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Research Article

FORMULATION AND EVALUATION OF DAPSONE NANOSPONGES BY SOLVENT EVAPORATION METHOD

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ABSTRACT:

In this study Nano sponges were prepared by the solvent evaporation technique and subsequently formulated in a gel form of Dapsone. The Nanosponges formulations were prepared by solvent evaporation method employing β-Cyclodextrin, Ethyl Cellulose and Pluronic F127 rate retarding polymers using PVA as a co polymer. The compatibility of the drug with formulation components was established by Fourier Transform Infra-Red (FTIR) spectroscopy. The surface morphology, particle size and drug entrapment efficiency of Nano sponges were examined. Shape and surface morphology of the Nano sponges were examined using scanning electron microscopy. Scanning electron microscopy revealed the porous, spherical nature of the Nanosponges.SEM photographs revealed the spherical nature of the Nano sponges in all variations; however, at higher ratios, drug crystals were observed on the nano sponge surface. Increase in the drug/polymer ratio (1:1 to 1:2) which is in increasing order due to the increase in the concentration of polymer but after certain concentration it was observed that as the ratio of drug to polymer was increased, the particle size decreased. The particle size was found in the range of 200-350 nm. The entrapment efficiency of different formulations were found in the range of 62.49±1.47% to 77.51±1.81%. The in vitro release studies revealed that the formulation with higher concentration of penetration enhancer showed greater drug release and it follows the Zero order drug release with Super case II transport mechanism.

Keywords: Dapsone, Ethyl Cellulose, Nano sponges Delivery System (NSDS), Scanning Electron Microscopy (SEM), FTIR.

INTRODUCTION

Nanosponges are mesh-like, minute structures that may contain a wide range of chemicals and pharmaceutical compounds. [1,2] They boost the solubilization ability of both water-soluble and lipid-soluble medicines and feature a spherical colloidal structure.[3] They enhance the bioavailability of medicines by extended drug release.[4] Nanosponges' amphiphile nature allows them to transport therapeutic compounds that are both hydrophilic and hydrophobic, thanks to their internal hydrophobic chambers and outside hydrophilic branching.[5] They are similar to a 3D network, with a backbone of long-chain polyesters in the solution and crosslinkers connecting different regions of the polymer.[6] It was discovered that cyclodextrins (cyclic oligosaccharides) may be treated with suitable crosslinking agents to generate nanosponges, a unique nanostructured material.[7]

Nanosponges may be manufactured as neutral or acidic materials and swell depending on the crosslinking agent used[8]. The end product is hollow spheres with holes that can store pharmaceutical compounds. During preparation, the cross-linking-to-cyclodextrin proportion can be adjusted to improve drug loading and offer a more tailored release profile. In comparison to the parent cyclodextrin molecules, their very permeable nanomeric nature allows drug molecules to organize themselves in nanosponge inclusion while also interacting with one another in a non-inclusion mode, resulting in efficient drug loading.

Leprosy, commonly known as Hansen's disease, is a global health issue that ranges from tuberculoid to lepromatous leprosy (paucibacillary to multibacillary illness) depending on the host immunological response. It

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is caused by Mycobacterium leprae and affects the skin, eyes, and nerves, resulting in skin sores, eye discomfort, vision loss, weakness, and numbness.[9] Skin biopsy, smear, and physical examination results are used to make the final diagnosis. The therapy choices range depending on the clinical symptoms. Leprosy can cause type 1 (reversal) and type 2 (erythema nodosum leprosum) immunologic responses, which can occur prior to, during, or following therapy. During this time, the condition might develop substantially, hence antibiotic medication should be continued.[10]

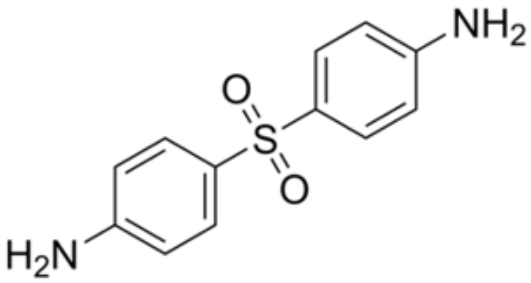

Figure.1 Structure of Dapsone

Since 1947, dapsone (4,4-diaminodiphenyl sulfone) has been known to be a useful drug in the treatment of leprosy. Its mechanism of action is via competitive inhibition of M. leprae dihydropteroate synthase, thus inhibiting the synthesis of folic acid.11,12 Dapsone (Figure 1) is classified as a class II drug according to the Biopharmaceutics Classification System, and has high permeability and low solubility in water (log P = 0.97).13 Thus, despite its therapeutic potential, the low solubility of dapsone in water results in low bioavailability and microbial resistance.[13] A viable alternative for dose reduction and therefore limitation of side effects, never described before in the literature for dapsone, is the use of an intestinal permeation enhancer system capable of maintaining the drug in a soluble state after administration.

