

Preparation and evaluation of famotidine nanosuspension**Marwa M. Malik*, Nidhal K. Maraie*.****Department of Pharmaceutics / College of Pharmacy/ Mustansiriyah University.**Marwa M. Malik meramom89@yahoo.com**dr-nidhal-khazaal @ yahoo.com*DOI: <https://doi.org/10.32947/ajps.18.02.0370>**Abstract:**

Famotidine (FM) is a potent H₂-receptor antagonist used for the treatment of peptic ulcer. It has a low and variable bioavailability which is attributed to its low water solubility. There are many methods used to increase dissolution rate of drug and in this study, the dissolution of the drug was enhanced by the preparation of nanosuspension. Famotidine nanosuspension was prepared by antisolvent precipitation method, where famotidine dissolved in methanol at room temperature and emptied into water containing different types of stabilizers (single and in combination). The optimum formula (F9) was selected according to particle size (362.8nm) and release profile (80 % of drug release within the 10 minutes) in comparison to pure famotidine powder release. The influence of formulation variables like the type and concentration of stabilizers Polyvinyl pyrrolidone K30, polyvinyl alcohol and poloxamer 188 (PVP K-30, PVA and poloxamer 188) in addition to combination of stabilizers on particle size of drug nanosuspensions were studied. The result showed that single stabilizer (poloxamer 188) has perfect surface affinity and could form a substantial mechanical and thermodynamic barrier at the interface of dug molecule. As the concentration of stabilizer increases the particle size decreases at fixed drug concentration (drug: stabilizer ratio 1:2).

Key words: Antisolvent precipitation method, famotidine nanosuspension.**تحضير وتقييم جسيمات النانو معلق لدواء الفاموتدين**

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الخلاصة:

فاموتدين من الادوية الفعالة لعلاج قرحة المعدة مضاد المستقبلات- H₂. فاموتدين يمتلك التوافر الحيوي ضعيف يعود السبب لقلّة ذوبانيته في الماء. هنالك عدة حلول لزيادة نسبة انحلال وذوبانية الدواء ومن هذه الطرق تحضيره بشكل النانو معلق. تحضر جسيمات نانوية للفاموتدين بطريقة الترسيب باستخدام المذيب المضاد، حيث يتم اذابة دواء الفاموتدين بكمية من الميثانول بدرجة حرارة الغرفة ثم يتم اضافته الى الماء الذي يحتوي على أنواع مختلفة من المثبتات ويتم اختيار أفضل عينة هي العينة 9 اعتمادا على حجم الجسيمة (362.8nm) وعلى التحرر الدوائي (80% من الدواء يتحلل خلال 10 دقائق) مقارنة مع تحلل باودر فاموتدين النقي. العوامل المؤثرة على عملية التحضير تتضمن نوع وتركيز المثبتات المختلفة مقارنة (PVPK-30، PVA و poloxamer188) بالإضافة الى استخدام أكثر من نوع (دمج) من المثبتات يؤثر على حجم جزيئة جسيمات نانوية التي يتم دراستها. اتضح من خلال النتائج ان استخدام نوع واحد من المثبتات (poloxamer188) لديه ميول جيد لسطح جزيئة الدواء ويمكن أن يشكل حاجزاً ميكانيكياً وديناميكياً هاماً على سطح جزيئة الدواء. كلما زاد تركيز المثبت قلّة حجم الجزيئة (نسبة الدواء: مثبت 1:2).

Introduction:

Solubility is an important factor for drug therapy in any route of administration. It possesses a major challenge for pharmaceutical technologists to progress new pharmaceutical products, since nearly half of the active pharmaceutical

ingredients (API) are either insoluble or poorly soluble in water. Nanosuspension has emerged as an essential tool in drug delivery to rectify these solubility conflicts [1] and has been developed as a promising elect for effective delivery of hydrophobic

drugs. The particle size distribution of the solid particles in nanosuspension was ordinarily less than one micron with an average particle size ranging between 200 and 600nm^[2]. Several methods applied for preparation of nanosuspension consist of precipitation technique, media milling, high-pressure homogenization in water, high pressure homogenization in non-aqueous media, and combination of precipitation and high-pressure homogenization^[3].

