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Development of sustained-release matrix tablets of BKP-01-041 (tilorone derivative) containing Hypromellose

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The objective of this research was to develop and evaluate sustained-release matrix tablets of BKP-01-041 (tilorone derivative) based on Hypromellose (hydroxypropyl methylcellulose, HPMC) as the matrix forming polymer. The sustained-release tablets were prepared by the wet granulation method. The influence of HPMC viscosity and ratios on drug release was investigated *in vitro*. Dissolution of the tablets developed with 26% HPMC K4 M/K100 M (1:2) (w/w) content showed a better drug release profile than the other batches tested in 12 h. Drug release from the optimal formulation was analyzed using release kinetics equations. The release kinetics parameters were determined and the value of the exponent (n) representing the apparent drug release mechanism determined from the Peppas equation was about 0.726. These results suggest that the drug release mechanism was non-Fickian ($0.45 < n < 0.89$), and drug release was dependent on both drug diffusion and polymer erosion.

1. Introduction

BKP-01-041 is a newly synthesized tilorone derivative used to treat pneumoconiosis which is a fibrosing pulmonary disease caused by long term inhalation of metallic or mineral dusts (Zhang et al. 2008). The water-soluble drug is an orally administered small molecular interferon inducer and has been shown to interact and influence the immune mechanisms in humans and animals (Dasari and Srikrishnan 2000). It is rapidly and widely distributed in the body and is excreted in urine following oral administration (Zhang et al. 2010). Furthermore, it has good solubility in solutions of various pH (the drug solubility in 0.1 N HCl, pH 6.8 phosphate buffer and pure water was 181 mg/ml, 184 mg/ml and 215 mg/ml at room temperature, respectively) and a short plasma elimination half-life which makes it a suitable candidate for delivery from sustained-release dosage forms as frequent drug administration may reduce patient compliance and thus therapeutic efficacy.

Sustained release (SR) oral delivery systems are designed to achieve therapeutically effective concentrations of drug in the systemic circulation over an extended period of time, thus can achieve optimum therapeutic responses, prolonged efficacy and a decreased incidence of adverse drug reactions (Sanchez-Lafuente et al. 2002; Gil et al. 2006; Krishnaraj et al. 2012). Hydrophilic matrix tablets are becoming the most popular delivery systems for oral sustained-release dosage forms *via* the gastrointestinal route. Various types of water soluble polymers are used as the gel forming agent in matrices, such as sodium carboxymethylcellulose, hydroxypropyl methylcellulose, hydroxypropylcellulose and methylcellulose (Hosny et al. 1997). Drug release from these types of systems is controlled by hydration of the polymer, which forms a gelatinous barrier layer at the surface of the matrix, through which the included drug diffuses. In addition, the resistance of such a gel layer to erosion is controlled by the viscosity grade of the matrix (Velasco et al. 1999).

Hypromellose (hydroxypropyl methylcellulose, HPMC) has a long history of application in marketed products with wide global regulatory acceptance (Pachuau and Mazumder 2012). Its popularity can be attributed to the polymer's non-toxic nature, small influence of processing variables on drug release, ease of compression, and its ability to accommodate high levels of drug loading (Taylan et al. 1996). Drug release from hydrophilic matrices is known to be a complex interaction between dissolution, diffusion and erosion mechanisms (Cox et al. 1999). The present study aimed to develop a BKP-01-041 sustained-release matrix tablet containing Hypromellose as the hydrophilic matrix to retard drug release and to elucidate the mechanism of its drug release *in vitro*.

2. Investigations, results and discussion

2.1. In vitro drug release characteristics

For the purpose of developing formulations of BKP-01-041 sustained-release tablets in 12 h, in the preliminary studies, we carried out single factor experiments to determine the effects of varying polymer levels (HPMC levels at 20, 26, or 32% (w/w)) and excipients (adhesive, lubricant and diluents including pregelatinized starch, microcrystalline cellulose and common starch) on drug release. These experimental results (data not presented) suggested that a greater drug release rate was observed for tablets with a lower HPMC level, and a better drug profile was obtained when the HPMC level was 26% (w/w) compared with the other levels tested. When the HPMC contents was > 32% (w/w), the drug release rates were not contents reduced by increasing the polymer concentration (the f_2 value of the HPMC 20%w/w

