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Multiparticulate Drug Delivery System for Gastrointestinal Tuberculosis

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ABSTRACT

Drug solubility poses numerous challenges in design of formulations for drugs with poor aqueous solubility. Ethionamide is an antitubercular drug belonging to biopharmaceutical classification system class II drug having less aqueous solubility. Nanosuspensions were prepared by using various solvents such as methanol, ethanol, acetone and chloroform and it was prepared using anti-solvent precipitation technique by using probe sonication. Various stabilizers such as tocopherolpolythytlene glycol succinate, polyvinylpyrrolidone and tween 80 singly or in combination were studied. A 3^2 factorial design was employed to study the effect of independent variables, concentration of stabilizers and stirring speed on particle size and cumulative percent drug release. The particle size of the optimized batch was 97.54 ± 8.47 nm with polydispersity index of 0.36 and zeta potential -10.1 ± 2.3 mV. The cumulative percent drug release of optimized batch was found to be 95.01 ± 1.16% in 60 min. Optimized batch was ultracentrifuged and evaluated for saturation solubility studies, stability and powder X-ray Diffraction studies. Optimized nanosuspension was loaded on Espheres by spraying in a coating pan and then coating of Eudragit controlled release polymers. The coated Espheres were evaluated for drug content, friability, scanning electron microscopy, *ex-vivo* permeation studies and drug release kinetics studies. The friability value for primary coated sphere was found to be 0.8 ± 0.12% and for secondary was 1% and the best fit model was found to be Korsmeyer-Peppas model which is indicative of diffusion controlled release. *Ex vivo* diffusion studies revealed a moderate increase in permeability.

Keywords: Solubility enhancement, Ethionamide, Nanosuspension, Coating, Multiparticulate system, Drug release kinetics.

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INTRODUCTION

Solubility is the property of substance in which solute molecules get dissolved in the different solvent to form a homogeneous solution. Particle size, polymorph, nature of solute and solvent are various factors that affect solubility. Drugs with poor water solubility (BCS class II) give some problems related to low solubility and low absorption. Different strategies to overcome

the problem include size reduction, cosolvency, solid dispersions, inclusion complexation and by using supercritical fluids. [1] Nanosuspensions are dispersions of nanoparticles that are stabilized by surfactants. Two methods commonly used for the preparation of nanosuspension are 'bottom-up technology' and 'top-down technology'. Bottom-up technology is basically a controlled crystallization process involving the use of solvent and antisolvent for the drug. [2] Effect of the flow rate of solvent and antisolvent, concentration of drug and stabilizers, solvent antisolvent ratio, temperature are important variables in the formulation of nanosuspension by this technique.

Oral multiparticulate drug delivery systems consist of granules, microspheres, pellets, spheroids and minitabs. They offer number a advantages in comparison to single unit systems in terms of uniformity of release and less probability of dose dumping. Hence they are most suited for controlled or sustained drug delivery. [3]

Tuberculosis (TB) is a life-threatening disease that can affect any organ in our system. Mainly TB is caused by *Mycobacterium tuberculosis* and exists in pulmonary and extra pulmonary form. Though pulmonary form is most common, the extra pulmonary sites include gastro intestinal tract (GIT), liver, spleen, pancreas, lymph nodes, etc. Nearly 64% cases of gastrointestinal TB occur in ileocecal region. [4] Ethionamide (ETH) is belonging to BCS class II and it is a nicotinic acid derivative related to isoniazid, widely used for treatment of tuberculosis. ETH undergoes intracellular modification and inhibits the synthesis of mycolic acids, an essential component of the tubercular bacterial cell wall. It has a high melting point and has a log p value 11.89 and it commercially available as conventional tablets and capsules. [5]

The present study was conducted to enhance the solubility of ETH by developing the nanosuspension using anti-solvent precipitation technique by using different type of stabilizers like Tocopherol polyethylene glycol succinate, polyvinyl pyrrolidone, Tween 80 and the solvents like acetone, methanol, ethanol, chloroform. Optimization was carried out using 3^2 factorial design and involved study of effect of independent variables like the concentration of stabilizers and stirring speed on particle size and percent drug release. The optimized nanosuspension was coated on Espheres by spray coating and then with Eudragit L100 and Eudragit S100 to achieve controlled release in small intestine. The coated Espheres were evaluated for drug content, friability, scanning electron microscopy (SEM), *in vitro* drug release and *ex vivo* permeation studies by everted sac method.

MATERIALS AND METHODS

Materials

Ethionamide (ETH) sample was gifted by Maneesh Pharma Ltd, Mumbai. Tocopherol polyethylene glycol succinate (TPGS) and polyvinyl pyrrolidone (PVP K30) were obtained from Loba Chemicals Pvt. Ltd. Mumbai, India. Espheres were obtained as a gift sample by Ideal Cures Mumbai, India. All the reagents and solvents used were of analytical grade.

Methods

Characterization of drug

The maximum wavelength (λmax) determination of the drug was done using UV spectrophotometer (Jasco V-550, Japan). The thermal compatibility of the drug was determined by using differential scanning calorimeter (Mettler Toledo DSC 821e, Mumbai, India). The compatibility of drug and excipients was checked by using Fourier Transform Infra-Red Spectrophotometer (JASCO-460 Plus, Japan), for this the drug and excipients.

UV spectrophotometric analytical method

Weighed quantity of ETH (250 mg) was added into methanol AR grade (100 ml). Different concentrations were prepared as 10-50µg/ml by diluting the stock solution with methanol, and analysis was done at 288 nm using UV spectrophotometer (Jasco V-550, Japan) against methanol as a blank. The validation parameters such as intra and inter day precision; accuracy, limit of detection (LOD) and limit of quantification (LOQ) were studied. [6]

Preliminary studies

ETH nanosuspensions (ETH-NS) were prepared using the anti-solvent precipitation technique. [7] The ETH solubility in various solvents such as acetic acid, ethanol, acetone and methanol was tested. Various surfactants and stabilizers such TPGS, PVP K30, SLS alone and in combination were used to analyze their stabilizing effect on nanosuspension. Surfactant concentration and solvents are important for producing minimum particle size. There are various parameters like the ratio of solvent to antisolvent, speed and time of stirring which affect the particle size of nanosuspension. ETH (250 mg) was dissolved in sufficient amount of solvent. The surfactant combination was dissolved in water separately. The drug solution was added to antisolvent in a drop wise manner under stirring by mechanical stirrer (Remi RQT124A, Mumbai, India) at 3500 rpm for 1 h. This solution was then subjected to probe sonication for 15 min. (55000 hz, pulse rate of 2 sec). Samples were analyzed for particle size and cumulative percent drug release.

Experimental design

A 3^2 factorial design was employed to investigate the effect of independent variables on particle size. Based on preliminary studies concentration of stabilizers and stirring speed were selected as factors. The level of stabilizers in the formulation was varied from 1% to 3% w/v in the ratio of (1:0.5 for PVP K30: TPGS). The levels for stirring speed were selected to be 2000, 3500 and 5000 rpm. The ten batches predicted by the optimization software were analyzed for particle size and cumulative percent drug release in 60 min. The factorial batches were also analyzed for zeta potential,

polydispersity index (PDI) and cumulative percent drug release. Optimized batch was selected from solutions given by software on the basis of constraints provided to software and 3D responses for all the responses.

