Chitosan and Sodium Sulfate as Excipients in the Preparation of Prolonged Release Theophylline Tablets

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ABSTRACT

The major objectives of this study were to monitor the effect of cross-linking of cationic chitosan in acidic media with sulfate anion during granules preparation by wet granulation method prior tableting using theophylline (TPH) as a model drug. The prepared granules and the compressed tablets were subjected to in vitro evaluation. The properties of the prepared matrix granules and the compressed tablets were dependent on chitosan:sodium sulfate weight ratios, chitosan content and molecular weight of chitosan. The prepared granules of all batches showed excellent to passable flowability and were suitable for compression into tablets. Most of the granules were hard and expected to withstand handling during the subsequent compression into tablets. Granules with high friabilities were only those prepared with high amount of sodium sulfate or low amount of chitosan. Compression of granule batches yield non-disintegrating tablets that showed a decrease in tensile strength with the increase of sodium sulfate content at high chitosan:sodium sulfate weight ratio or with decrease of chitosan content. On the other hand, friability of tablets was increased in presence of excessive amount of sodium sulfate and low chitosan content as observed with

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granules. Slow TPH release from the formulated tablets was achieved at 1:0.5 and 1:1 chitosan:sodium sulfate weight ratios where all or most of the cationic chitosan and sulfate anions were utilized in a cross-linking reaction during wet granulation. Ratios of 1:2 and 1:3 showed fast drug release, which support the hypothesis that excessive unreacted water soluble sodium sulfate, might increase the porosity of the nondesintigrating tablets during dissolution. Slow drug release was also obtained with high molecular weight chitosan while changing the hardness of the tablets did not significantly change the release profile of the drug as long as the tablets are intact during dissolution. Furthermore, slow drug release was observed as the total amount of chitosan was increased in the formulated tablets. A comparative in vivo study between the chosen formulated tablets (1:1 chitosan:sodium sulfate ratio that contains 10% high molecular weight chitosan) and the commercial Quibron® tablets indicated prolonged appearance of the drug in dogs plasma for both formulations with no significant differences ($p>0.05$) in rate and extent of drug absorption. The formulated tablets showed 103.16 % bioavailability relative to that of the commercial tablets.

Key Words: Chitosan; sodium sulfate; theophylline; wet granulation; prolonged-release tablets.

INTRODUCTION

Chitosan is a natural polymer that has received a considerable attention as a drug carrier in the last few years. Chitosan is a particular N-deacetylated product obtained from the natural polymer, chitin, which is found widely in nature[1]. The polymer has been recently used for various biomedical and pharmaceutical applications[2,3]. At low pH, chitosan is present as a cationic polymer, with a high charge density, and therefore may function as a good flocculent for negatively charged ions in an ionic cross-linking process and can be used in the development of dosage forms with controlled release properties[4]. The principle of ionic cross-linking and precipitation of chitosan with sodium sulfate was previously utilized for the preparation of oral microspheres with slow drug release properties[5,6].

Chitosan has been formulated into different dosage forms such as membranes[7], microspheres[8]. It was also used in tablet formulations as one of the tablet components for different functions. As a diluent, chitosan was used for preparation of direct compressed tablets[9-11] where drug release was controlled. Chitosan also showed higher binder efficiency than other tablet binders such as methylcellulose and sodium carboxymethylcellulose[12] and used as a binder for colon specific drug delivery tablets with slow drug release compared with other polysaccharides or synthetic polymers[13]. Chitosan was utilized as tablets disintegrant at a concentration less than 10%[14] and showed bioadhesive properties in mixture with sodium alginate and in the form of thiolated chitosan derivative with slow drug release for intra-oral drug delivery tablets[15,16]. Furthermore, the solubilizing and amorphizing properties of low molecular weight chitosan toward naproxin made it an optimal carrier for developing fast release oral tablet[17]. Depending mainly on ionic

interaction, chitosan was also used for the preparation of tablets matrix to control drug release[18,19].

In this study, prolonged release tablet formulations were designed by cross-linking of chitosan in acidic media with sulfate ions during granules preparation by wet granulation method. The granules were then compressed into tablets using TPH as a model drug. Both the prepared granules and compressed tablets were subjected to in vitro evaluation. The bioavailability of TPH from the most promising tablet formulation was compared with one of the commercially available tablets formulation.

EXPERIMENTAL

Materials

Theophylline (TPH) and β-hydroxy-ethyl theophylline (Sigma Chemicals, Company, St. Louis, MO, USA); anhydrous sodium sulfate, calcium sulfate, acetic acid, ethanol 96% (BDH Laboratory Supplies, Poole, England); Chitosan high, medium and low molecular weights (Fluka Chemie AG, Buchs, Switzerland) were used as received. Solvents used for chromatographic determinations were high-performance liquid chromatography (HPLC) grade; all other reagents and solvents were of analytical grade.

Methods

Preparation of Granules

The calculated amounts of TPH, calcium sulfate and sodium sulfate were mixed together using a turbula mixer (Erweka, Germany). An aliquot of 1.5% w/v

chitosan solution (prepared by dissolving chitosan in equivolume of 5% v/v acetic acid and ethanol) was added to the powder blend. The volume of chitosan solution was precisely chosen to end up with the required chitosan:sodium sulfate weight ratio. The produced wet mass was then passed through a sieve of 1250 μm opening size and placed on Teflon coated foil. The produced granules were then dried in an air oven for 8 hours at 60 $^{\circ}$C. The size fraction of the granules that passed through the 800 μm sieve and retained on 500 μm one was used in the study.

Flow Properties of Granules

Flow properties of the prepared granules were evaluated by determining the angle of repose and compressibility index. Static angle of repose (θ^{o}) was measured according to the fixed funnel and freestanding cone method of Banker and Anderson[20].

Compressibility index (I) values of the granules were determined by measuring the initial volume (V_o) and final volume (V) after subjecting to 100 tapings in a graduated measuring cylinder using the equation:

$$I = [1\text{-}V/V_o)] \text{ x } 100$$

Each of the reported value of angle of repose and compressibility index was the average of three determinations.

Friability of Granules

The friability of the granules was determined using a Roche friability tester (TA3R, Erweka, Apparatebau, Germany). The drum of the friabilator was loaded with 10 gm of the granules being tested, and the tester was run for 4 minutes at 25 rpm. The sample was then shaken through a 500 μm sieve for 2 minutes, with the help of

the shaker, and the weight of the retained granules was assessed. The friability of the granules was calculated as a percentage according to the following equation:

(Initial weight - Final weight) / Initial weight x 100

Preparation of Tablets

The amount of 2% magnesium stearate was mixed with the prepared granules for 10 minutes using the Turbula mixer. The compositions of different tablet formulations are shown in Table 1. Theophylline tablets were prepared by compressing the treated granules using a flat-faced 11-mm punch tableting machine (Korsch, type EKO, Frankfort, Germany). Tablet weight was adjusted to contain 100 mg TPH.

