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SOLUBILITY ENHANCEMENT OF MESALAMINE BY NANOCRYSTAL TECHNIQUE

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ABSTRACT
Nanocrystals, a carrier-free colloidal delivery system in nano-sized range, is an interesting approach for poorly soluble drugs. With the advancement in modern pharmaceutical technologies, Nanotechnology is the one of the most establish technology which is used to improve the therapeutic index and to overcome the formulation challenges of poorly water-soluble compounds. Mesalamine is Anti-inflammatory drug used for the treatment of inflammation of bowel i.e. ulcerative colitis and crohn's disease. In the present study an attempt is made to formulate the colon targeted drug delivery system of mesalamine using Eudragit -S 100 and HPMC K4M as a release retarding polymer. Various formulations were developed by varying concentration of Eudragit R 100 and HPMC K15M by using wet granulation technique. Developed formulations were evaluated for various parameters like hardness, friability etc. The formulation F3 was found to be optimum based on all evaluation parameters.

KEYWORDS: *Nanocrystals, HPMC K15M, Eudragit R 100 colloidal delivery system etc.*

INTRODUCTION

Now days, the most important issue in drug discovery and development is the poor solubility. Nanocrystals are crystalline nanoparticles with size ranging from 200-500 nm stabilized by surface stabilizers[2]. The drug Nanocrystals constitute a versatile formulation approach to enhance the pharmacokinetic and pharmacodynamic properties of poorly soluble drugs. Over the past several years there has been a dramatic increase in bowel diseases[2]. In the recent years, colon targeted delivery systems have been focus point of formulation laboratories because of colon is considered as a sutaible site for the delivery of both conventional and labile molecules, and it is also site for some specific diseases such as ulcerative colitis, crohn's disease, bowel cancer, some infections and constipation which require local delivery of drugs[3]. Mesalamine is a derivative of salicylic acid it is usually a first line anti-inflammatory agent to act locally on colonic mucosa and reduce inflammation for the treatment of colon related disease[4]. Mesalamine is categorized as BCS class IV drug (low solubility & low permeability) according to the biopharmaceutical classification systems[5].

MATERIALS AND METHODS

Mesalamine (5-amino salicylic acid) was obtained from Dhamtec Pharma & Consultants Mumbai. HPMC K15M and Eudragit R 100 were obtained from Yarrowchem products, Mumbai. Citric acid, talc, magnesium sterate, lactose were obtained from S.D.Fine chem, Mumbai. All reagents and solvents used were of analytical grade satisfying pharmacopoeial standards.

PREPARATION OF MESALAMINE NANOCRYSTALS[8]

The Nanocrystals were prepared by Antisolvent Precipitation technique. In this technique drug is dissolved in an organic solvent ethanol in which it is soluble and this solution is mixed with a miscible Antisolvent for precipitation. In the water solvent mixture the solubility is low and the drug precipitate out. Precipitation has also been combined with high shear processing. This is accomplished by a combination of rapid precipitation and high-pressure homogenization. Sudden super saturation occurs by rapid addition of drug solution to Antisolvent and generation of fine crystalline material may be favored at high super saturation when the solubility of the amorphous state is exceeded. The solution was filtered through a 0.45µm whatman's filter paper to remove the impurities. The formed mesalamine nanocrystals were filtered and dried at room temperature.

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PREPARATION OF MESALAMINE NANOCRYSTALS LOADED TABLET[9]

Weighed amount of mesalamine nanocrystals, Eudragit R 100 and HPMC K15M, lactose were geometrically mixed. Sufficient quantity of ethanol was added to get uniform wet mass. Then, sieve No. 10 was used for granulation and prepared granules were kept for drying for 2 hours in hot air oven at 40°C. The dried granules were again pass through the sieve no 12. Sufficient quantity of lubricant and glidant were added just before compression. The granules were compressed into tablets by single station compression machine.