Characterization of factorial batches

Particle size and zeta potential

The particle size of all ten ETH-NS batches was determined by particle size analyzer (Malvern ZS 90, India). For particle size and PDI determination, 1 ml of the sample was diluted to 10 ml with distilled water. The instrument setting for the refractive index was kept at 1, laser wavelength was set at 527 nm and cell temperature was maintained at 25°C and the mean diameter and the polydispersity index of each batch were recorded. For zeta potential measurement, determination was done utilizing laser diffraction with beam length 2.40 mm, range lens of 300 RF mm, and at 14.4% obscuration, the field strength applied was 20 Vcm^{-1}. [8] Triplicate measurements of particle size and zeta potential were taken.

Cumulative percent drug release

By using dialysis membrane method the drug release of ETH-NS was studied. The dialysis membrane was previously soaked for 8 h in the dissolution medium. The dialysis membrane (Dialysis membrane 70, HIMEDIA; MWCO 12,000-14,000 daltons; pore size 2.4 nm) was cut of 5cm length. It was tied at one end and 2 ml of ETH-NS was added into a dialysis bag and other end was sealed and tied to paddles of USP type II dissolution apparatus. It was placed in a USP dissolution vessel containing 900 ml of 0.1 N HCl. The temperature was maintained at 37°C with stirring speed at 50 rpm. At 15 min intervals 5 ml of aliquots were collected for 1 h and diluted with 0.1 N HCl. Sink conditions were maintained. The diluted sample was filtered by Whatman filter paper and samples were analyzed by UV-Vis spectrophotometer at 288 nm to determine the cumulative percent drug release. [9]

Evaluation of optimized batch

Ultracentrifugation

Ultracentrifugation is a technique to generate dried optimized nanosuspension. Formulations were centrifuged at 8000 rpm for 15 min using bench top centrifuge (Make: Remi Laboratories RM-12CBL, India) and observed for physical changes like separation of phases or precipitation of ETH-NS. The precipitate was collected and dried in the oven at 60°Cs.

Entrapment efficiency

About 10 mg of nanocrystals was accurately weighed and dissolved in methanol and filtered. Then drug content was estimated by UV visible detection at 288 nm. Entrapment Efficiency was calculated according to the following equation:

Entrapment Efficiency (%) = [Actual drug content in nanocrystals/Theoretical drug content] × 100

Fourier Transform Infrared Spectroscopy (FTIR)

The IR spectrum of ETH and ETH-NS were recorded using Fourier Transform Infra-Red spectrophotometer (JASCO-460 plus, Japan) with diffuse reflectance principle. The sample was scanned over a frequency range 4000-400 $cm^{-1.}$ The sample was ground with potassium bromide and pressed into a disc shape for measurement. [10]

Powder X-ray Diffraction studies (PXRD)

The PXRD spectra of ETH and ETH-NS were recorded using powder X-Ray diffractometer (D8 ADVANCE, BRUKER, India). The target filter was copper, voltage/current of 40 KV/40 mA and scan speed was 4°/min. The sample was analyzed at 2-theta angles between 5° to 50°. [11]

Compatibility Studies

Drug-excipients interactions were studied by recording FTIR spectra of pure ETH and ETH-NS. All the ingredients were uniformly mixed in a 1:1 ratio kept at 40 ± 2°C and 75 ± 5% RH. The samples were observed after 30 days for adverse physical interactions. [12]

Saturation solubility studies

Excess of ETH and ETH nanoparticles were placed in 20 ml of D/W, 0.1N HCl and phosphate buffer 6.8 for studying saturation solubility. The flask was kept in temperature controlled orbital shaker (CIS-24 Remi, India) for 24 h at 37±0.5°C. Dispersions were filtered using Whatmann filter paper no. 41. The filtrates were analyzed using UV spectrophotometer. [13]

Stability studies

According to ICH guideline stability studies of optimized ETH-NS was determined by using stability chamber (Make:Remi Equipments Ltd, Mumbai, Model- CHM-6S) and the samples placed in screw-capped vials under ambient conditions, at 40°C & 75% RH and 4°C in refrigerator for 1 month. The samples were inspected for discoloration, flocculation, sedimentation, and particle size. [14]

ETH-NS primary coating on Espheres

Optimized ETH-NS containing 250 mg of nanonized ETH in 100 ml water was prepared. Preliminary trials involved coating over Espheres with the optimized nanosuspension which revealed good adherence of NS on the Espheres. This was attributed to the TPGS which promoted wettability of the Espheres surfaces and PVP K 30 in the NS which facilitated adherence of the NS on the Espheres. Hence there was no need to add additional excipients to enable adherence of the NS. ETH-NS primary coating on Espheres was accomplished using pan coater. Espheres (# 18-22) were selected since it enables the uniform drug release compared to other solid unit dosage forms. ETH-NS were carefully sprayed on 10 g of Espheres using pan coater (Make: Instacoat) at the rate of 1ml/min. Talc was added intermittently as required to prevent the sticking of Espheres. Pan rotation speed was maintained at 60 rpm and the temperature was kept at 70°C. It was followed by rolling of layered Espheres for an additional 1h at 50°C to facilitate drying. [15] At the end of the study primary coated batch was evaluated for drug content, friability and cumulative percent drug release.

Secondary coating of Espheres for controlled release

Preliminary trials were conducted to select polymers and their concentration. Eudragit S 100 and L 100 were coated onto primary coated Espheres singly and in combinations of both. [16-17] The ratio of Eudragit S100 to L 100 was varied at 1:1 and 1:2. Based on the release properties Eudragit S100 and Eudragit L100 (1:1 ratio) was selected for controlled release coating. Coating solution was prepared in isopropyl alcohol: acetone (1:1) mixture. The weight gain achieved was 10%.

Evaluation of coated Espheres

ETH-NS loaded and secondary coated Espheres were evaluated for drug content, friability, drug release and scanning electron microscopy (SEM).

Drug content and entrapment efficiency

Drug content was evaluated at the last stage of the coating so as to understand coating efficiency at various stages**.** Espheres containing 10 mg drug was crushed and dissolved in methanol and filtered. Then drug content was estimated by UV visible detection at 288 nm. [18] Entrapment Efficiency was calculated according to the following equation:

Entrapment Efficiency (%) = [Actual drug content in microparticles/Theoretical drug content] × 100

Friability

Primary and secondary coated Espheres were subjected to friability testing. The friability of Espheres was done by using a rotating drum apparatus (Roche friabilator) at drum speed of 100 rpm for 10 min. Weight of Espheres was measured after the test to determine handling properties of the formulation. [19]

In vitro dissolution studies

Dissolution studies of ETH-NS primary and secondary coated Espheres were carried out in a USP type II (paddle type) dissolution apparatus in 900 ml medium at 37±0.5°C at 50 rpm in 750 ml of 0.1 N HCl for first 2 h. Subsequently 150 ml of a 0.2 M solution of trisodium phosphate dodecahydrate was added to adjust the pH to 6.5. The pH was adjusted if needed, with 2 M hydrochloric acid or 2 M sodium hydroxide to a pH of 6.8 ± 0.05. Subsequently pH was changed from 6.5 to 6.8 and finally to 7.2. Change in pH of dissolution medium was used to mimic GI conditions as specified in IP 2013 for controlled release preparations. [20] Aliquots (5 ml) were withdrawn and filtered at predecided intervals. Sink conditions were maintained by replacing medium with equal volume of fresh dissolution fluid and the absorbance was measured by UV spectroscopy at 288 nm. The pharmacokinetics studies of secondary coated Espheres were done by using PCP Disso software (V3, India).

