

EVALUATION OF *IN VIVO*-*IN VITRO* RELEASE OF THEOPHYLLINE FROM NOVEL CONTROLLED RELEASE MICROSPHERES

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ABSTRACT

Objective: The study is focused on the development of a new particulate drug delivery system using a modified chitosan matrix containing theophylline as a model drug molecule for drug prolonged release.

Methods: The polymeric beads of HKCTS-Zn loading theophylline are first prepared by zinc cross-linked. Modifications in matrix structure and physicochemical behaviour caused by the cross-linking reaction were assessed during particle formation and drug release. The correlation between the dissolution *in vitro* and the absorption *in vivo* of the sustained-release theophylline beads were then investigated. Rotating basket method was used to determine *in vitro* release rate. The drug plasma was determined by HPLC after a single oral dose of 50mg sustained beads was given to guinea pigs. The *in vivo* absorption percentage was calculated by Wagner-Nelson equation.

Results: The data showed that the drug dissolution coincided with Higuchi equation. The good linear regressive equation established between the absorption percentage *in vivo* and the dissolution percentage *in vitro* of the sustained-release theophylline beads.

Conclusions: The generated beads proved to be successful in prolonging drug release. The dissolution *in vitro* of the sustained-release theophylline beads in the stimulated gastric fluid proves to be related to *in vivo* absorption percentage.

Keywords: Beads, Modified chitosan, Cross-linking, *In vivo-in vitro* correlation

INTRODUCTION

Oral solid dosage forms for sustained drug release has the major attention amongst all the controlled drug delivery systems due to its conventional usage. Polymeric film coatings have been utilized widely for controlled release of an active substance from pharmaceutical dosage forms because the coated dosage forms enable the sustained and precise release of drug with good reproducibility[1-2]. Chitosan is a biopolymer produced from chitin by partially deacetylating its acetamido groups with a strong alkaline solution, it is the second most abundant polysaccharide found on earth next to cellulose. As a natural renewable resource, chitosan has a number of unique properties such as antimicrobial activity, nontoxicity, biocompatibility and biodegradability, which attract scientific and industrial interest in such fields as biotechnology, pharmaceuticals, wastewater treatment, cosmetics, agriculture, food science, and textiles[3-4]. Furthermore, many recent reports discussed the use of chitosan and modified chitosan in sustained release formulations and colon-targeting delivery systems[5-6]. However, in the thrust of our interest in this natural polymer we came across some contradicting reports regarding the drug release from inter matrices[7].

Chemical modifications by using crosslinking reagents are necessarily applied to find more versatile applications of biopolymers by the demonstration of improvements. The commonly used crosslinking agents are aldehydes such as glutaraldehyde and formaldehyde [8-10] or carbodiimides such as dicyclohexyl carbodiimide (DCC) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) [26-35]. Recent reports indicating the use of ion-crosslinked carboxymethylchitin and carboxymethylcellulose as drug releasing polymeric matrices[11-12], prompted us to develop, and evaluate the drug releasing properties of polymeric beads based on ion-crosslinked chemically modified chitosan. The presence of amine groups on the polymeric chain possibly masks a great number of chemical modifications, especially Schiff's reaction with aldehydes and ketones[13]. Chitosan can be modified by alpha-ketoglutaric acid and Hydroxylamine hydrochloride to obtain chitosan functional polymer containing Schiff-base group, these novel CTS derivatives would have stronger complexation ability and better adsorption characterization for

metal ions because of the synergistic effect of high molecular weight. Recent reports indicating the indefinite stability of zinc (II) amide complexes in various protic solvents including water[14] suggested the possibility of producing pharmaceutically useful polymeric matrix based on chitosan- ketoglutaric acid crosslinked with zinc ion.

