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A novel controlled porosity osmotic pump system for sodium ferulate

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A controlled porosity osmotic pump (CPOP) delivery system for sodium ferulate was prepared with cellulose acetate (CA) as semipermeable membrane, polyethyleneglycol 400 (PEG 400) as channeling agent and dibutylphthalate (DBP) as plasticizer and release controller. Effects of coating levels, PEG and DBP content and amount of sodium chloride on *in vitro* release were studied. Coating formulations were optimized by a L_9 (3^4) orthogonal array design (OAD) with three factors at three levels using statistical analysis. Controlled porosity osmotic pump tablets of sodium ferulate made with the optimal formulation were found to have good *in vitro* and *in vivo* release characteristics.

1. Introduction

Sodium ferulate derives from ferulic acid which is known primarily in the Chinese medicine Szechwan Lovge Rhizome, Chinese Angelica, (Lin and Chen 1994). In recent times, it is used widely and effectively in China for clinical treatment of coronary heart disease, unstable angina pectoris, ischemic stroke, renal glomerulus diseases, cerebral thrombosis, hemicrania and arteriosclerosis (Lin and Chen 1994; Liu 2005). Its effects include expanding blood vessels, improving neurological function deficits, reducing cerebral infarct volume (Lin and Chen 1994; Liu 2005; Wei et al. 2003), anti-oxidant and inhibiting arachidonic acid metabolism and NF-κB expression (Wang et al. 2003). However, the oral dosage forms of sodium ferulate marketed at present are all immediate release ones, which have undesirable characteristics when treating cardiovascular and cerebrovascular diseases, as a sharp increase or decrease of the blood drug concentration may cause unstable blood pressure. A controlled drug delivery system is thus very much required.

Osmotically actuated drug delivery has been extensively investigated. Drugs can be delivered with zero-order kinetics over a long period of time by osmosis, which provides a constant drug plasma concentration and hence safety in clinical use. Various types of osmotic pumps have been introduced and investigated (Santus and Baker 1995; Verma et al. 2002). Among them, the most basic is the elementary osmotic pump (EOP) (Santus and Baker 1995; Theeuwe 1975; Verma et al. 2002; Zentner et al. 1985). This device is formed by compressing the drug and an osmotic agent having suitable osmotic pressure into a core tablet. The core is then coated with a semipermeable membrane, and a small hole is drilled through the membrane coating. However, in the gastrointestinal system, the drug delivery orifice tends to become blocked by micro particulate material, which can result in uncontrolled drug release. In addition, because drug solution of high concentration can only be released through the unique small orifice some stomach irritation problems may occur. An alternative to the EOP is the Controlled Porosity Osmotic Pump (CPOP) (Zentner et al. 1985; Makhija and Vavia 2003), which has numerous micro pores instead of a single orifice in the semipermeable membrane. The delivery pores are formed by the incorporation of a water-soluble channeling agent in the coating. In this tablet, the holes through which the drug is released are formed when the water-soluble component of the coating is leached out after the tablet is swallowed and comes in contact with water. The release rate from systems of this type is dependent on factors including coating thickness, level of channeling agent in the coating, solubility of the drug in the tablet core, and osmotic pressure difference across the membrane. Therefore, in this study, CPOP was chosen as the delivery system.

In order to optimize the coating formulation, an orthogonal array study design was adopted. It is commonly recognized nowadays that the one-factor-at-a-time method is often unreliable and liable to give a false optimum (Lan et al. 1994). Therefore, the orthogonal array design has been introduced as an alternative. Orthogonal array design is believed to incorporate the advantages of the simplex method and factorial design (Zhu and Ju 2004). It balances different factors for effective optimization of experimental conditions. The results of the OAD experiment can be statistically treated by analysis of variance (ANOVA) and direct observation analysis to evaluate the significance of the effects of different factors on response functions and to find the optimal level for those factors. OAD has been widely applied to the optimization of experimental conditions in recent years (Onuki et al. 2004; Huang et al. 2002). In the present study, a three-level orthogonal array design was used to optimize the coating formulation.

2. Investigations, results and discussion

2.1. Influence of drug sodium chloride ratio on drug release

To determine the effect of the drug sodium chloride ratio on release, the core tablet formula a I–IV (Table 1) were used, while the coating formulation was kept constant: coating level 2.5%w/w; PEG 400 content, 55% (w/w); DBP content, 8% (w/w).