Formulation of tablets

Sr. No	Ingredients (mg)	F1	F2	F3	F4
1	Mesalamine	400	400	400	400
2	Microcrystalline cellulose	-	60	70	80
3	HPMC K15M	-	2	3	4
4	Eudragit R 100	0.5	1	2	3
5	Citric acid	14	15	10	10
6	Talc	0.25	0.25	0.30	0.3
7	Magnesium sterate	6	2	3	2
8	Lactose	79.25	19.75	11.7	0.7

Table No. 1: Formulation table for F1 to F4

EVALUATION OF PRE-COMPRESSED GRANULES OF MESALAMINE[11-16]

- **Bulk density**

  5 gm of mesalamine powder was weighed and gently poured through a short stemmed glass funnel into 100 ml graduated glass cylinder. The volume occupied by granules was read and the bulk density of the powder can be determined by the formula given below. It was measured in terms of $g/cm^{3.}$

  Bulk density = Weight of powder/ Bulk volume

  The results mentioned in table no. 2

- **Tapped density**

  Tapped density was determined by USP method II. Tablet blend was filled in 100 ml graduated cylinder of tap density tester ETD-1020 which was operated for fixed number of taps until the powder bed volume has reached a minimum. It was calculated by the following formula. It was measured in terms of g/cm^3.

  The results mentioned in table no. 2

- **Hausner's Ratio**

  Hausner's ratio is used for predicting flow characteristics. The Hausner's ratio is calculated by using following formula.

  Hausner's ratio = Tapped density/ Bulk density

  The results are mentioned in table No. 2

- **Compressibility Index**

  The Carr's Compressibility index were calculated by using following formula:

  Compressibility Index (%) = Tapped density – Bulk density / Tapped density X 100

  The results are mentioned in table No. 2

- **Angle of repose**

  The angle of repose measured to enhances the flow properties of granules. A funnel was fixed to a stand and the bottom of the funnel was fixed at a height of 3 cm from the plane. The powder was placed in the funnel and allowed to flow freely and the height and radius of the heap of the powder was measured.

  $\tan \Theta = h/r$

  The results are mentioned in table No. 2

EVALUATION OF TABLETS:[12-16]

- **Weight variation**

  20 tablets were randomly selected from every batch and average weight was calculated. Then the deviation of individual weights from the average weight and standard deviation were calculated.

  The results are mentioned in table No. 3

- **Hardness**

  10 tablets were randomly selected from each batch. In tablets, the crushing strength is additionally transformed into a tensile strength. It was measured in terms of kg/cm^2

  The results are mentioned in table no. 3

- **Thickness**

  Thickness of 5 randomly selected tablets from each batch were measured with digital Vernier calipers. Then the average thickness should be controlled within 5% variation of standard value.

  The results are mentioned in table No. 3

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- **Friability**

To check the strength of the tablets by using Rouche friability testing apparatus.Were20 tablets are weighed and placed in the plastic chamber which revolves at 25 rpm for 4 min. The tablets are then reweighed to find out the % loss in weight. The friability of the tablets was determined by the formula given below. The average hardness and standard deviation were calculated.

% Friability = Final weight – Initial weight / Initial weight X 100

The results are mentioned in table No. 3

- **Drug content**

Five tablets were selected randomly from every batch, weighed and powdered in a mortar. An accurately weighed quantity powdered tablets equivalent to 10 mg was taken in standard flask and make up the volume of flask with 6.8 buffer solution and the solution was filtered through 0.45 µ membrane paper. Each extract was sutaibly diluted and analyzed spectrophotometrically at 232 nm. Concentration of solution was calculated from the standard calibration curve.
The results are mentioned in table No. 3

- **Solubility study**

Solubility of Mesalamine loaded NCs 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 6.8) and shaken for 24 hours. The saturation is confirmed by 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 analyzed by UV spectrophotometer at 232 nm.
The results are mentioned in table no. 4

- **Fourier Transform Infrared Spectroscopy (FTIR)**

FTIR checks out the compatibility between the drug and the excipients. FTIR analysis of the pure drug and nanosphere formulations was carried out by FTIR spectroscopy instruments. The samples were dispersed in the Kbr powder and the pellets were made by applying the pressure. The FTIR spectra was obtained by powder diffuse reflectance. The spectrum was scanned from 4000 to 400cm^{-1}.
The results are mentioned in Table No.6