Evaluation of Tablets

The hardness of prepared tablets was adjusted according to the required values (Erweka TBH 28, Frankfort, Germany). The tablets were evaluated for uniformity of thickness, friability (Erweka friabilator, model A3R, Frankfort, Germany), and disintegration time (Erweka, model ZT4, Heusenstamm, Germany) according to USP XXIV tests. The diametral compression test was used to determine the tensile strength T, using the formula [21]

$$T = 2P/\pi Dt$$

Where P is the applied stress, D is the diameter of the tablet, and t is the tablet thickness. Three tablets of each batch were subjected to tensile strength determination.

In Vitro Dissolution Studies

Determination of TPH release from different formulated tablets was performed using USP XXIV dissolution apparatus 2 at 50 rpm. Dissolution was tested either in 750 ml distilled water for eight hours or in simulated gastric fluid (without pepsin) at pH 1.2 for the first hour followed by dissolution in simulated intestinal fluid (without enzymes) at pH 7.5 for the remained seven hours at 37 ± 0.5 $^{\circ}$C. Drug release was monitored at 272 nm as a function of time using a Philips PU 8620 spectrophotometer connected with IBM computer model P530 equipped with PU 9605/60 tablet dissolution system soft ware (Pye Unicom Ltd., Cambridge, England). The data shown are the mean of three tablets.

Animal Experiments

Five male beagle dogs weighing 11.6 ± 1.4 Kg were used in this study in a crossover design. After fasting overnight, during which water was allowed freely, each dog was placed in an upright position in a restrainer stand. The dog's legs were shaven and cannulated through the cephalic vein using an 18-gauge cannula. A washout period of one week was allowed between successive dosing.

Dosing and Sampling

Doses of 200 mg of TPH in the form of the most promising formulated sustained release tablets (two 100-mg tablets) or as sustained-release commercial tablets (Quibron®) were given by oral intubations on two different occasions to dogs starved for 18 hours prior to the experiment. Blood samples (3ml) were collected just prior to tablet administration and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0 and 24 hours post-administration into heparinized tubes. The samples were

immediately centrifuged at 4000 rpm for 7.0 minutes and the plasma was separated and frozen at -20 °C pending analysis. The in vivo study adhered to the principles of laboratory animal care that is approved by the Research Center at the College of Pharmacy, King Saud University, Riyadh Saudi Arabia.

Analysis of Plasma Samples

Plasma concentration of TPH was assayed using a modified HPLC method [22]. The method involved the use of 300 µl aliquots of β-hydroxy-ethyl theophylline solution as the internal standard (5 µg /ml in 70% acetonitrile in water) to be added to 200 µl plasma samples in small plastic centrifuge tubes. The tubes were vortexed for 30 seconds and then centrifuged at 5000 rpm for 5 minutes. An aliquot of 25 µl of the supernatant solution for each sample was injected into the HPLC system (Waters Inc., Bedford, MA, USA). The HPLC separation was achieved with a µ-Bondapak C_{18} cartridge column (Waters Assoc.; 10 µm, 10 cm x 8 mm id) using a mobile phase consisting of acetonitrile:acetate (0.01 M) buffer (7:93) adjusted at pH 4.2 with a flow rate of 3 ml/minute. The effluent was monitored at 270 nm (Waters Assoc. M-481 variable wavelength ultraviolet detector). The concentration of TPH was determined using a constructed calibration curve prepared on the day of sample assay.

Pharmacokinetic Analysis

The maximum plasma concentration C_{max} and the time to reach that maximum T_{max} were obtained directly from the plasma concentration time curve. AUC_{0-24} was determined for each dog by the linear trapezoidal rule for the period of plasma sampling. The area of the tail was calculated using the plasma concentration at the last time point and the elimination rate constant K_{el} for the determination of $AUC_{0-\infty}$. The

values of K_{el} and accordingly $t_{½}$ were estimated from the least square regression analysis of the elimination segment of the curve. All results were expressed as mean ± standard deviation (mean ± SD).

Statistical Analysis

The significance of the differences between the in vitro data of the formulated granule and tablet batches as well as the differences between in vivo data of the chosen formulated tablets and the commercial sustained-release tablets were evaluated using analysis of variance (ANOVA) at a significant level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Although chitosan is insoluble in ethyl alcohol, chitosan in the acetate form (in presence of acetic acid) was soluble in the hydro-alcoholic solution at the given concentration (1.5% w/v). Addition of alcohol was necessary to facilitate the drying of the formed granules. The dissolved cationic chitosan, in acidic media, was cross-linked and precipitated with the aid of sulfate anion during wet granulation to produce matrix granules that may control drug release on tableting.

Preliminary results showed that dried granules of particle size range 500-800 μm were suitable to produce granules with acceptable physical characteristics. The prepared granules for all different batches, at this particle size range, showed excellent to passable flowability as indicated from the values of angel of repose and compressibility index (Table 2). These values ranged between 22.9 ± 0.12 and 35.2 ± 0.10 degrees for angles of repose, while they were between 10.0 ± 0.06 and 23.0 ± 0.15 % for compressibility indices. This may indicate that all prepared granules of different batches, regarding flowability, are suitable for compression into tablets. It

was also observed that batches of the prepared granules were having different friabilities that ranged between 1.47 and 4.53 (Table 2). Most of the prepared granules were hard with friability less than 3% and expected to withstand handling during the subsequent processing into tablets. Evaluation of friability of granules was previously utilized to determine the suitability of granules for tableting[21]. These results may also indicate a successful cross-linking interaction in most cases. Granules of high friability were only those prepared with high amount of sodium sulfate or low amount of chitosan. Granules prepared at chitosan:sodium sulfate weight ratios of 1:2 and 1:3 at 10% chitosan showed 3.53 and 4.43 friabilities respectively, while the friability was 4.53 for granules contained only 3% chitosan at 1:1 chitosan:sodium sulfate weight ratio. These results also indicate the role of chitosan for matrix formation and the possible binding effect that are efficient at high chitosan content (more than 3%). Those effects may be altered in the presence of high amount of unreacted sodium sulfate. Furthermore, table 2 shows that high molecular weight chitosan can produce more flowable and less friable granules compared with those prepared with low and medium molecular weight chitosan.

