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Research Article

Preparation, Characterization and *In Vitro* / *In Vivo* Evaluation of Oral Time-Controlled Release Etodolac Pellets

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Abstract. The objective of this study was to prepare time-controlled release etodolac pellets to facilitate drug administration according to the body's biological rhythm, optimize the drug's desired effects, and minimize adverse effects. The preparation consisted of three laminal layers from center to outside: the core, the swelling layer, and the insoluble polymer membrane. Factors influenced the core and the coating films were investigated in this study. The core pellets formulated with etodolac, lactose, and sodium carboxymethyl starch (CMS-Na) were prepared by extrusion-spheronization and then coated by a fluidized bed coater. Croscarmellose sodium (CC-Na) was selected as the swelling agent, and ethyl cellulose (EC) as the controlled release layer. The prepared pellets were characterized by scanning electron microscopy and evaluated by a dissolution test and a pharmacokinetic study. Compared with commercial available capsules, pharmacokinetics studies in beagle dogs indicated that the prepared pellets release the drug within a short period of time, immediately after a predetermined lag time. A good correlation between *in vitro* dissolution and *in vivo* absorption of the pellets was exhibited in the analysis.

KEY WORDS: etodolac; time-controlled release; pellets; extrusion-spheronization; pharmacokinetics.

INTRODUCTION

Most of the traditional design of the drug delivery system has always been based on the concept of homeostasis originated by Claude Bernard (1). These drug dosage forms typically provide an immediate or rapid medicine release. Frequent administration is required to maintain the effective plasma drug concentration, which lead to poor patient compliance and low drug efficacy. Accordingly, modified drug release dosage forms, such as sustained-release and controlled-release drug delivery system emerge on this basis (2).These preparations are featured with continuous or constant release to maintain drug concentrations in the human body. However, long-term constant drug concentrations in the blood and tissue can cause problems such as resistance, tolerance, and drug side effects (3,4).Moreover, studies have shown that lots of diseases, such as arthritis, asthma, and angina, could outbreak circadianly and rhythmically (5,6). The modified drug release dosage forms could not fulfill the clinical treatment for these diseases. With the development of chronobiology and chronopharmacology, oral chronophopharmacologic drug delivery system (OCDDS) has become a topic of interest within pharmaceutical formulation in recent years.

OCDDS is a drug delivery system based on chronoth erapeutics, which pharmacodynamic is timed to match rhythms of disease in order to minimize side effects and optimize therapeutic outcomes (7). This system is characterized by a predetermined lag time generally brought by multi-layer coating method (8,9). Gastrointestinal fluids penetrate through the polymer coating, the swelling layer expands until the outer polymer coating ruptures and the drug makes a pulsatile release and then release rapidly and completely (10). The impact dosage helps to avoid first-pass effect and increases drug absorption. An improved drug bioavailability leads to an evident reduction of administration frequency, an increase of patient compliance, and an enhancement in therapeutic efficacy. Moreover, researchers have found that these drugs generally release in the terminal of the gastrointestinal tract, such as distal ileum and colon (11). Not only can it avoid the damage **by** gastric acid, but also reduce the irritation to stomach.

Chronobiology has a prominent role in rheumatoid arthritis (RA), with major symptoms such as joint pain and stiffness being most pronounced in the morning, possibly mediated by circadian rhythms of cytokine and hormone levels. Etodolac is a potent non-steroidal anti-inflammatory drug (NSAID) (12,13) employed in the management of chronic pain such as patients with inflammatory arthritis (14,15). Etodolac could be used as an analgesic due to its inhibition of cyclo-oxygenase enzyme and prostaglandin

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synthesis (16,17). By high selectivity and inhibiting of COX-2 (18–20), etodolac exerts anti-inflammatory effect and protects the gastric mucosa at the same time (21). Etodolac has also been shown to have the capability of retarding the progression of the skeletal changes associated with rheumatoid arthritis (22). The anti-inflammatory efficacy of etodolac, together with its safety profile, reveals that etodolac is distinguished among anti-inflammatory agents.

OCDDS can be classified as single-unit and multi-unit drug delivery system. Differ from tablets, pellets are multi-particulate dosage forms qualified with many new characteristics. The potential benefits related to pellets are that they enlarge the drug release area generally and enable drug absorption not to affect by gastric emptying. Dispersed widely *in vivo*, pellets could reduce the risk of systemic toxicity and local irritation. All the merits of pellets make it an ideal for pulsatile preparation.

In this study, a two-layered pulsatile release pellets system containing etodolac was studied. Taken before bedtime as a principle, the pellets released after a lag time before dawn in the next day, which made it possible to coincide with the circadian rhythm of RA and resulted in reduced morning stiffness and pain. The drug-loaded core pellets formulated with microcrystalline cellulose (MCC), CMS-Na, and lactose were prepared by extrusion-spheronization, and then layered with a swelling layer followed by a water-insoluble control layer through a fluidized bed coater. The effects of pellets with various coating types and coating levels on the lag time and the drug release time were studied by *in vitro* dissolution tests. The pellets were also evaluated *in vivo* by studying the pharmacokinetics after oral administration in beagle dogs**.**

MATERIALS AND METHODS

Materials

Etodolac (99.5% purity) was purchased from Shouxin Pharmaceutical Chemicals Co. Ltd., Zhejiang, China. Lactose, monopotassium phosphate, and diethyl phthalate were bought from Bodi Chemicals Co. Ltd., Tianjin, China. CMS-Na and low substituted hydroxypropyl methyl cellulose 21 (L-HPC 21) was obtained from Aoda Pharmaceutic Adjuvant Industry, Yingkou, China. CC-Na, cross-linking polyvingypyrrolidone (PVP), and microcrystalline cellulose PH101 (MCC PH101) were kindly supplied by J.Rettenmaier & shone GmbH & Co. KG, Rosenberg, Germany. Ethyl cellulose (EC, **10 cps**) was received as a gift from DOW Chemical Company, New York, USA. Triethyl citrate (TEC) was obtained from Rohm, Germany. Hydroxypropyl methylcellulose E5 (HPMC E5, **5 cps**) was kindly supplied by Colorcon, USA. All other chemicals and reagents used in the study were of analytical grade.

