PREPARATION AND *IN-VITRO* EVALUATION OF SUSTAINED RELEASE THEOPHYLLINE MICRO-SPHERES

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تم تحضير كرات دقيقة طويلة المفعول تحتوى على دواء الثيوفيللين بواسطة طريقة تبخير مذيب المستحلب الناتج وقد تم إستخدام نوعين من البوليمرات هما سيليلوز البروبيونات وإثيل السيليلوز بنسب   :   :
:   من الثيوفيللين للبوليمر المستخدم. وقد تم تقييم الكرات الناتجة من ناحية الإستعادة الكاملة محتوى الدواء   نسب وحجم الكرات الدقيقة   الفحص الظاهرى والميكروسكوبي وكذلك معدل انطلاق الدواء من الكرات الدقيقة المحضرة فى وسطي معامل اﻷس الهيدروجيني   ,   ,   بالمقارنة لمستحضر موجود في السوق المصري هو (Theo SR 100) الذي يحتوي   مجم من دواء الثيوفيللين وقد وجد من الدراسة أن سيليلوز البروبيونات بنسب   :   :   : قد أعطي   ,   % من   الإستعادة الكاملة للنسب المذكورة علي التوالي. كما أعطي
   % من محتوى الدواء علي التوالي. وعند إستخدام إثيل السيليلوز كبوليمر بنسب   :   :   : كانت نسب الإستعادة الكاملة   %
للنسب المذكورة علي التوالي كما أظهرت نسب محتوى الدواء
   % علي التوالي   مما يدل علي كفاءة الطريقة المستخدمة. وقد أظهرت دراسة معدل انطلاق الدواء من الكرات الدقيقة المحضرة تأثر بنوع البوليمر المستخدم حجم الكرات الدقيقة اﻷس الهيدروجيني   ضافة إلي نسبة الدواء للبوليمر المستخدم. كما وجد أن صغر حجم الكرات الدقيقة المحضرة أدى الى سرعة انطلاق الدواء ومع ذلك فان الكرات الدقيقة المحضرة أظهرت بطء في معدل انطلاق الدواء مع البوليمرات المستخدمة وبالنسب المختلفة في وسطي معامل اﻷس الهيدروجيني   ,   ,   بالمقارنة بالمستحضرالموجود في السوق المصري. وقد أوضحت دراسة حركية انطلاق الدواء من الكرات الدقيقة المحضرة أن انطلاق الدواء تم عن طريق انطلاق من رتبة الصفر.

A modified emulsion-solvent evaporation technique was utilized to prepare sustained release microspheres containing theophylline (TH). Two polymers were used for microspheres preparation, namely cellulose propionate (CP) and ethyl cellulose (EC). The two polymers were used at 1:1, 2:1, and 3:2 (drug - polymer ratios).

*The prepared microspheres were evaluated for their total recovery, drug loading, particle size distribution, surface morphology, drug content and drug release rate characteristics. The results have shown that, the total percentages drug recovery reached 96, 97.7and 90.3 for cellulose propionate at 1:1, 2:1 and 3:2 (drug - polymer ratios), and that of drug loading reached 89, 90, and 92 respectively. However, with ethyl cellulose, the total % drug recovery reached 99, 100, and 94 using the same drug − polymer ratios, and the total % drug loading reached, 85, 90, and 90 respectively. The results obtained have shown a significant complete recovery with an excellent drug loading and thus the efficiency of the procedure utilized to encapsulate the drug. The drug release characteristics from the prepared microspheres in simulated gastric fluid (pH 1.2) and phosphate buffer (pH 6.8) were compared with commercial sustained release capsules of theophylline (*Theo SR 100*). Results have revealed that, the release rate of theophylline was influenced by the type of polymer, microsphere size, pH as well as drug to polymer ratio. The decrease in particle size of the prepared microspheres led to increase in the release rate. However, the prepared microspheres showed more retarded release of theophylline than from the tested commercial product. Moreover, ethyl cellulose as a polymer was more effective for sustained effect. The release data were fitted to Peppas diffusion equation. Results have indicated that the release pattern of theophylline followed zero-order kinetics.*

