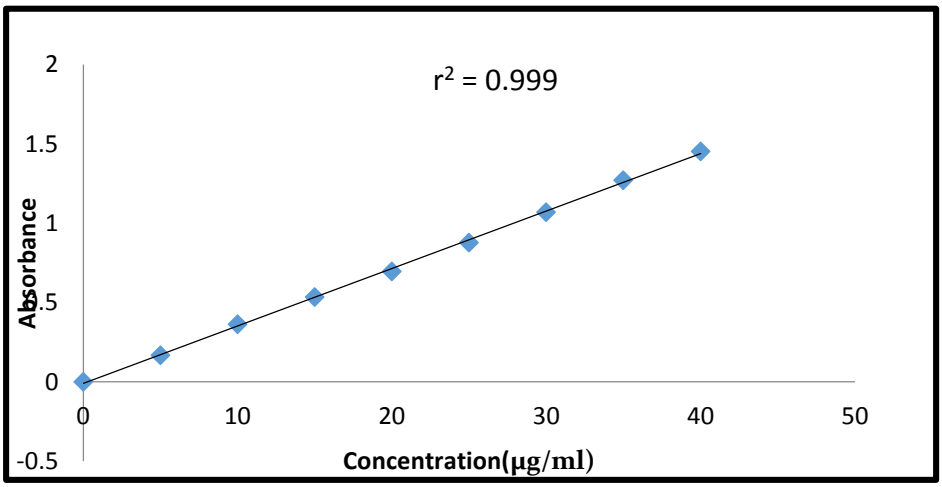


Figure 2: Calibration graph of Doxorubicin

Excipient Compatibility Studies

Fourier Transform Infrared (FT-IR) spectra of the samples were obtained using a SHIMADZU Spectrometer by KBr disc method. The spectrums were recorded for the pure drug and physical mixture of drug and polymer and are shown in Figures 3,4, and 5.

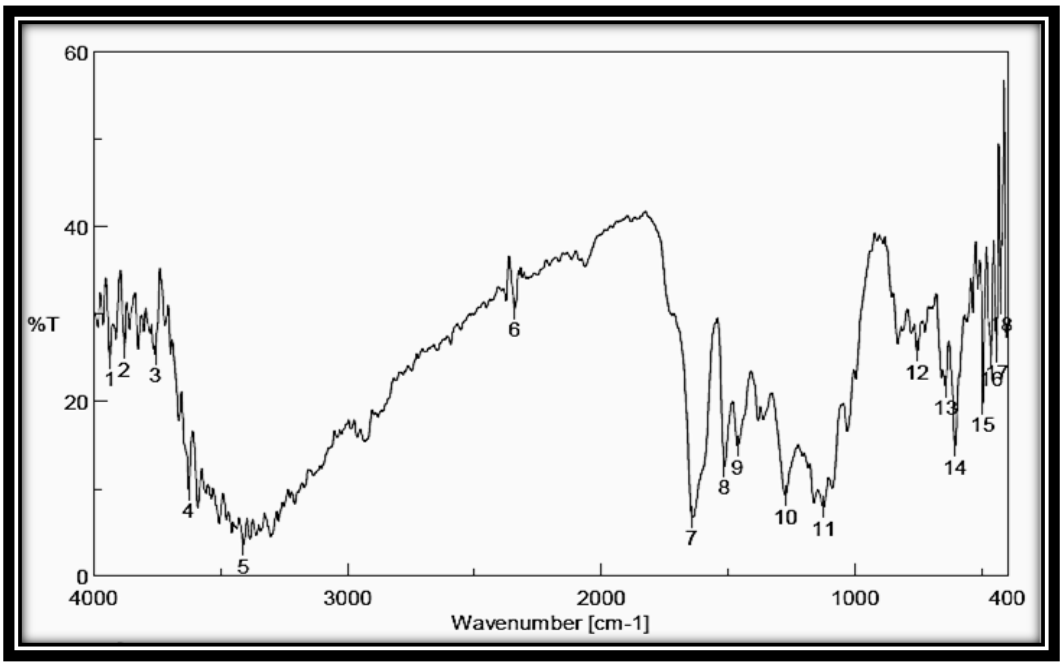


Figure 3: FTIR – spectrum of doxorubicin

Table 3: FTIR interpretation of Doxorubicin

Materials	**Standard wave number (cm^{-1})**	**Test wave number (cm^{-1})**	**Functional group assignment**
Doxorubicin	3650-3200	3410.49 3625.52	OH stretching
	1820-1665	1643.05	C=O stretching
	1320-1210	1273.75	C-O-C stretching
	1161-1029	1121.4	In plane =C-H bending

