INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

FORMULATION AND EVALUATION OF ANTICANCER DRUG (DOXORUBICIN) LOADED NANOSPONGES

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ARTICLE INFO

Article history

Received 09/12/2019

Available online

31/12/2019

Keywords

Doxorubicin,

Nanosponge Gel,

Nanosponge Etc.

ABSTRACT

The purpose of this research was to prepare Doxorubicin loaded Nanosponge gel for Sustained release of drug, increase the drug solubility, and increase the drug permeability, to reduce the dosing frequency and side effects. The FTIR studies proved that there were no interaction between the drug and Polymers. Homogenization technique followed by centrifugation was employed to prepare Nanosponge using various polymers. The formulation were prepared using different Polymers (Ethyl cellulose and Poly methyl methacrylate) in different ratios (Drug: Polymer–1:1, 1:2, 1:3, 1:4 and 1:5,) Using dichloromethane as cross linker as well as solvent. The formulations were characterized for drug entrapment efficiency. The entrapment efficiency of the formulations was observed to be from 97.85 to 99.21. The highest entrapment efficiency was observed with 99.21 and 98.94 for the formulations F3 and F7. The formulations were characterized for drug content. The Drug content of the formulations was observed to be from 82.90 to 95.71. The particle size analysis done by Malvern Zeta sizer showed that the average particle size of Doxorubicin loaded Nanosponge F3 and F7was 231.1nm and 370.3nm respectively. The SEM analysis of Nanosponge shows the spherical surface of the particles. The *in-vitro* release of Doxorubicin Nanosponge optimized formulation F3 was found to be 36.28% and F7 was 45.66% at the end of 24 hours. The drug content of the Gel G1and G2 was found to be 25.15% and 28.88% respectively. The *in-vitro* release of Doxorubicin Nanosponge Gel formulation G1was found to be 23.15% and G2 was 28.88% at the end of 24hours. The pH of the gels G1and G2 was found to be 4.89 and 4.92 respectively. The Viscosity of the gels G1 and G2 was found to be $2.939x10^6$ cps and $2.853x10^6$ cps respectively. It was concluded that the Doxorubicin loaded Nanosponge Gel may have increased the solubility, drug release and Antifungal activity (Increase in Zone of Inhibition), and provide sustained effect The FTIR studies proved that there were no interaction between the drug and Polymers.

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*Please cite this article in press as **B. Raja Narender** et al. Formulation and Evaluation of Anticancer Drug (Doxorubicin) Loaded Nanosponges. Indo American Journal of Pharmaceutical Research.2019:9(12).*

INTRODUCTION

The aim of Novel Drug Delivery System is to provide a therapeutic amount of drug to the appropriate site in the body to accomplish promptly and then maintain the desired drug concentration. The drug-delivery system should deliver drug at a rate control by the necessarily of the body over a specified term of treatment.[1]

This idealized objectives witch to the two aspects most important to drug delivery are as follows[2],

Because of their nanoporous structure, nanosponges can advantageously carry water insoluble drugs (Biopharmaceutical Classification System class-II drugs). These complexes can be used to increase the dissolution rate, solubility and stability of drugs, to mask unpleasant flavors and to convert liquid substances to solids. β-Cyclodextrin based nanosponges are reported to deliver the drug to the target site three to five times more effectively than direct injection[2]. Drugs which are particularly critical for formulation in terms of their solubility can be successfully delivered by loading into the nanosponges.

The nanosponges are solid in nature and can be formulated as Oral, Parenteral, Topical or Inhalation dosage forms. For the oral administration, the complexes may be dispersed in a matrix of excipients, diluents, lubricants and anticaking agents suitable for the preparation of capsules or tablets [9]. For the parenteral administration the complex may be simply carried in sterile water, saline or other aqueous solutions [9]. For topical administration they can be effectively incorporated into topical hydrogel [7, 8].

ADVANTAGES OF NANOSPONGES [11-14]

- Targeted site specific drug delivery.
- This Technology offers entrapment of wide variety of ingredients and reduced side effects.
- Improved Stability, increased elegance and enhanced formulation flexibility.
- Nanosponge systems are non-irritating, non-mutagenic, non-allergenic and non-toxic.
- A Nanosponge provides continuous action up to 12 hours i.e. extended release.
- It minimizes the irritation and it gives better tolerance which leads to improved patient compliance.
- Allows incorporation of immiscible liquids which improves material processing. Liquid can be converted to powders.
- These formulations are stable over wide range of PH (1-11) and temperature (up to 130^{o}c).
- These are self-sterilizing as their average pore size is 0.25µm where bacteria cannot penetrate.
- These are free flowing, highly compatible with wide variety of ingredients and cost effective.
- They have better thermal, physical and chemical stability.
- Nanosponge particles are soluble in water, so encapsulation can be done within the nanosponge, by the Addition of chemical called an adjuvant reagent.