On the other hand, hydroxamic acids are known to form particularly stable and relatively pH-independent complexes with zinc ions, i.e., within physiological limits[15]. Such complexes were utilized for analytical[16] and clinical purposes[17], as well as in the development of novel sustained release polymeric matrices [18]. Consequently, it seemed logical to generate zinc (II)-crosslinked hydroxamated chitosan-ketoglutaric acid, to be evaluated as a polymeric matrix in sustained release oral formulations.

The focus of the current paper was to explore applications of zinc-crosslinked modified chitosan as potential matrix material for theophylline as the model drug. In this study, release kinetics of theophylline sustained-release beads was evaluated. The correlation between the *in-vivo* plasma concentration profile and the *in-vitro* dissolution profile was established through the external predictability approach.

MATERIALS AND METHODS

Materials

Chitosan (CTS, MW4.9x10⁵, degree of deacetylation: 95%) was procured from Dalian Xindie Chitin Co., Ltd. Theophylline was provided by Qingdao Medicine Institute. α -ketoglutaric acid was purchased from Qianshan Science and Technology Development Company, Zhuhai of China. The other reagents were of analytical grade and used without further purification. All solutions were prepared with distilled, deionized water.

Preparation of the loaded polymeric beads and crosslinking with Zn

The particular polymer (HKCTS 1.0 g) was dissolved in 0.1 N sodium hydroxide solution (20 ml)[19]. Theophylline (1.0 g) was added to the viscous polymeric solution and the mixture was stirred for 40

min. The resulting viscous suspension was carefully dropped, using glass dropper, into a stagnant aqueous solution of zinc chloride (0.5%, w/v, 100 ml). The viscous droplets were left to cure in the zinc chloride solution over 2 h to generate beads. Bead preparation and curing was carried out under ambient room temperature. The beads were then collected and washed twice with deionized water (50 ml) and left to dry at room temperature over 24–48 h. The beads were evaluated using microscopy.

Determination of theophylline's dissolution profiles from HKCTS-Zn beads

A rotating basket apparatus fitted with a stainless steel basket was used. Dry beads (0.3 g) were placed in the basket. Buffered dissolution medium were used: pH 7.4 (phosphate buffer) for 5-6 h. The dissolution mediums (900 ml) were maintained at 37°C. The basket was rotated at 100 round/min. Samples (2 ml) were withdrawn appropriately every proper time for the analysis of released theophylline. The withdrawn volume was immediately replaced with an equivalent volume of the fresh medium maintained at the same temperature. The absorbencies of the undiluted samples were evaluated at 272 nm using a Cary 1E UV-visible spectrophotometer. Unloaded beads were utilized as blanks. The measurements were repeated 3 times and the average was reported. The concentrations of the released drug were converted to percent-release by dividing the released quantities over the total loaded drug.

In Vitro release

Five milligrams of microspheres was suspended in 3mL of 0.01M phosphate buffered saline (PBS), pH 7.4, containing 0.01% Tween 80, 0.05% sodium azide and 0.04M sodium chloride in a 5mL plastic vial (n = 3), and then the suspension was placed in a shaking bath (HZS-H, DongLian Electronic Co., Harbin, China) at 40 rpm and 37°C. Sink conditions were maintained during this study. At preset intervals, the vials were centrifuged at 3000×g for 30 min and then 2mL of the supernatant was drawn and replaced by fresh buffer[20]. Theophylline in supernatant was determined by HPLC method described above.

Dosing and sample collection

A single oral dose of 50 mg sustained beads was given to guinea pigs. Blood samples (3 mL) were collected into heparinized tubes before and at 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h after drug administration. Plasma was separated by centrifugation for 10 min at 3000×g and stored at -20°C until analysis. Theophylline medicine was analyzed using LC-MS/MS, according to the previous method[21].

Recovery

Spiked plasma samples were assayed using five replicates at three concentration levels of 1µg/ml, 10µg/ml and 30 µg/ml of theophylline and extracted as previous. Recovery (extraction efficacy) was calculated by comparing the peak-area of the extracted sample to that of unextracted pure standard solutions.