Preparation of Pellets

Preparing of Drug-Loaded Pellets

The drug-loaded pellets were prepared by extrusion-spheronization process. Firstly, powders consist of etodolac-MCC-lactose-CMS-Na (40%:40%:10%:10%, *w*/*w*/*w*/*w*) were mixed uniformly, and appropriate quantity of water was used as adhesive to combine powder mixture together. In order to achieve appropriate consistency of the wet mass, the adhesive needs to be added slowly and stirred evenly. Then the wet mass was immediately extruded at 30 rpm with a sieve plate of 0.8-mm diameter through an extruder (Yingge Pharmaceutical Machinery Co., Ltd., Chongqing, China). The extrudate was collected and spheronized at 600 rpm for 5 min on a 30-cm diameter plate of spheronizer (Yingge Pharmaceutical Machinery Co., Ltd., Chongqing, China). The prepared pellets were spread out on plates in a thin layer and dried in a hot-air oven (101-2AB, Taisite Instrument Co., Ltd., Tianjin, China) at 40°C for 24 h. The size fraction was separated by dry sieving with a set of standard screens with square openings (Hongxing Instrument Factory, Zhejiang, China).

Coating of the Pellets

The formulation together with process parameters of swelling and controlled layers was shown in Table I as follows. Swelling layer coating solution: CC-Na was used as disintegrants in this study, since it possess an excellent swelling property (23,24). HPMC E5 was first dissolved in hot water with continuous stirring until complete dissolution and then blended with **80% ethanol** to 100 ml. CC-Na was added gradually to the solution with intensive agitation. The solution was homogenized by means of a magnetic stirrer (Lecheng Electric Appliance Factory, Zhejiang, China). Gentle stirring was continued during the entire coating process using the magnetic stirrer. Controlled layer coating solution: EC, as a kind of macromolecule polymer, with its low water permeability, can prevent water from pouring into the pellets core. Allowing for its poor ductility, TEC was used as plasticizer to make the film more flexible and more difficult to rupture. Plasticizer was added based on the total solids content of the solution (25). EC and TEC were dispersed in 80% ethanol to 100 ml and homogenized with constant stirring.

Thirty to 35 mesh drug-loaded pellet cores were sieved and coated in a fluid bed coater (DPL1/3 Multi-processor, Jinggong Pharmaceutical Machinery Co., Ltd., Chongqing, China) until 35% weight gain of swelling layer and 8% weight gain of controlled layer. After coating, the pellets were dried in the oven for further 12 h at 40°C and stored in sealed container until analysis.

Characterization of the Pellets

Sphericity and Roundness

Sphericity and roundness are important characters of pellets. Highly spherical pellets flowed easily, which made them ideal for the following processes, coating, tableting, and packaging (26). One-plane-critical-stability (OPCS) method (27,28) was used to evaluate the quality of the pellets. Put 10 g of pellets on a flat plate, lift one side of the plate until the pellets rolled down, measure the length of the plate and the raised height before rolling. The critical angle was calculated as: $\alpha = arc\ sin(h/l)$,where h and l were the height and length of the plate, respectively. The smaller the critical angle was, the better the roundness of the pellets.

Density

Based on the USP 40, weigh 30–35 mesh pellets 50 g, put them into a 100-ml plastic graduate, the bulk density was calculated as the ratio of weight to the occupied volume. Drop down from the height of 2 cm at different times until there was no variation in volume was observed, and then measure the volume

Table I. Formulation and Process Parameters of Swelling and Controlled Layer

Coating layer	Ingredient	Blower frequency	Atomizing pressure	Inlet temperature	Rotational speed
Swelling layer	CC-Na (5 g) HPMC E5 (1 g) 80% ethanol (100 ml)	35 Hz	0.15 MPa	45°C	0.75 ml/min
Controlled layer	EC (3 g) TEC (0.3 g) 80% ethanol (100 ml)	35 Hz	0.15 MPa	35°C	0.5 ml/min

(29). Bulk density was calculated as: $\rho_{bulk} = W/V_{bulk}$, where W was the weight of the pellets and V_{bulk} was the initial unsettled apparent volume. Tapped density was calculated as: $\rho_{tapped} = W/V_{tapped}$, where W was the weight of the pellets and V_{tapped} was the final tapped volume. Compressibility index (*CI*) and Hausner ratio (*HR*), which were calculated from bulk and tapped densities, were significant parameters for evaluation of flowing properties, as follows, $CI = 100 \times [(\rho_{tapped} - \rho_{bulk})/\rho_{tapped})]$, $HR = \rho_{tapped}/\rho_{bulk}$.

Friability Test

Friability test was performed using a friabilator (CJY-300B, Huanghai Pharmaceutical Control Equipment Co. Ltd., Shanghai, China). **The** pre-weighted pellets sample of 10 g were placed and run in the friabilator for 4 min at 25 rpm (29). Collect the remaining pellets and sieve them through 35 mesh screens. Reweigh the sample and the friability (*Fr*) was calculated as: $Fr = (W_i\text{-}W_f)/W_i$, where W_i is the initial weight before the test and W_f is the final weight after the test of the sample.

Process Yield

The process yield (*Yd*) was calculated by the weight (*W*) of pellets sieved through 30–35 mesh in the end and the theoretical weight (W_t) of the formulation (30) . The formula was as follows, $Yd(\%) = W/W_t$.

Scanning Electron Microscopy

Both surface and cross-section morphology of the pellets were carried out through scanning electron microscopy (SEM) (S-3700N, Hitachi, Japan).

