Formulation development and *in vitro* evaluation of theophylline microcapsules

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Abstract: The study was aimed to investigate microencapsulation of theophylline using different ratios of eudragit S 100 as wall material by the emulsion solvent evaporation technique.
The release profiles, effect of stirring speed and different pH of dissolution medium on release profiles and stability were also studied. Various formulations of microcapsules were compressed in to tablets. In vitro dissolution studies indicated that the rate of drug release was reduced with an increase in the amount of Eudragit S 100. Moreover, the release data of various formulations were fitted to Zero order, First order, Higuchi, Hixson Crowell and Korsmeyer Peppas kinetic models.
It was observed that the release of drug from all the formulations followed Higuchi's kinetic model as its value of coefficient of determination is greater than that of others. The release profiles of the test formulation in distilled water and various pH media were compared and decreased release rate was seen at lower pH i.e. pH 1.0. Slight or no change was apparent in the release rate at higher stirring speed compared to lower stirring speed. No significant changes were also observed in the drug release profiles of test tablets stored at different temperatures.
Test tablet (F2) was found to produce similar and sustainable release rate compared to commercial product, Quibron-T/SR tablet on the basis of their $T_{70\%}$. Similarly, f_2 values of reference versus F2 is 66.98 which show less difference between these two as compared to the comparison of reference with F1 and F3.

Keywords: Theophylline, *in vitro* study, eudragit, microencapsulation.

INTRODUCTION

Present era has put forward many new drug delivery systems including enteric formulations to release drug at required site and rate by different techniques e.g. microencapsulation. Enteric microparticles constitute a dosage form that is capable to release drug slowly to get a safe therapeutic window in blood and reduce the chances of gastrointestinal irritation. The objective of such formulation is to deliver the drug in a sustained fashion after its administration to maintain therapeutic level (Widder *et al.*, 1979).

The therapeutic window of theophylline, an anti-asthmatic alkaloid, ranges from 5 to 20 mg/ml. Its biological half life is 4-6 hours. This drug is therefore, a suitable candidate for its formulation into controlled release formulation (Stithit *et al.*, 1998). In present study, theophylline is microencapsulated into eudragit S 100, an acrylic microencapsulating polymer, to develop enteric microparticles by solvent evaporation system. The microparticles were analyzed mathematically and statistically. Scanning electron microscope was used to determine microparticle shape.

EXPERIMENTAL

Materials
Micronized theophylline anhydrous was gifted by

Shanghai Wandai Pharmaceutical, China. Methacrylic acid copolymer Eudragit S 100 was purchased from Pharmacia LKB GmbH, Germany. Magnesium stearate, talc, acetone, n-hexane, dipotassium hydrogen phosphate and phosphoric acid were purchased from Merck, Germany. Mineral oil (Acros Organics, USA) and span 85 (Sigma Aldrich, Germany) were also purchased through commercial sources.

Instruments
Hot plate magnetic stirrer (Velp Scientifca, Germany), pH meter (Inolab, Germany), digital weighing balance (Precisa, Switzerland), automatic dissolution apparatus USP (Pharma Test, Germany), double beam spectrophotometer (Shimadzu, Japan), sieves with different mesh numbers (Mughal Traders, Pakistan), single punch tablet machine (Emmay, Pakistan), Digital hardness tester (Curio, Pakistan), friabulator (Emmay, Pakistan), sonicator (Elma, Germany)

Preparation of microcapsules
Theophylline-eudragit S100 enteric microparticles were prepared by solvent evaporation technique. A weighed quantity of polymer was added to acetone to make its homogenous solution. A weighed quantity of theophylline was dispersed to this solution by continuous mixing to make dispersion. This dispersion was slowly added at 15°C into liquid paraffin (50 ml) having 1% (w/w) of Span-85 with continuous stirring at 1000 rpm to form a

Pak. J. Pharm. Sci., Vol.25, No.1, January 2012, pp.15-19

15

homogenous emulsion using magnetic stirrer. The emulsion was stirred continuously to achieve room temperature, evaporate acetone and obtain fine microparticles by decantation. The microparticles were washed with n-hexane and water, dried at room temperature and oven for three hours and passed through sieve no. 60. The sieved microparticles were stored in air tight glass bottles. Three batches were prepared with different proportions of core to coat materials (drug: polymer, 1:1, 1:1.5 and 1:2 (w/w) named as M1, M2 and M3 microparticles, respectively and F1, F2 and F3 tablets, respectively).

