Original Articles

SOLID DISPERSION OF FAMOTIDINE: FACTORIALLY DESIGNED CAPSULE FORMULATION AND *IN VIVO* EVALUATION OF ANTIULCER ACTIVITY

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تم تصميم دراسة مفكوكية لاستخدامها كوسيلة لاختيار السواغ المناسب لصياغة كبسولات دواء فاموتيدين. وقبل صياغة الكبسولات ومن أجل تحسين ذوبانية دواء فاموتيدين، فقد تم تحضير المبعثرات الصلبة للدواء باستخدام بولي إيثيلين جليكول كمادة حاملة للداوء. وقد تم تجربة كل من بولي إيثيلين جليكول 4000 وبولي إيثيلين جليكول 6000 بنسب مختلفة من كل من الدواء وحامل الدواء. وتبين أن بولي إيثيلين جليكول 6000 كان أكثر فاعلية في تعزيز ذوبان دواء فاموتيدين. وأما التصميم الذي تم تطبيقه فقد كان من نوع 2×3×4 وتم استخدامه لتقييم تأثير نسبتي بولي إيثيلين جليكول 6000 المستخدمتين في تحضير كل من مبعثر دواء فاموتيدين الصلب، وثلاث مزلقات وأربع مخففات على التوالي على T_{30} للدواء. وكل العوامل التي درست تبين مدى أهميتها، فقد كان أكثرها أهمية هو ما يتعلق بالمزلق وكان أقلها أهمية هو النسبة بين الدواء والبوليمر. كما وجد أن الأنواع المختلفة للتداخلات بين العوامل المختلفة كانت مهمة أيضاً. وطبقاً للتداخل الثلاثي الاتجاهات فقد تبين أن النسبة الأكثر اقتصادية هي 1:1 بين كل من الدواء وبولي إيثيلين جليكول 6000 والتي أعطت نسبة إطلاق لدواء فاموتيدين مقدارها 99.92% مع ايروسيل كمادة مزلقة ومانيتول كمادة مخففة. ولقد تم حساب قياس خاصية السريان للبودرة وذلك للصيغة الأكثر مناسبة. كما تم تقييم كل من الفاعلية المضادة للقرحة وذلك للصيغة التي أظهرت T_{30} الأعلى وأفضل الخواص للسريان.

A factorially designed study has been developed as a tool for choosing the most suitable excipients for formulating famotidine capsules. Prior to capsule formulation, and to improve the solubility of famotidine, drug solid dispersions (SD) have been prepared using polyethylene glycol (PEG) as a carrier. Both PEG_{4000} and PEG_{6000} were tried in different drug to carrier ratios. PEG_{6000} was found to be more effective in enhancing famotidine dissolution. The design applied was a 2 ×3 ×4 type to evaluate the effect of two ratios of PEG_{6000} used in the preparation of famotidine SD, three lubricants and four diluents, respectively, on the T_{30} of the drug. All the factors under study were found to be significant with the highest one being that of lubricant and the lowest one the ratio between the drug and the polymer. Different types of interactions between the different factors were also found significant. According to the three-way interaction which showed that the more economical 1:1 drug to PEG_{6000} ratio gave 99.92% of famotidine release with aerosil as lubricant and mannitol as diluent. The flow property measurement of the powder fill of the most promising formulae was calculated. The anti-ulcer activity of the formulae exhibiting the highest T_{30} and the best flow characteristics was evaluated as well.

Keywords: Famotidine, solid dispersions, polyethylene glycol, factorial design, enhancement of dissolution, capsules, anti-ulcer activity

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Introduction

Famotidine is 3 - [[[2 - [(Aminoiminomethyl) amino]–4 thiazolyl]methyl] thio]–N-(aminosulfonyl) propanimidamide. It is a potent H_2–receptor antagonist used to treat peptic ulcer. When compared with other H_2 – receptor antagonists it was shown to be 7.5 and 20 times more potent than ranitidine and cimetidine, respectively (1). In spite of its benefits, famotidine suffered from low and variable bioavailability which was attributed to its low water solubility (2). Several ways have been used to improve the oral bioavailability of poorly soluble drugs as an example solid dispersion (SD) technique with water soluble carriers (3,4). The increase in dissolution rate of poorly water soluble drugs from SDs can be attributed to one or combination of different factors (5). Among the popular carriers used in the formulation of SD are polyethylene glycols (PEGs). They are widely used because of their hydrophilicity, low melting point, and low toxicity (4).

The development of a pharmaceutical formulation is usually a trial and error technique including a careful control of the variables one at a time in a series of logical steps. This is generally a time consuming method in which the effect of each experimental variable will be investigated separately, while keeping all others constant. Besides, variables may interact with each other and the magnitude of the effect caused by altering one factor will depend on the magnitude of one or more other factors. Such interactions cannot be elucidated by classical methods.

