Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 333 (2007) 87–94
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Sustained-release tablets of indomethacin-loaded microcapsules: Preparation, in vitro and in vivo characterization

Bin Lu [a,b,c,*], Rong Wen [a], Hong Yang [a], Yingju He [a]

[a] West China School of Pharmacy, Sichuan University, Chengdu 610041, China
[b] Key Lab of Drug Targeting of the Ministry of Education of China, Sichuan University, Chengdu 610041, China
[c] National Key Lab of Biotherapy of Human Diseases, Sichuan University, Chengdu 610044, China

Received 1 February 2006; received in revised form 17 September 2006; accepted 1 October 2006
Available online 6 October 2006

Abstract

Indomethacin (IDM) was encapsulated in gelatin-cellulose acetate phthalate (CAP) microcapsules (A) by complex coacervation method and in CAP microcapsules (B) by simple coacervation method. Microcapsules A and B, having mean diameters of 38.24 and 35.74 μm, respectively, were used to prepare sustained-release tablets A and B. The activation energy of thermal degradation for tablets A and B was calculated based on differential scanning calorimetry (DSC) to be 258.9 and 284.8 kcal/mol, respectively. In vitro release profiles showed no burst effect and release $t_{1/2}$ of the two sustained-release tablets were found to be 41.30 ± 1.86 and 33.25 ± 2.84 min, respectively, while that of IDM plain tablets C was 6.30 ± 0.39 min ($P < 0.01$). In vitro release of IDM from tablets A and B could be described by Higuchi equation and zero-order kinetics, respectively. After per os (po) administration with physiological saline, their irritation to rat stomach was obviously reduced in comparison with tablets C. Pharmacokinetic study in rabbits showed that t_{max} was delayed and C_{max} lowered compared with tablets C and the values of $AUC_{0-24\,h}$ of the three tablets were very close.

Keywords: Coacervation; Oral drug delivery; Microencapsulation; Calorimetry (DSC); Pharmacokinetics; Tableting

1. Introduction

Indomethacin (IDM) is a very effective anti-inflammatory and antipyretic drug with analgesic property. But, when taken orally against chronic inflammatory and pain conditions, adverse events often occur, such as general serious gastrointestinal reaction (even stomach perforation), central nervous system symptoms, liver function damage, inhibition of hematopoeitic system and allergic reaction (Chen et al., 2003). Meanwhile, its inconvenience in use is also a problem, such as pretty high frequency of administration (25 mg, tid) and long period of treatment (Mason et al., 2004; O'Brien, 1968). Hundreds of papers have been published in terms of its new carriers and new dosage forms to reduce its side effects and to enhance its therapeutic efficiency, such as nano-encapsulated microparticles (Chen and Lin, 2005), chitosan microspheres (Aggarwal et al., 2001), spray-dried powders of polymeric nanocapsules (Guterres et al., 2000) and suppository (Uzunkaya and Bergisadi, 2003). Entric-coated tablets, cream, patches, capsules (Lu et al., 1986), suppository and liniment of IDM are all collected in the Pharmacopoeia of China (2005 Ed.).

IDM microcapsules prepared by coacervation method have also been reported. Rowe and Carless (1981) prepared IDM microcapsules by gelatin-acacia complex coacervation method and found that all formulations showed a zero-order release pattern after an initial burst phase. Daniels and Mittermaier (1995) studied the coacervation behavior of a mixture of commercial grade gelatin and acacia using five different acids to adjust the coacervation pH and examined the barrier properties of the capsule shells of corresponding microcapsules with IDM as a model drug. They found that IDM microcapsules showed the slowest release rate when the coacervation pH was adjusted to the electrical equivalence pH value and not to the pH of maximum coacervate yield and that dissolution profiles of the microcapsules were quite similar even when different acids were used for pH adjustment. The above authors all used acacia, of which only an imported product is available in China.

* Corresponding author at: #17, 3rd Block, Renmin South Road, West China School of Pharmacy, Sichuan University, Chengdu 610041, China. Tel.: +86 28 85502664; fax: +86 28 85503689.
E-mail addresses: lubin@wcums.edu.cn, lubinxc@163.com (B. Lu).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.10.002

Now that oral absorption of IDM is fast and complete (Luo et al., 2001), oral administration is preferable provided that its side-effects, especially serious gastrointestinal reactions, could be avoided.

In an attempt to reduce the side-effects of IDM, sustained-release tablets of two kinds of IDM microcapsules without using acacia were prepared, characterized and compared in vitro and in vivo, and IDM plain tablets used for comparison.

