



DESIGN AND CHARACTERIZATION OF IDARUBICIN NANOSPONGES FOR TARGETED DRUG DELIVERY

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ABSTRACT

The current study's goal and purpose were to create and assess Idarubicin nanosponges for targeted distribution. Ethyl cellulose was used as the polymer and polyvinyl alcohol as the stabilising agent to create nanosponges through the solvent evaporation process. The manufactured Nanosponges were assessed for many factors, including the drug: polymer ratio, stirring rate, and time. The formulation underwent encapsulation and a diffusion investigation. It was discovered from this study that the nanosponges were spherical in shape and had a porous nature after doing particle size analysis and scanning electron microscopy on them. The produced nanosponges were put into the carbapol gel basis. An analysis of the nanosponge gel's diffusion was done. Similar to F5, F6 and F7 showed the best release among the five Idarubicin-eudragit nanosponges formulations, with respective rates of 90.19 and 87.10 percent after 48 hours..

Keywords: *Idarubicin, Nanosponges, Solvent Evaporation Method.*

1. INTRODUCTION

Nanosponges have emerged as one of the most promising fields of science because of their perceived application in controlled drug delivery. Nanosponge delivery system can precisely control the release rates or target drugs to a specific body site and have an enormous impact on the health care system. This nanosized delivery system has definite advantages for the purpose of drug delivery because of its high stability, high carrier capacity and feasibility of incorporation of both hydrophilic and hydrophobic substances. The application of nanosponges for targeted and localized delivery of therapeutic agents is the driving force for the research in this area (2). The sponge acts as a three-dimensional network or scaffold. The backbone is long-length polyester. It is mixed in solution with cross-linkers to form the polymer. The net effect is to form spherically shaped particles filled with cavities where drug molecules can be stored. The polyester is biodegradable, so it breaks down gradually in the body. As it breaks down, it releases its drug payload in a predictable fashion. The nanosponges can be synthesized to be specific size and to release drugs over time by varying proportions of crosslinker to polymer. The main limitation of nanosponges is their ability to include only small molecules (3). Nanosponges are solid in nature and are small particles with porous surface can be formulated as oral, parenteral, topical or inhalational dosage forms.

I. DRUG EXCIPIENT COMPATIBILITY STUDIES:

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

II. FORMULATION OF IDARUBICIN NANOSPONGES BY EMULSION SOLVENT DIFFUSION METHOD

Emulsion solvent diffusion method was used to formulate Idarubicin 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 Idarubicin 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 Idarubicin and polymers.

A few mg of sample (Idarubicin 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:

Idarubicin 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 Idarubicin 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 Idarubicin 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 Idarubicin in phosphate buffer were measured by spectrophotometer at 288 nm. Aliquots withdrawn were assayed at each time interval for the drug released at λ_{max} 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.

2. RESULTS AND DISCUSSION

I. PREFORMULATION STUDIES PHYSICAL CHARACTERISTICS:

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

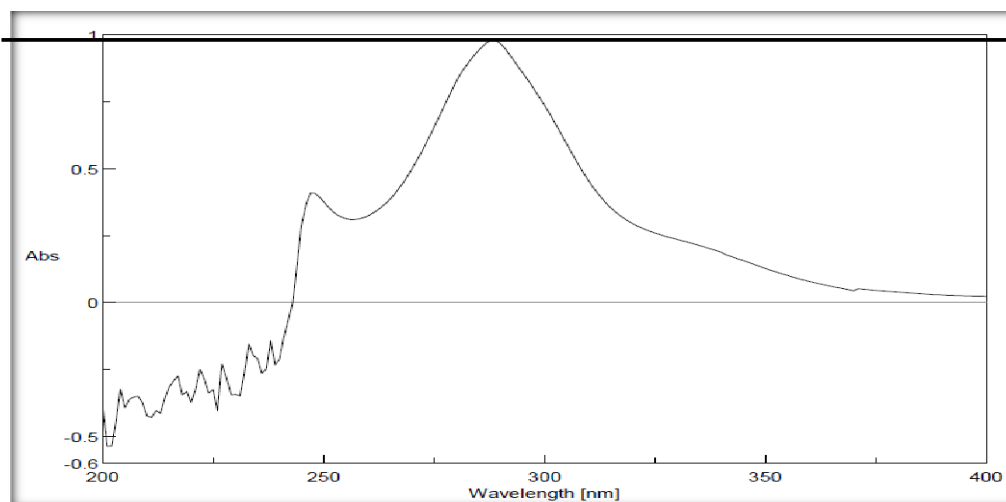
Solubility:

Solubility test for Idarubicin 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 Idarubicin in different solvents

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

Selection of Wavelength:



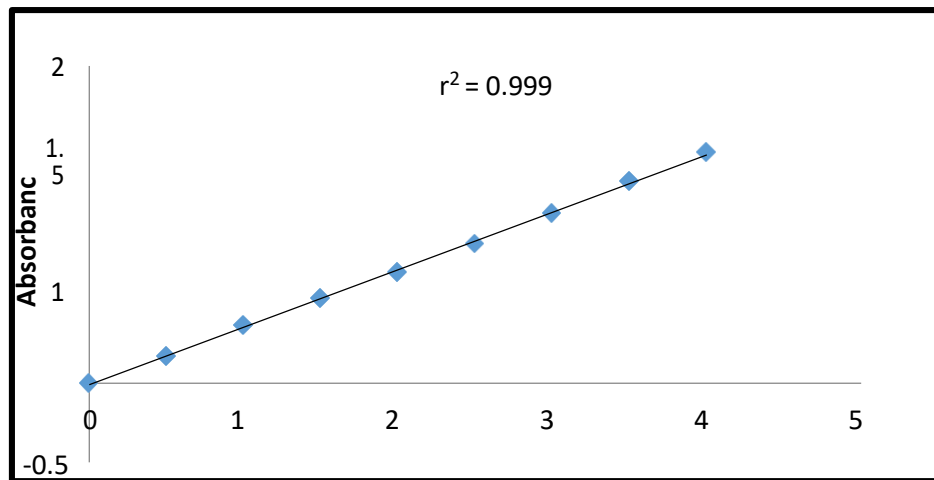
The Idarubicin stock solution of concentration 100µg/mL was scanned in the range of 200- 400nm for λ_{max} . using double beam UV Spectrophotometer. The absorption peak obtained is shown in Figure 1.

Figure 1: UV spectra of Idarubicin

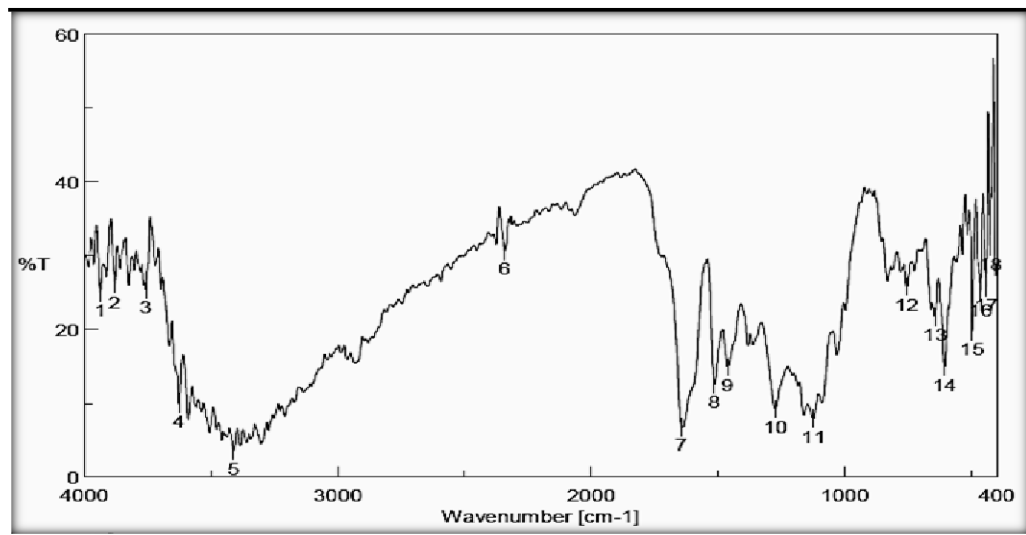
The maximum absorption of Idarubicin was found to be at 232nm and hence it is selected as the wavelength for further studies.

