Application of an excipient made from chitosan and xanthan gum as a single component for the controlled release of Ambroxol.

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Original Paper

ABSTRACT

An oral controlled release (CR) matrix system of Ambroxol hydrochloride was developed using a binary hydrophilic polymer mixture of chitosan (CH) and xanthan gum (XG) (1:1 w/w ratio). Two test tablet formulations were prepared using drug to polymer mixture ratios (D:P) of 1:1 and 1:3 (w/w), designated as T1 and T2, respectively. The *in vitro* drug release data was best fitted to the Higuchi equation. The 1:1 ratio (T1) demonstrated *in vitro* dissolution similarity with the commercial product, Mucosolvan LA. A preliminary *in vivo* study was performed using six volunteers. The study was designed to include open, randomized, single-dose, three-treatment, six-sequence, crossover (Williams design) under fasting conditions. The data showed that T1 was bioequivalent to Mucosolvan LA $^®$ after the administration of a single 75mg oral dose. Two *in vivo, in vitro* correlations (IVIVC) were established between C_{max} versus the fraction of drug dissolved (FRD) after 4 hours, and AUC versus the ratio of fraction of drug dissolved (FRD) after 10 hours where a multiple point level C correlation of IVIVC was obtained.

KEY WORDS: Ambroxol hydrochloride, xanthan gum, chitosan, controlled release, bioequivalence

INTRODUCTION

Matrix type formulations are the most commonly used controlled release (CR) systems. These are prepared from either swellable hydrophilic polymers or non-swellable lipophilic excipients or combinations thereof.

Currently, hydrophilic matrices are the most widely used CR systems. They control drug release by mechanisms that include swelling/erosion and dissolution/diffusion (1-5). Most of the hydrophilic matrices utilize the synergistic binary mixture of anionic and cationic polymers. An interesting example is the binary matrix system consisting of xanthan gum (XG) combined with galactomannans or glucomannans. Such a system was found to be applicable for a wide range of drugs. The

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mechanism of retardation was simply based on the formation of a tight gel in an aqueous environment (6).

The combination of anionic, e.g., alginate (ALG) or XG and cationic polymers, e.g., chitosan (CH), have been reported to form an insoluble hydro gel which slowly dissociates in acidic medium (7-8). Polymeric platforms such as spray dried matrices (9), microcapsules (10), microspheres (11), mucosal adhesive tablet matrices (12), chitosan-reinforced alginate gel beads (13) , direct-compression tablets (14) , hot melt extruded tablets have been developed utilizing CH/ALG or CH/XG mixtures (15). In a previous study a universal CR matrix system made from a binary CH/XG polymer mixture was patented (16). Such combinations have been widely reported in the literature (17-19). Chitosan, shown in Figure 1, is a natural cationic polysaccharide obtained by the N-deacetylation of chitin. It consists of β-1, 4-Linked D-glucosamine units (20). Xanthan gum (XG) also shown in Figure 1, is an anionic exocellular polysaccharide produced by aerobic fermentation of sugars by the bacterium, *Xanthomonas campestris*. Its main chain consists

Figure 1 Chemical structure of chitosan, xanthan gum and Ambroxol

of a linear 1, 4-linked β-D-glucose backbone substituted on every two units with a charged trisaccharide side chain (21). In the present study Ambroxol HCl was used as a model drug. Ambroxol HCl (Figure 1) is an active metabolite of the mucolytic agent bromhexine and is used for the treatment of bronchitis to improve expectoration. The drug is rapidly absorbed after oral administration with an elimination half-life of 3–4 hours requiring three doses per day for optimum therapeutic efficacy. Several sustained release formulations have been developed based on tablet, pellet or capsule dosage forms allowing once daily administration of Ambroxol HCL with the aim of improving patient compliance (22-25).

The aim of the current study was to examine the potential for the sustained delivery of Ambroxol HCl from a matrix tablet formulation comprising a hydrophilic polymer mixture of CH and XG and to correlate the *in vitro* release profiles with *in vivo* results. In addition, a preliminary pilot bioequivalence fasted study was performed using Mucosolvan LA® as a control drug.