Scanning electron microscopy (SEM)

SEM was performed for primary and secondary coated Espheres to study surface morphology. The particles were fixed on a carbon stub using two ways adhesive tape and then coated in vacuum with thin layer of platinum (3–5 nm), for 100 s and at 30 W. [21] to enable conductivity.

Permeation studies using everted sac study

Everted intestinal sac method was performed to study the absorption across intestinal membrane. Goat intestine was opened by incision along the middle and the mesenteric attachments were carefully removed without rupturing the intestinal structure. The intestinal segment was transferred to a petri dish containing Kreb's solution. The apparatus consisted of a U tube glass chamber with a break in one arm of the U-tube to facilitate the mounting of the everted intestine. This entire assembly was clamped in place in the dissolution vessel. The inside of the U tube serves as the intestinal lumen and the dissolution vessel became the serosal compartment. Krebs solution (900 ml) and was used as dissolution media and secondary coated batches were subjected to in vitro dissolution studies at 37^{o}C and 50 rpm stirring speed in the USP type II dissolution apparatus. The aliquots were collected at different time points (at intervals of 2 h for 8 h) and analyzed by UV spectroscopy at 288 nm. [22]

RESULTS AND DISCUSSION

UV spectrophotometric Analytical Method

The calibration curve showed good linearity in the Beer's law limit of 10-50µg/ml in methanol AR grade for ETH with correlation coefficient (r^2) of 0.996. Intra and inter-day precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) were computed to validate the method. The percent relative standard deviation (% RSD) values of interday and intraday precision were found to be 0.925 and 1.43 for 10µg/ml. The recovery (n= 3) was found to be 98.18% for the drug ETH showing the accuracy of the method. Limit of detection (LOD) and limit of quantification **(**LOQ) was found to be 0.2062µg/ml and 0.625µg/ml, respectively.

Drug- Excipients compatibility studies

Compatibility study of ETH was carried out to determine any drug-excipients interaction. IR spectra showed the specific types of peaks in graph which shows minor changes in frequency and height. Drug-excipients compatibility studies were done to ensure absence of chemical reaction between drug and excipients. No undesirable change in peak size and value were observed indicating absence of untoward reactions. All the important drug peaks were retained (C-H stretching of alkane at 2874, C=O bending of ketone at 1725 cm^{-1}, N-H stretching of amine at 3329 cm^{-1}). [23]

Preliminary studies

ETH is a BCS class-II drug displaying low solubility and high permeability. Nanosuspensions were prepared by using various solvents such as methanol, ethanol, acetone and chloroform using anti-solvent precipitation technique by probe sonication. In this method the solvent vaporization takes place so that for the preliminary trials study used the volatile solvents. Solvents should have low boiling point and preferably be miscible with water to enable faster desolubilization. These factors combined with high stirring speed leads

to formation of nanoparticles. As compared to other solvents under study, acetone is highly miscible with water, and migrates rapidly to the water phase (antisolvent) and also evaporates rapidly due to constant stirring. Faster evaporation of acetone results in higher supersaturation and rapid nucleation, all of which are essential for nanometer crystal size. Thus ETH-NS with minimum particle size was obtained with acetone as solvent. Various stabilizers such as PVP K 30, TPGS, tween 80 were used for preparing ETH-NS individually and in combination. Nanosuspension prepared using single stabilizers showed particle size > 3000 nm in the form of precipitate. Combination of PVP K30 and TPGS in 1% in ratio of (1:0.5) gave nanosuspension with particle size (130 ± 10.9 to 218 ± 15.35 nm) but increase in particle size was observed visually in the form of floccules after 15 days at room temperature. Higher strength of 2% this combination gave smaller particle size with better stability. The total concentration of stabilizers in these trial batches was 1, 2 and 3% w/v in different ratios. Other stabilizers like tween 80 gave suspensions in size range greater than 1500 nm and hence no further studies were conducted using this stabilizer.

Higher concentration (3%) in ratio of (1:0.5) PVP K30 and TPGS combination yielded nanosuspension with particle size in range of (250 to 550 nm). Minimum particle size was found at concentration of 2% in ratio of (1:0.5) (97 ± 3 nm to 101 ± 5 nm) of both the stabilizers. It can be attributed to fact that stability of nanosuspension totally depends upon the presence of proper type and amount of stabilizers. Insufficient stabilizer induces particle agglomeration or incomplete barrier on particle surface. Excess stabilizer may promote Oswald's ripening wherein small particles of nanoparticles dissolve and then redeposit on the larger particles in the formulation that changes the structure particle as per time. [24]

ETH-NS with 2% of PVP K 30: TPGS ratio (1:0.5) was further evaluated for effect of solvent and stirring speed. ETH-NS were prepared using various solvents such as methanol, ethanol and acetone by method discussed previously. Stirring speed is another factor that affects the nanoparticles by promoting rapid nucleation, rapid evaporation and crystal breakdown and it also affects the particle size and stability of formulation. Higher stirring times may lead to formation of smaller particles; however the cohesion between the particles increases due to higher surface free energy which leads to crystal growth. It was observed that when formulation was stirred at 3500 rpm for 1h solvent gets evaporated. Further sonication of the formulation for 15 min by probe sonicatior (Make: Sonics vibra cell, Model: VCX750) caused decrease in particle size of nanoparticles.

Experimental design

A 3 level factorial design (3^2) was used for the optimization of nanosuspension. Based on preliminary studies optimization design was employed to study the effect of concentration of stabilizers and stirring speed on particle size and cumulative percent drug release. Concentration of stabilizers in a ratio of (1:0.5) was varied from 1 to 3% and stirring speed was varied from 2000 to 5000 rpm for 1has enlisted in (table 1).

Evaluation parameters of 3^2 factorial design

Particle size

Stabilizers like TPGS and PVP K 30 produce adsorption layer at the surface of nanoparticles, creating a steric or electrostatic barrier which prevents crystal growth. Concentration of stabilizers was selected as one of the independent variable because stabilizers play an important role in formation of stable nanosuspension. PVP forms a macromolecular sheath around nanoparticles that ensures steric stabilization. [25] This stabilization affects physical stability of formulation and physical properties like particle size.

