FORMULATION AND EVALUATION OF MESALAMINE LOADED NANOSPONGES

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ARTICLE INFO	ABSTRACT
Key Words Nanosponges, Mesalamine, Quasi emulsion solvent diffusion method,Eudragit RS 100, Ethylcellulose, Dimethyl sulfoxide **Access this article online Website:** https://www.jgtps.com/ **Quick Response Code:**	Mesalamine loaded Nanosponges was prepared by using Quasi emulsion solvent diffusion method. Mesalamine was selected as a model drug because of its short half-life, low water solubility. Nanosponges are non- irritating, non- mutagenic, non- allergenic. Mesalamine loaded Nanosponges were prepared by using polymers such as Eudragit RS 100 and Ethylcellulose and DMSO as a solvent. Mesalamine loaded nanosponges were characterized for particle size, practicle yield, entrapment efficiency, FTIR, SEM, solubility study and *in- vitro* drug release study. The optimized batch (F4) contained Ethylcellulose as a polymer. Batch F4 exhibited %EE of 79.58%, Percentage yield of 78.06%, Average particle size of 4.5 μm. The solubility of nanosponges was found to be 3.15 mg/ml in water. The FTIR spectra showed stable character of Mesalamine in mixture of polymer and revealed the absence of drug polymer interactions. The SEM studies confirmed their porous structure. The drug release from nanosponges was found to be 65.93% upto 6 hrs. It was concluded that the Nanosponges developed by this method showed increase in solubility and dissolution rate of Mesalamine.

INTRODUCTION

For administration of drugs to patients, the oral route is considered to be the most convenient among all. Dosage forms that deliver drugs into the colon rather than upper GIT offers various advantages such as oral delivery of drugs to the colon in the treatment of diseases of colon (i.e. Ulcerative colitis, Chron's disease, Infections and Carcinomas), minimizing side effects that occur because of release of drugs in upper GIT or unnecessary systemic absorption.[1] Inflammatory bowel diseases (IBD) include two major forms of chronic intestinal disorders namely Crohn's disease and Ulcerative colitis. Ulcerative colitis is the inflammatory disease of the colonic mucosa usually treated with salicylates and glucocorticoids.[2] Mesalamine (5-aminosalicylic acid) is an anti-inflammatory drug used to treat inflammatory bowel diseases (IBD). Mesalamine belongs to Biopharmaceutical Classification System (BCS) class IV drug, with low permeability and solubility. Elimination half life of mesalamine is 5 hrs. Mesalamine is an anti-inflammatory agent which is structurally related to the salicylates and NSAIDs like acetylsalicylic acid and is active in IBD. Mesalamine is considered to be the active moiety of sulphasalazine. It shows 20 to 30% of oral bioavailability.[3]"Nanosponges" means the nanoparticles having porous structure. Nanosponges are tiny sponges with size of about a virus i.e. diameter below 1μm.. The sponge simply acts as a three dimensional network or scaffold. These scaffolds are generally composed of polymers and other materials which have been used in drug

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delivery system. The backbone is a long length polyester mixed in solution with cross-linkers to form the polymer.. These sponges circulate around the body until they encounter the specific target site and bind to the surface and begin to release the drug in a controlled and predictable manner.Nanosponges can control the release rate of the drug or target drug to a specific body site.[4] Nanosponges are solid in nature and can be formulated as oral, topical, parentral or inhalational dosage form. For oral administration, these nanosponges may be dispersed in a matrix of excipients, diluents, lubricants, suitable for the preparation of tablets or capsules with the major benefits of reducing total dose, reduction in toxicity, retention of dosage form and improving patient compliance.[5]
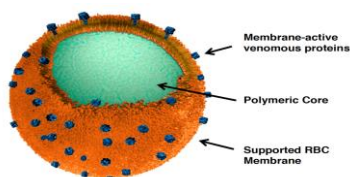