The aim of the present work is to prepare Nanosponges loaded with anti cancer drugs (doxorubicin) for topical delivery

Doxorubicin is a cytotoxic anthracycline antibiotic isolated from cultures of *Streptomyces peucetius var. caesius*. Doxorubicin binds to nucleic acids, presumably by specific intercalation of the planar anthracycline nucleus with the DNA double helix.

Doxorubicin is an antineoplastic in the anthracycline class. General properties of drugs in this class include: interaction with DNA in a variety of different ways including intercalation (squeezing between the base pairs), DNA strand breakage and inhibition with the enzyme topoisomerase II. Most of these compounds have been isolated from natural sources and antibiotics. However, they lack the specificity of the antimicrobial antibiotics and thus produce significant toxicity. The anthracyclines are among the most important antitumor drugs available. Doxorubicin is widely used for the treatment of several solid tumors while daunorubicin and idarubicin are used exclusively for the treatment of leukemia. Doxorubicin may also inhibit polymerase activity, affect regulation of gene expression, and produce free radical damage to DNA. Doxorubicin possesses an antitumor effect against a wide spectrum of tumors, either grafted or spontaneous. The anthracyclines are cell cycle-nonspecific. The purpose of the work i to Improving the Solubility of Poorly soluble drug to enhance its Dissolution and Bio-availability. Thereby its dose can be minimized to reduce the intensity and frequency of adverse effects associated with long term usage. The objective of this study is to consider the development and feasibility of innovative technology such as Solid Dispersion preparation and drug complextion for immediate release delivery of poorly soluble Drugs.

Limitations

The major inconvenience of these nanosponges is their capability to contain only minute molecules. The nanosponges could be whichever Para crystalline or in crystal-line appearance. The load ability of nanosponges depends primarily on extent of crystallization. Para crystalline N.S. can prove unlike loading capacity. The nanosponges can be synthesized to be of unambiguous size and to leave go of drugs in due course by unstable the quantity of cross linker to polymer. Nanosponges have the capacity of encapsulate small molecules, not fitting for superior molecules. Dose clearance may happen at times

List of Materials Used

Doxorubicin, Ethyl cellulose, Polymethyl methacrylate, Polyvinyl alcohol, Dichloromethane, Dimethyl sulfoxide, Potassium dihydrogen ortho phosphate, Disodium hydrogen ortho phosphate, Methanol, Dialysis Bag (LA653), Carbopol 934, Glycerin, Triethanolamine. All the ingredients are the gift samples received from Karthikeya drugs and pharmaceuticals pvt. Ltd. Hyderabad.

METHODOLOGY

Solubility analysis

Preformulation solubility analysis is to be done, which include the selection of a suitable solvent, to dissolve the respective drug as well as various excipients used since the invention of Nanoparticles.

COMPATIBILITY STUDIESFOR DRUG ANDEXCIPIENTS

For the development of formulation dosage form, preformulation studies were carried out to confirm no interaction between the drug and excipients. It gives information needed for selection of excipients with the drug for the formulation of Nanosponge. Physical compatibility of the drug with excipients were done. The possibility of drug excipients (Ethylcellulose and Polymethyl methacrylate) interaction was investigated by FT-IR Spectrum study.

Physical Compatibility

Physical compatibility of the drug and excipients were carried at Room temperature and at $40^{o}C\pm2^{o}C/75\pm5\%$ RH (in days) with the physical admixture of drug and excipients.

Fourier Transforms Infrared (FT-IR) Spectroscopic studies

The spectroscopic studies were carried out to find out the interaction between pure drug, excipients and its physical mixture by KBr pellet technique using FT-IR spectrophotometer. The IR spectrum of the physical mixture is then compared with the spectrum of pure drug (Doxorubicin) to assess the compatibility of the excipients and drug. The scanning range is 450-4000 cm^{-1} and the resolution is $4cm^{-1}$.

Differential Scanning Calorimetry (DSC) studies

Thermograms were obtained by using a scanning calorimeter (New Castle, United States TA 60) at a heating rate 10°c/min. Over a temperature range of 35- 400°c. The sample should be hermetically sealed in an aluminum crucible. Nitrogen gas will be used to purge at a rate of 10 ml/min for maintaining inert atmosphere

STANDARDCURVE OFDOXORUBICIN

100mg of drug was accurately weighed and dissolved in 30ml methanol and made upto100ml with phosphate buffer pH 5.5. Calibration curve was prepared in a mixture of phosphate buffer and methanol (7:3) at λmax 305 nm.

