PREPARATION AND EVALUATION OF THEOPHYLLINE LOADED BOVINE SERUM ALBUMIN MICROSPHERES

K. I. Saleh, M. A. Ibrahim and T. M. Faris

Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al- Azhar University at Assiut, Assiut, Egypt

تم   هذا البحث تحضير الكريات الدقيقه من زلال مصل الأبقار والمحمله بعقار ثيوفيللين باستخدام طريقة البلمره من المستحلب عن طريق الجلوترالدهيد كعامل ربط بين جزيئات الزلال كما تم تقييم تأثير عوامل الصياغه المختلفه مثل تركيز زلال مصل الأبقار تركيز المواد ذات النشاط السطحي   لزوجة الوسط وتركيز الجلوترالدهيد على كفاءة تحميل الكريات الدقيقة وكذا معدلات الانطلاق المعملي والحيوي للعقار من هذه الكريات. تم في هذا الصدد أيضا استخدام نسب مختلفه من العقار إلى الزلال هى :

:   :   :   :   :   و :   كما تم استخدام أوساط زٍ لتحضير المستحلبات مثل أيزوأوكتان زيت البرافين الخفيف زيت الزيتون وزيت بذر الكتان وقد دلت النتائج المستقاه من البحث على أن الكريات المحضرة قد تميزت بٍ   كروية الشكل ذات أسطح ملساء وكان متوسط أحجامها ما بين   إلى   ميكرومتر. أذ نت الدراسة أيضا أن كفاءة تحميل الكريات بعقار ثيوفيللين قد زاد زيادة مضطردة بزيادة تركيز الزلال زيادة تركيز المادة ذات النشاط السطحي زيادة لزوجة الوسط الزيتي وكذلك زيادة تركيز الجلوترالدهيد. وقد تميز الانطلاق المعملي للعقار بٍ كان سريعا في البدايه ثم كان بطيئاً عد ذلك. وقد علل ذلك بسبب وجود ؛ ٍ من العقار بالقرب من أو علي أسطح الكريات الدقيقه. وقد أوضحت نتائج دراسة الإتاحه الحيويه للعقار باستخدام الأرانب أن الكريات الدقيقه المحضرة من زلال مصل البقر والمحمله بعقار ثيوفيللين أعطت تركيزًا للعقار فى البلازما فى حدود المستوى العلاجى علي مدار حوالي   ساعات.

Theophylline-loaded bovine serum albumin (BSA) microspheres were prepared by an emulsion polymerization method using glutaraldehyde as the crosslinking agent. The study was designed to evaluate the effects of different formulation parameters as BSA concentration, surfactant concentration, hydrophilic lipophilic balance (HLB), dispersion medium viscosity and glutaraldehyde concentration on the extent of drug loading, size of microsphere and the in-vitro as well as the in-vivo release rates of theophylline from such microspheres. Drug polymer ratios of 1:1, 1:2, 1:3, 1:4,

1:5 and 1:6 were investigated. Span 80 was used as a surfactant at different concentrations. Moreover, Different span 80 / tween 80 blend concentrations; 0.5, 1.0 and 2.0% w/v were used to study the effect of HLB. Also different dispersion media; isooctane, light paraffin oil, olive oil and Linseed oil were used for microspheres preparation. In addition, different glutaraldehyde concentrations; 5, 10 and 25% v/v were used. It was found that all micro-spheres were spherical with the mean particle size of 90-180 µm. The results revealed also that: as the concentration of BSA increases, the drug loading is increased. Increasing surfactant concentration and viscosity of the dispersion medium has led to decrease the particle size, while increasing glutaraldehyde concentration nearly had no effect. Furthermore, encapsulation efficiency was found to be directly proprortional to albumin content, surfactant concentration, viscosity of the dispersion medium and glutaraldehyde concentration. Drug release from the prepared microspheres displayed a biphasic pattern characterized by an initial burst, followed by a slower release period, which may be attributed to the presence of theophylline material near to or onto the microspheres surfaces. The bioavailability of theophylline from the prepared microspheres was evaluated in rabbits. The prepared microspheres were found to control theophylline release even up to 10 hrs. The peak serum concentrations of such microspheres were within the therapeutic level. The results indicate also that the previoiusly mentioned formulation parameters have a prnounced impact to control the release of the entrapped drug.

INTRODUCTION

Albumin microspheres have attracted considerable attention for several years as a matrix for controlled and sustained delivery of many drugs. These protein microspheres provide a potentially useful vehicle for drug delivery to endocytic cells since they are physically and chemically stable, rapidly removed from the vascular system by phagocytosis, amenable to large-scale preparation, readily metabolized and capable of accommodating a broad variety of the drug molecules in a relatively nonspecific fashion[1-12].

Glutaraldehyde cross-linked albumin is relatively non- immunogenic in nature, and studies have shown that albumin micro-spheres are bio-degradable in the muscle in about two months without causing any adverse tissue reactions[13]. Suspension crosslinking of albumin can be accomplished either by direct reaction between functional groups on the

polypeptide side chains (self-crosslinking), or by the use of the crosslinking agents. Drug release from albumin microspheres can take place via various routes, such as total microsphere disintegration, microsphere hydration, surface erosion, particle diffusion and leaching[14]. Most of the *in-vitro* studies on the release of drugs incorporated into microspheres focused on biphasic profile describes an initial fast release (burst effect) followed by a slower first order release profile[15-19]. In this study, we have investigated the effect of five process factors, drug: polymer ratio, surfactant (span 80, HLB 4.3) concentration, blend of surfactants (span 80 / tween 80, HLB 5) concentration, dispersion medium viscosity and glutaraldehyde concentration on theophylline loaded albumin microspheres characteriza-tion and on the *in-vitro* release profiles of theophylline. This study also reports the bioavailability of theophylline from the prepared microspheres in rabbits.

MATERIALS AND METHODS

Materials
- Bovine serum albumin (BSA), glutaraldehyde 25%, and diethyl ether were purchased from Fluka Chemie GmbH, Germany.
- Anhydrous Theophylline was purchased from Sigma Chemie GmbH, Germany.
- All other ingredients and chemicals used were of reagent grade.