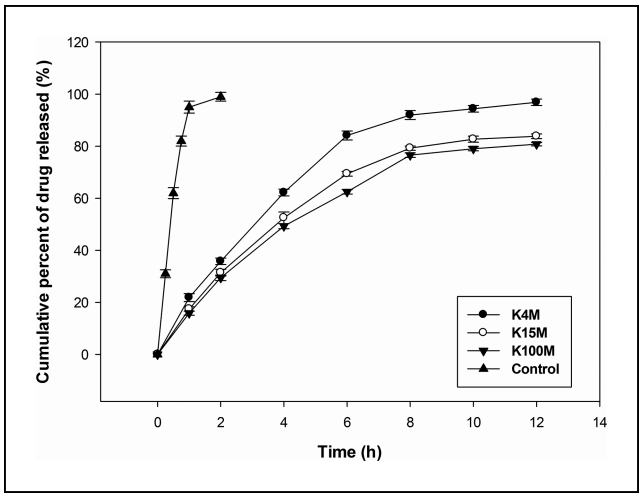

Fig. 1: Cumulative percent of BKP-01-041 released *vs.* time profiles for formulations with various HPMC grades: (●) K4 M (F1); (◯) K15 M (F2); (▼) K100 M (F3); (▲) Control, mean ± SD, n = 6.

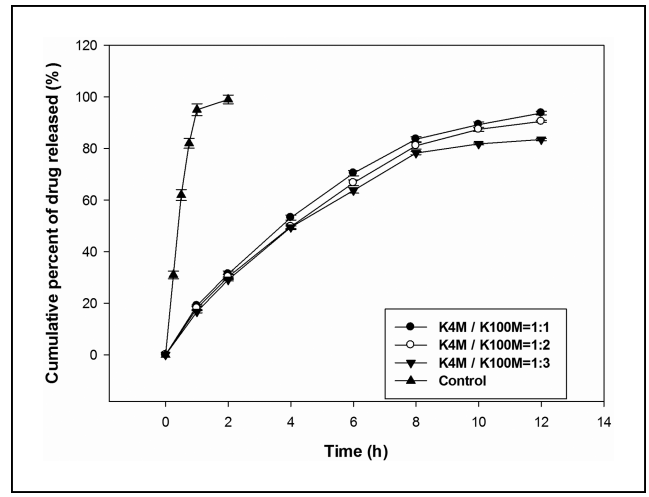

Fig. 2: Cumulative percent of BKP-01-041 released *vs.* time profiles for formulations with various ratios of METHOCEL K4 M/K100 M: (●) K4 M/K100 M = 1:1 (F4); (◯) K4 M/K100 M = 1:2 (F5); (▼) K4 M/K100 M = 1:3 (F6); (▲) Control, mean ± SD, n = 6.

and 26%w/w was 44.1 but the f_2 value of the HPMC 32%w/w and 26%w/w was 72.3). Furthermore, the influence of excipients on the BKP-01-041 release rate was not significant and pregelatinized starch showed better compressibility and fluidity than other diluents in our experiments. Thus in subsequent drug release experiments, we selected a constant HPMC level (26%) and pregelatinized starch in the formulations, while the viscosity grade of HPMC was varied.

Figure 1 shows the release profiles of BKP-01-041 from various SR formulations, with the immediate release (IR) tablet as a control. The viscosities of METHOCEL K4 M, METHOCEL K15 M and METHOCEL K100 M in a 2% solution (20 °C) were 4 000, 15 000 and 100 000 mPa s, respectively. It was obvious that drug release values gradually decreased when the viscosity increased. The METHOCEL K4 M formulation exhibited a significantly greater drug release rate than K15 M (f_2 = 48.2) and K100 M (f_2 = 42.1). The higher viscosity gel layers provided a more tortuous and resistant barrier to diffusion (Nellore et al. 1998) which resulted in slower release of BKP-01-041 from these matrices. The non-significant difference in the drug release profiles for the K15 M and K100 M formulations (f_2 = 70.9) suggests the existence of a 'limiting HPMC viscosity' (Sung et al. 1996). That is, the drug release rate no longer decreased when the viscosity was increased above 15 000 mPa s.

We then selected mixtures of METHOCEL K4 M/K100 M as the matrix for the BKP-01-041 SR tablets and compared the release profiles of the BKP-01-041 SR formulations containing various weight ratios (1:1, 1:2 and 1:3) of METHOCEL K4 M/K100 M. Figure 2 shows the BKP-01-041 release profiles with various weight ratios of METHOCEL K4 M/K100 M. The release profiles indicated that the drug release rate decreased when the ratio of K4 M/K100 M decreased or the content of K100 M increased. The f_2 values suggested that the dissolution profiles of F4, F5 and F6 were similar. That is, at the ratio range of METHOCEL K4 M/K100 M from 1:1 (w/w) to 1:3 (w/w), the drug release rate of the SR formulations showed no significant fluctuations. Formulation F5 (K4 M/K100 M = 1:2) had an intermediate drug release rate compared with F4 and F6, but showed a better release profile according to the target profile design parameters. Therefore, we selected F5 as the optimal formulation and studied the drug release mechanism further.