The use of factorial design experiment is an efficient method of indicating the relative significance of a number of variables in the production of a given result. In addition it offers the advantage to provide a way of analyzing the results to decide on most significant variables. Analysis of variance (ANOVA) is generally used. A maximum outcome can be drawn out of theses models with the use of a small number of experiments. In addition they allow a mean of assessing interactions which exist between different variables over the response (6,7). This method is well documented in a variety of fields including pharmaceutical formulation (8- 12).
The work described here was concerned with the formulation of famotidine SD in capsules, using factorially designed experiment. Being an important parameter for industrial purpose the flow properties of the powder fill of the most promising famotidine capsules were evaluated,. The antiulcer activity of the formulae with the best dissolution and flow properties was evaluated as well and compared with that of drug powder and marketed product.

Experimental

Materials and methods:

Famotidine was kindly supplied by (Memphis Egypt), polyethylene glycol (PEG_{4000} and PEG_{6000}) (Aventis, Egypt), starch (Alamia Company for chemicals, anhydrous lactose (El-Gomhouria Company, Egypt), microcrystalline cellulose (Avicel® PH 101), mannitol, talc, aerosil, glacial acetic acid, potassium dihydrogen orthophosphate (El-Nasr Pharmaceutical Chemicals, Egypt), croscarmellose sodium (Ac-di-sol®), sodium starch glycolate (Primojel®) (Glaxosmithkline, Egypt), magnesium stearate (Mgst.) (Winlab, England).

Preparation of the solid dispersions:

The melting method was used to prepare famotidine SDs with PEG_{4000} and PEG_{6000}. Each of the PEGs grade, was mixed separately with famotidine in the ratios of 1:1, 1:2 and 1:3 drug to polymer ratio, melted with constant stirring on thermostatically controlled hot plate (100°C), then quenched on ice with vigorous stirring. The prepared SDs were then stored at room temperature in a desiccator until solidification. After complete dryness, the famotidine SDs were ground then sieved between sieves having mesh size ranging from 0.2 – 0.25mm.

Dissolution studies:

Dissolution studies for pure famotidine with a solubility of 0.53mg/ ml, famotidine - PEG SD powders with a solubility of 0.65 mg/ml, and capsules were carried out, using 20mg of famotidine, or an equivalent amount of famotidine-PEG SD as powder or formulated as capsule, in 900ml phosphate buffer pH 4.5 as dissolution medium (USP 23, 1995). The dissolution was performed using USP dissolution test apparatus I for powders and apparatus II for capsules. The baskets or the paddles were rotated at 50 rpm. The temperature of the dissolution medium was maintained at 37±0.5°C. Samples were withdrawn at different time intervals and analysed spectrophotometrically at λ_{max} 265 nm.

Differential scanning calorimetry study (DSC):

The SD of famotidine with the carrier exhibiting the highest drug dissolution as well as famotidine powder and the carrier itself were subjected to DSC study using differential scanning calorimeter (Schimadzu DSC 50) at a scanning speed of 10°C/min in the temperature range of 30 – 400°C under nitrogen gas flow. Indium was used as standard for calibrating the apparatus.

X-ray diffraction (X-RD):

The crystallinity of the same samples chosen for DSC was investigated. X ray diffraction patterns were obtained with a Philips's X'Pert MPD diffractometer under ambient conditions over the 2 θ range of 10-60 ^{o}. The diffractometer operated by a computer system so as to calculate the inter planar distance of different crystals, the relative peak intensities and the 2 θ angle at which diffraction occurred.

Preparation and evaluation of capsules using a full factorial design experiment:

Amounts equivalent to 20 mg of famotidine of the drug SDs prepared with PEG_{6000} in 1:1 and 1:2 drug-polymer ratio, were used in the capsule preparation. The capsules were manually filled and a full factorial design experiment was built up to study the effect of three types of additives (three factors) namely: the amount of PEG_{6000} in the prepared SD at two levels (1:1 and 1:2 drug- polymer ratios), the lubricant at three levels (Mgst, talc and aerosil) and the diluents at four levels (starch, lactose, *Avicel® PH 101*, and mannitol) leading to 2 x 3 x 4= 24 experiments. Typical design of the full factorial experiment with the composition of different capsules is shown in table 1.

Analysis of data obtained from the factorial design experiment.:

The difference between percents of famotidine dissolved after 30 min (T_{30}) from its various formulations (the chosen response for analysis) were statistically evaluated by the analysis of variance (ANOVA) using the statistical software (Statview abacus concept version 4.57).