Figure 1: Structure of Nanosponges

These nanosponges can bind poorly water soluble drug within the matrix leading to improve in their solubility and bioavailability. Nanosponges are able to entrap both hydrophilic and lipophilic drug molecules because of their external hydrophilic branching and inner hydrophobic cavities.[6]Nanosponges are a novel class of hyper-cross linked polymer based colloidal structures consisting of solid nanoparticles with colloidal and nanosized cavities.[7]

MATERIALS AND METHODS:

Materials: Mesalamine, a gift sample was obtained from Nova Chem Drugs Pvt Ltd (Pune, Maharashtra, India). Eudragit RS 100, a gift sample obtained from CP Kelco India Pvt Ltd (Mumbai, Maharashtra, India). Ethylcellulose, DMSO, Polyvinyl alcohol used in the preparation were of laboratory grade.

Method: Mesalamine loaded nanosponges were prepared by Quasi emulsion solvent diffusion method using different polymer amounts. Two phases are prepared in this method i.e. inner phase and outer phase. The inner phase is prepared by dissolving polymer (such as Eudragit RS 100 and Ethylcellulose) in suitable solvent (DMSO), then the drug is added to the inner phase and dissolved under ultrsonication at 35ºC. The inner phase is then poured into the outer phase which contain PVA solution in water and stirred for 1 hr. Then the mixture is filtered and dried in air at room temperature or in oven at 40ºC for 12 hrs. Composition of Mesalamine loaded Nanosponges is shown in Table no. 1:[8]

CHARACTERIZATION OF NANOSPONGES:

Percentage yield: The production yield can be determined by calculating the initial weight of raw materials and final weight of nanosponges:

$$\text{Percentage yield} = \frac{\text{Practical mass of nanosponges}}{\text{Theorotical mass}} \times 100$$

The percentage yield of batches was determined by weighing the Mesalamine loaded nanosponges after drying.

Entrapment efficiency: UV Spectrophotometric method was used to determine entrapment efficiency of Mesalamine loaded nanosponges. The amount of the drug in the suspension was analysed by centrifugation at 1000 rpm for 30 mins and measuring the concentration of drug in the supernatant layer. The amount of drug was analysed by using UV spectrophotometric method at 300 nm. The concentration of drug is analysed by using calibration curve data after necessary dilutions. The percentage of drug entrapped was calculated by the following equation:[9]

$$\% \text{ Entrapment Efficiency} = \frac{Actual\ drug\ content\ in\ the\ nanosponges}{Theorotical\ drug\ content} \times 100$$

Fourier transforms infrared spectroscopy studies (FTIR): FTIR checks out the compatibility between the drug and the excipients. FTIR analysis of the pure drug and nanosponges formulations was carried out by FTIR spectroscopy instrument. The samples were dispersed in the KBr powder and the pellets were made by applying the pressure. The FTIR spectra were obtained by powder

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diffuse reflectance. The spectrum was scanned from 4000 to 400 cm^{-1}.[10]

Average particle size: The average particle size and size distribution of batches of Mesalamine loaded Nanosponges was determined by using SAGLO SOFT- SGL Micro- Imaging Adaptor.

Scanning Electron Microscopy (SEM): The shape and morphology of nanosponges were examined by using Scanning Electron Microscopy (FEI NOVA Nano FESEM 650). A suspension was spread on a glass slide and kept under vacuum. The scanning electron microscope was operated at 15 kV acceleration voltage. Photographs were elaborated by an image processing program and diameters of individual nanoparticles were measured to obtain mean particle size.[11]

Solubility study: Solubility of Mesalamine loaded nanosponges was studied by using shake flask method. According to this shake flask method, the compound was added in surplus to a solvent (Water and Phosphate buffer pH 6.8) and shaken for 24 hrs. The saturation is confirmed by observing the presence of un-dissolved material. The un-dissolved material is removed by filtration. Filtration and analysis should be performed under the same temperature. The amount of solute contained in the sample was analysed by UV spectrophotometer at 300 nm.