Zeta potential between +25 mV to -25 mV confers high degrees of stability to nanosystems. Zeta potential predicts the long term stability of the nanoparticles. However, in steric stabilization there is a reduction of the zeta potential as the shear plane is shifted away from the particle surface [26], which is not an indication of a reduced electrostatic repulsion. A PDI value of 0.1-0.4 for nanosystem reflects monodispersity while PDI value >0.6 value indicated that the system has highly polydispersity. The nanoparticles of optimized batch displayed PDI value of 0.36 which indicated that the nanosuspensions were reasonably monodisperse. [27]

Factorial design batches yielded particle size in range of 97.54 ± 8.47 nm to 552 ± 5.45 nm size range. PDI value for the factorial batches is reported in Table 1 and the measured zeta potential was between -2.79 ± 1 to -15.8 ± 4.5 mV. ANOVA studies revealed that the quadratic model was significant in the study due to the high F-value of 9.68 and the "R-Squared" value was found to be 0.96.

Table 1: Experimental batches of eth-ns obtained from design of experiment (doe) software (n=2, Mean ± SD)

Run	Conc of stabilizer (%)	Stirring speed (rpm)	Particle size (nm)	Cumulative Drug release (%)	Zeta potential	PDI
F1	3	5000	552.00 ± 5.45	60.01 ± 2.08	-5.50 ± 1.2	1.00
F2	2	3500	97.54 ± 8.47	95.01 ± 1.16	-7.65 ± 1.3	0.36
F3	1	2000	218.00 ± 17.91	79.55 ± 3.29	-2.79 ± 1.1	0.80
F4	1	5000	109.00 ± 12.89	88.67 ± 5.01	-7.41 ± 2.1	0.28
F5	2	3500	101.20 ± 8.63	94.85 ± 5.53	-10.1 ± 2.3	0.50
F6	1	3500	133.70 ± 14.88	88.67 ± 3.88	-10.6 ± 2.2	0.06
F7	3	3500	247.00 ± 17.619	77.35 ± 4.84	-12.6 ± 2.3	0.73
F8	2	5000	218.00 ± 26.09	74.98 ± 3.08	-12.9 ± 2.1	0.35
F9	2	2000	178.20 ± 35.16	89.1 ± 3.61	-13.2 ± 2.2	0.65
F10	3	2000	137.40 ± 22.30	85.02 ± 5.16	-15.8 ± 4.5	0.98

Coded equation for particle size was found to be as follows:

$$R1 = +109.36 +79.28A -57.57B +130.90AB +70.99A^2 +78.74B^2 \quad \text{----------Eq (1)}$$

The higher and positive coefficient for A (concentration of stabilizers) was indicative of its pronounced effect on particle size than stirring speed. Increase in stabilizers' concentration led to increase in particle size of the nanosuspension. Sign on each term indicates nature of relationship with response. Consequently, concentration of stabilizers had direct relationship and stirring speed had inverse effect on particle size. (Fig. 1) shows 3D response surface plot of particle size.

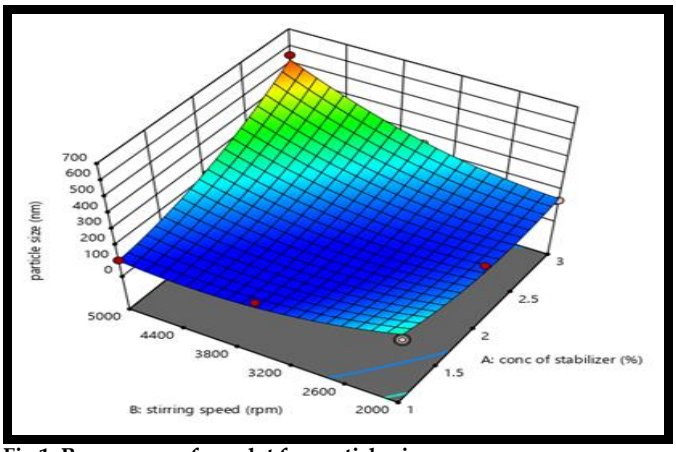


Fig 1: Response surface plot for particle size

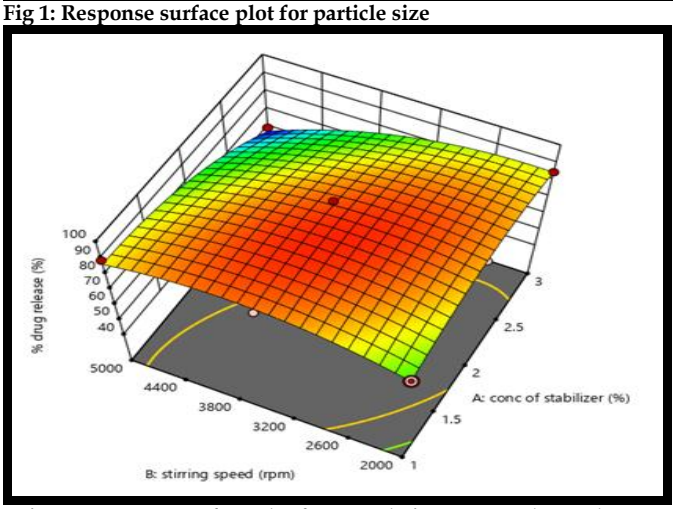


Fig. 2: Response surface plot for cumulative percent drug release.

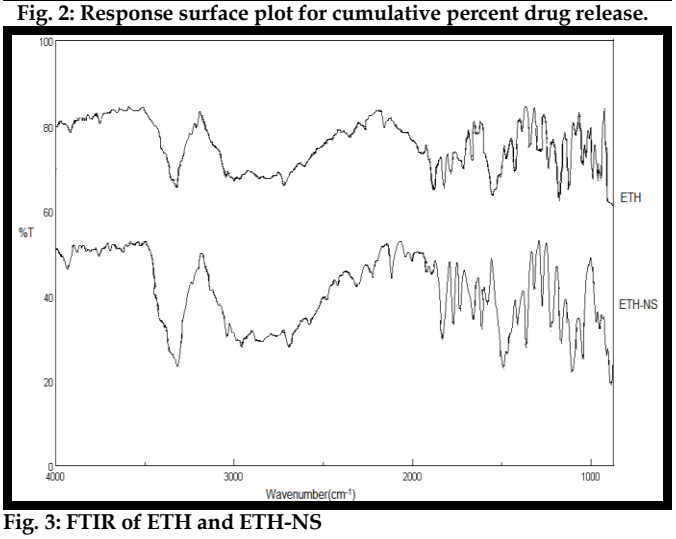


Fig. 3: FTIR of ETH and ETH-NS

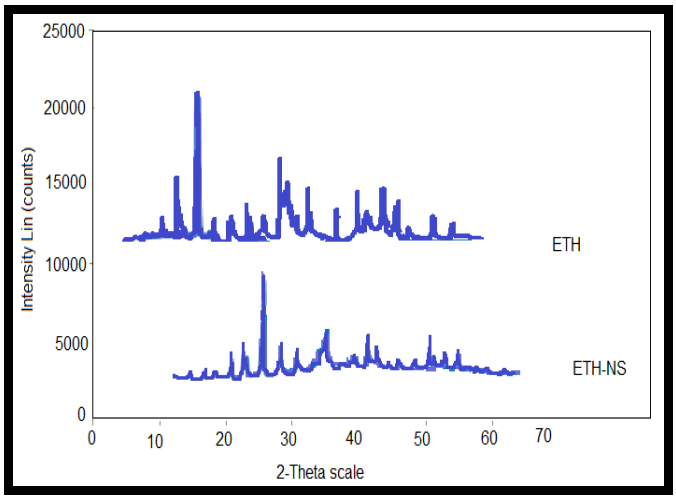


Fig. 4: PXRD of ETH and ETH-NS

Minimum particle size was seen in the region where stabilizers concentration was in between 1 to 3% and stirring speed of 3500 rpm (blue region). As stirring speed was increased particle size also increased and as stirring speed was decreased, particle size increased as shown by green region. Minimum speed of stirring and high concentration of stabilizers gave rise to particles in size range of 97.54 ± 8.47 to 552 ± 5.45 nm depicted by red region. From the results of all ten batches it was concluded that 2% in ratio of (1:0.5) TPGS and PVP K30 as stabilizers and stirring speed of 3500 rpm give rise to minimum particle size.