2. Materials and methods

2.1. Instruments, reagents and animals

Spectrophotometer (UV-250) was produced by Shimazu, Japan, Differential scanning calorimeter (CDR-1) by Analytical Instrument Factory of Shanghai, China, and Automatic Release System with DU-65 Spectrophotometer by Beckmann, U.S.A. One-punch tablet machine (type TDP) was produced by the 1st Pharmaceutical Machine Factory of Shanghai, China.

IDM was obtained from the Pharmaceutical Factory of Shijiazhuang, China, cellulose acetate phthalate (CAP, or cellacefate, $C_8H_5O_3$ 34–36%, C_2H_3O 20–24%) from the 19th Pharmaceutical Factory of Shanghai, China, gelatin A with electrical equivalence pH value of 8.6 from Gelatin Factory of Shanghai, China. Sodium sulfate was chemically pure and other reagents of reagent grade. IDM plain tablets were prepared in this lab.

Animals: male Sprague–Dawley rats and rabbits were obtained from West China Experimental Animal Center of Sichuan University.

2.2. Preparation and characterization of the microcapsules

Two kinds of IDM microcapsules were prepared.

2.2.1. Microcapsules A

IDM (150 mesh) was encapsulated in gelatin-CAP microcapsules by complex coacervation method, the weight ratio of gelatin:CAP:IDM being kept to 1:1:1. Typically, 200 mg CAP was pulverized and mixed with 0.6 g $Na_2HPO_4{\cdot}12H_2O$ and 15 ml water and stirred in a water-bath at 50 ± 0.5 °C. The solution was filtered and the insoluble impurities discarded (pH of the CAP solution being 7.5). Two hundred milligrams of IDM was suspended in 6.6 ml 3% (w/v) gelatin solution and stirred, and the mixture added to the CAP solution thermostatted at 50 ± 0.5 °C. Then 5% (w/v) acetic acid was added dropwise to the thermostatted mixture under stirring to adjust pH to 4.6. At this pH value, gelatin was positively charged and CAP negatively charged, thus coacervation of gelatin and CAP occurred due to static electric attraction forming IDM-loaded microcapsules. The microcapsule formation process was monitored visually under an optical microscope. When a great number of microcapsules of round shape were formed, 30 ml 30–40 °C water was added to improve the morphology of the microcapsules and to prevent the microcapsules from adhesion. The warm mixture was added 6.6 ml 37% (w/w) formaldehyde. After stirring for 1.5 h at room temperature (around 20 °C), 20% (w/v) NaOH was added to adjust pH to 8.0 and the mixture stirred for another 1 h, and then put in refrigerator overnight. The microcapsules were separated by sedimentation, and thoroughly washed off residue of formaldehyde with water (tested with Schiff's reagent).

2.2.2. Microcapsules B

IDM was encapsulated in CAP microcapsules by simple coacervation method, using sodium sulfate as the coacervation agent. Typically, 1 g IDM (150 mesh) was suspended in 120 ml 4% (w/v) $Na_2HPO_4{\cdot}12H_2O$ (containing 1 g CAP) and stirred while the mixture was thermostatted at 65 °C. Then 55 ml 40% (w/v) $Na_2SO_4{\cdot}10H_2O$ was added dropwise, upon stirring, to make coacervation of CAP (the final $Na_2SO_4{\cdot}10H_2O$ concentration being 12.6%, w/v). When microcapsules were formed (examined under an optical microscope), 525 ml 12.6% (w/v) $Na_2SO_4{\cdot}10H_2O$ (containing 10 ml pure acetic acid) of 65 °C was added to improve the microcapsule shape and solidify the microcapsules. The mixture was stirred and kept at 65 °C for 30 min. At this step, if water was used to take the place of Na_2SO_4 solution for dilution, CAP would dissolve and the microcapsules disappear. Filtration was then conducted to discard the large residues and sulfate was thoroughly washed off with water of pH 4.0 (tested with $BaCl_2$ solution) with centrifugation at 3000 rpm for separation.

Both gelatin and CAP showed a molecular weight distribution. Upon coacervation, molecules of higher molecular weight would give rise to coacervation first. Usually excessive amount of coacervation agent was needed, and some molecules of lower molecular weight would still remain in the solution. But too much coacervation agent would be harmful due to a too large surface tension of the microcapsules which was not preferable for formation of microcapsules of small diameter. Thoroughly stirring was also necessary upon coacervation of microcapsules to prevent the microcapsules from cohesion.