Famotidine is designated for the treatment of ulcers and hypersecretory situations. Mechanism of action, pharmacological effects, site of the action, and clinical uses are the parallel as for the other H₂-receptor antagonists, but famotidine is reported to be about 7.5 and 20 times more effective than ranitidine and cimetidine, respectively, in preventing gastric acid secretion. Although famotidine has minimum first-pass metabolism but its oral bioavailability reported to be low and changeable, ranging from 40% to 50% because of its poor aqueous solubility, high polarity of compound, and stomach degradation. Since famotidine is poorly water-soluble drugs, the dissolution rate is often the rate-limiting step for its bioavailability. The dissolution rate is a function of the solubility and the surface area of the drug; thus, dissolution rate will be increased when the solubility of the drug is increased^[4].

The aim of this work is to prepare nanosuspension of famotidine and study

the variables that affect the particle size and dissolution rate of the drug in order to optimize the final formula.

Materials and methods:

Materials:

Famotidine and Polyvinyl pyrrolidone K-30 (PVP K-30) were provided by (Sinopharm Chemical Reagent Co.,Ltd, China), Poloxamer 188 (pluronic F68) ® was gained by (HIMEDIA, Mumbai, India) and Polyvinyl alcohol (PVA) was supplied from (Disto Pharmaceutical PVT. LTD. India). All the materials were used as received.

Methods:

Preparation of famotidine

Nanosuspension:

Famotidine nanosuspension was prepared by antisolvent precipitation method, where famotidine was dissolved in (6mL) methanol at room temperature to form organic solution. The stabilizers (PVP, PVA and poloxamer) were separately dissolved in (10 mL) distilled water to form aqueous solution. After that organic solution containing drug added by means of a syringe drop by drop to the aqueous solution with stirrer for about 2hrs to allow the organic solvent to evaporate and maintained at temperature of 30-40°C to get aqueous suspension. The ratios of famotidine to the surfactant to prepare nanosuspension were, 1:1 and 1:2^[5]. Table (1) Shows the composition of each formula.

Table (1): Composition of famotidine nanosuspension in different formulas.

Formulas	Famotidine (mg)	PVP K30 (mg)	PVA (mg)	Poloxamer 188 (mg)	Methanol (mL)	Water (mL)
F1	20	20			6	10
F2	20		20		6	10
F3	20			20	6	10
F4	20	10	10		6	10
F5	20	10		10	6	10
F6	20		10	10	6	10
F7	20	40			6	10
F8	20		40		6	10
F9	20			40	6	10

PVP K30: Polyvinyl pyrrolidone K30, PVA: polyvinyl alcohol and poloxamer 188

Variables Affecting the Formulation of Nanosuspension

Different factors were studied during the formulation which are:

Effect of type of stabilizer on the particle size

The effect of using single stabilizer type was studied in F1-F3 which contain PVP K30, PVA and poloxamer 188 at drug: stabilizer ratio 1:1.

Effect of combination of stabilizers on the particle size

The combination of two stabilizers were also studied to determine their effect on particle size. F4-F6 incorporated different stabilizers combination at drug: stabilizer ratio 1:1.

Effect of the concentration of stabilizers on the particle size

F1 and F7 were utilized to study the effect of concentration of PVP on the particle size F1 and F7 had drug: stabilizer ratio 1:1 and 1:2 respectively. Formula F2 and F8 were used to study the effect of PVA concentration at drug: stabilizer ratio 1:1 and 1:2 respectively. While F3 and F9 containing poloxamer 188 were used for the same purpose.