FORMULATION DEVELOPMENT

FORMULATION OF DOXORUBICIN LOADED NANOSPONGE

Doxorubicin Nanosponges were prepared by Emulsion solvent evaporation method. Two different polymers were used in the formulation. Ethylcellulose (EC) and Polymethyl methacrylate (PMMA) were the Polymers used. Polyvinylalcoholin Distilled water is used as the aqueous phase. The Drug is dissolved in the required solvent (Dimethyl sulphoxide) and the Polymers (1:1, 1:2, 1:3, 1:4, and 1:5) were dissolved in Dichloromethane. The Drug solution was poured in to the polymer solution and the mixture was shaken well. Then the Drug polymer mixture was poured into the aqueous phase and the mixer is subjected to homogenization using High speed homogenizer in 1500rpm for 2 hours at $35^{o}C$. The formed Nanosponges were centrifuged by high speed cooling centrifuge and the residue was freeze dried.

Table-1: Formulation of Nanosponge.

S.No	INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1.	Drug(mg)	100	100	100	100	100	100	100	100	100	100
2.	EC(mg)	100	200	300	400	500	-	-	-	-	-
3.	PMMA (mg)	-	-	-	-	-	100	200	300	400	500
4.	PVA (g)	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3
5.	DCM (ml)	20	20	20	20	20	20	20	20	20	20
6.	Distilled water(ml)	100	100	100	100	100	100	100	100	100	100

EC-Ethyl cellulose, PMMA–Polymethyl methacrylate, DCM–Dichloro methane, PVA- Polyvinyl Alcohol.

CHARACTERIZATION OF DOXORUBICIN LOADED NANOSPONGE

All the formulated Doxorubicin loaded Nanosponge were evaluated for its Drug content, entrapment efficiency, particle size distribution, poly dispersity index, *in vitro* drug release and kinetics of drug release.

Determination of drug content

The total drug content of Nanosponge was determined by spectrophotometric analysis. 10milligram equivalent of Doxorubicin loaded Nanosponge taken in a beaker (closed to avoid evaporation) containing (10 ml) of Methanol and stirred for 30 minutes in magnetic stirrer, 3ml of that solution is pipette out and that volume was made up to10ml by using pH5.5 phosphate buffer to make1µg/ml concentration. The absorbance was measured at 305nm λ_{max} using UV spectrophotometer. From the absorbance drug content was calculated.

The percentage Drug Content is calculated by following formula:

$$\%\ \text{Drug content} = \frac{\text{Practical Drug Content}}{\text{Theoretical drug content}} \text{x100}$$

[13]

Determination of drug entrapment efficiency

The entrapment efficiency was determined by measuring the concentration of the drug in the supernatant after centrifugation. The unentrapped Doxorubicin were determined by adding 10 mg Doxorubicin loaded Nanosponge in 10 ml of methanol and then the dispersion were centrifuged at 9,000rpm for 30minutes at 4^{o}C using a cooling centrifuge in order to separate entrapped from the unentrapped drug. The free drug concentration in supernatant layer after centrifugation is determined at λ_{max} (265nm) using UV Spectrophotometer The percentage entrapment efficiency (%EE) is calculated by following formula:

$$\%EE = \frac{\text{Weight of Initial drug} - \text{Weight of free drug}}{\text{Weight of Initial drug}} \text{x100}$$

***in- vitro* release studies**[13]

The *invitro* release of Doxorubicin from Nanosponge was evaluated by the Dialysis Bag diffusion technique. The release studies of Doxorubicin from Nanosponge were performed in Phosphate buffer of pH5.5 and methanol (70:30) 18mg equivalent Doxorubicin Nanosponge were suspended in 10ml of buffer pH 5.5 mixture and placed in the dialysis bag (donor compartment) and sealed at both ends. The dialysis bag were immersed in receptor compartment containing 100ml of buffer mixture, which was stirred at 100rpm and maintain 32±2^{o}C.The receptor compartment was covered to prevent evaporation of the diffusion medium. Samples were taken from the receptor compartment and the same amount was replaced with the diffusion medium. Samples are taken upto24hrs.Doxorubicin in the samples were measured spectrophotometrically at λ 305nm.

Same procedure was carried out for pure drug and *invitro* release were measured spectrophotometrically at λ305nm.

SELECTION AND EVALUATION OF OPTIMIZED FORMULATION

The best formulation selection based on the results obtained from particle size, entrapment efficiency, *in-vitro* release studies and kinetics of drug release.

