The following study involves formulation and evaluation of simple highly drug loaded matrix tablets of theophylline anhydrous (THF) containing ethylcellulose (EC) polymer as a release retardant at low concentrations (1-9% w/w) using wet granulation technique. An optimum formula was chosen on the basis of tablet physical properties and in vitro drug release. A kinetic study on theophylline release from selected matrix formula was established including zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas kinetic models. The drug release was found to be non-Fickian (n=0.54) due to both tablet diffusion and matrix erosion. The effects of certain formulation variables on drug release rate such as formulation technique, tablet geometrical shape and granule size were studied. The results have shown significantly different dissolution rates in case of applying one of the first two variables only. Finally, a comparative in vitro in vivo study was done between the selected formula and two theophylline sustained release (SR) dosage forms commercially available in the Egyptian market. The formulated tablets have shown the slowest release rate (86.68% after 8h) compared to the other two products of Quibron®/T/SR tablets and Theo SR® 300 mg capsules which have released 100% of their drug content after that time. According to the in vivo absorption profile, a significant difference in the means of Cmax, Tmax,1/2, and MRT was detected between the innovator and the two reference preparations. Such data provides strong evidence that the formulated THF tablets have better therapeutic sustaining effects than the two market products.

Keywords: Theophylline, Sustained release, Matrix tablets, Ethylcellulose.

INTRODUCTION

Theophylline (1,3-dimethylxanthine) has always remained the cornerstone for asthma management. Although the introduction of new potent anti-inflammatory inhaled steroids has decreased its use, theophylline is still an important drug in the treatment of this disease, especially for patients with moderate to severe symptoms.

In addition, Theophylline is the most widely prescribed drug in the treatment of asthma in the underdeveloped world because of its low cost and relative absence of other effective remedies. Its therapeutic concentration range is narrow (from 10 to 20 μg/mL) while toxicity usually appear at concentration above 20 μg/mL and the fluctuations of its serum concentrations can result in variability in clinical response. Therefore, there is an obvious need for SR dosage form which will be able to maintain therapeutic serum levels of theophylline throughout 24h using once or twice administered dose. As a result, many clinical advantages are offered including reduced dosing frequency with improved patient compliance and reduced fluctuations in drug plasma concentrations with lower incidence of side effects. In addition, according to the International Asthma Report, the use of long-acting bronchodilators is recommended for basic symptomatic treatment. These long acting preparations are also able to control night time symptoms because of its prolonged action.

Fabrication of a controlled release device is a process of turning a bioactive agent into a finished product that has been widely used in preparing microcapsules and microspheres and as a matrix forming material. Hydrophilic polymers are suitable matrix agents for developing SR dosage forms especially for drugs with high water solubility. EC is a non-toxic, stable, compressible and inert hydrophobic polymer that has been widely used in preparing microcapsules and microspheres and as a matrix forming material. Therefore, the current study was performed to prepare sustained release THF matrix tablets with low amounts of EC as a release-modifying agent and, to compare the in vitro and in vivo performance of the selected formula with commercially available SR tablets and capsules.

MATERIALS AND METHODS

Materials

Theophylline anhydrous (THF) (Gift from MUP Pharmaceutical Company, Abu Sultan, Egypt), Ethylcellulose (EC) (45cp) (Carl Roth GmbH, Chemical Company, Germany), Lactose (Aldrich Chemical Company, St. Louis, USA), Magnesium Stearate (MgSt) (NF, Merck, Dramstadt, Germany), Methanol HPLC grade (NICE-chemicals, India), Acetonitrile HPLC grade (SDS, France). All other chemicals were of analytical grades.