Particle size and poly dispersity index analysis:

The measurement of the Z-average diameter and polydispersity index (PDI; size range of particles) was done for all prepared formulas by using dynamic light scattering process (Malvern, UK Zetasizer Nano ZS) in which the light scattering fluctuations were investigated due to the particles Brownian motion of nanosuspension formulations [6]. Diluted nanosuspension was added to the sample cell (folded capillary zeta cell) and monitored the light scattering at 22°C (173° angle).

In vitro release study:

The release of three formulas (F7, F8, F9) were studied (where these formulas selected according to smallest particle size) using USP dissolution test apparatus-paddle method using dialysis membrane (MWCO 2000 Da) and 900 mL of dissolution media (0.1N HCl pH 1.2) with speed of 50 rpm at 37 ± 0.5°C. Ten mL of each formula was added to the dialysis membrane and put in dissolution media (0.1N HCl pH 1.2) then 5 mL samples were withdrawn at suitable time intervals and 5 mL fresh solution were added for replenishing the media, the samples were determined spectrophotometrically using a UV-Vis spectrophotometer at the (265 nm) λ_{max}, calibration curves of famotidine in

0.1N HCl (pH 1.2) was made by preparing number of dilutions of the drug through transferring (1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 mL) from stock solutions (0.05 mg/mL)^[7, 8].

Fourier transform infrared (FTIR) spectroscopy

The Fourier transform infrared spectroscopy (FTIR) spectra was used to obtain the FTIR spectroscopy for the following samples: Pure famotidine powder, Poloxamer 188 alone and physical mixture of famotidine and the selected stabilizer (poloxamer 188).

The samples were grounded and mixed thoroughly with potassium bromide. The spectrum obtained was in between the wave number of (4000-400 cm⁻¹)^[9, 10].

Drug Entrapment Efficiency of Nanosuspension (EE) determination:

Formula F9 was centrifuged at 20,000 rpm for about 20 minutes using ultracentrifuge instrument. The percentage of drug entrapment efficiency was determined by subtracting the amount of free drug in the supernatant from the initial amount of drug taken. Using UV-visible spectrophotometer for determining the absorbance of the appropriately diluted supernatant solution at 265 nm. The work was repeated in triplicate and the mean \pm SD was calculated^(11,12)

The percentage of drug entrapment efficiency (% EE) was obtained by the following equation:

$$\text{Entrapment Efficiency (\%)} = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100$$

Results and Discussions:

Results:

Nine formulas of nanosuspension (F1-F9) were prepared according to the antisolvent precipitation method and subjected to characterization. The following parameters were estimated

particle size and poly dispersity index analysis:

The particle size and size distribution are very important parameter in evaluation of nanosuspension. The Z- average particle size for formulations (F1-F9) is shown in the Table (2) (diameter by intensity). Polydispersity index is a parameter used to explain the particle size distribution of nanoparticles obtained from a particle analyzer. PDI is an index of width or spread or difference within the particle size distribution and provides an indication about the long-term stability of nanosuspension. Monodisperse system has a lower PDI value (homogenous), whereas higher values of PDI indicated polydisperse system (heterogeneous)^[13]. Figure (1) shows particle size distribution by intensity for the selected formula F9.

Table (2): Mean intensity diameter and PdI of prepared nanosuspension formulas. (n = 3)

drug: stabilizer ratio 1:1	Z-average diameter (nm)	PdI
F1	689±0.4	0.557±0.6
F2	1446±0.3	0.752±0.8
F3	981.8±0.6	0.628±0.9
F4	861.9±0.2	0.527±0.7
F5	986.7±0.7	0.717±0.9
F6	1036±0.4	0.303±0.5
Drug: stabilizer ratio 1:2	Z-average diameter (nm)	PdI
F7	297±0.3	0.624±0.1
F8	307±0.6	0.440±0.7
F9	362.8±0.8	0.107±0.5

In vitro release study:

The release profile of famotidine from F7, F8 and F9 formulas in 0.1N HCl pH 1.2 are shown in Figure (2), formulas showed initial burst effect where >40 % of drug released within the first 5 minutes and >80 % of drug released within 10 minutes.