Morphology of Nanosponge by scanning electron microscopy (SEM) technique

The Surface Morphology of the Nanosponge can be measured by SEM. The formulations are poured in a circular aluminum stubs using double adhesive tape, and coated with gold in HUS–5GB vaccum evaporator and observed in HitachiS–3000NSEMatan acceleration voltage of 10 Kv and a magnification of 5000X.

Particle size distribution:

Particle size (z-average diameter), and poly dispersity index (as a measure of the width of the particle size distribution) of Doxorubicin loaded Nanosponge dispersion is performed by dynamic light scattering also known as photon correlation spectroscopy(PCS) using a Malvern Zeta sizer 3000NanoS (Malvern instruments, UK) at 25°C.

Prior to measurements all samples were diluted using ultra–purified water to yield a suitable scattering intensity. The diluted nanosponge dispersion was poured into disposable sizing cuvette which is then placed in the cuvette holder of the instrument and analyzed. Air bubbles were removed from the capillary before measurement.

Kinetic drug release

To analyze the drug release mechanism, *in-vitro* release are fitted into a

Zero-order

First order

Higuchi,

Hixon-Crowellcube root law,

Korsmeyer-peppas model.

STABILITY STUDIES
STABILITY STUDIES

The stability studies of the optimized Nanosponge were performed at different conditions of temperature and the effect on physical characteristic, entrapment efficiency and drug content was noted. The Nanosponge were kept in the airtight container and stored at 40±2°C and in Relative humidity 75±5% for 45 days. The samples were analyzed for the above parameter in 15 days, 30 days and 45days.

The samples were withdrawn on 15 days, 30days and 45days and checked for changes in Physical appearance and drug content as per ICH QIA (R_2) guidelines.

FORMULATION OF DOXORUBICIN LOADED NANOSPONGE GEL [11, 12]

The formulation of Nanosponge prepared using the optimized ratio of Polymer containing Doxorubicin equivalent to18mg was incorporated into the gel base composed of Carbopol 934 (1%), Glycerol (15%), Triethanolamine (q.s.) and distilled water up to 1g.

Table-2: Preparation of Gel.

S.No.	INGREDIENTS	GELBASE-1	GELBASE-1
1.	Carbapol 934	1 %	2 %
2.	Glycerol	15 %	25 %
3.	Triethanolamine	Quantity Sufficient	Quantity Sufficient
4.	Distilled water	Up to 15g	Up to 20g

EVALUATION OF NANOSPONGE GEL

Physical Appearance

The prepared gel was examined for clarity, color, homogeneity and the presence of foreign particles.

pH

2.5g of gel was accurately weighed and dispersed in 25ml of distilled water. The pH of the dispersion was measured by using a digital pH meter.

Rheological study

Viscosity measurement:
Viscosity measurement:

Viscosity was determined by Brook field viscometer. In the present study, spindle no.S64 with an optimum speed of 0.6rpm was used to measure the viscosity of the preparation.

Content Uniformity:

The drug content of the prepared gel was carried out by dissolving accurately weighed quantity of gel equivalent to 9mg of the drug in a beaker containing 10ml of methanol, stir the solution for 30minutes and centrifuged in High speed cooling centrifuge and 3ml of the solution is made upto10ml with phosphate buffer pH5.5. The samples were analyzed spectrophotometrically at λmax 265nm against blank using UV-Visible spectrophotometer.

***In-vitro* Drug Diffusion study**

In-vitro drug diffusion study was studied using dialysis bag. The Nanosponge gel equivalent to 18mg of the drug was placed in a Dialysis Bag having 8cm length and 3cm breadth; both the sides are tied with thread. This acted as the donor compartment. Then the bag was placed in a beaker containing 100ml phosphate buffered methanol pH5.5, which acted as receptor compartment. The temperature of the receptor medium was maintained at 37°±2°C and the medium was stirred at a speed of 100 rpm using a magnetic stirrer.

5ml of the samples were collected at a predetermined time and replenished immediately with the same volume of fresh buffer PB mixture pH5.5. The sink condition was maintained throughout the experiment. The collected samples were analyzed spectrophotometrically at 265nm using UV-Visible spectrophotometer.

RESULTS AND DISCUSSION

Solubility:

Table:3.

Solvent	Solubility (mg/ml)
Ethanol	62.35±0.21
Water	50.23±0.12
DMSO	41.27±0.14
Alcohol	52.16±0.54
pH 6.8 Phosphate buffer	49.37±0.24

PRE-FORMULATIONSTUDIES

The optimization of a formulation can be done only after a thorough investigation of its physicochemical properties of the drug and excipients. The drug and the polymer must be compatible for a successful formulation.