- Rabbits weighing 1.75-2.25 kg were used in the bioavailability studies.

Equipment
- Spectrophotometer UV.1601 (Shimadzu Co., Japan).
- Sieve Shaker, Rx-86-1 (Cole-Parmer Instrument Co., USA).
- Dissolution test apparatus, SR11 6 Flask (Hanson Co., USA).
- Electrical digital balance (Precisa XB 220, Swisserland).
- Over head stirrer (Heidolph, Germany).
- Centrifuge (Cole-Parmer 8890, USA).

Methodology
Preparation of BSA microspheres

BSA microspheres were prepared using emulsion polymerization technique slightly modified as described by Tomlinson *et al.*[20]. About 500 mg of BSA powder were dissolved in 1 ml phosphate buffer. The solution of BSA was dropped to 100 ml light paraffin oil during stirring at 500 rpm. The microspheres were stabilized by dropping about 0.6 ml of a 25% (v/v) glutaraldehyde with continual stirring for 60 min. to ensure microspheres formation. After stabilization of the microspheres, 80 ml of anhydrous diethyl ether was added and stirring was continued for another 5 min. then set aside and the supernatant layer was decanted. Complete removal of residual oil was achieved by washing the albumin

microspheres in anhydrous diethyl ether (100 ml) three times followed by washing once with petroleum ether (100 ml).

Theophylline loaded BSA microspheres were prepared using the same previous preparation method except that theophylline powder (100 mg) was dispersed in the BSA solution where theophylline has a low aqueous solubility (1 g in about 120 ml). In this study we followed the experimental design which studies the effect of each process factor individually through different levels. The level at which the drug is highly entrapped was chosen and established during studying the second process factor and vice versa. To study the effect of drug: polymer ratio, different ratios were selected (1:1, 1:2, 1:3, 1:4, 1:5 and 1:6). To study the effect of the surfactant concentration, different span 80 (has HLB value of 4.3) concentrations were selected; 0.5, 1 and 2% w/v. To study the effect of achieving a surfactant of HLB value of 5 (which is essential for proper emulsification when mineral oil or vegetable oil is used as a dispersion medium)[21], different span 80 / tween 80 blend concentrations were selected; 0.5, 1 and 2% w/v. To obtain a blend of HLB 5, span 80 (HLB 4.3) and tween 80 (HLB 15) were used in the percentage ratio of 93.46: 6.54, respectively. Also different batches were prepared using different dispersion media; isooctane, light paraffin oil, olive oil and Linseed oil as well as different glutaraldehyde concentrations; 5, 10 and 25% v/v were used.

Characterization of microspheres
Particle size distribution of the prepared BSA microspheres

Determination of microspheres particle size was performed through sieving the yield of the prepared microspheres using a set of sieves (40 μm – 515 μm) and specifying the weight of the fractions that passed through each sieve. The particle size distribution curve was constructed through plotting percentage of weight fraction against microsphere particle size[13].

Drug encapsulation efficiency

An amount of 10 mg of theophylline microspheres was weighed accurately. The amount of drug loaded was determined by digesting the amount of microspheres in 5 ml of glacial acetic acid for 24 hrs (4°C) followed by centrifugation (5000 rpm) to completely separate the precipitated mass. The amount of theophylline in the supernatant of each sample was determined by measuring the absorbance in the spectrophotometer at 271.5 nm. Corrections for albumin contribution to absorbance were made using reference solutions of placebo microspheres also in glacial acetic acid.

In-vitro drug release from albumin microspheres

In-vitro release of theophylline from BSA microspheres was carried

out using the USP XXII paddle method. The dissolution media were 900 ml of simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) maintained at $37 \pm 0.5°C$. The paddles were positioned 2.5 cm from the bottom of the vessel and rotated at speed of 100 rpm. Ten mg of the prepared microspheres was transferred to the stirred dissolution medium in each vessel. At a predetermined time intervals, samples were withdrawn by pipette fitted with a filter and measured spectrophotometerically at 271.5 nm for determining drug concentration. Blanks were used.

Kinetics of the *in-vitro* drug release

The data obtained from the experiments were analyzed by means of computer, using linear regression analysis to specify the mechanism of drug release. The correlation coefficient obtained after applying the zero order, the first order and Higuchi's diffusion model were compared. The model which showed the highest correlation coefficient is assigned as the mechanism of release.

Theophylline bioavailability studies

Rabbits weighing 1.75-2.25 kg were used for this study. Each rabbit was given a dose of 15 mg/kg body weight of theophylline powder or an equivalent dose in microspheres form with 10 ml water orally through a catheter. In intravenous administration, a dose of 19.04 mg of aminophylline injection USP (25 mg/ml), equivalent to 15 mg of theophylline/kg, was administered through a catheter into the marginal ear vein of the rabbit over 5 minutes. Venous blood samples (1 ml each) were collected at 1, 2, 4, 6, 8, 12, 18 and 24 hrs from the Jugular vein after administration. Samples were incubated at 37°C till clotted then centrifuged at 2500 rpm for 10 min and the serum was separated. Theophylline in the serum was extracted and analyzed in the following way; 0.4 g of ammonium sulphate were added to 0.5 ml of serum and theophylline was extracted using 15 ml of chloroform/hexane (7:3 mixture) by stirring the contents using magnetic stirrer for 40 min. The organic layer was separated and 10 ml of which were extracted using 3 ml of 0.1 M carbonate buffer (p 9.0) after shaking for 30 min. The aqueous layer was then separated. The absorbance of this layer was measured at 271.5 nm[22].

The results of the bioavailability studies were used to determine the pharmacokinetic parameters. The elimination rate constant K_{el} was determined from the slope of the terminal linear portion of the semi-logarithmic curve when serum concentration was plotted against time using linear regression analysis as reported by Maruyama *el al*[23]. Also the elimination half-life values were determined by dividing 0.693 by the elimination rate constant K_{el}. The area under the concentration-time curve AUC is calculated from the following equation:

$$AUC_{0-} = AUC_{0-t} + C_t / k_{el}$$

where AUC $_{0-t}$ is the area under the curve from time 0 to t calculated using trapezoidal rule and C_t is the concentration at time t.