MATERIALS AND METHODS

Materials

Chitosan (CH) (degree of deacetylation 93%, viscosity (1.0%, in 1.0% acetic acid) < 20 mPa.s, at 25°C, particle size passes through 80/100 mesh, molecular weight of 250 KDa pharmaceutical grade, (Batch No. F000802), was purchased from Xiamen Xing DA Co. Ltd., (China). Xanthan gum (XG) viscosity of 1% in 1% KCl solution of 1492 mPa.s at 25 ºC, particle size passes through 80/200 mesh, food & pharmaceutical grade, (Lot No. 1949/ 01.05), was purchased from Jungbenzlauer Ges. M.B.H. Handelsgericht Wien, (Germany). Ambroxol HCl (particle size passes through 80/100 mesh was purchased from Sifavitor Company, (Italy). The commercially available Mucosolvan LA® capsule (containing 75mg Ambroxol HCl and the following excipients:
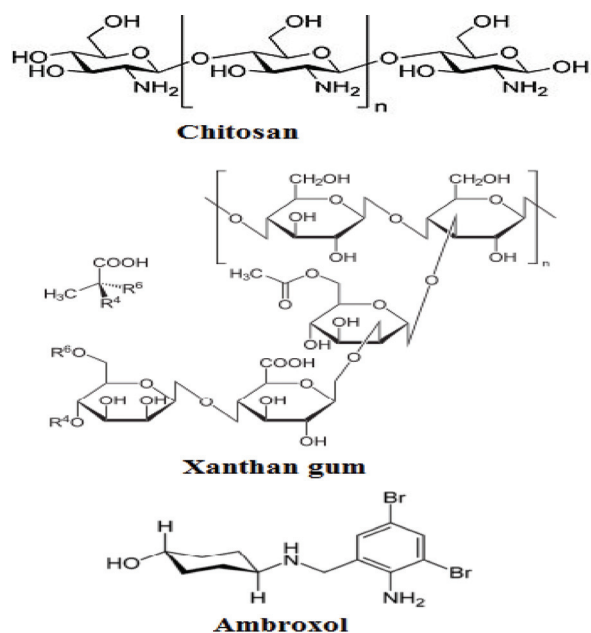
carnuba wax, gelatin, magnesium stearate, crospovidone, stearyl alcohol and coloring agents, B.N. 104708, Boehringer Ingelheim Pharma KG, Germany) was used as a reference (R) and were purchased locally.

Tablet preparation

Based on a preliminary study, two test formulations (T1 and T2), each containing 75 mg Ambroxol HCl and a polymer mixture of CH:XG (1:1 w/w) were prepared. The drug to polymer mixture ratio (D:P) was set at 1:1 and 1:3 w/w for (T1) and (T2), respectively. The Composition of the hydrophilic matrix tablets (T1 and T2) is listed in Table 1. The tablets were prepared by direct compression. The components of each tablet were gently mixed using a porcelain mortar and pestle for 10 minutes. The mixture was then transferred manually into the die of a hydraulic press. At least 40 biplanar tablets with a 11 mm diameter were manufactured by applying a pressure of 450 MPa for 15 seconds.

Table 1 Tablet formulations of T1 and T2

Tablet Reference	Ambroxol Hcl (mg)	CH (mg)	XG (mg)
T1	75	37.5	37.5
T2	75	112.5	112.5

Physical characterization of tablets

Testing for variation in tablet weight, drug content, hardness and friability were performed according to the directions in the General Chapters of USP 24/NF 19.

Weight variation was determined based on 20 tablets using an electronic balance (using a Mettler Toledo balance, AG135, Mettler Toledo, GmbH, Greifensee, Switzerland). Tablet hardness was determined using a minimum of 6 tablets of each formulation using a Monsanto (Standard type) tablet hardness

tester. Friability was determined based on 20 tablets using a Cambell Electronic Friabilator for 4 minutes at 25 RPM. Drug content uniformity of the formulated tablets was determined in triplicate. 20 tablets were crushed using a mortar and pestle. Accurately weighed quantities of the resulting powder equivalent of 75 mg Ambroxol HCl was dissolved in a phosphate buffer at pH 6.8. After suitable dilution, the drug amount was determined using a UV spectrophotometer (PDA Multi-spec UV-1501, Shimadzu, Japan). All calculations were performed with reference to a prepared calibration curve at λ_{max} 306 nm.

In vitro release study

Ambroxol HCl release rates from test formulations (T1 and T2) tablets and a reference (R), Mucosolvan LA® capsules were determined using USP dissolution apparatus I (Basket method) in 500 ml of 0.1M HCL with stirring speed set at 75 RPM for 2 hours at 37°C ± 0.5 using a dissolution tester (Erweka DT-6, Germany). To simulate the GI tract, the acidic medium was decanted and replaced with 500 ml of phosphate buffer solution at pH 6.8 for the remainder of the dissolution time. The amount of drug released was determined as stated earlier. The results were based on an average of three experiments.

