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Research Paper

Effect of Formulation variables on Nanosuspension Containing Famotidine Prepared by Solvent Evaporation Technique

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ABSTRACT: Low oral bioavailability of poorly water-soluble drugs poses a great challenge during drug development. Poorly water-soluble compounds are difficult to develop as drug products using conventional formulation techniques and are frequently abandoned early in discovery. The aim of the this study was to improve the dissolution rate of a poorly water-soluble drug famotidine, by a nanoprecipitation technique. Selected parameters of the nanoprecipitation method, such as the amount of Lutrol F-68 and stirring speed were varied so as to obtain drug nanoparticles. The combination of lowest amount of stabilizer with low speed yield bluish white transparent nanosuspensions with the smallest average particle size (566 nm). In contrast to the very slow dissolution rate of pure famotidine, the nanosuspension of the drug considerably enhanced the dissolution rate. Nanosuspension prepared with 0.25% Lutrol F-68 with 1000 rpm showed the most improvement in dissolution rate of famotidine. The formulation of famotidine as a nanosuspension was very successful in enhancing dissolution rate, more than 42% of the drug being dissolved in the first 10 min (batch F_1) compared to less than 2.5% of the micronized drug (batch F_7).

KEYWORDS: Famotidine, Poorly water-soluble drug, Nanoprecipitation, Drug Nanoparticles

Introduction

Design and formulation of a dosage form requires consideration of the physico-chemical and biological characteristics of the drug substances and pharmaceutical ingredients to be used in fabricating the product. An important physico-chemical property of a drug substance is solubility, especially aqueous system solubility. It also poses a major challenge for companies developing new pharmaceutical products, since nearly half the active substances being identified are either insoluble or poorly soluble in water (Seedher et al., 2003).

The solubility/dissolution behaviour of a drug is key determinant to its oral bioavailability. An improvement of oral bioavailability of poor water-soluble drugs remains one of the most challenging aspects of drug development. The techniques that have commonly been used to overcome drawbacks associated with poorly water-soluble drugs in general, include micronization, salt formation, use of surfactant and use of pro-drug. However, all these techniques have potential limitations. Over the last 8-10 years, nanoparticle engineering processes have been developed and reported for pharmaceutical applications (Kocbek et al., 2006; Jacobs et al., 2000). Nanosuspension engineering processes currently used are precipitation, pearl milling and high pressure homogenization, either in water or in mixtures of water and water-miscible liquids or nonaqueous media (Trotta et al., 2001; Liversidge et al., 1995; Kayser et al., 2003).

Famotidine is a H_2-receptor antagonist widely prescribed in gastric ulcers, duodenal ulcers, Zollinger-Ellison syndrome and gastro-esophageal reflux disease. Famotidine is reported to be 7.5 and 20 times more potent than ranitidine and cimetidine, respectively. Famotidine reportedly undergoes minimal first-pass metabolism and its oral bioavailability in man is reported to be low and variable, ranging from 40 to 50% due to its poor aqueous solubility. Since for poorly water-soluble drugs (like famotidine) the dissolution rate is often the rate-limiting step for bioavailability, and the dissolution rate is a function of the solubility and the surface area of the drug, thus, dissolution rate will increase if the solubility of the drug is increased, and it will also increase with an increase in the surface area of the drug (Hassan et al., 1990; Rania et al., 2008). Solvent evaporation method presents numerous advantages, in that it is a straightforward technique, rapid and easy to perform. In this method, the

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drug is dissolved in an organic solvent such as dichloromethane, chloroform, methanol or ethyl acetate. Drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form oil in water (o/w) emulsion. After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type of stabilizer, concentrations of stabilizer, and homogenizer speed. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed. The super saturation was further accentuated by evaporation of drug solvent. This leads to the precipitation of the drug. High shear force prevents nucleus growth and Oswald's ripening (Bilati et al., 2005; Kassema et al., 2007). The present study addresses famotidine suspension in sub micron range (nano-range) in the form of nanosuspension using solvent evaporation method. In this respect, the present work essentially focuses on parameters of the nanoprecipitation procedure that might be affected in order to lead the formation of drug nanoparticles.