- **Scanning Electron Microscopy (SEM)**

The shape and morphology of nanosphere were examined by using SEM. A suspension was spread on a glass slide and kept under vacuum. The SEM was operated at 15 KV acceleration voltage. Photographs were elaborated by an image processing program and diameter of individual nanoparticles were measured to obtain mean particle size.
The results are mentioned in Fig.No.1

- **Differential Scanning Calorimetry (DSC)**

When a material is heated or cooled, there is a change in its structure or its composition. These changes are connected with heat exchange. Some of these changes are endothermic (i.e. heat consuming process such as melting) and others are exothermic (i.e. heat producing process such as crystallization). DSC is used for measuring the differences in heat flow between a sample and a reference during a controlled change of temperature (74-75). DSC analysis allows quantitative and qualitative information to be obtained about the physical and chemical changes that occur in the sample.
DSC is used extensively in pharmaceutical industry to determine the melting points, purity and glass transition temperature of materials. In the solid dispersion area, DSC is a powerful tool in evaluating the drug-carrier interactions, determining the solubility of a drug in a polymeric carrier and detecting polymorphic modifications. The broadening of the drug melting peak in the DSC thermal profile of a solid dispersion indicated that the drug is dispersed molecularly or it exists in the amorphous form.
The results are mentioned in Fig.No.3

- **X-Ray Diffraction (XRD)**

Diffraction of X-rays is the basic technique for obtaining information on the atomic structure of crystalline solids. The phenomenon of XRD by crystals results from a scattering process in which x-rays are scattered by the electrons of the atoms without change in wavelength. The crystallinity in a sample is reflected by a characteristic fingerprint region in the diffraction pattern. In a Nanosphere the crystallinity in the drug is molecularly dispersed and nanosphere in which the drug is present in the crystalline form. However, crystallinities of under 5-10% cannot generally be detected with x-ray diffraction.
The results are mentioned in Fig No.4

- **In- vitro release studies**

In-vitro release studies were carried out in USP type I dissolution apparatus (i.e. Basket apparatus) with stirring speed 50 rpm at 37± 0.5°C. Initially, the drug release was carried out in 900 ml of phosphate buffer 6.8 for the next 6 hours. 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 analyzed spectrophotometrically at a wavelength of 232nm.
The results are mentioned in table No. 7

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RESULTS AND DISCUSSION

Pre-compressed granules

Formulation Code	Bulk density (gm/cc)	Tapped density (gm/cc)	Angle of repose (°)	Compressibility index (%)	Hausner's Ratio
F1	0.413	0.516	38.65	19.9	1.24
F2	0.3435	0.4079	30.45	15.78	1.18
F3	0.3207	0.3790	31.60	15.38	1.18
F4	0.2944	0.3533	31.21	16.67	1.20

Table No. 2: Pre-compressional parameters for F1 to F4

Evaluation of tablets

Formulation code	Thickness (mm)	Hardness (Kg/cm^2)	Weight variation (mg)	Friability (%)	Drug Content (%)
F1	2.717	3.9	506	0.19	81.90
F2	2.644	3.8	501	0.19	86.84
F3	2.687	4	497	0.18	91.77
F4	2.606	3.9	475	0.19	90.13

Table No. 3: Evaluation parameters for F1 to F4

- **Solubility study**

Solubility study of all batches of tablets were performed in distilled water and phosphate buffer 6.8. The solubility was determined by shake flask method. Prepared Mesalamine loaded nanocrystals showed increased solubility than the pure drug in phosphate buffer and water. F3 batch showed increased 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.