Data treatment of in vitro release

The *in vitro* release profiles of Ambroxol HCl from the test and reference formulation were compared using similarity factors (f_2) (US FDA, 1995). Dissolution profiles were considered similar when $f_2 > 50$.

Elucidation of release kinetics

The mechanism of Ambroxol HCl release from the CR matrix tablets was determined by fitting the release data to different release kinetic models.

In vivo measurement of Ambroxol HCl absorption

In vivo study protocol

As a preliminary *in vivo* study, 6 healthy, male volunteers (ages between 18 and 48, body-mass index 19 to 30 kg/m^2, shown in Table 2) participated in this study in accordance with a protocol approved by the Ethical Committee of Al-Isra Hospital, Amman, Jordan. The consent of the volunteers was obtained in concordance with the Declaration of Helsinki and Good Clinical Practice.

Table 2 Demographic characteristics and formulation sequence of six healthy volunteers participating in a bioequivalence study of 75mg Ambroxol HCl using two test formulations (T1) and (T2) and Mucosolvan LA® as control drug, administered as a single oral dose

Volunteer	Age Years	Weight Kg	Height cm	MBI Kg/m^2	Sequence
1	21	65	175	19.92	RT2T1
2	38	75	180	22.45	T1RT2
3	40	81	182	29.56	T2T1R
4	27	78	167	25.00	T1T2R
5	48	86	179	30.00	T2RT1
6	23	76	172	29.56	RT1T2

The study was designed to include open, randomized, single-dose, three-treatment, six-sequence, crossover (Williams design) under fasting conditions. The hospitalization period was a total of 24 hours, including 12 hours before dosing and 12 hours after dosing, in each period.

The dosing periods were separated by a washout period of sufficient length (5 days) which was greater than ten elimination half-lives of the drug. The Ambroxol HCl preparations were administered in the morning with 240 ml of water following 12 hours of fasting. A total of 20 blood samples were collected according to a predetermined sample collection schedule.

Sample treatment and analysis

All samples were collected in ethylene diamine tetracetic acid (EDTA) blood tubes, and centrifuged at 1500 x g for 10 minutes at room temperature (Sigma 4K15, Sigma Laborzentrifugen, Osterode, Harz). The supernatant plasma samples were transferred to screw top polypropylene tubes. These were capped and immediately stored at –30°C ± 5 until analysis.

The plasma concentration of Ambroxol was determined using a validated high-performance liquid chromatography (HPLC) method with electrochemical detection. In the present work, the chromatographic system consisted of stationary phase (Synegi MAX-RP, 150x4.6 mm, with a 4 mm particle size) and guard column (Synergi MAX-RP, 10x4.0 mm, 4 mm particle size). The mobile phase comprised of a filtered and degassed mixture of acetonitrile, methanol and phosphate buffer (0.05M) in the ratio of 26:34:40.0 (v/v). The mobile phase was adjusted to pH 7.4 using a potassium hydroxide solution (5.0M) before filtration. The flow rate was set at 1.0 (ml/min). Detection was based on cyclic voltammograms recorded from Ambroxol solutions in the mobile phase. The detector was a digital electrochemical amperometric detector, and the following instrumental settings were employed: Applied potential (E_1) +0.85 V (vs Ag/AgCl), applied time (t_1): 800ms, first cleaning potential (E_2): +1.05 V, cleaning time at E_2 (t_2): 420ms, second cleaning potential (E_3): -0.85 V and cleaning time at E_3 (t_3): 400ms. The whole cycle lasted for 1620 ms, with a sampling time (ts) of 20 ms and a current range of 50 nA. The oven temperature was kept at 35°C. Sample injection volume was 100 ml. The limit of quantitation (LOQ) of Ambroxol HCl was set at a signal to noise ratio of 10.

Pharmacokinetic and bioavailability studies

The pharmacokinetic parameters: C_{max}, T_{max}, AUC_{0-24}, AUC_{0-inf} were estimated using

WINNONLIN (Ver. 5.0.1, Pharsight Corp. CA).

Relative bioavailability (RB) was determined based on the test to reference ratio (T/R) of the geometric means of the main pharmacokinetic metrics of interest such as AUC_{0-24}, AUC_{0-inf} and C_{max}.