***In Vitro* Dissolution Test**

In vitro dissolution test was performed in phosphate buffer solution (pH 7.4) using a ZRCD6-B dissolution tester (Huanghai Drug Testing Instrument Factory, Shanghai, China) based on the USP 40 apparatus 2 (paddle apparatus). Maintain **37 ± 0.5°C** and rotation speed of 100 rpm. Each sample (5 ml) was withdrawn at predetermined times at l, 2, 3, 4, 6, 8, 10, and 12 h. The solubility of etodolac in pH 7.4 buffer was 9.60 g/L by experiment, which could met the determination requirement of sink condition completely. Each sample was immediately passed through a 0.8-μm millipore filter and analyzed by a UV spectrophotometer (UV-9100, Ruili Analytical Instrument Co., Ltd., Beijing, China) at 278 nm. Each batch of pellets was prepared in triplicate and the experiment was repeated thrice.

***In Vivo* Pharmacokinetics Studies**

Experiment Design

A random, cross-over, and single-dose pharmacokinetic study was conducted on a total of six healthy male beagle dogs, weighting **18 ± 2 kg**. The dogs were divided randomly into two groups, and fasted overnight for at least **12 h** prior to the experiment with free access to water. Each group was orally administered with the test pellets and the reference pellets (Guangzhou Nanxin Pharmaceutical Co. Ltd., batch number: EBT10143) at a dose equivalent to 200 mg, respectively. Each blood sample (5 ml) was collected from antebrachium vein at predetermined time intervals: 0 (pre-dose), 0.5, 1, 1.5, 2 ,3 ,4 ,6 ,8 ,10 ,12 ,16, and 24 h (post dose), and put into heparinized tubes immediately. After separated by centrifugation at 3000 rpm for 10 min, plasma samples were withdrawn and stored at − 4°C for subsequent analysis. There was a 7-day washout period between two treatments. All animal procedures were approved by University Ethics Committee for the use of experimental animals and carried out in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Table II. Effects of Types of Disintegrant and Drug Content on Repose Angle and Yield of Time-Controlled Etodolac Pellets

Formulation			Critical angle (degree)	Yield (%)
Type of disintegrant	M1	CMS-Na	17.5	72.3
	M2	CC-Na	22.3	55.4
	M3	L-HPC	12.6	80.3
Drug content (%)	N1	30% etodolac	15.7	85.6
	N2	40% etodolac	17.2	78.4
	N3	50% etodolac	25.3	56.3

HPLC Assay

A validated HPLC system was used to determine etodolac plasma concentration. The mobile phase was made up of methanol-potassium dihydrogen phosphate buffer (88:12, *v/v*, pH 4.5) (30). Chromatographic separation was performed at a flow rate of 1.0 ml/min, wave length of 278 nm, using a diamonsil C_{18} column (4.6 × 200 mm, 5 μm) and column temperature maintained at 30°C.

Pharmacokinetic Data Analysis

Pharmacokinetic analysis was conducted using the software program DAS 2.1.1.The most suitable compartment model was determined by the smallest AIC value (31,32). Etodolac plasma concentration was plotted against time to obtain the concentration-time profiles which was used to determine the peak blood concentration (C_{max}) and time to achieve the peak concentration (T_{max}). Non-compartmental pharmacokinetic analysis was conducted to calculate the area under the curve. The relative bioavailability (F) was determined by the ratio of AUC for the test formulation (AUC_T) and the reference formulation (AUC_R).

Bioequivalency Analysis

Two one-sided *t* tests were used to evaluate whether the 90% confidence interval of the geometric mean ratios (test: reference) for these parameters were within the range of 80.00 ~ 125.00% (using log transformed data).

In Vitro-In Vivo Correlation Analysis

In this study, DAS 2.1.1 pharmacokinetic software package was employed to *in vitro-in vivo* correlation (IVIVC) analysis which can be used to describe the relationship between the *in vitro* property and the *in vivo* response of an oral dosage form. Percent dissolved (F_t) values were taken from *in vitro* release, and percent absorbed (F_a) was determined by the Wanger-Nelson method using the following equation:

$$F_a\ (\%) = \frac{Ct + k\ AUC0\text{-}t}{k\ AUC0\text{-}\infty} \times 100\%$$

where F_a is the fraction of drug absorbed, C_t is the drug plasma concentration at time t, k is the elimination rate constant, AUC_{0-t}, and $AUC_{0-\infty}$ are areas under the curve between time zero and time t and between time zero and infinity, respectively.

RESULT AND DISCUSSION

Preparing of Pellets Cores

Types and Amounts of Adhesives

Different adhesive agents had different effects on properties of pellets. In this study, with the ratio of powder and binder 1:1(*w/w*), 20, 40, 60, and 80% ethanol and water were investigated to find the effect of adhesive agents on the formulation of pellets. The study indicated that pellets had good formability, low friability, and good flow property when water was used as a binder. When the weight of water and powder reached a ratio of about 1:1, the extruded strip had moderate humidity and can be made into pellets of uniform particle size. However, pellets made from ethanol or ethanol-water mixture as adhesives were more fragile and less suitable for coating.