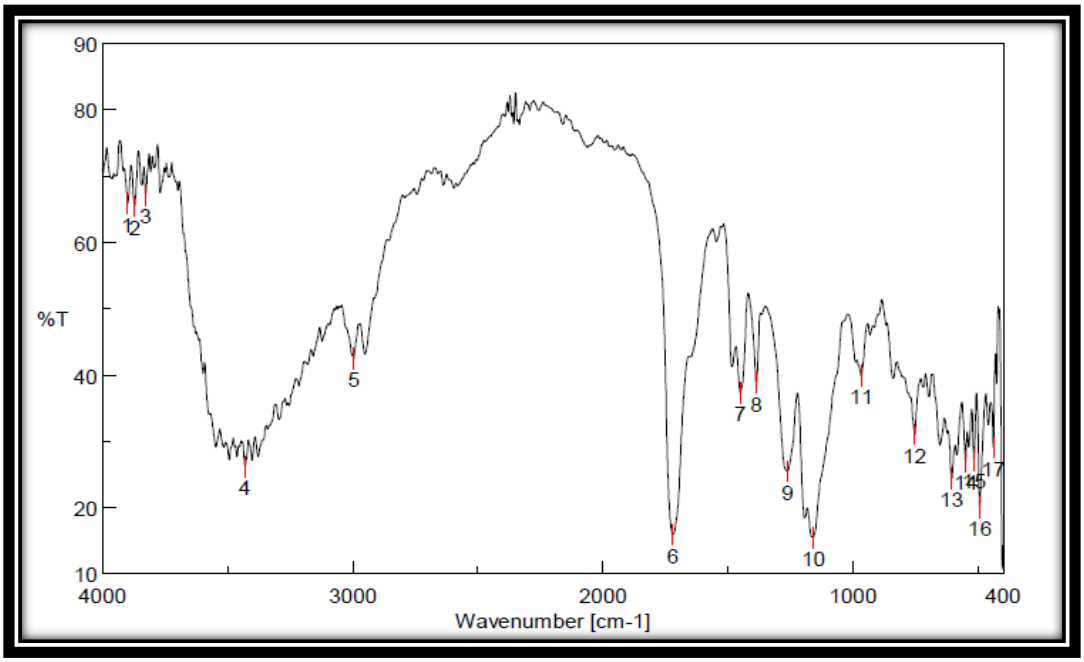


Figure 4: FTIR spectrum of Eudragit

Table 4: FTIR interpretation of Eudragit

Materials	Standard wave number(cm^{-1})	Test wave number (cm^{-1})	Functional group assignment
EUDRAGIT	3000-3700	3430.74	O-H stretching
	1500-1800	1720.19	N-H bending
	2700-3300	2995.87	C-H stretching
	1300-1500	1451.17 1386.57	C-H bending
	1000-1300	1262.18 1159.01	C-O stretching

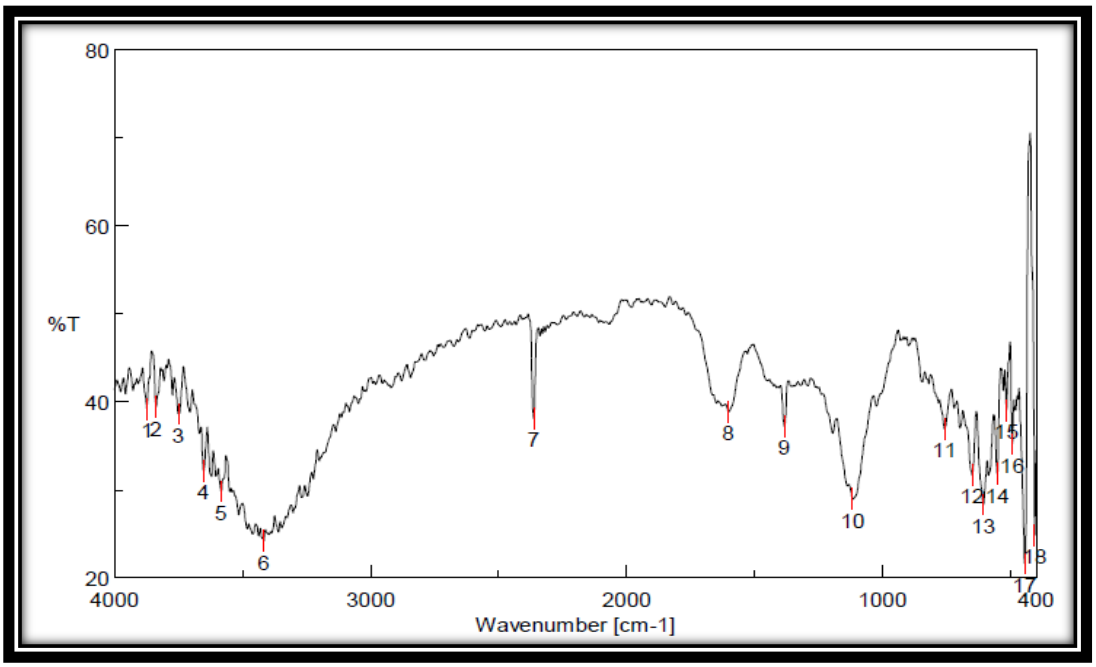


Figure 5: FTIR spectrum of Poly Vinyl Alcohol (PVA)

Table 5: FTIR interpretation of Poly Vinyl Alcohol

Materials	**Standard wave number(cm^{-1})**	**Test wave number(cm^{-1})**	**Functional group assignment**
POLYVINYL ALCOHOL	3300-3600	3584.06	OH stretching
	2850-2970	2862.37	CH_3 stretching
	1500-1760	1600.63	COOH
	1340-1470	1383.68	Alkanes bending
	1000-1300	1116.58	C-O stretching
	600-800	757.888 648.929	C-H rocking

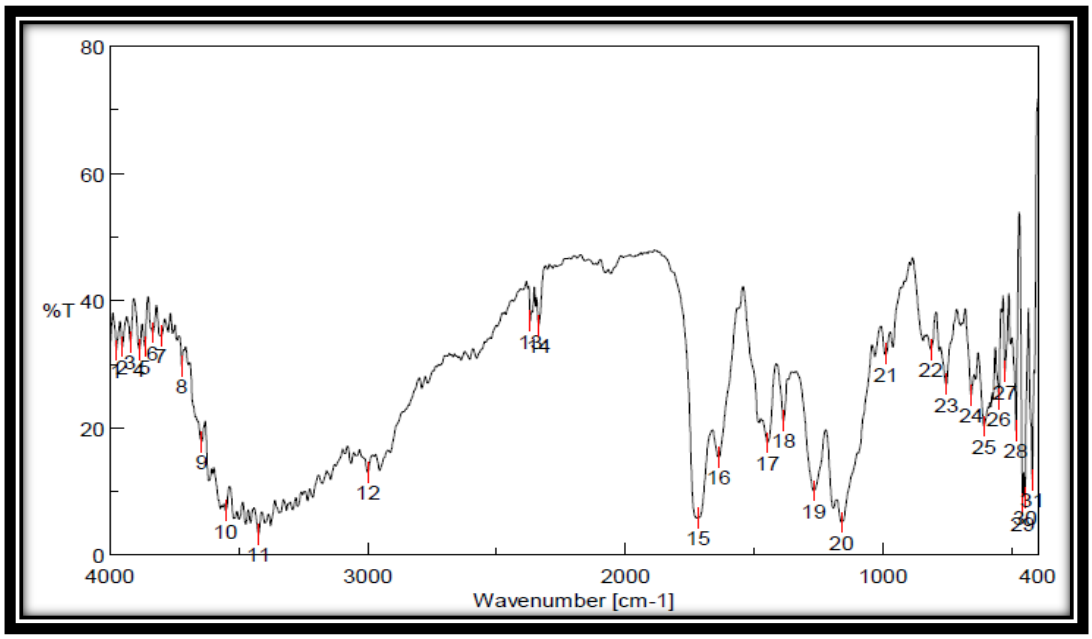


Figure 6: FTIR spectrum of physical mixture containing Doxorubicin, Eudragit and PVA