IDM is insoluble in water, but when $pH > 7$, its solubility increases with pH. For complex coacervation, when the microcapsules were solidified with formaldehyde, the pH value should not be adjusted directly to 7–8 (the preferable pH value for solidification by formaldehyde). Otherwise IDM would go into aqueous phase and vacant microcapsules formed. After reaction for 1.5 h at room temperature at lower pH values, the surface of microspheres was solidified and the pH was then adjusted to 8 for further solidification.

After the formation of microcapsules by simple coacervation method, dilution with a Na_2SO_4 solution of the same concentration was necessary to improve the morphology of the microcapsules and to prevent the microcapsules from cohesion. The CAP microcapsules were then solidified by acetic acid. When washing off the sulfate residue, acidic water was used to prevent the microcapsules from decoacervation.

Size of microcapsules and its distribution were determined as follows. Microcapsule samples were examined under an optical microscope with microscale, 600 particles being measured each batch, and the mean value of diameter obtained. Histogram was obtained as follows: frequency, the percentage of microcapsules within a diameter difference of 5 μm, was plotted versus the particle size with 5 μm as a unit.

2.3. Preparation of sustained-release tablets

Tablets A and B were prepared the same way. Typically, microcapsules were centrifugalized at 3000 rpm to obtain a slurry of microcapsules (the moisture content being about 45%, w/w). Then 128 g of the slurry was mixed with 15 g starch and 15 g lactose to make a damp mass (soft wood). The damp mass was used to make granules of 14 mesh. After being dried in air (around 20 °C) for 6 h, the granules (100 g) were mixed with 0.5 g magnesium stearate, 4 g talc and 0.02 g carboxymethyl starch and sieved with a 16 mesh sieve and finally compressed into tablets of 25 mg IDM/tablet. The punch (and dye) used was Ø8 mm for tablets A and Ø6 mm for tablets B.

IDM plain tablets (25 mg IDM/tablet) were prepared almost the same way, the only difference was, when 35 g IDM was used, 60 ml thick liquid of about 25% (w/w) starch was used to take the place of 15 g starch and a punch (and dye) of Ø5.5 mm was used.

2.4. Quantification of IDM

Based on UV absorption spectra, IDM was determined in 20% (v/v) ethyl alcohol at the absorbance maximum 321 nm free from the interference of gelatin, CAP and excipients of the tablets. Absorbance at 321 nm of IDM of different concentrations in a mixture of anhydrous alcohol/pH 7.4 PBS (v/v, 20/80) was measured to give the standard curve and regression equation ($C = 8$–40 μg/ml) with $r = 0.9999$. Microcapsules were dried at 60 °C in a vacuum desiccator (under 600 Hg mm pressure) for 3 h. Ten milligrams of microcapsules was mixed with anhydrous alcohol to make 50 ml, of which 5 ml was added pH 7.4 PBS to make 25 ml. The mixture was used for measurement of absorbance at 321 nm. The drug content was calculated according to the regression equation.

Twenty sustained-release tablets were pulverized and mixed and 30 mg weighed out, to which anhydrous alcohol was added to make 50 ml. Five millilitres of alcoholic solution was added pH 7.4 PBS to make 25 ml. The mixture was used for measurement of absorbance at 321 nm and calculation of drug content.

For in vitro release tests, IDM was determined at the absorbance maximum 318 nm in pH 7.4 PBS. The calibration equation was obtained in the concentration range 5.03–30.18 μg/ml with $r = 0.9999$.

For blood samples, 3.0 ml of whole blood (with heparin) was extracted with ethyl ether for seven times, each time 5.0 ml. Ethyl ether extracts were collected and evaporated to dryness in a 50 °C water-bath. Five millilitres of 95% ethanol was added and the mixture vortexed for 1 min. After staying in the refrigerator overnight in a sealed test tube, the mixture was centrifugalized for 5 min at 3000 rpm. The supernatant was used for determination of absorbance at 319 nm (absorbance maximum) and the blood concentration calculated from the following standard regression equation. To obtain the standard regression equation for blood samples, anhydrous alcoholic standard solutions (15–180 μl) of IDM was mixed with 3.0 ml rabbit blood containing heparin. The mixture was vortexed and stayed at room temperature for 30 min, then extracted with ethyl ether for seven times, 5.0 ml each time. The ethyl ether extracts were collected and treated as above. The absorbance at 319 nm of the supernatant was measured and the regression equation obtained in the concentration range of 1.5–18.0 μg/ml with $r = 0.9991$.