Bioequivalence statistical considerations

The statistical treatment of data was based on a statistical model suited for a K-sequence and J-period crossover design as shown in Equation 1.

$$Y_{ijk} = \mu + G_k + S_{ik} + P_j + F_{(j.k)} + C_{(i-1K)} + e_{ijk} \qquad \text{Eq. 1}$$

where;

$i = 1,...,nk$; μ = the overall sample mean; $J = 1,...,J$; $k = 1,...K$; G, P and F are the respective fixed sequence (k), period (P) and formulation (F) effects; $C_{(i-1, K)}$ is the fixed first order carryover effect (C) of the treatment in the kth sequence administered in the $(j-1)^{th}$ period; e_{ijk} is the within subject random error of Y_{ijk}. The number of periods and number of sequences were set at three and six respectively. The analysis of the variance for this model has been performed on the natural log -transformed data with and without adjustment for the first order carryover effect. Consequently, pair-wise contrasts for different treatments were undertaken so that a 95% two one-sided t-test (Schuirmann), as well as, a 90% shortest confidence interval are established. Results thus obtained were verified by those obtained by the analysis of individual subject ratios consequent to log-transformation of the plasma raw data (multiplicative model).

Bioequivalence statistical evaluation based on pharmacokinetic parameters (C_{max} and AUC) of the test products (T1 and T2) and the reference Mucosolvan LA (R) was established using Statistical Application Systems (SAS) Ver. 6.12.

In vivo and in vitro correlation (IVIVC)

An IVIVC was developed using dissolution and absorption data. The fraction of drug dissolved (FRD) after 4 and 10 hours of in vitro dissolution was compared with in vivo parameters of C_{max} and AUC.

RESULTS AND DISCUSSION

Physical characteristics of tablets

The physical appearance, weight variation and drug content uniformity of tablet formulations were within compendial limits. The crushing strength varied between 5 and 6.0 N. The percentage friability was ≤ 1%. The tablets showed low weight variation (SD ± 6% of the average weight of the tablet) and a high degree of drug content uniformity (± 8% of the theoretical value). Overall, the physical characteristics of the processed tablets indicated a suitable matrix capable of being compressed into tablets.

In vitro dissolution profiles and release kinetics

In the 0.1 M HCl media, the carboxylic acid groups of XG are nonionized while the amino groups of CH are ionized and protonated and positive charges ($NH3^+$) appear inside the gel, while in the pH 6.8 buffer medium, CH is neutralized and negative charges (COO^-) from the ionization of XG appear inside the gel. The mutual repulsion between positive or negative charges and the entry of water together with counter ions that neutralize these charges cause a swelling of the matrix and the formation of a subsequent hydrogel layer (19-22).

The differences in release profiles of the formulations are probably due to the hydration behavior and the (inter- and intra-) molecular interactions of the hydrophilic polymers in the matrices. The high degree of gum hydration with simultaneous swelling resulted in the lengthening of the drug diffusion pathway and the reduction of drug release rate. The strong
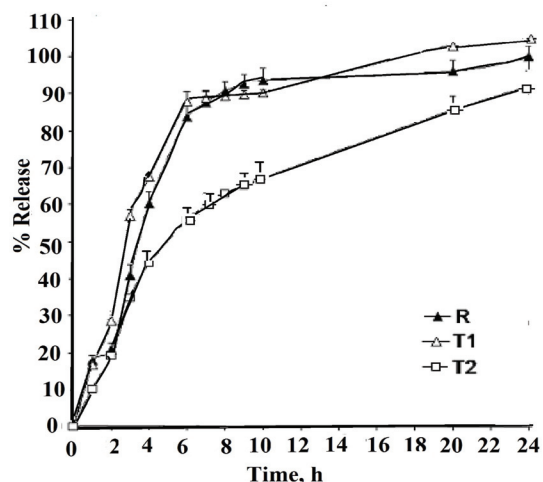
Figure 2 Dissolution profiles of Ambroxol HCl from R: Mucosolvan LA $^®$, T2 (D: P 1:1) and T1 D:P (1:3).

synergistic interactions between polymers resulted in the formation of a tight network that retarded the release of the dissolved drug. As Ambroxol HCl is soluble in the pH range tested, its release from the hydro gel matrix is dependent on the swelling and the dissolution/erosion of the matrix.