Study of the flow properties of the powder fills of the capsules:

As the flow properties are of major concern in industry, the famotidine capsules exhibiting the best drug dissolution, were chosen for the evaluation of the flow characters of their powder fill using the following parameters: Carr's compressibility index (13), Hausner's ratio (14) and angle of repose (15). To measure the first two flow parameters values, the volume of 10 gm weight of the bulk powder in a 25 ml cylinder was measured (V_b) and the volume of the same powder after being tapped to a constant height (V_t) was recorded. The Carr's compressibility index and the Hausner's ratio were calculated according to the respective equations:

Carr's compressibility index =

$$[\,1-(V_t / V_b)\,] \times 100 \qquad \text{Eq. (1)}$$

$$\text{Hausner's ratio} = V_b / V_t \qquad \text{Eq. (2)}$$

The angle of repose was evaluated adopting the fixed height cone method using a glass funnel tightened at 2.5 cm height according to the equation:

$$\tan\theta = 2h / D \qquad \text{Eq. (3)}$$

Where: θ is the angle of repose.
h is the height of the funnel (2.5 cm)
D is the diameter of the area of the base of the formed cone

In vivo evaluation of the antiulcer activity:

Indomethacin induced gastric ulcer method (16) was applied to test the antiulcer activity of the prepared famotidine formulations showing the best flow properties and the highest T_{30}. In this method, 36 male albino rats were used following the approval of the experimental protocol by the Ethics Committee of EAPRU (Experimental and advanced pharmaceutical research unit, faculty of pharmacy Ain Shams university). The animals, of average weight 150-200 gm, were divided into six groups each containing 6 rats. The following oral treatments were given to the animals of different groups: group I (control A) and group II (control B) received saline, groups III, IV, V and VI received each plain famotidine powder, crushed commercial tablets and the chosen capsule formulae respectively. The last three treatments were administered to the animals each as a 1% suspension in carboxymethyl cellulose. After 30 minutes (17), all groups, except group I, were injected subcutaneously with a dose of 25mg/kg of indomethacin (18). Seven hours later, the animals were sacrificed, and the stomach of each rat was isolated and examined at its inner surface, for ulceration and erosions, with a magnifying lens.

The ulcer index (19) was evaluated for each rat according to the following equation:

Ulcer index (UI) = mean number of ulcer/rat + mean severity/rat + incidence of ulceration in each group x 100/10.

The data for the indices were expressed as mean of six animals ± SE. Multiple comparison was carried out by using one way analysis ANOVA followed by Tukey-Kramer test as post hoc test. A computer program SPSS (version 8) was used for the purpose.

The average severity of ulcers for a given stomach was determined by visual observation using an arbitrary scale from 0 to 5 indicating the degree and number of ulcers (19), where (0) was given for no lesions, (1) for just a hyperemia or the presence of one or two slight lesions, (2) for more than two lesions, (3) for severe lesions, (4) for very severe lesions and (5) for lesions involving the whole mucosa with hemorrhage.

The preventive effect of different tested formulae against gastric ulceration was expressed according to the following equation:

Preventive index (PI%) =

$$\frac{(\text{UI of control gp} - \text{UI of drug treated gp}) \times 100}{\text{UI of control gp}}$$

Histopathological examination was done to all the rats' stomach to detect any unseen changes. Scores were given for each specimen under study according to the severity of reaction as follows: (+ + +) for severe reaction; (+ +) for moderate reaction; (+) for mild reaction, (±) was considered as an almost normal tissue and (−) a tissue totally free from any inflammatory reaction.

Results and Discussion

Dissolution of famotidine SD:

Figure 1 shows that all the prepared SD systems increased drug dissolution in comparison with the plain drug. According to Verheyen *et al* (20), the mechanisms involved are solubilization and improved wetting of the drug in PEG rich micro-environment formed at the surface of drug after the dissolution of the polymer. These findings are, also, in accordance with previous reported articles (21, 22) on the enhancement of the solubility and dissolution of different drugs by preparing PEG SD. From the same figure, it was also noticed that drug dissolution was increased upon increasing the molecular weight of the polymer from PEG_{4000} to PEG_{6000}. This might be due to the increase in the hydrophilic character of the carrier, by increasing its chain length helping in a better drug wetting. Similar findings were reported by other workers, studying the effect of the use of different PEGs for the improvement of the dissolution of different drugs (23-25). It is worthy to note that, regardless the polymer chain length, the ratio of 1:2 drug to polymer, showed the highest percent of dissolved famotidine after 90 minutes, with values of 83.23% and 89.96% in case of PEG_{4000} and PEG_{6000} respectively. Further increase in polymer content was accompanied by a decrease in drug dissolution. According to Kellaway and Najib (26), this can be attributed to a viscosity delaying effect on drug dissolution caused by higher polymer concentrations. Hence it can be deduced that famotidine-PEG_{6000} SD in 1:2 ratio is the best system for formulating the drug capsules. But since it was expected that the addition of some excipients might have an enhancement effect on drug dissolution and it is more economic to use the 1:1 ratio, this latter was also used in capsule preparation.