MATERIALS

Dapsone was procured from B.M.R Chemicals, Hyderabad, Poly vinyl alcohol (PVA), PLURONIC F127 were procured from COLORCON GOA, β cyclodextrin, Ethyl Cellulose were procured from B.M.R.Chemicals, Hyderabad, Methanol, Distilled Water(ml) were procured from Narmada Chemicals, Hyderabad.

METHODOLOGY

Spectroscopic study:

Identification of pure drug:

Solubility studies:

Solubility of Dapsone was carried out in different solvents like- distilled water, 0.1 N HCL 7.4 pH & 6.8 pH buffers and organic solvents like Ethanol & Methanol. Solubility studies were performed by taking excess amount of drug in different beakers containing the solvents. The mixtures were shaken for 24 hrs at regular intervals. The solutions were filtered by using whattmann's filter paper grade no. 41. The filtered solutions were analyzed spectrophotometrically.

Determination of absorption maximum (λmax):

The wavelength at which maximum absorption of radiation takes place is called as λmax. This λmax is characteristic or unique for every substance and useful in identifying the substance. For accurate analytical work, it is important to determine the absorption maxima of the substance under study. Most drugs absorb radiation in ultraviolet region (190-390nm), as they are aromatic or contain double bonds.

Accurately weighed 10mg Dapsone separately was dissolved in 2-3 ml of methanol in a clean 10ml volumetric flask. The volume was made up to 10ml with 6.8 pH phosphate buffer which will give stock solution-I with concentration 1000µg/ml. From the stock solution-I, 1ml was pipette out in 10ml volumetric flask. The volume was made up to 10ml using 6.8 pH phosphate buffer to obtain stock solution-II with a concentration 100µg/ml. From stock solution-II, 1ml was pipette out in 10ml volumetric flask. The volume was made up to 10ml using 6.8 pH phosphate buffer to get a concentration of 10µg/ml. This solution was then scanned at 200-400nm in UV-Visible double beam spectrophotometer to attain the absorption maximum (λ-max).

Construction of calibration curve:

Accurately weighed 10mg Dapsone was dissolved in methanol taken in a clean 10ml volumetric flask. The volume was made up to 10ml with 6.8 pH phosphate buffer which gives a concentration of 1000µg/ml. From this standard solution, 1ml was pipette out in 10ml volumetric flask and volume was made up to 10ml using 6.8 pH phosphate buffer to obtain a concentration of 100µg/ml. From the above stock solution, aliquots of 0.2, 0.4, 0.6, 0.8 , 1.0 and 1.2 ml each was transferred to a separate 10ml volumetric flask and solution was made up to 10ml using 6.8 pH phosphate buffer to obtain a concentration of 2, 4, 6, 8, 10 and 12µg/ml respectively. The absorbance of each solution was measured at 291 nm.

Drug excipient compatibility study:

The drug and excipient compatibility was observed using Fourier Transform – Infra Red spectroscopy (FT-IR). The FT-IR spectra obtained from Bruker FT-IR Germany (Alpha T) was utilized in determining any possible interaction between the pure drug and the excipients in the solid state. The potassium bromide pellets were prepared on KBr press by grounding the solid powder sample with 100 times the quantity of KBr in a mortar. The finely grounded powder was then introduced into a stainless steel die and was compressed between polished steel anvils at a pressure of about 8t/in2. The spectra were recorded over the wave number of 4000 to 400cm-1.

PREPARATION OF NANOSPONGES:

Table.1 Formulation table of Dapsone loaded Nano Sponges

Ingredients(mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Dapsone	100	100	100	100	100	100	100	100	100	100	100	100
Poly Vinyl Alcohol	100	100	100	100	100	100	100	100	100	100	100	100
β-cyclodextrin	50	100	150	200	--	--	--	--	--	--	--	--
Pluronic F127	--	--	--	--	50	100	150	200	--	--	--	--
Ethyl Cellulose	--	--	--	--	--	--	--	--	50	100	150	200
Methanol (ml)	20	20	20	20	20	20	20	20	20	20	20	20
Ratio (Drug:Polymer)	1:0.5	1:1	1:1.5	1:2	1:0.5	1:1	1:1.5	1:2	1:0.5	1:1	1:1.5	1:2
Water (ml)	100	100	100	100	100	100	100	100	100	100	100	100

Method of Preparation of Nanosponges

Solvent Evaporation Method:

Nanosponges using different proportions of β-cyclodextrin, Pluronic F127, Ethyl cellulose as rate retarding polymer and co-polymers like polyvinyl alcohol were prepared by solvent evaporation method. Disperse phase consisting of Dapsone (100mg) was dissolved in 20ml solvent (methanol) and was slowly added to a definite amount of PVA in 100ml of aqueous continuous phase, prepared by using magnetic stirrer. The reaction mixture was stirred at 1000 rpm for three hours on a magnetic stirrer for 2hours. The nanosponges formed were collected by filtration through whatman filter paper and dried in oven at 50ºC for 2 hours. The dried nanosponges were stored in vaccum desicator to ensure the removal of residual solvent.

