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Formulation and Evaluation of Melphalan Nanosponges

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ABSTRACT

Nanosponges are a new type of nano structured materials which have been widely used for resolving many physical biological and chemical problems related with the treatment of disease. Melphalan is a drug which has been used for the formulation of novel drug delivery systems (NDDS) called nanosponges. The synthesized nanoparticles have been characterized by X-ray diffraction (XRD), scanning electron microscope (SEM), transmission electron microscopy (TEM), and infrared spectrophotometer (IR) spectroscopy. The morphology of the nanostructures has been studied by using SEM, TEM, and IRspectrometry.Theresultsshowthattheobtainednanoscalestructuresare sub-micron in dimension and with a spherical shape. It is found that the drug is soluble in the alkaline solution and Ethanol and insoluble in Acetone and Water. The in-vitro release of the drug was found to be higher in the inclusion complex. The absorption values of Melphalan were 0.1686, 0.3624, 0.5357, 0.6963, 0.8770, 1.0693, 1.2700and1.4516respectively.In the calibration curve, linearity was obtained between5-40g/ml concentration of Melphalan and the regression value was R^2 = 0.9996. The nano scale characterization technique should be used to a larger extent to identify the nano scales at disease sites in affected organs or tissues and to establish relevant interaction mechanisms.

Keywords: *Nanosponges, Melphalan, Eudragit, Ethyl Cellulose, Emulsion Solvent Diffusion Method, Dialysis Bag Diffusion Method.*

1. INTRODUCTION

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 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 andhydrophilic substances and thereby improving the solubility of poorly water-soluble molecules. The nanosponges are encapsulating type of nanoparticles which encapsulates the drug molecules within it score. Based on the method of associating with drugs,the nanoparticle sare classified into encapsulating nanoparticles, conjugating nanoparticles and complexing nanoparticles.The encapsulating

nanoparticles are presented by nanosponges and nanocapsules.Nanosponges such as alginate nanosponge, which are sponge like nanoparticles may contains holes that carry the drug molecules. The second category is conjugating nanoparticle, which links to drugs through covalent bonds. The third tube 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[1].

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 hydrogel. 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 300ºC. 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[2].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.

2. CHARACTERISTICFEATURESOFNANOSPONGES

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 300oC. 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[4].

3. MATERIALSANDEQUIPMENTS

Melphalan was a drug used to prepare the nanosponge formulation which was purchased as a gift sample. Then the excipients like Ethylcellulose was purchased from Hi media pharmaceutical,Mumbai.Eudragit and Polyvinyl Alcohol were purchased from Yarrow Pharma and Sigma Aldrich. Then we have used Di methyl Formamide, Disodium hydrogen Orthophosphate and Potassium dihydrogen orthophosphate and those were purchased from Zhubai Chemico Industries, SD Fine Chemical Ltd and Qualigens Fine Chemicals, Mumbai.

REMI – 2 MLH model Magnetic Stirrer, MOTICB 1 SERIES Optical microscope and JASCOV – 530 model UV Spectrophotometer were used for the experiment. Then, further FT-IR spectrometer with the model of FTIRJASCO-4100, pH meter with the model of Phtester1,2 (EUTECH) and Zeta sizer with the model of MALVERN was used for the experiment. For the evaluation of structural morphology, the instrument HITACHIX650, TOKYO and Dialysis membrane of 50 mm with the model of Himedia, Mumbai were employed. Minitab18, INC, USA was the software tool used for the experiment.

4. EXPERIMENTALMETHODS

Preparation of stocksolution

The standard stock solution of melphalan was prepared by transferring accurately weighed quantity(10 mg)of melphalan raw material in 100ml of volumetric flask.The drug was dissolved in few ml of ethanol and the volume was made up to 100 ml with ethanol to get a stock solution of 100 µg/ml[5].

Selection of Wavelength

The standard stock solution was scanned in the range of 200to400nminUVspectrophotometer using methanol as blank.

Construction of calibration curve of Melphalan:

From the standard stock solution of melphalan 0.5, 1, 1.5, 2, 2.5, 3,3.5 and 4 ml were withdrawn to 10 ml volumetric flask and then made up volume with methanol to get a concentration range of 5-40 µg/ml. The absorbance of these solutions was measuredat288nm usingJASCOV-530 UV 1600UV- visible spectrophotometer methanol was used as blank. The calibration curve was plotted between concentration and absorbance.