Particle size and external morphology determination
The mean size and shape of microparticles was studied by optical microscopy and scanning electron microscope, respectively.

Drug loading
Twenty five milligram ground microparticles were added to 100 ml phosphate buffer pH 6.8 and sonicated for three hours. Then the solution was filtered, appropriate dilutions were made from the filtrate and analyzed using UV absorption spectroscopy at 272 nm.

Preparation of theophylline tablets
The microparticles were blended with magnesium stearate (1 %) and talc (0.5 %) and compressed into tablets to study the variation in their dissolution behavior.

Physical tests of Tablets
A digital weighing balance was used to determine weight uniformity and weight variation (%) of tablets. Digital hardness and thickness tester was used to determine tablet hardness and thickness. The friabulator was employed to determine tablet friability. Each test was preformed thrice.

Dissolution studies
Dissolution studies of various microparticles were performed using USP apparatus II, 900 ml distilled water as the dissolution medium with the temperature maintained at 37.0 ± 0.5 ℃ stirred at 50 rpm. Samples of about 5 ml each were filtered through 10 μm sinter filters, collected at predetermined time points with an automated sample collector and analyzed using UV absorption spectroscopy at 272 nm. Dissolution test was performed thrice.

Influence of pH, stirring speed and storage condition on drug release rate of tablets
Tablet formulation (F2) was tested at different pH values i.e. pH 1.0 (0.1 M HCl), distilled water and pH 6.8 (phosphate buffer). Test tablet was also tested at various stirring speeds i.e. 50, 100 and 150 rpm. Moreover, test tablets were packed in an airtight glass bottles, stored at 25 °C and 40 °C and tested by dissolution after 1, 2 and 3 months to check their stability. Dissolution test was performed thrice.

Analysis of drug release data
To study the release kinetics, data obtained from in vitro drug release studies were plotted in various kinetic models: zero order (Eq. 1) as cumulative amount of drug released vs. time, first order (Eq. 2) as log cumulative percentage of drug remaining vs. time, and Higuchi's model (Eq. 3) as cumulative percentage of drug released vs. square root of time.

$$F = K_0 t \qquad \text{Eq. 1}$$
$$\text{Log } F = \text{Log } F_0 - k_1 t / 2.303 \qquad \text{Eq. 2}$$
$$F = K_H t^{1/2} \qquad \text{Eq. 3}$$

Where K_0 and K_1 are the zero- and first-order constants, respectively. While K_H is the constant reflecting the design variables of the system and t is the time in hours.

Hixson-Crowell cube root law was employed to evaluate drug release with changes in surface area and diameter of the particles/tablets:

$$F_0^{1/3} - F_t^{1/3} = K_{HC} \times t \qquad \text{Eq. 4}$$

Where F_t is the quantity of drug released in time t, F_0 is initial quantity of drug in the tablet, and K_{HC} is rate constant for the Hixson-Crowell rate equation, as the cube root of percentage of drug remaining in matrix vs. time.

$$F = K t^n \qquad \text{Eq. 5}$$

Where K is a constant of Korsmeyer rate equation, n is release exponent that indicates drug release mechanism and F represents the fraction of drug dissolved at time t. Coefficient of determination (r^2).

Moreover, the similarity factor (f_2) (Moore *et al.*, 1996) was used to compare dissolution profiles.

$$f_2 = 50 \text{Log} \left\{ \left[1 + 1/n \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \qquad \text{Eq. 6}$$

Where n is dissolution data points and R_t & T_t represent dissolution profiles at same time point t for the reference and test dissolution profiles, respectively. The f_2 value between 50 and 100 elaborates that the two dissolution profiles are identical.

RESULTS

Theophylline-eudragit S 100 tabletted microparticles (100 mg) were prepared by compressing microparticles designed by solvent evaporation method and characterized by dissolution. Eudragit S 100 was choosed on the basis of its enteric coating and wall forming properties. It also provides the desired characteristics of microencapsulation. The microparticle size ranged between 165-180 μm. The microcapsules were white and free flowing with rough surface (fig. 1).
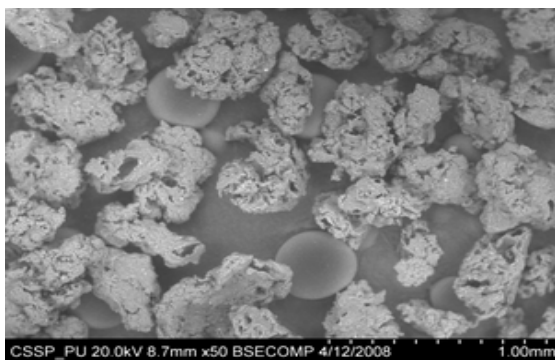
Fig. 1: scanning electron microscopic view of M1