Table 6: FTIR interpretation of mixture containing Doxorubicin, Eudragit and PVA

Materials	Standard wave number (cm^{-1})	Test wave number (cm^{-1})	Functional group assignment
MIXTURE CONTAINING DOXORUBICIN, EUDRAGIT and PVA	3650-3200	3642.87 3423.033	OH stretching
	3300-2700	2999.73	C-H stretching
	1820-1665	1718.26	C=O stretching
	1800-1500	1639.2	N-H bending
	1500-1300	1386.57	C-H bending
	1320-1210	1268.93	C-O-C stretching
	1161-1029	1161.9	In plane =C-H bending
	800-600	814.777 658.571	C-H rocking

The peaks present in the FTIR spectra of pure Doxorubicin are present in the FTIR spectra of physical mixture containing Doxorubicin with ethyl cellulose and doxorubicin with eudragit. It is therefore evident that the Doxorubicin is compatible with the excipients ethyl cellulose

eudragit and poly vinyl alcohol and can be chosen for the formulation of Doxorubicin nanosponges.

II. FORMULATION OF NANOSPONGES

Selection of polymers for the formulation of doxorubicin nanosponges by emulsion solvent diffusion method was based on the trial batches carried out by using different polymers such as ethyl cellulose, eudragit, sodium alginate, HPMC, Carbopol, hydroxyl ethyl cellulose, chitosan and pectin and details are depicted in table 15. Drug: polymer ratio was selected based on the literature. The results indicated that ethyl cellulose and eudragit was found to be suitable for the formulation of doxorubicin nanosponges.

Table 7: Trial batches for formulation of doxorubicin nanosponge

Drug	Polymer	Ratio	Result observed
DOXORUBICIN	Ethyl cellulose	1:2	Product obtained
	Eudragit	1:2	Product obtained
	Hydroxy propyl methyl cellulose	1:2	Less yield
	Hydroxyl ethyl cellulose	1:2	Less yield
	Carbopol	1:2	Gel like product
	Sodium alginate	1:2	Gel like product
	Chitosan	1:2	No product
	Cyclodextrin	1:2	No product
	Pectin	1:2	No yield

Total ten formulations (F1 – F5 and F6 – F10) of doxorubicin nanosponges with two different polymers ethyl cellulose and eudragit in different ratios were formulated by emulsion solvent diffusion method as given in Table 16 and Table 17.

Table 8: Formulation of Doxorubicin nanosponges

S. No	Formulation code	Drug	Polymer	Drug: polymer ratio
1	F1	DOXORUBICIN	Ethyl cellulose	1:0.5
2	F2		Ethyl cellulose	1:1
3	F3		Ethyl cellulose	1:1.5
4	F4		Ethyl cellulose	1:2
5	F5		Ethyl cellulose	1:3
6	F6		Eudragit	1:0.5
7	F7		Eudragit	1:1
8	F8		Eudragit	1:1.5
9	F9		Eudragit	1:2
10	F10		Eudragit	1:2.5

Table 9: Formulation of Doxorubicin nanosponges by emulsion solvent diffusion technique

S. No	Formulation code	Weight of drug (mg)	Weight of polymer (mg)	Weight of polyvinyl alcohol(mg)
1	F1	100	50	200
2	F2	100	100	200
3	F3	100	150	200
4	F4	100	200	200
5	F5	100	300	200
6	F6	100	50	200
7	F7	100	100	200
8	F8	100	150	200
9	F9	100	200	200
10	F10	100	250	200

III. CHARACTERISATION OF DOXORUBICIN NANOSPONGES

FTIR Spectroscopy of doxorubicin nanosponges

FTIR Spectrum of doxorubicin nanosponges using ethyl cellulose is given in figure 7.

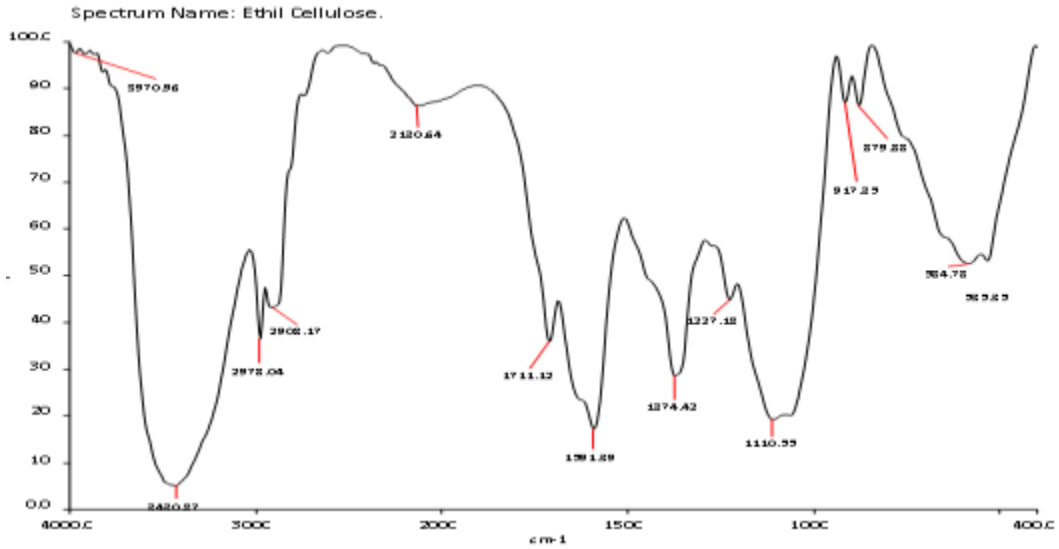


Figure 7: FTIR interpretation of doxorubicin nanosponges using Ethyl cellulose

Table 10: FTIR interpretation of doxorubicin nanosponges using Ethyl cellulose

Materials	Standard wave number (cm-1)	Test wave number (cm-1)	Functional group assignment
FORMULATION F4	3650-3200	3615.88 3478.95	OH stretching
	2970-2850	2876.31	C-H stretching
	1725-1665	1668.2	C=O stretching
	1161-1029	1114.65	Inplane =C-H bending
	800-600	876.488 643.144	C-H rocking

FTIR Spectroscopy of Doxorubicin Nanosponges

FTIR spectrum of Doxorubicin nanosponge using eudragit is given in Figure 8.