Precision and accuracy

Precision and accuracy of this method were evaluated using three different concentrations of 1µg/ml, 10µg/ml and 30 µg/ml of theophylline. The experiment was carried out six times in a day (intra-day) and for six consecutive days (inter-day).

In Vivo release and pharmacokinetic analysis

The terminal elimination rate constants (K_e) after oral administration were estimated with least-squares regression of values in the terminal log-linear region of plasma concentration-time curves at the time points above. The terminal elimination half-life ($T_{1/2}$) was calculated as $0.693/K_e$. The areas under the curve from time zero to last sampling time (AUC_{0-t}) after drug administration were determined by the linear trapezoidal rule. The area under the curve from time zero to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + C_t/K_e$, where C_t is the last detectable plasma concentration and t is the time at which this concentration occurred.

The mean plasma concentration-time data after oral administration of the theophylline solution was used to obtain the best fitted compartmental model by Drug and Statistics (DAS) version 2.0 (Hunan Provincial Center for Drug Clinical Evaluation, China) program. All data were expressed as mean±standard deviation (S.D.).

IVIVC

The data generated in the pharmacokinetic study were used to develop the IVIVC (Level A). The relationship between percent in vitro dissolution in PBS at 37°C and the fraction of drug absorbed in vivo (F_t) was examined. The F_t was determined using the Wagner-Nelson method (WN) by the following equation: $F_t = (C_t/K_e + AUC_{0-t})/AUC_{0-\infty}$ [22]. The relationship between percent in vitro dissolution in PBS at 37°C and percent $AUC(AUC_{0-t}/AUC_{0-\infty})$ was also examined. Linear regression analysis was applied to the IVIVC plots. The values of correlation coefficient (R^2), slope and intercept were calculated, respectively.

RESULTS AND DISCUSSION

FTIR analysis

The FTIR spectrum of HKCTS-Zn polymeric matrix(Fig.1)shows both carboxyl and amide carbonyls were shifted to lower frequencies upon complexation to zinc, i.e., from 1665 to 1560 cm^{-1} for amide carbonyls and from 1733 to 1680 cm^{-1} for carboxylic carbonyls, and the newly formed hydroxamic carbonyl groups at 1636 cm^{-1} in HKCTS-Zn were also shifted to lower frequencies upon complexation to zinc. Such shifts indicate that hydroxyl group in hydroxylamine, carboxyl group and amide group are coordinated to zinc ion. The band at 1110 cm^{-1} , 660 cm^{-1} assigned to ionic SO_4^{2-} was found in all complexes' spectra, which indicated that SO_4^{2-} existed in the complexes in the ionic form.

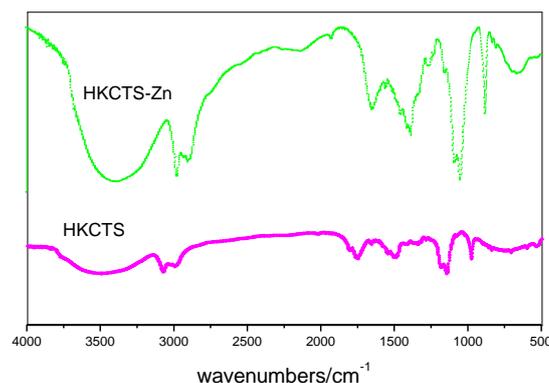


Fig. 1: FT-IR Spectra for HKCTS and HKCTS-Zn

Particle size analysis

Size distributions can be seen from Fig. 2. To HKCTS-Zn-T microspheres, the particle size of 50% of the spheres was below 0.165 µm and 10% was below 0.075. About 90% of the spheres were below 1.868 µm. The range of HKCTS-Zn-T microspheres was found to be small. Aggregation of smaller spheres could be attributed to the very small amounts of very large particles in the size distribution curve of HKCTS-Zn-T microspheres. Bimodal size distributions of this type are common in microsphere studies: the large particles are microspheres, and the smaller particles fragments of nonencapsulated material and fragments of the microspheres themselves.