In vitro drug release profiles

Figs. 2 and 3 shows the effect of increasing amount of eudragit S 100 on in vitro release of theophylline from various formulations. At lower concentration of Eudragit S 100 (M1), almost 80% of drug was released within 180 minutes while about 63% and 33% of drug was released in formulations containing higher amounts of eudragit S 100 (M2 and M3, respectively) during the same interval.
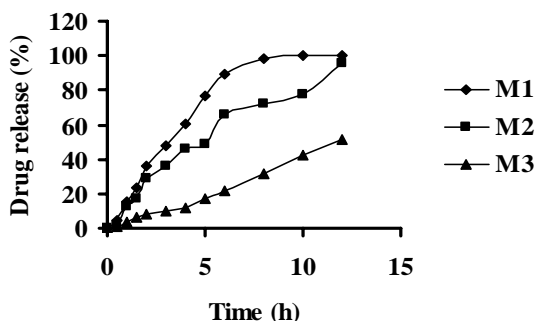

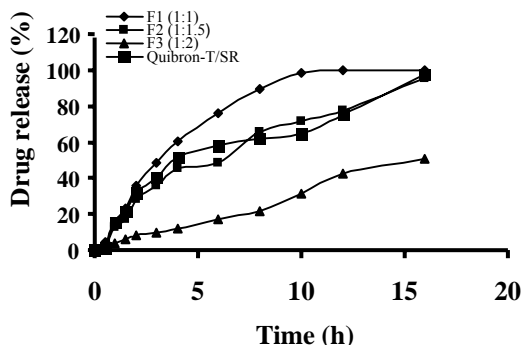**Fig. 2**: *In vitro* release of theophylline from its microcapsules (M1, M2 and M3)

Fig. 3: *In vitro* release of theophylline from its test (F1, F2 and F3) and reference tablets (Quibron-T/SR)

Evaluation of release kinetics

It was clearly seen that the data could be comparatively better fitted in Higuchi model as the R^2 values of Higuchi model was found to be greater as compared to that obtained from zero order, first order, Hixson Crowell and Korsmeyer Peppas equation.

Table 1: Values of release rate constant '*k*', correlation coefficient "*R^2*" and $T_{70\%}$ obtained from data of Theophylline tablets (F1, F2 and F3) containing various drug to polymer ratios and at different pH and stirring speed

Formulations with their dissolution conditions	K	R^2	$T_{70\%}$
F1 in distilled water at 50 rpm	10.504	0.9771	7.9968
F2 in distilled water at 50 rpm	8.6057	0.9875	9.7857
F3 in distilled water at 50 rpm	7.3022	0.7566	13.1873
Quibron-T/SR in pH 1.0 at 50 rpm	8.3274	0.9777	9.7340
F2 in pH 1.0 at 50 rpm	5.9116	0.7481	14.8222
F2 in pH 6.8 at 50 rpm	8.0773	0.9513	10.6832
F2 in distilled water at 100 rpm	8.5486	0.9527	10.2997
F2 in distilled water at 150 rpm	9.0974	0.9656	10.0256

Influence of pH on drug release of test tablets

F2 was selected as test formulation and further evaluated. Fig. 4 shows the release profiles of theophylline from the test tablets at different pH values i.e., pH 1.0 and 6.8. Difference was seen in the release characteristics of test tablets in both pH.
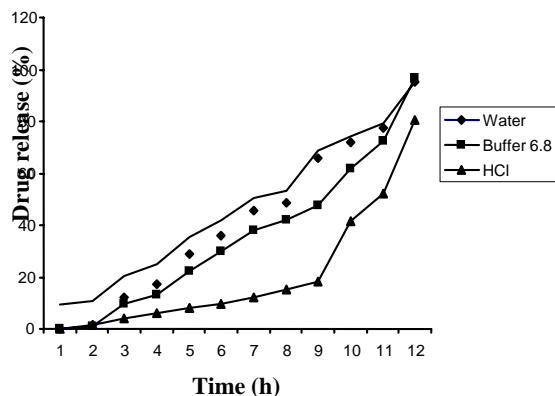

Fig. 4: *In vitro* drug release from theophylline test tablets (F2) at different pH media.