***In- vitro* drug release studies:** *In- vitro* drug release studies were carried out in USP type I dissolution apparatus (i.e. Basket apparatus) with stirring speed 50 rpm at 37 $\pm$ 0.5ºC. Initially, the drug release was carried out in 900 ml of 0.1 N HCl for 2 hrs followed by in 900 ml Phosphate buffer pH 6.8 for the next 6 hrs. Samples were withdrawn at regular intervals of time and each time equal volume of fresh dissolution medium was added to maintain the sink condition. The samples were analysed spectrophotometrically at a wavelength of 300 nm.[12]

RESULTS AND DISCUSSION:

Percentage yield: The percentage yield of F1 and F4 batch was found to be the best. By further increasing the concentration of polymers the percentage yield decreases because of the sticky nature of the product. Percentage yield of Mesalamine loaded nanosponges ranged between 63.42% to 72.77% for formulation with Eudragit RS 100, 69.55% to 78.06% for formulation with Ethylcellulose. The percentage yield value of all batches of Mesalamine loaded nanosponges is tabulated in Table No. 2:

Entrapment efficiency: From the results given in the table, it has been observed that F4 batch has higher entrapment efficiency than other five batches. Different formulations showed entrapment efficiency values that ranged between 55.28% to 79.58%. F4 batch has 79.58% entrapment efficiency, while F1, F2, F3, F5 and F6 has 69.76%, 55.28%, 63.98%, 72.76% and 62.48%. The percentage of entrapped drug was determined spectrophotometrically. The percentage entrapment efficiency of all batches of Mesalamine loaded nanosponges is tabulated in Table No. 2:

Fourier transform infrared spectroscopy (FTIR): Infrared spectroscopy was one of the method used for the authentication of the compound. The FTIR spectra of pure Mesalamine and Mesalamine loaded nanosponges are shown in Figure 2 and 3. IR spectrum of pure drug Mesalamine displayed absorption peaks of functional group such as C$-$H aromatic stretching, C$=$C aromatic stretching, O$-$H stretching of carboxyl, C$-$O stretching of carboxyl and N$-$H bending of amine was found to be 2980 cm^{-1}, 1645 cm^{-1}, 2521 cm^{-1}, 1313 cm^{-1} and 1617 cm^{-1} respectively. Various absorption peak of functional group at C$-$H aromatic stretching, C$=$C aromatic stretching, O$-$H stretching of carboxyl, C$-$O stretching of carboxyl and N$-$H bending of amine group of F4 batch was found to be 2972 cm^{-1}, 1645 cm^{-1}, 2520 cm^{-1}, 1313 cm^{-1} and 1617 cm^{-1} respectively. Hence, Mesalamine loaded nanosponges showed similar absorption peaks which indicates its good compatibility with polymers.

Table No. 1: Composition of Mesalamine loaded Nanosponges.

Formulation code	Mesalamine (mg)	Eudragit RS 100 (mg)	Ethylcellulose (mg)	DMSO (ml)	PVA (mg)	Distilled Water (ml)
F1	500	100	-	10	400	40
F2	500	300	-	10	300	40
F3	500	500	-	10	200	40
F4	500	-	100	10	400	40
F5	500	-	300	10	300	40
F6	500	-	500	10	200	40

Table No. 2: Percentage yield and Entrapment efficiency of Nanosponges.