Drug Excipient Compatibility Studies

FT-IR spectrum of drug was recorded using FT-IR Spectro photometer(ShimadzuJASCO4100). 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 MELPHALAN, polymers ethyl cellulose and eudragitwererecorded.ThespectrumofphysicalmixtureofMelphalan,polymer and co-polymer

were also documented to check for their compatibility[6].

Determination of Percentage Yield

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

$$\% = \frac{\text{Practical weight of Nanosponges obtained}}{\text{Theoritical Weight (drug + polymer)}} \times 100$$

Scanning Electron Microscopy(SEM)

SEM analysis was performed to determine their microscopic characters (shape & morphology) of prepared Melphalan 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[10].

ParticleSize 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 Melphalan nanosponges.

DeterminationofZetaPotential

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[12].

Determination of EntrapmentEfficiency

The entrapmentefficiency of nanosponges were determined by adding10ml of phosphate buffer of pH 7.4 and sonicated in a bath sonicator and filtered. 1 ml of filtrate is made up to 10 ml with phosphate buffer and was assayed spectrophotometrically at 288nm (UVvisiblespectrophotometer,modelUV- 1601 PC, Shimadzu). The amount of entrapped drug was calculated from the equation[15].

Entrapment efficiency = (Practical drug content/Theoretical drug content) X 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 25ml 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 melphalan released in phosphate buffer were measured by spectrophotometer at 288 nm[18]. 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 pH6.8 as blank and the amount of released drug was estimated by the standard curve[20].

5. RESULTSANDDISCUSSION

Construction of calibration curve of Melphalan

For the estimation of the UV absorbance of Melphalan at a wavelength of 288 nm, eight different concentrations of Melphalan drug solution were prepared such as 5, 10, 15, 20, 25, 30, 35, 40 µg/ml and the absorbance values of 0.1686, 0.3624, 0.5357, 0.6963, 0.8770, 1.0693, 1.2700 and 1.4516 were observed respectively. In the calibration curve, linearity was obtained between 5-40 µg/ml concentration of Melphalan and the regression value was found to be r2=0.9996.Hence, we can conclude that Melphalan obeys Beer Lambert's Law at a concentration between 5-40 µg/ml.

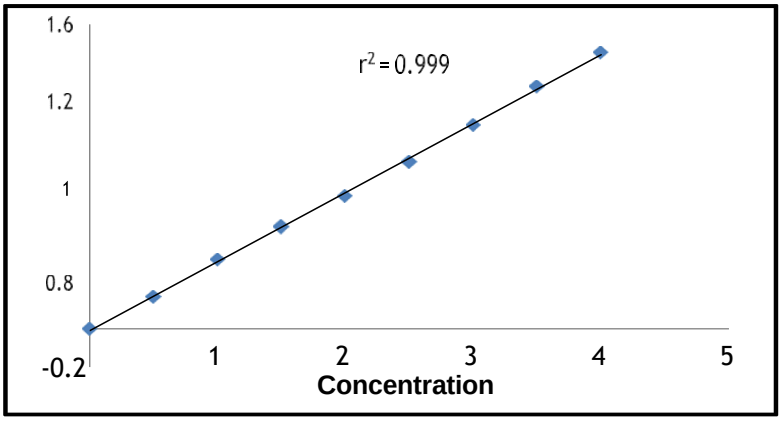

Figure1 :Calibration graph of Melphalan

Table1: Formulation of Melphalan nanosponges

Formulation code	Drug in mg	Polymer in mg	Drug:polymer ratio
F1	**MELPHALAN (100)**	Ethylcellulose (50)	1:0.5
F2		Ethylcellulose (100)	1:1
F3		Ethylcellulose (150)	1:1.5
F4		Ethylcellulose (200)	1:2
F5		Ethylcellulose (300)	1:3
F6		Eudragit (50)	1:0.5
F7		Eudragit (100)	1:1
F8		Eudragit (150)	1:1.5
F9		Eudragit (200)	1:2
F10		Eudragit (250)	1:2.5

Table2 :Percentage yield of melphalan 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