Fourier transform infrared (FTIR) spectroscopy

FTIR is one of the most widely reported spectroscopic techniques. Figure (3) showed the main absorption bands of pure famotidine at stretching (NH₂) guanidine and stretching (NH₂) sulfonamide at 3505.95 cm⁻¹, stretching (C= N) at 1637 cm⁻¹, and stretching (SO₂) at 1321.00 cm⁻¹ [14].

Poloxamer 188 showed a strong absorbance bands for stretching (O-H) at 3421.10 cm⁻¹, stretching (C-H) at 2886.92 cm⁻¹, in plane (O-H) at 1342.21 cm⁻¹ and stretching (C-O) at 1109.83 cm⁻¹ that agreed with reported data [15]. As in Figure (4)

The spectrum of physical mixture of pure famotidine and poloxamer 188 at ratio 1:1 was equivalent to the spectrum of pure drug and poloxamer 188 each separately as shown in Figure (5)

Drug Entrapment Efficiency of Nanosuspension(EE):

The selected F9 (according to its higher release and smallest particle size) was subjected for calculation of entrapment efficiency and it was found to be about 96.5±0.75 [16].

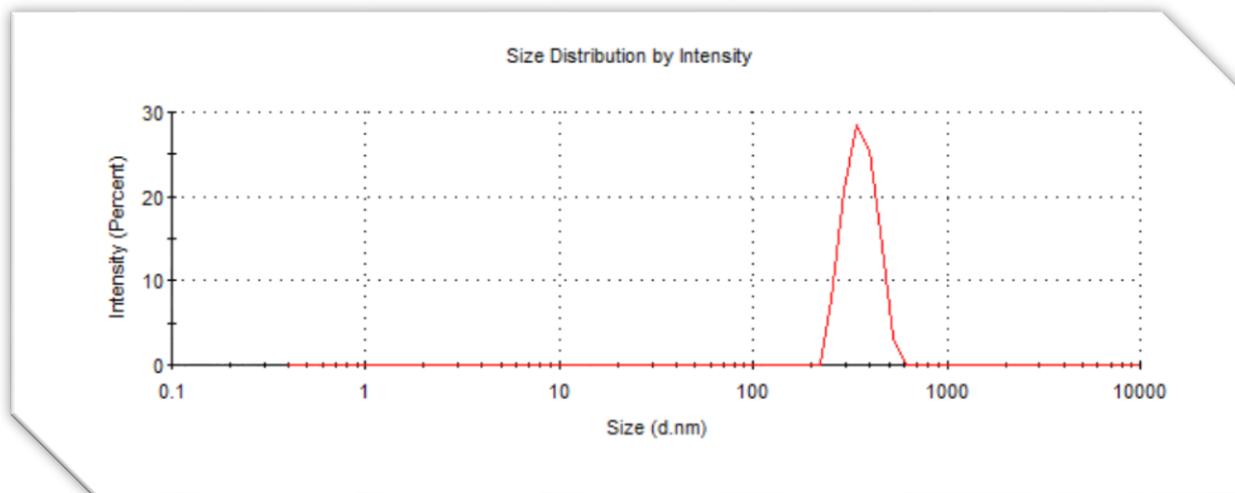


Figure (1): Particle size distribution by intensity of F9.