The significant low particle size obtained with optimum TPGS and PVP combination may be attributed to these properties. At lower levels of stabilizers, adsorption layer formed around nanoparticles may be insufficient to provide adequate protective barrier around the nanoparticles hence particle size was higher. On the other hand at higher level, increase in particle size was evident which could be because of bridging of chains of TPGS in adjacent particles which promoted particle agglomeration. [27] Also at higher level, TPGS increases the viscosity of suspension medium and provide effective micro-mixing of solvent and antisolvent. Inverse relationship between stirring speed and particle size can be explained with help of the Reynold's number. [28] At the highest stirring speed of 5000 rpm Reynolds's number attained highest value of 21000 which is indicative of turbulent flow. This turbulent flow is indicative of effective micro mixing of solvent and antisolvent, giving rise to rapid nucleation. Based on the input constraints in the software, the suggested optimized batch was prepared using PVP K30 and TPGS 2% in (1:0.5) ratio and stirring speed of 3500 rpm. The particle size of the optimized batch was found to be 97.54 ± 8.47 nm.

Cumulative percent drug release

USP type II (paddle type) dissolution apparatus was used for determining cumulative percent drug release of factorial batches. ETH is BCS class II drug and expected to have higher affinity for the lipid than

surrounding aqueous medium so that it reduces the cumulative percentage of drug release. A high dissolution rate in GIT facilitates oral absorption of poorly water-soluble drugs which is achieved through nanosizing.

$$R_2 = + 92.59 - 5.75\ A - 5.00\ B + 8.53\ AB - 7.24\ A^2 - 8.21B^2$$

----------Eq(2)

Cumulative percent drug release (R2) of factorial batches of nanosuspension was found to be between 60.01-95.01% in 60 min. Increase in stirring speed increases the drug release as it reduces the particle size. The concentrations of stabilizer also affect the release of drug. TPGS is ampiphilic in nature and its polyethylene glycol chain (lipophilic alkyl tail) and the tocopherol succinate portion (hydrophilic polar head) have large surface areas which provide excellent steric stabilization. [29] PVP minimizes aggregation of nanoparticles as the long chain polymer gets adsorbed on the particle surface leading to steric stabilization. Polymer chains may undergo interpenetration and some compression as the particles approach each other. This increases the local segment density leading to strong repulsion. [30] The decrease in particle size increases the drug release rate. The negative coefficient B (concentrations of stabilizer) implied a decrease in release with increase in cumulative percent drug release. Stirring speed was found to have direct relationship and concentration of stabilizers had an inverse effect on drug release as shown in (Fig. 2).

Selection of optimized formulation

The selection of optimized formulation for nanosuspension was based on the concentration of stabilizers, particle size and cumulative percent drug release. The optimized batch was chosen by numeric optimization using desirability function. The constraints set for numerical optimization included particle size and cumulative percent drug release. Based on these two responses (R_1 and R_2), there were three different optimized batches of given by the software and among these three batches, one batch was selected as an optimized batch based on their desirability function. Hence, the selected optimized batch of nanosuspension contained optimum concentrations of stabilizers (2%) in a ratio of (1:0.5) (A) and stirring speed (B) exhibiting a particle size of 97.54 ± 8.47 nm, and cumulative percent drug release of 95.01 ± 1.16. Thus, the formulation batch giving minimum particle size (R_1) and maximum cumulative percent drug release (R_2) was chosen as the optimized batch. Based on solutions given by Design expert software optimized nanosuspension was prepared to check the desirability of statistical design.

Evaluation of optimized batch

Entrapment efficiency

Entrapment efficiency was calculated by using UV visible detection (Lab India 3000+) at 288 nm. By dissolving nanocrystals in methanol and it was found to be 94.58 ± 1.73%.

Particle size and zeta potential

Particle size and zeta potential were analyzed in triplicate by using Malvern zetasizer (ZS90). Particle size of optimized batch was found to be 109.00 ± 2.89 nm and zeta potential was -10.65 ± 2.25 mV.

Fourier Transform Infrared spectra

Characteristic peaks for ETH were observed of N-H stretching of amine at 3273cm^{-1}, a sharp peak at 1594 cm^{-1} due C=N , aromatic C-H stretching at 941cm $^{-1}$. All of the peaks were retained in ETH-NS spectrum but with diminished intensity as seen in (Fig. 3). This could be due to presence of stabilizers in formulation.

Powder X-ray Diffraction studies (PXRD)

PXRD was performed to determine the changes in crystalline structure of ETH in the nanosuspension. The spectrum of ETH showed that drug is highly crystalline in nature with prominent peaks at 2θ angle of 12, 19, 21, 22, 24 and 28°. Minor shifting of peaks to 29, 31, 33, 37, 45 and 49° along with decreased peak intensity were evident in diffractogram of dried ETH-NSas seen in (Fig. 4). The extremely poor solubility of ETH can be due to its crystalline nature. Reduced intensity of peaks can be attributed to partial amorphization of drug which could be one of the reasons for increase in solubility of the nanoform of ETH. [31]

Saturation solubility studies

Saturation solubility studies were done using orbital shaker (CIS-24 Remi, India) for 24 h and this study of plain drug and the optimized batch were carried out in distilled water (DW), 0.1N HCl and phosphate buffer pH 6.8. Poor solubility of drug in water leads to various biopharmaceutical disadvantages. This study was comparison between saturation solubility of ETH and ETH-NS formulation. ETH solubility in distilled water, 0.1N HCl and phosphate buffer pH 6.8 was found to be 0.572 ± 0.04µ/ml, 2.389 ± 8.49µg/ml and 1.154 ± 0.6µg/ml respectively as enlisted in (Table 2).

A 13.77 fold rise in saturation solubility of ETH-NS in distilled water was seen, whereas in 0.1N HCl and phosphate buffer, the increase was 3.75 fold and 2.87 folds respectively. This increased solubility can be attributed to increased surface area, decreased diffusion path length and hence increase in concentration gradient as well as increased dissolution velocity of nanoparticles. [32]

The Ostwald-Freundlich equation explains the link between increase in saturation solubility and particle size as follows:

$$\rho/\rho_{eq} = \exp(R_{critical}/\ R) \qquad \text{----------Eq(3)}$$

ρ_{eq}= Equilibrium partial pressure

ρ= Partial pressure

R=radius of particle

Equation co-relates solubility with surface tension, temperature, partial vapor pressure and particle dimensions. It is observed that drug nanosuspension provide a number of physicochemical advantages over large-sized drugs, majorly being increased solubility and bioavailability. [33] Therefore, ETH-NS can overcome above mentioned problems associated with low aqueous solubility.