The release profile of Ambroxol HCl from test (T1 and T2) and reference formulations is shown in Figure 2 which shows that the release
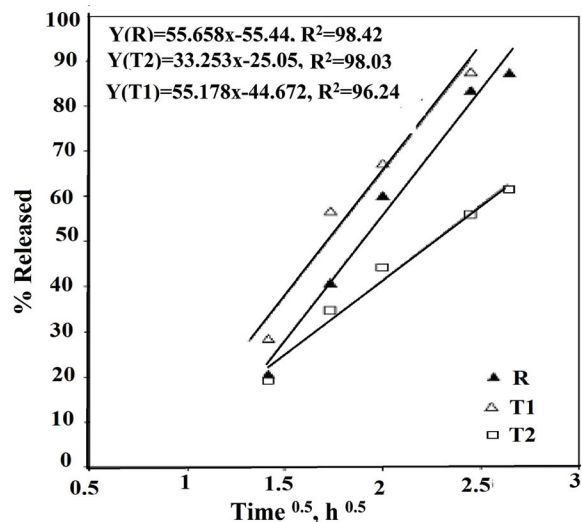

Figure 3 Higuchi plot of dissolution profiles of Ambroxol HCl controlled release preparations

rate decreased with an increase in polymer proportion as a more viscous tight hydrogel layer may have been formed in the case of T2 which further retarded the drug release. The T1 tablets showed a more similar release profile to the Mucosolvan LA$^®$ capsules than did the T2 tablets. This was further confirmed by the f_2 values of 58.4 and 35.6 for T1 and T2, respectively.

The dissolution release kinetics were elucidated using different release kinetic models. The data best fitted the Higuchi model (Figure 3). The data showed a good fit ($R^2 > 0.96$) to the Higuchi equation, indicating that the release mechanism might be governed by a simple diffusion process. The release rate of T1 was similar to that of R as indicated by the slope of the Higuchi equations. T2 showed a slower release rate than T1 inicative of a greater drug release retardation.

In vivo assessment of the controlled release products

The quantification limit for ambroxol in human plasma was determined to be 6 ng ml^{-1} The intra- and inter-day coefficients of variation were <8.61 and 7.50%, respectively, for Ambroxol plasma concentrations of 5 and 200 ng ml^{-1}. Ambroxol is reported to have a biological half-life of 3.72 hours, a volume of distribution of 1.52 l/kg body weight and a systemic clearance of 565 ml/min. These parameters were estimated from IV dosing and were used to construct a simulated plasma concentration-time profile by considering a bioavailability factor between 0.73 and 0.81 following administration as oral solution or tablets (26). The C_{max} value from this simulation approximates 180 ng. ml^{-1}. This value is close to what was reported as 163 ng. ml^{-1} at about 3.5 hours following a 75 mg oral dose (22).

The bioavailability parameters shown in Table 2 and Figure 4 shows that test formulation T1 showed greater similarity with the bioavailability profile and pharmacokinetic parameters of R than T2 did.
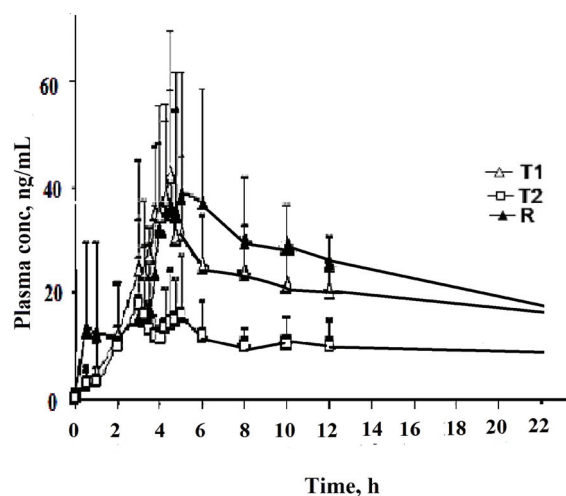
Figure 4 The mean plasma concentration of T1, T2 and R containing 75 mg Ambroxol HCl given to the six subjects following oral administration.

Increasing the polymer ratio from 1:1 to 1:3 w/w (D:P) resulted in a decrease in the percent of *in vitro* drug release rate. The ratio of fraction of drug dissolved (FRD) of T2 divided by the FRD of T1 for the release data between the time intervals from 1 to 10 hours was almost a constant value (0.672 ± 0.044). This indicates that D:P ratio was the controlling factor in the release process, such that an increase in the D:P ratio resulted in a constant increase in FRD. The comparison between C_{max}, AUC_{0-24}, and AUC_{0-inf}, of the T2/T1 yielded the following ratios 0.574 ± 0.208, 0.61 ± 0.272 and 0.599 ± 0.173, respectively. This is indicative of the fact that the polymer ratio was the rate-controlling factor in the *in vivo* drug release rate as well and demonstrated a good correlation with the *in vitro* data.

T1 showed higher bioavailability than T2. The average relative bioavailability values of T1/R and T2/R with respect to C_{max}, AUC_{0-24}, and AUC_{0-inf} are presented in Table 3. These results indicate that T1 could be considered bioequivalent to the reference formulation. The non-bioequivalence of T2 when compared with R could be attributed to the slower release of ambroxol from T2 caused by the greater ratio of polymer mixture to drug.