2.2. *Drug release mechanism*

The *in vitro* release kinetics of the optimal formulation (F5) containing the matrix of METHOCEL K4 M/K100 M = 1:2 (w/w) was analyzed using various equations and the kinetic parameters are presented in Table 1. For the zero-order equation, first-order equation, Higuchi equation and Hixson-Crowell equation, the release data obtained from dissolution profiles in 0 ∼ 12 h were fitted to the equations. As for the Peppas equation, the release data from 0 to 4 h were fitted because the equation was applied during the early stages of release (fraction released, < 0.6). The correlation (r^2) was used as an indicator of the best fit for each of the models considered. Evaluation of drug release kinetics and application of best fit using correlation coefficients showed that the first-order (r^2 = 0.996), Higuchi (r^2 = 0.993) and Hixson-Crowell (r^2 = 0.994) equations seemed to be a better fit than the zero-order equation (r^2 = 0.971). This indicated that the drug release was both diffusion and erosion dependent. The release exponent value (n = 0.726) of F5 obtained from the Peppas model suggested that the release mechanism was anomalous (non-Fickian) for the first part of the release curve (up to 60% of drug dissolved).

For the case of non-Fickian transport, the percentage contributions of Fickian diffusion (F) and relaxation (R) over 12 h of BKP-01-041 release from matrix tablets (formulation F5) are shown graphically in Fig. 3. The contribution of Fickian diffusion predominated throughout the entire dissolution time

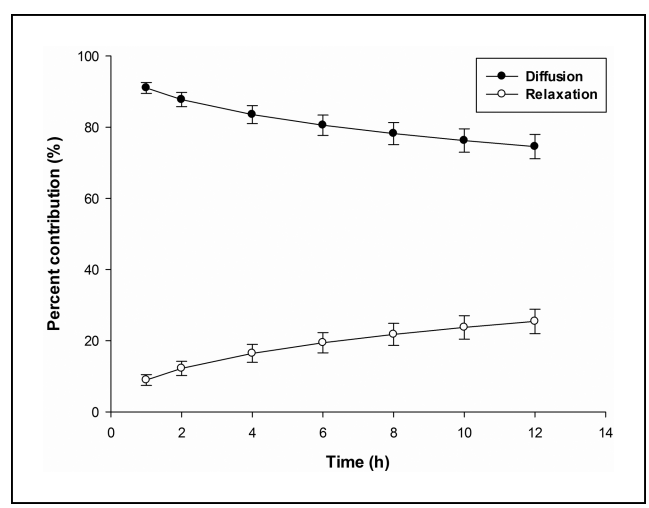

Fig. 3: Percentage contributions of Fickian diffusion and the polymer relaxation mechanisms valid throughout 12 h of drug release from F5 SR tablets (mean ± SD, n = 6).

Table 1: Parameters obtained from kinetic equations of the BKP-01-041 sustained-release tablets (optimal)

Parameter	Peppas	zero-order	First-order	Higuchi	Hixson-Crowell	Diffusion-relaxation
k	18.229	6.776	0.206	31.250	-0.212	20.762 (k_2) 2.044 (k_3)
r	0.999	0.971	0.996	0.993	0.994	0.993
n	0.726	/	/	/	/	/

All values are mean values for 6 samples. *k*, kinetic constant; *r*, coefficient of correlation; *n*, release exponent.

period, and the ratios gradually decreased from 91.04% for 1 h to 74.57% for 12 h. In contrast, the ratios of the polymer relaxation gradually increased from 8.96% for 1 h to 25.43% for 12 h. Owing to slow erosion of the outer gel layer of HPMC matrices, the diffusional path length for the drug increased with time and hence the release rate decreased with time.

The above results show that for the formulation F5 containing METHOCEL K4 M/K100 M 1:2 (w/w), the BKP-01-041 release mechanism was non-Fickian ($0.45 < n < 0.89$), and drug release was predominantly dependent on drug diffusion and supplemental polymer erosion. According to the literature (Gil et al. 2006), drug release from a hydrophilic matrix is governed by the following sequential processes: firstly, hydration or swelling of the matrix which results in the formation of a gel; secondly, dissolution of the drug into the hydrated matrix/gel; thirdly, diffusion of the drug molecules through the hydrated matrix; and finally surface erosion and/or dissolution of the formed gel-matrix.