The release of sodium ferulate from the coated tablets was determined (Fig. 1). It was obvious that in the absence of sodium chloride, the drug release rate was low; while on the other hand, a high ratio of sodium chloride actually retarded release. The results indicate that sodium chloride actually acts not only as an osmotic propellant but also as a retardant, as its Na^+-ion could both increase the osmotic pressure in the core solution and decrease the solubility of sodium ferulate. Initially, sodium chloride dissolved quickly in contact with water, and the osmotic pressure in the tablet core was relatively high, while the solubility of sodium ferulate was comparatively low. Gradually, the core pressure decreased but the solubility of sodium ferulate increased with the reduction of sodium chloride concentration. These inversely related changes of sodium ferulate solubility and osmotic pressure tended to produce the desired zero-order release profile. The general expression for the drug delivery rate dM/dt from an elementary osmotic pump tablet can be described by the following equation (Lia et al. 2004):

$$dM/dt = (A/h)\,Lp(\delta\Delta\pi - \Delta\rho)\cdot C \qquad (1)$$

Where A is the membrane area and h is its thickness; Lp is the mechanical permeability; δ is the reflection coefficient; $\Delta\pi$ and $\Delta\rho$ are the differences in osmotic and hydrostatic pressure, respectively, between the inside and outside of the system; and C is the concentration of drug in the fluid released. In this study, A, h, Lp, and δ were kept constant during the release interval. $\Delta\pi - \Delta\rho$ and C were the only parameters changed. As the size of the delivery micropores increases, the hydrostatic pressure inside the system is minimized and $\Delta\pi \gg \Delta\rho$. Since, the osmotic pressure of the release medium is negligible compared to that of the core, π can be safely substitued for $\Delta\pi$, and the following equation is obtained (Verma et al. 2002):

$$dM/dt = (A/h)\,Lp\,\delta\pi\cdot C \qquad (2)$$

Therefore the inverse relation of π and C tends to keep dM/dt constant.

Table 1: Composition of core tablets

Ingredients (mg/tablet)	Drug: Sodium chloride (1 : 0) (I)	Drug: Sodium chloride (1 : 0.5) (II)	Drug: Sodium chloride (1 : 1) (III)	Drug: Sodium chloride (1 : 1.5) (IV)
Sodium ferulate	75.0	75.0	75.0	75.0
Sodium chloride	0	37.5	75.0	112.5
Lactose	123.0	85.5	48.0	10.5
Magnesium stearate	2.0	2.0	2.0	2.0

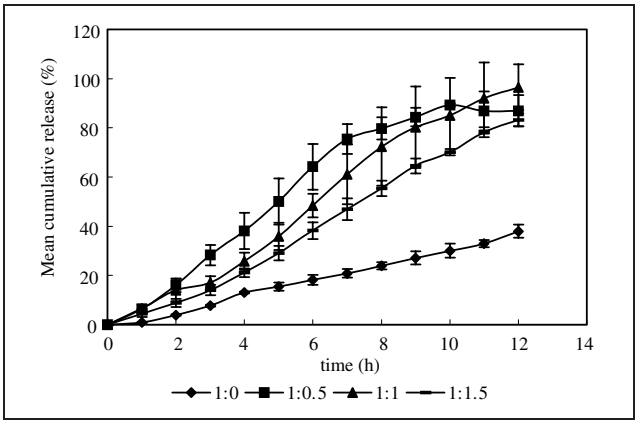

Fig. 1: Influence of drug: sodium chloride ratio on drug release. Each point represents mean ± S.D. (n = 6)

2.2. Influence of coating thickness on drug release

In order to establish the controlled porosity osmotic pump as a reliable system, it was important to investigate membrane changes and the role of various factors responsible for drug release. Therefore, the tablets were coated with different amounts of solutions with varied contents of channeling agent and plasticizer. These tablets were analyzed *in vitro*.

Tablets with a hardness of 5 kg were coated to a range of different thicknesses with 55% PEG and 8% DBP as channeling agent and plasticizing agent respectively. The weight gain for the coated tablets was determined as %w/w. The *in vitro* release of sodium ferulate from the coated tablets was determined (Fig. 2). It can be seen that the release rate from the tablets decreased dramatically with increasing weight. A lag time was seen after 2% weight gain, and the greater the weight gain the longer was the lag time: in the case of 2% coated tablets, the lag time was about 2 h, while for 4% tablets it was about 6 h. This may because in highly coated tablets, it would take a relatively longer time for water to penetrate the membrane both to dissolve the PEG, producing channels, and to change the tablet core into a saturated solution; in addition, the core solution would also take a longer time to pass through the holes in the membrane. A zero-order release profile over 12 h tended to be obtained with highly coated tablets. This was because with a weight gain below 2%, the core solution was released so quickly that not much of the drug and osmotic agent were left by the end of the release period, causing a reduction in core osmotic pressure and hence a lower driving force to push the drug solution from the system. However, an extremely high weight gain was not found to give the desired release profile over 12 h, as the ultimate cumulative release percentage was relatively low.