Morphology of Zn crosslinked polymeric beads

Upon qualitative visual inspection using microscopy, the prepared beads were dark brown in color, spherical biconcave in shape. It

could be found that the surface of polymeric beads was smooth. They underwent approximately 5-fold reduction in their size upon drying. A photograph illustrating the generated beads is shown in Fig. 3. From the SEM photographs, these spheres were found to have regular spherical geometry and the mean diameter of microspheres was about 2~2.5µm.

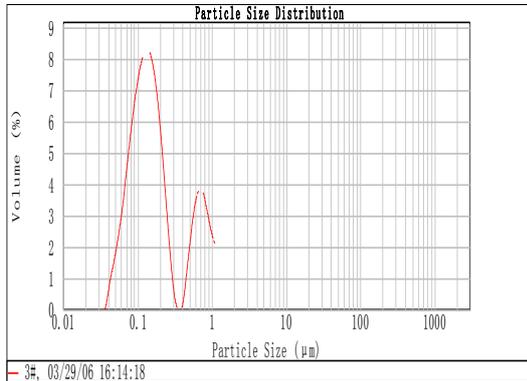


Fig. 2: Particle Size Distribution of HKCTS-Zn-T

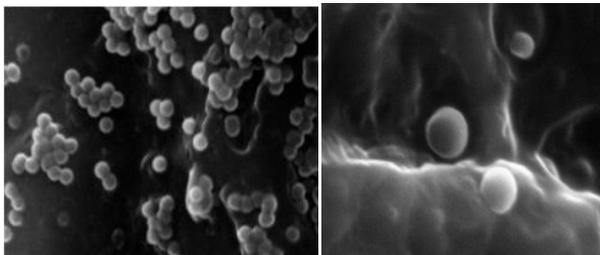


Fig. 3: The Optical Microscope Image for Polymer Beads

In Vitro study

Fig. 4 illustrates the dissolution profiles of theophylline from HKCTS-Zn beads. We found that the release rates vary depending upon the amount of drug present in the matrices, i.e. release was slower for those formulations having lower amount of the drug. However, the burst release is again prevalent for these systems, suggesting the rigidity of the polymer matrices. The burst release may help to maintain the patient therapeutic regime, while the sustained release will maintain the plasma concentration level. Release of the drug from chitosan derivatives involves three different mechanisms: (a) release from the surface of particles, (b) diffusion through the swollen rubbery matrix and (c) release due to polymer erosion. Initially, higher release rates are observed due to the dissolution of surface-adhered drug. At longer time, drug release is due to the diffusion process, which is much slower when compared to the initial release. Complete release of the drug from the matrix occurs only after complete erosion or degradation of the chitosan derivatives matrix.

For the mathematical evaluations, we characterized drug release kinetics by fitting standard release equations (zero-, first-, Higuchi equation, etc.) to the experimental data [23]. Table 1 shows the release equations obtained from these kinetic models. Following exclusion from the analysis of the first phase (<10% of drug released) and the last phase (>60% of drug released), it was clearly

seen that Higuchi square-root of time model ($r^2 > 0.99$) showed significantly better fitting than first-order ($r^2 < 0.990$), zero-order ($r^2 < 0.981$) and Ritger-Peppers model ($r^2 < 0.981$). The best fit was obtained with the Higuchi equation

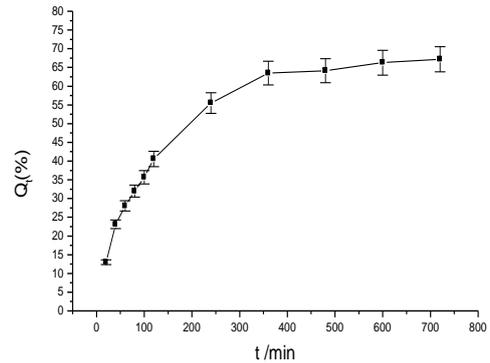


Fig.4: Released Behaviours of Chitosan Derivatives Loaded Theophylline Aggregates in PBS (pH 7.4, 0.01 M) Solutions at 37 °C