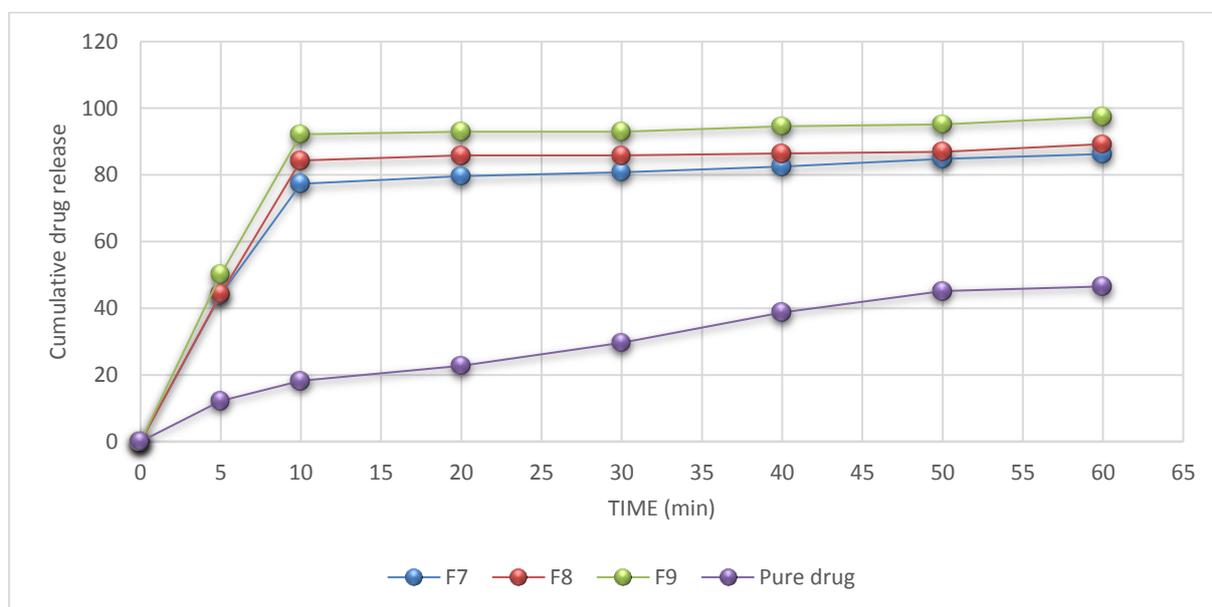


Figure (2): The release profile of famotidine from different nanosuspension formulas in 0.1N HCl pH 1.2