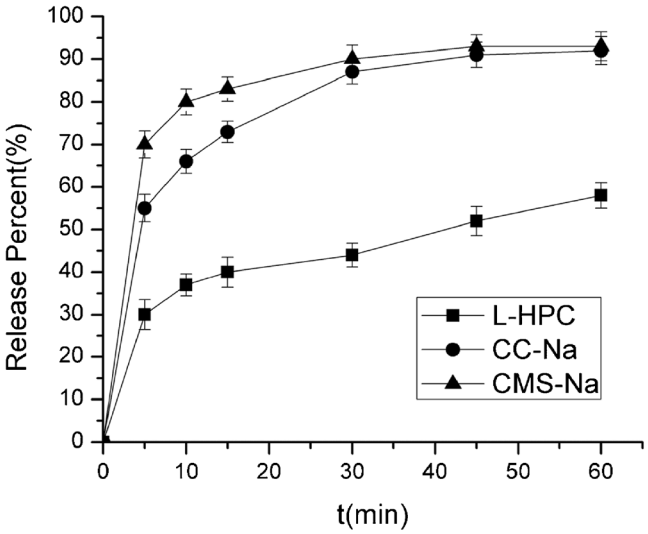

Fig. 1. Effects of types of disintegrantson release behavior of etodolac pellets

Types and Ratio of Disintegrants

Maintain the drug loading of 40%, together with a constant ratio of MCC and disintegrants, add varieties of disintegrants to get the different pellets. Sphericity, release behavior, and yield of target pellets were investigated in the study. Table II revealed that CC-Na pellets were worst both in roundness and yield while L-HPC pellets showed best performance. However, according to Fig. 1, CMS-Na pellets reached nearly 90% drug release within the shortest time of 30 min among the three, and followed by CC-Na pellets with the time of 45 min, while L-HPC pellets

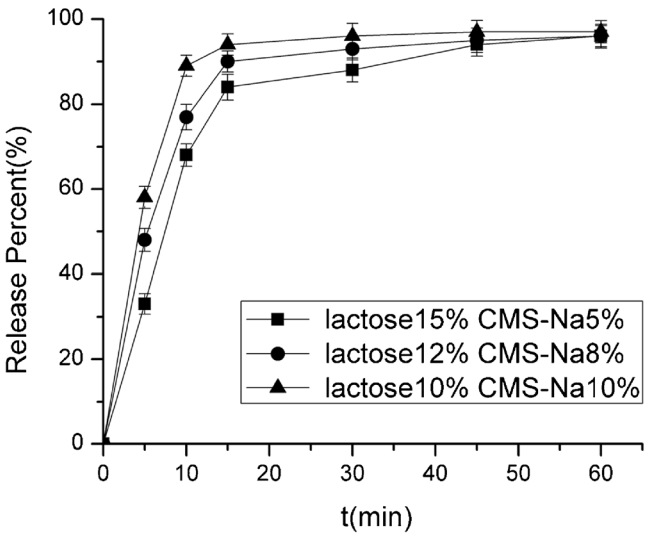

Fig. 2. Effects of CMS-Na and lactose content on release behavior of etodolac pellets

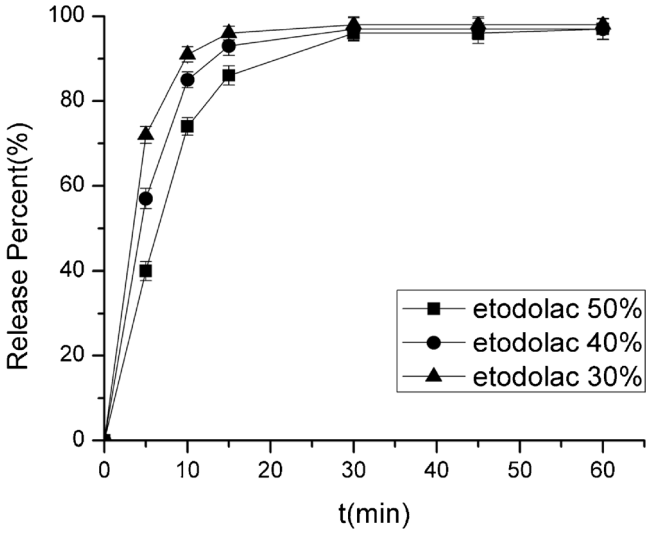

Fig. 3. Effects of drug content on release behavior of etodolac pellets

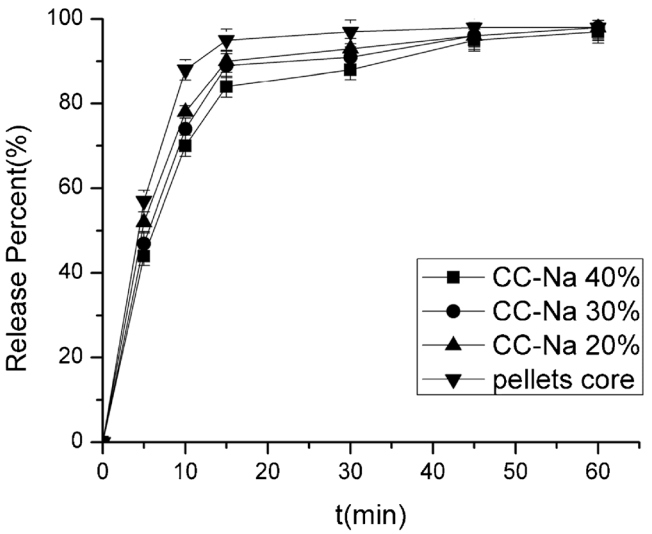

Fig. 4. Effects of the coating level of swelling layer on release behavior of etodolac pellets without EC

reached only 58% drug release in 1 hour. Therefore, the drug release of L-HPC pellets is slower than the other two, which is not suitable to serve as the pellets core. CMS-Na pellets had faster release behavior, and relatively proper sphericity and yield. Take these factors into consideration; CMS-Na was selected as the desired disintegrants.

It was reported that the effect of disintegrants on dissolution rate of MCC pellets made by extrusion-spheronization was moderate (33–35). Therefore, lactose, with its hydrotropic property, was considered to be added to optimize the performance of pellets together with the disintegrants (8,10,36–38). At the beginning of the experiment, fact was that pellets were difficult to form when the ratio of MCC was below 40%. Therefore, keep a ratio of 40% etodolac, 40% MCC, and a total 20% proportion of CMS-Na and lactose unchanged, pellets with 10% lactose and 10% CMS-Na (P1), pellets with 12% lactose and 8% CMS-Na (P2), and pellets with 15% lactose and 5% CMS-Na (P3) were tested in this study. From Fig. 2, P1 reached nearly 90% drug release within the shortest time of 15 min, while P2 of 30 min, and P3 of 45 min. P1 had the fastest drug release among the three. Pulsatile drug release required in the treatment was expected in this study, therefore, 10% lactose and 10% CMS-Na were selected as a proper proportion.