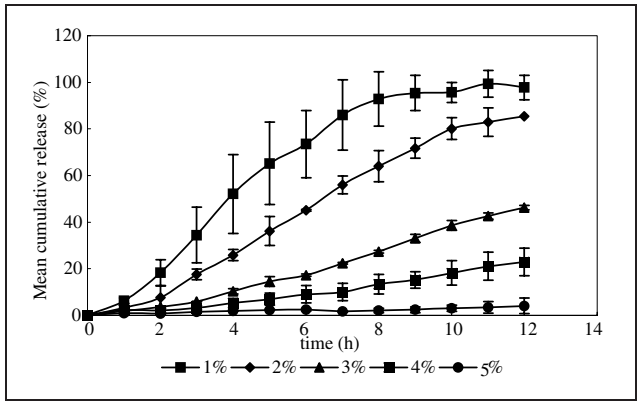

Fig. 2: Effect of different coating levels on *in-vitro* release of sodium ferulate. Each point represents mean ± S.D. (n = 6)

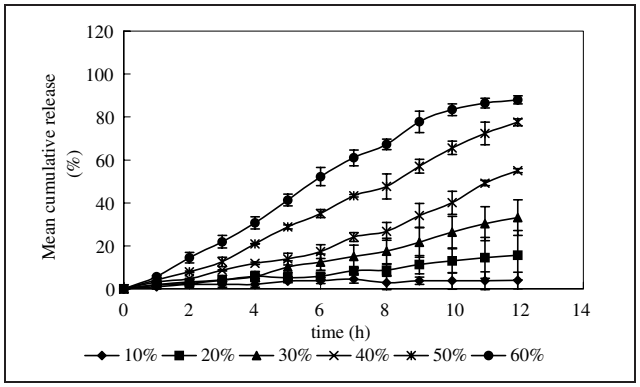

Fig. 3: Effect of PEG on *in vitro* release of sodium ferulate. Each point represents mean ± S.D. (n = 6)

2.3. Influence of PEG on drug release

Tablets with a hardness of 5 kg were coated with 8% DBP and a series of different amounts of PEG in the coating formulation to give a 2.5% increase in weight. The *in vitro* release was determined (Fig. 3). The *in vitro* release studies indicated that as the amount of PEG 400 in the polymeric coating increased, the drug release rate also increased, as did the mean cumulative release percentage over 12 h. As a hydrophilic channeling agent, PEG dissolved quickly when contacted with water, forming micropores for drug delivery. As the content of PEG 400 increased, the permeability of the polymeric coating increased. Therefore, by adjusting the amount of PEG in the coating solution the desired release rate could be achieved.

2.4. Influence of DBP on drug release

As a plasticizer, DBP could increase the flexibility of the polymeric coating so that it can withstand high osmotic pressure, which is of great importance for the safety of drug administration. In addition, because of its hydrophobic characteristics, DBP had the effect of preventing water from contacting the membrane, thus decreasing the drug release rate. The *in vitro* release of tablets with a hardness of 5 kg, 55% PEG, 2.5% weight gain and a series of different levels of DBP was investigated (Fig. 4). The results showed that as the amount of DBP in the coating formulation increased, the release rate decreased in consequence. Therefore, the desired release rate could be achieved by changing the DBP content of the coating solution.