All the other pharmacokinetic parameters for *in-vivo* study of theophylline are calculated and tabulated.

RESULTS AND DISCUSSION

Particle size analysis

It was found that the drug: polymer ratio of 1:1 did not form microspheres. Increasing the polymer content has shown to increase the size of the microspheres. The results (Fig. 1) reveal that by decreasing the drug concentration via decreasing the drug: polymer ratio from 1:2 to 1:6 (i.e, increasing the polymer content), the amount of the microspheres having particle size more than 130 µm was increased from 20% to 74%.
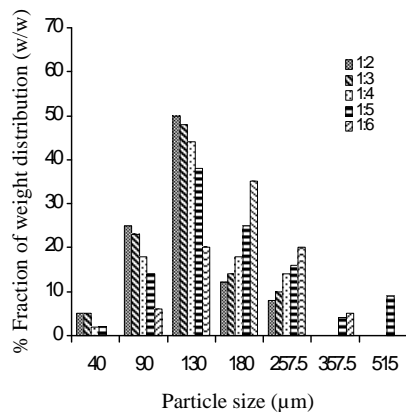
Fig. 1: Effect of different drug: polymer ratios on the % fraction of weight distribution of theophylline loaded BSA microspheres.

It was noticed that increasing span 80 concentration led to decrease in particle size[24]. The results reveal that upon increasing span 80 concentration from 0% to 0.5, 1 and 2%, the amount of the microspheres having particle size less than 130 µm was increased from 54 to 84, 91 and 92%, respectively (Fig. 2).
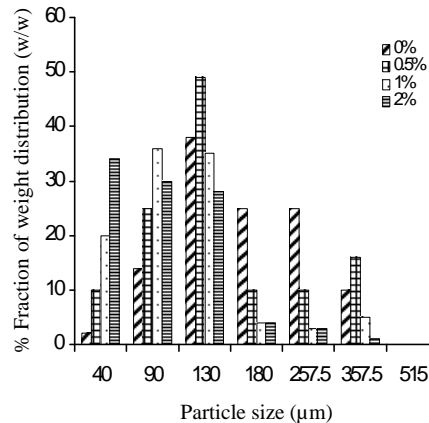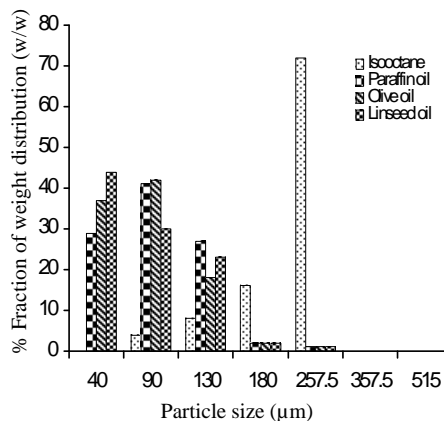

Fig. 2: Effect of different span 80 concentrations (% w/v) on the % fraction of weight distribution of theophylline loaded BSA microspheres prepared using drug: polymer ratio of 1:5.

Figure 3 shows that there is a slight increase in the amount of the microspheres having particle size less than 130 µm (from 82 to 97%) upon increasing span 80 / tween 80 blend concentration from 0.5 to 1% w/v. By increasing the blend concentration from 1 to 2% w/v, there was no change in the amount of the microspheres having particle size less than 130 µm but there was an increase in the amount of the microspheres having particle size less than 40 µm from 29% to 41%, respectively.
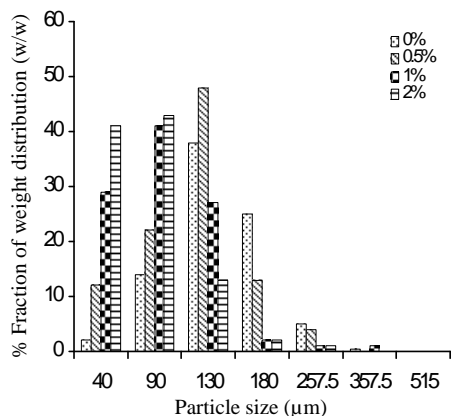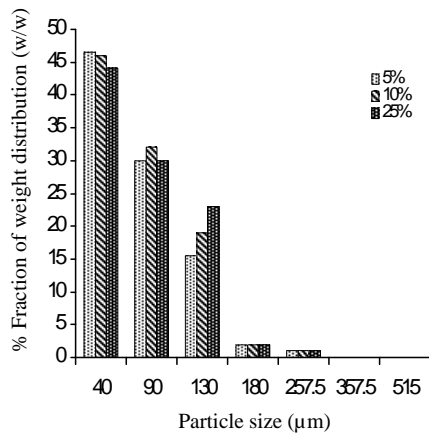
Fig. 3: Effect of different span 80 / tween 80 blend concentra-tions (% w/v) on the % fraction of weight distribution of theophylline loaded BSA microspheres prepared using drug: polymer ratio of 1:5.

Fig. 4: Effect of different types of dispersion media on the % fraction of weight distribution of theophylline loaded BSA microspheres prepared using drug: polymer ratio of 1:5 and span 80 / tween 80 blend concentration of 1% w/v.

It is noticed that upon increasing the viscosity of the dispersion medium, there is a corresponding decrease in the particle size, (Fig. 4). The used dispersion media were isooctane, light paraffin oil, olive oil and linseed oil having viscosities of 0.5, 66.2, 75 and 94 cp., respectively. Albumin microspheres having very large diameter were produced upon using isooctane as the dispersion medium[25&26].