Effect of Drug Content

The forming and releasing of pellets were also subjected to drug content. Keep the proportion of MCC, CMS-Na, and lactose unchanged, 30, 40, and 50% (*w*/*w*) drug content were designed to produce the pellet cores. From Table II and Fig. 3, we elucidated that, with the increasing ratio of etodolac, the drug release slowed down. The critical angle was similar between 30 and 40% drug content pellets, and had a slight increase of 50% drug content. The smaller critical angle was, the better the roundness. The yield of the pellets was also similar between 30 and 40% drug content pellets, but it was difficult to form ground and smooth pellets when the drug content reached 50%, along with more challenges in its preparation process. However, take the clinical dosage into account, a higher drug loading of 40% etodolac pellets, whose yield and roundness was no better than 30% etodolac-loaded pellets, but still reasonable and acceptable, was selected as an appropriate dose.

Table III. The Results of Swelling Degree with Different Disintegrants

Disintegrant	Swelling degree
CMS-Na	13.6
CC-Na	9.3
L-HPC	6.1
PVPP	2.3

Coating of the Pellets

Both types and thickness of coating layer had significant effect on the release of drugs. Since it is difficult to measure the

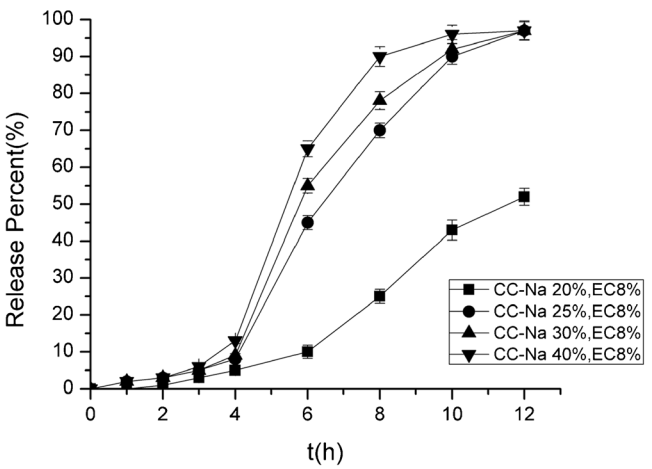

Fig. 5. Effects of the coating level of swelling layer on release behavior of etodolac pellets with different coating level of EC

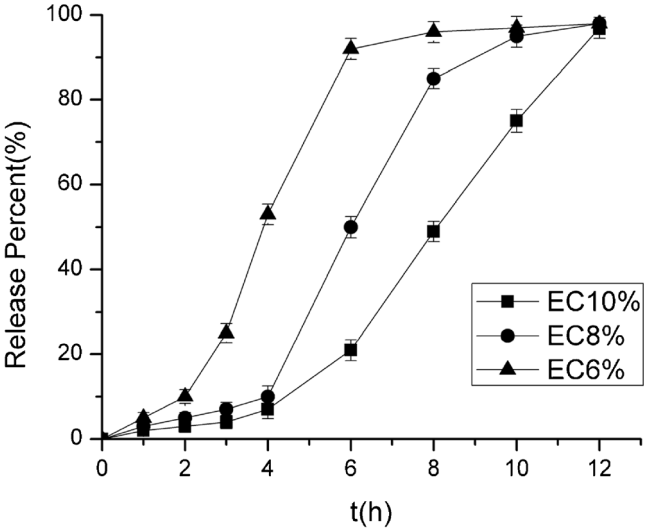

Fig. 6. Effects of different levels of EC on release behavior of etodolac pellets

thickness of coated film directly, an indirect method (39)based on the percentage of weight gain was employed in this study.

$$\omega_s(\%) = (W_s - W_c)\Big/W_c \times 100\%$$
$$\omega_c(\%) = (W_f - W_s)\Big/W_s \times 100\%$$

Where ω_s (%) is percentage of swelling layer weight gain, ω_c (%) is percentage of controlled layer weight gain, W_c is the weight of the core, W_s is the weight of pellets after coated with swelling layer, W_f is the final weight after coating controlled layer.

Types and Weight Gain of Swelling Layer

Materials with a rapid expansion after water absorption were qualified to be used as the swelling layer. As a consequence of the expansion, the outer film would have a sudden burst, and then followed immediate drug release. The

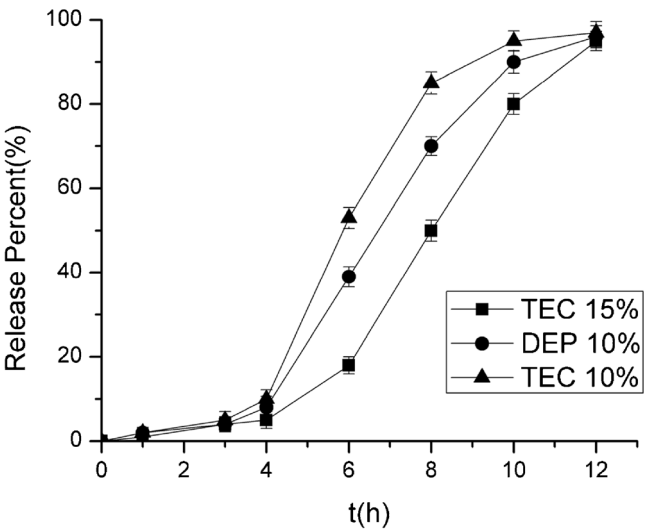

Fig. 7. Effects of palsticizers on release behavior of etodolac pellets

Table IV. The Results of Full Factors Test

Formulation	A(CCNa) (%)	B(EC) (%)	Index(h)	
			T_{lag}	T_r
F1	25	7	3.68	3.75
F2	30	7	3.27	3.31
F3	35	7	3.18	3.06
F4	40	7	3.08	2.76
F5	25	8	4.73	4.68
F6	30	8	4.42	3.87
F7	35	8	4.02	3.41
F8	40	8	3.86	3.32
F9	25	9	6.43	5.78
F10	30	9	5.21	5.37
F11	35	9	4.91	4.85
F12	40	9	4.18	4.26