Construction of calibration curve of Idarubicin:

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

**Figure 2: Calibration graph of Idarubicin****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 Idarubicin**

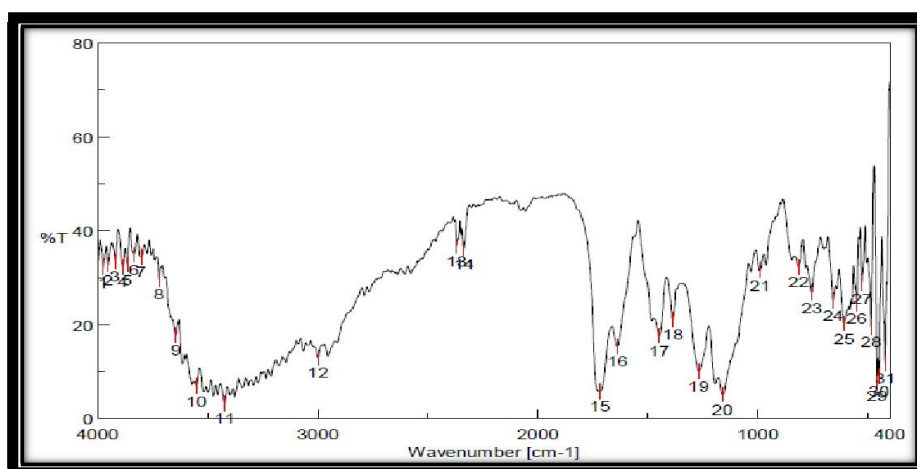


Figure 4: FTIR spectrum of physical mixture containing Idarubicin, Eudragit and PVA Table

The peaks present in the FTIR spectra of pure Idarubicin are present in the FTIR spectra of physical mixture containing Idarubicin with ethyl cellulose and Idarubicin with eudragit. It is therefore evident that the Idarubicin is compatible with the excipients ethyl cellulose eudragit and poly vinyl alcohol and can be chosen for the formulation of Idarubicin nanosponges.

II. FORMULATION OF NANOSPONGES:

Selection of polymers for the formulation of Idarubicin 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 Idarubicinnanosponges.

Table 7: Trial batches for formulation of Idarubicin nanosponge

Drug	Polymer	Ratio	Result observed
IDARUBICIN	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 Idarubicin 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 Idarubicin nanosponges

S. No	Formulation code	Drug	Polymer	Drug: polymer ratio
1	F1	IDARUBICIN	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 Idarubicin 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 IDARUBICIN NANOSPONGES

Percentage yield analysis:

Percentage yield of the formulated Idarubicin nanospheres were calculated using the formula:

$$\text{Percentage Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

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.