Materials

Famotidine was obtained as a gift sample from Cadila Pharmaceutical Ltd., Ahmedabad, India. Lutrol F-68 (Poloxamer-188) was obtained as a gift sample from Torrent Pharmaceutical Ltd. Ethyl Acetate, methanol, was obtained as a gift sample from S.D.Fine Chemicals Ltd., Mumbai, India.

Procedures

Determination of Famotidine Solubility

The solubility of famotidine in water and in the organic solvents: ethyl acetate, methanol were determined by adding an excess of the drug to the solvent. The suspensions were stirred using a magnetic stirrer at 25°C for 24 h, filtered (0.22 µm) and the content of dissolved famotidine was analyzed by UV method at 267nm (Systronic 2203, Japan).

Preparation of Famotidine Nanosuspensions by Nanoprecipitation

Nanosuspensions were prepared by the solvent evaporation technique. Famotidine was dissolved in a methanol (6 ml) at room temperature. This was poured into 10 ml water containing different amounts of Lutrol F-68 maintained at a temperature of 30–40°C and subsequently stirred at ranging agitation speed for 1 hr to allow the volatile solvent to evaporate (Remi, High speed stirrer, India.). Addition of organic solvents by means of a syringe positioned with the needle directly into surfactant containing water. Organic solvents were left to evaporate under a slow magnetic stirring of the nanosuspensions, at room temperature for 2 hours (Table 1).

Particle Size and its Morphology

The particle size of the nanosuspension obtained was analyzed by laser diffractometeretry (Sympatec Helos, Japan) which yielded the mean particle diameter of the suspension. The diameters were calculated using the volume distribution. Diameters 50 and 90% mean that 50% (respectively, 90%) of the particles are below the given size. All samples were measured appropriately after diluting with bidistilled water. The nanoparticle surface appearance and shape were analyzed by scanning electron microscopy (SEM).

Drug Release Studies from Nanosuspension

Famotidine release from nanosuspension was taken in modified diffusion cell apparatus (Figure 1). The drug release from nanosuspension was determined using a dialysis tube (donor compartment) containing the known quantity (10 ml) of the nanosuspension in a water-jacketed beaker containing 300 ml of 0.1N HCl (pH 1.2) at 37 ± 1°C. The contents of the beaker were agitated on a magnetic stirrer. Samples were withdrawn periodically and replaced with an equal volume of fresh 0.1N HCl (pH 1.2). Samples were diluted suitably and filtered through a filter paper (0.22 µm). Famotidine content was determined by UV method at 267nm (Systronic 2203, Japan).

Table 1. Formulation of famotidine nanosuspension.

Ingredients	F_1	F_2	F_3	F_4	F_5	F_6	F_7
Famotidine	40	40	40	40	40	40	40
Methanol (ml)	6	6	6	6	6	6	-
Lutrol F-68 (%w/v)	0.25	0.5	0.75	1	0.25	0.25	-
Volume of aqueous solvent	10	10	10	10	10	10	10
Stirring Speed(Rpm)	1000	1000	1000	1000	1500	2000	
Mean particle size (µm)	0.566	2.28	2.72	4.63	1.09	5.02	12.8