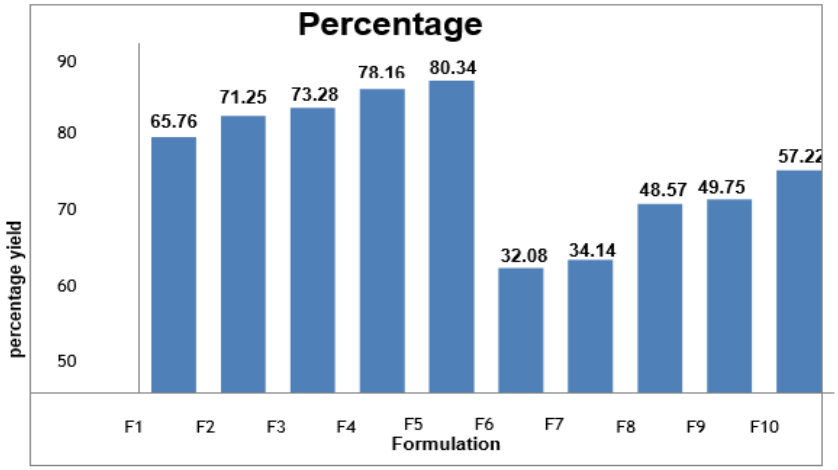

Figure2 :Percentageyieldanalysisofmelphalannanosponges

Figure 3 : SEM images of Melphalan Nanosponges using Ethyl Cellulose

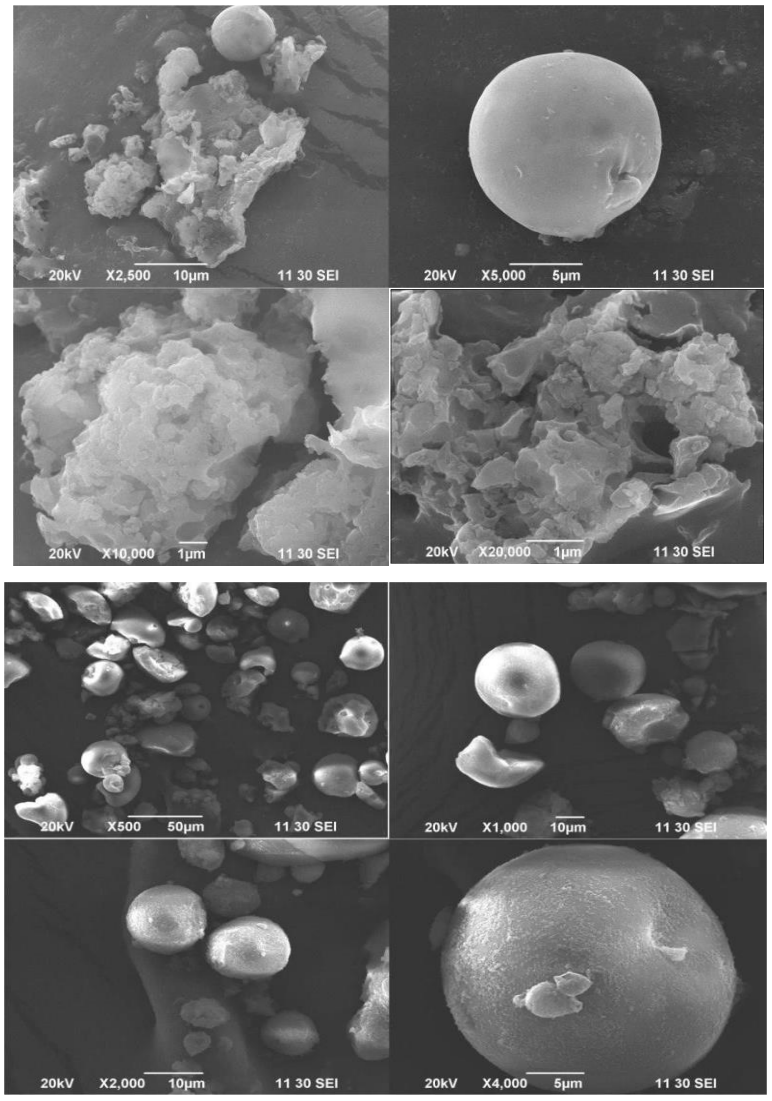