In conclusion, the drug in the developed BKP-01-041 tablets (Formulation 5) was successfully released *in vitro* for 12 h compared with the IR tablets. The correlations (r) of Higuchi (diffusion) and Hixon–Crowell (erosion) kinetic equations achieved 0.993 and 0.994, respectively, and consequently, a co-dependent diffusion/erosion mechanism is suggested as the main drug release mechanism from these tablets. Further studies are needed to investigate the pharmacokinetics of the SR tablets in beagle dogs, and to correlate the *in vivo* and in vitro results.

3. Experimental

3.1. Materials

BKP-01-041 was supplied by Berkgen Biotechnology Co., Ltd. (Tianjin, China). HPMC (METHOCEL K4 M/K15 M/K100 M) and pregelatinized starch (Starch 1500) were purchased from Colorcon (UK). Polyvinylpyrrolidone (KoVidone K30) was obtained from Boai NKY Pharmaceuticals Ltd. (China). Microcrystalline cellulose (MCC 101) was purchased from ILE (Tianjin, China). Magnesium stearate was provided by Kermel Chemical Reagent Co., Ltd. (Tianjin, China). All other materials were of analytical reagent grade.

3.2. Preparation of sustained-release tablets

In our preliminary steps, HPMC was used as the matrix forming polymer in this investigation and the matrix tablets (each batch size was 100 tablets and about 33 g) were prepared by the wet granulation (WG) method and direct compression (DC) method. The results suggested that the drug release was faster from tablets produced by the direct compression method than from those prepared by the wet granulation method. The wet granulation method used in the preparation of granules significantly improved the compressibility and flowability of the powder for all the batches of formulations (Table 2). Furthermore, the blend powder of the high drug loading formulations (>45%w/w) had better content homogeneity and bulk density using the wet granulation method. Thus the matrix tablets (Table 3) were manually prepared using the wet granulation method according to the experimental design. First, all materials after drying to a constant weight, with the exception of lubricants, were thoroughly mixed and then wetted using PVP-K30 in aqueous ethanol as the granulating fluid. The mixed product was passed through a 24 mesh (ChP 2010). The granules were dried at 55 °C for 2 h until the loss on drying of the granules was between 1% and 2% w/w to achieve limited moisture content and uniform drug content in the tablets. The dried granules were then passed through a 20 mesh (ChP 2010). The prepared granules were blended with magnesium stearate and then compressed using 10 mm flat punches in a single punch tablet press (Shanghai Zhouxiang Pharmaceutic Machinery Co., Ltd. China) under a constant compression force which was obtained using the same distance between the upper and lower punches. The hardness of the matrix tablets was 40 - 50 N and determined by the hardness tester (Tianjin Xintianguang Instrument Co., Ltd. China).

Table 2: Properties of the granules (WG) and the powder mixtures (DC)

Properities	Granules of WG	Powder mixtures of DC
Angle of repose (○)	$25.09 \pm 0.43^*$	32.26 ± 0.51
Bulk density (g/ml)	0.47 ± 0.01	0.45 ± 0.01
Tapped density (g/ml)	0.54 ± 0.03	0.56 ± 0.02
Compressibility index (%)	$13.69 \pm 0.19^*$	19.65 ± 0.08

$^*P < 0.05$, when compared with the powder mixtures

3.3. In vitro dissolution test

The *in vitro* dissolution test was performed on ChP appendix XC dissolution apparatus 1 (basket method) (ZRS-8G dissolution tester, Tianjin, China), with 900 ml of pure water as the dissolution medium maintained at 37 ± 0.5 °C and rotated at 100 rpm. The drug content release at predetermined time intervals was measured using a UV–Vis spectrophotometer (UV-1750 spectrophotometer, shimadzu, Japan) at 263 nm. The cumulative percent of drug released was calculated and plotted *versus* time. Experiments were performed on six tablets of each formulation and mean values and standard deviation were calculated. These release studies were also compared with immediate release (IR) tablets (Table 2).

A comparison of dissolution profiles was made according to the similarity factor method (Moore and Flanner 1996). A similarity factor can be defined as:

$$f_2 = 50 \log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \qquad (1)$$

In Eq. (1) f_2 is the similarity factor, n is the number of the time point, R_t is the mean percent drug dissolved of the current formulation, and T_t is the mean percent drug dissolved of the changed composition.

An f_2 value between 50 and 100 suggests that two dissolution profiles are similar. In this study, experimental data corresponding to 1, 2, 4, 6, 8, 10, and 12 h were considered.