The results reveal that upon increasing glutaraldehyde concentra-tion from 5 to 10 and 25% v/v, the amount of the produced microspheres having particle size less than 130 µm were nearly of the same values, (Fig. 5). These data indicate that increasing glutaraldehyde concentra-tion had no effect on the particle size range[24].
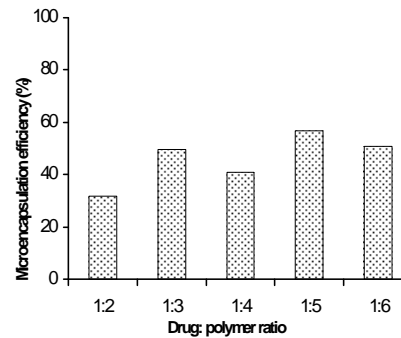
Fig. 5: Effect of different glutaraldehyde solution concentrations (% v/v) on the % fraction of weight distribution of theophylline loaded BSA microspheres prepared using drug: polymer ratio of 1:5, span 80 / tween 80 blend concentration of 1% w/v and linseed oil as the dispersion medium.
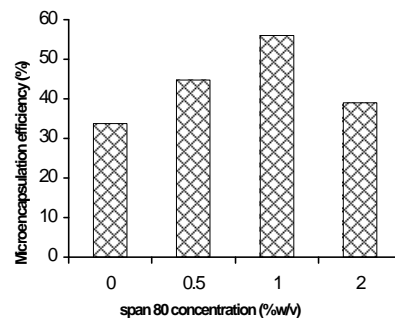
Encapsulation efficiency of theophylline

It is obvious from (Fig. 6) that upon increasing albumin content in the aqueous phase during microspheres preparation, there is a remarkable increase in the loading efficiency till the drug: polymer ratio of 1:5 then the entrapment efficiency is decreased. The highest drug loading was found to be with the drug-albumin ratio of 1:5; so it was chosen and used in the next investigations. It is noticed from (Fig. 7) that increasing span 80 concentration from 0 to 1% increased the encapsulation efficiency from 33.7 ± 8.11 to 56.8 ± 3.66%. The

encapsulation efficiency decreased to 38.9 ± 7.05% for span 80 concentration 2% w/v. The microspheres prepared using span 80 concentration of 1% w/v had the highest encapsulation efficiency so it was chosen and used in the next investigation.
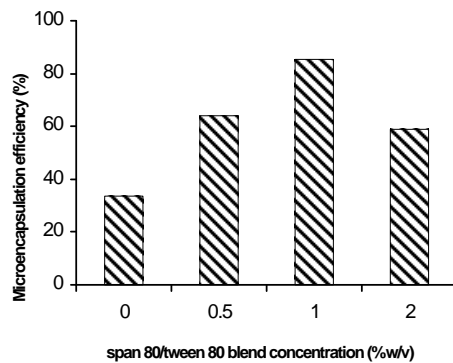

Fig. 6: Effect of different drug: polymer ratios on the encapsulation efficiency of theophylline loaded BSA microspheres.

Fig. 7: Effect of different span 80 concentrations (% w/v) on the encapsulation efficiency of theophylline loaded BSA microspheres prepared using drug: polymer ratio of 1:5.
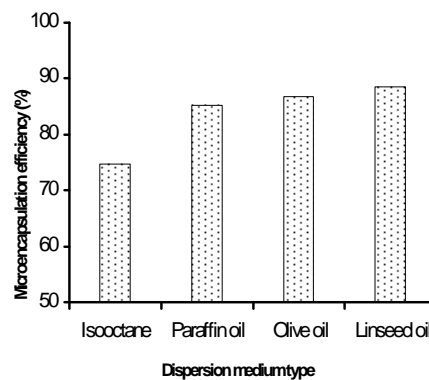
The microencapsulation efficiency of the microspheres prepared using span 80 / tween 80 blend is much more than those prepared with span 80 alone and ranged from 63.5 ± 5.9 to 85.3% ± 4.5, (Fig. 8). The microspheres prepared using blend concentration of 1% w/v has the highest encapsulation efficiency so it was chosen and used in the next investigation.
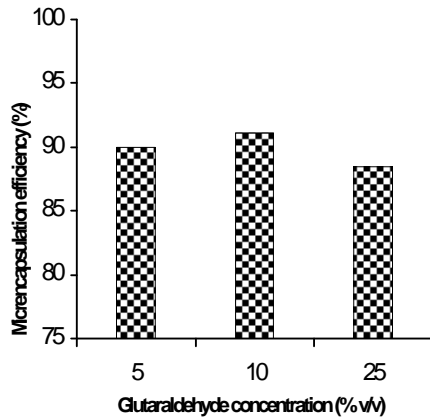

Fig. 8: Effect of different span 80 / tween 80 blend concentrations (% w/v) on the encapsulation efficiency of theophylline loaded BSA microspheres prepared using drug: polymer ratio of 1:5.

The lower encapsulation efficiency (74.64 ± 2.4%) is noticed with the batch prepared with isooctane (low viscosity of 0.5 cp.) as a dispersion medium. The highest (88.43% ± 2.52) encapsulation efficiency is noticed with batch prepared with linseed oil (high viscosity of 94 cp.) as the dispersion medium (so it was chosen and used in the next investigation) (Fig. 9). The batch prepared with glutaraldehyde concentration of 10% w/v has the highest encapsulation efficiency (91.1% ± 1.92) (Fig. 10). With the increase in the glutaraldehyde concentration from 5 to 10% w/v, there was a slight increase in the encapsulation efficiency from 90 ± 1.92 to 91.1% ± 1.92. The encapsulation efficiency was decreased from 91.1 ± 1.92 to 88.43% ± 2.52 by increasing glutaraldehyde concentration from 10 to 25% v/v.