Should the IDM concentration in blood samples be found higher than the linear range, dilution was conducted and determination carried out again.

2.5. Examination of stability of sustained-release tablets

2.5.1. Examination of physical stability

Observation of tablets A, B and C after storage at room temperature (15–25 °C) was conducted in a duration of half a year. Visual inspection of the appearance and test of hardness by hand were conducted.

2.5.2. Examination of chemical stability

Tablets A, B and C were tested for IDM contents after every month of storage at 37 °C and 75% RH in a duration of 3 months. After heating the tablets at 95 °C for 15 h, IDM content was also determined.

Parameters of thermal degradation kinetics were calculated based on DSC. Twenty tablets A, B and C, respectively, were pulverized and put in an aluminum crucible and DSC curves were obtained at a scanning speed of 1, 2, 5 and 10 °C/min (speed of paper 60, 120, 300 and 600 mm/h), respectively, in an atmosphere of static air and α-$A1_20_3$ used as the reference. The DSC peak temperatures for tablets A, B and C were recorded.

Activation energy was calculated according to Kissinger's equation (see Section 3.4.2).

2.6. Test for in vitro release rate of the tablets

The in vitro release test was carried out by basket method with the Automatic Release System. Six tablets were placed in six baskets, respectively, stirred at 96 ± 1 rpm in 900 ml of pH 7.4 PBS and the device was thermostatted at 37 ± 0.5 °C. Samples were taken and determined at 318 nm automatically every 5 min.

2.7. Test for irritation to rat stomach

Thirty two rats, weighing 180–200 g each, after 19 h of fasting, were divided into four groups, eight rats each group, administrated po tablets A, B, C at a dose of 25 mg IDM/kg (pulverized and suspended in 0.7% (w/v) carboxymethyl cellulose solution of physiological saline), and the same volume of physiological saline, respectively. The animals were sacrificed 5 h later and the stomach washed with physiological saline and then filled with 4% (w/v) formaldehyde 10 ml each. Fifteen minutes later, the stomach was washed thoroughly and cut open along the external bend and examined for ulcers.

2.8. Determination of bioavailability

Nine rabbits, weighing (2.39 ± 0.18) kg each, were deprived of food for 24 h and divided into three groups, three rabbits each

group, and administrated po tablets A, B and C at a dose of l00 mg IDM/kg (pulverized and suspended in 0.7% (w/v) carboxymethyl cellulose solution of physiological saline), respectively. Three millilitres of blood was taken from aural vein at designated time intervals and IDM concentration determined.

3. Results and discussion

3.1. Preparation of microcapsules

After preliminary tests, single factor tests were used to optimize the preparative technology of microcapsules.

The temperature during microcapsule formation was a key condition. Round shape of smooth microcapsules could be obtained only in proper temperature range: 48–52 °C for complex coacervation and 60–70 °C for simple coacervation.

For complex coacervation, the weight ratio of gelatin/CAP/IDM, the amount of Na_2HPO_4 and water amount for CAP, temperature and pH were chosen as the independent variables and microcapsule morphology and drug content as the evaluation indexes. The best optimized procedures were as described in Section 2.2. The microscopic photograph of microcapsules A is shown in Fig. 1(A). The average diameter was 38.24 μm, with the number of microcapsules in the diameter range of 15–60 μm amounting to over 88.4%. The distribution of the particle size is shown in Fig. 2(A).

Microcapsules B were made by simple coacervation method with simple factor tests for optimization. Weight ratio of IDM/CAP, the amount of water and $Na_2HPO_4{\cdot}12H_2O$, temperature and amount of 40% (w/v) $Na_2SO_4{\cdot}10H_2O$ were the independent variables with drug content and morphology of microcapsules as the evaluation indexes. The optimized procedures were as described in Section 2.2.

The microscopic photograph of microcapsules B is shown in Fig. 1(B). The average diameter was 35.74 μm, with the number of microcapsules in the diameter range of 15–60 μm amounting to over 94.6%. The distribution of the particle size is shown in Fig. 2(B).

The IDM content for three batches of microcapsules A was determined to be $(34.4 \pm 0.19)\%$, $(45.9 \pm 0.53)\%$ and $(50.0 \pm 0.75)\%$ (all $n = 5$), respectively, and for three batches of microcapsules B to be $(64.3 \pm 0.39)\%$, $(70.4 \pm 0.75)\%$ and $(61.8 \pm 0.40)\%$ (all $n = 5$), respectively. Since the ratio of IDM used to be 1/3 and 1/2 during preparation of microcapsules A and B, respectively, the relatively high IDM contents might be a consequence of incomplete coacervation of the materials.