Methods

Formulation of theophylline tablets

All formulated tablets were of average weight of 340 mg. Each formula contained 300 mg of theophylline anhydrous (THF) and 2% w/w MgSt as a lubricant. Table 1 shows the composition of the prepared formulae. THF and lactose were individually sieved (300 μm), geometrically mixed with a pestle and mortar for 15 min to obtain a well-mixed composition then granulated using EC alcoholic solution (95%) solution. The formed granules were dried, sieved and sized between 500-250 μm. MgSt was then sieved, added to the dried granules and mixed. Each formula was compressed using press tablets machine (Chamunda Pharma Machinery Pvt. Ltd, Ahmedabad, India), oblong punches, 18 L, 8 W mm die, at constant compression force (3.5 tons).
The effects of the following variations in preparation of tablet formulae on dissolution rates were examined:

a) **Formulation technique:** For F-3D tablets, the components were individually sieved, mixed as before and then directly compressed.

b) **Geometrical shape:** F-3S1 and F-3S2 tablets were prepared similarly to F-3 tablets except that they were compressed using circular flat punches (12 mm diameter die) and circular concave punch (10 mm diameter die), respectively. To separate the effect of tablet shape from hardness, F-3S1 and F-3S2 tablets were compressed to the constant hardness of F-3 by changing the compression force.

c) **Granule size:** F-3G1 and F-3G2 tablets were prepared similarly to F-3 tablets except that they were prepared from granules sizes ranging from 710 to 500 µm and less than 250 µm, respectively.

**Physical tests**

The formulated tablets were evaluated according to their physical properties. All the tests were done according to the USP XXX standards.

**Thickness**

The thickness of ten tablets was measured using multi-purpose thickness tester (Shanghai, China).

**Hardness**

The Hardness of ten tablets was determined using digital tablets hardness tester (Campbell Electronics, Maharashtra, India).

**Tablet weight variation**

Ten tablets were randomly selected and accurately weighed using an electronic balance. The results are expressed as mean values of 20 determinations.

**Friability**

A sample of twenty tablets was placed in the drum of the friabilator (S.B.S. Instruments, Barcelona, Spain). The drum was adjusted to rotate 100 times in 4 minutes. The tablets were then collected, dedusted and reweighed. The percentage of weight loss was calculated.

**In vitro Dissolution studies**

Dissolution tests were performed in triplicate using six cups dissolution tester (S.B.S. Instruments, Barcelona, Spain) dissolution apparatus I (basket at 100 rpm) in 900 ml of simulated intestinal fluid (SIF). The amount of drug released was determined using a Shimadzu (UV/160A) spectrophotometer at 272 nm. Theophylline release profiles from different formulations were interpreted according to the criteria of USP XXX test number 8 for sustained release theophylline capsules.

**Kinetics of theophylline release**

In order to study the kinetics of drug release from the formulated matrices, the dissolution data were kinetically analyzed according to zero order, first order, Higuchi diffusion model, Hixson-Crowell model as well as Korsmeyer-peppas model.
In vivo studies

Protocol

The experimental protocol in human volunteers was performed according to the guidelines issued by ethical committee of Faculty of Pharmacy, Suez Canal University, Egypt. Six healthy volunteers, 2 male and 4 females ranging 25-40 (mean± standard error (S.E): 30.5±4.4) years old and weighing 65-100 kg (85.5 ± 3.3) participated in this study. The subjects were non smokers and had not taken any drugs during the testing period. None of them had a history of any serious or chronic disease. They were allowed no xanthine-containing beverages for 3 days before each administered dose and for the duration of the sampling schedule. No food was permitted the night before and for 3 h after dosing. Each volunteer received a 300 mg single dose of selected F-3 formula, Quibron®-T/SR tablets and Theo SR® 300 mg capsules in a cross over manner. The respective dose of the drug was given with 150 mL of water and the participants were instructed to rinse the mouth during drinking.