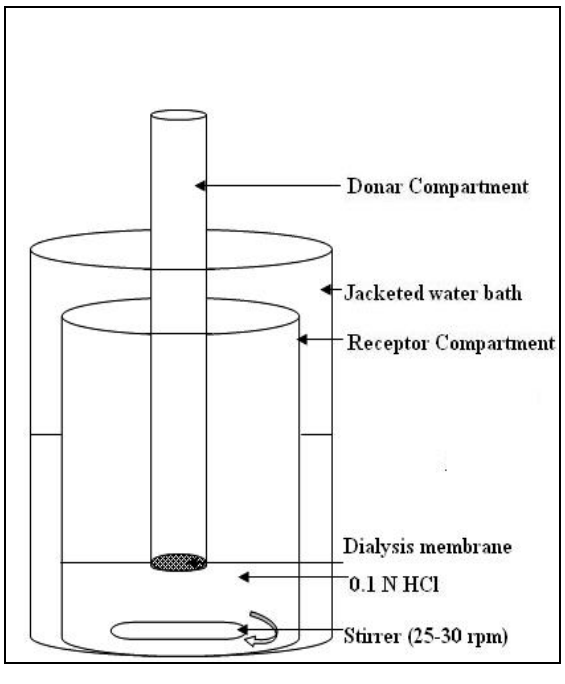

Fig 1. Schematic representation of modified diffusion cell apparatus.

Results and Discussion

Solvent evaporation with homogenization has been employed to produce nanosuspension of famotidine. The different formulative variables (1) amount of stabilizer (2) stirring speed were contribute largely towards the change in particle size in nanosuspension preparation. Nanosuspension of famotidine was prepared by as formulation shown in table 1. F_1 -F_4 formulations were containing different concentration of Lutrol F-68 (0.25% - 1%) at 1000 rpm on high speed stirrer. F_5 and F_6 were containing 0.25% of Lutrol F-68 at 1500 rpm and 2000 rpm on high speed stirrer respectively. Amount of organic solvent was kept constant for all batches. Bluish white transparent nanosuspension was successfully prepared which was compared with distilled water (figure 2).

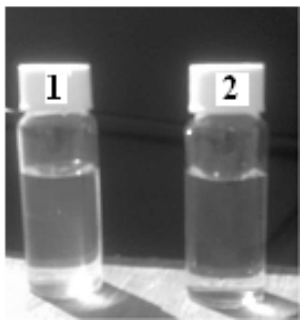

Fig 2. (1) Bluish white transparent nanosuspension (batch F_1) (2) Distilled water.

Famotidine Solubility

The selection of solvent is critical to obtain drug particles in the nanometer range. The solubility of famotidine was determined in water, methanol, and ethyl acetate. According to experimental data shown in table 2, the solubility of famotidine in water is very low, in ethyl acetate low, while solubility in methanol is higher. The water miscibility of the solvent is determining factor of the efficiency of the process. When ethyl acetate was used, famotidine was present in less soluble form. When methanol as a solvent was used, drug was completely dissolved. Methanol could miscible with water. It directly goes up to the water surface when it is injected into water. A part of the methanol could also move into the outer aqueous phase, contributing to faster precipitation of drug. The rapid diffusion of methanol into the aqueous phase causes a remarkable decrease in interfacial tension between the organic and aqueous phase and hence finer drug nanoparticles are obtained. To obtain a homogenous mixture, some outside forces were introduced. Sucker and coworkers used vigorous stirring during the precipitation preparation (List et al., 1995). To avoid the crystallization on surface, methanol should quickly diffuse. In our experiment, different stirring speed was adopted to enhance the diffusion rate of methanol in to aqueous phase.

Table 2. Solubility of famotidine in water and organic solvents at 25°C.

Solvents	Famotidine solubility in solvents
Methanol	65.55 mg/10ml
Ethyl acetate	2.79 mg/10 ml
Water	1.03 mg/10ml

Influence of Amount of Stabilizer on Particle Size

The stabilizer character and concentration play an important role in creating a stable formulation. It must be capable of wetting the surface of the drug crystals and providing a steric or ionic barrier. Too little stabilizer induces agglomeration or aggregation and too much stabilizer promotes Oswald's ripening. First of all a screening of formulations was designed with different concentrations of commonly used surfactant, Lutrol F-68. Lutrol F-68 is a well known efficient steric stabilizer forming adsorption layers on drug nanoparticles. Important function of Lutrol F-68 is that it can form a substantial mechanical and thermodynamic barrier at the interface that retards the approach and coalescence of individual emulsion droplets at their optimum level. From F_1-F_4, amount of Lutrol F-68 were increase, with increasing particle size due to formation of thick adsorption layer onto particles. This was leading to formation of aggregation of particles. It was shown that higher concentration of steric stabilizer (Lutrol F-68) did not influence the particle size significantly. Table 3 shows the mean particle diameter and particle size distribution of F_1-F_4 formulations. It was

shown that 0.25% of Lutrol F-68 was sufficient for nanosuspensions produced by solvent evaporation method. Particle size distribution of batch F_1 is depicted in graphical form (fig. 3).