Formulation code	Solubility in water (mg/ml)	Solubility in Phosphate buffer pH 6.8 (mg/ml)
F1	0.95	1.72
F2	0.89	3.12
F3	1.90	6.23
F4	1.49	4.25

Table No. 4: Solubility for F1 to F4

- **Scanning Electron Microscopy (SEM)**

The surface morphology of nanocrystals were investigated by SEM. The SEM image of Mesalamine nanocrystals loaded tablets are shown in figure. From this image observe that the nanocrystals were crystalline in shape. The average nanocrystals was found to be 5µ.

Fig No.1 Scanning Electron Microscopy

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- **Fourier Transform Infrared Spectroscopy (FTIR)**

The FTIR analysis was implemented to assume the compatibility of assorted excipients blend with the pure drug. Spectral examination were execute using FTIR to explore the generation f new compound or any chemical change in the functional portion of the admixtures among the blends. IR spectroscopy was utilized in pharmaceutical investigation for its authentication and structure elucidation of drug. The peaks given in the table could be considered as the characteristic peaks of mesalamine.

IR spectroscopy was one of the method used for the authentication of the compound. The FTIR spectra of pure mesalamine and mesalamine nanocrystals loaded are shown in the figure. IR spectrum of pure drug mesalamine displays 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 2535 cm^{-1}, 2982.46 cm^{-1}, 1655.19 cm^{-1}, 1328.6 cm^{-1}, 1619 cm^{-1} respectively.

Functional group	Absorbance frequency region	Mesalamine cm^{-1}	F3 Batch cm^{-1}
O-H Carboxyl Streching	3000-3200	3085	2535
C-H aromatic stretching	2800-3100	2980.01	2982.46
C=C aromatic streching	1400-1700	1645.09	1655.19
O-H carboxyl streching	2500-3300	2521.20	2510.87
C-O carboxyl streching	1210-1320	1313.53	1328.60
N-H Bending of Amine	1600-1650	1617.03	1619.07