Table 2: Solubility of ETH and ETH-NS in 0.1 N HCl, distilled water, phosphate buffer 6.8

Media	Plain drug	Formulation
Distilled water	0.572 ± 0.105	7.88 ± 4.26
0.1 N HCl	2.389 ± 1.31	8.96 ± 5.15
Phosphate buffer pH 6.8	1.154 ± 0.916	3.32 ± 2.53

Table 3: Comparative processing parameters for primary and secondary coating

Processing parameter	Drug loading	Polymer coating
Nozzel internal diameter	1 mm	1 mm
Atmospheric air pressure	0.4 bar	0.4 bar
Air flow	0.8 ml/min	0.8 ml/min
Temperature	70°C	30°C
Spray rate	1 ml/min	1 ml/min

Stability studies

As per ICH guidelines, by using stability chamber (Remi- CHM-6S, India) the optimized ETH-NS were subjected to different environmental conditions for 1 month to observe any physical changes. There was no evidence of discoloration, flocculation and sedimentation in the optimized batch. After exposure to ambient conditions the particle size of the optimized batch was increased by 2.33% while after exposure to 40°C & 75% RH was increased up to 6.42%. Particle size was seen to have increased after refrigeration (220 ± 5.5 nm) which can be considered as nominal. The optimized stability batch had a PDI less than 0.60 that indicates absence of Ostwald ripening.

ETH-NS Primary coating on Espheres

By using suspension layering technique the optimized ETH-NS batch was loaded on Espheres (#18-22) which is composed of micro crystalline cellulose and lactose. The uniform shape, smooth surface and mechanical resistance of Espheres make them ideal for loading large amount of drugs. Also Espheres enable uniform drug absorption profile. Due to spherical nature of Espheres, ETH-NS was loaded uniformly on its surface. Multiple-unit dosage forms are able to pass the stomach and spread in intestinal pH, thus reducing the gastrointestinal transit variability. Also, subdivision of the dose reduces the possibility of dose-dumping. From the properties of Espheres are very critical as ETH-NS loaded Espheres were further coated with controlled release polymer coating so as to give controlled release of drug throughout the GIT. [34]

Secondary coating on ETH-NS loaded Espheres

Eudragit L and S coating polymers confer protection to drugs in gastric environment besides providing uniform and controlled release. Preliminary trials for secondary coating were done on pan coater as per selected parameters (Table 3). Studies entailed coating with Eudragit S100 and L100 singly and in combination. Level of coating was kept constant at 10% weight gain. Eudragit S 100 dissociates at pH 7 but singly it gave the minimum release in colon and with Eudragit L 100 alone drug release started from pH 5.5 to 6.5. Thus it was observed that the polymers singly did not show uniform drug release throughout the intestine. Combining Eudragit polymers in different ratios allowed drug release in desired narrow pH range in the GIT. In present study combination of 1:1 and 1:2 were studied. Drug release was minimum in the combination of 1:2 as compared to the ratio1:1. Drug release was found to be 87.84 ± 2.75% at the end of 8 h in the case of Eudragit S 100: Eudragit L100 at 1:1 ratio hence this ratio was selected.

Evaluation of coated Espheres

The primary and secondary coated Espheres prepared in pan coater was evaluated for drug content, friability; drug release and scanning electron microscopy (SEM).

Drug content and entrapment efficiency

Drug content was estimated by UV visible detection (Lab India 3000+, India) at 288nm. By dissolving the crushed primary coated spheres drug content was found to be 96.32 ± 2.96% and in case of secondary coated Espheres drug content was found to be 95.20 ± 1.51%.

Friability

The friability of primary coated Espheres was found to be 0.8 ± 0.12% and for secondary, it was 1%. This value indicated that the percent weight loss of the product is insignificant. It was carried out to determine if the Espheres are strong enough to withstand the rigours of pan cpating process. A weight loss of less than 0.5% to 1% of weight is acceptable. The low percent loss also indicates that the nanoparticles in the primary coated Espheres remain affixed and will ensure that they do not get detached during the secondary coating process.

In vitro dissolution studies [35]

Primary study

Drug release studies of the primary and secondary coated formulations were carried out using USP dissolution apparatus II. Primary coated Espheres were subjected to dissolution studies in 0.1 N HCl. Drug release was up to 92.41 ± 3.16% in case of optimized ETH-NS loaded Espheres in 60 min percent release shown in (Fig. 5). ETH-NS shows the release behaviour of drug in stomach acidic area. Nanoparticles have high Brownian motion which is responsible for faster dissolution velocity.

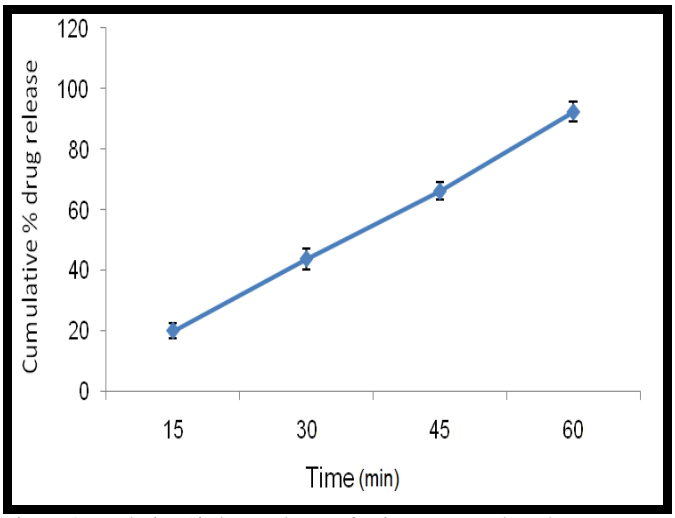


Fig. 5: Cumulative % drug release of primary coated espheres

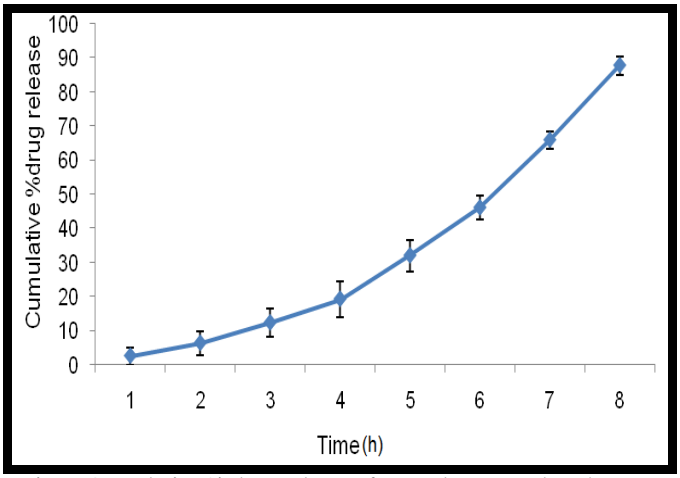


Fig. 6: Cumulative % drug release of secondary coated espheres.