Influence of Stirring Speed on Particle Size

The water miscibility of the solvent is determining factor for nanosuspension preparation. For nanosuspension of famotidine, methanol was selected (Table 1). From Table 1, F_1-F_4 containing total amount of organic solvent was 6 ml at stirring speed 1000 rpm. At 1000 rpm, high solubility of methanol in water enables their fast diffusion from dispersed droplets into aqueous phase. Thus, as soon as the dispersed phase comes in contact with a large amount of aqueous phase during the emulsion dilution, fast diffusion of organic solvent occurs, leading to fast drug precipitation and particle formation. Amount of surfactants was the main governing parameter in F_1-F_4 rather than stirring speed. For determination of effect of stirring speed on particle size, variation in stirring speed could be done at 1500 and 2000 rpm in subsequent batches (F_5-F_6). All the nanoparticles obtained at 1000 rpm (batches F_1-F_4) showed lower mean sizes than those obtained at 1500 and 2000 rpm (batches F_5-F_6). These findings are quite unusual but could be explained by considering that at the higher agitation speed greater foaming occurred in the mixture. This could cause an earlier separation of solid nanoparticles from the aqueous medium that limited the size reduction effect induced by stirring. For an alternative explanation of the above results, the role of methanol during nanoparticle formation must be taken into account. Higher agitation speeds made easier the evaporation of the solvent, with the concomitant rapid precipitation of the drug upon contact with the aqueous phase and a partial coalescence of particles in larger aggregates (Kawashima et al., 1989). Reducing the stirring speed increased the size of methanol droplets but slowed down the evaporation rate and rendered more homogeneous the conversion of the nanoemulsion into nanoparticles (Table 4). Scanning electron microscope analysis of batch F_1 at 1000 rpm with 0.25% Lutrol F-68 was shown in Figure 4.

Table 3. Particle size distribution of F_1-F_4 formulations.

Batch code	Mean particle diameter	Particle size distribution
F_1	566 nm	90% < 0.65 µm 50% < 0.55 µm
F_2	2.28 µm	90% < 2.78 µm 50% < 1.49 µm
F_3	2.72 µm	90% < 3.91 µm 50% < 1.31 µm
F_4	4.63 µm	90% < 6.13 µm 50% < 2.77 µm

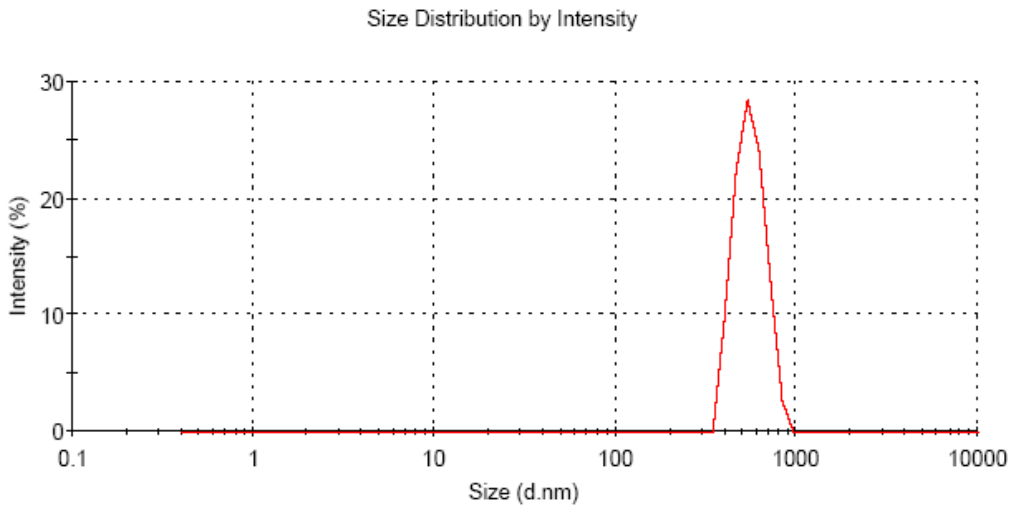

Fig 3. Particle size analysis of batch F_1.