The DCS thermograms of famotidine, PEG_{6000} and famotidine-PEG_{6000} SD are shown in figure 2. Famotidine and PEG_{6000} each gave an endothermic peak at 166.52°C and 65.04°C, respectively, corresponding to their melting point. The thermogram of the solid dispersion of famotidine-PEG_{6000} (1:1) show an endotherm at 61.7°C which is close to the PEG melting temperature, while the fusion endotherm of famotidine broadened, shifted to a lower value (145.54°C), reduced in intensity and lost its sharp distinct appearance. On the other hand the drug and the carrier crystallinity peaks were evident in the X-RD traces of the prepared drug solid dispersion (figure 3) indicating that no amourphization of the drug had occurred by SD preparation.

Table 1. Factors and levels used for factorial design

Factor	Level
Ratio of Famotidine-PEG_{6000}	Famotidine-PEG_{6000} (1:1)
	Famotidine-PEG_{6000} (1:2)
Lubricants	Magnesium Stearate
	Talc
	Aerosil
Diluents	Starch
	Lactose
	Mannitol
	Avicel®PH101

Table 2. Composition of different famotidine-PEG_{6000} SD capsules according to the factorial design.

Ratio of Famotidine-PEG_{6000}	Lubricant	Diluent			
		Starch	***Lactose***	***Avicel®PH101***	***Mannitol***
S.D 1:1	Magnesium Stearate	1	7	13	19
	Talc	2	8	14	20
	Aerosil	3	9	15	21
S.D 1:2	Magnesium Stearate	4	10	16	22
	Talc	5	11	17	23
	Aerosil	6	12	18	24

Table 3. ANOVA of the data analysis of the T_{30} of famotidine -PEG_{6000} SD capsules following a full factorial design.

Source of Variation	DF[+]	Sum of Squares	Mean Square	F-Value
Ratio of drug to PEG_{6000}	1	413.131	413.131	3063.537**
Lubricant	2	1157.52	578.76	4291.746**
Diluent	3	556.435	185.478	1375.399**
Ratio of drug to PEG_{6000} * Lubricant	2	182.228	91.114	675.647**
Ratio of drug to PEG_{6000} * Diluent	3	204.627	68.209	505.797**
Lubricant * Diluent	6	584.793	97.466	722.748**
Ratio of drug to PEG_{6000} * Lubricant * Diluent	6	305.498	50.916	377.566**
Residual	24	3.237	0.135	

[+] DF = degrees of freedom, ** the main effect of the factor or the interaction under study is significant.

Table 4. Flow properties of powder fills of famotidine-PEG_{6000} SD capsules.

Formula no.	*Carr's Index* ± SD	*Hausner's Ratio* ± SD	Angle of Repose θ ± SD
6	30.50 ± 0.5	1.51± 0.27	39.40 ±0.52
11	44.50 ± 0.92	1.80 ± 0.36	48.70 ± 0.64
18	28.80 ± 2.92	1.40 ± 0.1	34.80 ± 0.26
21	25.20 ± 2.98	1.34 ± 0.23	32.30 ± 0.28
22	36.90 ± 1.85	1.58 ± 0.22	42.10 ± 0.85
23	44.00 ± 4.58	1.79 ± 0.11	48.80 ± 2.36
Famotidine powder	45.1± 0.96	1.86 ± 0.2	49.21± 0.72

Table 5. Ulcer and preventive indices recorded with different treatments.

Animal groups	Mean n° of ulcers ± SD*	Mean Severity ± SD*	Mean incidence	Ulcer Index ± SE**	Preventive index
Group I	zero	zero	zero	zero	-
Group II	36 ± 4.73	4.83 ± 0.4	10	50.83 ± 2.01	-
Group III	11.16 ± 1.47	2.33 ± 0.51	10	23.50 ± 0.72	53.76
Group IV	2.83 ± 1.47	1.6 ± 1.03	8.33	12.66 ± 0.99	75.09
Group V	1± 1.26	0.5 ± 0.54	5	6.5 ± 0.72	87.21
Group VI	1.8 ± 1.16	1.3 ± 0.81	8.33	11.5 ± 0.70	77.37

* SD = standard deviation. ** SE = standard error

Table 6. Results of test of significance of ulcer indices of different treatments done using the one way ANOVA followed by Tukey Kramer test.

	Control B	Plain drug	Commercial tablet	Formula 18	Formula 21
Control B	---	S	S	S	S
Plain drug	S	---	S	S	S
Commercial Tablet	S	S	---	S	NS
Formula 18	S	S	S	---	S
Formula 21	S	S	NS	S	---

S = significantly different.
NS = Not significantly different.