Evaluation parameters of Nanosponges:[72-79]

The Nanosponges was evaluated for various parameters:-

1. Entrapment efficiency
2. Scanning electron microscopy
3. Particles size and shape
4. In-vitro drug release studies
5. Drug release kinetics studies

Entrapment efficiency

The 100 mg of the Dapsone weight equivalent nanosponge was analyzed by dissolving the sample in 10ml of distilled water. After the drug was dissolved 10ml of clear layer of dissolved drug is taken. Thereafter the amount of drug in the water phase was detected by a UV-spectrophotometric method at 291 nm (U.V Spectrophotometer, systronics). The test was repeated with another nanoparticulate sample. The amount of the drug in the suspension was analyzed by centrifugation at 500rpm for 5 mins and by measuring the concentration of the drug in the clear supernatant layer by the UV-spectrophotometric method. The concentration of the drug is determined with the help of calibration curve. The amount of drug inside the particles was calculated by subtracting the amount of drug in the aqueous phase of the suspension from the total amount of the drug in the nanoparticle suspension. The entrapment efficiency (%) of drug was calculated by the following equation.

$$\%\ \text{of Drug entrapmen} = \frac{\text{Mass of drug in nanosponge}}{\textit{Mass of drug used in formulation}}\ x100$$

Scanning electron microscopy

The morphological features of prepared nanospongess are observed by scanning electron microscopy at different magnifications.

Particle size and shape

Average particle size and shape of the formulated nanospongess was determined by using Malvern Zetasizer ZS using water as dispersions medium. The sample was scanned for determination of particle size.

Dissolution Parameters

Medium : 900ml, 6.8 pH phosphate buffer for 12hrs.
Apparatus : Basket (USP-I)
RPM : 50
Temperature : 37° C±0.5
Time Points : 1,2, 3,4,5,6,7,8,9,10,11,12 hr

Procedure:

For the oral dosage forms the in vitro dissolution study must be conducted in the dissolution medium which simulate the in-vivo conditions (actual physiological conditions). The in vitro drug release studies for the prepared formulation were conducted for a period of 12 hrs using an Electro lab model dissolution tester USP Type-1 apparatus (rotating basket) set at 50 RPM and a temperature of 37± 0.5°C weight equivalent to 100mg of Dapsone nano sponge was filled in capsule and kept in basket apparatus and placed in the 900ml of the medium. At specified intervals 5ml samples were withdrawn from the dissolution medium and replaced with fresh medium to keep the volume constant. The absorbance of the sample solution was analyzed at 291 nm for the presence of model drug, using a UV-visible spectrophotometer.

Modelling of Dissolution Profile

In the present study, data of the in vitro release were fitted to different equations and kinetic models to explain the release kinetics of Dapsone Nanosponges. The kinetic models used were Zero order equation, First order, Higuchi release and Korsmeyer-Peppas models.

Kinetic Studies: Mathematical models:

Different release kinetic equations (zero-order, first-order, Higuchi's equation and Korsmeyer-peppas equation) were applied to interpret the release rate of the drug from matrix systems for the optimized formulation. The best fit with higher correlation (r2) was calculated.

Zero-order model:

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be representedby the equation

$$Qt = Q0 + K0t$$

First Order Model:

The first order equation describes the release from systems where the dissolution rate is dependent upon the concentration of the dissolving species.

$$Log\ C = Log\ C_o - kt/2.303$$

Higuchi model: The first example of a mathematical model aimed to describe drug release from a system was proposed by Higuchi in 1961. Initially conceived for planar systems, it was then sustained to different geometrics and porous systems. This model is based on the hypothesis that

$$Q = K_H - t^{1/2}$$

Korsmeyer-Peppas model: Korsmeyer et al.(1983) derived a simple relationshipwhich described drug release from a polymeric system equation. To find out the mechanism of drug release, first60% drug release data were fitted in Korsmeyer-Peppas model,

$$Mt / M\infty = Kt^n$$

RESULTS AND DISCUSSION

Dapsone Characterization:

Solubility: Solubility of Dapsone was carried out in different solvents like, methanol, Ethanol, 7.4 pH phosphate buffer, 0.1N HCl and 6.8 pH phosphate buffer.

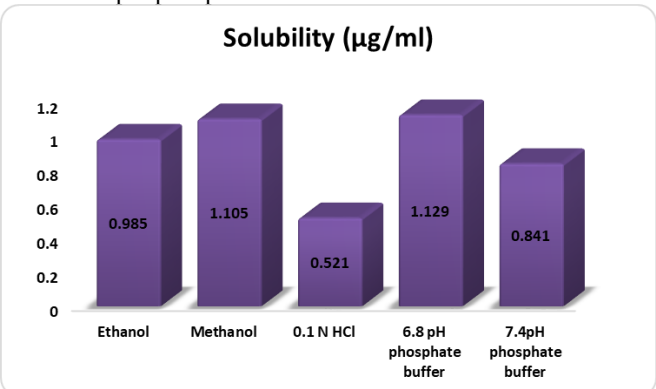

Figure.2 Solubility studies of Dapsone

Discussion:
From the above obtained solubility studies we can say solubility of the drug is more in 6.8 pH phosphate buffer than the other buffers. In organic solvents the solubility was found more in Methanol than other organic solvents.