Table 1: The Release Equations and r^2 of Dissolution Results According to the Zero-order, First-order, Higuchis and Ritger-Peppers models

| Kinetic | Equation | r^2 |
|----------------|------------------------------------|--------|
| Zero-order | $Q_t = 10.77 + 0.26 t$ | 0.9806 |
| First-order | $\ln(100 - Q_t) = 4.51 - 0.0036 t$ | 0.9895 |
| Higuchi | $Q_t = -3.98 + 4.05 t^{1/2}$ | 0.9950 |
| Ritger-Peppers | $\ln Q_t = -0.77 + 0.61 \ln t$ | 0.9809 |

Recovery

Absolute recoveries for theophylline were more than 98%. Low concentration (1µg/ml) showed more than 99% recovery. Internal standard (caffeine) followed the same result. Combination of more than 99% recovery and 98% recovery were obtained from medium concentration (10µg/ml). On the other hand, more than 98% recovery was possible in high concentration (30µg/ml). These results indicated that the extraction procedure was very selective.

Accuracy and precision measurement

Intra-day precision and accuracy of the assay was measured for theophylline at concentration levels of 1µg/ml, 10µg/ml and 30µg/ml. Method intra-day accuracy and precision (%CV) ranged from 99.26 to 99.72 and 1.13 to 2.06 respectively. Method inter-batch accuracy and precision (%CV) ranged from 98.92 to 99.78 and 1.89 to 2.09 respectively. The results so obtained were within the acceptance criteria for accuracy and precision designed by USFDA[24].

The calculation of the fraction of drug absorbed In Vivo

Table 2 shows the measured plasma concentrations of theophylline in HKCTS-Zn-T. The measured plasma concentrations were used to calculate the area under the plasma concentration-time profile from time zero to the last concentration time point (AUC_{0-t}).

Table 2: The Measured Plasma Concentrations of Theophylline in HKCTS-Zn-T

| Time(min) | C ₁ | C ₂ | C ₃ | C ₄ | C ₅ | \bar{C} (µg/ml) |
|-----------|----------------|----------------|----------------|----------------|----------------|-------------------|
| 40 | 1.019 | 1.021 | 1.028 | 1.026 | 1.026 | 1.024 |
| 80 | 1.293 | 1.299 | 1.301 | 1.294 | 1.298 | 1.297 |

| | | | | | | |
|------|-------|-------|-------|-------|-------|-------|
| 120 | 1.619 | 1.619 | 1.616 | 1.627 | 1.629 | 1.622 |
| 240 | 2.001 | 1.977 | 1.978 | 1.986 | 1.989 | 1.984 |
| 360 | 2.254 | 2.274 | 2.264 | 2.262 | 2.271 | 2.265 |
| 480 | 2.434 | 2.432 | 2.435 | 2.443 | 2.441 | 2.437 |
| 720 | 2.460 | 2.451 | 2.455 | 2.461 | 2.463 | 2.458 |
| 1080 | 0.691 | 0.683 | 0.682 | 0.681 | 0.693 | 0.686 |
| 1620 | 0.111 | 0.099 | 0.097 | 0.108 | 0.100 | 0.103 |
| 1860 | 0.053 | 0.051 | 0.045 | 0.043 | 0.048 | 0.048 |

The Wagner–Nelson method [25] was used to calculate the percentage of theophylline dose absorbed:

$$F_t = C_t + K_e AUC_{0-t} \quad (1)$$

where F_t is the amount absorbed. The percent absorbed is determined by dividing the amount absorbed at any time by the plateau value, $K_e AUC_{0-\infty}$ and multiplying this ratio by 100:

$$F_t (\%) = \frac{C_t + K_e AUC_{0-t}}{K_e AUC_{0-\infty}} \times 100\% \quad (2)$$