Secondary coated Espheres

Secondary coating on the Espheres has controlled release system that was aimed in such a way that it will release drug in intestine. Eudragit S100 and Eudragit L 100 were selected for controlled release coating over Espheres. One of the advantages of drug loading onto Espheres was increase in dissolution rate of drug as well as uniform drug release. Controlled release system show as the release of drug in hydrophilic matrix. Assuming an intestinal transit time of 3–5 h, the pH conditions are varied with pH 6.5 and residence time of 1 h reflecting proximal small intestine, pH 6.8 and residence time 2 h in lower part and terminal ileum with pH 7.2. Thus, the dissolution tests were carried out in media with pH 1.2, 6.5, 6.8 and 7.2. For controlled release of drug throughout the GIT, combination Eudragit S100 and Eudragit L100 was used in ratios of 1:1. Eudragit L and Eudragit S are obtained by copolymerisation of methacrylic acid and methyl methacrylate and the ratio of carboxyl to ester group is in the combination of 1:1 and 1:2. Eudragit S solubilized rapidly at and above pH 7 due to lower ratio of carboxyl group which resulted in lower degree of ionization in neutral to alkaline media than Eudragit L. Eudragit L100 solubilized at and around pH 6. Secondary coating of Eudragit copolymers was done on primary coated Espheres using pan coater. Dissolution data revealed that ETH-NS secondary coated Espheres released 87.84 ± 2.75% of drug in 8h as seen in (Fig. 6). However, the drug release from the nanosuspension coated Espheres was more uniform, regardless of the coating method. This can be ascribed to the increased saturation solubility and dissolution velocity achieved due to nanosizing. Also, the PDI of the nanosystem was lower which may confirm the absence of Ostwald ripening means there was no changes in the internal structure of nanosuspension.

The release data of secondary coated Espheres were subjected to mathematical modeling using PCP Disso (V3.0, India) and best fit model was found to be Korsmeyer-Peppas model which is indicative of diffusion controlled release. The kinetic constants were found to be n = 0.27 and k = 0.08. According to Korsmeyer-Peppas model the equation derived as follow [36]

$$M_t / M_\infty = Kt^n \qquad \text{----------Eq(4)}$$

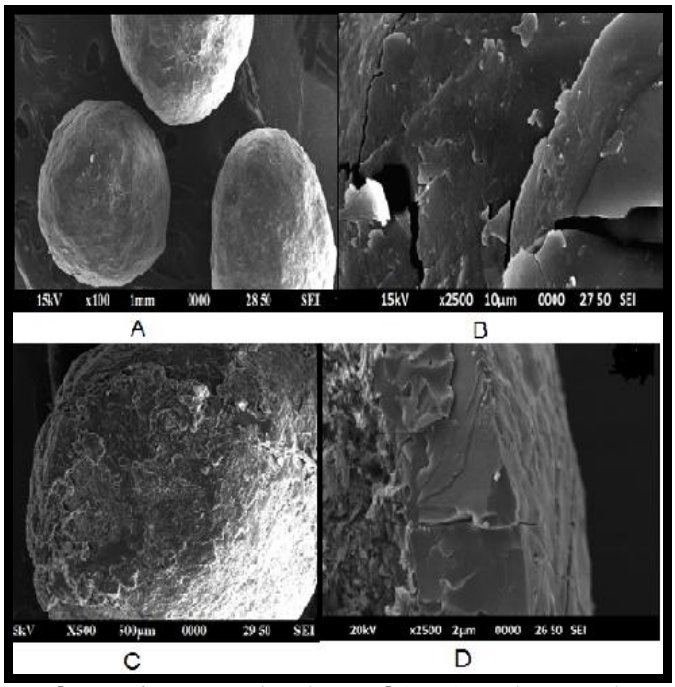
Where, M_t / M_∞ = A fraction of drug released at time t

k = Release rate constant and n = Release exponent.

Release exponent of 0.45 ≤ n points to a Fickian diffusion mechanism, 0.45 < n <0.89 to non-Fickian transport, n = 0.89 to Case II (relaxation) transport, and n >0.89 to super case II transport. Fickian diffusional release occurs by the usual molecular diffusion of the drug against a concentration gradient through the polymeric membrane. Korsmeyer and Peppas developed an empirical equation to analyze both Fickian and non-Fickian release of drug from swelling and non-swelling polymeric delivery system. [37]

Scanning electron microscopy (SEM)

SEM images at various stages of coating were taken to observe any morphological changes. SEM images of primary coated Espheres were distinctly different from that of the secondary coated Espheres. ETH-NS coated Espheres showed the presence of nanosized drug particles coated on the surface in (Fig. 7).



I] A, B-Primary coated Espheres; II] C, D-Secondary coated Espheres

Fig. 7: Scanning electron microscopy images

Permeation studies using everted sac method

Everted sac study using goat intestine was performed to study the permeability of drug and its formulation. This method was used to evaluate the drug transport mechanism. Plain ETH and secondary coated Espheres were subjected to this test. Plain ETH showed permeation up to 33.26 ± 0.65% whereas it was found to be 53.27 ± 0.65% in the case of secondary coated Espheres in 8 h (Fig. 8). This enhanced permeation can be attributed to the presence of stabilizers in the formulation which acts as a permeation enhancer. The drug permeated across the intestine wall and its

concentration was measured in the Kreb's-Ringer solution. [38] The drug diffused through polymeric pores and permeated across the intestinal membrane. The secondary coated Espheres exhibit 2.20 times increase in flux and 1.06 times increase in apparent permeability than pure drug. An increase in the dissolution rate causes an increase in flux. Flux determined the rate of diffusion or permeation of drug across the intestinal membrane.

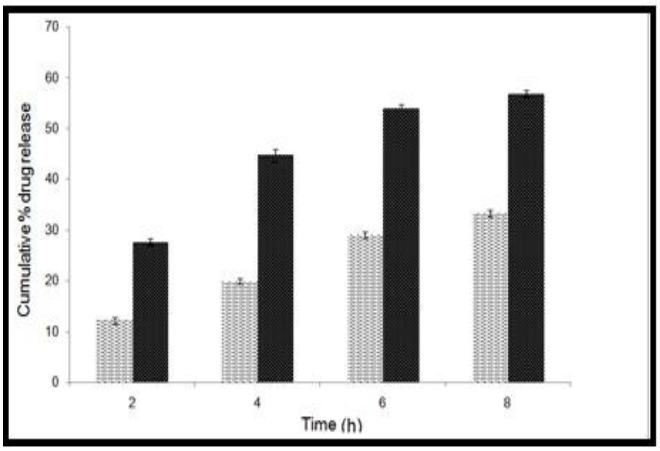


Fig. 8: Permeation studies: everted sac method

Based on the study it can be concluded that nanosuspensions can be an effective approach to enhance solubility and dissolution rate of ethionamide. Antisolvent precipitation technique is simple and efficient technique for producing nanosuspension. The optimized batch of nanosuspension shows good stability, drug release, compatibility and particle size in nanometer size range. Furthermore nanosuspension layering on Espheres converts nanosuspension into deliverable multiparticulate solid dosage forms. Eudragit polymers can be used in combination to achieve controlled release of drug in intestinal lumen. *Ex vivo* diffusion studies revealed a moderate increase in permeability.