Generally, all the prepared TPH tablet batches showed good appearance and were non-disintegrating. The diameter of tablets was chosen to be 11 mm in order to minimize differences in surface area. The average surface area of tablets of all tested batches was 3.48 ± 0.128 cm^2 (deviations were less than 4%). The other physical characteristics of the prepared tablets are shown in Table 3. It was clear that changes of chitosan:sodium sulfate ratio is accompanied with changes in the physical properties of the produced tablets. Tablets showed a significant decrease in tablets tensile strength ($p<0.05$) with the increase of chitosan:sodium sulfate ratio although all tablets were adjusted at same hardness (6.0 Kp). Tablets of chitosan:sodium sulfate

weight ratios 1:2 and 1:3 showed the most decrease in tensile strengths where excessive amount of sodium sulfate is present in formulations. On the other hand, friability of tablets was increased in presence of excessive amounts of sodium sulfate and clearly exceeded 1.5% at chitosan:sodium sulfate weight ratios of 1:2 and 1:3.

The increase in percentage of chitosan in tablets from 3% up to 10% (at 1:1 chitosan:sodium sulfate ratio) was also associated with a decrease in the tensile strength of the tablets from 8.75 ± 0.210 at 3% chitosan content to 7.44 ± 0.141 at 10% which could be due to increase of tablet thickness although matrix intensity was obviously increased with increase of chitosan content. It was also interesting to observe that as the tablet content of chitosan was increased the friability of the tablets decreased and this could be due to the increase in matrix intensity at high concentration of chitosan and/or due to the binding effect of chitosan. The same observation was noticed for friability of the granules before tableting (Table 2).

Table 3 shows also the effect of molecular weight of the used chitosan on some physical properties of tablets. At hardness 6.0 Kp the tablets showed only small but non-significant change ($p>0.05$) in both tensile strength and friability on using chitosan of low, medium or high molecular weights. This could indicate that thickness of the tablets and the firmness of matrix are not significantly affected by changing chitosan molecular weights. On the other hand, the change of tablets hardness (using high molecular weight chitosan) showed to change tablets' thickness, tensile strength and friability (Table 4). The increase of tablet hardness from the value of 4.0 up to 10.0 Kp was accompanied with significant ($p< 0.05$) increases of tensile strength of tablets from 4.55 ± 0.111 up to 13.37 ± 0.207 Kp/cm^2 with decreases in tablets' thickness while, the increase of tablets' hardness made the tablets less friable as expected.

Figures 1-4 shows the dissolution properties of tablets prepared from different tablet batches. The dissolution properties were dependent on chitosan:sodium sulfate weight ratios, chitosan content and type of chitosan use. Figure 1 shows that in absence of sodium sulfate, chitosan was not sufficiently able to retard TPH release. As the ratio of chitosan:sodium sulfate increased, the release of the drug slowed down till we reached the slowest drug release at 1:1 ratio. At low sodium sulfate content fast drug release as well as high burst effect (initial drug release) was observed because of limited cross-linked matrix. On the other hand, in presence of high sodium sulfate content at high chitosan:sodium sulfate ratio (1:2 and 1:3) the drug release was fast again and all drug was released within about one hour with high initial drug release. This could indicate that, excessive un-reacted water-soluble sodium sulfate might increase the porosity of the non-disintegrating tablets during dissolution which allowed for fast drug release as well as high burst effect. While, at ratios 1:0.5 and 1:1 controlled theophylline release was observed where most or all of the cationic chitosan and sulfate ions were utilized in a cross linking reaction during wet granulation with higher initial drug released ,25 %, for 1: 0.5 ratio compared with 14.4 % for 1:1 ratio (Figure 1). This indicates also that optimizing chitosan:sodium sulfate ratio is an essential requirement for control of drug release.

The dissolution study revealed also that, at 1:1 chitosan:sodium sulfate ratio, the change of total amount of chitosan in the tablets showed to significantly affect the dissolution properties of the tablets ($p<0.05$). Figure 2 shows that as the chitosan content was increased, the release of drug was decrease and that could be attributed to the role of chitosan for matrix formation with the aid of sulfate ions and possible binding effect of chitosan. These mutual effects are expected to be responsible to slow down the release of the drug. Furthermore, high molecular weight chitosan, at 1:1

chitosan:sodium sulfate ratio, was the most suitable to give slow drug release (Figure 3) compared with both medium and low molecular weight chitosan. It was also interesting to notice that increasing in tablets' hardness, although it changed their physical properties such as the tablets' tensile strength and friability, it did not show significant effects on drug release ($p>0.05$) at tested dissolution times as shown in figure 4. At all tested hardness, tablets did not disintegrate during dissolution even at low hardness value (4.0 Kp). Thus, changing of tablets hardness could be utilized only to optimize the physical properties of the tablets with no effect on drug release as long as the tablets are intact during dissolution.

Formula D (1:1 chitosan:sodium sulfate ratio contained 10% high molecular weight chitosan) was selected for further in vivo study as it showed good acceptable physical properties for both granules and tablets and showed slow TPH release from the tablets in vitro. Formula D was subjected to comparative study with one of the commercially available TPH tablets (Quibron®). The selected formula and commercial tablets were first compared for in vitro dissolution in both distilled water for 8 hours and in simulated gastric fluid (without pepsin) pH 1.2 for the first hour followed by dissolution in simulated intestinal fluid (without enzymes) pH 7.5 for the remained seven hours. It was interesting to observe that changing the dissolution medium did not significantly change the release of TPH for both formulations at all tested dissolution times ($P>0.05$). Although chitosan is known to be soluble in acid and form viscous gel, the formulated tablets remained intact during dissolution course where cross-linked chitosan was compressed in presence of the other selected excipients**.** The proposed formulated tablets also showed much slower theophylline release profile than that of commercial tablets (Quibron®) in both dissolution media which is essential for the design of controlled release drug delivery system depending

on diffusion of drug through a matrix especially when it is a biodegradable. Most dissolution studies for theophylline release from matrix dosage form require long dissolution time (mostly for 8 hours). Thus, In vivo comparative studies of such two formulations could be essential.