INTRODUCTION

Microencapsulation is a process by which a gas, liquid, or solid material (core) is packaged inside micronized capsule of a second material (shell)[1]. The process embodies a series of techniques for the entrapment of solids or liquids within polymer coats or matrices. It has been used for the improvement of drug stability, taste masking, providing sustained release, and for producing targetable drug carriers[2&3]. Microencapsulation by emulsification / solvent evaporation technique is conceptually a simple procedure. This technique can be tailored to produce microspheres over a wide size range, and by choice of suitable solvent systems, many drugs with different solubility characters can be encapsulated[4-6]. The structure of microspheres produced by emulsification / evaporation is essentially drug dispersed through a matrix as a solid or a molecular dispersion[7-9]. Oral sustained release preparations can be classified into multiple-unit preparations and single-unit preparations. Multiple-unit preparations distribute granules relatively widely

200

in the digestive tract after administration and have been reported to be relatively unaffected by gastric emptying rate, increase in bioavailability, and less variation in the results of pharmacokinetic studies[10-13]. Theophylline has long been used as a treatment of bronchial asthma[14]. It has a relatively short half-life and narrow therapeutic index, with 5-20 µg/ml serum concentrations. The desirability of maintaining theophylline plasma levels have led to the development of sustained release formulations. Thus sustained-release formulations that can produce more uniform serum concentrations with less fluctuation in peak-trough levels are useful for the oral delivery of theophylline. In addition, sustained release theophylline formulations can ensure good patient compliance since it is difficult for a patient to take oral medication repeatedly during an acute asthma attack[15-23]. Hence the objective of this study aims to encapsulate theophylline using enhanced emulsion-solvent evaporation process for the rapid and efficient microencapsulation. Commercial slow release theophylline capsules were used for *in-vitro* comparison of drug release.

MATERIALS AND METHODS

Materials

Theophylline, ethyl cellulose, and cellulose propionate (Sigma Chemical Co. St.Louis, MO, United States), liquid paraffin (S&C Chem.,

Germany). All other reagents and chemicals were of analytical grades. Theo SR 100; commercial sustained release theophylline capsules produced by gsk GlaxoSmithKline (Cairo, Egypt). The product was used as a reference for *in-vitro* drug release.

Apparatus

Mechanical mixer (ER10, MLW, Germany), pH meter (Hanna pH213), electron scanning microscope (Jeol, JSM-5400 LV, Japan), set of standard sieves (Gilson Company SS-15, USA), double beam spectrophotometer (Shimadzu UV-1601PC, Japan), dissolution apparatus USP 20 type II (Pharmatest Germany).

Methods
Preparation of theophylline microspheres

Theophylline microspheres were prepared by utilizing a modified emulsion/solvent technique[23&24]. The polymer was dissolved in acetone and various amounts of the drug was added to the polymer solution to produce 1:1, 2:1, and 3:2 drug to polymer ratios. The mixture was emulsified into 100 ml liquid paraffin at 600 rpm for 2 hrs at room temperature, and then the emulsification temperature was raised to 40ºC on water bath to complete acetone evaporation. The formed microspheres were separated, washed three times with n-hexane to remove any adsorbed mineral oil and collected by filtration. The microspheres were then dried at room temperature.

Calculation of the total recovery

The percentage total recovery was determined by the following equation:

% Recovery =

$$\frac{\text{Weight of collected microspheres}}{\text{total weight of drug and polymer used}} \times 100$$

Drug loading

A weighed amount of microspheres equivalent to 50 mg theophylline was extracted with the dissolution medium after dissolving the polymer with 5 ml ethyl acetate. Drug concentration was determined after filtration spectrophotometrically at 272 nm wavelength[23]. At the specified wavelength, no spectrophotometric interferences were observed from blank microspheres.

Determination of surface characteristics of the prepared microspheres.

The surface characteristics of the prepared microspheres were observed with a scanning electron microscope.

Determination of particle size range (sieve analysis)

Microspheres size distribution was determined by using a set of standard sieves (USP) and the following size ranges were determined: 1500-1000 µm, 1000-500 µm, 500-355 µm, 355-250 µm, and 250-180 µm.

***In-vitro* drug release characteristics**

In-vitro release studies of theophylline from the prepared microspheres were carried out using USP dissolution apparatus II.

Accurately weighed amounts of the dried microsphere equivalent to 50 mg theophylline were suspended in 900 ml of dissolution media at 37°C and 100 rpm. The dissolution media used were simulated gastric fluid (pH 1.2) and phosphate buffer (pH 6.8). At specified time intervals, 5 ml of dissolution fluid was withdrawn to assay the released drug spectrophotometrically at 272 nm and replaced by 5 ml of fresh medium. Each graphical data point was an average of three determinations. Corrections were made for the removal of samples.