Determination of absorption maximum (λmax): Determination of Dapsone λ-max was done in 6.8 pH phosphate buffer for accurate quantitative assessment of drug dissolution rate.

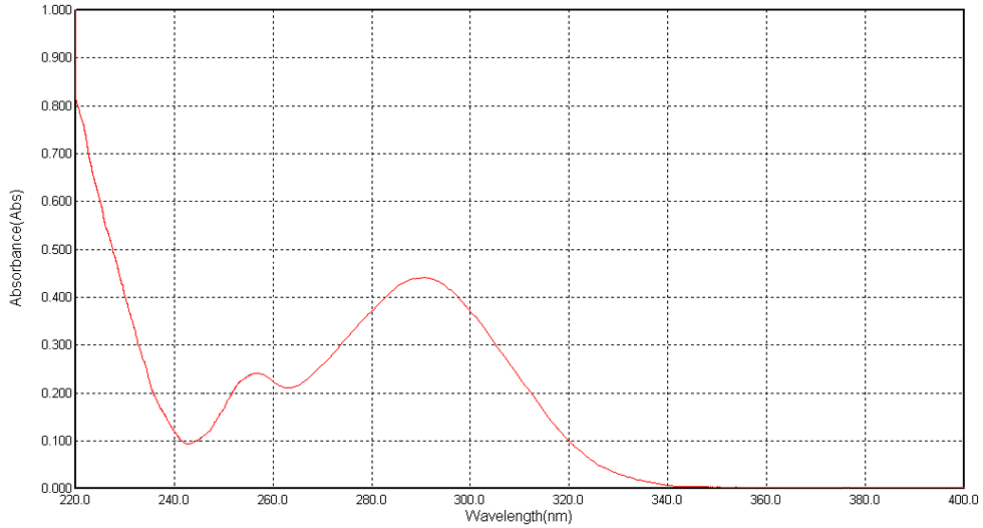

Figure.3 λ-max in 6.8 pH phosphate buffer

Discussion: The maximum absorbance of the Dapsone in 6.8 pH phosphate buffer was found to be 291 nm for 100% concentration solution as shown in Fig. Hence, the wavelength of 291 nm was selected for analysis of drug in dissolution media.

Calibration curve:

Table.2 Calibration curve data of Dapsone

Concentration(µg/ml)	Absorbance
0	0
2	0.106
4	0.212
6	0.331
8	0.445
10	0.557
12	0.673

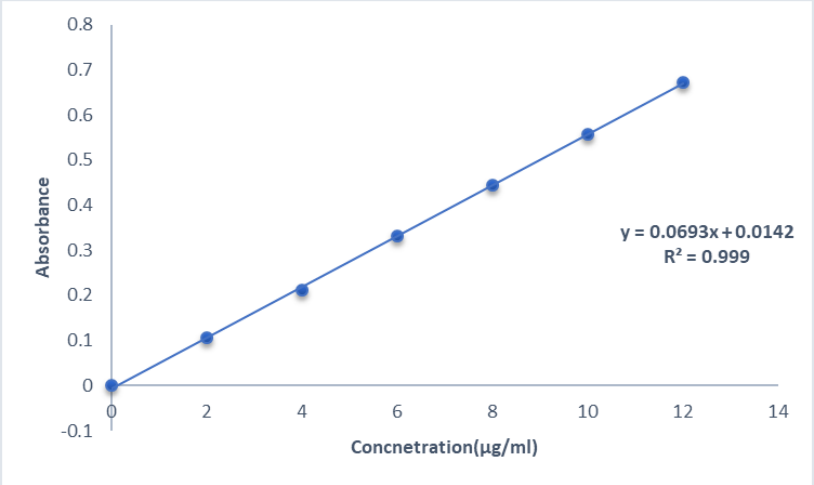

Figure.4 Calibration Curve of Dapsone in 6.8 pH phosphate buffer

Discussion:

The linearity was found to be in the range of 2-12µg/ml in 6.8 pH phosphate buffer. The regression value was closer to 1 indicating the method obeyed Beer-lambert's law.

Drug excipient compatibility:

Drug and excipient compatibility was confirmed by comparing spectra of FT-IR analysis of Pure drug with that of various excipients used in the formulation.