Table 2: f_2 - values determined from drug release data of test tablets (F2) at different pH and stirring speeds (rpm).

Comparison	f_2 – value
Distilled water Vs pH 1.0	29.05
Distilled water Vs pH 6.8	65.24
100 rpm Vs 150 rpm	61.24
100 rpm Vs 50 rpm	57.42
50 rpm Vs 150 rpm	57.52

Influence of stirring speed on drug release of tablets

Fig. 5 shows the release profile of the test formulation at three different stirring speeds. As the stirring speed of test tablets was increased, the disintegration of test tablets was

Pak. J. Pharm. Sci., Vol.25, No.1, January 2012, pp.15-19

17

unaffected indicating similar erosion of the micro-encapsulation system. Therefore, no significant change in the release rates of test tablets at three different stirring speeds was noticed. Table 2 shows the f_2 values obtained from in vitro release data of test microencapsulated tablets at 100 rpm vs. 150 rpm and 100 rpm vs. 50 rpm. The values obtained from f_2 test for 100 rpm vs. 150 rpm were greater than 50 and therefore dissolution data were similar due to erosion and disintegration of matrix at higher speed. The f_2 value at 100 rpm vs. 50 rpm was less than 50 and the dissolution data could not be matched due to gradual swelling and erosion of matrix.

$T_{70\%}$ was also calculated and it is seen from table 1 that there is no significant difference present between all three stirring speed values. Same results are found in analysis of Hayashi *et al.* (2005).
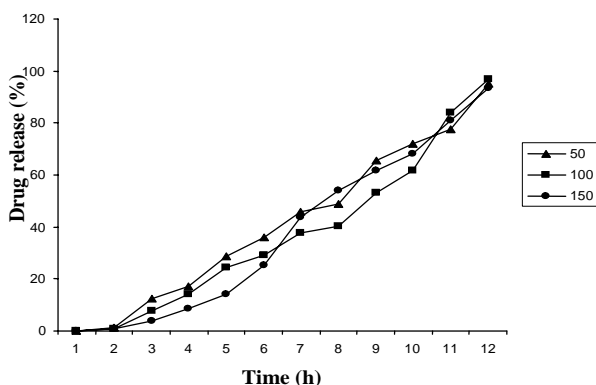
Fig. 5: *In vitro* drug release from theophylline test tablets (F2) at various stirring speed.

Stability studies

Although eudragit S 100 has no aging effect during storage at different temperatures, however changes in release rate of test tablets stored at room temperature, 25°C and 40°C versus time were also studied. A slight difference in the release profiles of test tablets after storage of 3 months at 40°C compared to the initial release profile are noticeable. Drug release rate was slightly reduced at 40°C from the initial value (fig. 6). No considerable change in the release profile was noted during 3 months.

DISCUSSION

Due to the exclusive solubility characteristics of polyacrylic resins, these polymers have been widely employed in the fabrication of controlled release formulations (Reddy *et al.* 2003). Eudragit S 100, an anionic copolymer of methacrylic acid and methacrylate, possess free carboxyl groups which endow it pH sensitive property. Eudragit S 100 is insoluble below pH 7 (Fassihi and Ritschel, 1993). This polymer has been used to encapsulate theophylline by solvent evaporation method.

Tabletted microcapsules of eudragit S 100 are expected to retard drug release at the gastric fluid pH and if possible control it at intestinal fluid pH. Figs. 2 and 3 exhibits that the time of dissolution is related to the amount of polymer used in microcapsules and dissolution time was increased with increasing amount of Eudragit S 100. By increasing the ratio of Eudragit S 100 in microcapsules may reduce the diffusion of water molecules inside the polymer, thus reducing the extent of swelling of microcapsules, resulting in slower release of drug. Hence, the presence of high percentage of Eudragit S 100 in microcapsules resulted in a more hydrophobic character. At very high ratio of polymer, the capsules become impermeable to the dissolution medium and give very slow release of drug as observed in the release profile of formulation M3 (1:3). The above facts indicate that reduction in drug release from the microcapsules may be due to a reduction in the diffusion of dissolution medium inside the microcapsules because of hydrophobicity of Eudragit S 100 in water.
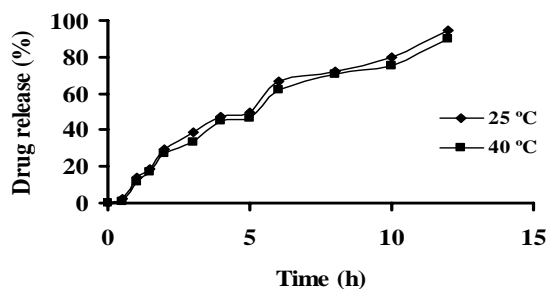
Fig. 6: *In vitro* drug release from theophylline test tablets (F2) at various temperatures for three months for their stability testing.