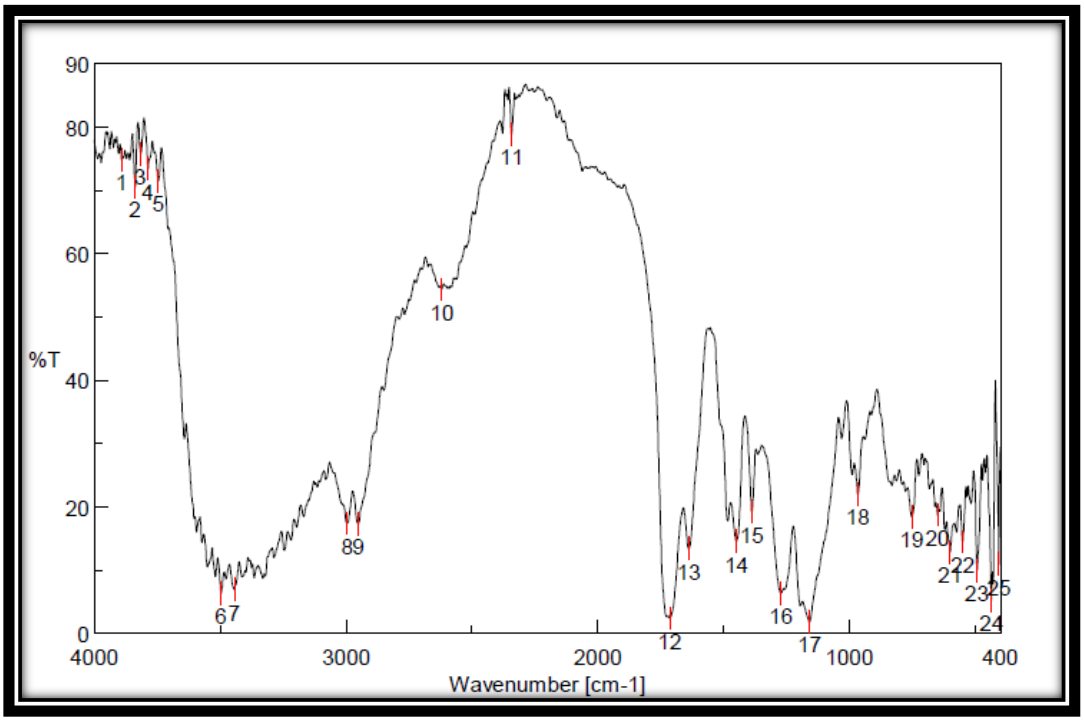


Figure 8: FTIR spectrum of Doxorubicin nanosponges using Eudragit

Table 12: FTIR interpretation of Doxorubicin nanosponges using Eudragit

Materials	Standard wave number(cm^{-1})	Test wave number(cm^{-1})	Functional group assignment
FORMULATION F9	3650-3200	3497.27 3444.24	OH stretching
	3300-2700	2993.94 2951.52	C-H stretching
	1820-1665	1714.41	C=O stretching
	1800-1500	1638.23	N-H bending
	1500-1300	1449.24	C-H bending
	1320-1210	1271.82	C-O-C stretching
	1161-1029	1159.01	In plane =C-H bending
	800-600	753.066 648.929	C-H rocking

The peaks present in the FTIR spectra of pure Doxorubicin are present in the FTIR spectra of formulations. The FTIR interpretations indicated that the Doxorubicin is compatible with the excipients eudragit and poly vinyl alcohol and no interactions observed in all formulations of nanosponges.

Percentage yield analysis

Percentage yield of the formulated Doxorubicin nanosponges were calculated using the formula:

$$Percentage\ Yeild = \frac{Practical\ yield}{Theoritical\ yield} \times 100$$

Table 13: Percentage yield of Doxorubicin nanosponges

S. No	Formulation code	Percentage yield (%)
1.	F1	65.76
2.	F2	71.25
3.	F3	73.28
4.	F4	78.16
5.	F5	80.34
6.	F6	32.08
7.	F7	34.14
8.	F8	48.57
9.	F9	49.75
10.	F10	57.22

The percentage yield was minimum for formulation F6 (32.08%) and maximum for formulation F5 (80.34%). From the results we can conclude that as the concentration of polymer increases the percentage yield also increases. It can also be noted that the yield obtained while using ethyl cellulose as polymer is much higher when compared with eudragit. The percentage yield of all formulations is depicted in Figure 9.

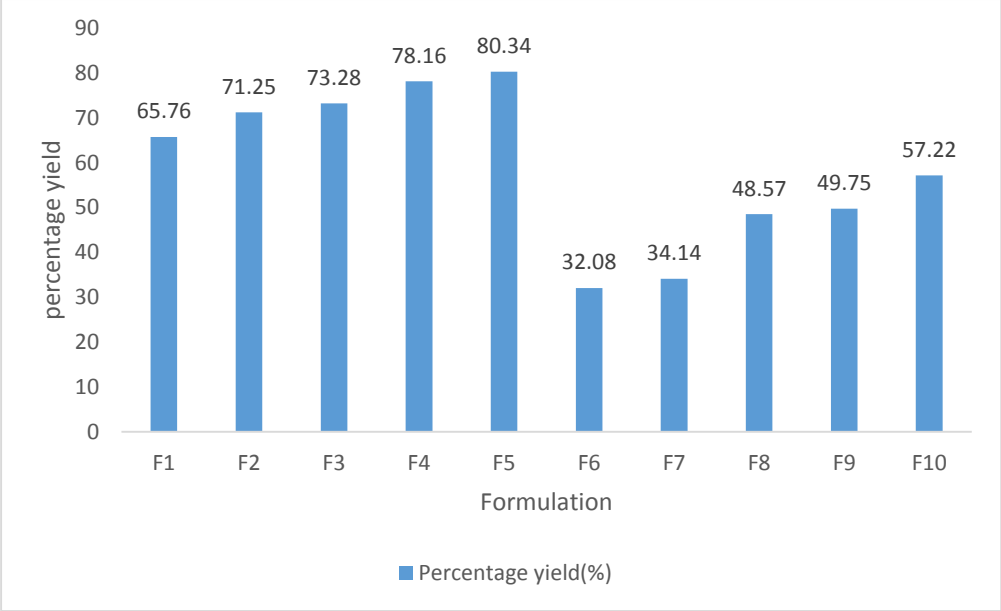


Figure 9: Percentage yield analysis of Doxorubicin nanosponges

Scanning Electron Microscopy

SEM analyses of the formulated Doxorubicin nanosponges were performed to evaluate the surface morphology of nanosponges. The SEM images of formulation F9 are shown in Figure 10.