3.2. Preparation of sustained-release tablets

The optimum excipients and formulation were optimized with the tablet appearance, time of disintegration (completely disintegrating within 15 min in water at 37 ± 1 °C, a standard specified in the Pharmacopoeia of China, 2005) and absence of interference with quantification of IDM as the evaluation indexes.

When dry microcapsules were used to take the place of the slurry, good appearance of tablets could not be obtained. When

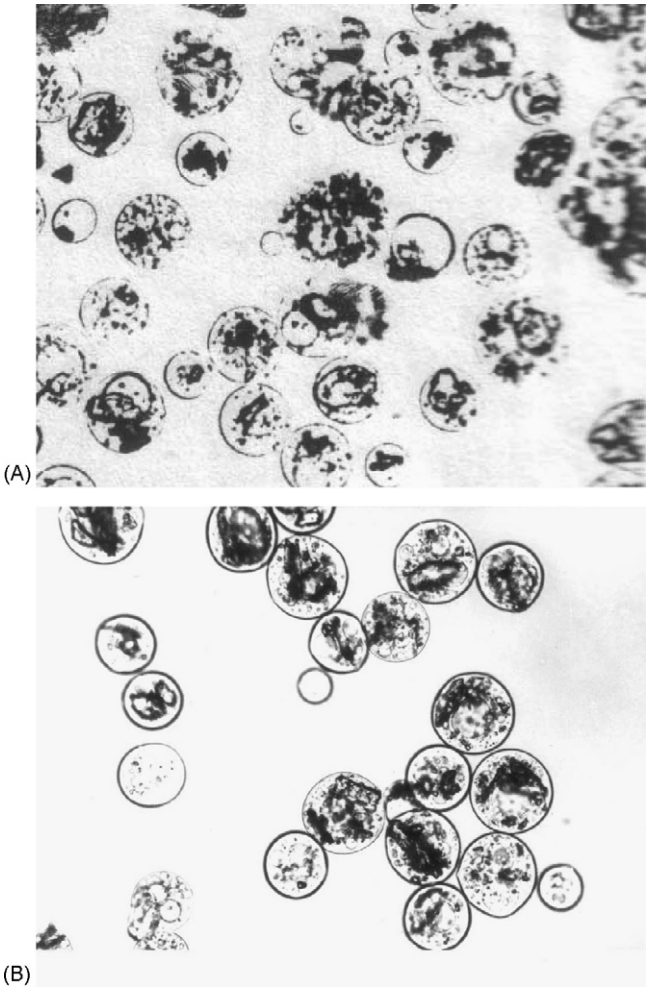

Fig. 1. Microscopic photos (×100) of microcapsules A (A) and microcapsules B (B).

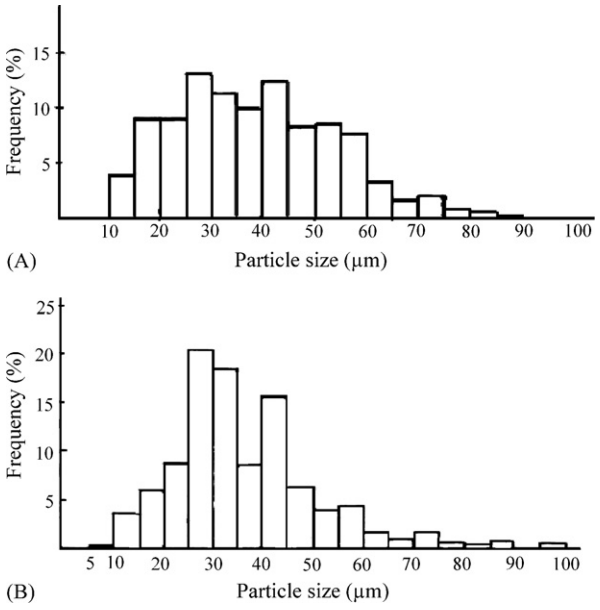

Fig. 2. Histogram of IDM microcapsules A (A) and microcapsules B (B).

starch/dextrin/sucrose (wt. ratio = 3:1.5:1) was used to take the place of starch and lactose, the tablets disintegrated too slowly. When only starch was used, the appearance was not good. While only lactose was used, its capability of absorbing water was limited, which made the preparation of the damp mass difficult. The optimized procedures were as described in Section 2.3. Many batches of microcapsules were mixed up for preparation of tablets. Tablets A, B and C all completely disintegrated in 3 min in water at 37 ± 1 °C.