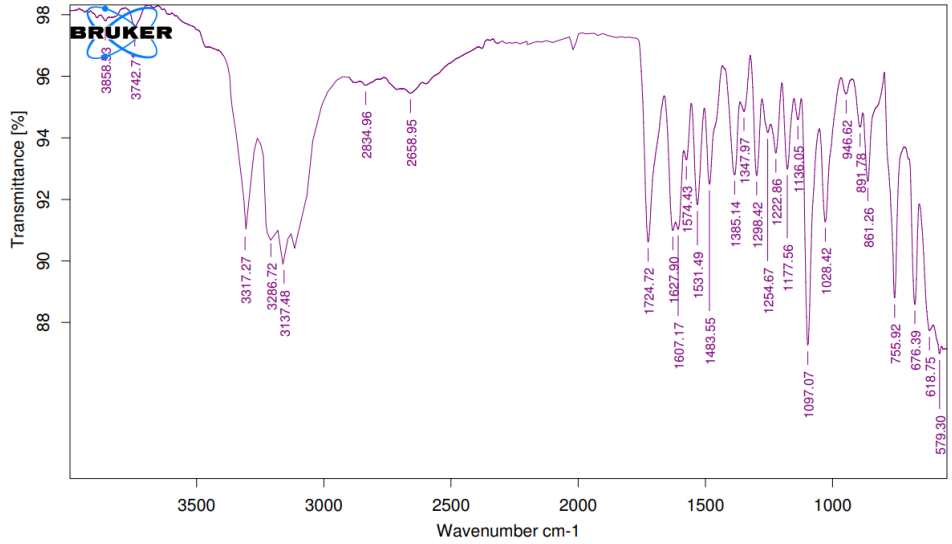

Figure.5 FTIR Spectra of Pure Drug

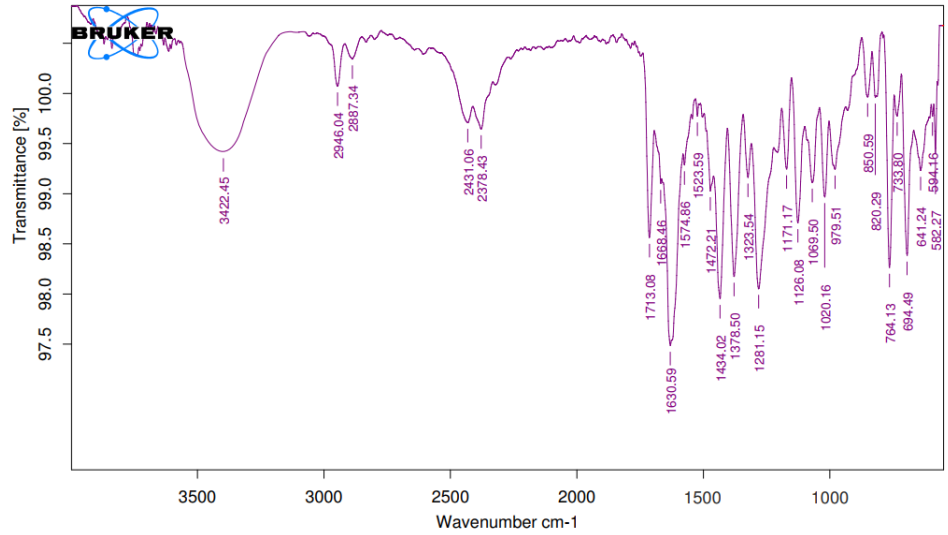

Figure.6 FTIR Spectra of drug and excipients

Discussion:-

From the compatibility studies it was concluded that the functional groups that were presented in the pure drug were present in the optimized formulation with very minute changes, from this we can concluded that the drug and excipients have no interactions.

Entrapment efficiency:

It is calculated to know about the efficiency of any method, thus it helps in selection of appropriate method of production. After the preparation of formulations the Practical yield was calculated as Nano sponges recovered from each preparation in relation to the sum of starting material (Theoretical yield). It can be calculated using following formula.

Table.3 % Entrapment Efficiency of Nano sponges

Formulation code	Entrapment efficiency %
F1	62.49±1.47
F2	65.20±1.26
F3	68.17±1.20
F4	72.74±1.19
F5	64.66±1.64
F6	67.84±1.75
F7	69.75±1.26
F8	74.95±1.95
F9	66.29±1.45
F10	69.45±1.10
F11	74.30±1.37
F12	77.51±1.81

Discussion: The entrapment efficiency of formulation F1-F12 was found in the range of 62.49±1.47% to 77.51±1.81%. Among all the formulations F12 shows high entrapment efficiency of 77.51±1.81%.

Morphology determination by scanning electron microscopy (SEM):

Scanning electron microscopy (SEM) was used to determine the Morphology of the prepared nanosponges. SEM is useful for characterizing the morphology and size of microscopic specimens with particle size as low as 10-10 to 10-12 grams. The sample was placed in an evacuated chamber and scanned in a controlled pattern by an electron beam. Interaction of the electron beam with the specimen produces a variety of physical phenomena that, when detected, are used to form images and provide elemental information about the specimens. It was observed that the nanosponges were spherical morphology, porous surface,, and uniform with no drug crystals on the surface. The shape of the nanosponges affects the surface area and surface area per unit weight of spherical nanosponges.