The areas under the curve from time zero to last sampling time (AUC_{0-t}) after drug administration were determined by the linear trapezoidal rule.

$$AUC_{0-t} = \sum_{i=0}^{n-1} \frac{C_{i+1} + C_i}{2} [t_{i+1} - t_i] \quad (3)$$

The area under the curve from time zero to infinity ($AUC_{0-\infty}$) was calculated.

$$AUC_{0-\infty} = AUC_{0-t} + C_t / K_e \quad (4)$$

where K_e was estimated by fitting the logarithm of the concentrations versus time to a straight line over the observed exponential decline, C_t is the last detectable plasma concentration and t is the time at which this concentration occurred. According to the measured plasma concentrations data in Table 2, the percent absorbed is determined by the Wagner–Nelson method. The result is shown as Table 3.

Table 3: The Parameter of Theophylline Absorbed *in Vivo*

| t(min) | C_t ($\mu\text{g/ml}$) | AUC_{0-t} | F_t (%) |
|--------|----------------------------|------------------|-----------|
| 40 | 1.024 | 20.48 | 30.45 |
| 80 | 1.297 | 66.90 | 40.360 |
| 120 | 1.622 | 125.28 | 52.29 |
| 240 | 1.984 | 341.64 | 72.17 |
| 360 | 2.265 | 596.58 | 91.39 |
| 480 | 2.437 | 878.7 | 98.06 |
| 720 | 2.458 | 1466.10 | 110.87 |
| 1080 | 0.686 | 2032.02 | 108.38 |
| 1620 | 0.103 | 2244.05 | 100.8 |
| 1860 | 0.048 | 2262.17 | 99.9 |
| K_e | 0.00151 | $AUC_{0-\infty}$ | 2293.95 |

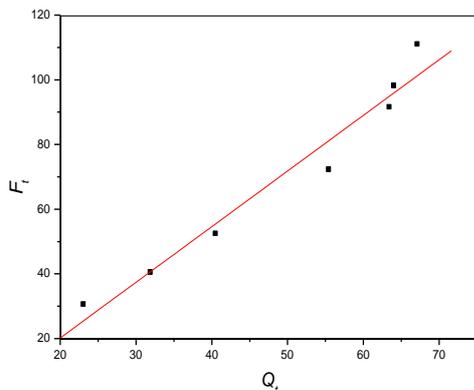


Fig. 5: Percentage of Fraction of Drug vs. Percentage of Drug Dissolved by *in Vitro* Method

***In-Vitro-In-Vivo* correlation**

A level A *in-vitro-in-vivo* correlation was investigated using the percent dissolved vs. the percent absorbed data using pH 6.8

phosphate buffer dissolution media at 50 rpm. The plot of F_t versus Q_t for the experimental data in Table 3 is shown in Fig. 3. As known from Fig.5, a good linear regression relationship between the percent *in vitro* dissolution in PBS at 37°C and the percent absorption ($R^2 = 0.9809$, $P < 0.001$) for the Wagner–Nelson method.

CONCLUSION

In this study, a novel modified chitosan-metal complexes loaded theophylline beads were successfully achieved by ion crosslinking. The release kinetics was evaluated by fitting the experimental data to standard release equations (zero-, first-, Higuchi and Ritger-Peppas equation). The guinea pig was administered into the theophylline dosage to obtain the absorption fraction (F_t) which was according to the plasma concentration determined by HPLC and estimated by Wagner–Nelson method. *In vivo-in vitro* correlation of the sustained-release theophylline beads was evaluated by Wanger-

Nelson method. The significant regression equation between the in vivo absorption and the in vitro release were got and in vivo-in vitro correlation was good. The release determination method created by the study was proved to be scientific and reasonable, which could guarantee the internal quality under the control.

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