Sampling of saliva

Salivary samples were collected at times of 0, 1, 2, 4, 6, 8, 10, 12 h and placed in dry stoppered glass tubes. A small amount of citric acid which is a salivary flow stimulant was put on tongue and held for one minute before sampling. A 3 mL sample was collected each time and frozen at -20° C until assay. Before the assay, the 3 mL saliva were first centrifuged then 1 mL of the supernatant was withdrawn and mixed with the mobile phase till a final volume of 10 mL. The mixture was vortexed using magnetic stirrer (Velp Scientifica, Italy) then 25 µL were injected directly into the column.

HPLC assay method

HPLC assay was performed using Knaur HPLC, Germany, equipped with Smartline UV detector 2500 version 7604 and Smartline pump 100 version 5010. The separation was achieved using C 18 reversed-phase analytical column 250 x 4.6 mm Discovery® (5 µm particle size) (Sigma-Aldrich Group, USA) at 25°C. The mobile phase was prepared by mixing 0.01 M ammonium acetate (pH was adjusted to 4 using acetic acid), and acetonitrile HPLC in ratio of (91:9 v/v). The U.V. detector was set at λ 272 nm. The flow rate: 1 mL/min. The separation time was 7 minutes.

Pharmacokinetic parameters

The pharmacokinetic parameters representing the maximum concentration (C max), the time corresponding to maximum concentration (T max), half life (t1/2), area under the curve from 0 to 12h (AUC0-12h), area under the curve from zero to infinity (AUC0-∞) and mean residence time (MRT) were calculated. A comparative study between the prepared formulation and the commercial products was established.

Statistical analysis

All the data represents the mean ± S.E. The differences were considered to be significant at a level of p < 0.05, using paired T test.

RESULTS AND DISCUSSION

Physical characteristics

The physical characteristics of the formulated tablets: thickness, weight variation, hardness and friability expressed as mean ± S.E are shown in Table 2. The produced tablets had nearly a uniform thickness of 2 mm. The values of weight variations for all formulae were within the acceptable range. Tablets hardness has obviously increased from 6.2±0.8 Kg for F-1 to 10.1±0.5 Kg for F-5 while all tablets have passed the friability test. This could be attributed to the increase in EC concentration from 1% w/w in F-1 formula to 9% w/w in F-5 tablets. This agrees with Friedman et al., 1988, who stated that in tablet formulations, EC either dry or wet-granulated with a solvent such as ethanol(95%) produces hard tablets with low friability.

Figure 1 illustrates the release data of theophylline from F-1 to F-5 formulae in SIF pH 7.5 using basket at 100 rpm. There was an obvious retardation in drug release rate as the concentration of EC increased from F-1 to F-5. In case of F-1 tablets, 90.1±1.1% of theophylline was released after 8 h compared to 75.0±9.6% in case of F-5 ones. Such retardation in dissolution was expected due to the hydrophobic nature of EC which prevents the penetration of the dissolution medium within the matrix. In addition, increasing EC concentration has increased tablet hardness leading to a slower release rate. During the dissolution testing, no tablet swelling was observed; however, tablets have eroded gradually while maintaining their geometrical shape. This coincides with the EC physical properties as a non swellable polymer which has a natural tendency to erode in water.

Table 2: Physical characteristics of theophylline anhydrous formulated tablets.

<table>
<thead>
<tr>
<th>Formulation no.</th>
<th>Thickness (nm)</th>
<th>Weight (mg)</th>
<th>Drug content (%)</th>
<th>Hardness (Kg)</th>
<th>Friability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-1</td>
<td>2.0±0.5</td>
<td>339.2±0.6</td>
<td>99.7±0.7</td>
<td>6.2±0.8</td>
<td>0.59±0.2</td>
</tr>
<tr>
<td>F-2</td>
<td>2.0±0.1</td>
<td>341.5±2.1</td>
<td>101.1±0.8</td>
<td>7.5±1.3</td>
<td>0.35±0.1</td>
</tr>
<tr>
<td>F-3</td>
<td>2.0±0.1</td>
<td>337.7±0.9</td>
<td>99.1±2.0</td>
<td>8.2±1.0</td>
<td>0.27±0.8</td>
</tr>
<tr>
<td>F-4</td>
<td>2.1±0.2</td>
<td>340.1±0.3</td>
<td>98.0±1.1</td>
<td>9.2±0.8</td>
<td>0.14±1.2</td>
</tr>
<tr>
<td>F-5</td>
<td>2.0±0.7</td>
<td>337.9±1.4</td>
<td>99.5±0.5</td>
<td>10.1±0.5</td>
<td>0.13±0.5</td>
</tr>
</tbody>
</table>