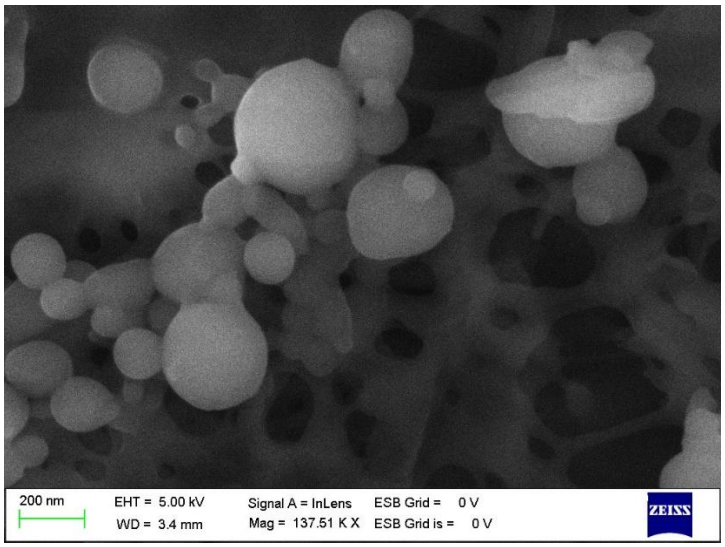

Figure.7 Nano sponges structure optimized formulation (F12)

Discussion: The morphology of the Nanosponges prepared by emulsion solvent evaporation method were investigated by SEM. The representative SEM photographs of the Nanosponges are shown in Fig.

Particle size analysis of Nano sponges:

Table.4 Mean Particle size of Nano sponges

Formulation code	Mean Particle size (nm)
F1	220
F2	340
F3	312
F4	270
F5	185
F6	302
F7	254
F8	349
F9	220
F10	240
F11	260
F12	200

Discussion: The particle size of the nanosponges was determined by optical microscopy and the nano sponges were found to be uniform in size. The average particle size of all formulations ranges from 180 nm to 350 nm which is in increasing order due to the increase in the concentration of polymer but after certain concentration it was observed that as the ratio of drug to polymer was increased, the particle size decreased. This could probably be due to the fact that in high drug to polymer ratio, the amount of polymer available per nanosponge was comparatively less. Probably in high drug-polymer ratios less polymer amounts surround the drug and reducing the thickness of polymer wall and nanosponges with smaller size were obtained. By performing the particle size analysis, it is concluded that the formulation has the particle size varies with the concentration of polymer drug ratio.

In-vitro dissolution studies of prepared Nanosponges:

In vitro release studies were performed in triplicate using USP basket method at 50 rpm and 37±0.2ºC in 900ml of phosphate buffer (pH 6.8 Phosphate Buffer). 10 mg of the formulated Nanosponges is used for each experiment. Samples were taken at appropriate time intervals for 1,2,3,4,5,6,7,8,9,10,11 & 12 hour. The samples were measured spectrophotometrically at 291.0 nm. Fresh dissolution medium was replenished each time when sample is withdrawn to compensate the volume.

Table.5 Percentage of drug release of Nano-sponges (F1-F6)

Time (hrs)	%Cumulative Drug Release					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	21.58±1.18%	19.63±1.22%	14.48±1.69%	12.79±1.69%	26.48±1.27%	29.16±1.27%
2	39.45±1.54%	25.42±1.75%	20.15±1.48%	24.13±1.47%	32.19±1.26%	35.45±1.59%
3	45.72±1.64%	39.76±1.69%	35.69±1.20%	39.48±1.20%	45.25±1.27%	47.28±1.24%
4	57.19±1.42%	50.98±1.71%	47.20±1.25%	48.61±1.27%	54.48±1.26%	58.15±1.64%
5	69.14±1.02%	65.39±1.02%	59.78±1.57%	56.82±1.54%	69.16±1.20%	71.42±1.45%
6	78.68±1.97%	79.24±1.34%	70.26±1.56%	62.68±1.36%	76.18±1.67%	78.29±1.46%
7	85.45±1.02%	85.45±1.52%	83.45±1.02%	68.79±1.02%	85.41±1.25%	82.38±1.27%
8	98.20±1.02%	94.61±1.29%	88.16±1.08%	76.39±1.74%	92.59±1.45%	90.49±1.20%
9		98.42±1.04%	94.32±1.45%	83.32±1.20%	98.45±1.24%	94.27±1.06%
10			98.42±1.06%	91.41±1.42%		99.46±1.27%
11				98.59±1.26%		
12						

Table.6 Percentage of drug release of Nano-sponges (F7-F12)