Figure 4 : SEM images of Melphalan Nanosponges using eudragit

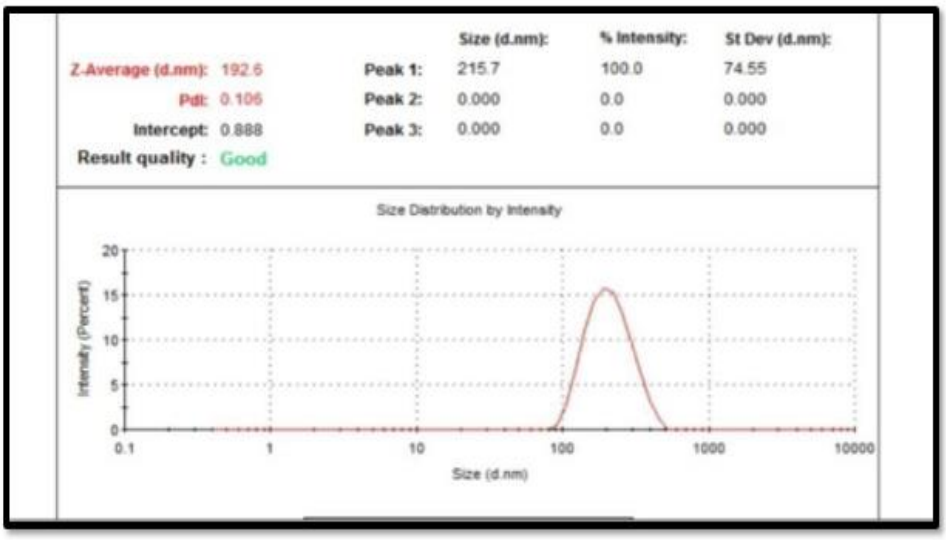

Figure5:Zeta size distribution of melphalan nanosponges(F4)

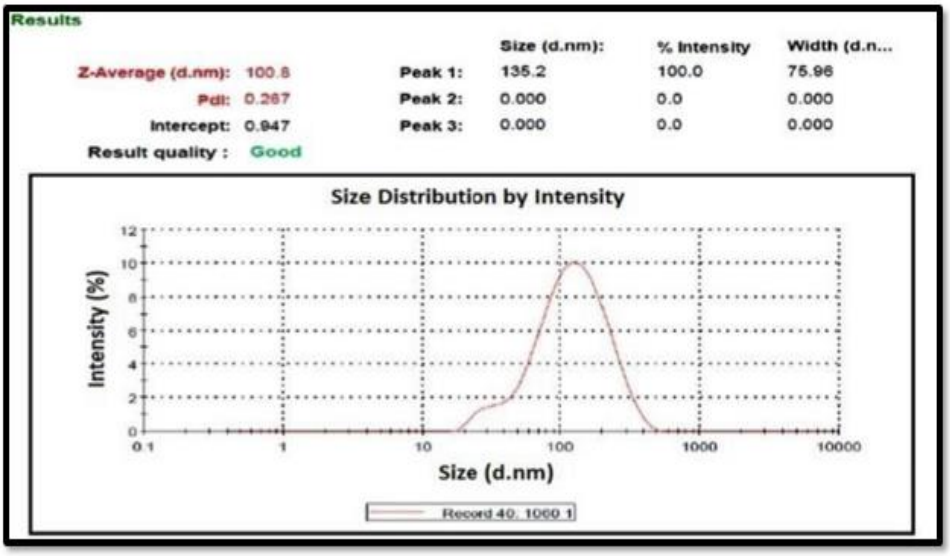

Figure6:Zetasize distribution of me lphalannanosponges(F6)