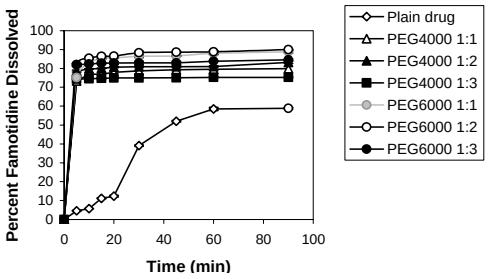


Fig.1. Dissolution profile of different famotidine PEGs solid dispersions in phosphate buffer pH

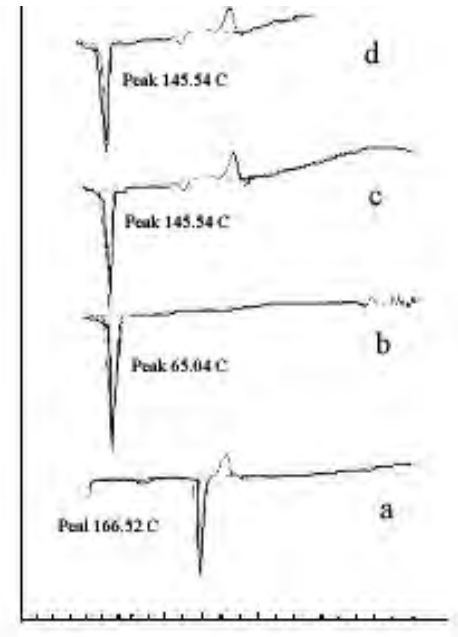


Fig.2. DSC of: a- Famotidine; b- PEG_{6000}; c- Famotidine-PEG_{6000} SD Fresh; d- Famotidine-PEG_{6000} Stored for One Year

Since the X-ray spectrum of the studied SD did not change and its DSC trace showed a change in the melting point of famotidine , it can be said that the increase in drug dissolution shown by the preparation of SD can be attributed to a sort of interaction between the drug and the carrier rather than a change in the crystalline form of the drug. Similarly, Zerrouk *et al* (27) noticed an improvement in naproxen dissolution when coground with chitosan without detecting a noticeable change in its crystalline nature. For famotidine-PEG SD we thought that a hydrogen bond might have been formed between the acidic hydrogen of both the aminosulfonyl and the iminomethyl groups of famotidine and the unshared electron pair of the oxygen of polyethylene glycol. The change in DCS thermograms due to hydrogen bond formation was previously proved by Ramakrishnan *et al* (28). From figures 2 and 3, it is obvious that the drug SD did not change neither the drug endotherm nor its crystallinity after storage for one year.

Analysis of data obtained from the factorial design experiment:

Analysis of variance for the T_{30} values of all the tested formulae shown in table 3 revealed that all the main factors under study namely: ratio of PEG_{6000} in different SDs, lubricants and diluents had a significant effect ($p < 0.0001$) on the percentage of drug dissolution. Based on the mean square values the most significant effect was that of lubricant and the lowest one was of the ratio between drug and PEG_{6000}.

Figures 4 (A-C) show in details the main effect of each variable. As a lubricant (figure 4A), aerosil had a significant higher effect on drug dissolution when compared to Mgst and talc. This may be due to the more hydrophilic nature of aerosil which favours water uptake and enhances drug dissolution.

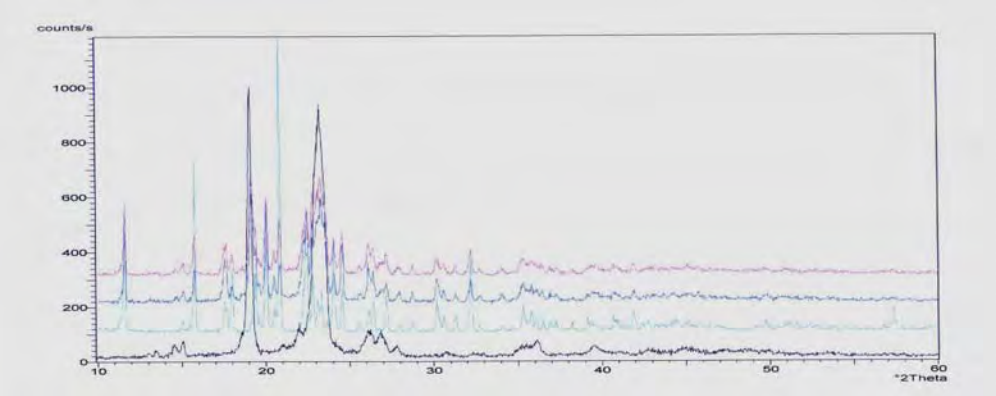


Fig.3. X Ray Diffraction of : PEG6000; Famotidine; Fresh famotidine- PEG6000 SD; Famotidine-PEG6000 SD Stored for One Year (from below to above)

The lowest drug dissolution noticed with talc might be due to its well known adsorptive power.