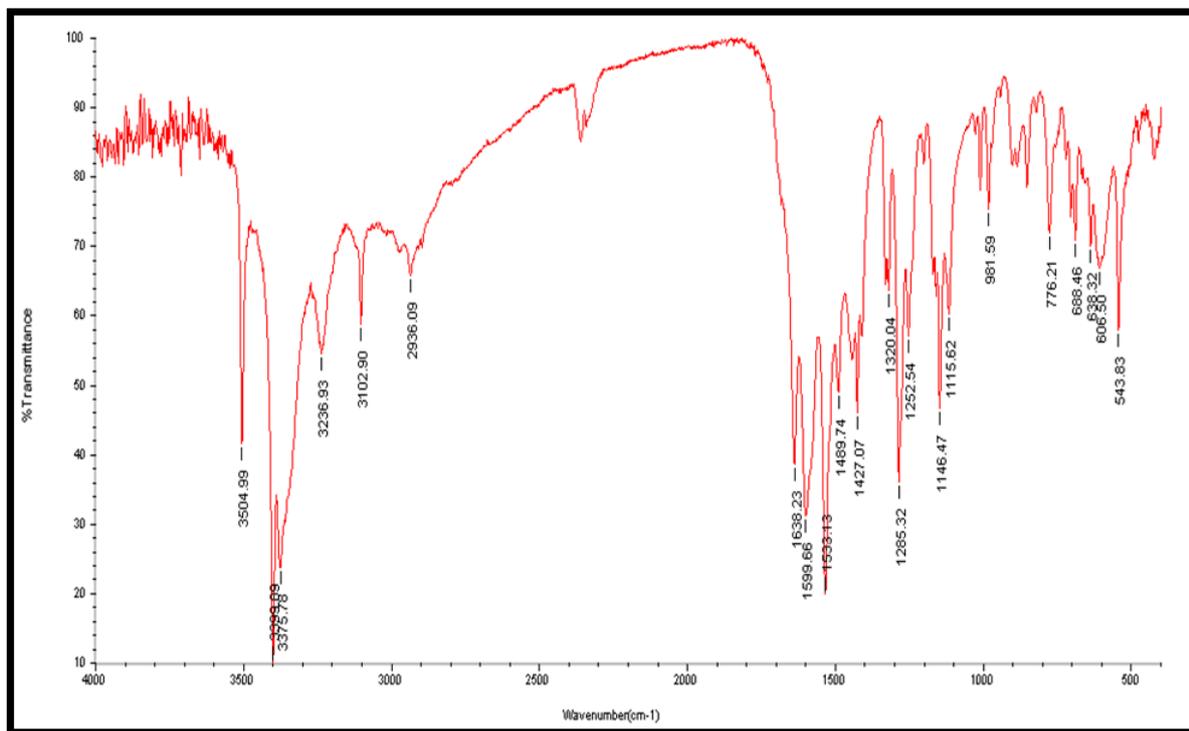


Figure (3): FTIR spectrum of pure famotidine

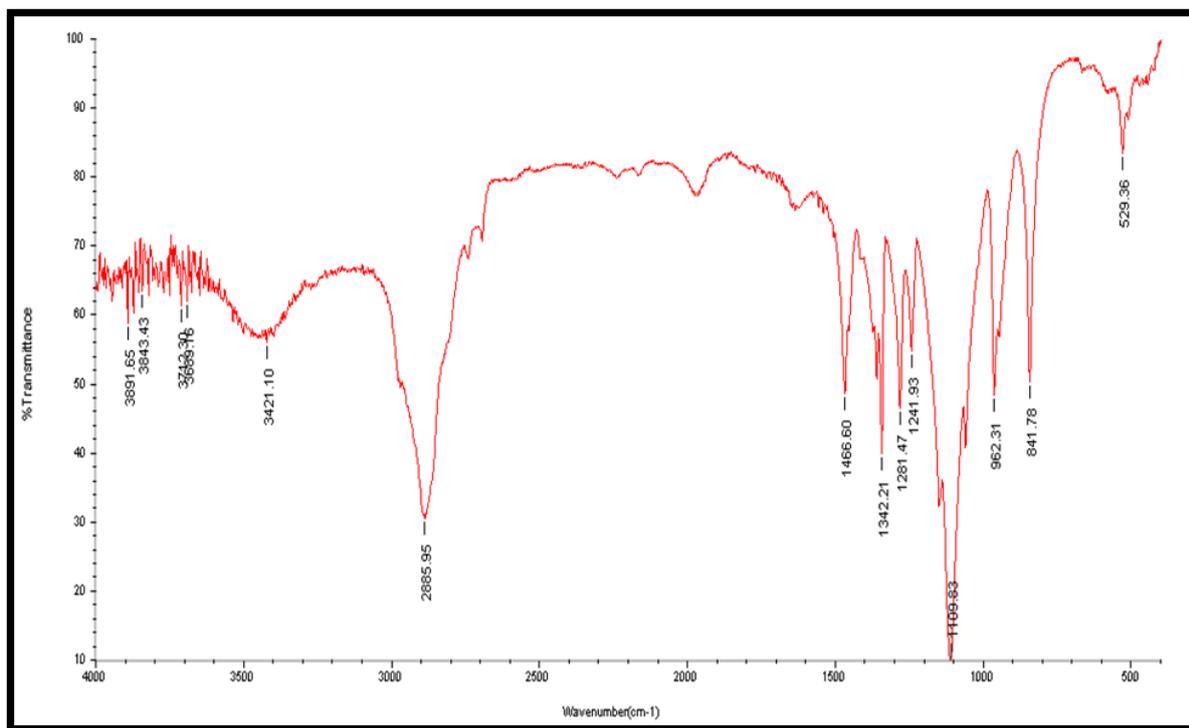


Figure (4): FTIR spectrum of pure poloxamer188

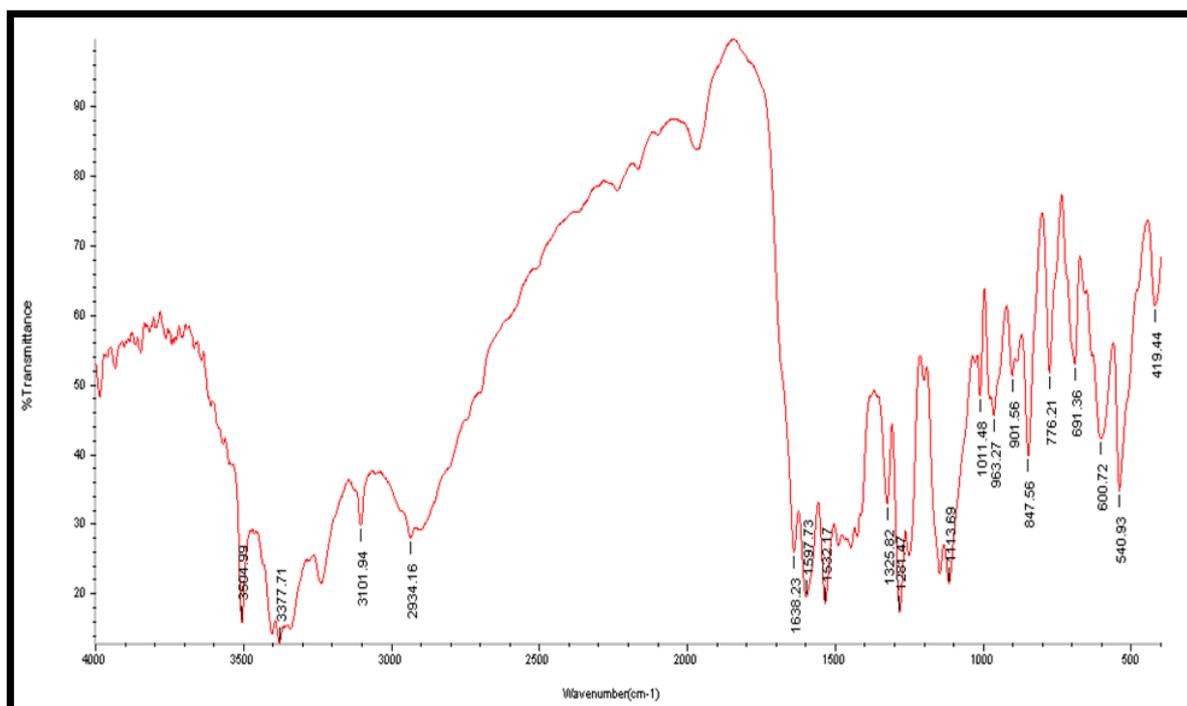


Figure (5): FTIR spectrum of physical mixture of famotidine and poloxamer 188 at ratio (1:1).

Discussion:

According to the Malvern Zetasizer Nano ZS series user manual, low value of poly dispersity index 0.08-0.7 is considered to be desirable for uniform distribution, high quality and homogeneity of nanosized particles within the preparation. While PdI value > 0.7 to less than 1 is considered to have broad distribution of particle size while PdI equal to 1 is considered so polydisperse and sample is not suitable for measurement by Malvern zetasizer Nano⁽¹⁷⁾. Formulas 1, 3, 4, 6, 7, 8 and 9 showed PdI within the desirable range but F7, F8 and F9 showed smaller particle size therefore, they were further evaluated.

The results showed that using single stabilizer (each separately) as in F1-F3, F7-F9 indifferent ratio (1:1 and 1:2) where PVP, PVA and poloxamer 188 were used as primary stabilizer. They stabilized the system by steric stabilization where the surfactant adsorbed onto the drug particle surface leading to reduce in the surface tension and increasing the nucleation rate. On the other hand, the adsorption of surfactant makes the particles less

hydrophobic and thereby reduces the hydrophobic forces of attractions (van der Waals interactions) and that reduced particle growth and aggregation^[18].