Physical Compatibility

Table-4: Physical compatibility.

S.No.	Description and condition							
	Drug (D) and excipients	Initial	Room temperature (in days)			40°C±2°C/75±5% RH(in days)		
			10	20	30	10	20	30
1.	Drug	NC	NC	NC	NC	NC	NC	NC
2.	D+EC	NC	NC	NC	NC	NC	NC	NC
3.	D+PMMA	NC	NC	NC	NC	NC	NC	NC
4.	D+PVA	NC	NC	NC	NC	NC	NC	NC

NC – No Change.

Inference

The drug and the excipients of the formulation are physically compatible with each other. They were evaluated for 10, 20 and 30 days at room temperature and at 40°C±2°C/75±5% Relative Humidity.

Chemical compatibility

FT-IR spectroscopy gives the possible information about the interaction between the drug and Polymer.

Drug-Polymer compatibility studies

FT-IR Spectroscopic studies

The compatibility between drug and Polymer was confirmed using FT-IR Spectroscopy. Infrared spectroscopic analysis for drug (Doxorubicin), Polymer, Drug-Polymer admixture was carried out

Table-5: FT-IR Data of Doxorubicin.

S. No.	Peak absorbed at Wavenumber cm^{-1}	Characteristics
1.	3787.90	OH-Stretching
2.	3402.18	NH -Stretching
3.	3008.73	Aromatic CH- Stretching
4.	2923.87	Aliphatic CH- Stretching
5.	1627.80	Carboxylic C=O-Stretching

Inference

The peak shows the presence of NH, OH, aromatic CH, aliphatic CH and Carboxylic C=O groups in the Doxorubicin sample.

Table-6: FT-IR Data of Ethyl cellulose.

S. No.	Peak absorbed at Wavenumber cm^{-1}	Characteristics
1.	2977.87	Aliphatic CH -Stretching
2.	1635.52	CH(methyl)-Stretching

Inference

The peak shows that the Ethyl cellulose has Characteristics Aliphatic CH and Methyl CH -stretching.

Table-7: FT-IR Data of Polymethyl methacrylate.

S. No.	Peak absorbed at Wavenumber cm^{-1}	Characteristics
1.	1735.81	C=O -Stretching
2.	2923.87	Aliphatic CH -Stretching
3.	1535.22	CH (methyl)-Stretching

Inference

The peak shows that the Characteristics C=O, Aliphatic CH and Methyl CH– stretching.

Table-8: FT-IR Data of Doxorubicin with PMMA.

S. No.	Peak absorbed at Wave number cm^{-1}	Characteristics
1.	3787.92	OH -Stretching
2	3433.04	NH-Stretching
3.	3001.02	Aromatic CH-Stretching
4.	2954.73	Aliphatic CH-Stretching
5.	2854.44	Carboxylic OH -Stretching
6.	1735.81	C=O-Stretching

Inference

The peak observed in the FTIR Spectrum of Doxorubicin pure drug with Polymer (PMMA) showed no shift and no disappearance of characteristic peaks of pure drug suggesting no interaction between the drug and Polymer.

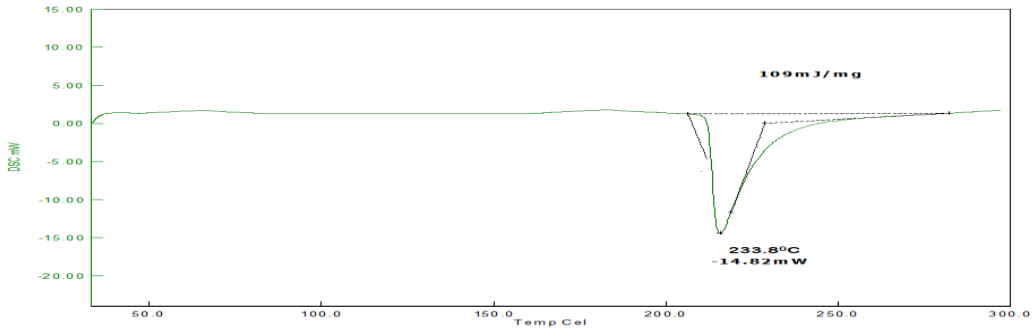
Table-9: FT-IR Data of Doxorubicin with Ethyl cellulose.