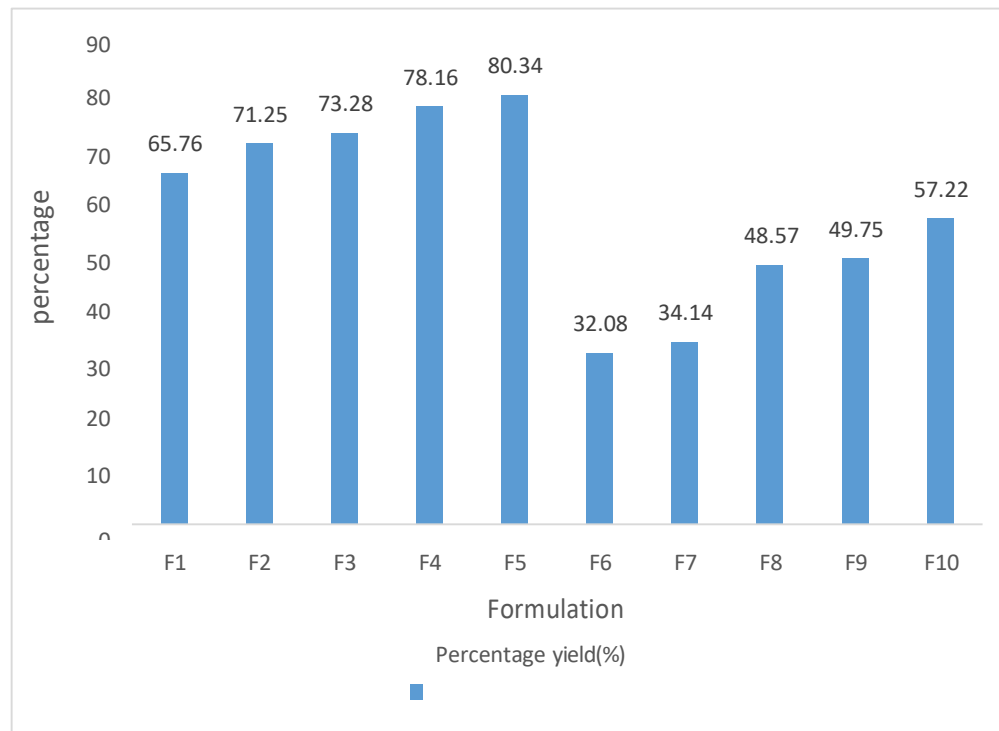


Figure 5: Percentage yield analysis of Idarubicin nanospheres

Scanning Electron Microscopy

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

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

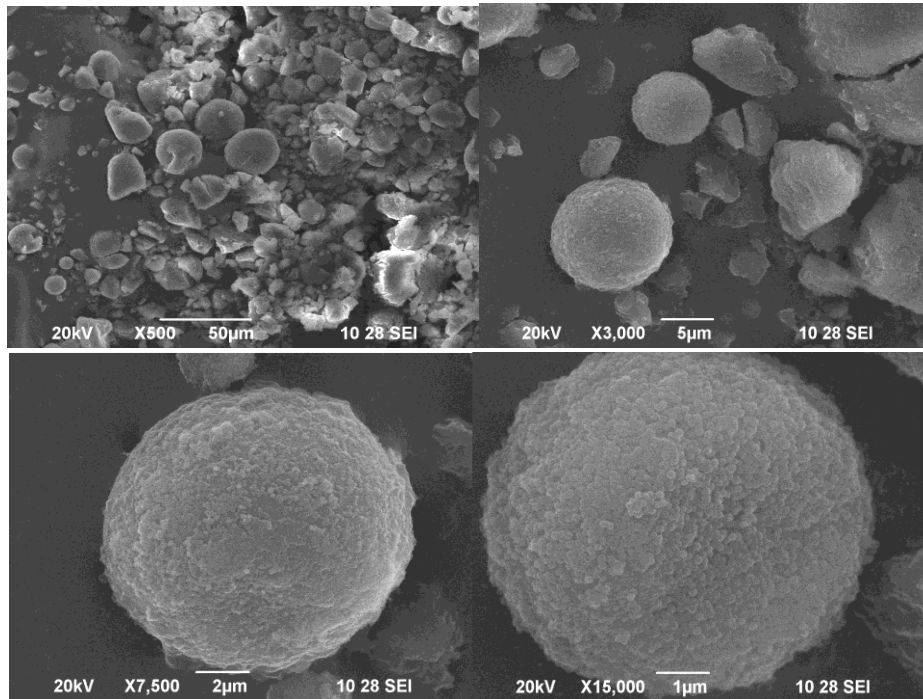


Figure 6: SEM images of Idarubicin nanospheres using eudragit

Particle Size Measurement

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

Particle size analysis showed that the average particle size of Idarubicin nanospheres formulated using eudragit (F9) was found to be 4097 nm with polydispersity index (PDI) value

1.00 and with intercept 1.41. The zeta size distribution of ethyl cellulose –Idarubicin nanospheres is depicted in Figure 11.