Detailed statistical evaluation for the Williams design is presented in Tables 4 and 5. The 90% confidence intervals together with the t-values for the two one-sided test procedures provided in these tables give further evidence to the results that formula T1 is bioequivalent, in the average AUC, to the reference formulation. However, it is worth noting that Williams' design lacks robustness especially when a small sample size is considered. Significant difference in the 90% confidence interval and the t-values of the two one sided test TOST procedures

Table 4 Summary of the two one-sided test procedure and the asymmetric confidence intervals calculated from the ln-transformed data for AUC_{0-24} after an oral administration of a single 75 mg dose of T1 and R

Pair-wise Contrasts	Carryover	Schuirmann two one-sided test			90% CI	
		T_L/T_U	Tests	p-Value	Lower	Upper
T1 vs. R	Yes	T_L	3.248	0.009	-0.209	0.229
		T_U	-3.072	0.011	81.11	125.77
	No	T_L	1.623	0.072	-0.393	0.172
		T_U	-3.076	0.008	67.53	118.76
T2 vs. R	Yes	T_L	-3.654	0.005	-0.988	-0.550
		T_U	-9.973	0.000	37.22	57.71
	No	T_L	-2.384	0.022	-1.001	-0.436
		T_U	-7.083	0.000	36.76	64.64

Table 3 Pharmacokinetic parameters for ambroxol HCl after oral administration of single doses of 75mg of T1, T2 and R in 6 healthy volunteers. Values are expressed as mean (SD).

PARAMETER	T1	T2	R	T1 / R	T2/ R	T1/T2
C_{max}, µg .ml^{-1}	46.7 (25.8)	27.6 (14.7)	41.4 (22.8)	1.139(0.3)	0.700(0.3)	0.59(0.208)
AUC_{0-24},µg .h .ml^{-1}	654.5(289.3)	371(178.7)	721.5(291.5)	0.910(0.2)	0.535(0.3)	0.61(0.272)
AUC_{0-inf} µg · h/ml^{-1}	758.3(332.4)	464.1(225.4)	796.5(367.9)	0.973(0.2)	0.606(0.3)	0.599(0.173)
T_{max} , h	4.21 (0.5)	4.13 (0.8)	5.80 (2.1)	---	---	----

Table 5 Summary of the two one-sided test procedure and the asymmetric confidence intervals calculated from the ln-transformed data for C_{max} after an oral administration of a single 75 mg dose of T2 and R.

Pair-wise Contrasts	Carryover	Schuirmann two one-sided test			90% CI	
		T_L/T_U	Tests	p-Value	Lower	Upper
T1 vs. R	Yes	T_L	2.643	0.019	-0.231	0.468
		T_U	-1.326	0.116	79.38	159.60
	No	T_L	2.961	0.009	-0.188	0.384
		T_U	-1.684	0.065	82.90	146.77
T2 vs. R	Yes	T_L	-0.869	0.209	-0.862	-0.164
		T_U	-4.838	0.001	42.23	84.91
	No	T_L	-0.578	0.290	-0.731	-0.160
		T_U	-5.222	0.000	48.14	85.23

$$Y(AUC_{0-24})=13.607x - 545.53, R^2=0.9821$$
$$Y(AUC_{0-inf})=12.662x-386.1, R^2=0.9999$$

Figure 6 Fraction of drug dissolved (FRD) after 10 hours of dissolution (*in-vitro* parameter) versus AUC (ng.h.ml-1) (*in-vivo* parameter)

were obtained when analysis was performed using linear and logarithmic scales. In addition, outlying observations were shown to have a significant impact on the outcome of the statistical evaluation. As it would be anticipated, these properties were exacerbated due to the small number of subjects in the present study. The C_{max} and the percent of the drug dissolved after 4 hours (FRD 4) for the T1 controlled release product correlated substantially ($R^2>0.99$, Figure 5). The importance of choosing this time is that it is close to T_{max} i.e., the point of C_{max}. The good correlation