All tests were done according to USPXXX. Each data represents the mean ± S.E.
Selection of formulation

The release profiles of theophylline from different formulated tablets were evaluated according to USP Test 8 for theophylline extended release capsules. According to USP XXX dissolution test requirements, only F-1, F-2 and F-3 formulations have fulfilled the requirements. The rest of formulations were out of the range of tolerance established in such test where F-4, F-5 formulations have shown much slower drug release.

Kinetics of theophylline release from selected formulae

In order to understand the release mechanisms of theophylline from EC matrices, mathematical models were applied. The release of drugs from tablets can be analyzed by release kinetics theories as follows:

Zero order kinetics: $F_t = K_0 t$  

First order kinetics: $\ln(1 - F) = -K_1 t$  

Higuchi model: $F = K_2 t^{1/2}$  

Hixson-Crowell model: $(1 - F)^{1/3} = 1 - K_3 t$  

Korsmeyer-Peppas model: $F = K_4 t^n$  

The model that best fitted the release data was determined by the highest $r^2$. The best fit with highest regression for the three formulae was found with Higuchi's equation indicating that the release of theophylline from the formulated hydrophobic matrix tablets was mainly due to drug diffusion. However, Higuchi's equation is applicable only to ideal conditions and doesn't take into consideration some of the actual matrix complications such as matrix swelling or erosion. In addition, the previous findings didn’t agree with the experimental observations where a noticeable erosion of the tablets was detected at the end of the dissolution test. Therefore, the dissolution data were also fitted according to Korsmeyer equation. F-1, F-2 and F-3 tablets have shown good correlation with Korsmeyer– Peppas model ($r^2 > 0.99$). The exponent ($n$) determined by the model's equation suggests that oblong tablets show Fick's diffusion (Case-I transport) when $n = 0.45$, non-Fick type release (anomalous transport) when $0.45 < n < 0.89$, Case-II transport when $n = 0.89$, and super Case-II transport when $n > 0.89$. The $n$ values of the three formulations were of values $0.45 < n < 0.89$ indicating that the drug release was due to non-Fickian release which includes both diffusion and matrix erosion. However, based on the $r^2$ values of Higuchi's model which are higher than those of Hixson-Crowell model and the $n$ values which are close to 0.45, Fickian diffusion may be considered as the primary release mechanism followed by matrix erosion. In addition, it was also observed that increasing the concentration of EC from F-1 to F-3 tablets has decreased $r^2$ value of Hixson-Crowell model indicating that the release is more likely to occur by diffusion rather than tablet erosion. This may be attributed to EC binding properties causing less tablet erosion.

Effect of certain formulation variables on theophylline release from selected formula

For further studies, F-3 formula was selected since it has shown the highest tablet hardness compared to F-1 and F-2 ones. Optimization of the F-3 formula was done by demonstrating the effect of certain formulation variables such as formulation technique, granule size and tablet geometrical shape. For an effective comparison between the release profiles of tablets, the ($f_2$) similarity equation was calculated as following:

$$f_2 = 50 \times \log\left[1 + (1 + n) \sum_{j=1}^{n} \left(\frac{R_j - T_j}{X_j}ight)^2\right]^{-0.5} \times 100$$  