Similar release pattern was observed from the compressed tablets. As the ratio of eudragit S 100 was increased, a corresponding decrease in the release of theophylline was perceptible because the microcapsules are fine particles and the drug can easily be diffused out. On the other hand, tablets are large compact masses of particles, therefore, sufficient time is required for the dissolution medium to penetrate into the tablets and dissolve the drug. Moreover, the hardness in all tablet formulation was kept constant (above 5.0 Kg) in order to prevent disintegration of tablets during the drug release study.

In vitro release profile of test tablets was compared with commercially available product, Quibron-T/SR tablet (Bristol Meyers Squibb) containing 100 mg of Theophylline. All the formulations used for in vitro evaluation had same drug contents. Fig. 3 shows the dissolution profile of the test formulations and Quibron-T/SR tablet. Graphical data shows that F1 is dissolved almost completely after 8th hour, while F3 was dissolved almost 51% even after 12 hours. The release pattern of formulation F2 is likewise seemed to be similar to reference product. Time for its 70% release is also similar

to the reference product release than the other two formulations that is F1 and F3. The f_2 values for comparison of dissolution data of test and reference tablet was found to be greater than 50 indicating that drug release profiles of the two formulations are comparable which is also described by Costa (2001). The initial part of release data of various tablet formulations containing increasing amount of eudragit S 100 were also evaluated using different kinetic models. The drug release constant (k) and regression coefficient (R^2) obtained from zero order; Higuchi and first order, Hixson Crowell and Korsmeyer-Peppas model are shown in table 1. It was clearly seen that the data could be comparatively better fitted in Higuchi model as the R^2 values of Higuchi model was found to be greater as compared to that obtained from zero order, first order, Hixson Crowell and Korsmeyer Peppas equation. The most probable mechanism of Theophylline release from the Eudragit S 100 microencapsulated system seemed to be by diffusion of drug from the insoluble porous matrices into the dissolution medium due to swelling of this system. It indicates that the release of drug from Eudragit matrix is directly proportional to the square root of time i.e., Higuchi Model which is also described by Obeidat and Price, (2006). Throughout drug release study, very marginal erosion of the matrix was observed. Korsmeyer plots indicated an *n* value of 0.74, which was indicative of an anomalous diffusion mechanism or diffusion coupled with erosion; hence, the drug release was controlled by more than one process. Reddy *et al*. (2003) observed similar results with a matrix tablet of nicorandil with an *n* value of 0.71 and Fassihi and Ritschel (1993) with a matrix tablet of theophylline with an *n* value of 0.7. These two groups of researchers also considered the corresponding *n* values to indicate an anomalous release mechanism. Hixson-Crowell plots indicated a change in surface area and diameter of the tablets with progressive dissolution of formulated tablets as a function of time.

The reason is that Eudragit S 100 did not dissolve completely in acidic media and usually used for enteric purposes. While at pH 6.8, its release profile is seemed to be comparable with that of distilled water. The f_2 values obtained from in vitro release data of water vs. pH 1.0 and water vs. pH 6.8 is presented in table 2. $T_{70\%}$ was also determined and it is seen that at pH 1.0, its value is 14.82 while at pH 6.8, it is 10.68 (table 1). This indicates that at pH 1.0, release profile of theophylline tablets is less than at pH 6.8 which is also described by Mastiholimath *et al*. (2007).

CONCLUSION

By increasing the ratio of Eudragit S 100 in the microcapsules reduced the rate of diffusion of water, thus reducing the extent of swelling of microcapsules, resulting in slower release of drug. The most probable mechanism of Theophylline release from the Eudragit

S100 microencapsulated system seemed to be by diffusion coupled with erosion from the insoluble porous matrices into the dissolution medium due to swelling of this system. It indicates that the release of drug from eudragit matrix is directly proportional to the square root of time. A slower release of drug from microparticles was seen in 0.1 N HCl compared to water and phosphate buffer pH 6.8 because of pH dependent solubility of eudragit S100. No significant difference in dissolution phenomenon was observed at various stirring speeds (50, 100 and 150 rpm). The microparticles exhibited good stability for three months with respect to the release profile.

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