Table No. 6: Fourier Transform Infrared Spectroscopy (FTIR) for F3

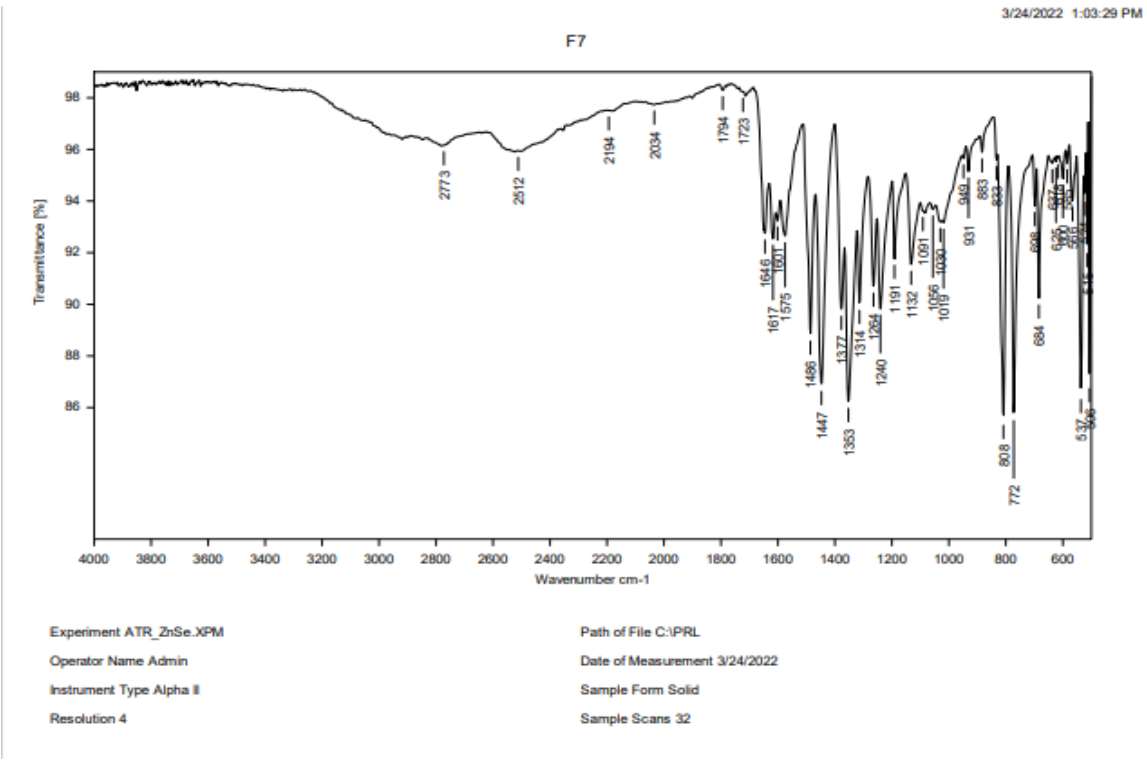

Fig. No: 2 Fourier Transform Infrared Spectroscopy (FTIR)

- **Differential Scanning Calorimetry (DSC)**

Thermal analysis of F3 batch was done through DSC. It was commonly used method within the pharmaceutical industry, which allows the assessment of any change in phase transition from crystalline to amorphous state of drug concurrently preformulation and formulation steps. This approach aid in the assessment of physical properties, compatibility and stability studies of the constituents of pharmaceutical advancements. It was observed from the thermo gram of x drug was implicated in the fig. The single

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sharp endothermic transition was characterized at a temperature of maximum of 275°C and these values found to be in acceptable range with the literature value of 275°C-280°C.

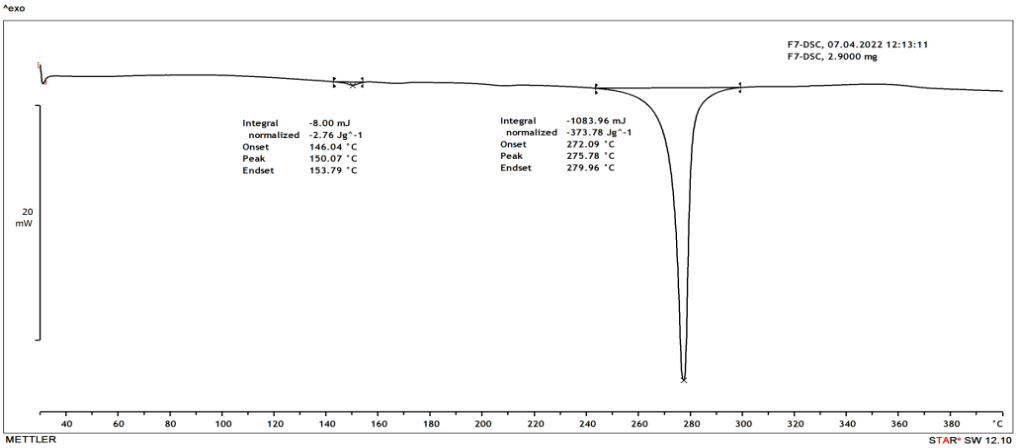

Fig No.3 Differential Scanning Calorimetry

- **Powder X-Ray Diffraction**

The P-XRD patterns of pure mesalamine showed numerous sharp peaks at 27.13, 16106.7,11.71 which are the characteristics of the crystalline compound and are compared with the P-XRD patterns of nanocrystals. F3 batch shows crystal peaks. P-XRD graphs of F3 batch are shown in fig. respectively.