The results in Table (2) showed that using single stabilizer (each one separately) as in F1-F3, F7-F9 in different ratio 1:1, 1:2, gave nanoparticles with different range. It was found that as the amount of stabilizer increased the particle size decreased indicating sufficient amount stabilizer adsorbed on the surface and reduced aggregation. While formula containing poloxamer 188 showed particles 981.8 nm (F3, 1:1 ratio), 362.8 nm (F9, 1:2 ratio), this could be explained on the basis of the viscosity of solution which is increased and the hydrophilic chains of one particle may have interacted with hydrophilic chains of the other particle and this inter-particle interaction of chains may have resulted in agglomeration at higher concentration of poloxamer 188 and this agreed with reported data⁽¹⁹⁾.

Less amount of stabilizer induces agglomeration or aggregation and too much stabilizer promotes Oswald's

ripening. Concerning combination of stabilizers, it is found that at drug: stabilizer ratio (1:1), using combination equal amount of PVP-PVA (F5), equal amount combination of PVP- poloxamer 188 (F5), and equal amount combination of PVA-poloxamer 188 (F6) lead to increase in particle size (in comparison to formulas containing single stabilizer), this could be attributed to either the amount of stabilizer (in the combination) is not sufficient to cover drug surface particles or due to poor stabilization in this combination^[20]. Therefore, this stabilizer ratio (1:1) is not appropriate to give suitable particle size for nanosuspension. According to the particle size obtained, it is found that the type as well as the concentration of stabilizer used have great effect. generally, when concentration of stabilizer increased, the particle size was decreased at fixed drug concentration, which indicated that the surface of drug particle size was adequately enveloped by suitable stabilizer molecule, therefore formulas F7, F8 and F9 showed suitable smaller particle size. According to particle size distribution (indicating by PdI); F9 showed best particle size distribution (with smaller PdI 0.107) than other formula.

The release profile of famotidine from different nanosuspension formulas in 0.1 N HCl pH 1.2 was shown in Figure (3) formulas (F7, F8, F9). When comparing release profile of pure famotidine with nanosuspension famotidine formulas, it was shown that all nanosuspension formulas have significant much higher release than pure famotidine indicating that reducing the particle size led to increase in surface area subjected to the dissolution media and as a result the release of the drug was increased^[21]. The variation in the dissolution rate in different formulation is due to variation in diffusion layer thickness. Poloxamer 188 is a non-ionic polymer, potential agglomeration was less liable to occur. The formation of a hydrodynamic boundary layer surrounding the nanocrystals resulted in the growth

inhibition and prevention of agglomeration as well as adsorption of the polymer molecules on the growing crystal faces that provide a steric hindrance to agglomeration of nanosuspension and also surface activity, solubilization and wetting effect^(22, 23).

Formula F9 (which contain poloxamer 188 at drug: stabilizer ratio 1:2) gave good drug release and suitable particle size due to presence of sufficient amount of poloxamer. Therefore, F9 was selected for further study.

The result of FTIR showed the main absorption bands of pure famotidine and Poloxamer 188 showed a strong absorbance bands, all these bands agreed with reported data⁽¹⁴⁾.

The spectrum of physical mixture of pure famotidine and poloxamer 188 at ratio 1:1 was equivalent to the spectrum of pure drug and poloxamer 188 each separately and that indicating no chemical interaction or complexation occur in physical mixing. The percentage of drug entrapment efficiency of F9 was 96.5 % entrapment efficiency defines the efficiency of the preparation method to incorporate drug into the nanoparticles. The drug was incorporated into nanoparticles either by hydrogen bonding, ionic interaction, dipole interaction, physical entrapment (or encapsulation), precipitation, covalent bonding or may be adsorbed to the surface. More than one loading mechanism was involved in most drug delivery systems.

Conclusion

The results of this study showed the capability of nanotechnology to reduce the particle size that led to increase in surface area subjected to the dissolution media and as a result the release of the famotidine was increased, which may improve its absorption.

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