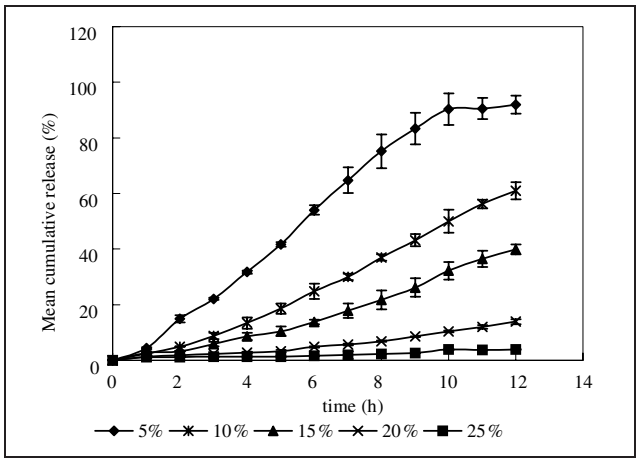

Fig. 4: Effect of DBP on *in vitro* release. Each point represents mean ± S.D. (n = 6)

2.5. Coating formulation optimization

The L_9 (3^4) orthogonal array designs for three factors were used to prepare the model formulations. Coating level, and content of channeling agent and plasticizing agent in the coating solution were selected as the factors in this study. As for any orthogonal array design, a matrix, which consists of columns and rows, with various numbers at the intersections of each column and row, was constructed. Table 2 shows an L_9 (3^4) matrix. This is a three-level orthogonal array matrix which is made up of three columns and 9 rows. Each column represents a factor, which is an independent variable. Each row represents an experimental trial. The numbers at the intersections show the level settings that apply to the factors for the experimental trials. Table 2 assigned to each factor at each level in the L_9 (3^4) OAD experiment. The core tablets were prepared with formula III in Table and coated. The *in vitro* release profile of the tablets with those formulations was tested to determine the best formulation. The tablets were prepared twice for each formulation.

As for the optimization study, the optimum formulation was assumed to be the one with a zero-order release profile, and a high cumulative release percentage. Therefore, four indexes were selected: r (correlation coefficient) of the linear regression for the dissolution curve, to estimate whether the release profile was zero-order; cumulative release percentage of 2, 6 and 12 hours (which were marked as L_1, L_2, and L_3, respectively), to determine whether there was a lag time, whether the median release rate was desirable and whether the drug was released completely, respectively. Hence, a response weighted summation was introduced to incorporate all the four indexes scientifically into one comprehensive contribution response L. It was calculated by the following equation:

$$L = |L_1 - 17\%| \times 100 \times 1 + |L_2 - 50\%| \times 100 \times 1 + |L_3 - 90\%| \times 100 \times 1 + |r - 1| \times 100 \times 2 \quad (3)$$

Here, the coefficients for L_1, L_2, L_3 and r were 1, 1, 1 and 2 respectively.

The smaller L was, the better the formulation would be. Results of the L_9 orthogonal array design for three factors were analyzed based on a statistical analysis (analysis of variance, ANOVA) technique using the computer program Orthogonality Experiment Assistant (Sharetop Software Studio, China).

The aim of the OAD was to select the optimal coating level, PEG content, and DBP content for obtaining the desired release profile. The factors and response function (L) are given in Table 3. Table 4 shows the significance (F ratio) analysis, and it can be seen that DBP content is statistically significant at $0.01 < P < 0.05$; PEG content

Table 2: Formulations for insulin emulsion based on orthogonal array design

Formulation number	A	B	C
	Table weight gain (%)	PEG content (%)	DBP content (%)
Rp. 1	2.0	50.0	5.0
Rp. 2	2.0	55.0	7.5
Rp. 3	2.0	60.0	10.0
Rp. 4	2.5	50.0	7.5
Rp. 5	2.5	55.0	10.0
Rp. 6	2.5	60.0	5.0
Rp. 7	3.0	50.0	10.0
Rp. 8	3.0	55.0	5.0
Rp. 9	3.0	60.0	7.5

Table 3: L_9 (3^4) OAD matrix with the experimental results

Formulation	Tablet weight gain (%) (A)	PEG content (%) (B)	DBP content (%) (C)	L
Rp.1	2.0	50.0	5.0	62.415
Rp.2	2.0	55.0	7.5	12.700
Rp.3	2.0	60.0	10.0	28.486
Rp.4	2.5	50.0	7.5	16.061
Rp.5	2.5	55.0	10.0	63.274
Rp.6	2.5	60.0	5.0	19.179
Rp.7	3.0	50.0	10.0	108.624
Rp.8	3.0	55.0	5.0	69.353
Rp.9	3.0	60.0	7.5	21.252
K_1	34.534	62.367	50.316	
K_2	32.838	48.443	16.617	
K_3	66.410	22.972	66.795	
R	33.572	39.395	50.124	

* K was the average of L at each level of a factor; R was the range of K of a factor

and tablet weight gain are significant at $P < 0.1$. These results were further confirmed by the analysis of percentage contribution (PC%) in Table 4. The PC indicated DBP content (43.9%) is the most important factor contributing to the L response, followed by PEG content (25.9%), and tablet weight gain (22.9%). The data analysis of PC% is clearly in good agreement with the conclusion of the significance analysis discussed above. The level for each factor that would result in the smallest response (L) should be adopted to make the optimal formulation. Therefore, the formulation for attaining optimal drug release profile is tablet weight gain (A) at level 2 (2.5%), PEG content (B) at level 3 (60%), and DBP content (C) at level 2 (7.5%), based on the response in the experiments listed in Table 3.