S. No.	Peak absorbed at Wave number cm^{-1}	Characteristics
1.	3787	OH -Stretching
2.	3400	NH-Stretching
3.	2954	Aliphatic CH-Stretching
4.	1700	Carboxylic OH -Stretching

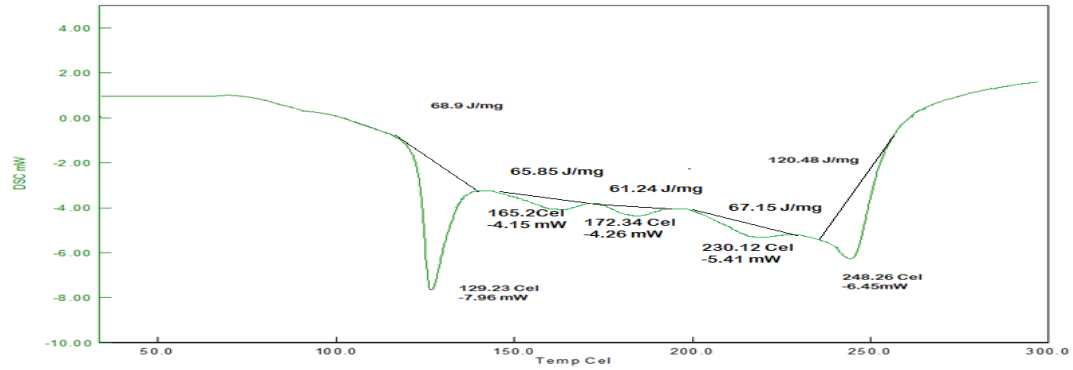
Inference

The peak observed in the FTIR Spectrum of Doxorubicin pure drug with Polymer (Ethyl cellulose) showed no shift and no disappearance of characteristic peaks of pure drug suggesting no interaction between the drug and Polymer.

DSC STUDIES



DSC spectrum of pure drug



DSC spectrum of pure drug with polymer mix

STANDARDCURVE OF DOXORUBICIN

The UV spectrometric method was used to analyze Doxorubicin. The absorbance of the drug in phosphate buffered methanol pH5.5. (70:30) was measured at a wavelength of 305nm. The results are given in the Table and Figure

CALIBRATION CURVE

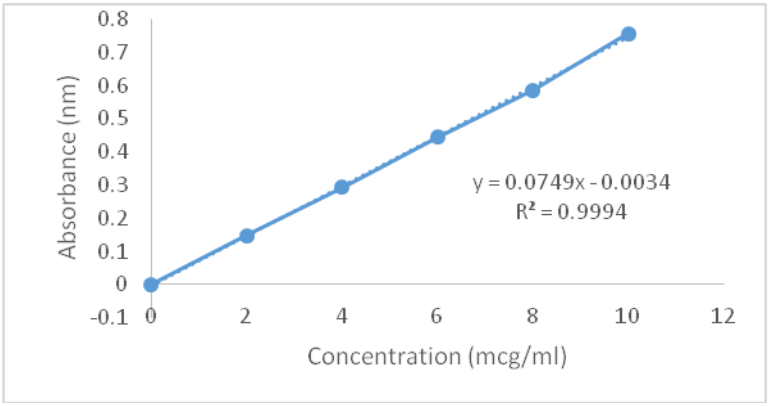


Figure-1: Calibration curve for Doxorubicin.

Inference

It was found that the solutions of Doxorubicin in Phosphate buffered methanol pH5.5 show linearity (R^2=0.999) in absorbance at concentrations of 2-10mcg/ml and obey Beer Lambert's law.

FORMULATION OF DOXORUBICIN NANOSPONGE

Doxorubicin Nanosponge was prepared by homogenization technique followed by centrifugation.

CHARACTERIZATION OF NANOSPONGE

Drug content and entrapment Efficiency

Table-10: Drug content and Entrapment Efficiency of Doxorubicin Nanosponge.

FORMULATION CODE	%DRUG CONTENT (%w/v)	% ENTRAPMENT EFFICIENCY (%w/v)
F1	94.52±0.109	98.79
F2	91.31±0.633	98.14
F3	90.26±0.266	98.94
F4	92.54±1.173	98.86
F5	82.90±1.239	97.85
F6	90.36±0.366	96.57
F7	95.71±0.231	99.21
F8	85.44±0.555	85.44
F9	88.95±0.524	88.95
F10	86.70±0.708	86.70

SURFACE MORPHOLOGY OF NANOSPONGE

Surface morphology of Nanosponge was measured by Scanning Electron Microscopy (SEM).