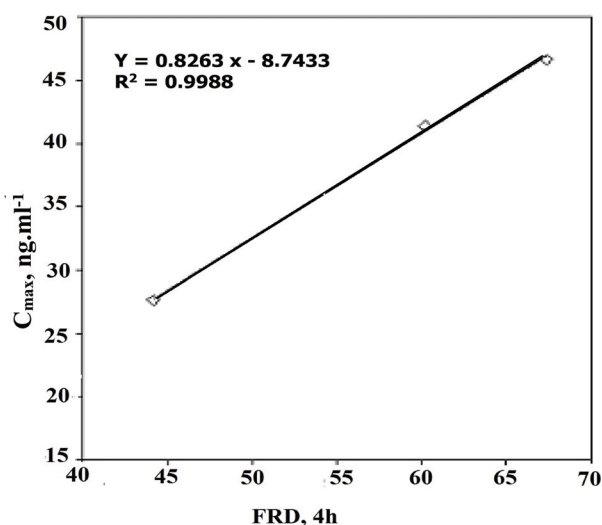$$Y = 0.8263 x - 8.7433$$
$$R^2 = 0.9988$$

Figure 5 Fraction of drug dissolved (FRD) after 4 hours of dissolution (*in-vitro* parameter) versus maximum drug level (C_{max}) (ng/ml) of oral absorption (*in-vivo* parameter)
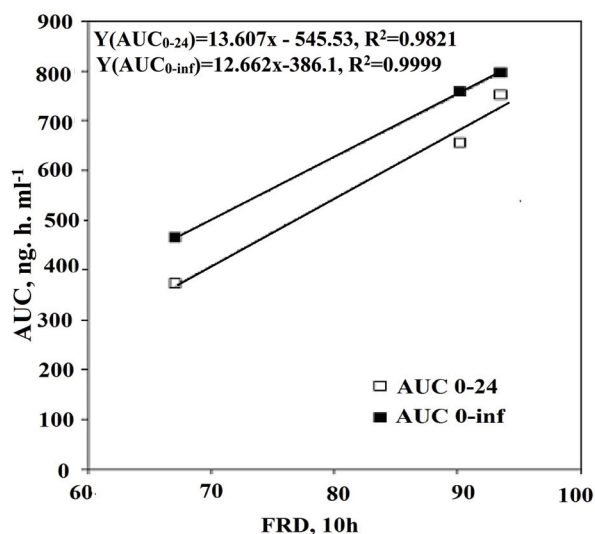
between C_{max} and the FRD shown by the *in vitro* dissolution data indicated that the *in vitro* dissolution data substantially reflected the *in vivo* release behavior. Similarly, the *in vivo* parameters (AUC $_{0-24}$ and AUC $_{0-inf}$) and *in vitro* parameter (FRD 10) were found to correlate significantly ($R^2>0.98$, Figure 6). This represents the time when the formulation releases most of its drug load to the dissolution medium (for T1 and R > 90% and for T2> 67%). The *in vivo* parameters, AUC $_{0-24}$ and AUC$_{0-inf}$ represent the extent of drug absorption in the total time period from 0 to 24 hours and 0 to infinity, respectively.

The linear relationship between *in vitro* and *in vivo* parameters may indicate that the *in vivo* dissolution process of the controlled release products can be adequately described using *in vitro* results.

This suggests a multiple point level C correlation of the IVIVC. In this way the *in vitro* parameters of FRD 4 and FRD 10 may be used as a predictive measure of the *in vivo* parameters, C_{max} and AUC, respectively. This measure can be used for developing other formulations, quality control and evaluating Ambroxol HCl CR dosage forms.

CONCLUSION

A matrix consisting of xanthan gum and chitosan was able to control the release of Ambroxol hydrochloride from compressed tablets. The release rate profile of the drug from the hydrophilic matrix was found to be significantly similar to that of Mucosolvan LA, the marketed formulation, under both *in vitro* and *in vivo* conditions. Additional *in vivo* studies using fed volunteers must be performed to confirm bioequivalence.

REFERENCES

1 Al-Akayleh F, Al Remawi, Rashid I, Badwan A. Formulation and In vitro assessment of sustained release terbutaline sulfate tablet made from binary hydrophilic polymer mixtures. Pharm Dev Technol, 4: 100-111, 2011.

2 Santos H, Veiga F, Pina M, Sousa Eugenia J. Compaction, compression and drug release properties of diclofenac sodium and ibuprofen pellets comprising xanthan gum as a sustained release agent. Int J Pharm, 295:15–27, 2005.

3 Sujja-areevath J, Munday DL, Cox PJ, Khan KA. Release characteristics of diclofenac sodium from encapsulated natural gum mini-matrix formulations. Int J Pharm, 139: 53–62, 1996.

4 Nunthanid J, Laungtana-anan M, Sriamornsak P, Limmatvapirat S, Puttipipatkhachorn S, Limc LY, Khor E. Characterization of chitosan acetate as a binder for sustained release tablets. J Control Release, 99: 15–26, 2004.