SEM images showed the nanosponge was porous with a smooth surface morphology and spherical in shape. The spongy and porous nature of the nanosponges can be seen in the above figures.The presence of pores was due to the impression of diffusion of the solvent dichloromethane.

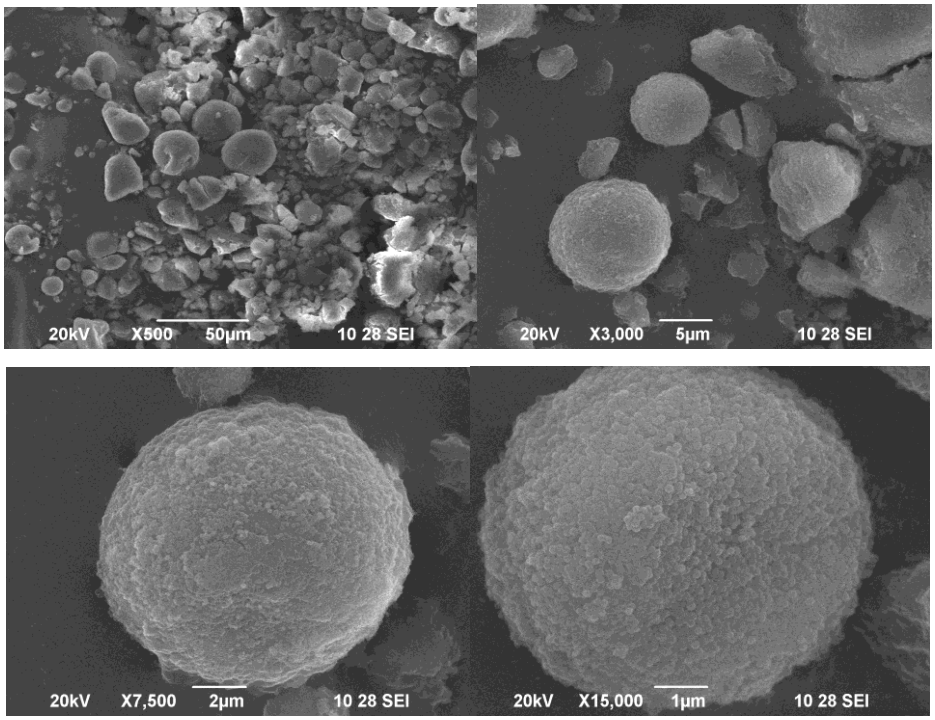


Figure 10: SEM images of Doxorubicin nanosponges using eudragit

Particle Size Measurement

The particle size is one of the most important parameter for the characterisation of nanosponges. The average particle sizes of the prepared Doxorubicin nanosponges were measured using Malvern zeta sizer.

Particle size analysis showed that the average particle size of Doxorubicin nanosponges formulated using eudragit (F9) was found to be 4097 nm with polydispersity index (PDI) value 1.000 and with intercept 1.41. The zeta size distribution of ethyl cellulose –Doxorubicin nanosponges is depicted in Figure 11.

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	1.708e4	Peak 1:	1.117	100.0	1.490e-8
PdI:	0.767	Peak 2:	0.000	0.0	0.000
Intercept:	1.46	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report

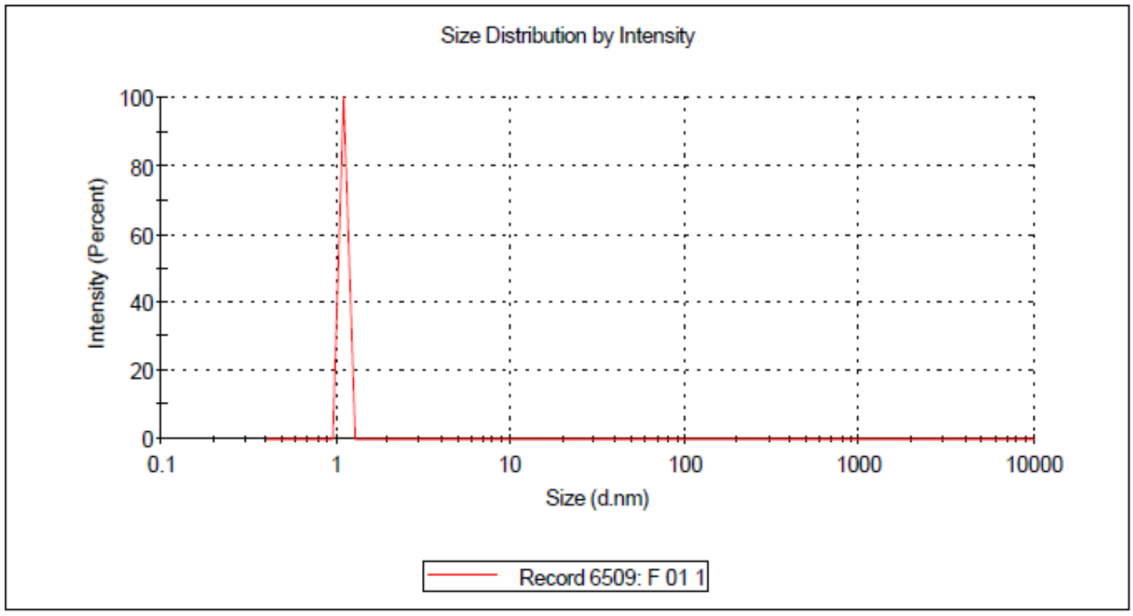


Figure 11: Zeta size distribution of Doxorubicin nanosponges

The average particle size analyses of eudragit-Doxorubicin nanosponges are 1.708which is lesser than 5µm.

Determination of Zeta Potential

Zeta Potential was determined using Malvern zeta-sizer instrument. Zeta potential analysis is carried out to find the surface charge of the particles to know its stability during storage. The magnitude of zeta potential is predictive of the colloidal stability. Nanoparticles with zeta potential value greater than +25 mV or less than -25 mV typically have high degrees of stability.