Time (hrs)	%Cumulative Drug Release					
	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0
1	20.46±1.27%	12.48±1.02%	25.78±1.27%	25.09±1.45	26.34±1.54%	18.94±1.58%
2	29.75±1.04%	19.64±1.24%	38.12±1.45%	34.47±1.02	34.75±1.48%	24.38±1.24%
3	34.29±1.47%	24.27±1.45%	49.05±1.27%	43.07±1.57	41.12±1.36%	29.79±1.46%
4	43.15±1.20%	31.69±1.65%	57.72±1.57%	51.63±1.36	49.28±1.45%	35.63±1.87%
5	45.28±1.64%	38.89±1.78%	66.34±1.26%	59.27±1.45	56.18±1.27%	41.78±1.52%
6	53.42±1.20%	46.75±1.45%	75.65±1.58%	65.39±1.78	67.34±1.45%	48.69±1.49%
7	62.45±1.25%	54.89±1.25%	82.67±1.45%	74.15±1.24	75.25±1.02%	55.12±1.58%
8	76.28±1.69%	69.75±1.64%	89.67±1.02%	81.36±1.59	81.84±1.36%	66.79±1.02%
9	85.26±1.45%	77.29±1.58%	91.95±1.69%	93.09±1.25	87.63±1.74%	78.42±1.54%
10	91.46±1.37%	85.42±1.57%	98.36±1.75%	99.02±1.45	92.65±1.20%	88.24±1.69%
11	98.48±1.85%	92.79±1.48%			98.12±1.02%	93.23±1.45%
12		98.26±1.63%				99.32±1.20%

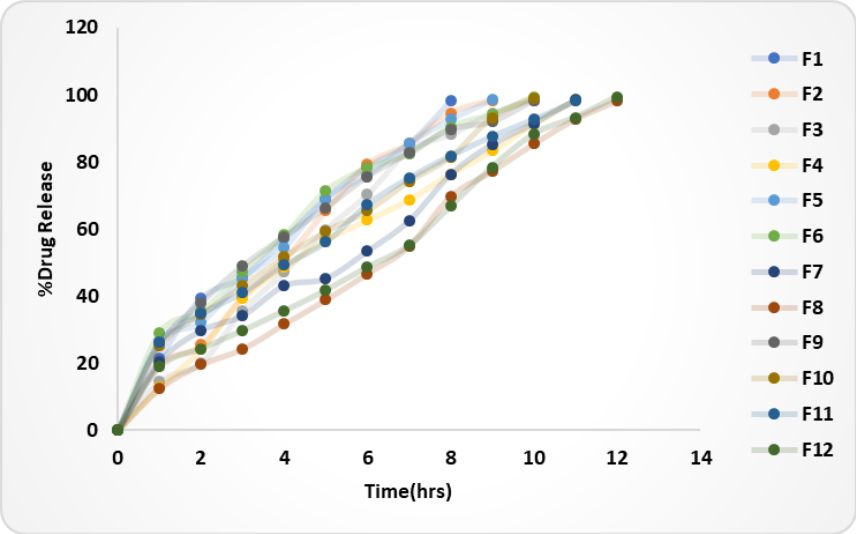

Figure.8 In vitro drug release of formulations F1-F12

Discussion:

By comparing the above dissolution studies of formulations F1-F12. Maximum drug release of 99.32±1.20% was found in F12 formulation containing Drug: Ethyl Cellulose in 1:2 ratio. So F12 formulation was taken as the optimized formulation, and drug release kinetics were performed for F12 formulation.

Kinetics Analysis for F12:

Zero Order:

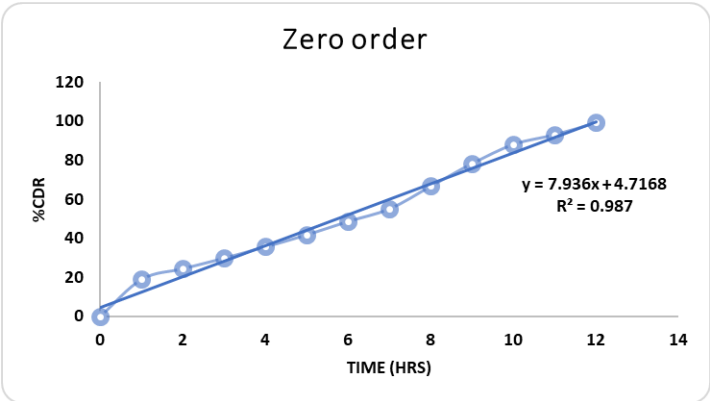

Figure.9 Zero Order Plot for F12

First Order:

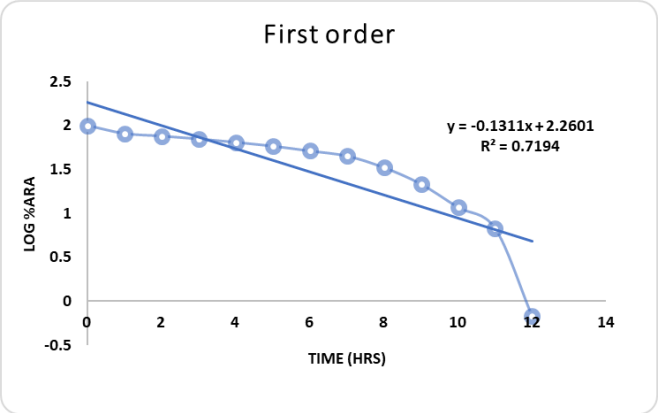

Figure.10 First Order Plot for F12

Higuchi Plot:

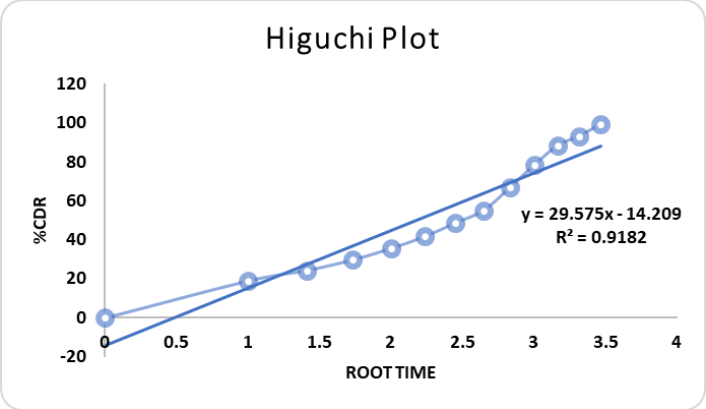

Figure.11 Higuchi Plot for F12

Korsmeyer Peppas Plot:

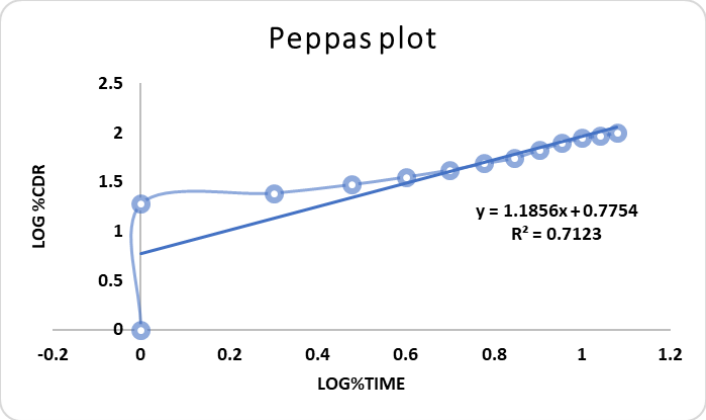

Figure.12 Peppas Plot for F12

Discussion:

The optimized formulation F12 has coefficient of determination (R2) values of 0.987, 0.719, 0.918 and 0.712 for Zero order, First order, Higuchi and Korsmeyer Peppas respectively. A good linearity was observed with the First order, indicates the rate of drug release through the mode of diffusion and to further confirm the diffusion mechanism, data was fitted into the Korsmeyer Peppas equation which showed linearity with n value of 1.1856 for optimized formulation. Thus n value indicates the Super case II transport mechanism. Thus, the release kinetics of the optimized formulation was best fitted into Zero order with Super case II transport mechanism.

SUMMARY AND CONCLUSION

The Dapsone Nanosponge was prepared by solvent evaporation method using β-cyclodextrin, Ethyl Cellulose and Pluronic F127 as rate retarding polymers and PVA as co polymer using Methanol as a solvent. The prepared nano sponges were evaluated for its different parameters which revealed many interesting results for efficient

preparation of the nano sponge. The formulation F12 has better results than other formulations. F12 have its particle size 200nm, entrapment efficiency of 62.49±1.47% to 77.51±1.81%., Among the polymers used such as β-cyclodextrin, Ethyl Cellulose and Pluronic F127 the drug polymers ratio of Dapsone: Ethyl Cellulose (1:2) ratio sustains the drug release up to 12 hours. The Formulation F12 drug release was found to be 99.32±1.20% in 12 hours, all these parameters are in optimized range for preparing a sustained release dosage form so showing itself as an optimized formulation in this project work. FTIR spectroscopy analyses indicated the chemically stable, amorphous nature of the drug in these nano sponge. SEM photographs revealed the spherical nature of the nanosponge in all variations. With the revealed results by different evaluation parameters, it is concluded that nanosponge drug delivery system has become highly competitive and rapidly evolving technology and more and more research are carrying out to optimize cost-effectiveness and efficacy of the therapy. The coefficient of determination (R2) values of 0.987, 0.719, 0.918 0.712 for Zero order, First order, Higuchi and Korsmeyer Peppas respectively. A good linearity was observed with the zero order, data was fitted into the Korsmeyer Peppas equation which showed linearity with n value of 1.185 for optimized formulation. Thus n value indicates the Super case II transport mechanism. Thus, the release kinetics of the optimized formulation was best fitted into Zero order drug release with Super case II transport mechanism.

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