Fig. 9: Effect of different dispersion media on the encapsulation efficiency of theophylline loaded BSA microspheres prepared using drug: polymer ratio of 1:5 and span 80 / tween 80 blend concentration 1% w/v.
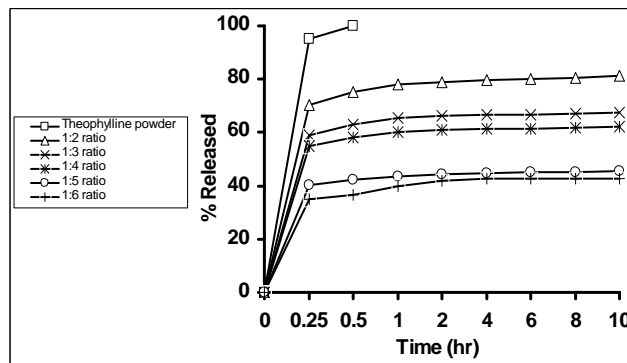
Fig. 10: Effect of different glutar-aldehyde concentrations (% v/v) on the encapsulation efficiency of theophylline loaded BSA microspheres prepared using drug: polymer ratio of 1:5, span 80 / tween 80 blend concentration 1% w/v and linseed oil as the dispersion medium.
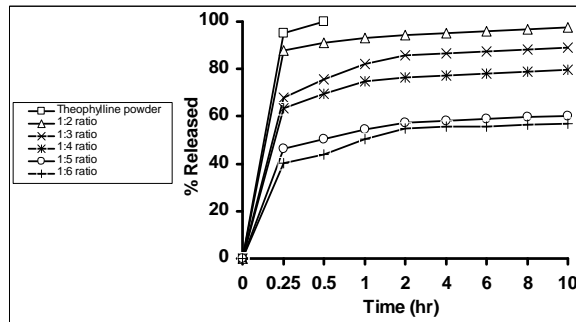
Theophylline *in-vitro* release study

Generally drug release from microspheres as reviewed by Tomlinson *et* al.[20] is characterized by an initial rapid release of the drug ("burst" release), followed by slower release of the remaining drug. The initial phase was completed within 2 hrs and about 35-92% of the loaded theophylline was released during this period. Also the release rate was higher in SGF than SIF.

It is noticed from (Figs. 11&12) that the theophylline release from the BSA microspheres was retarded with an increase in albumin content. Moreover, changing drug to polymer ratio from 1:2 to 1:6 decreased the amount of theophylline released from 79 to 40% after 2 hrs of the experiment time course. In (Figs. 13&14), when the BSA was used as a self-emulsifying agent (no Span 80 was added), the amount of theophylline released after 2 hrs was 44.2% while this amount was increased to 53.4, 58.6 and 60.6% when span 80 was used at concentrations of 0.5, 1 and 2% w/v, respectively after the same period.
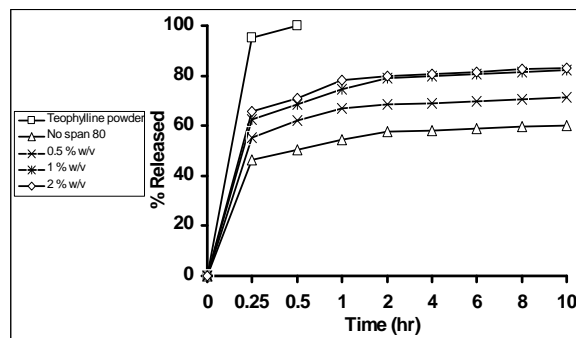

Fig. 11: Effect of different drug: polymer ratios on the *in-vitro* release of theophylline loaded BSA microspheres in SIF at 37°C.

74

Fig. 12: Effect of different drug: polymer ratios on the *in-vitro* release of theophylline loaded BSA microspheres in SGF at 37°C.
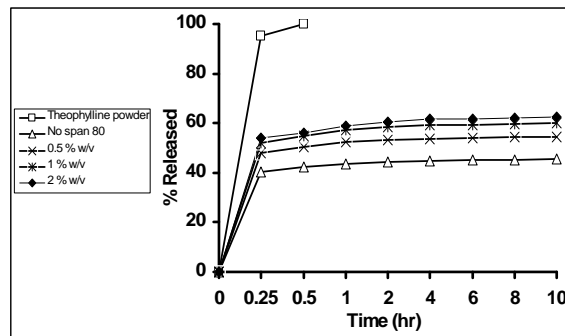
Fig. 13: Effect of different span 80 concentrations (% w/v) on the *in-vitro* release of theophylline loaded BSA microspheres prepared using drug: polymer ratio of 1:5 in SIF at 37°C.
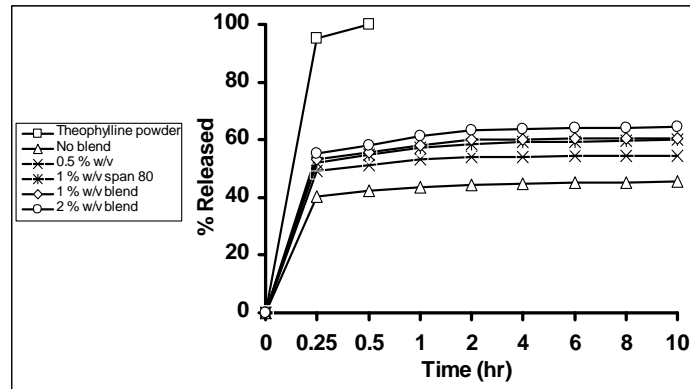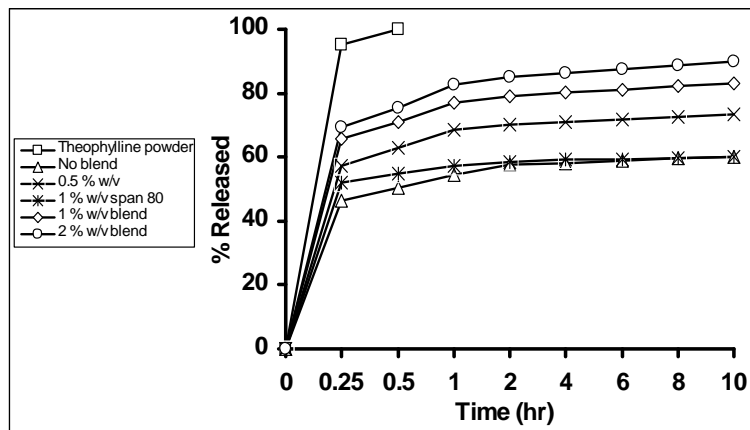

Fig. 14: Effect of different span 80 concentrations (% w/v) on the *in-vitro* release of theophylline loaded BSA microspheres prepared using drug: polymer ratio of 1:5 in SGF at 37°C.