Table 4: Particle size distribution of F_5-F_7 formulations

Batch code	Mean particle diameter	Particle size distribution
F_5	1.03µm	90% < 1.78 µm 50% < 0.96 µm
F_6	5.02µm	90% < 13.69 µm 50% < 1.90 µm
F_7	12.8µm	90% < 33.69 µm 50% < 2.82 µm

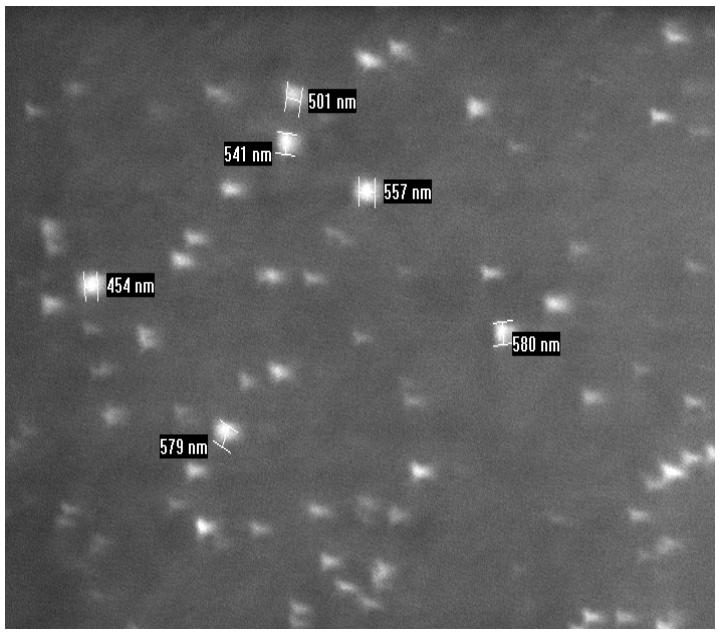

Fig 4. Scanning electron microscope analysis of batch F_1

Drug Release Studies from Nanosuspension

Fig. 5 shows the dissolution behaviour of famotidine alone, and from nanosuspensions. The release rate profiles were drawn as the percentage famotidine dissolved from the nanosuspension and pure drug versus time. Dissolution studies of pure famotidine (F_7) and all other prepared nanosuspension (F_1-F_6) were carried out in 0.1N HCl (pH 1.2). $DP_{10\ min}$ (percent drug dissolved within 10 minutes), mean dissolution time (MDT) and $t_{50\%}$ (time to dissolve 50% drug) values calculated from release profile are reported in Table 5. From this data, it was evident that onset of dissolution of pure famotidine was very low ($DP_{10\ min}$ value 2.54% and $t_{50\%}$ >>2 h).

Dissolution of famotidine nanoparticles were affected by different surfactant concentrations and stirring speed. Both formulation parameters generate different particle sizes which are correlate with dissolution of nanoparticles. To evaluate the influence of the four different surfactant concentrations of Lutrol F-68 (0.25%, 0.5%, 0.75%, and 1%, wt/wt) were used. The results demonstrate that with 0.25% of Lutrol F-68, the mean particle size remains below nanometer range (566nm). Increasing the Lutrol F-68 concentration determines a significant change of the mean particle diameter (~2.27 µm for 0.5% wt/wt Lutrol F-68 concentration and ~2.72 µm and 4.63 µm for 0.75% and 1% wt/wt respectively). Lutrol F-68 exhibited remarkable influence on particle size when the concentration was as above as 0.25%. The dissolution profiles of famotidine nanosuspensions, in comparison with a reference mixture of micronized famotidine, are shown in Fig. 5. The dissolution rate was markedly enhanced in the nanometer-sized system, as more than 100% of the drug dissolved in 50 min (batch F_1), as opposed to only 12% of micronized drug (batch F_7). This could be due to the increased surface area of the drug.

