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FORMULATION AND EVALUATION OF DOXORUBICIN NANOSPONGES

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ABSTRACT

The main objective of the study is to formulate Doxorubicin loaded nanosponges. The nanosponges were prepared by Emulsion solvent diffusion method using various concentrations of Eudragit and Ethyl cellulose. 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 in putrate. 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 prepared nanosponges were evaluated for particle size, zeta potential, entrapment efficiency and *in vitro* drug release. The prepared nanosponges have the average particle size was found to be 4097nm with PDI value 1.000 zeta potential was found to be -24.3mV. The above results are confirmed the prepared nanosponges is used for the breast cancer treatment.

KEYWORDS

Formulation Doxorubicin and Nanosponges.

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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 nanosize 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 'crosslink' 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 it score. 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 orotheraqueous 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 other 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.

EXPERIMENTALMETHODS

Pre-formulation 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 (10mg) of doxorubicin raw material in 100ml of volumetric flask. The drug was dissolved in few ml of ethanol and the volume was made up to get a stock solution of 100µg/ml.

Selection of Wavelength

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

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 4ml were withdrawn to 10ml of volumetric flask and then made up volume with phosphate buffer pH7.4 to get a concentration range of 5-40µg/ml. The absorbance of these solutions was measured at 288nm using JASCOV-530UV1600 UV-visible spectrophotometer. Phosphate buffer pH7.4 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 20.209gm of disodium hydrogen phosphate and 8gm 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 JASCO4100). 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.

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 20ml of an organic solvent dichloromethane. Aqueous phase consist of specified amount of poly vinyl alcohol dissolved in 100ml distilled water. Disperse phase was added drop by drop into aqueous phase by stirring on magnetic stirrer at 1000rpm 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.

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 in traction between the doxorubicin and polymers.

A few mg of sample (doxorubicin nanosponges) was weighed and mixed with 100mg 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 4000-400cm-1 in IR spectrophotometer.

Determination of percentage yield

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

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

Scanning electron microscopy (SEM)

SEM analysis was performed to determine their microscopic characters (shape and morphology) of prepared doxorubicin nanosponges. Nanosponges were prepared and dried well to remove the moisture content and images were taken using scanning electron microscopy (HitachiX650, Tokyo, Japan) in different magnifications. Samples were placed on glass slide keptundervacuum and then by using sputter coater unit, samples were coated with a thing old 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 characterization of stability of the nanosponges.

Determination of Entrapment Efficiency

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

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

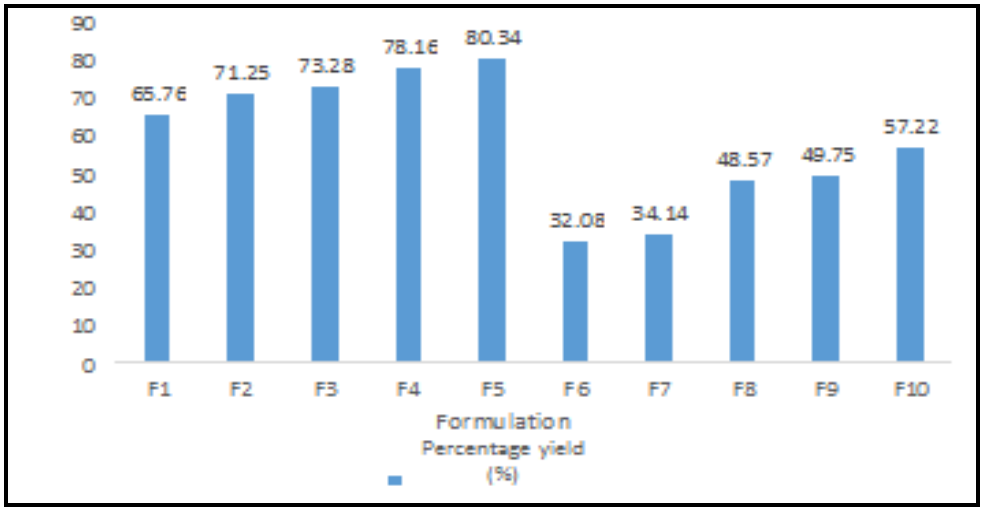
***In-vitro* release studies**

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 (100rpm, room temperature). At predetermined time intervals, 2ml 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 288nm. Aliquots withdrawn were assayed at each time interval for the drug released at λmax of 288nm 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.