Theophylline release from the BSA microspheres prepared using span 80 / tween 80 blend as a surfactant was delayed somewhat and not greatly, (Figs. 15&16). The amount of theophylline released after 2 hrs was 54%, when span 80 / tween 80 blend was used at a concentration of 0.5% (w/v). With increasing the amount of span 80 / tween 80 blend from 0.5 to 1 and 2%, the amounts of theophylline released were 60 and 63.5%, respectively.

Fig. 15: Effect of different span 80 / tween 80 blend concentrations (% w/v) on the *in-vitro* release of theophylline loaded BSA microspheres prepared using drug:polymer ratio of 1:5 in SIF at 37°C.
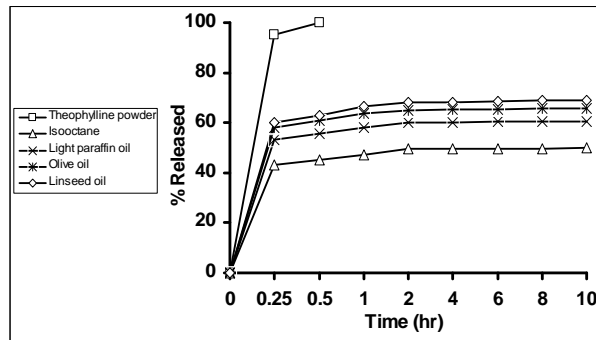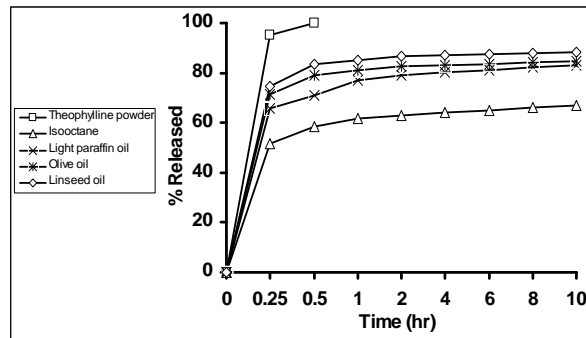

Fig. 16: Effect of different span 80 / tween 80 blend concentrations (%w/v) on the *in-vitro* release of theophylline loaded BSA microspheres prepared using drug:polymer ratio of 1:5 in SGF at 37°C.

76

Theophylline release from the BSA microspheres was increased with an increase in the viscosity of the dispersion medium. When isooctane was used, the amount of theophylline released after 2 hrs was 49.5%, while by increasing the viscosity using light paraffin oil, olive oil and linseed oil as dispersion media, the amounts of theophylline released were 60, 65 and 68% at the same selected period, respectively, (Figs. 17&18). It is obvious from (Figs. 19&20) that the release of theophylline was decreased with increasing glutaraldehyde concentration. With decreasing glutaraldehyde concentration from 25 to 5% v/v, the amount of theophylline released after 2 hrs increased from 68 to 85.46%.

Fig. 17: Effect of different dispersion media on the *in-vitro* release of theophylline loaded BSA microspheres prepared using drug: polymer ratio of 1:5 and span 80 / tween 80 blend concentration of 1% w/v in SIF at 37°C.

Fig. 18: Effect of different dispersion media on the *in-vitro* release of theophylline loaded BSA microspheres prepared using drug: polymer ratio of 1:5 and span 80 / tween 80 blend concentration of 1% w/v in SGF at 37°C.

77
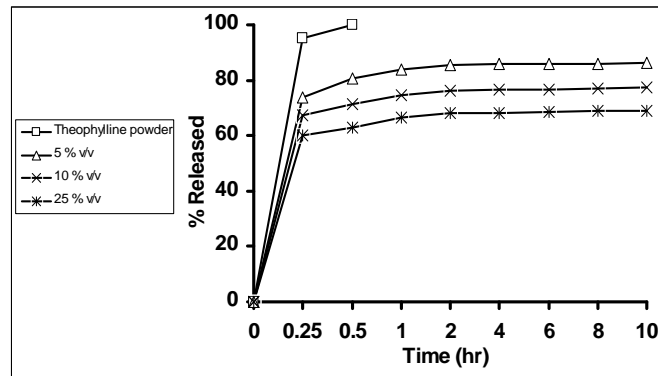
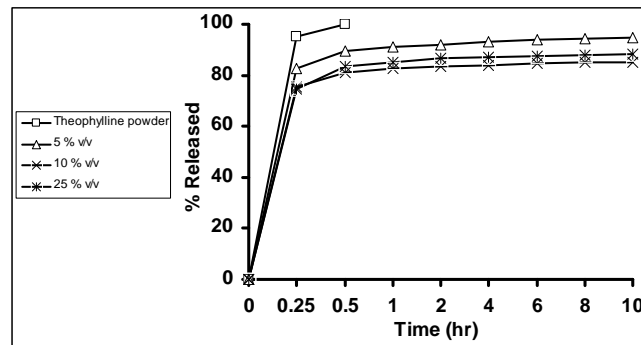**Fig. 19:** Effect of different glutaraldehyde solution concentrations (% v/v) on the *in-vitro* release of theophylline loaded BSA microspheres prepared using drug: polymer ratio of 1:5, span 80 / tween 80 blend concentration of 1% w/v and linseed oil as the dispersion medium in SIF at 37°C.

Fig. 20: Effect of different glutaraldehyde concentrations on the *in-vitro* release of theophylline loaded BSA microspheres prepared using drug: polymer ratio of 1:5, span 80 / tween 80 blend concentration of 1% w/v and linseed oil as the dispersion medium in SGF at 37°C.

Kinetics of the *in-vitro* drug release

Tables (1-5) show that theophylline release from albumin microspheres was according to Higuchi's diffusion model where it shows the highest correlation coefficient.

78

Table 1: Kinetic data for *in-vitro* release of theophylline from albumin microspheres using different drug: polymer ratios in simulated intestinal fluid (SIF) at 37°C.