swelling degree was an index to reflect the swelling property of the disintegrants and was calculated as follows (40):

$$Q = V_t\Big/V_0$$

Where Q is swelling degree, V_0 is drying volume of **1.0 g** disintegrants, V_t is the hydration volume of the disintegrants after immersed into the water for **48 h**.

CMS-Na, CC-Na, L-HPC, and cross-linking PVP were investigated in this study. From Table III, we found that CMS-Na had a swelling degree of 13.6, which meant the volume became 13.6 times of the original volume after water absorption, and then followed by CC-Na, L-HPC, and cross-linking PVP. However, the viscosity of CMS-Na coating solution made it more difficult in atomizing and caused pellets adhesion as well. In contrast, CC-Na had a high efficiency in coating process due to its fine powder properties, and does not easily plug the spray gun. Therefore, CC-Na was identified as the best choice for the rupturing release polymer membrane. These results were in good agreement with results from other studies, where CC-Na had a superior effectiveness as the swelling layer when compared with other materials (23,41).

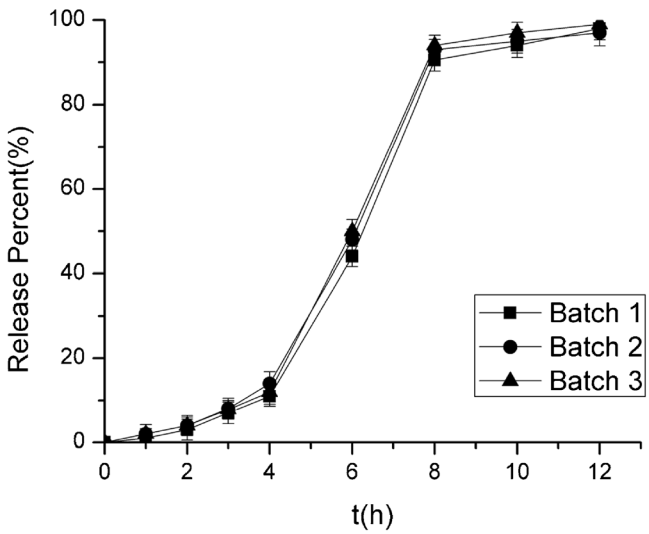

Fig. 8. Release profile for the three batches of time-controlled release etodolac pellets

Table V. The Micromeritics Properties and The Yield of Etodolac Pellets

Batch no.	Critical angle (degree)	Bulk density (g/ml)	Tapped density (g/mL)	CI (%)	HR	Fr (%)	Yield (%)
1	24.1	0.791	0.888	10.9	1.12	0.7	69.5
2	20.8	0.797	0.896	11.0	1.12	0.6	74.5
3	22.6	0.761	0.838	9.1	1.10	0.7	71.3

HPMC E5 was used as the binder to achieve a better adhesion effect of the coating solutions. First, we explored the effect of the coating level of swelling layer on drug release without the EC layer. Twenty, 30, and 40% coating weight of swelling layer were tested; Fig. 4 conveyed a message that the release of drug decreased slightly with the increase of the swelling layer, so it could be considered that the swelling layer had no effect on the release of immediate-release pellets. Next, we explored the effect of the coating level of swelling layer with different coating level of EC. Figure 5 manifested that, in the case of 8% weight gain of controlled layer, swelling layer increased by 20%, the drug release was slow, and the release rate of 12 h was only 60%; when swelling layer increased by 25%, drug release increased significantly. However, with continously increasing of the thickness (30, 40%), neither lag time nor release rate had evident changes. It was illustrated that, with the thickening of swelling layer, the time lag was reduced and the release rate increased, but when it increased to a certain degree, the changes were not obvious anymore. This can be explained by swelling pressure. The swelling layer needs to reach a certain thickness, only to this degree can it absorb enough moisture to meet and surpass the swelling pressure, so as to cause the rupture of the outer film. However, excessively increasing might affect the release behavior, and the extra weight was meaningless at this time.

Types and Weight Gain of Controlled Layer

EC, cellulose acetate (CA), and polyacrylic resin were widely used as coating films in controlled release (37). Differ from cellulose, polyacrylic resin had a better ductility, which made the film robust and not break easily. Consequently, it was more suitable for time-delayed preparations. CA and EC were two kinds of cellulose materials. Compared with EC, CA was slightly higher in intensity. Film formed by CA was more stable and tougher, which would not expand to a large extent in contacting with solutions. EC was a kind of water-insoluble polymer, which also had good performance in coating process (42). Its extensibility was relatively weak and more easily fracture among the three. Allowing for time-controlled release mechanism, EC was a better material for the controlled release layer.

EC, the earliest material used in the process of controlled-release polymeric membranes, had good film-forming properties that enable flexible coatings to be produced (43). EC used as coating material was usually dispersed in organic dispersion. Additionally, Aquacoat and Surelease were two currently listed ethyl cellulose water dispersion product. It was reported that EC organic dispersion and Surelease were not affected by pH *in vivo* (44–47). However, Surelease coating process was hard to control and Aquacoat needed to be extra sufficient heated before the acid-alkali medium had no effect on it (48,49). Moreover, the water permeability of pure EC was very low, and long-time heat treatment was uneconomic. Therefore, 80% ethanol was selected as the dissolvant in this study after trial and error.