In Table 4 SS_x is the sum of squares due to the factor X; df is the degrees of freedom; MS_x is the mean square due to the factor X; MS_{error} is the mean square due to error; F is the random variable of the F distribution; SS′ is the purified sum of squares; PC is the per cent contribution, calculated by the following equation. SS_x were calculated by the Orthogonality Experiment Assistant software, and other parameters were calculated by the following equations:

$$MS_x = \frac{SS_x}{df} \quad (4)$$

$$MS_{error} = \frac{SS_{error}}{df_{error}} \quad (5)$$

$$F = \frac{MS_x}{MS_{error}} \quad (6)$$

$$SS'_x = SS_x - 2MS_{error} \quad (7)$$

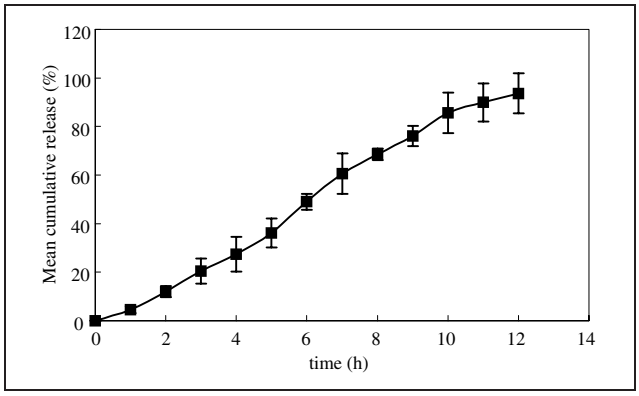

Fig. 5: Drug mean release profile of tablets with optimal formulation. Each point represents mean ± S.D. (n = 6)

$$SS'_{error} = SS_{total} - \sum SS'_x \quad (8)$$

$$PC = \frac{SS'_x}{SS_{total}} \times 100\% \quad (9)$$

Tablets were produced with the optimized formulation, and tested *in vitro* (Fig. 5). The desired zero-order release profile over 12 h was obtained with $r = 0.9955$ and 93.6% of the drug was released in 12 h.

First-order and Higuchi models were compared (Table 5). As shown, r was greatest for the zero-order model, which indicated that the zero-order model gave the best fit.

2.6. In vivo studies

A calibration curve for the determination of sodium ferulate in dog plasma ($A = 0.3327\,C - 0.0073$, $r = 0.9990$) was prepared over a linear range of 0.084 μg/ml − 16.128 μg/ml, and plasma concentrations were measured by an HPLC method (see 3.6). Mean sodium ferulate plasma concentrations versus time profiles for CPOP and immediate − release tablets are illustrated in Fig. 6. Pharmacokinetic parameters, listed in Table 6, were obtained using the DAS pharmacology software package.

The observed difference in mean pharmacokinetic parameters of sodium ferulate from controlled porosity osmotic pump and immediate release (reference) tablet dosage forms was assessed by analysis of variance (ANOVA). Significance was tested by Student's t-test and a value of $P < 0.05$ was considered statistically significant. The relative bioavailability was calculated by the equation:

$$\text{Relative bioavailability} = \frac{(AUC_{0\to t})_{cpop}}{(AUC_{0\to t})_{ref}} \times 100\% \quad (10)$$

Table 4: ANOVA analysis for L in the L_9 (3^4) OAD matrix

Source	SS	df	MS	F	SS′	PC(%)
Tablet weight	2146.021	2	1073.011	10.492*	1941.491	22.96
PEG content	2394.574	2	1197.287	11.708*	2190.044	25.90
DBP content	3915.953	2	1957.977	19.146**	3711.423	43.89
Error	204.530	2	102.265		613.590	7.20
Total	8456.548	8			8456.548	100.00

* Critical value is19.0 (** $0.01 < P < 0.05$) and 9.00 (* $P < 0.10$)