Drug: polymer ratio	The correlation coefficient (r) according to the mechanisms of release		
	Zero order	First order	Higuchi's diffusion
1:2	0.723	0.774	0.816
1:3	0.708	0.714	0.802
1:4	0.729	0.747	0.826
1:5	0.743	0.779	0.874
1:6	0.724	0.749	0.847

__ The underline indicates the mechanism of best fit.

Table 2: Kinetic data for *in-vitro* release of theophylline from albumin microspheres using a drug: polymer ratio of 1:5 at different span 80 concentrations in simulated intestinal fluid (SIF) at 37°C.

Span 80 concentrations (% w/v)	The correlation coefficient (r) according to the mechanisms of release		
	Zero order	First order	Higuchi's diffusion
0.5	0.726	0.735	0.858
1	0.763	0.776	0.857
2	0.767	0.798	0.896

__ The underline indicates the mechanism of best fit.

Table 3: Kinetic data for *in-vitro* release of theophylline from albumin microspheres using a drug: polymer ratio of 1:5 at different span 80 / tween 80 blend concentrations in simulated intestinal fluid (SIF) at 37°C.

Span 80 / tween 80 blend concentrations (% w/v)	The correlation coefficient (r) according to the mechanisms of release		
	Zero order	First order	Higuchi's diffusion
0.5	0.689	0.782	0.794
1	0.724	0.769	0.845
2	0.708	0.740	0.849

__ The underline indicates the mechanism of best fit.

Table 4: Kinetic data for *in-vitro* release of theophylline from albumin microspheres using a drug: polymer ratio of 1:5 and span 80 / tween 80 blend concentration of 1% w/v in different types of dispersion media in simulated intestinal fluid (SIF) at 37°C.

Dispersion medium	The correlation coefficient (r) according to the mechanisms of release		
	Zero order	First order	Higuchi's diffusion
Isooctane	0.696	0.719	0.842
Light paraffin oil	0.744	0.769	0.845
Olive oil	0.725	0.727	0.825
Linseed oil	0.678	0.731	0.792

__ The underline indicates the mechanism of best fit.

Table 5: Kinetic data for *in-vitro* release of theophylline from albumin microspheres using a drug: polymer ratio of 1:5, span 80 / tween 80 blend concentration of 1% w/v and linseed oil as the dispersion medium at different glutaraldehyde solution concentrations (% v/v) in simulated intestinal fluid (SIF) at 37°C.

| Glutaraldehyde solution concentrations (% v/v)	The correlation coefficient (r) according to the mechanisms of release		
	Zero order	First order	Higuchi's diffusion
5	0.724	0.738	0.824
10	0.724	0.725	0.824
25	0.678	0.731	0.792

__ The underline indicates the mechanism of best fit.

Bioavalability

The obtained data are graphically illustrated in (Fig. 21). The pharmaco- kinetic parameters are represented in (Table 6).
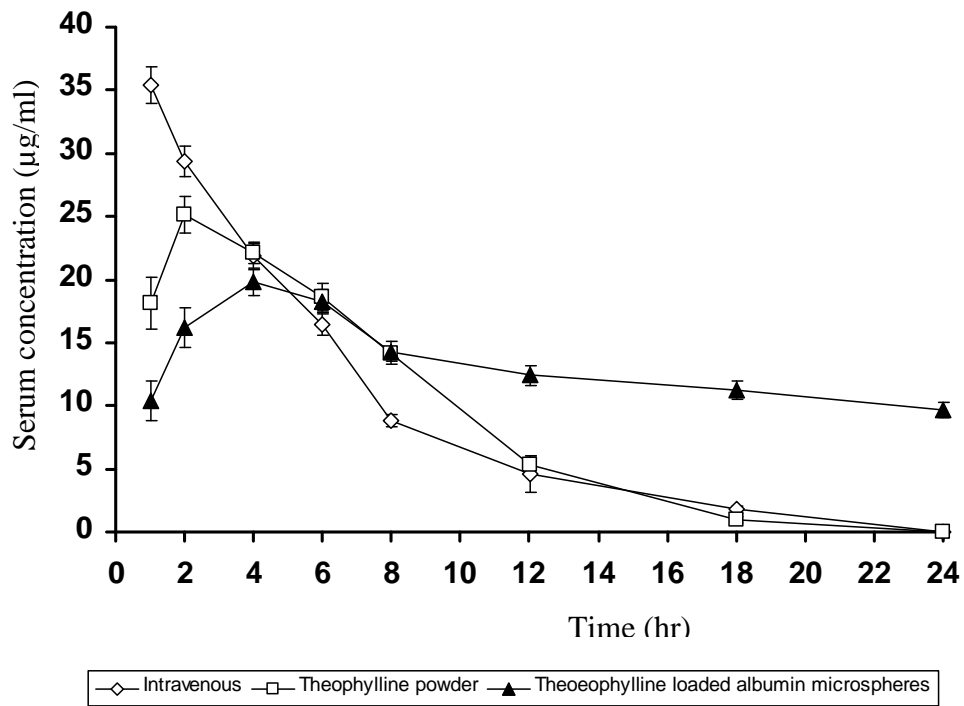
Fig. 21: Serum theophylline concentration (μg/ml) after intravenous injection and oral administration of theophylline powder and theophylline loaded BSA microspheres to rabbit.

Table 6: Pharmacokinetic parameters for *in-vivo* study of theophylline after intravenous injection and oral administration of theophylline loaded BSA microspheres and powder.