Release study of theophylline from commercial theophylline product

The commercial theophylline product (**Theo SR**), each capsule containing 100 mg of theophylline was used as a reference for *in-vitro* drug release study. The release also was carried out in both pH 1.2 and pH 6.8.

Kinetic Studies of drug release

To investigate the mechanism of drug release from the prepared microspheres and commercial product, the release data were fitted to the following mathematical equation developed by Ritger and Peppas[24-26].

$$Q = k\,t^{n} \qquad \text{(equation 1)}$$

By taking the log scale
$$\text{Log } Q = \log k + n \log t \quad \text{(equation 2)}$$

where Q is the fractional drug released at time t, k is a kinetic constant and n is an exponent indicative of the release mechanism.

When n approximates 0.5, a Fickian/diffusion controlled mechanism is implied, with $0.5 < n < 1$ indicating non Fickian transport, and $n=1$ for zero order release mechanism.

RESULTS AND DISCUSSION

Size distribution of the prepared microspheres

Figure 1 (A&B) shows the size distribution (histograms) of the prepared microspheres of theophylline with cellulose propionate and ethyl cellulose respectively at 1:1, 2:1, and 3:2 drugs to polymer ratios. The obtained microspheres with size ranges of 1500-1000 µm and 1000-500 µm were used to demonstrate the release characteristics of theophylline.

Scanning electron microscopy

Figure 2 shows the scanning electron micrographs of theophylline microspheres prepared with cellulose propionate at 1: 1 drug to polymer ratio. The prepared microspheres were spherical, showing few pores on their surfaces due to rapid evaporation of the organic solvent.

Total percentage drug recovery

The Total % drug recovery values reached 96, 97.7 and 90.3 from theophylline microspheres prepared with cellulose propionate at 1:1, 2:1 and 3:2 (drug - polymer ratios) respectively. However, when ethyl cellulose was used as a polymer in microspheres preparation, the total % drug recovery reached 99, 100 and 94 respectively using the same drug-polymer ratios (Fig. 1A&B). The

results obtained show complete recovery and thus the efficiency of the procedure used to encapsulate the drug.

Figure 1 (A&B) also shows that the particle size of the prepared microspheres was influenced by polymer type and concentration. The obtained results are in agreement with Mohammed and Hassan[24]. The authors have found that the particle size of the prepared microspheres of ciprofloxacin and nor-floxacin with cellulose acetate were increased with polymer concentration.
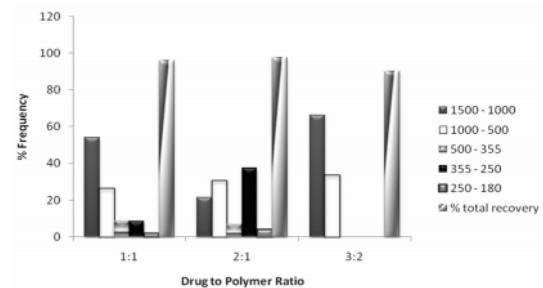
Fig. 1A: Effect of drug to polymer ratio on the percent frequency size distribution and percent recovery of theophylline microspheres prepared using cellulose propionate.
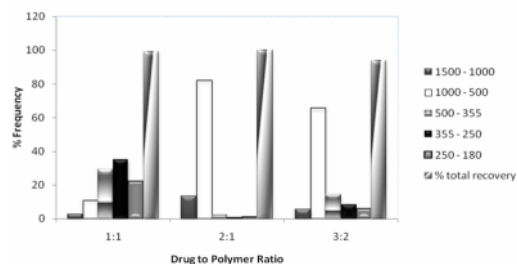
Fig. 1B: Effect of drug to polymer ratio on the percent frequency size distribution and percent recovery of theophylline microspheres prepared using ethyl cellulose.

203
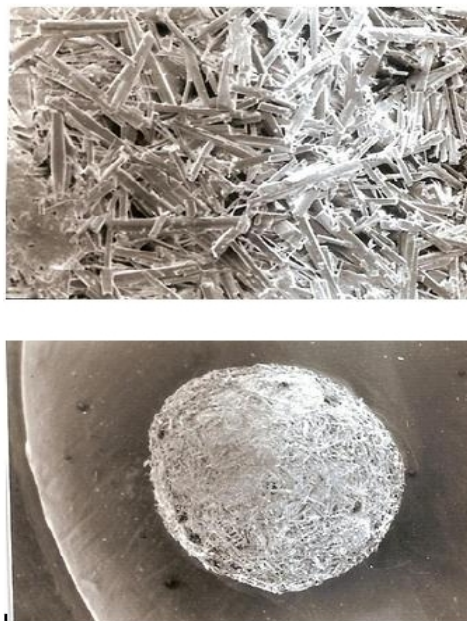
Fig. 2: Scanning electron micrograph of theophylline microspheres prepared with cellulose propionate (1:1) drug to polymer ratio. (Up): high magnification, (down): low magnification.