Both formulations (The selected formula D and commercial tablets) were administered orally in two separate studies to beagle dogs. The mean (± SD) plasma concentration profiles for both tablet formulations are shown in Figure 6. The results showed comparable plasma concentration profiles with prolonged appearance of drug in plasma in detectable amounts for up to 24 hours. The mean pharmacokinetic parameters of TPH following oral administration of the formulated and commercial tablets are shown in table 5. The maximum plasma concentration C_{max} and the time to reach this maximum concentration T_{max} obtained from the mean of the individual plasma concentration-time data for each dog were 17.20±3.21 μg/ml and 6.0±0.89 h. respectively, for the proposed tablet formulation, while they were 19.36±5.6 μg/ml and 5.2 ±1.09 h. respectively, for the commercial Quibron® tablets. No significant differences in C_{max} and T_{max} for the two formulations were noticed ($p>0.05$) which could indicate comparable absorption of TPH from both formulations. This was also supported with the determined absorption rate which is defined as $C_{max}/AUC_{0-\infty}$ that was 0.081 ±0.019 and 0.0978 ± 0.018 h^{-1} for formulated and commercial tablets, respectively, with no significant differences ($p>0.05$). This ratio has been used for evaluation of absorption of prolonged-release formulations[23]. The area under plasma concentration curve, that indicates extent of drug absorption, was 184.40 ± 45.32 μg.h/ml in 24 hours and 213.10 ± 62.11 μg.h/ml up to infinite time for formulated tablets compared with 187.57 ± 65.78 and 206.57 ± 71.56 μg.h/ml, respectively for commercial tablets with no significant differences between the two formulations

($p>0.05$). Furthermore, the relative bioavailability of the formulated to the commercial tablets was 103.16 %. Table 5 also shows that the half life ($T_{1/2}$) of TPH was 6.90 ± 1.54 h for the formulated tablets and 5.53 ± 2.04 for the commercial tablets with no significant differences ($p>0.05$). Thus, the above results revealed that there were no significant differences in rate and extent of TPH absorption between the formulated and commercial tablets. Moreover, in this unbalanced crossover study (5 dogs), period and sequence effects were tested and found to have no influence on the final conclusion of no significant differences between treatments.

In conclusion, this study is offering a simple method for the preparation of a prolonged release tablets depending on the cross-linking of chitosan with sulfate anion during wet granulation process. The proposed tablets showed physical and release characteristics that can be controlled to obtain an optimized system. One of the proposed formulations showed comparable bioavailability with a commercial sustained release tablets.

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TABLE 1

Composition of Formulated Tablets [a]

Formula	TPH (mg)	CH (mg)	Na_2SO4 (mg)	$CaSO_4$ (mg)	Mg Stearate (% w/w)
A	100	75.0	0.0	500	2.0
B	100	75.0	18.75	500	2.0
C	100	75.0	37.5	500	2.0
D	100	75.0	75.0	500	2.0
E	100	75.0	150	500	2.0
F	100	75.0	225	500	2.0
G	100	18.75	18.75	500	2.0
H	100	37.5	37.5	500	2.0
I	100	56.3	56.3	500	2.0

[a] The particle size range of compressed granules was 500 – 800 μm for all batches.

TABLE 2

Effect of Chitosan:sodium Sulfate Weight Ratio, Chitosan % (w/w) and Molecular Weight of Chitosan on Granules Characteristics (n =3) [*].

Process Variables	Values of Variables	Angle of repose (°)	Compressibility Index (%)	Friability (%)
[a]Chitosan:sodium Sulfate Weight Ratio (w/w)	1 : 0.00	29.4 ± 0.32	14.0 ± 0.06	1.47
	1 : 0.25	32.6 ± 0.21	15.0 ± 0.06	1.79
	1 : 0.50	31.2 ± 0.10	10.0 ± 0.06	1.53
	1 : 1.00	22.9 ± 0.12	14.0 ± 0.03	2.19
	1 : 2.00	34.6 ± 0.21	17.0 ± 0.10	3.53
	1 : 3.00	35.2 ± 0.10	17.0 ± 0.03	4.43
[b]Chitosan % (w/w)	3.0 %	32.0 ± 0.17	23.0 ± 0.15	4.53
	5.0 %	30.5 ± 0.08	14.0 ± 0.10	2.83
	8.0 %	30.9 ± 0.06	16.0 ± 0.13	2.39
	10.0 %	22.9 ± 0.12	14.0 ± 0.03	2.19
[c]Molecular Weight of Chitosan	Low	32.8 ± 0.01	16.0 ± 0.10	2.56
	Medium	33.1 ± 0.06	18.0 ± 0.09	2.30
	High	22.9 ± 0.12	14.0 ± 0.03	2.19

[*] The particle size range was 500 – 800 μm for all batches.
[a] Chitosan content was 10% using high molecular weight chitosan.
[b] Chitosan:sodium sulfate weight ratio was fixed at 1:1(w:w) using high molecular weight chitosan.
[c] Chitosan:sodium sulfate weight ratio was fixed at 1:1 (w:w) and Chitosan content was 10% w/w.

TABLE 3

Effect of Chitosan:sodium Sulfate Weight Ratio, Chitosan % (w/w) and Molecular Weight of Chitosan on Tablets' Characteristics (n =3) [*].

Process Variables	Values of Variables	Tablets Formula	Thickness (mm)	Tensile Strength (Kp/cm^2)	Friability (%)
[a]Chitosan:Sodium Sulfate Weight Ratio (w/w)	1:0.00	A	4.47 ± 0.067	7.78 ± 0.117	1.13
	1:0.25	B	4.51 ± 0.081	7.69 ±0.131	1.22
	1:0.50	C	4.59 ± 0.097	7.57 ± 0.158	1.35
	1:1.00	D	4.67 ± 0.089	7.44 ± 0.130	1.57
	1:2.00	E	4.99 ± 0.199	6.96 ± 0.145	1.91
	1:3.00	F	5.23 ± 0.131	6.64 ± 0.138	2.12
[b]Chitosan % (w/w)	3.0	G	3.97 ± 0.095	8.75 ± 0.210	2.21
	5.0	H	4.25 ± 0.093	8.17 ± 0.179	2.15
	8.0	I	4.48 ±0.087	7.75 ± 0.151	2.03
	10.0	D	4.67 ± 0.089	7.44 ± 0.130	1.57
[c]Molecular Weight of Chitosan	Low	D	4.74 ± 0.090	7.33 ± 0.139	1.59
	Medium	D	4.70 ± 0.087	7.39 ± 0.136	1.61
	High	D	4.67 ± 0.089	7.44 ± 0.130	1.57

[*] The hardness of all tablets was kept at 6.0 Kp.
[a] Chitosan content was 10% using high molecular weight chitosan.
[b] Chitosan:sodium sulfate weight ratio was fixed at 1:1(w:w) using high molecular weight chitosan.
[c] Chitosan:sodium sulfate weight ratio was fixed at 1:1 (w:w) and Chitosan content was 10% w/w.

TABLE 4

Effect of Hardness on tablets characteristics (n=3).