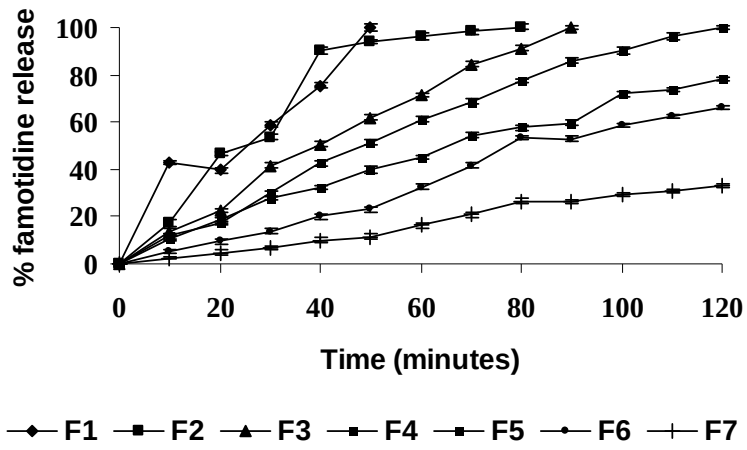

Fig 5. Percentage famotidine dissolved from the nanosuspension and pure drug versus time

Influence of stirring speed was identified on drug release from nanosuspension. At fixed amount of surfactant (0.25% Lutrol F-68) batches F_5 and F_6 were prepared to study the effect stirring speed on drug release from nanosuspension. Nanosuspension of famotidine at 1000 rpm (batch F_1) significantly enhanced dissolution rate of famotidine (100%) within 120min as compared to 1500 rpm and 2000 rpm (78.24% and 66.17% of batch F_5 and F_6 respectively). Rapid evaporation of solvents takes place at high speed which causes the larger aggregates of drug nanoparticles and release rate in diffusion medium to decrease. Highest improvement was obtained at slow speed (1000 rpm) with 0.25% Lutrol F-68 in solvent evaporation techniques.

MDT reflects the time for the drug to dissolve and is the first statistical moment for the cumulative dissolution process that provides an accurate drug release rate (Reppas et al., 2000). It is accurate expression for drug release rate. A higher MDT value indicates greater drug retarding ability (Vueba et al., 2004). MDT values were calculated using equation

$$MDT_{\text{in vitro}} = \frac{\sum_{i=1}^{n} t_{mid}\ \Delta M}{\sum_{i=1}^{n} \Delta M} \qquad (1)$$

Here, i is dissolution sample number, n is number of dissolution times, t_{mid} is time at the midpoint between times t_i and t_{i-1}, and ΔM is the amount of famotidine dissolved (μg) between times t_i and t_{i-1}.

From table 5, it was shown that batch F_1 have very high $DP_{10\ min}$ with lowest $T_{50\%}$ (42.8%, 20 min respectively). MDT value of pure famotidine is high (15.11 min). This value decreased to a greater extent after preparing its nanosuspensions. F_1 showed lowest MDT value (13.71 min). MDT values of batch F_1 was lower than other prepared nanosuspension.

Conclusion

A nanoprecipitation method was developed to prepare famotidine nanoparticles using Lutrol F-68 as stabilizer. In this process, the particle size of famotidine can be obtained in the micron and nano-size ranges, by adjusting the operation parameters, such as the stabilizer concentration (%w/v) and the stirring rate (rpm). The best nanosuspension of famotidine can be obtained by 0.25% w/v Lutrol F-68 at 1000 rpm using solvent evaporation technique. The drug particle size decreases with the decreasing of the stirring rate and the amount of stabilizers. The dissolution of nanosized famotidine is significantly enhanced compared to the pure famotidine suspension. In conclusion, the nanoprecipitation method offers a direct process to obtain drug nanoparticles of desirable size, amenable for continuous and consistent production.

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