Table 5: Results of model fitting analysis

Model	Regression equation	Max. absolute error (%)	Max. relative error (%)	Re	r
Zero-order	$Y = 0.086t - 0.033$	0.057	0.061	0.012	0.9955
First-order	$Ln(1-y) = -0.224t + 0.395$	0.484	4.726	0.3658	0.9577
Higuchi	$Y = 0.316t^{1/2} - 0.230$	2.621	6.867	27.852	0.9500

Table 6: Mean pharmacokinetic parameters for single dose administration (300 mg) of controlled porosity osmotic pump and immediate-release tablets of sodium ferulate

Parameters	Controlled porosity osmotic pump	Immediate-release tablets	t-test
$AUC_{0\rightarrow t}$ ($\mu g \cdot h \cdot mL^{-1}$)	14.747 ± 2.87	14.136 ± 4.605	$P > 0.05$
T_{max} (h)	4.833 ± 0.726	1.000 ± 0	$P < 0.05$
C_{max} ($\mu g \cdot mL^{-1}$)	2.227 ± 0.5834	12.942 ± 1.568	$P < 0.05$
$T_{1/2}$ (h)	12.892 ± 2.879	5.824 ± 1.660	$P < 0.05$
Relative bioavailability (%)		104.3%	

Data represented as mean ± SD

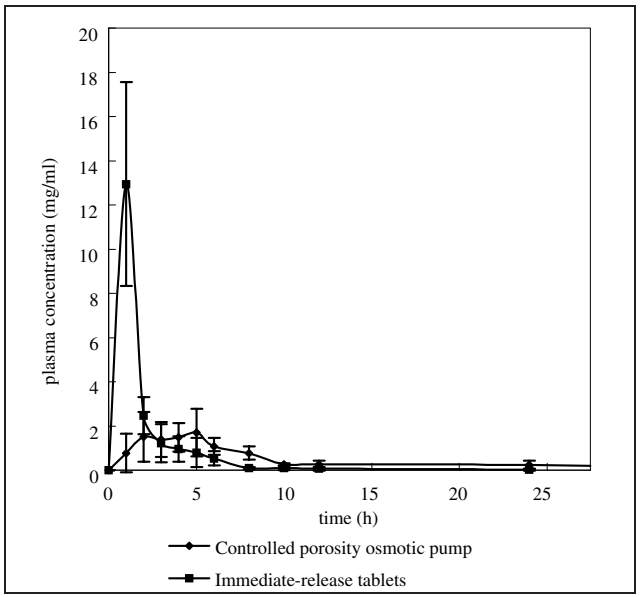

Fig. 6: Drug plasma concentration-time profile for controlled porosity osmotic pump and immediate-release tablets of sodium ferulate. Each point represents mean ± S.D. (n = 6)

Where $(AUC_{0\rightarrow t})_{cpop}$ and $(AUC_{0\rightarrow t})_{ref}$ were $AUC_{0\rightarrow t}$ of controlled porosity osmotic pump and immediate release reference tablets, respectively.

The *in vivo* release of sodium ferulate from controlled porosity osmotic pump formulations was compared with release from conventional tablets. The two types of tablet showed totally different release profiles: peak concentration was reached almost immediately (within 1 h of oral administration) for immediate release tablets, while it took about 5 h when controlled porosity osmotic pump tablets were administrated; and the peak concentration with conventional tablets was significantly higher than that with CPOP tablets (Fig. 6). The calculated $AUC_{0\rightarrow t}$ values were not significantly different ($p > 0.05$) between the two formulations. The C_{max} value for CPOP tablets was significantly lower ($p < 0.05$) compared with the reference tablets, while their T_{max} was significantly longer ($p < 0.05$). The serum half life $T_{1/2}$ was also significantly prolonged ($p < 0.05$) when the formulation was changed from conventional tablets to CPOP. Relative bioavailability was 104.3%. All these pharmacokinetic parameters are listed in Table 6.

From these results it can be concluded that sodium ferulate CPOP tablets yielded a more constant plasma concentration and similar bioavailability compared to conventional tablets.

3. Experimental

3.1. Materials

Sodium ferulate was chosen as the model drug (Chengdu First Medical Co., China). Beagle dogs were supplied by the Laboratory Animal Center of Sichuan Antibiotic Institution. Sodium chloride (Chengdu Chemical Co., China) and lactose (Huaxi Medical Company of Sichuan University, China) were used as release-controlling agent and diluent, respectively. Cellulose acetate with 54.5% ~ 56% acetyl content (Medical Company of China) was used as a semipermeable membrane. Dibutylphthalate (DBP, Tianjin Bodi Chemical Co., China) and polyethyleneglycol 400 (PEG 400, Chengdu Guanghua Chemical Co., China) were employed as plasticizer and channeling agent respectively. Methanol (HPLC grade) was purchased from Sanli Chemical Factory (Zhejiang, China). Water for HPLC was prepared in a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals used were of analytical grade.