Formula	Hardness (Kp)	Thickness (mm)	Tensile strength (Kp/cm^2)	Friability (%)
D	4.0	5.09 ± 0.119	4.55 ± 0.111	1.83
D	6.0	4.67 ± 0.089	7.44 ± 0.130	1.57
D	10.0	4.33 ± 0.067	13.37 ± 0.207	1.41

- Tablets contained high molecular weight chitosan.

TABLE 5

Pharmacokinetic Parameters (Mean ± SD) of Theophylline after Oral Administration of Formulated Sustained Release (SR) and Commercial SR (Quibron®) Tablets to Dogs (n = 5).

Pharmacokinetic Parameters	Formulated SR Theophylline Tablets	Commercial SR Theophylline Tablets
C_{max} (μg/ml)	17.20 ± 3.21	19.36 ± 5.60
T_{max} (h)	6.0 ± 0.89	5.2 ± 1.09
C_{max}/AUC (h^{-1})	0.081 ± 0.019	0.098 ± 0.018
K_e (h^{-1})	0.100 ± 0.099	0.1513 ± 0.079
$T_{½}$ (h)	6.90 ± 1.54	5.53 ± 2.04
AUC_{0-t} (μg . h/ml)	184.40 ± 45.32	187.57 ± 65.78
$AUC_{0-\infty}$ (μg . h/ml)	213.10 ± 62.11	206.57 ± 71.56
Relative bioavailability	103.16 %	-

Figures Captions

Figure 1. Effect of chitosan:sodium sulfate weight ratio on theophylline release from formulated tablets (n = 3).

Figure 2. Effect of % of chitosan content on theophylline release from formulated tablets (n = 3).

Figure 3. Effect of chitosan molecular weight on theophylline release from formulated tablets (n = 3).

Figure 4. Effect of tablets hardness on theophylline release from formulated tablets (n = 3).

Figure 5. Release of theophylline from selected tablets formula (formula D) and commercial tablets(Quibron®) in different dissolution media.

(I) Distilled water for 8 hours.

(II) Simulated gastric fluid (without pepsin) pH 1.2 for 1 hour followed by simulated intestinal fluid (without enzymes) pH 7.5 for 7 hours.

Figure 6. Mean (± SD) plasma concentration-time curves of theophylline following oral administration of formulated and commercial sustained release (Quibron®) tablets.

Figure 1

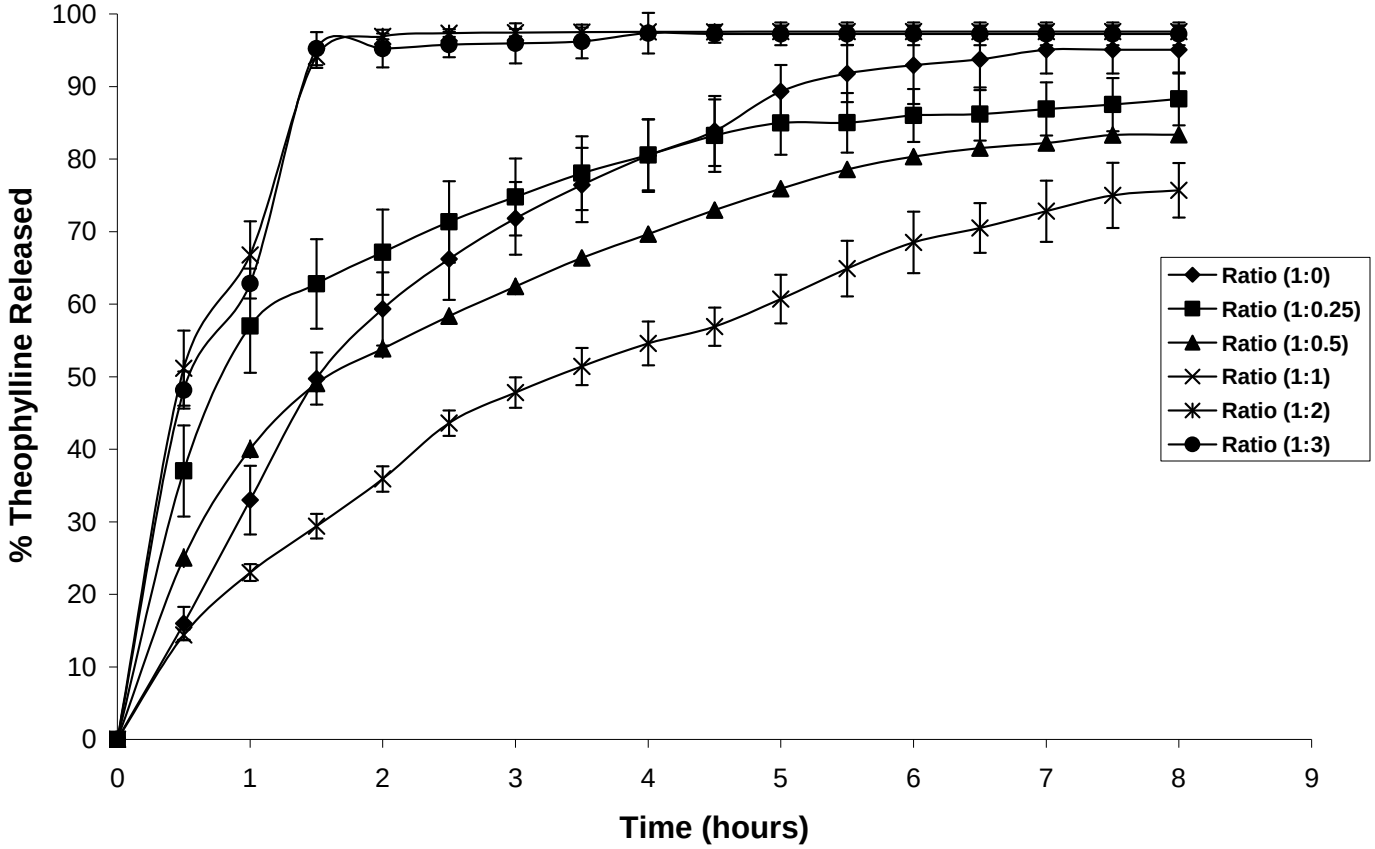

% Theophylline Released
Time (hours)
Ratio (1:0)
Ratio (1:0.25)
Ratio (1:0.5)
Ratio (1:1)
Ratio (1:2)
Ratio (1:3)