Percentage drug loading of the prepared microspheres

The % drug loading reached 89, 90 and 92 for theophylline microspheres prepared with cellulose propionate at 1:1, 2:1 and 3:2 (drug - polymer ratios) respectively. However, the corresponding % drug loading reached 85, 90, and 90 for theophylline microspheres prepared with ethyl cellulose at the same drug - polymer ratios respectively. These findings indicate excellent drug loading.

Drug release studies of the prepared microsphere and commercial (Theo SR 100)

Effect of microspheres size on theophylline release

Figure 3 (A-C) and figure 4 (A-C) show the effect of particle size on theophylline release from the prepared microspheres using cellulose propionate with different ratios in (pH 1.2) and (pH 6.8) respectively. The results obtained reveal that the release rate of theophylline was markedly retarded with microspheres prepared with cellulose propionate (3:2) drug to polymer ratio with size range 1500-1000 μm in both pH values compared with commercial product (Theo SR 100). The relatively rapid release from the smaller microspheres size range 1000-500 μm when compared with larger microspheres could be attributed to the larger surface area of the smaller microspheres, which is in agreement with Mohammed and Hassan[24]. In addition, figure 5 (A&B) show the effect of particle size on theophylline release from the prepared microspheres prepared with ethyl cellulose (2:1) drug - polymer ratio in (pH 1.2) and (pH 6.8) respectively compared with commercial (Theo SR 100). The results illustrate similar effects obtained with cellulose propionate, i.e., the prepared microspheres showed a retarded release profiles and the smaller particles show a relatively rapid release than lager microspheres due to available lager surface area[24].
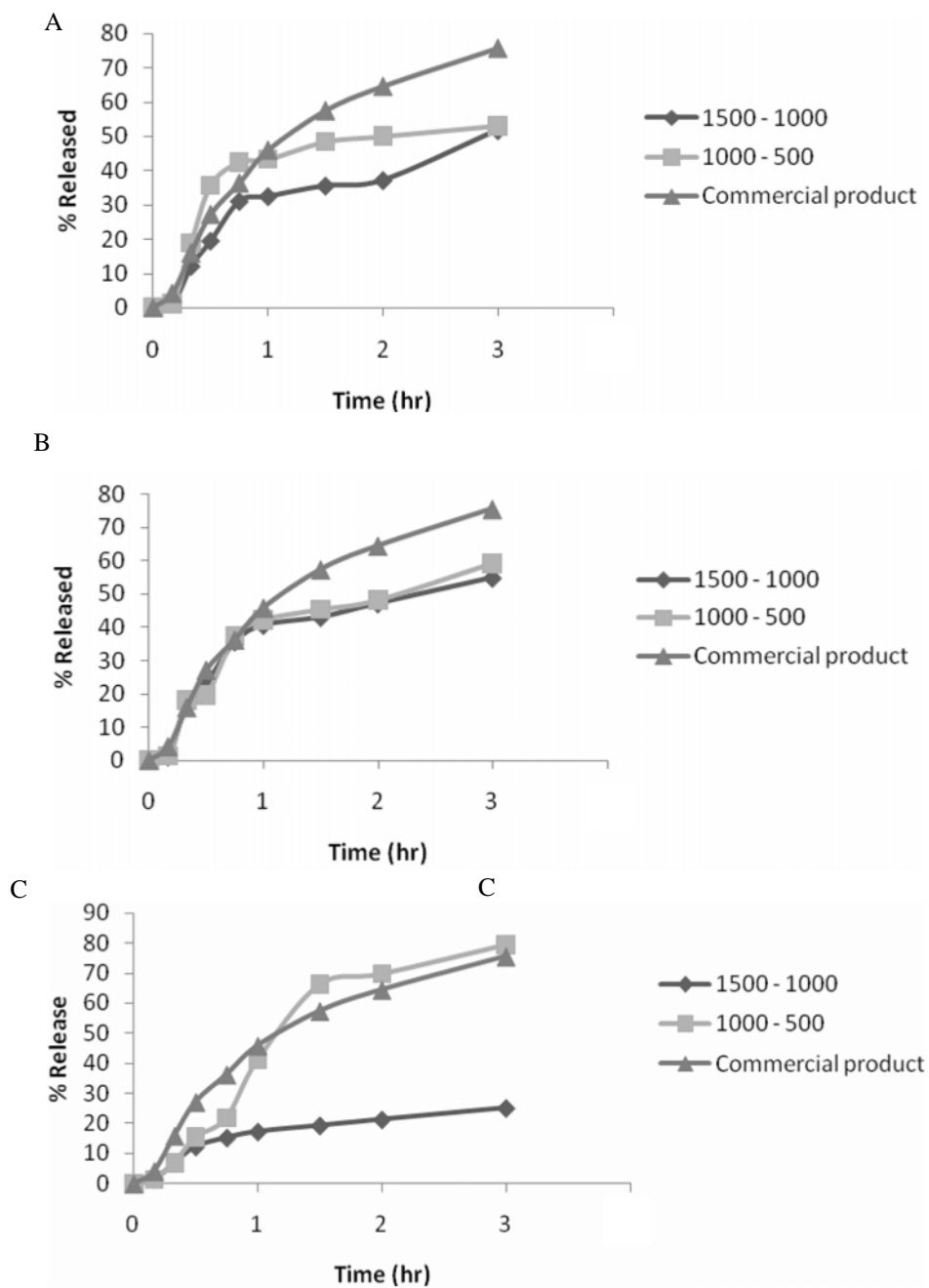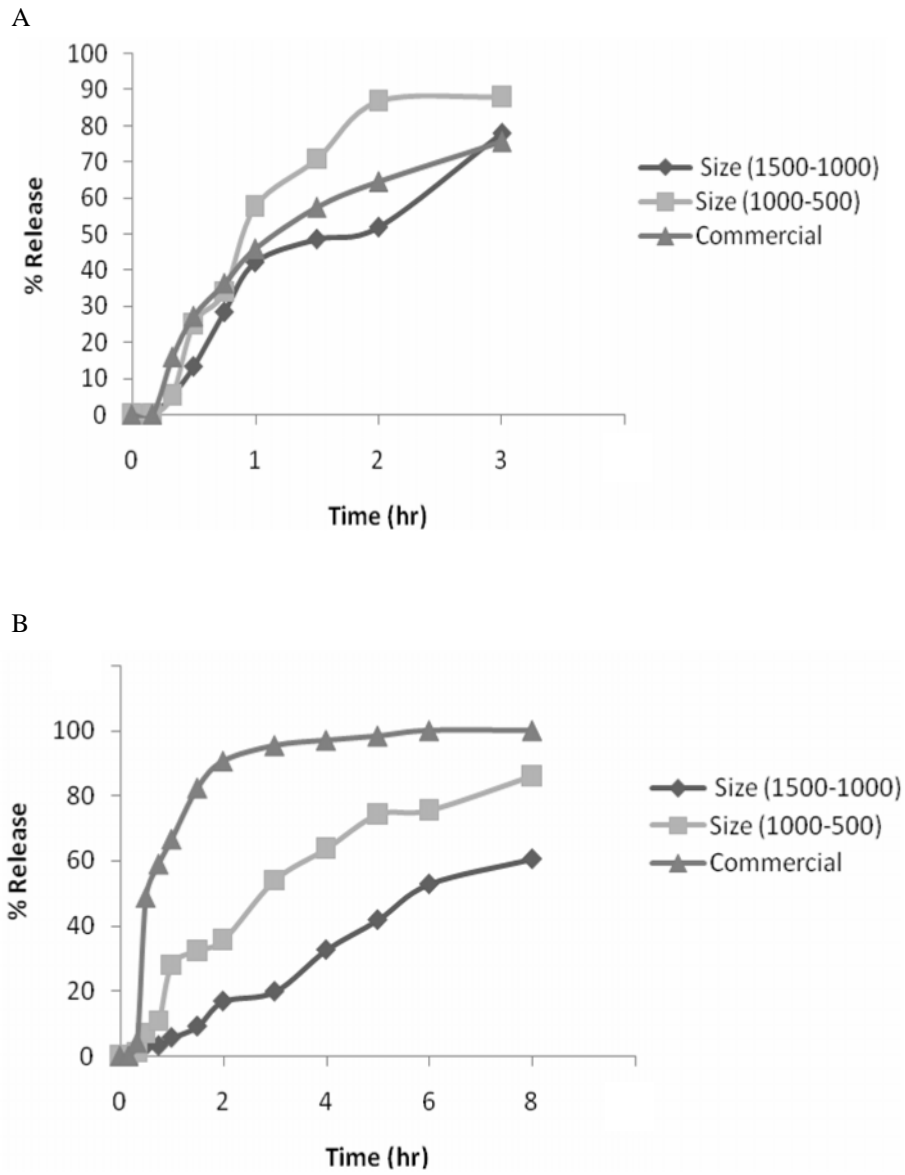
Fig. 3: Effect of particle size on theophylline release from microspheres prepared with cellulose propionate pH 1.2 compared with the commercial product, (A) 1:1, (B) 2:1 and (C) 3:2.
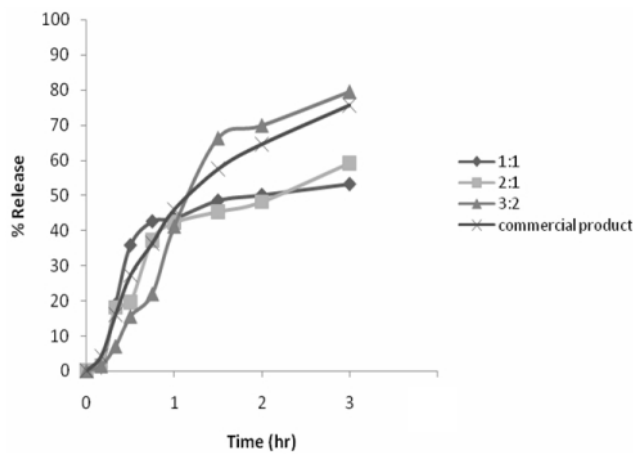
A