5 Maderuelo C, Zarzuelo A, Lanao JM. Critical factors in the release of drugs from sustained release hydrophilic matrices. J Control Release, 1549: 2–19, 2011.

6 Williams PA, Day DH, Langdon MJ, Phillips GO, Nishinar K. Synergistic interaction of xanthan gum with glucomannans and galactomannans. Food Hydrocolloids, 4:489–493, 1991.

7 Pasparakis G, Bouropoulos N. Swelling studies and in vitro release of verapamil from calcium alginate and calcium alginate–chitosan beads. Int J Pharma, 323: 34-42, 2006.

8 Li Y, McClements DJ. Controlling lipid digestion by encapsulation of protein-stabilized lipid droplets within alginate–chitosan complex coacervates. Food Hydrocolloids, 25: 1025-1033, 2011.

9 Liu X, Xue W, Liu Q, Yu W, Fu Y, Xiong X, Ma X, Yuan Q. Swelling behaviour of alginate–chitosan microcapsules prepared by external gelation or internal gelation technology. Carbohydrate Polymers, 56: 459-464, 2004.

10 António JR, Silva C, Ferreira D, Veiga F. Chitosan-reinforced alginate microspheres obtained through the emulsification/internal gelation technique. Eur J Pharm Sci, 25: 31-4, 2005.

11 Polk A, Amsden B, Yao K, Peng T, Goosen M. Controlled Release of Albumin from Chitosan-Alginate Microcapsules. J Pharm Sci, 83:178-185, 1994.

12 Miyazaki S, Nakayama A, Oda M, Takada M, Attwood D. Drug Release from Oral Mucosal adhesive Tablets of Chitosan and Sodium Alginate. Int J Pharm, 118: 257-263, 1995.

13 Murata Y, Maeda T, Miyamoto E, Kawashima S. Preparation of Chitosan-Reinforced Alginate Gel Beads-Effects of Chitosan on Gel Matrix Erosion. Int J Pharm, 96:139-145, 1993.

14 Yomota C, Miyazaki T, Okada S. Sustained Release Effect of the Direct-Compressed tablet Based on Chitosan and Na-alginate)abstract(, Yakuqaku Zasshi, 114:257-263, 1994

15 Fukuda M, Peppas N, McGinity J. Properties of sustained release hot-melt extruded tablets containing chitosan and xanthan gum. Int J Pharm, 310: 90–100, 2006.

16 Badwan A, Al Remawi M, Salem M. European patent EP1512394, Apr. 09, 2005

17 Bernkop-Schnürch A, Dünnhaupt S. Chitosan-based drug delivery systems. Eur J Pharm Biopharm, 81: 463–469, 2012.

18 Desplanques S, Renou F, Grisel M, Malhiac C. Impact of chemical composition of xanthan and acacia gums on the emulsification and stability of oil-in-water emulsions. Food Hydrocolloids, 27:401- 410, 2012.

19 Kubo W, Miyazaki S, Dairaku M, Togashi M, Attwood D. Oral sustained delivery of ambroxol from in situ-gelling pectin formulations. Int J Pharm, 271: 233-240, 2004.

20 Vergin H, Bishop-Freudling G, Miczka M, Nitsche V, Matzkies, F. Pharmacokinetics and bioequivalence of different formulations of ambroxol, Arzneim-Forsch, 35:1591-1595, 1985.

21 Nieder M, Jaeger H. Selective quantification of ambroxol in human plasma by HPLC. Journal of High Resolution Chromatography, 2:305-307, 1998.

22 Chi N, Guo JH, Zhang Y, Zhang W, Tang X., An oral controlled release system for ambroxol hydrochloride containing a wax and a water insoluble polymer. Pharm Dev Techno, 15:97-104, 2010.

23 Argin–Soysal S, Kofinas Y. Martin Lo Y . Effect of complexation conditions on xanthan–chitosan complex gels polyelectrolyte. Food Hydrocolloids, 23: 202–209, 2009.

24 Martı´nez-Ruvalcab A, Chornet E, Rodrigue D. Viscoelastic properties of dispersed chitosan/xanthan hydrogels. Carbohydr Polym, 67: 586–595, 2007

25 Fukuda M, Peppas NA, McGinity JW. Properties of sustained release hot-melt extruded tablets containing chitosan and xanthan gum. Int J Pharm, 310: 90–100, 2006

26 Vergin H, Bishop-Freudling GB, Miczka M, Nitsche V, Strobel K, Matzkies F. The pharmacokinetics and bioequivalence of various dosage forms of ambroxol. Arzneimittelforschung, 35:1591–5, 1985