Figure 2

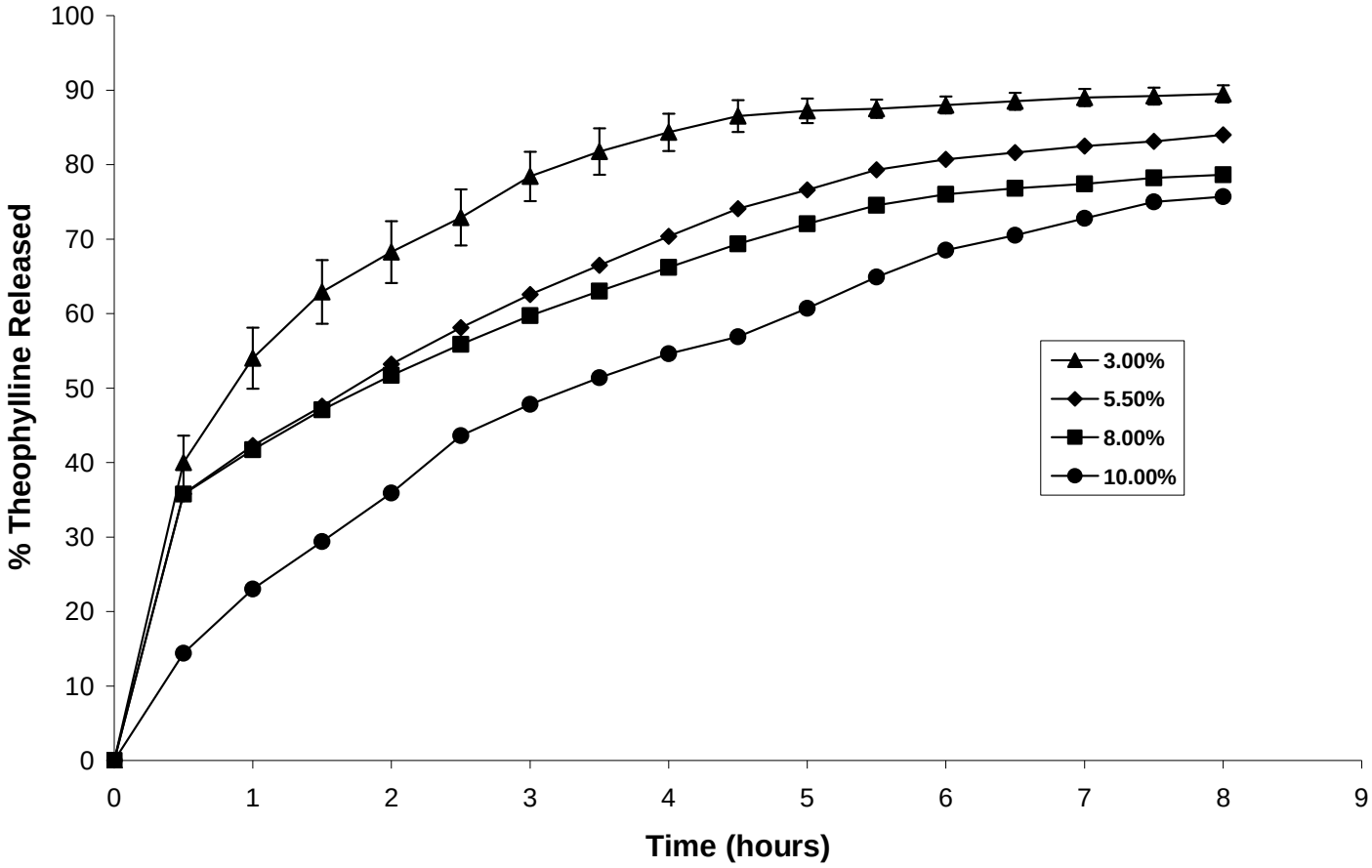


Figure 3

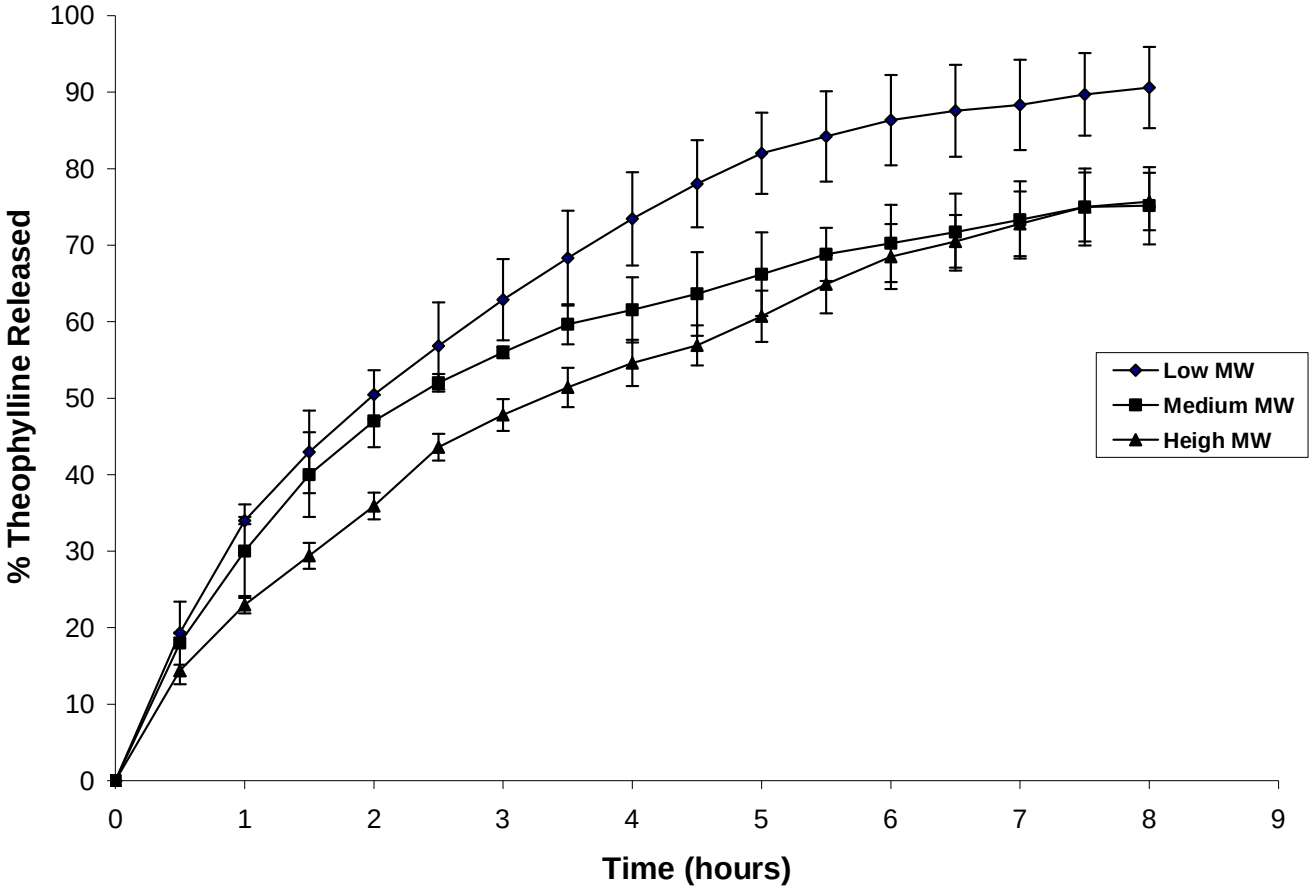

% Theophylline Released
Time (hours)
Low MW
Medium MW
Heigh MW
0
10
20
30
40
50
60
70
80
90
100
0
1
2
3
4
5
6
7
8
9