Figure 7: Zeta size distribution of Idarubicin nanospheres

The average particle size analyses of eudragit-Idarubicin nanospheres are 1.708 which 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 Idarubicin nanospheres using eudragit zeta potential was found to be -24.3mV with peak area of 100% intensity. These values indicate that the formulated Idarubicin nanospheres are stable. Zeta potential distribution of Idarubicin nanospheres prepared using eudragit is depicted in Figure 12.

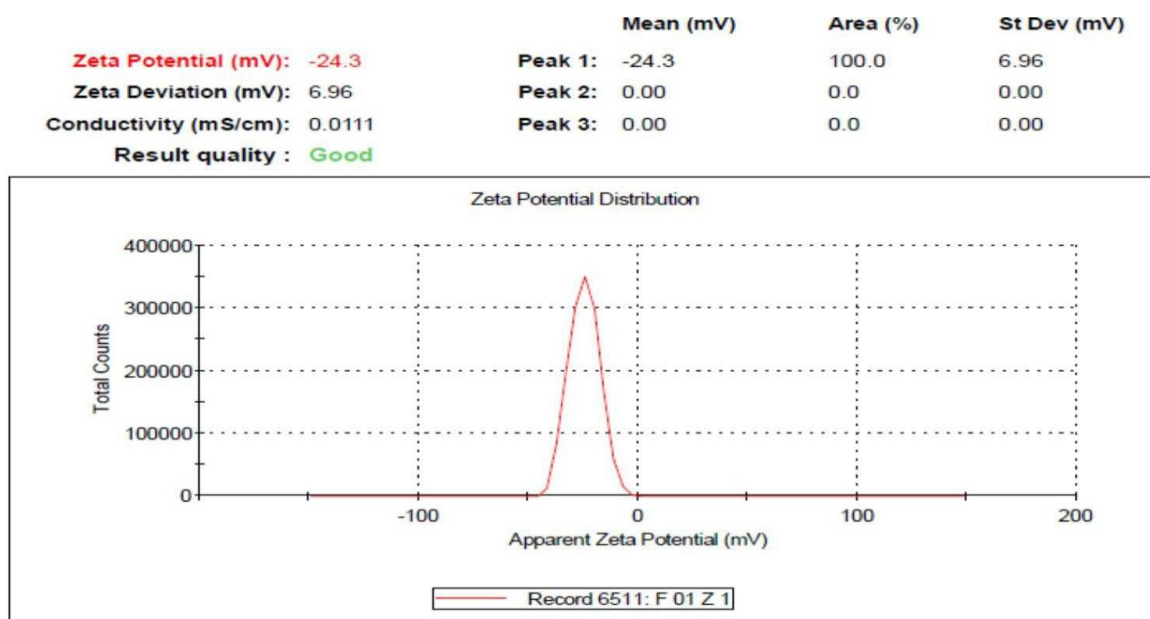


Figure 8: Zeta potential of Idarubicin nanospheres Entrapment efficiency

The amount of entrapped drug was calculated from the equation:

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

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

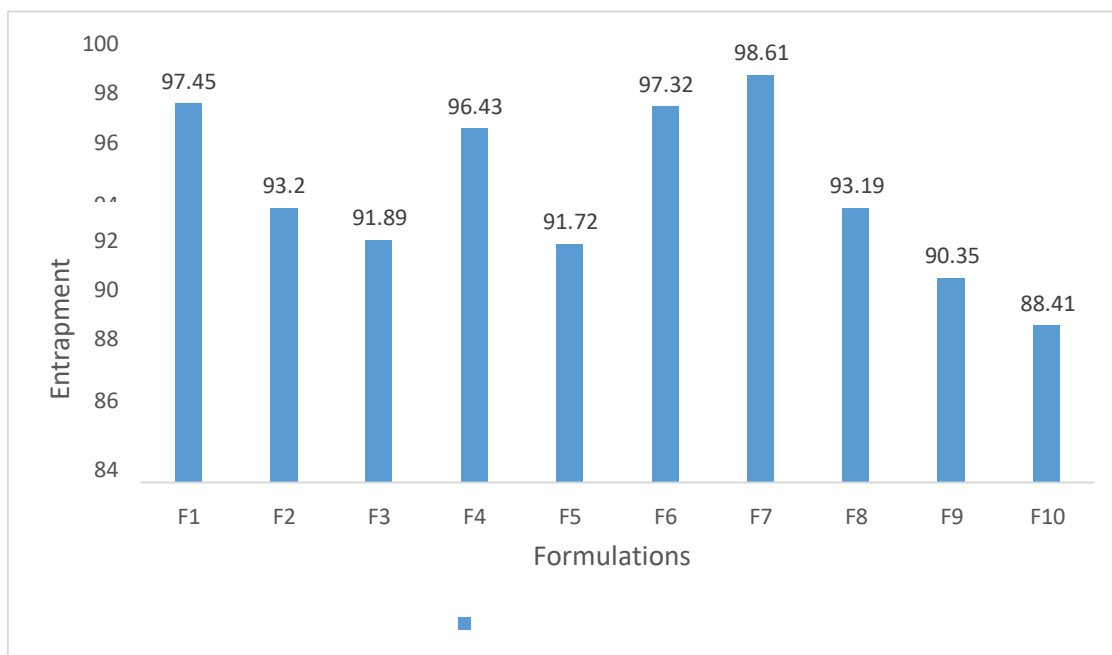


Figure 9: Entrapment efficiencies of Idarubicin nanosponges**Table 15: *In vitro* drug release profile of Idarubicin nanosponges (F1-F5)**

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

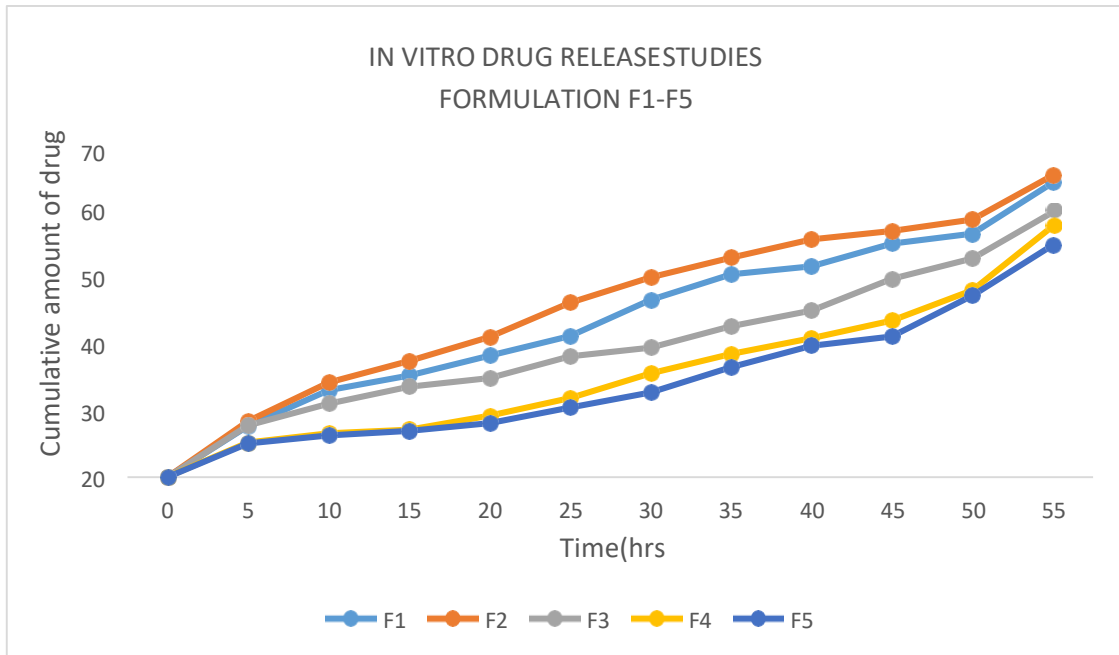


Figure 10: *In vitro* drug release profile of Idarubicin nanosponges (F1-F5)