For Doxorubicin nanosponges using eudragit zeta potential was found to be -24.3mV with peak area of 100% intensity. These values indicate that the formulated Doxorubicin nanosponges are stable. Zeta potential distribution of Doxorubicin nanosponges prepared using eudragit is depicted in Figure 12.

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -24.3	**Peak 1:**	-24.3	100.0	6.96
Zeta Deviation (mV): 6.96	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm): 0.0111	**Peak 3:**	0.00	0.0	0.00
Result quality : Good				

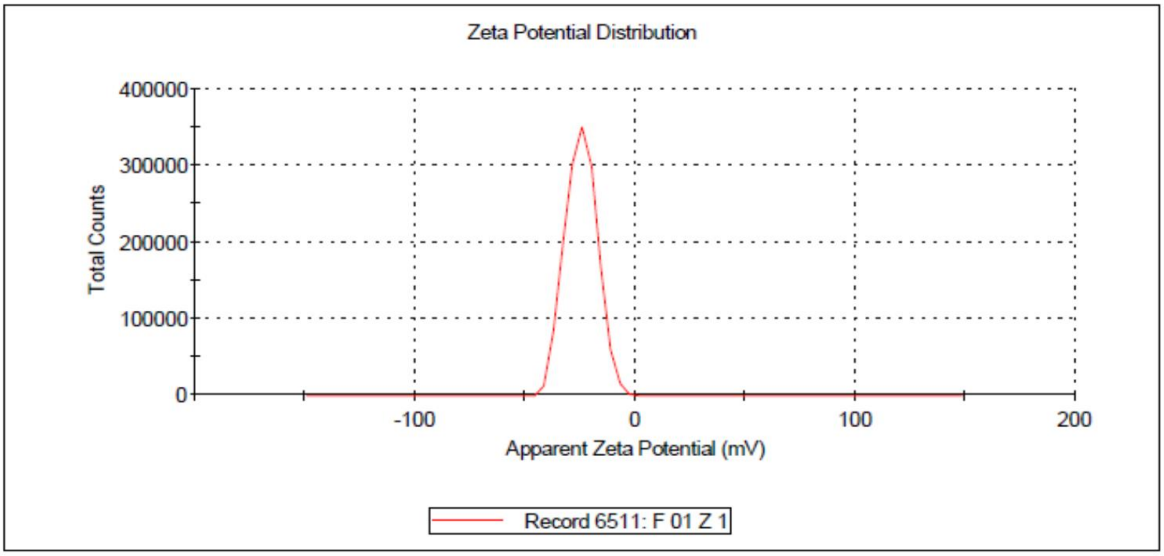


Figure 12: Zeta potential of Doxorubicin nanosponges

Entrapment efficiency:

The amount of entrapped drug was calculated from the equation:

$$\%\ Drug\ Entrpment = \frac{Practical\ drug\ content}{Theoretical\ drug\ content} \times 100$$

Entrapment efficiency of prepared formulation is given in Table 14 and Figure 13.

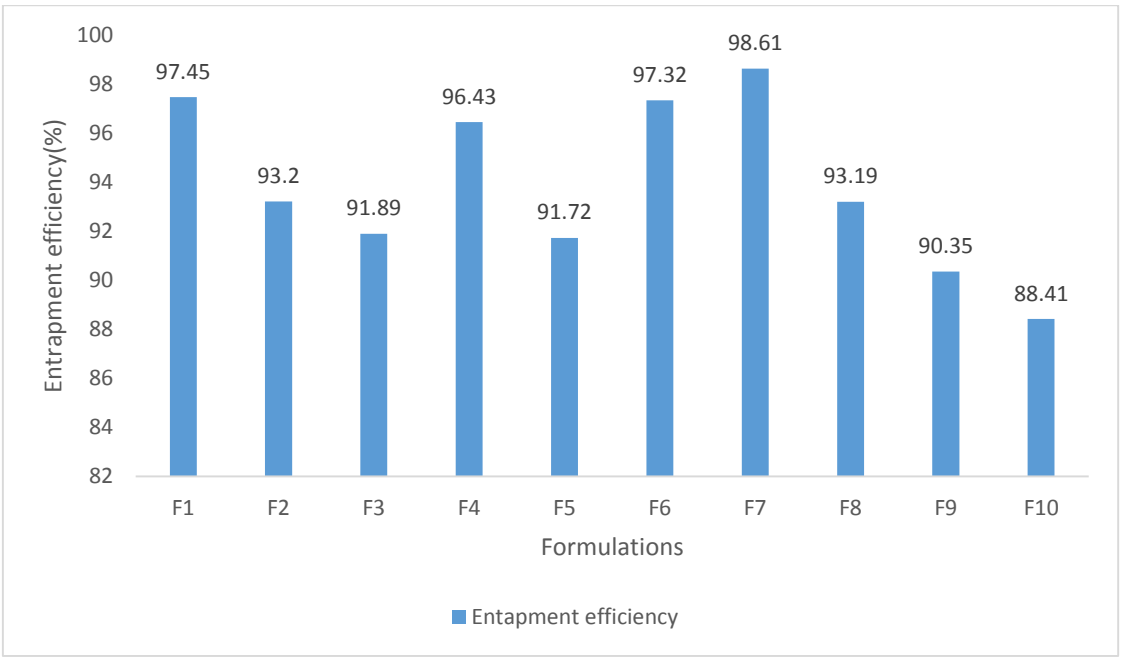


Figure 13: Entrapment efficiencies of Doxorubicin nanosponges

Table 14: Entrapment efficiencies of Doxorubicin nanosponges

S. No	Formulation code	Entrapment Efficiency (%)
1.	F1	97.45
2.	F2	93.20
3.	F3	91.89
4.	F4	96.43
5.	F5	91.72
6.	F6	97.32
7.	F7	98.61
8.	F8	93.19
9.	F9	90.35
10.	F10	88.41

The entrapment efficiency was found to be highest for F7 formulation which is 98.61 and the lowest entrapment of drug was found for F10 formulation. This might be due to the fact that the variation in entrapment efficiency was due to the changes in the polymer concentration and difference in the degree of cross linking. The prepared nanosponges possess high drug entrapment efficiency and were found to be in the range of 88.40%-98.61%.