Figure 4

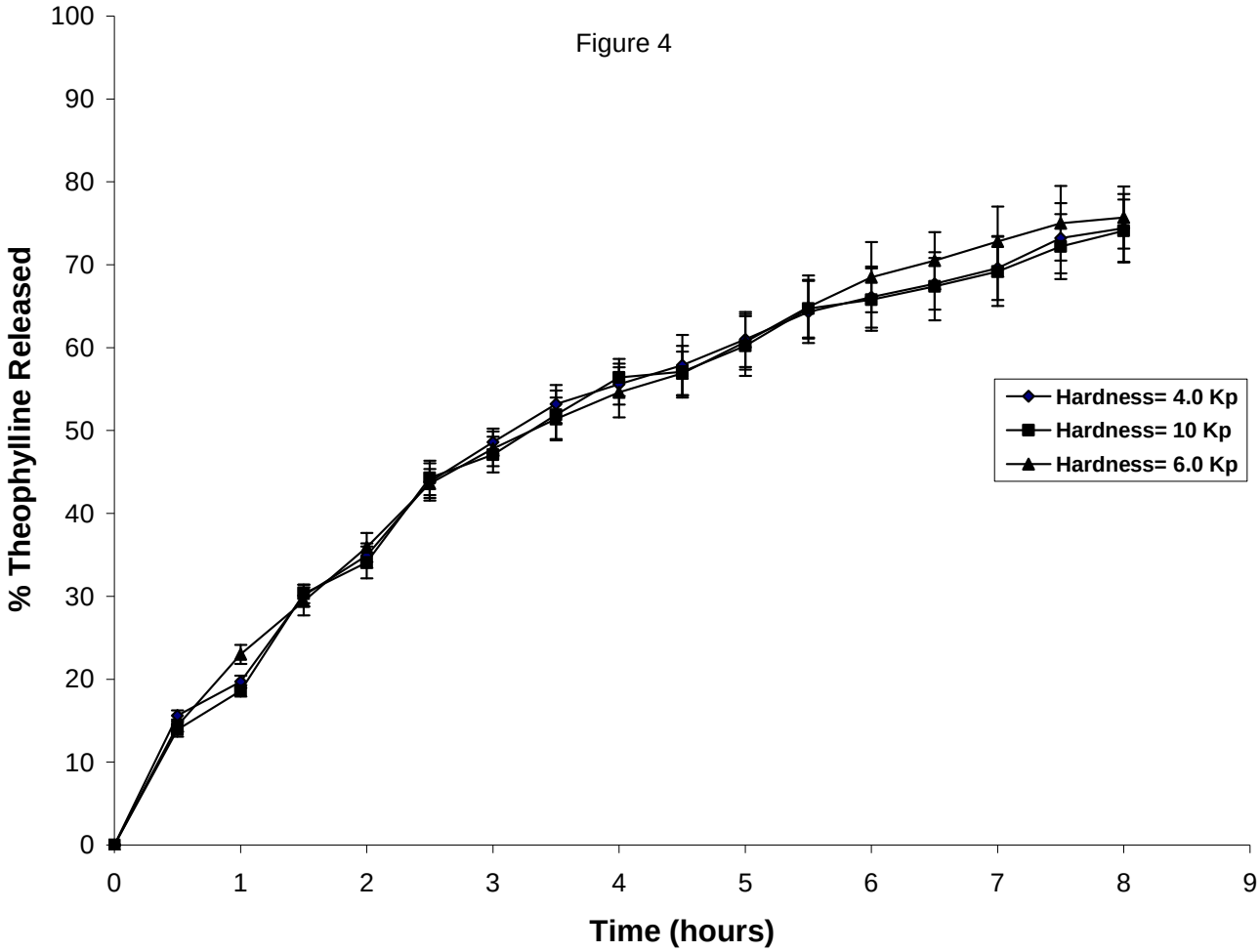

% Theophylline Released
Time (hours)
Hardness= 4.0 Kp
Hardness= 10 Kp
Hardness= 6.0 Kp
0
10
20
30
40
50
60
70
80
90
100
0
1
2
3
4
5
6
7
8
9