B

C
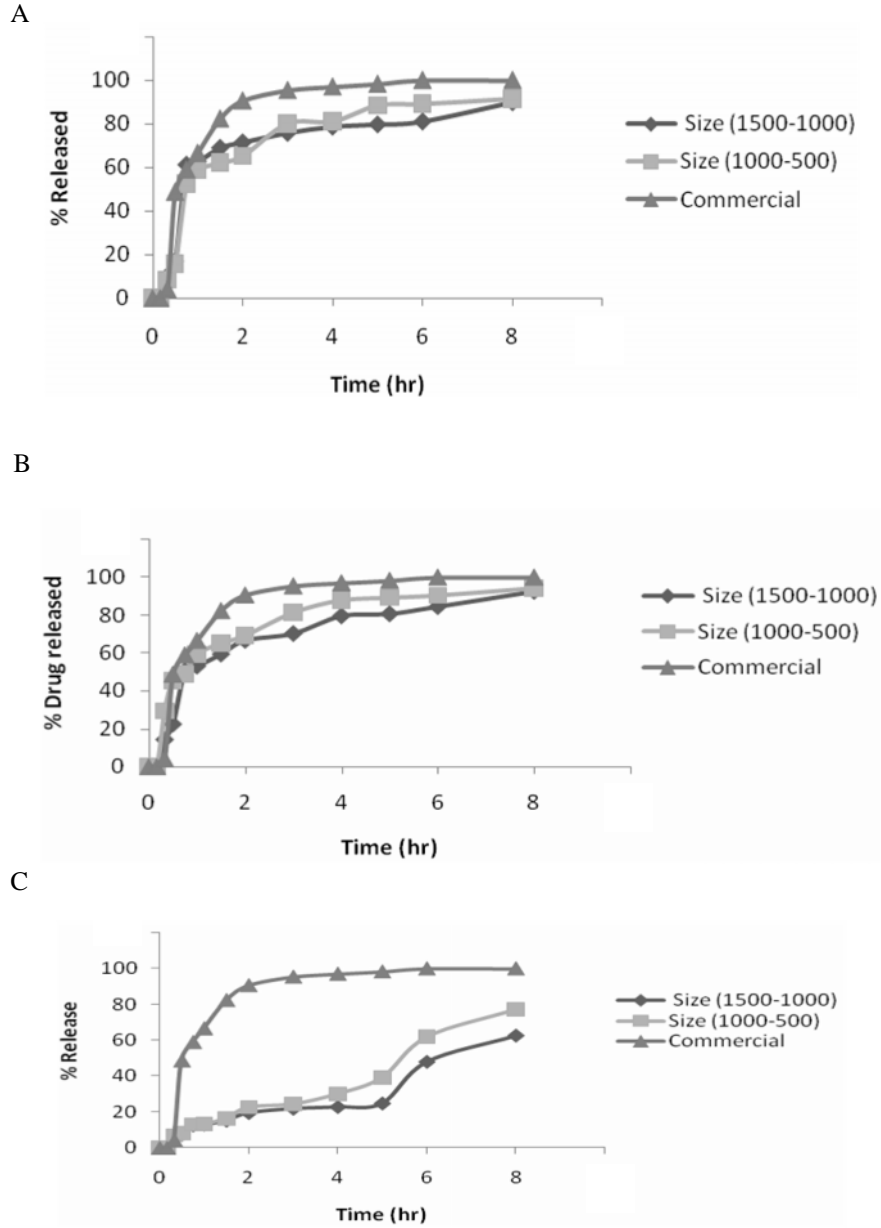
Fig. 4: Effect of particle size on theophylline release from microspheres prepared with cellulose propionate pH 6.8 compared with the commercial product, (A):1:1, (B):2:1 and (C):3:2.