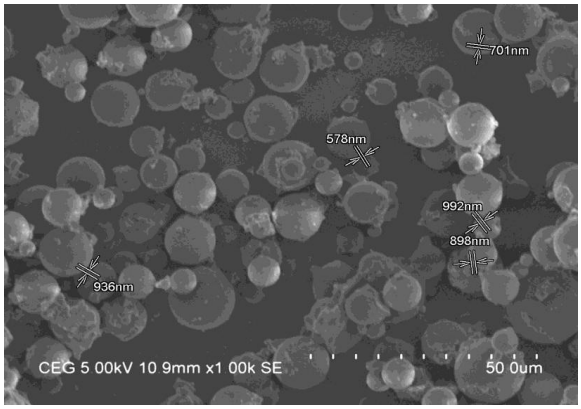


Figure-2: SEM image of optimized Formulation (F7) Inference.

SEM picture shows the formation of Spherical Nanoparticles.
The average particle size of Doxorubicin Nanosponge F7 was 370.3nm and the poly dispersity index was found to be1.000.

in- vitro drug release of Doxorubicin

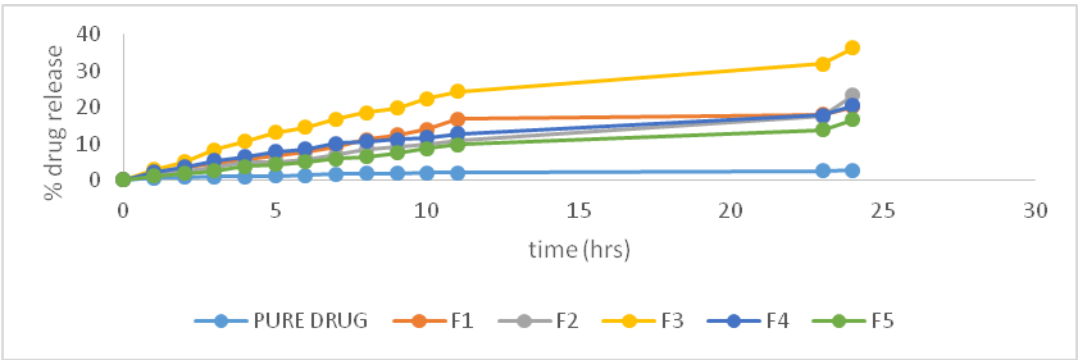


Figure-3: *in- vitro* drug release of Doxorubicin Nanosponge (EC) and pure drug *in-vitro* drug release of pure drug and doxorubicin Nanosponge.

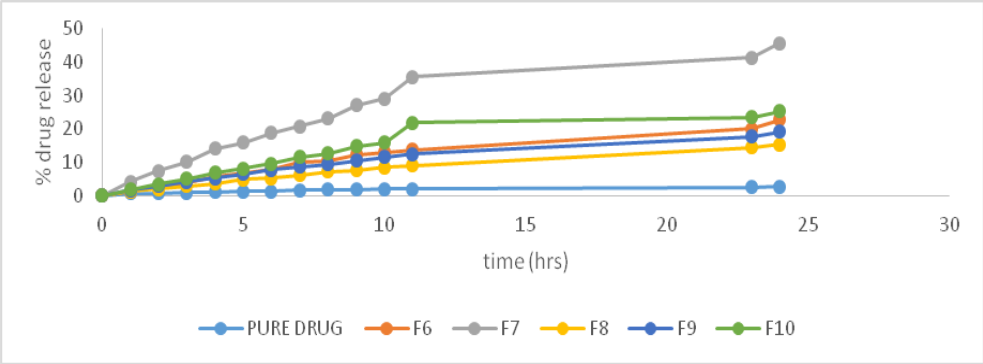
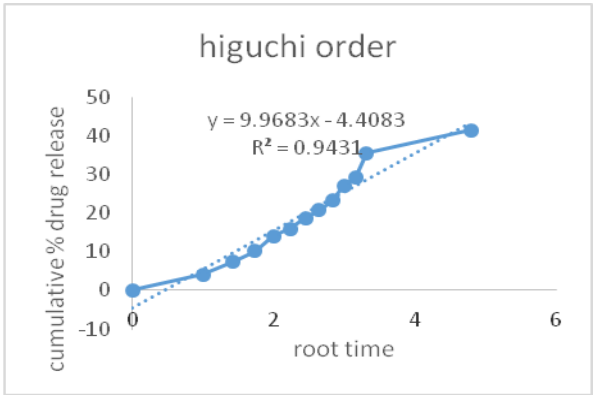


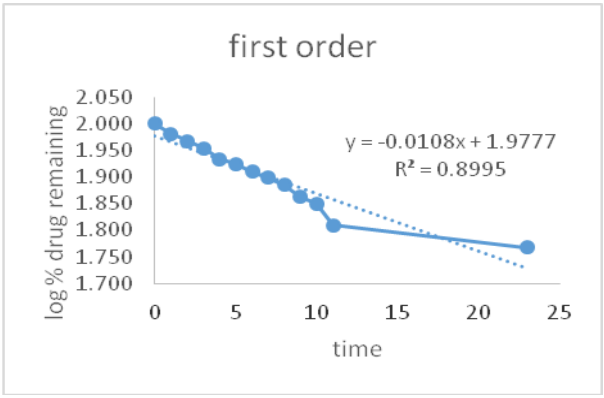
Figure-4: *in-vitro* drug release of Doxorubicin Nanosponge (PMMA) with pure drug. Inference.