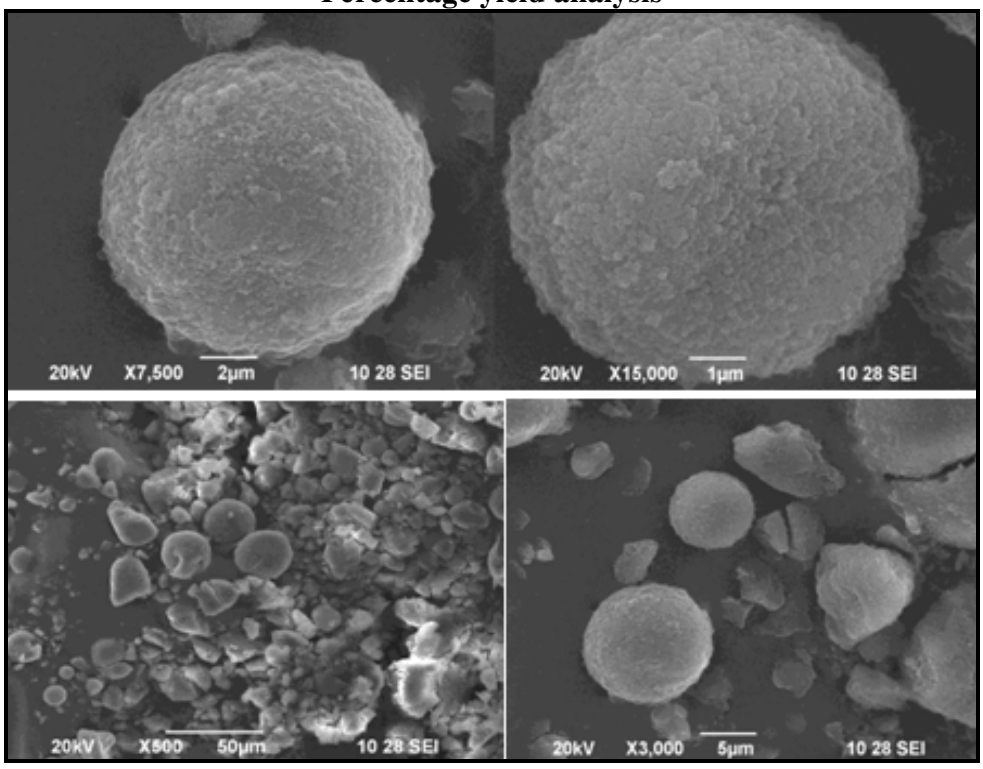
RESULTS AND DISCUSSION

Solubility

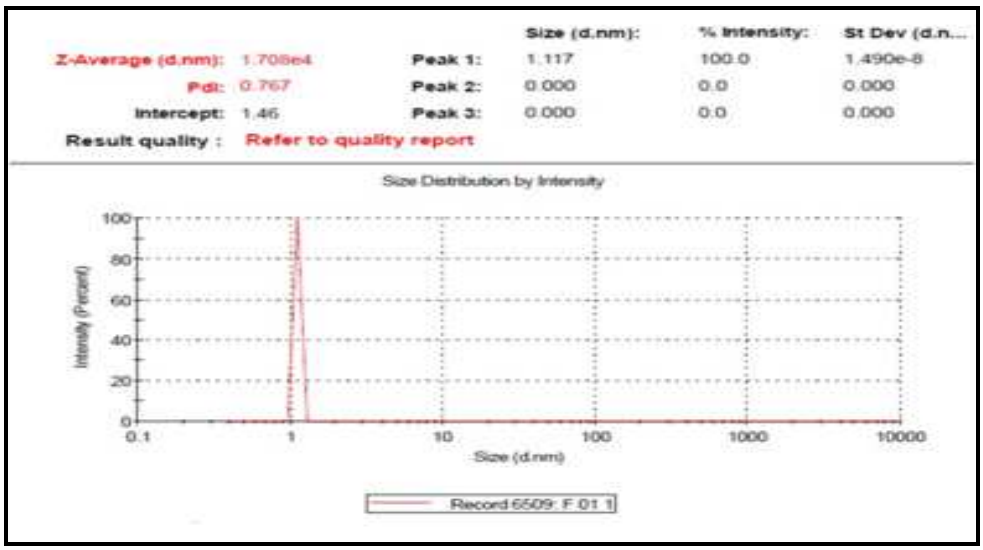
S.No	Solvent	Soluble	Sparingly Soluble	Insoluble
1	Ethanol	✓	-	-
2	Dichloromethane	✓	-	-
3	Chloroform	-	✓	-
4	Water	✓	-	-