A

B

Fig. 5: Effect of particle size on theophylline release from microspheres prepared with ethyl cellulose (2:1) compared with the commercial product, (A) pH 1.2 and (B) pH 6.8.

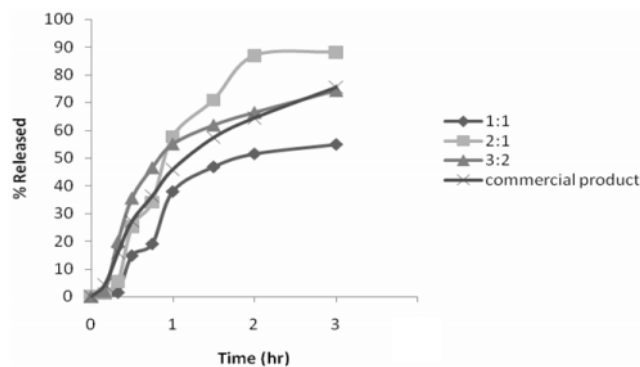**Effect of drug to polymer ratio on theophylline release**

Figure 6 (A&B) shows the effect of drug to polymer ratio on theophylline release from the prepared microspheres prepared with cellulose propionate and ethyl cellulose respectively in pH 1.2 compared with the commercial product (Theo SR 100). The microspheres with size range 1000-500 µm were selected to study this effect. The results reveal that, the release profiles from both microspheres prepared with cellulose propionate and ethyl cellulose (1:1) drug to polymer ratio show the most retarded release patterns when compared with the commercial product (Theo SR 100). Moreover, this effect was more with microspheres prepared with ethyl cellulose.
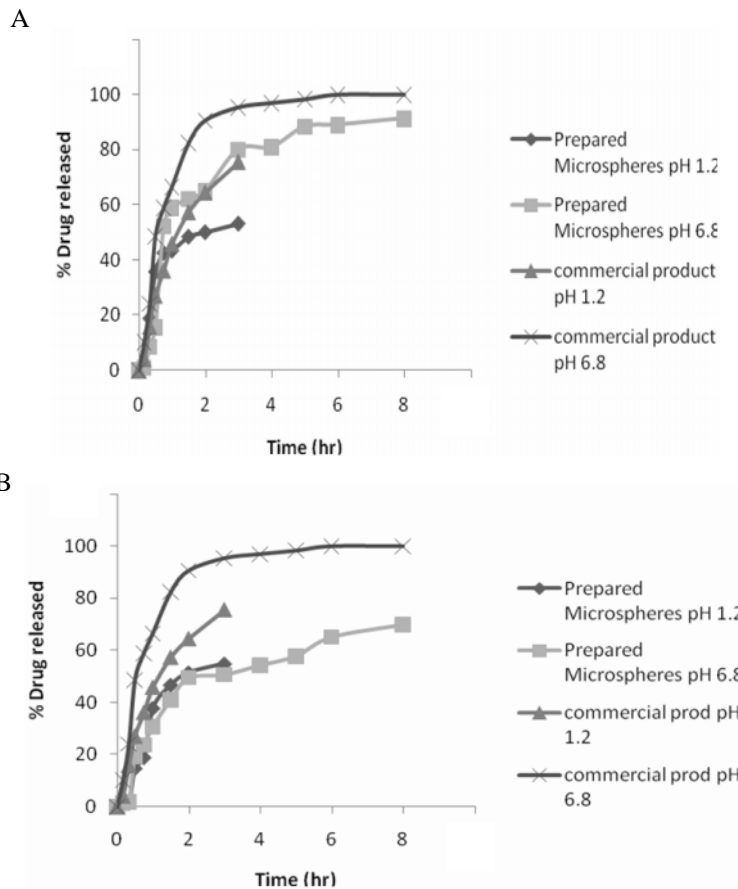
A

B

Fig. 6: Effect of drug to polymer ratio on theophylline release from microspheres prepared with (A) cellulose propionate and (B) ethyl cellulose (P.S 1000 – 500 µm) pH 1.2 compared with the commercial product.