The *in-vitro* release of the formulations was observed to be between 15.53% to 45.66%. The results shown that the increase in polymer concentration increases the drug release and then it is decreased as described by Dr Prathima Srinivas *etal*. From the result it was observed that the formulations F3and F7 Showed sustained release of drug.

Table-11: Evaluation of Gel.

S. No.	Code	Physical appearance	%Drug content	Viscosity (cps)	pH	%Drug release
1.	G1	Good	91.10	$2.939x10^6$	4.89	25.15
2.	G2	Good	94.55	$2.853x10^6$	4.92	28.88





Following the above data it was observed that the formulation F7 followed first order release with higuchi mechanism

TABLE 12: STABILITY STUDY OF OPTIMIZED FORMULATION.

Temperature	Room temperature (30 ± 2°C)		Refrigerator (4 ± 2°C)		Accelerated (40 ± 2°C, 75 ± 5% RH)	
Parameters	Physical appearance	Percentage drug content (%)	Physical appearance	Percentage drug content (%)	Physical appearance	Percentage drug content (%)
Freshly prepared	Transparent and smooth	95.836	Transparent and smooth	95.836	Transparent and smooth	95.836
7^{th} day	Transparent and smooth	94.512	Transparent and smooth	95.421	Transparent and smooth	95.515
15^{th} day	Transparent and smooth	94.123	Transparent and smooth	95.053	Transparent and smooth	95.098
30^{th} day	Transparent and smooth	93.883	Transparent and smooth	94.899	Transparent and smooth	95.001
60^{th} day	Transparent and smooth	93.338	Transparent and smooth	94.788	Transparent and smooth	94.879
90^{th} day	Transparent and smooth	93.063	Transparent and smooth	94.593	Transparent and smooth	94.749

Based on the results from the stability studies it was confirmed that the prepared formulation is stable throughout the entire testing period as the drug content levels were constant for most of the time.

CONCLUSION

The purpose of this research was to prepare Doxorubicin loaded Nanosponge gel for Sustained release of drug, increase the drug solubility, and increase the drug permeability, to reduce the dosing frequency and side effects. The drug and the excipients of the formulation are physically compatible with each other. They were evaluated for 10, 20 and 30 days at room temperature and at $40^{o}C\pm2^{o}C/75\pm5\%$ Relative Humidity. The UV spectrometric method was used to analyze Doxorubicin. The absorbance of the drug in phosphate buffered methanol pH5.5. (70:30) was measured at a wave length of 305nm. The average particle size of Doxorubicin Nanosponge F7 was 370.3nm and the poly dispersity index was found to be1.000. The *in-vitro* release of the formulations was observed to be between15.53% to 45.66%. The results shown that the increase in polymer concentration increase the drug release and then it is decreased as described by Dr Prathima Srinivas *etal* .From the result it was observed that the formulations F3and F7 Showed sustained release of drug. Following drug release kinetic data it was observed that the formulation F7 followed first order release with higuchi mechanism.

The future recommendation is that Nanosponges will be conventional as drug delivery arrangement to recap or construct awake for both deliquescent and lip tropic drug by figure a composite. They can productively transport the drug in a forbidden behavior at an intend spot. Nanosponges might be included smitten with topical research such as liniment, cream, ointments etc. And liquid or concentrate form. The profit of this cleverness offer goal the drug to exacting site reduces side effects, get enhanced steadiness, and look up phrasing flexibility and better stable compliance. Nanosponges offer piece of apparatus in other region such as cosmetics, biomedicine, bioremediation method, and catalysis etc. This here study resolves give details a number of calls for of cyclo-epta-amylose underneath Nanosponges as delivery service for anticancer drugs. Present stylish Nanosponges, bright towards be alive amenable to an outside inducement, point be too talk regarding. In vitro and in vivo trial results get hold at present worn objects. Cyclo-epta-amylose -based Nanosponges are able to be watchful a challenging knowledge preordained for the spreading out of pioneering phrasings, suitable for a variety of management direct for anti-cancer drugs.

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