3.2. Preparation of core tablets

The drug, osmotic agent and diluents were weighed accurately, passed through an 80 mesh sieve and mixed for 15 min. This mass was granulated using 10% PVP (w/v) in 95% ethanol as binder. Compression was done on a single punch tablet machine (Tianhe, Shanghai, China) using 8.0 mm standard concave punches. The weight of the tablets was 200.0 mg. The compression force was controlled at 5.0 ± 0.5 kg. Tablet hardness was monitored using a tablet hardness tester (Model 78X-2, Shanghai, China). Different ratios of drug: release-controlling agent tried were 1 : 0, 1 : 0.5, 1 : 1 and 1 : 1.5. Compositions of core tablets are listed in Table 1.

3.3. Coating with semipermeable membrane

Cellulose acetate (2%, w/v) in acetone: ethanol (2 : 1) containing different levels of channeling agent (ranging from 10% to 60% w/w) of the CA amount and plasticizing agent (raging from 5% to 25% w/w) of the CA amount was used as the coating solution. The level of coating in % (w/w) (ranging from 1% to 5%) was determined from the weight gain of 20 tablets coated at the same time, divided by the initial tablet weights.

After being warmed for 10 min, core tablets were spray-coated with the membrane by a conventional pan-spray method (BY300A pan-coater, Shanghai, China) under operating conditions as follows: batch size, 40 g; spraying rate, 1 ml/min; drying temperature, 35–40 °C; spray air pressure, 0.6 kg/cm^2. The tablets were dried at 50 °C for 4 h to remove residual solvent after coating (Zhang et al. 2003). The surface morphology of the tablets was smooth and uniform in appearance.

3.4. In vitro release

In vitro drug release studies were performed according to the P.R. China Pharmacopoeia Dissolution Apparatus method at 37 ± 0.5 °C. The release medium was distilled water according to the P.R. China Pharmacopoeia. During the release studies, 5 ml samples were withdrawn every hour up to 12 h, being replaced with an equal volume of medium. The rotation speed was 50 rev./min. The concentration of sodium ferulate released from the coated tablets was analyzed by an UV spectrophotometer (UV-2201, Shimadzu, Japan). This was done in triplicate.

Dissolution tests were performed on four specific types of tablet with formulation I–IV as listed in Table 1 to investigate the role of sodium chloride in drug release behavior. Tablets with a series of coating levels (Zhang et al. 2003; Agrawal and Panchagnula 2004), and contents of channeling agent and plasticizer were tested to estimate the effects of all these factors on drug release.

3.5. In vivo studies

A group of three male and three female beagle dogs with a mean weight of 7.5 ± 1.3 kg (ranging from 6.2 to 8.8 kg) were included in this study. To compare the relative bioavailability of controlled porosity osmotic pump tablets and commercially available immediate-release tablets, the following study using an open randomized crossover design was performed. Four tablets (containing sodium ferulate 300 mg in total) produced with the optimal formulation were administered to dogs fasted for 12 h and immediately followed by an oral gavage of 50 ml water. As a control, commercially available immediate-release tablets with the same dosage were administered. The dogs were fed their normal diet 4 h after dosing.

Whole blood samples (3.0 ml) were taken from the leg vein of each dog using 5-ml disposable syringes before dosing and at 1, 2, 3, 4, 5, 6, 8, 12, 24, and 36 h after dosing for the controlled porosity osmotic pump tablets

and at 1, 2, 3, 4, 5, 6, 8, 12, and 24 h for the reference tablets. The samples were immediately transferred to heparinized (sodium heparin) centrifuge tubes. Samples were spun in a centrifuge at 3000 rpm for 5 min. The plasma samples were poured into 2-ml plastic tubes. Samples were frozen and stored in a freezer until they were analyzed, usually within a period of 1 week. Plasma was thawed before analysis. Then, 0.4 ml plasma was acidified with 0.1 ml 0.1 M HCl and vortex-mixed for 1 min. Acidified plasma was vortex-mixed with 3 ml ethyl acetate and 15 μl tinidazole (100 μg/ml, acting as internal standard) for 2 min and centrifuged at 3000 rpm for 10 min. 2.5 ml of the organic layer was taken out and dried at 50 °C under a stream of nitrogen and the residue was dissolved in 100 μl of the mobile phase for injection to the chromatographic system. All the above operations were carried out avoiding light.