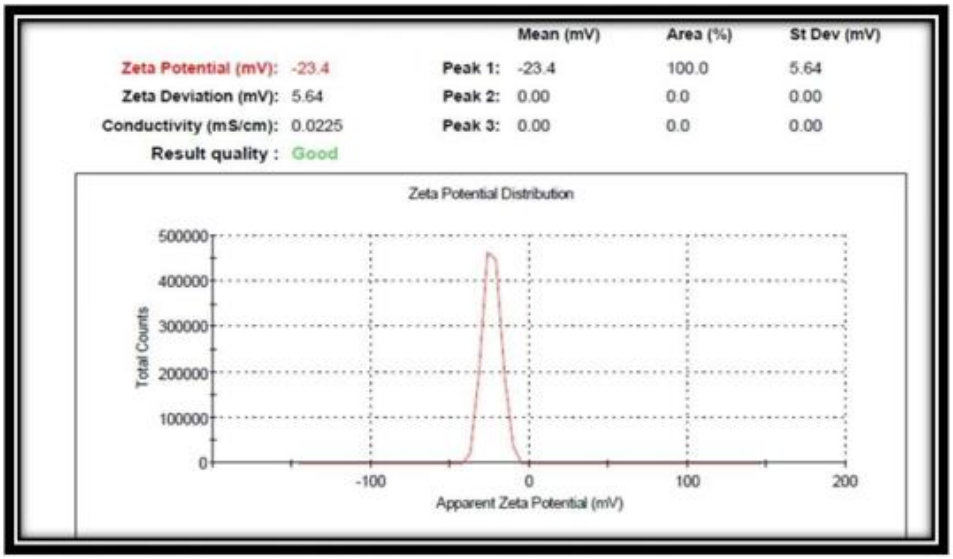

Figure7 :Zeta potential of melphalan nanosponges(F9)

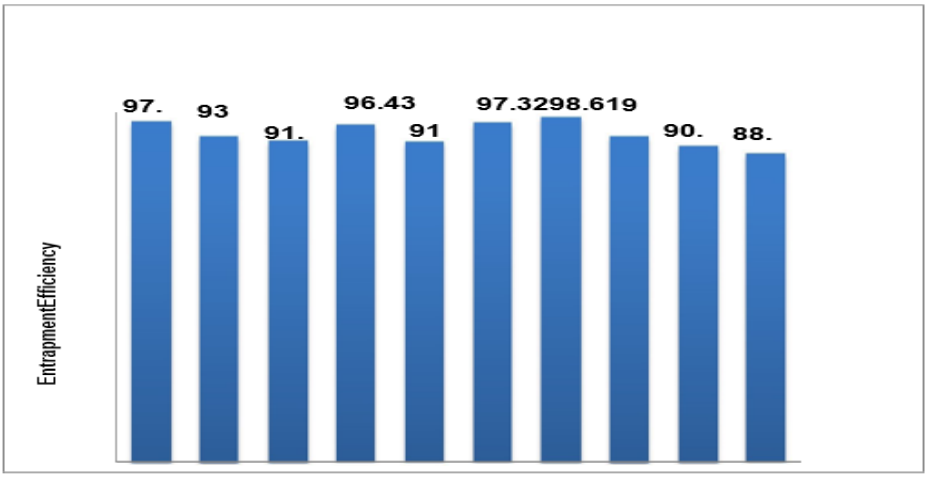

Figure8 :Entrapmentefficiencies of melphalan nanosponges

Table 3: In-vitro drug release profile of melphalan 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