For the effect of PEG_{6000} content on drug dissolution shown in figure 4B, it is obvious that doubling the amount of PEG_{6000} resulted in a significant increase in drug dissolution because of the increased amount of the hydrophilic carrier.

As depicted from figure 4C, the different diluents used can be arranged in the following descending order: mannitol > lactose > Avicel® > starch. According to Fisher's PLSD test (pair wise least significant difference), the difference between the mean percent of drug dissolved when using lactose or mannitol was not significant, but they were both significantly different from those of Avicel® PH 101 or starch ($P < 0.0001$). The higher drug dissolution with mannitol and lactose can be attributed to their water soluble nature.

According to the data shown in table 3, all the interactions between the variables under study were significant ($p < 0.0001$).

Figures 5 (A-C) illustrate the details of the two-way interaction between the different pairs of variables. From figure 5A, it is obvious that with all the used diluents, except starch, the 1:2 ratio of drug - PEG_{6000} in the SD resulted in higher famotidine dissolution than the 1:1 ratio. In addition, with both ratios the water soluble diluents, lactose and mannitol, increased drug dissolution more than the water swellable *Avicel®* PH101. Increasing the content of PEG_{6000} did not affect famotidine dissolution when the diluent used was starch.

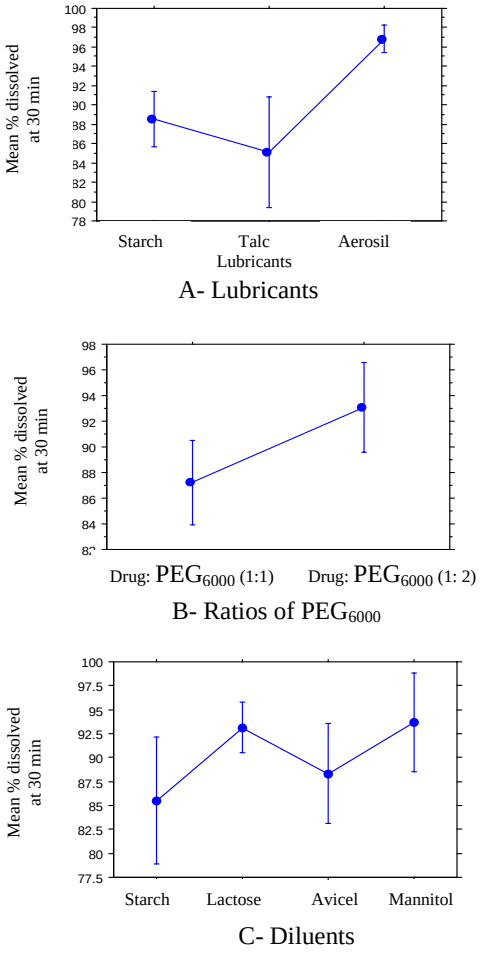


Fig. 4. Plot of the main effects of the different factors.

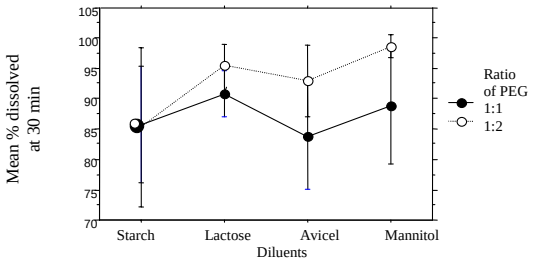
From figure 5B, it is clear that among the lubricants used, aerosil, at the level of the four tested diluents gave the highest drug dissolution. The use of talc with starch diluent gave capsules with an extremely low percent of famotidine dissolution (73.96%), hence such combination should not be recommended.

For the interaction between the level of PEG_{6000} used in SD and lubricant seen in figure 5C, it was noticed that with the different levels of lubricant used, changing the drug to PEG_{6000} ratio from 1:1 to 1:2 gave better famotidine dissolution, especially with talc and Mgst. Meanwhile, with aerosil, both ratios of PEG gave famotidine dissolution percent of very close values.