The plasma-concentration-time profiles were analyzed using standard pharmacokinetic analysis techniques. The following parameters were assessed: area under the plasma concentration-time curves ($AUC_{0\rightarrow t}$), peak concentration (C_{max}), and peak time (T_{max}), using the DAS ver1.0 (Drug And Statistics for Windows) program. Relative bioavailability was calculated subsequently.

3.6. HPLC analysis

A HPLC system (SPD-10A vp, Shimadzu, Japan) with UV detector (SPD-10A vp, Shimadzu, Japan) was used to quantify the amount of drug in plasma. Data integration was done using the CLASS-VP 5.0 software package (Shimadzu, Japan). Chromatography column was Shimadzu Shim-Pack C reverse phase column (200 × 6 mm I.D.) with 5-μm pore size column (Chiyoda-Ku, Kyoto, Japan) protected by a Shimadzu Shim-Pack G guard column (4 × 1 mm I.D.) (Chiyoda-Ku, Kyoto, Japan).

The mobile phase consisted of methanol : acetic acid aqueous solution (0.05%) = 35 : 65(v/v) at a flow rate of 1.0 ml/min. The mobile phase was filtered, degassed by sonication and pumped through the system at a flow rate at 1.0 ml/min at 35 °C. The normal operating pressure was 100–130 Mpa and the analytical time was ∼20min. Analysis was done at a wavelength of 324 nm.

References

Agrawal S, Panchagnula R (2004) *In vitro* evaluation of fixed dose combination tablets of anti-tuberculosis drug after real time storage at ambient conditions. Pharmazie 59: 782–783.

Huang C, Su Y, Hsieh Y (2002) Optimization of the headspace solid-phase microextraction for determination of glycol ethers by orthogonal array designs. J Chromatogr A 977: 9–16.

Lan WG, Wang MK, Chen N, Sin YM (1994) Four-level orthogonal array design as achemometric approach to the optimization of polarographic reaction system for phosphorus determination. Talanta 41: 1917–1927.

Lia X, Pana W, Nie S, Wu L (2004) Studies on controlled release effervescent osmotic pump tablets from traditional chinese medicine compound recipe. J Control Release 96: 359–367.

Lin Y, Chen W (1994) Pharmacological effects and molecule alteration prospect of sodium ferulate. Acta Pharm Sinica 29: 717–720.

Liu H (2005) Pharmacological effects and clinical use of sodium ferulate. China Pharmaceuticals 14: 78–79.

Makhija SN, Vavia PR (2003) Controlled porosity osmotic pump-based controlled release systems of pseudoephedrine I. Cellulose acetate as a semipermeable membrane. J Control Release 89: 5–18.

Onuki Y, Morishita M, Takayama K (2004) Formulation optimization of water-in-oil-water multiple emulsion for intestinal insulin delivery. J Control Release 97: 91– 99.

Santus G, Baker RW (1995) Osmotic drug delivery: a review of the patent literature. J Control Release 35: 1–21.

Theeuwe F (1975) Elementary osmotic pump. J Pharm Sci 64: 1987–1991.

Verma RK, Krishna DM, Garg S (2002) Formulation aspects in the development of osmotically controlled oral drug delivery systems. J Control Release 79: 7–27.

Wang Q, Chen S, Xiong L, Jin W, Hu S, Dong H (2003) Ameliorative effects of sodium ferulate on experimental colitis and their mechanisms in rats, World J Gastroenterol 9: 2533–2538.

Wei G, Shao P, Bao P, Dong F, He S, Jie P (2003) Effect of sodium ferulate on activation of postsynaptic density-95 after transient facoi cerebral ischemia. J Medical Colleges Pla 18: 201–202.

Zentner GN, Rork GS, Himmelstein KJ (1985) The controlled porosity osmotic pump. J Control Release 1: 269–282.

Zhang Y, Zhang Z, Wu F (2003) A novel pulsed-release system based on swelling and osmotic pumping mechanism. J Control Release 89: 47–55.

Zhu G, Ju H (2004) Determination of naproxen with solid substrate room temperature phosphorimetry based on an orthogonal array design. Anal Chim Acta 506: 177–181.