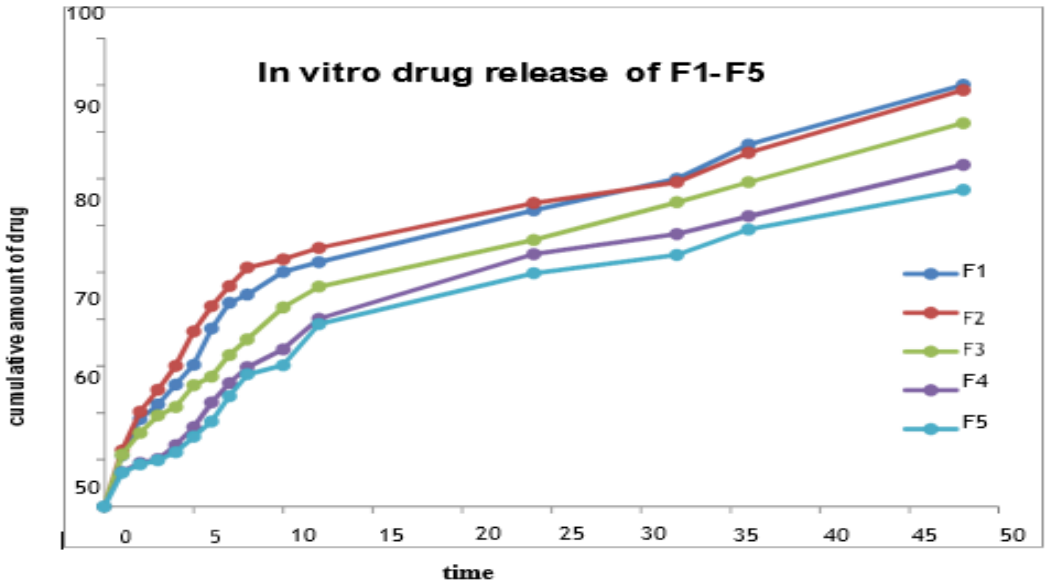

Figure 9 : In-vitro drug release profile of melphalan nanosponges(F1-F5)

Table 4 : In-vitro drug release profile of melphalan nanosponges(F6-F10)

Sl.No	Time (hrs)	Cumulative percentage drug release(%)				
		F6	F7	F8	F8	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

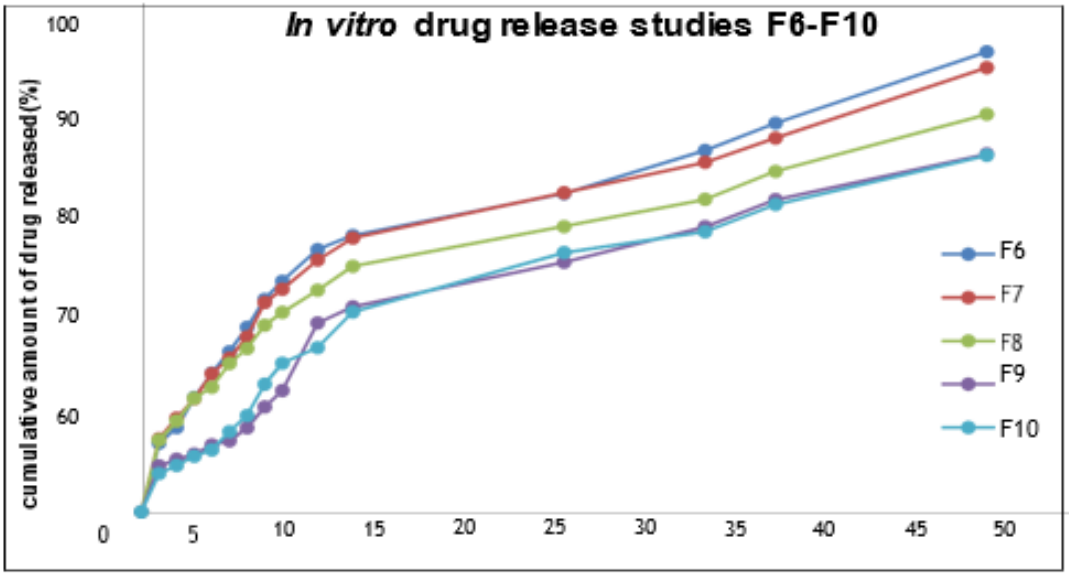

Figure 10 : In-vitro drug release profile of melphalan nano sponges(F6-F10)

6. SUMMARY AND CONCLUSION

The present work aimed at formulating melphalan nanosponges with two different types of polymers namely hydrophilic and hydrophobic polymers using emulsion solvent diffusion method. This method was simple and cost effective.Even though the drug release from both – melphalan ethyl cellulose nanosponges (F5) and melphalan- eudragit nanosponges (F10) are similar, the F5 formulation is selected asthe optimum formulation due to its comparatively higher yield. The in vitro release model best fitted to Higuchi release order. This was confirmed by plotting percentage cumulative drug release and square root of time and R^2 value ranges between 0.8477 and 0.9888. It is observed that formulation F1, F2, F6, F7 and F8 followed Fick's law of diffusion and rest showed an anomalous behaviour. The formulated melphalan nanosponges can be used in the treatment of cancer such as leukemia and other cancers. This can be targeted to the cancerous cells and produce sustained drug delivery which in turn reduces the dose, frequency of administration and the side effects.

7. ACKNOWLEDGEMENT

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8. CONFLICT OF INTEREST

We declare that we have no conflict of interest

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