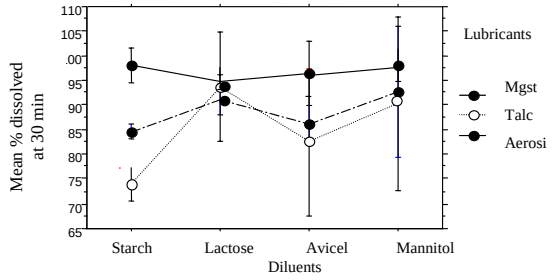
By examining the three-way interactions, shown in figure 6, it is obvious that the drug SD with PEG_{6000} in 1:2 ratio gave higher famotidine dissolution when compared to the SD prepared in 1:1 ratio with the following lubricant- diluent combinations: Mgst - mannitol, talc – lactose, talc – mannitol, aerosil - starch and aerosil - *Avicel*® PH101. On the contrary the formulations containing the 1:1 drug – PEG_{6000} ratio gave higher dissolution with the following combinations: Mgst - starch, aerosil - lactose and aerosil – mannitol, reaching a value of 99.92% drug dissolution with the last combination. This finding can be of beneficial importance for cost reduction in the industrial field.

Concerning the choice of the proper combinations of lubricant, diluent and ratio of drug to PEG_{6000} for formulating famotidine capsules, it can be concluded that the talc-starch mixture is not a recommended combination whatever the amount of PEG_{6000} used in the preparation of drug SDs. On the other hand, not less than 99% of drug release was achieved with the formulations including starch lactose, avicel, and mannitol as diluents respectively combined with aerosil, talc, aerosil and Mgst or talc as lubricants with the 1:2 famotidine-PEG_{6000} ratio. On the other hand as previously said when mannitol was combined with aerosil, the 1:1 ratio of drug-PEG_{6000} gave almost a 100 % famotidine release.

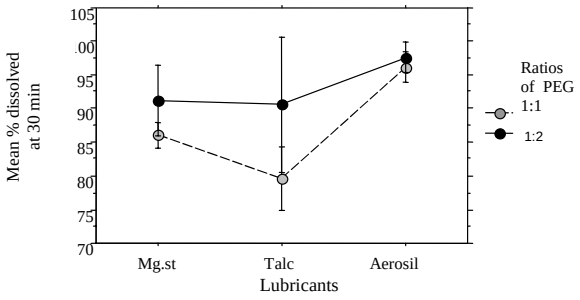
From the previous results and discussion, it is obvious that formulae 6, 11, 18, 21, 22, and 23, gave the highest drug dissolution, with not less than 99% of famotidine dissolved and consequently better bioavailability will be expected.



A- Effect of Ratios of PEG_{6000} *Diluents



B- Effect of Lubricants *Diluents



C- Effect of Ratios of PEG_{6000} *Lubricants

Fig. 5. Two way interaction plot for the combined effect of different factors

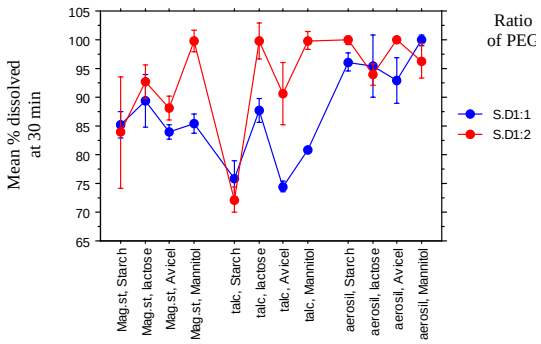


Fig. 6. Plot of the three way interactions between the three factors.

Study of the flow properties of the powder fill of the capsules:

According to table 4, formulae 18 and 21 gave the lowest Carr's indices, Hausner's ratios and angle of repose indicating better flow properties. This reflects the superiority of aerosil in improving the flow properties of capsule fill over talc and Mgst. So these two formulae can be considered suitable for industrial use and were further tested for their anti-ulcer activity.

In vivo evaluation of the antiulcer activity:

The stomach of the rats in group I revealed no inflammation, no hemorrhage or any patches of hyperemia and it was noticed that the animals were healthy during the experimental work. However, rats in group II, injected with indomethacin and receiving no treatment seemed ill, drowsy and kept stagnant without movement in their cages till the end of the experiment. The mucosa of their stomach revealed marked red elongated patches of ulcers and lesions. Animals of other groups were healthy and nearly of normal activity during the experiment.

Table 5 shows that the ulcer index in the rats of group II was significantly the highest, reaching a value of 50.83 ($P < 0.05$). Moreover, the ulcer index calculated for the stomach rats treated with plain drug equaled 23.5, a value significantly lower than that of group II ($P<0.05$). It is to be noted that the preventive index calculated for this group was lower than for other rat groups receiving famotidine treatment. The ulcer indices, noticed with the groups of rats treated with the commercial tablet and with formula 21 did not differ significantly from each other with respective values 12.66 and 11.50 and their preventive indices were 75.09 and 77.37 respectively. However rats treated with formula 18 had an ulcer index of 6.5 which was significantly lower than all other ulcer indices ($P<0.05$) and accordingly the preventive index noticed with this formula was the highest with a value of 87.21.