As declared in Fig. 6, with the progressively increasing (6, 8, 10%) of EC layer, drug release decreased and lag time delayed. Eight percent EC was found to provide the best release characteristics in terms of appropriate lag time and drug release profile.

Types and Amounts of Plasticizers

TEC equivalent to 10, 15% (*w*/*w*) of the solid content and diethyl phthalate (DEP) equivalent to 10% (*w*/*w*) of the solid content were applied for plasticizers selection in this study. The results of Fig. 7 revealed that increasing TEC content (from 10 to 15%) reduced the release rate and extended lag time as a result. This was mainly because the plasticizer contributed to enhancing the tensile strength and ductility of EC film, which made it difficult to break down

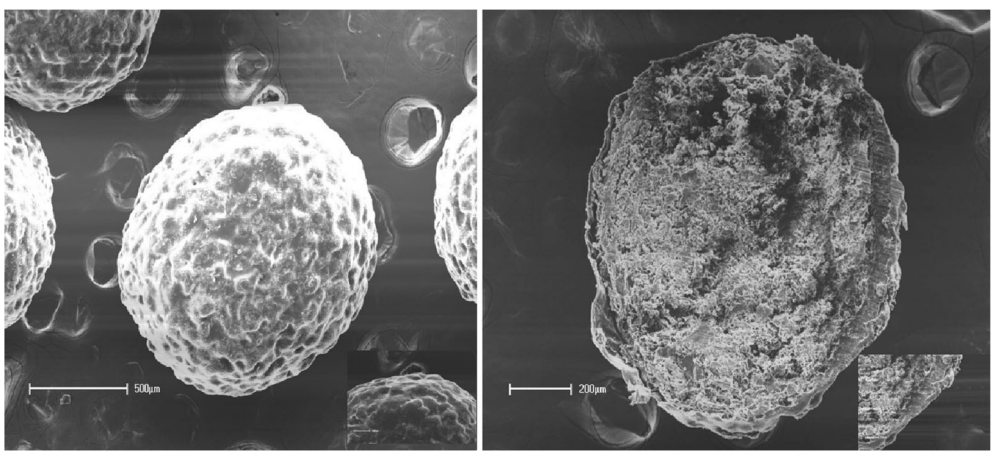

Fig. 9. Scaning electroscope photographof time-controlled release etodolac pellets

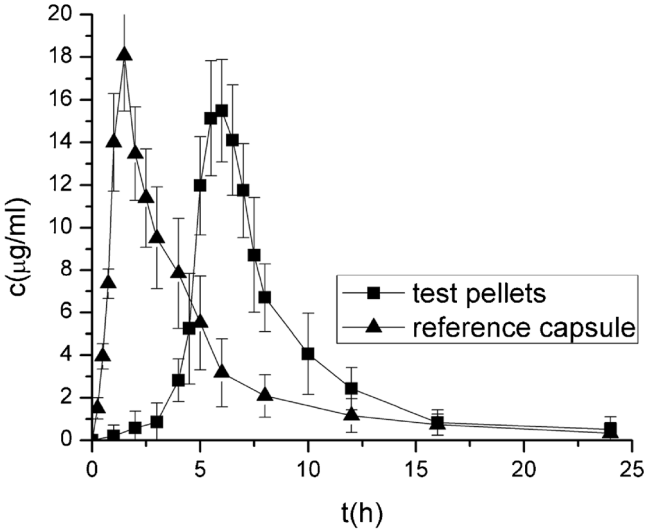

Fig. 10. Mean plasma concentration of etodolac after oral administration of test pellets and reference pellets

(50). Compared with DEP, the hydrophilic property of TEC gave an approach to improving the release behavior. At the same time, the quality of controlled film needed to be taken into account so as not to be too fragile, TEC equivalent to 10% of the solid content was opted as a proper amount.

Formulation Optimization

Two important factors including the weight gain of swelling layer (A) and controlled layer (B) were selected in order to find the best prescription. Based on the single factor investigations above, 25, 30, 35, and 40% weight gain were selected as factor levels of A, 7, 8, and 9% weight gain were selected as factor levels of B. In the same circumstances of other variable parameters, the comprehensive full factors experiment was designed to optimize the prescription, with lag time (T_{lag}) and drug release time (T_r) selected as the evaluation index. The details were shown in Table IV.

It was reported that drug release at 2 a.m. was more effective than other times, and more effective in controlling morning stiffness and pain (51). In theory, it takes nearly 4 h between taking the medicine from bedtime to two o'clock in the morning, so formula with a 4 h delay was considered as the ideal preparation. The result in Table IV indicated that, F1–F4 had less lag time than 4 h, while F5, F6, F9–F11 had longer lag time. F7, F8, and F12 had a time lag close to 4 h and there was no significant difference in T_r. From a productive perspective, in comparision with F8 (CC-Na: EC=40:8, *w*/*w*) and F12 (CC-Na: EC=40:9, *w*/*w*), F7 (CC-Na: EC=35:8, *w*/*w*) had less weight gain of coating layer, which was more economize in pharmatheutical excipients and saved more production time. Therefore, pellets with 35% swelling layer weight gain and 8% controlled layer weight gain was considered as the optimal formula.