IN VITRO DRUG RELEASE STUDIES

In vitro drug release study of the prepared. Doxorubicin nanosponges was carried out using dialysis bag diffusion method. Amount of drug released in different time intervals were observed.

In vitro drug release profile data of Doxorubicin nanosponges containing ethyl cellulose (F1-F5) are given in Table 15 and Figure 16.

Table 15: *In vitro* drug release profile of Doxorubicin nanosponges (F1-F5)

Sl.No	Time (hrs)	Cumulative percentage drug release (%)				
		F1	F2	F3	F4	F5
1	0	0	0	0	0	0
2	1	10.90	11.93	11.08	7.36	7.23
3	2	18.62	20.26	15.7	9..33	8.96
4	3	21.76	24.89	19.39	10.13	9.89
5	4	26.00	30.01	21.24	13.11	11.54
6	5	30.23	37.37	25.86	16.93	14.89
7	6	37.94	42.73	27.71	22.19	18.16
8	7	43.47	47.03	32.33	26.35	23.54
9	8	45.18	50.96	35.68	29.71	28.18
10	10	50.04	52.74	42.46	33.53	30.13
11	12	52.14	55.16	46.89	40.05	38.91
12	24	63.17	64.73	56.86	53.83	49.75
13	32	69.90	69.16	64.90	58.12	53.67
14	36	77.18	75.44	69.17	61.92	59.11
15	48	89.90	88.79	81.75	72.86	67.56

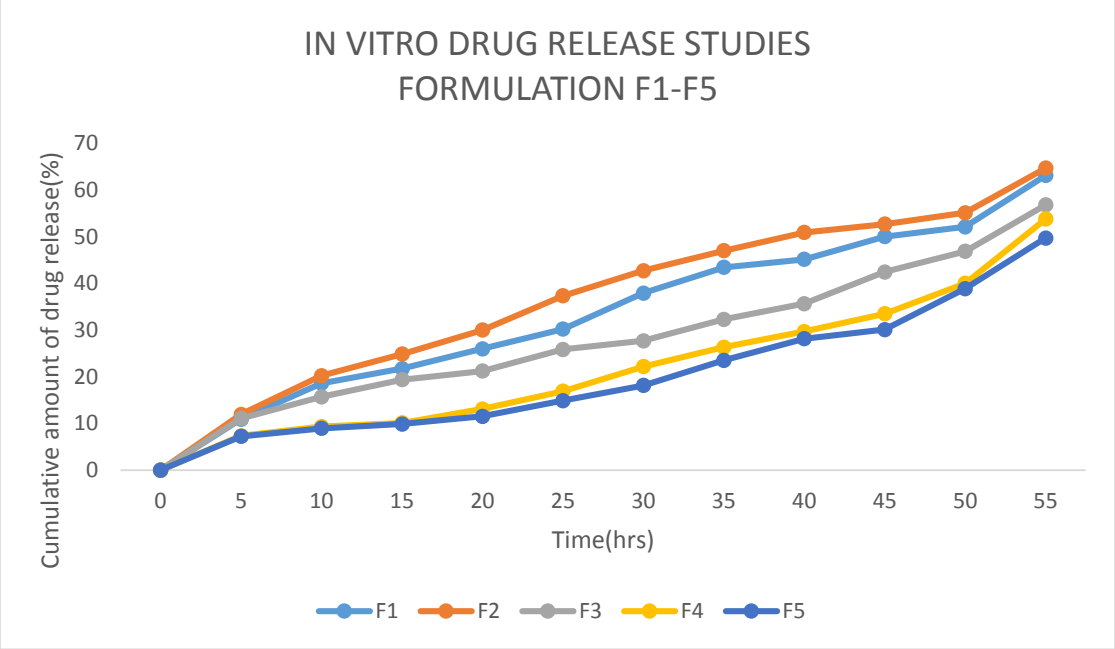


Figure 14: *In vitro* drug release profile of Doxorubicin nanosponges (F1-F5)

In vitro drug release profile data of doxorubicin nanosponges containing eudragit (F6-F10) are given in Table 16 and Figure 15.

Table 16: In vitro drug release profile of doxorubicin nanosponges (F6-F10)

Sl. No	Time (hrs)	Cumulative percentage drug release (%)				
		F6	F7	F8	F9	F10
1	0	0	0	0	0	0
2	1	13.44	14.32	14.06	8.99	7.45
3	2	16.48	18.35	17.77	10.27	9.06
4	3	22.39	22.14	22.26	11.30	10.87
5	4	27.18	27.04	24.41	13.10	12.12
6	5	31.4	30.05	29.05	13.87	15.68
7	6	36.16	34.24	32.02	16.44	18.86
8	7	41.64	41.08	36.57	20.55	24.98
9	8	45.19	43.61	39.09	23.76	29.12
10	10	51.4	49.35	43.43	36.99	32.19
11	12	54.16	53.67	48.13	40.18	39.16
12	24	62.41	62.53	55.89	48.91	50.80
13	32	70.85	68.51	61.24	55.16	54.89
14	36	76.18	73.27	66.75	61.19	60.23
15	48	90.18	87.10	77.94	70.14	69.86