The target profile design parameters (Cohen et al. 1990) of a sustained-release tablet of BKP-01-041 were as follows: $30 \pm 5\%$ release in 2 h, $65 \pm 5\%$ release in 6 h and $85 \pm 5\%$ release in 10 h.

Table 3: Composition of the BKP-01-041 sustained released tablet formulations

Ingredients	Formulation quantity (mg)						
	F1	F2	F3	F4	F5	F6	IR
BKP-01-041	150	150	150	150	150	150	50
METHOCEL K4M	86	/	/	43	28.7	21.5	/
METHOCEL K15M	/	86	/	/	/	/	/
METHOCEL K100M	/	/	86	43	57.3	64.5	/
Pregelatinized starch	76	76	76	76	76	76	63
$CaCO_3$	/	/	/	/	/	/	10
Magnesium stearate	3.3	3.3	3.3	3.3	3.3	3.3	1
PVP K30	14.7	14.7	14.7	14.7	14.7	14.7	6
Total	330	330	330	330	330	330	130

3.4. Release kinetics

The release data obtained from *in vitro* dissolution studies were fitted to various kinetic equations to identify the mechanism of drug release from the matrix tablets. The kinetic models used included the Peppas equation (Korsmeyer et al. 1983; Peppas 1985), zero-order equation (Gibaldi and Feldman 1967), first-order equation (Wagner 1969), Higuchi equation (Higuchi 1963) and Hixson-Crowell equation (Hixson and Crowell 1931).

$$M_t/M_\infty = Kt^n \quad \text{(Peppas equation)} \tag{2}$$

$$M_t/M_\infty = k_0 t \quad \text{(Zero-order equation)} \tag{3}$$

$$\ln M_t = \ln M_\infty + k_1 t \quad \text{(First-order equation)} \tag{4}$$

$$M_t/M_\infty = k_H t^{1/2} \quad (\textit{Higuchi equation}) \tag{5}$$

$$M_t/M_\infty = 1 - (1 - k_s t)^3 \quad \text{(Hixson-Crowell equation)} \tag{6}$$

In the Peppas equation (Korsmeyer et al. 1983; Siepmanna and Peppas 2001; Mohammadi-Samani et al. 2005), M_t and M_∞ are the absolute cumulative amount of drug released at time t and infinite time, respectively; K is a kinetic constant incorporating the structural and geometric characteristic of the device and n is the release exponent indicating the type of drug release mechanism. The release exponent 'n' calculated from the Peppas equation for a cylinder tablet was < 0.45 for Fickian release, > 0.45 and < 0.89 for non-Fickian release, 0.89 for case II release and > 0.89 for super case II type release (Peppas 1985; Velasco et al. 1999). Fickian diffusional release occurs by the usual drug molecular diffusion due to a chemical potential gradient. Case-II relaxational release is the drug transport associated with stresses and state-transition in hydrophilic glassy polymers which swell in water or biological fluids and this process includes polymer disentanglement and erosion. In the zero-order equation (Eq. 3), first-order equation (Eq. 4), Higuchi equation (Eq. 5) and Hixson-Crowell equation (Eq. 6), k_0, k_1, k_H and k_s are constants. It is clear from Eq. (2) that when the exponent n has a value of 1.0, the drug release rate is independent of time. This case corresponds to zero order release kinetics. When the exponent n equals 0.5, it corresponds to the Higuchi equation. The Hixon–Crowell equation indicates an erosion-depended release mechanism. On the other hand, the Higuchi equation expresses a diffuse release mechanism.

For non-Fickian transport, Peppas and Sahlin proposed a model which incorporated the rate effects of diffusion and relaxation phenomena. The diffusion and erosion equations (Eq. 5 and Eq. 6) mentioned above were coupled with the diffusion/relaxation model as follows to describe the release data in a more appropriate way.

Diffusion/relaxation equation (Peppas and Sahlin 1989; Upadrashta et al. 1993):

$$M_t/M_\infty = k_2 t^{1/2} + k_3 t \tag{7}$$

In Eq. (7), the first term describes the Fickian contribution and the second term, case-II relaxational contribution. It essentially corrects the Higuchi pure diffusion model for relaxation of the matrix by adding the linear term. The percentage of drug release due to the Fickian diffusion mechanism (F) and relaxation mechanism (R) are respectively calculated as:

$$\mathrm{F} = 1/(1 + k_2 t^{1/2}/k_1) \tag{8}$$

$$\mathrm{R} = 1/(1 + k_1/k_2 t^{1/2}) \tag{9}$$

Therefore, Eq. (7) indicates that solute release from its geometric shape can be written in terms of a Fickian and a relaxational contribution. It represents release by both diffusion and relaxation mechanisms.

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