Table VI. The Pharmacokinetic Parameters of Reference Capsule and Test Pellets After Single Oral Administration in Beagle Dogs (n = 6)

Parameters	Units	Reference	Test
$AUC_{(0-t)}$	mg/L*h	71.451	74.450
$AUC_{(0-\infty)}$	mg/L*h	74.763	76.878
K_a	h^{-1}	0.684	0.475
$t_{1/2}$	h	5.989	4.059
T_{lag}	h	0.291	3.295
T_{max}	h	1.5	5.7
C_{max}	mg/L	20.23	18.46

Table VII. ANOVA Analysis of $\ln(AUC_{0-\infty})$

Source	*df*	*SS*	*MS*	F	$\alpha = 0.05$
Individual	5	0.423	0.114	7.49	$F_{0.05(5,4)} = 6.26$
Period	1	0.030	0.030	0.27	$F_{0.05(1,4)} = 7.71$
Formulation	1	0.018	0.018	0.16	$F_{0.05(1,4)} = 7.71$
Error	4	0.0452	0.113		
Total	11	0.6472			

Characterization of the Pellets

The critical angle, bulk density, tapped density, CI, HR, friability, and yield for the same three batches of etodolac time-controlled release pellets were investigated in this study; their particular properties were shown in Table V.

In Vitro Dissolution Test

Refer to the optimal formulation above, three batches of the same etodolac time-controlled release pellets were prepared for dissolution test. The release profiles for three batches pellets were shown in Fig. 8. It was evident that the pellets expressed time-lag characteristics and good reproducibility. The prepared pellets released the drug after a lag time about **4 h** and had a release time of **3.5 h**.

Scanning Electron Microscopy

Scanning electron microscopy showed the surface and cross-section structure of the coated pellets. From Fig. 9, we could see the pellets were spherical and intact in shape; the outer surface of the coated pellets was smooth and continuous. However, the cross-section view indicated that the layers could not be distinguished from the cores, and the boundary between the two layers was not obvious. This was mainly because of the migration of water during the process of drying.

In Vivo Pharmacokinetics Studies

Pharmacokinetic Data Analysis

Figure 10 illustrated the mean etodolac concentration and time curve after single-dose administration in beagle dogs (n = 6). Both the reference and the test pellets were fit to one-

Table VIII. Two One-Sided Test for $AUC_{0-\infty}$

Statistical parameters	Values	$T_{1-0.05/2}$	90% confidence
T_1	3.533	2.132	89.7% ~ 117.2%
T_2	2.75		

Table IX. Drug Release of Etodolac Test Pellets *In Vitro* and *In Vivo*

T(h)	4	5	6	8	10
Ft (%)	7.77	25.92	42.82	85.54	94.85
Fa (%)	3.85	12.08	31.28	61.28	75.33

compartment models according to AIC value and R^2. The main pharmacokinetic parameters were summarized in Table VI.

As learned from Table VI, the T_{lag} of the test pellets and the reference were 3.259 and 0.291 h, and the T_{max} of the test pellets and the reference were 5.7 and 1.5 h. Obviously, the test pellets had a time lag and longer mean residence time compared with the reference, which illustrated that the drug could release before dawn after taking at bedtime and reach its peak concentration in the morning, so as to meet the treatment demand of morning stiffness (MS).

The relative bioavailability of etodolac compared with the reference was 96.90%, which was calculated using the following equation:

$$F(\%) = AUC_T \Big/ AUC_R \times 100\%$$

Bioequivalency Analysis

The area under the blood concentration curve of the test preparation and reference preparation was analyzed in Table VII. ANOVA analysis demonstrated that there was no significant difference between the test and reference pellets in $AUC_{0\sim\infty}$. The result of two one-sided *t* tests and *(1-2α)* confidence interval analysis was showed in Table VIII $T_1 > T_{1-0.05/2}$, $T_2 > T_{1-0.05/2}$, the test preparation was bioequivalent to the reference preparation, and its 90% confidence interval was from 89.7 to 117.2%.

In Vitro-In Vivo Correlations Analysis

The regression equation was $F_a = 0.8142Ft\text{-}5.0711$, and a correlation coefficient *r* of 0.994 suggested a good linear regression relationship between the percent *in vitro* release and *in vivo* absorption. The high correlations indicated that the drug absorption in the physiological conditions could be illustrated by the *in vitro* release test under the current conditions. The regression equation and coefficient of correlation between percent dissolved and percent absorbed were summarized in Table IX and Fig. 11.

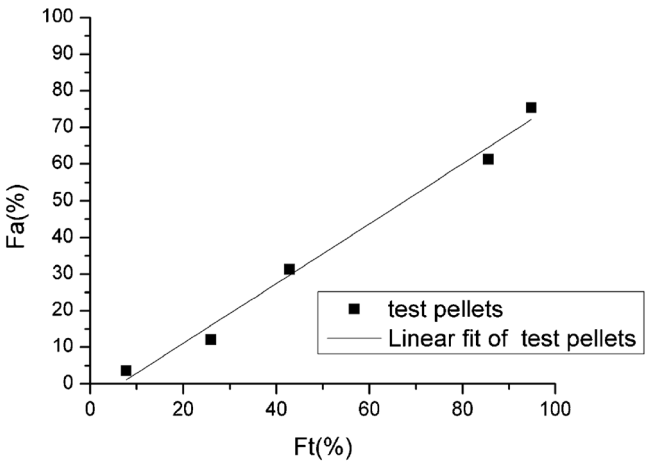

Fig. 11. The profile of IVIVC for etodolac test pellets

CONCLUSION

The addition of CMS-Na, which acted as the disintegrants, together with appropriate proportion of lactose and etodolac, has a considerable effect on the formation of pellets made by the process of extrusion-spheronization. Coated in a fluid bed, CC-Na was used as swelling layer and EC as controlled layer. The release profiles *in vitro* showed the pellets had a lag time about **4 h** and release time about **3.5 h**, which basically achieved the expected goal. Pharmacokinetic studies in beagle dogs indicated that the test preparation had better time-lag characteristics and longer mean residence time compared with commercial available capsules. The two preparations are bioequivalent, the relative bioavailability of the test pellets was 96.90% calculated according to $AUC_{0\text{-}t}$. IVIVC model exhibited a good linear regression relationship. Based on these results, it is evident that time-controlled release etodolac pellets were likely to be a more suitable formulation in treating with morning stiffness of rheumatic arthritis disease.

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