Figure 5

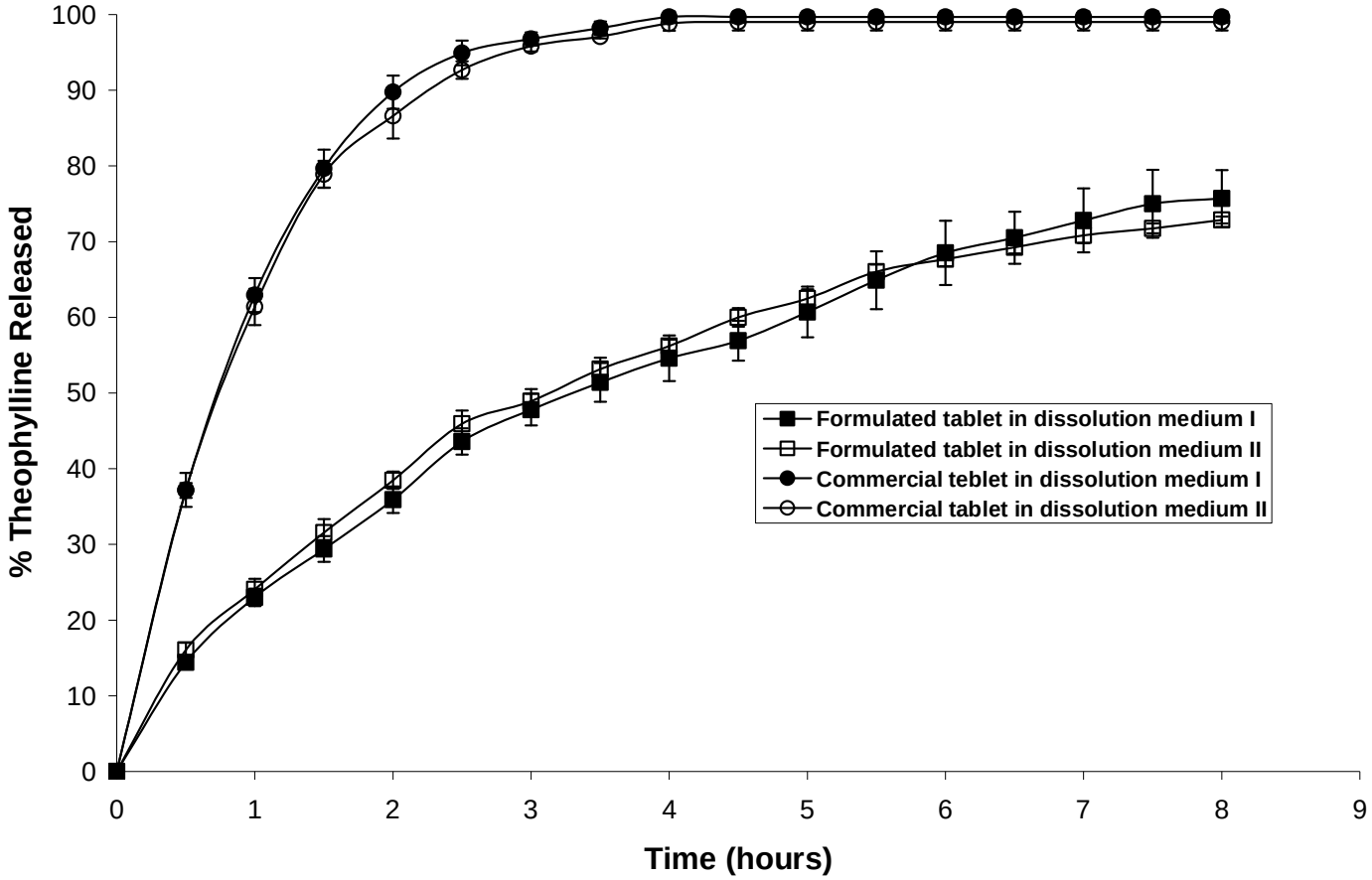
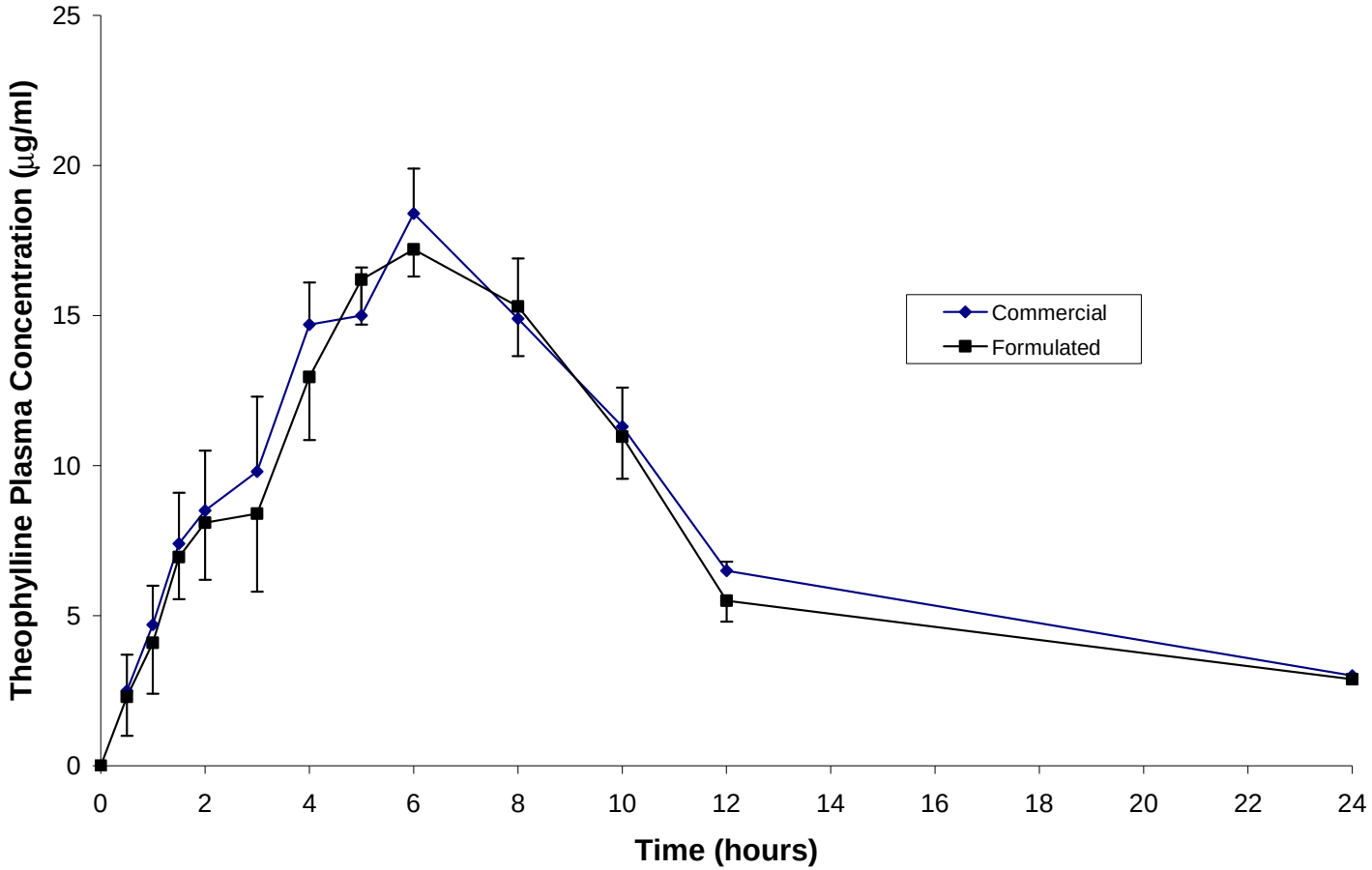

% Theophylline Released
Time (hours)
0
10
20
30
40
50
60
70
80
90
100
0
1
2
3
4
5
6
7
8
9
Formulated tablet in dissolution medium I
Formulated tablet in dissolution medium II
Commercial teblet in dissolution medium I
Commercial tablet in dissolution medium II

Figure 6


Theophylline Plasma Concentration (μg/ml)
25
20
15
10
5
0
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (hours)
Commercial
Formulated