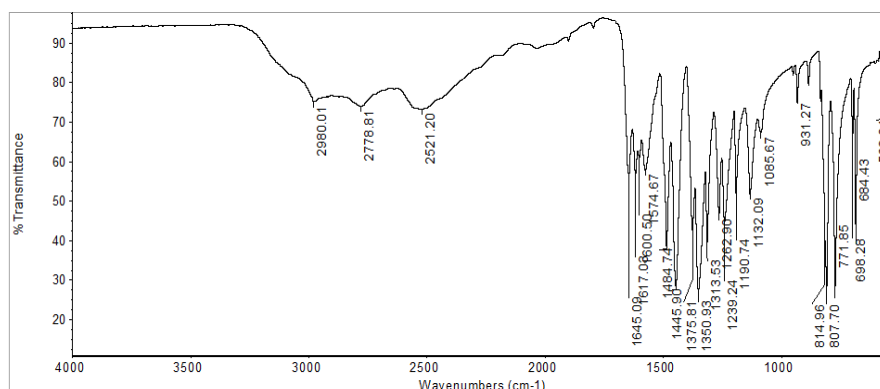| Formulation code	Percentage yield	Entrapment efficiency
F1	72.77%	69.76%
F2	63.42%	55.28%
F3	69.11%	63.98%
F4	78.06%	79.58%
F5	71.48%	72.76%
F6	69.55%	62.48%

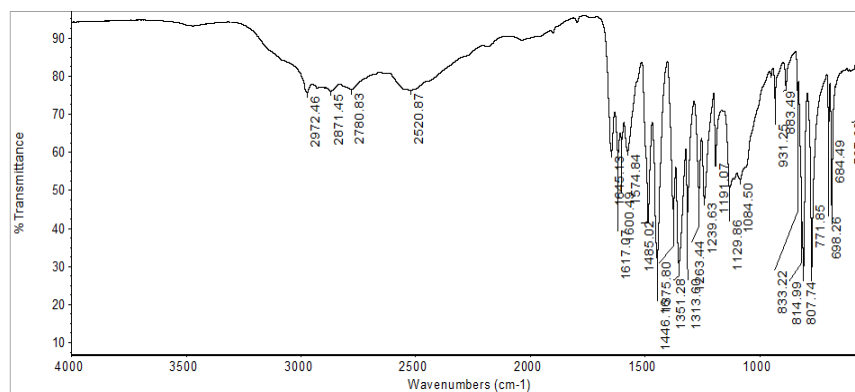**Figure 2: FTIR spectrum of pure drug Mesalamine.**

Figure 3: FTIR spectrum of F4 batch of Nanosponges.

Average particle size: Particle size of Mesalamine loaded nanosponges was analysed by simply using SAGLO SOFT- SGL Micro- Imaging Adaptor. Particle size of all six batches of Mesalamine loaded nanosponges was determined. Nanoparticles have size in nanometres whereas nanosponges have pores in nanometers while their overall size can extend upto micrometers and are usually smaller than 5 μm. Average particle size of nanosponges of F4 batch was found to be 4.533 μm. Microscopic image of F4 batch is shown in Figure 4:
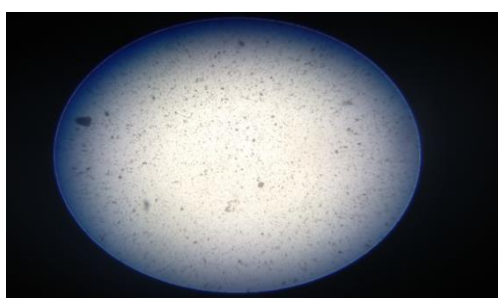

Figure 4: Microscopic image of F4 batch.

Scanning Electron Microscopy (SEM): The surface morphology of nanosponges was investigated by scanning electron microscopy. The SEM images of Mesalamine loaded nanosponges are shown in Fig 5 and 6. From these images it was observed that the nanosponges were uniformly spherical in shape, spongy and porous in nature.
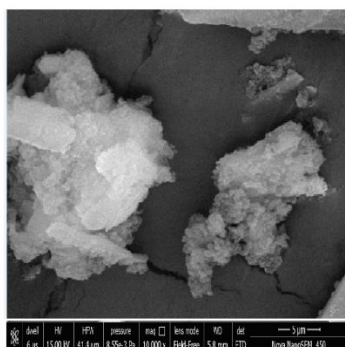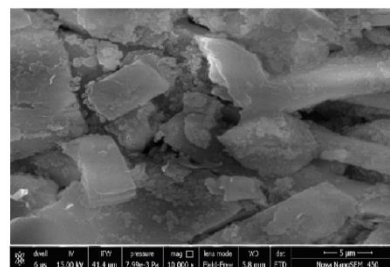

Figure 5: SEM image of F4 batch.