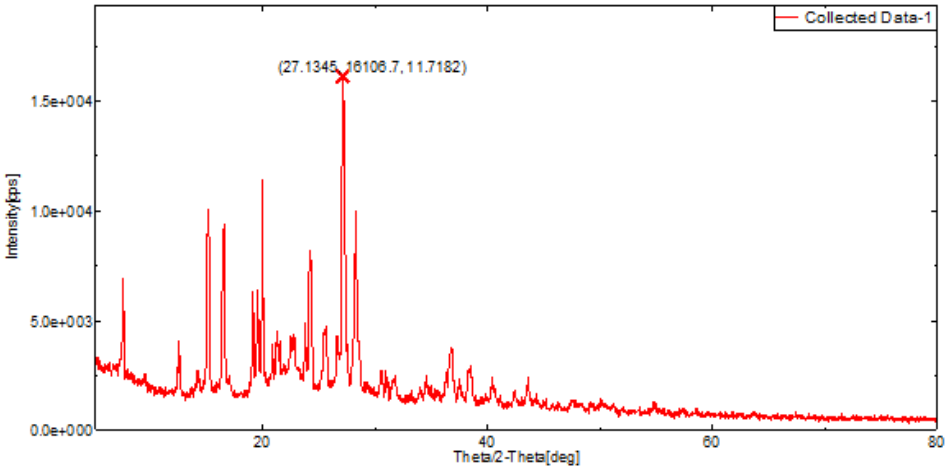

Fig.No: 4 X-Ray Diffraction

- ***In- vitro* study ofF1to F4 batch**

Time (hours)	F1	F2	F3	F4
0	0.00	0.00	0.00	0.00
0.5	2.19	3.26	7.09	6.51
1	5.65	6.19	15.75	11.10
2	7.41	9.30	24.51	19.42
3	11.18	14.45	31.39	26.65
4	17.50	18.24	45.68	33.97
5	22.47	24.25	48.77	39.19
6	28.90	30.64	56.88	43.88
7	36.34	36.81	64.38	52.24

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8	42.14	42.85	72.63	55.39
9	46.08	50.38	77.11	61.82
10	48.59	52.38	84.39	69.79
11	53.26	57.28	88.27	76.93
12	57.81	61.00	94.93	83.93

Table No. 7: *in-vitro* for F1 to F4

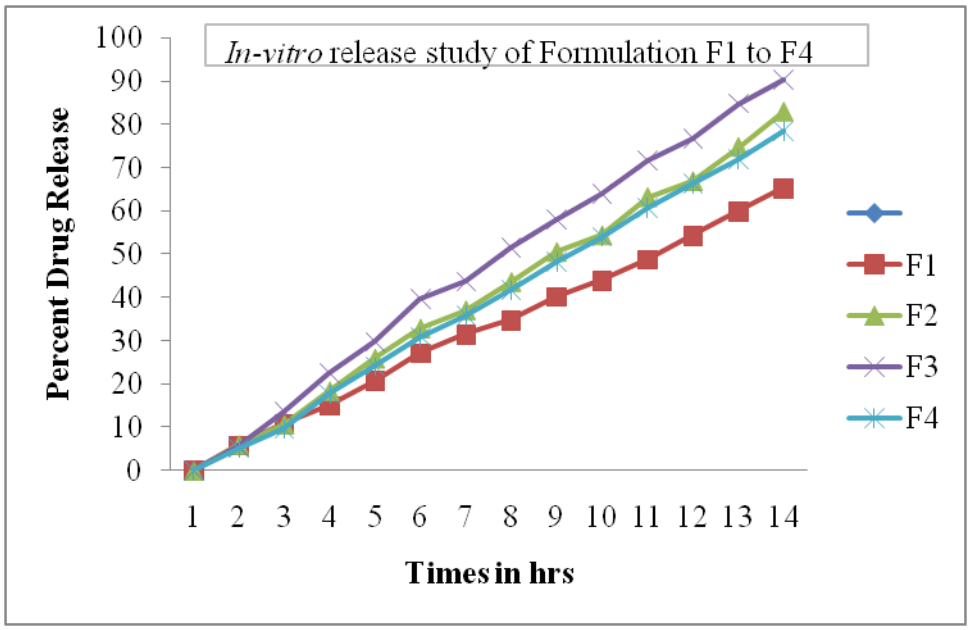

Fig. No. 5 In-vitro release study

CONCLUSION

From the above results we can conclude that mesalamine formulations prepared with HPMC K15M and Eudragit R 100 showed acceptable properties like friability, weight variation, hardness and in-vitro drug release which remained unchanged upon storage for 3 months. However, HPMC K4M, Eudragit S 100 based mesalamine tablets with the formulation code F3 proved to be the formula choice, since it showed the highest drug release and lag time when compared to the marketed formulation.

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