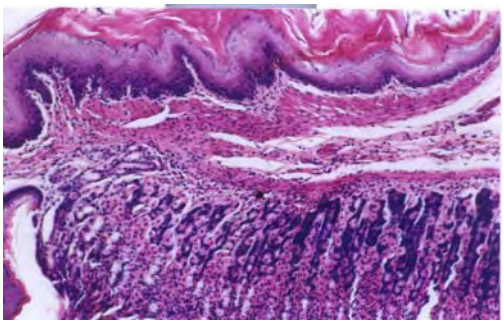


Fig.7. Histopathology of stomach rat group I (control A).

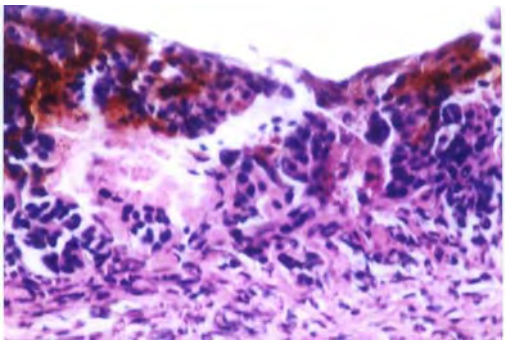


Fig. 8. Histopathology of Stomach of Rats of Control B.

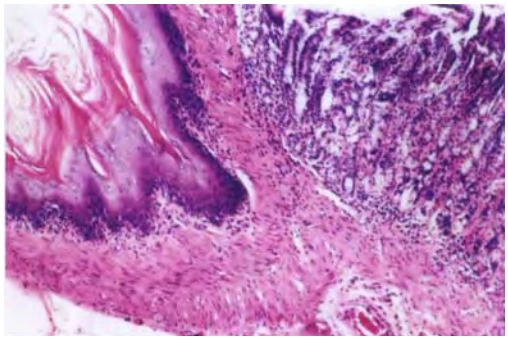


Fig. 9. Histopathology of Stomach of Rats Receiving Formula 21.

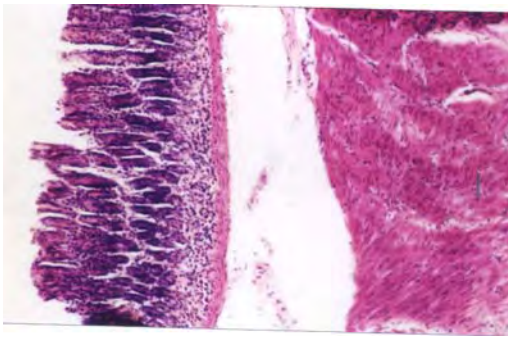


Fig. 10. Histopathology of Stomach of Rats Receiving Formula 18.

Since the results of the ulcer indices were subjective i.e. depending on visual observation, histopathological examination was done. Figure 7 shows intact histological structure of the mucosal layer lined by columnar epithelium with underlined lamina propria consisting of loose fibrous connective tissue of the stomach of the rats of group I. On the other hand figure 8 revealed a severe inflammatory reaction, manifested by oedema with local mononuclear leucocytic infiltration in the lamina propria, muscularis mucosa and submucosal layers of the tissue of the stomach of the group control B. A detachment with pigmentation (due to rupture of red blood corpuscles) in the superficial epithelial mucosa, accompanied by necrosis of the underlying lamina propria and inflammatory cells infiltration was also seen. This is a typical acute phase of ulcer and can be considered as a severe inflammatory reaction.

The tissue of the stomach of the group III receiving plain drug, showed moderate inflammatory reaction, where oedema was noticed with massive mononuclear leucocytic inflammatory cells infiltration as well as extravasated red blood cells in the connective tissue of the lamina propria and submucosal layer. The severity of reaction was mild in case of animals receiving commercial formula, and formula 21 where figure 9 showed massive number of mononuclear leucocytic inflammatory cells infiltration in the submucosal layer and muscularis mucosa.

Finally the reaction was within the normal limit in case of animals group VI receiving formula 18 as seen in figure 10 i.e. only few mononuclear leucocytic inflammatory cells in the submucosal layer and hyperemic red blood cells with minor changes if compared to the tissue of the stomach of rats of group control A (the healthy group of animals with no ulcer induction).

Conclusion:

Factorial design has been used as a rapid method for choosing the best excipients needed for preparing famotidine capsules, containing the drug as a SD with PEG_{6000}. It was possible to use both the lower and higher drug to polymer ratios, combined with the suitable excipients, to formulate drug capsules with drug dissolution of 99%, in addition to good flow properties noticed with their powder fill and a comparative antiulcer activity as the commercial product.

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