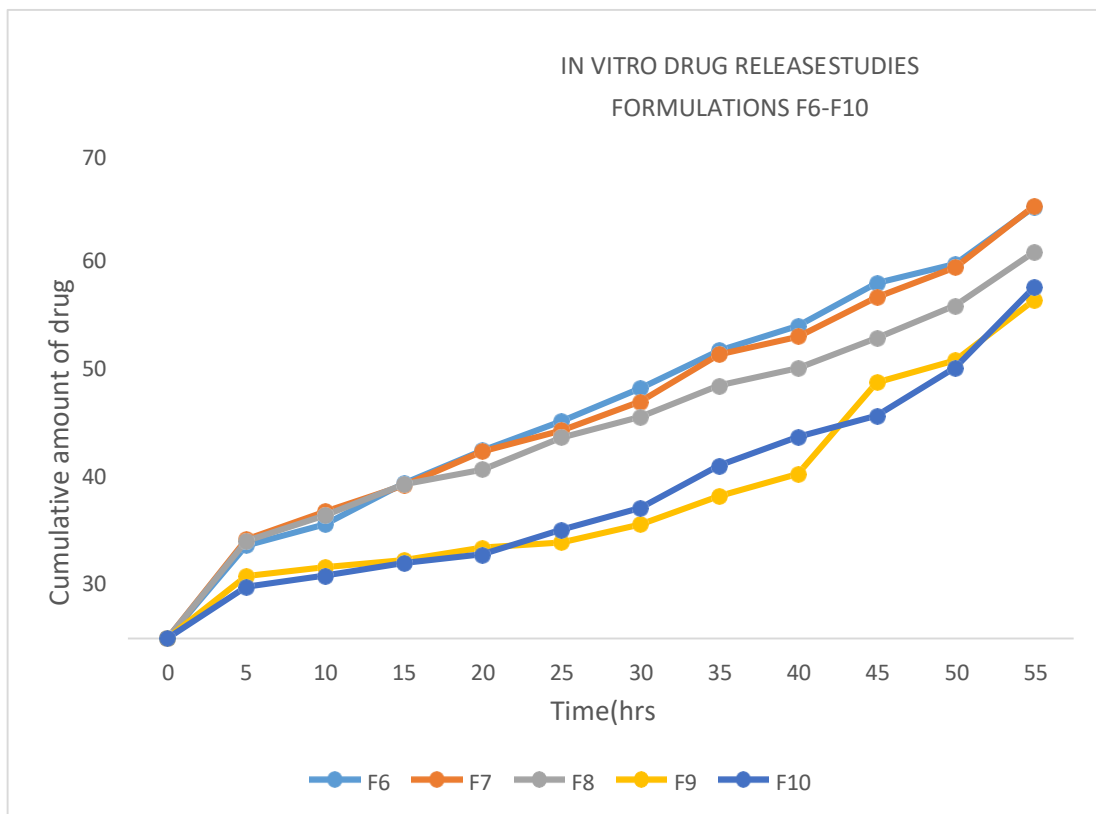


Figure 11: *In vitro* drug release profile of Idarubicin nanosponges (F6-F10)

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

Table 16: In vitro drug release profile of Idarubicin 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

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 Idarubicin 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 Idarubicin nanosponges containing ethyl cellulose (F1-F5) are given in Table 15 and Figure 16.

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 Idarubicin – ethyl cellulose nanosponges. Similarly F6 and F7 exhibited the best release of 90.19% and 87.10% respectively at the end of 48 hours among all the five formulations of Idarubicin – 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 Idarubicin 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 untrapped 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.

3. CONCLUSION

The Idarubicin nanosponges can be formulated by cost effective and easy emulsion solvent diffusion method using hydrophobic polymers such as eudragit. The formulated Idarubicin 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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