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REFERENCES

1. Jagtap S, Magdum C, Jadge D. Solubility enhancement technique: A review. Int J Pharm Sci Res. 2018; 10(9): 2205-11.
2. Prasanna L, Kumar A. Nanosuspension Technology: a review. Int J Pharm Pharm Sci; 2: 4-10.
3. Gandhi B, Baheti J. Multiparticulates drug delivery systems: a review. Int J Pharm Chem Sci. sep 2013;2 (3):1620-27.
4. Debi U, Ravisankar V, Prasad KK, Sinha SK, Sharma AK. Abdominal tuberculosis of the gastrointestinal tract: Revisited. World J Gastroenterol. Oct 2014; 20(40): 14831-40.
5. https://www.drugbank.ca/drugs/DB00609
6. Kumar S, Saisivam S, Debnath M. Validated UV-Spectrophotometric Method for the Ethionamide Estimation in Bulk, Tablet and Nanoparticles. Int J Drug Dev Res 2017; 9(1): 20-23.
7. Taneja S, Shilpi S, Khatri K. Formulation and optimization of efavirenz nanosuspensions using the precipitation-ultrasonication technique for solubility enhancement. Art cells, Nanomed Biotech. 2015;1-15.
8. Kakran M, Sahoo NG, Li L, Judeh Z. Particle size reduction of poorly water soluble artemisinin via antisolvent precipitation with a syringe pump. Powder Tech. 2013; 237: 468-476.
9. Souza S. A review of *In Vitro* drug release test methods for nano-sized dosage forms. Hindawi Publishing Corporation. Adv in Pharmaceu. Volume 2014:1-12.
10. Liu D, Xu H,Tian B,Yuan K. Fabrication of carvedilol nanosuspensions through the anti-solvent precipitation–ultrasonication method for the improvement of dissolution rate and oral bioavailability. American Asso Pharm Sci. March 2012; 1(13): 295-305.
11. Danish M, Shetsandi A, Bhise KS. Development and Taste Masking of Rifaximin Nanosuspension Pharm Tech. 2013;1-16.
12. Swaminathan S, PasteroL, Serpe L, Trotta F, Vavia P, Aquilano D 2009.Cyclodextrin-based nanosponges encapsulating camptothecin: Physicochemical characterization, stability and cytotoxicity. Eu J Pharma Biopharma;74(2):193-201.
13. Kassem M, Elmesh1ad A, Fares A. Enhanced solubility and dissolution rate of lacidipine nanosuspension: formulation via antisolvent sonoprecipitation technique and optimization using box–behnken design. AAPS Pharm Sci Tech 2016;1-14.
14. Cartensen J. 1995. ICH Guidelines. In: Drug Stability Principles and Practices, 2nd ed., New York, Marcel Dekker, pp.514-546.
15. Akhgari A, Afrasiabi GH, Sadeghi F, Azimaie M 2005. Statistical optimization of indomethacin pellets coated with pH-dependent methacrylic polymers for possible colonic drug delivery. Int J Pharm. 305:22–30.
16. Evonik product amnual Eudragit L 100 Evonik.com accessed on 1/4/2019.
17. Evonik product amnual Eudragit S 100 Evonik.com accessed on 1/4/2019.
18. Sharon K. Formulation and evaluation of floating microspheres of Gabapentin by using solvent evaporation method. Int J App Pharm Res. 2010; 1(1):12-16.
19. Pisek R, Korselj V, Vrecer F. Comparison of direct rotor pelletization (Fluid Bed) and high shear pelletization method for pellet production. Pharm Biopharm. 2002; 53: 327-33.
20. Singh K, Borkhataria H, Seth N, Formulation and in vitro evaluation of lansoprazole Micropellets. Int J Pharm Tech Res. 2009,1(4) :1530-40.
21. Sharon K. Formulation and Evaluation of Floating Microspheres of Gabapentin By using Solvent Evaporation Method. Int J Applied Pharma Res. 2010; 1(1):12-16.
22. Freag M, Elnaggar Y, Ossama Y. Development of novel polymer-stabilized diosmin nanosuspensions: In vitro appraisal and ex vivo permeation. Int J Pharma. 2013;1-10.
23. Cheng-Hung H. Rapid examination of the kinetic process of intramolecular lactamization of gabapentin using DSC–FTIR. Thermochimica Acta. 2009; 486: 5-10.
24. Lindfors L, Skantze P, Skantze U. Amorphous Drug Nanosuspensions Inhibition of Ostwald Ripening. Langmuir. 2006; 22: 906-910.
25. Dandekar P, Jain R, Kumar C, Subramanian S, Samuel G, Venkatesh M, Patravale V. Curcumin loaded pH-sensitive nanoparticles for the treatment of colon cancer. J Biomed Nanotechnol. 2010; 5(5): 445-55.
26. Honary S, Foruhe Z. Effect of zeta potential on the properties of nano-drug delivery systems - A review (Part 2). Trop J Pharm Res. 2013;12 (2): 265-273.
27. Nakarani M, Misra AK, Patel JK, Vaghani SS. Itraconazole nanosuspension for oral delivery: Formulation, characterization and *in vitro* comparison with marketed formulation. Daru. J Pharm sci. 2010; 18(2):84–90.
28. Mishra PC, Mukherjee S. A brief review on viscosity of nanofluids. Int Nano lett. 2014; 4(4):109–120.

29. Sunyaprasert V, Morakul B. Nanocrystals for enhancement of oral bioavailability of poorly water-soluble drugs. Asian J Pharma Sci. 2015; 10: 13-23.
30. Rosen J. Surfactants and interfacial phenomenon. Edn 3,wiley interscience, New jursey, Canada,pp.120-121.
31. Murshedkav T. Effect of crystalline to amorphous conversions on solubility of cefuroxime axetil, University of rhode island. 2002; 2-118.
32. Shchekina AK, Rusanov AI. Genaralization of gibbs Kelvin kohler and Oswald frendlich equations of liquid film on soluble nanoparticles. J chem phy. 2008; 129-34.
33. M Costa, A Villa, R Barros, E Junior, E Santos. Development, characterization and evaluation of the dissolution profile of sulfasalazine suspensions, Brazilian Journal of Pharm Sci. 2015; 2(51):450-459.
34. Dey NS, Majumdar S, Rao M. Multiparticulate Drug Delivery Systems for Controlled Release, Tropical Journal of Pharmaceutical Research, September 2008; 7 (3): 1067-75.
35. Galia E, Nicolaides E, Hörter D, Löbenberg R, Reppas C, Dressman JB. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. Pharm Res. 1998;15: 698-705.
36. Paarakh MP, Jose PA. Release kinetics – concepts and applications. Int J Pharm Res Tech. 2018;8:12-21.
37. Shah JC, Deshpande A. Kinetic modeling and comparison of *in-vitro* dissolution profiles World J Pharm Sci. 2014; 2(4): 302-309.
38. Freag MS, Elnaggar YS, Abdallah OY. Development of novel polymer-stabilized diosmin nanosuspensions: In vitro appraisal and ex vivo permeation. Int J Pharma. 2013;454(1):462-71.

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