Where $n$ is the sampling number, $R_j$ and $T_j$ are the percentages of the dissolved drug from the reference and the test ($F$-3), respectively, at each time point $j$. If $f_2$ value is greater than 50 (50–100), therefore, dissolution curves is considered to be equivalent.
Formulation technique

Figure 2 shows the release of F-3 and F-3D formulae in SIF pH 7.5 using basket at 100 rpm. According to $f_2$ values ($f_2 < 50$), the release profiles of F-3 and F-3D tablets were not comparable where the release of directly compressed tablet was much faster than that prepared by wet granulation. This indicates that EC which is the main cause of the prolonged release of theophylline in F-3 formula was more effective in release retardation when used in wet condition rather than dry one. Similar results have been reported indicating decreased drug release rates from tablet matrices when wet granulation technique was used. This decrease can be attributed to decreased porosity and greater interparticle cohesion that causes resistance to the penetration of the dissolution medium and a more tortuous matrix for drug diffusion, resulting in a slower drug release rate.

Table 3: Mathematical modeling and drug release kinetics of theophylline F-1, F-2 and F-3 tablets in simulated intestinal fluid using basket at 100 rpm.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi</th>
<th>Hixson-Crowell</th>
<th>Korsmeyer-Peppas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K</td>
<td>$r^2$</td>
<td>K</td>
<td>$r^2$</td>
<td>K</td>
</tr>
<tr>
<td>F-1</td>
<td>0.09</td>
<td>0.951</td>
<td>0.17</td>
<td>0.982</td>
<td>0.3</td>
</tr>
<tr>
<td>F-2</td>
<td>0.08</td>
<td>0.963</td>
<td>0.16</td>
<td>0.987</td>
<td>0.3</td>
</tr>
<tr>
<td>F-3</td>
<td>0.08</td>
<td>0.974</td>
<td>0.20</td>
<td>0.986</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Tablet shape

Several attempts were made to regulate the dissolution behavior of drug matrices by controlling their geometry. Figure 3 illustrates the release of F-3, F-3S1 and F-3S2 tablets prepared in the three different geometrical shapes in SIF pH 7.5 at basket at 100 rpm. Both F-3S1 and F-3S2 have shown a significantly slower release rate than that of F-3 ($f_2 < 50$). Such results agree with the literature findings that state that the size and shape of the matrix tablets can affect the drug dissolution rate.

The release difference between those shapes could be due to difference in surface area exposed to the dissolution medium although the hardness of the three shapes was kept the same.

Fig. 2: Release profile of F-3 and F-3D formulae in simulated intestinal fluid pH 7.5 using basket at 100 rpm. Each data point represents mean ± S.E. (n=3).

Fig. 3: Release profile of F-3, F-3S1 and F-3S2 tablets in simulated intestinal fluid pH 7.5 using basket at 100 rpm. Each data point represents mean ± S.E. (n=3).
Granule size

Figure 4 shows the release of the F-3, F-3G1 and F-3G2 formulae in SIF using basket at 100 rpm. The granule size can alter the porosity of the matrix tablets and thus the release from the formulations. However, changing the granule size in the range from 710 µm to less than 250 µm didn't change much the porosity leading to similar drug release profiles ($f_2 > 50$).

In vitro comparative study between selected formula and commercially available theophylline solid dosage forms

Figure 5 shows the release data obtained for F-3 tablets, Quibron®-T/SR tablets and Theo SR® 300 mg capsules in SIF pH 7.5 basket apparatus at 100 rpm. According to $f_2$ values, there was no similarity in dissolution profiles of F-3 tablets and the two reference products since they have shown faster dissolution rates. Quibron®-T/SR tablets have demonstrated an unexpected fast release in the dissolution medium where 100%±4.9 of its drug content was released after 2 h only compared to 89.6%±4.5 in case of Theo SR® 300 mg capsules and 41.32%±5.6 F-3 matrix tablets. Since the formulated tablets showed much slower drug release than that of Quibron tablets and Theo SR capsules, it was necessary to conduct an in vivo study for F-3 formula and compare it with that of the two commercial products. It might be assumed that the slower drug release rate of F-3 formulated tablets would result in lower peak plasma concentration and longer duration time.