Parameter	Administered form		
	Intravenous injection	Oral	
		Powder	BSA microspheres
C max (μg/ml)	35.1	25.11	19.78
C min (μg/ml)	0.05	0.97	9.7
t max (hr)	1	2	4
K abs $(hr)^{-1}$	–	0.363	1.797
$t_{1/2}$ abs (hr)	–	1.908	0.385
K el $(hr)^{-1}$	0.201	0.230	0.030
$t_{1/2}$ el (hr)	3.013	3.440	22.61
MRT* (hr)	5.037	5.921	17.55
Volume of distribution (L)	0.648	1.145	1.565
Total clearance rate (ml/min)	2.177	4.391	0.799
AUC_{0-24} (μg. hr/ml)	184.6	211.84	311.65
AUC_{24-} (μg. hr/ml)	0	0	316.52
AUC_{0-} (μg. hr/ml)	184.6	211.84	628.18
Peak-to-trough difference** (μg/ml)	35.05	24.14	10.08
Relative bioavailability***%	–	–	147.11
Absolute bioavailability**** %	–	–	168.82
$AUMC_{0-24}$ (μg.hr^2/ml)	929.95	1254.45	3429.46
$AUMC_{24-}$ (μg.hr^2/ml)	0	0	7596.69
$AUMC_{0-}$ (μg.hr^2/ml)	929.95	1254.45	11026.16
C max / AUC_{0-24} $(hr)^{-1}$	0.190	0.118	0.063

– = Not relevant.

MRT* = Mean residence time.

Peak-to-trough difference** = C max – C min.

Relative bioavailability*** = (AUC_{0-24} for oral formula/AUC_{0-24} for powder)$\times$100.

Absolute bioavailability**** = (AUC_{0-24} for oral formula/AUC_{0-24} for intravenous) $\times$ 100.

Conclusion

Increasing the polymer content of the aqueous phase, span 80 concentration and viscosity of the dispersion medium decreased the size of the prepared microspheres. It was found that increasing glutaraldehyde concentration has no effect on the particle size range. Encapsulation efficiency is increased by increasing albumin content, surfactant concentration, viscosity of the dispersion medium and glutaraldehyde concentration. Drug release from microspheres is characterized by an initial rapid release of the drug ("burst" release), followed by slower release of the remaining drug. The controlled release formulae of theophylline BSA microspheres show good *in-vitro* controlled release characteristics, continued to exhibit a good *in-vivo* controlled release behavior. They show good relative bioavailability, a longer duration of action, a small peak-to-trough difference and a lower fluctuation compared to the intravenous theophylline or theophylline powder.

REFERENCES

1- J. J. Burger, E. Tomlinson, E. M. A. Mulder and J. G. McVie, Int. J. Pharm., 23, 333 (1985).

2- Y. Nishioka, S. Kyotani, M. Okamura, Y. Mori, M. Miyazaki, K. Okazaki, S. Ohnishi, Y. Yamamoto and K. Ito, Chem. Pharm. Bull., 37, 1399 (1989).

3- S. P. Vays, S. Bhatnagar, P. J. Gogol and N. K. Jain, Int. J. Pharm., 69, 5 (1991).

4- P. K. Gupta, C. T. Hung and D. G Perrier, ibid., 33, 147 (1986).

5- P.K. Gupta, C.T. Hung, F.C. Lam and D.G Perrier, ibid., 43, 167 (1988).

6- K. Egbaria and M. Frieddman, J. Control. Rel., 14, 79 (1990).

7- K. Sugibayashi, Y. Morimoto, T. Nadai, Y. Kato, A. Hasegawa and T. Arita, Chem. Pharm. Bull., 27, 204 (1979).

8- C. Jones, M. A. Burton and B. N. Gray, J. Pharm. Pharmacol., 41, 813 (1987).

9- I. Zoll, B. A. Rhodes and H. N. Wagner, Int. J. Appl. Radiat. Isot., 21, 155 (1970).

10- P. A. Kramer, J. Pharm. Sci., 63, 1646 (1974).

11- K. J. Widder, G. Flouret and A. Senyei, ibid., 68, 79 (1979).

12- B. Farhadieh, US Patent, 3, 922, 379 (1975).

13- T. K. Lee, T. D. Sokoloski and G. P. Royer, Science, 213, 233 (1981).

14- J. J. Burger, E. Tomlinson, E. M. A. Mulder and J. G. McVie, in: P. Buri and A. Gumma (Eds.), "Drug Targeting", Elsevier, Amsterdam, 1985, pp. 81-94.

15- A. E. Senyei, S. D. Reich, C. Gonczy and K. J. Widder, J. Pharm. Sci., 70, 389 (1981).

16- T. Kojima, M. Hashida, S. Muranishi and H. Sezaki, Chem Pharm. Bull., 26, 1818 (1978).

17- M. El-Samaligy and P. Rohdewald, Pharm. Acta Helv., 57, 201 (1982).

18- A. F. Yapel, US Patent, 4, 147, 767 (1979).

19- H. Sezaki, M. Hashida and S. Muranishi, in: H. Bund-Gaard, A.B. Hansen and H. Koford (Eds.), "Optimization of Drug Delivery", Munksgard, Copenhagen, 1982, pp. 316-339.

20- B. Tomlinson, J. J. Burger, E. M. A. Schoonder-Woerd, J. Kuik, F. C. Scholtz and J. G. McVie, "Human Serum Albumin Microspheres For Intraarterial Drug Targeting of Cytostatic Compounds. Pharmaceutical Aspects and Release Characteristics". In S.S. Davis, L. IIIum, J.G. McVie and E. Tomlinson (Eds.), "Microspheres and Drug Therapy", Elsevier, Amesterdam, 1984, pp.75-89.

21- M. J. Stoklosa and H. C. Ansel, in: "HLB System: Problems Involving HLB Values, Pharmaceutical Calculations", Middle East Ed., Mass Publishing Co., Egypt, 1997, pp 129-139.

22- M. S. Latha, K. Rathinam, P. V. Mohanan and A. Jayakrishnan, J. Control. Rel., 34, 1 (1995).

23- K. Maruyama, Y. Pongpaibul and M. Iwatsuru, ibid, 10, 17 (1989).

24- Wen-Ho Chuo, Tong-Rong Tsai, Shu-Hui Hsu and Thau-Ming Cham, Int. J. Pharm., 144, 241 (1996).

25- W. I. Higuchi, R. Okada and A. P. Lemberger, J. Pharm. Sci., 51, 683 (1962).

26- G. Q. Chen, W. Lin, A. G. A Coombes, S. S. Davis and L. Illum, J. Microencap., 11, 395 (1991).