208

Effect of pH on theophylline release

Figure 7 (A&B) shows the release profiles of theophylline from microspheres prepared with cellulose propionate (Fig. 7A) and ethyl cellulose (Fig. 7B) respectively. This study was carried out using microspheres prepared in ratio (1:1) drug to polymer ratio with 1000-500 µm size range. The data reveal that the prepared microspheres show retarded release of the drug compared to that from the commercial product (Theo SR 100) in both pH values (1.2 and 6.8). This may indicate that the use of the prepared microspheres could control the release of the drug. Moreover, ethyl cellulose when used as a polymer show more retardation effect than cellulose propionate (Fig. 7A&B).

A

B

Fig. 7: Effect of pH on release of theophylline from microsphere prepared with (A) cellulose propionate and (B) ethyl cellulose (1:1) particle size (1000-500 µm) in comparison with the commercial product.

Release kinetics

Tables (1A and 1B) show the kinetic parameters obtained from fitting the release data of theophylline microspheres prepared with cellulose propionate and ethyl cellulose respectively to the Ritger and Peppas kinetic equation[24-26]. It is evident that the fitting curves are almost linear, as indicated by the higher linear correlation coefficient (r) and determination coefficient (r^2) values, which reached almost > 0.9 (table 1A and 1B). The n values approach 1 indicting zero order release mechanism of theophylline from the prepared microspheres[24-26]. However, the n values of the commercial capsules reported 0.633and 0.799 in pH (6.8) and pH (1.2) respectively are an indication to a non-Fickian transport mechanism of theophylline release from the commercial capsules.

Table 1A: kinetic parameters of the release data of theophylline microspheres prepared with cellulose propionate compared to the commercial product.

pH	Particle size (µm)	Drug: polymer ratio	Kinetic parameters			
			r	r^2	K	n
6.8	1500-1000	1:1	0.835	0.698	26.13	0.911
		2:1	0.840	0.710	27.11	0.855
		3:2	0.955	0.911	11.02	0.737
	1000-500	1:1	0.857	0.736	24.96	0.969
		2:1	0.710	0.510	224.56	1.060
		3:2	0.976	0.953	12.48	0.800
1.2	1500-1000	1:1	0.883	0.779	24.57	1.050
		2:1	0.846	0.710	28.98	1.110
		3:2	0.906	0.821	13.80	0.858
	1000-500	1:1	0.803	0.646	32.01	1.104
		2:1	0.869	0.755	28.74	1.070
		3:2	0.968	0.937	29.36	1.170
*6.8			0.842	0.709	36.00	0.633
*1.2			0.728	0.529	24.00	0.799

*Data of the commercial capsules.

210

Table 1B: kinetic parameters of the release data of theophylline microspheres prepared with ethyl cellulose.

pH	Particle size (µm)	Drug: polymer ratio	Kinetic parameters			
			r	r^2	K	n
6.8	1500-1000	1:1	*	*	*	*
		2:1	0.990	0.982	6.618	1.091
		3:2	0.977	0.955	8.595	1.291
	1000-500	1:1	0.887	0.786	16.78	0.939
		2:1	0.990	0.982	6.61	1.091
		3:2	0.984	0.968	5.537	1.247
1.2	1500-1000	1:1	*	*	*	*
		2:1	0.946	0.896	25.41	1.501
		3:2	0.936	0.877	27.58	1.122
	1000-500	1:1	0.931	0.866	20.05	1.516
		2:1	0.936	0.8.77	34.65	1.519
		3:2	0.936	0.877	27.58	1.122

*Not used for release studies because too small amounts obtained with this size range.

Conclusion

Controlled release microspheres of theophylline were successfully prepared utilizing a modified emulsion-solvent evaporation technique and using cellulose propionate and ethyl cellulose as polymers. The release rate of the drug from the prepared microspheres was markedly retarded when compared with the commercial theophylline capsules (Theo SR 100). Moreover, ethyl cellulose as a polymer shows a more retarded effect of the drug release.

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