Fig. 4: Release profile of F-3, F-3G1 and F-3G2 formulae in simulated intestinal fluid pH 7.5 using basket at 100 rpm. Each data point represents mean ± S.E. (n=3).

Fig. 5: Release of F-3 tablets, Quibron®-T/SR tablets and Theo SR® 300 mg capsules in SIF pH 7.5 using basket at 100 rpm. Each data represent mean ± S.E. (n=3).

Pharmacokinetic comparative study between selected formula and commercially available theophylline solid dosage forms

Good correlations were found between theophylline plasma and salivary concentrations. Figure 6 shows the mean salivary drug concentration-time curves for F-3 tablets, Quibron®-T/SR tablets and Theo SR® 300 mg capsules. No lag time in absorption was observed for any of the three preparations indicating that theophylline was released and absorbed in the stomach although theophylline is slightly acidic. It has been reported that theophylline salivary concentrations are approximately 50% of those in the plasma and its therapeutic range is 5-20 µg/mL. Therefore, it is possible to consider that theophylline levels have reached minimum effective concentration after 1 h of Quibron administration compared to 2 h in case of Theo SR capsules and F-3 tablets. Therefore, Quibron tablets can be a better choice for treating acute conditions. As for F-3 tablets, from its theophylline salivary concentrations, it can be anticipated that for every 12 h dosing, theophylline plasma concentrations will probably range from 5-10 µg/mL. Patients with mild disease or with chronic cases such as chronic obstructive pulmonary disease may benefit from these lower concentrations with minimal side effects.

For an effective comparative study, the main pharmacokinetic parameters for each of the tested formula and the commercial products were calculated. The $C_{max}$ and $T_{max}$ were obtained directly from the data. The half-life of the terminal elimination phase was obtained using the relationship $t_{1/2} = 0.693/K_e$ where $K_e$ is calculated by the linear regression of the log-transformed concentrations of the drug in the terminal phase. The area under the salivary concentration versus time curve $AUC_0-t$ was calculated by the linear trapezoidal method. The $AUC_0-\infty$ was extrapolated to infinity ($AUC_0-\infty$) by adding the equation of $C_{last}/K_e$, where $C_{last}$ represents the last measured concentration. The MRT was calculated by the ratio of AUMC/ $AUC_0-\infty$ where AUMC is the area under the first moment curve.

Table 4 shows the values of different pharmacokinetic parameters for the tested formula and the two commercial products. There was a significant difference between F-3 formulated tablets and each of Quibron tablets and Theo SR capsules in both $C_{max}$ and $T_{max}$ values which both represent the absorption rate. Quibron®-T/SR tablets have shown the highest $C_{max}$ and the shortest $T_{max}$ values which coincides with its rapid
dissolution rate. F-3 tablets had a significantly higher t_{1/2} and MRT values than both references indicating more sustained drug release ability of such formula for oral controlled release systems. This was expected since the formulated F-3 tablets have differed significantly in the dissolution testing from both commercial products showing the slowest drug release rate.

**CONCLUSION**

Matrix sustained release tablets of THF were prepared successfully using low amounts of EC by conventional wet granulation technique. Increasing its concentration has increased tablet hardness, decreased tablet friability and retarded the drug dissolution rate. Changing the formulation technique from wet granulation to direct compression or altering tablet geometrical shape was found to influence theophylline release rate from formulated tablets. However, no significant difference was found in the dissolution rate for tablets prepared from granule size ranging from (710 µm to less than 250 µm). Finally, comparing the selected formula of 5%w/w EC to two commercially available theophylline products has revealed that the test formula had slower in vitro release rate and better in vivo sustaining effects that the two references.

**ACKNOWLEDGMENT**

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