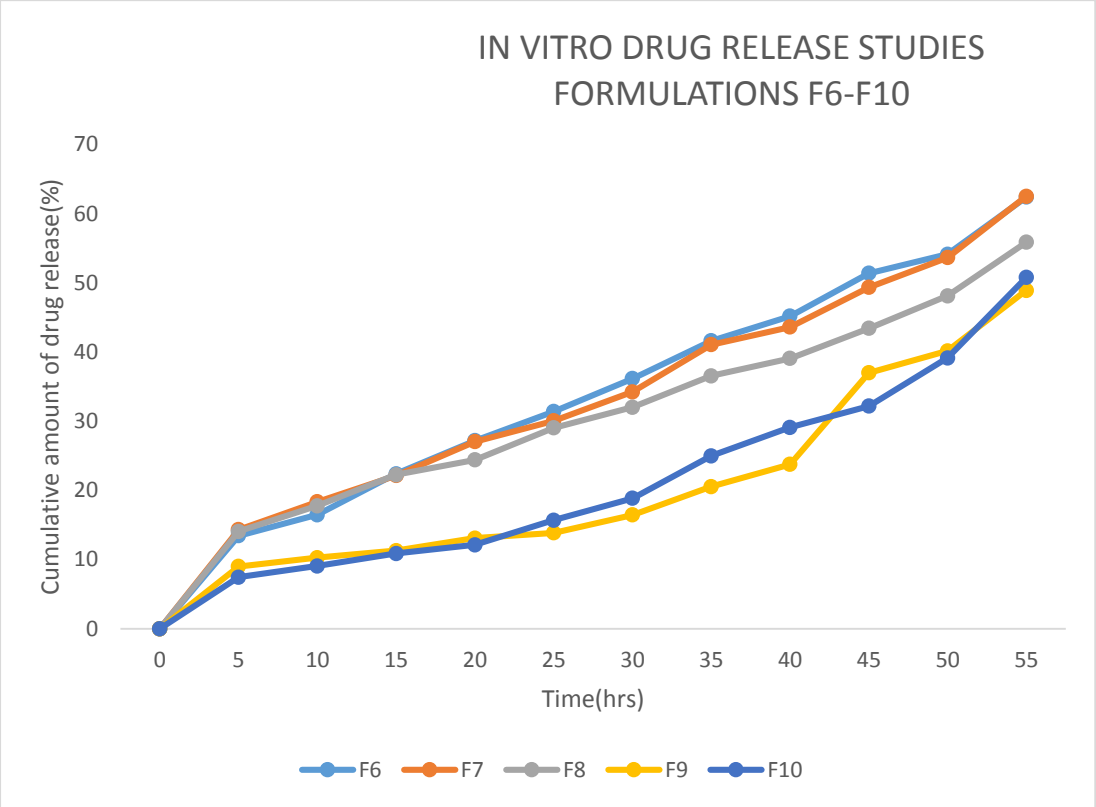


Figure 15: In vitro drug release profile of doxorubicin nanosponges (F6-F10)

From the in vitro release data it was found that formulation F1 and F2 showed the best release of 89.90% and 88.79% respectively at the end of 48 hours among all the five formulation of doxorubicin – ethyl cellulose nanosponges. Similarly F6 and F7 exhibited the best release of 90.19% and 87.10% respectively at the end of 48 hours amoung all the five formulations of doxorubicin – eudragit nanosponges. The release rate was related to drug: polymer ratio. Increase of drug release was observed as a function of drug: polymer ratio. It was observed that the drug release decreased with an increase in the amount of polymer for each formulation. This may be due to the fact that the release of drug from the polymer matrix takes place after complete swelling of the polymer and as the amount of polymer in the formulation increases the time required to swell also increases. These result are in agreement with the release pattern of doxorubicin nanoparticles observed by Hui-ping-sun et al (2016).

The newly developed nanosponges exhibit a core shell structure with a hydrophobic core formed by either ethyl cellulose (F1-F5) and eudragit (F6-F10) and a hydrophilic shell formed by PVA macromolecules. The release showed a bi-phasic pattern with an initial burst effect may due to the unentrapped drug adsorbed on the surface of the nanosponges, while remaining drug released for further few hours say around 7-8 hours may stem from drug molecule physically entrapped with in hydrophilic outer shell. At the same time, hydrophilic PVA molecules that from the shell could also solubilize within aqueous medium and release part of drug. Remaining drug is probably entrapped within the core of nanosponges and are released in the later time period.

SUMMARY AND CONCLUSION

Nanosponges are microscopic particles with few nanometers wide cavities, in which a large variety of substances can be encapsulated. These particles possess the ability to carry both lipophilic and hydrophilic substances and thereby improving the solubility of poorly water soluble molecules. Drugs encapsulated within the nanosponge pores are shielded from premature destruction and stability of drug is enhanced.

Main objective of this study was to formulate doxorubicin loaded nanosponges using polymer to target cancer cells (breast cancer, colorectal cancer or oesophageal cancer) and release the drug in a controlled manner. This formulation reduced the side effects, minimized the dosing frequency and dose.

The present work aimed at formulating doxorubicin nanosponges with polymer name hydrophobic polymer using emulsion solvent diffusion method. This method was simple and cost effective.

Preformulation studies were carried out to find out the solubility of doxorubicin. Solubility test gave an idea that doxorubicin is water soluble and soluble in solvents like acetone, dichloromethane etc.

FTIR and UV spectral studies authenticate the spectra obtained with the sample drug matched with standard pure drug. UV spectra gave the maximum absorption peak at 232nm.

The comparison of FTIR spectra of doxorubicin and mixture of doxorubicin and polymer confirms that there is no appearance of additional new peaks and disappearance of existing peaks from that of the drug. This indicates that there is no interaction between the drug and polymer used in the study.

Formulation was carried out by emulsion solvent diffusion method. Trial batches indicated that hydrophilic polymers are not suitable for the doxorubicin nanosponges. The hydrophilic polymers produced no yield or very less yield. Hydrophobic polymers produced good formulations.eudragit were selected for further studies.

Scanning electron micrograph of the prepared nanosponges at different magnification showed that the nanosponges were porous with a smooth surface morphology and spherical shape. The spongy and porous nature of nanosponges was clearly observed in the SEM images.

Particle size and zeta potential was determined by Malvern Zeta sizer. The particle size analysis confirmed that the prepared sample were in the nanometer range. Average particle size obtained for the formulations F9 is 1.708e4. Zeta potential values of nanosponges indicated that the formulated nanosponges are stable.

The amount of drug being entrapped in nanosponges was calculated and all the prepared nanosponges were found to possess very high entrapment efficiency.

From the *in-vitro* release data from the dialysis bag diffusion method it was found that formulations F1 to F5 & F6 to F10 showed the best release of 89.90, 88.79, 81.75, 72.86, 67.56 and 90.18, 87.10, 77.94, 70.14, 69.86 respectively at the end of 48 hours. Increase of drug release was observed as a function of drug: polymer ratio. It was observed that the drug release decreased with an increase in the amount of polymer for each formulation. This is because the newly developed nanosponges is believed to exhibit a core shell structure with a hydrophobic core formed by eudragit and a hydrophilic shell formed by PVA macromolecules.

CONCLUSION

The doxorubicin nanosponges can be formulated by cost effective and easy emulsion solvent diffusion method using hydrophobic polymers such as eudragit. The formulated doxorubicin nanosponges can be used in the treatment of breast cancer. This can be targeted to the cancer cells and produce sustained drug delivery which in turn reduces the dose, frequency of administration and the side effects.

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