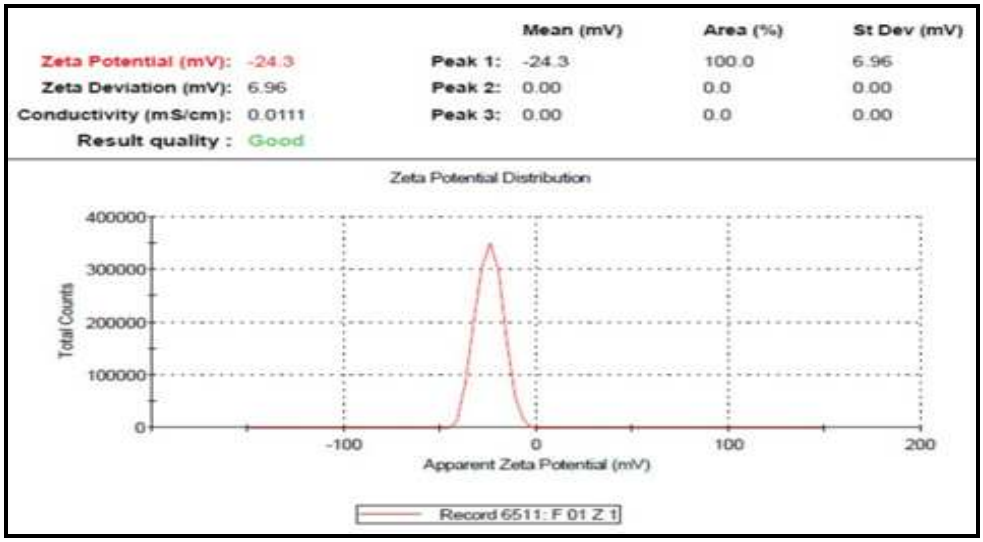
Percentage yield analysis



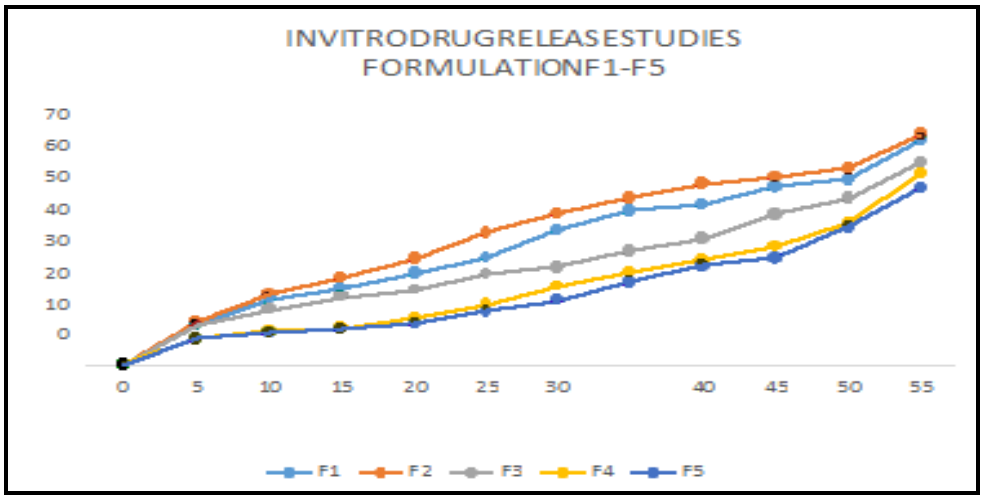
Scanning Electron Microscopy

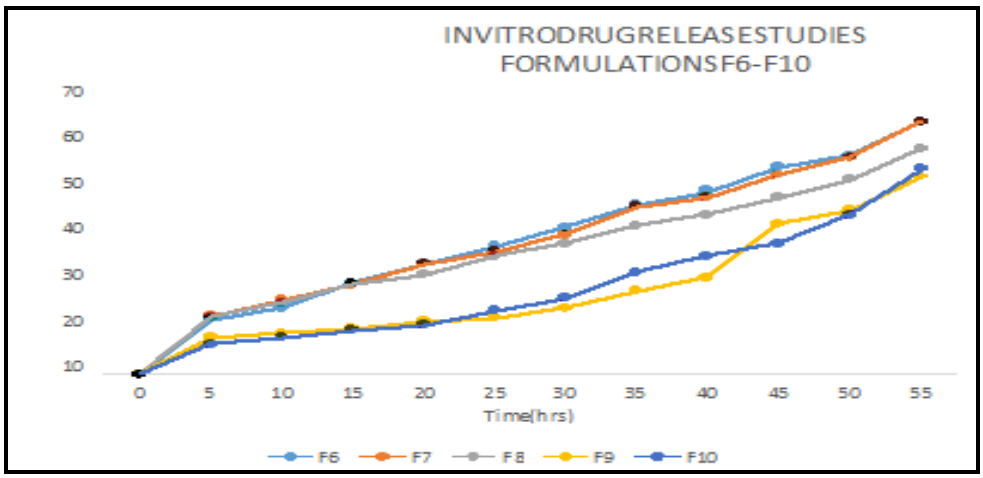


Particle Size Measurement



Determination of Zeta Potential





***In Vitro* Drug Release Studies**

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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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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