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Figure 6: SEM image of F4 batch

Solubility study: Solubility study of all batches of nanosponges was performed in distilled water and Phosphate buffer pH 6.8 was determined by shake flask method. Prepared Mesalamine loaded nanosponges showed increased solubility than the pure drug in phosphate buffer and water.F4 batch showed increase in solubility both in water and in phosphate buffer pH 6.8. The increase in solubility of drug may be due to reduction in particle size. Data of solubility is represented in Table No. 3:

***In- vitro* drug release study:** The *in- vitro* drug release profile of Mesalamine loaded nanosponges is shown in the Table No. 4. The *in- vitro* drug release of Mesalamine loaded nanosponges was found in the range of 48.54% to 65.93% in 360 mins respectively. From the dissolution study it was concluded that all the formulation batches show more than 40% drug release in 300 mins. In all batches, F4 batch shows maximum drug release i.e. 65.93% and was selected as the optimized batch. Hence, graph was plotted which is given in the Figure 7 to determine the drug release of the drug.
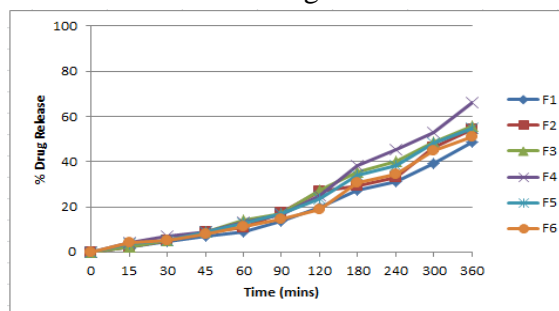

Figure 7: *In- vitro* drug release of Mesalamine loaded nanosponges.

Table No. 3: Solubility of Nanosponges in water and phosphate buffer.

Formulation code	Solubility in water (mg/ml)	Solubility in Phosphate buffer pH 6.8 (mg/ml)
Pure drug	0.84	2.84
F1	0.97	1.89
F2	0.89	3.10
F3	0.85	2.91
F4	3.12	7.81
F5	1.91	6.55
F6	1.11	3.21

Table No. 4:*In- vitro* drug release study of Nanosponges.

Time (min)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
15	2.14	2.51	2.30	4.38	3.83	3.96
30	4.68	5.32	5.18	7.23	5.29	5.07
45	7.08	8.80	9.12	9.05	8.93	7.74
60	9.07	10.73	13.97	13.06	12.78	11.05
90	13.51	17.52	16.87	16.67	16.89	14.61
120	19.56	26.87	27.46	25.11	23.59	18.74
180	27.57	29.07	35.17	38.25	33.75	30.67
240	31.15	33.24	40.28	45.28	38.25	34.61
300	39.29	46.20	48.78	52.84	48.08	44.83
360	48.54	54.09	55.56	65.93	54.57	51.13

CONCLUSION: Quasi emulsion solvent diffusion method was useful for the incorporation of poorly water soluble drug i.e. Mesalamine. The particle size of prepared nanosponges were in nanometer range. The *in-vitro* drug release from nanosponges is much higher than the pure drug. Based on entrapment efficiency, solubility study, FTIR study, SEM, etc F4 batch containing polymer (Ethylcellulose) was considered as the optimized batch. The results of the above study show that the research work was satisfactory for improved solubility and drug release profile.

Conflict of interest: The authors declare that there is no conflict of interest.

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