IDM content was (17.9 ± 0.37)% for tablet A and (32.4 ± 0.78)% (both $n = 5$) for tablet B, or (25.03 ± 0.38) mg/tablet for tablet A and (24.76 ± 0.30) mg/tablet (both $n = 5$) for tablet B. Tablets C gave a IDM content of (24.83 ± 0.32) mg/tablet ($n = 5$).

3.3. Quantification of IDM

Recovery of IDM in a mixture of anhydrous alcohol/pH 7.4 PBS (v/v, 20/80) was determined to be (100.8 ± 1.2)% ($n = 4$) for a mixture of CAP, gelatin and IDM (wt. ratio = 1:1:1), and (l01.7 ± 0.79)% ($n = 4$) for a mixture of CAP and IDM (wt. ratio = 1:1). Recovery of IDM in a mixture of anhydrous alcohol/pH 7.4 PBS (v/v, 20/80) for a mixture of excipients of tablets A and microcapsules A was determined to be (100.9 ± 1.0)%, and that for a mixture of excipients of tablets B and microcapsules B was (99.1 ± 0.76)% (both $n = 5$).

The absolute extraction recovery (seven times with ethyl ether) of IDM in blood samples was determined to be (80.8 ± 6.1)% ($n = 4$), e.g. only (80.8 ± 6.1)% IDM was extracted when different quantities of IDM were added to the blank blood samples.

3.4. Test for stability

3.4.1. Physical stability

After 6 months of storage at room temperature, no change was observed in the appearance and hardness of tablets A, B and C.

3.4.2. Chemical stability

After 3 months of storage at 37 °C and 75% RH, tablets A, B and C were examined and results, shown in Table 1, indicated that no significant change happened in the content of IDM ($P < 0.01$). IDM content after heating at 95 °C for 15 h also showed insignificant change (data not shown).

From DSC curves with different rate of rise in temperature (β) of tablets A, B and C, the peak temperatures (T_p) of the curves are shown in Table 2. Activation energy was calculated according to Kissinger's equation:

$$\ln\left(\frac{\beta}{T_p^2}\right) = -\frac{E_a}{RT_p}$$

in which R stands for gas constant and E_a activation energy.

In Fig. 3 is shown the straight line of a plot of $-\ln(\beta/T_p^2)$ versus $1/T_p$, which indicates that the thermal degradation conformed to Kissinger's equation. The values of E_a for tablets A, B and C were calculated to be 258.9, 284.8 and 254.8 kcal/mol, respectively. The large values of activation energy together with the results of storage tests showed that chemical stability of the sustained-release tablets was satisfactory.

Table 1
IDM contents of sustained-release tablets A and B and tablets C before and after storage at 37 °C and RH 75% ($n = 6$)

Time (months)	Tablets A (%)	Tablets B (%)	Tablets C (%)
0	17.9 ± 0.21	32.3 ± 0.12	28.3 ± 0.20
1	17.9 ± 0.20	32.7 ± 0.30	28.5 ± 0.55
2	17.5 ± 0.20	32.2 ± 0.29	28.2 ± 0.38
3	17.5 ± 0.17	32.1 ± 0.31	28.0 ± 0.33

$P < 0.01$ when IDM contents of 0 month compared with those of 3 months.

Table 2
DSC results of rate of rise in temperature and peak temperatures

Samples	Rate of rise in temperature (°C/min)	Peak temperature (°C), mean ± S.D.
Tablets A	1	159.8 ± 0.84
	2	161.0 ± 0.71
	5	162.0 ± 0.00
	10	164.2 ± 0.45
Tablets B	1	154.9 ± 0.85
	2	155.4 ± 0.85
	5	156.8 ± 0.96
	10	157.7 ± 0.45
Tablets C	1	155.8 ± 0.84
	2	157.0 ± 0.71
	5	158.0 ± 1.00
	10	159.2 ± 0.84

3.5. Test for in vitro release

Six curves of cumulative release rate versus time were obtained for each of tablets A, B and C, from which release $t_{1/2}$ was calculated as follows: 41.30 ± 1.86 min for tablet A, 33.25 ± 2.84 min for tablet B and 6.30 ± 0.39 min (all $n = 6$) for tablet C. The results of Student's t-test and analysis of variance (F-test) showed that the difference among the three data or between any two was significant ($P < 0.01$).

Average results of the test for in vitro release are shown in Fig. 4 with S.D. bars. The cumulative release rate Q of tablet A

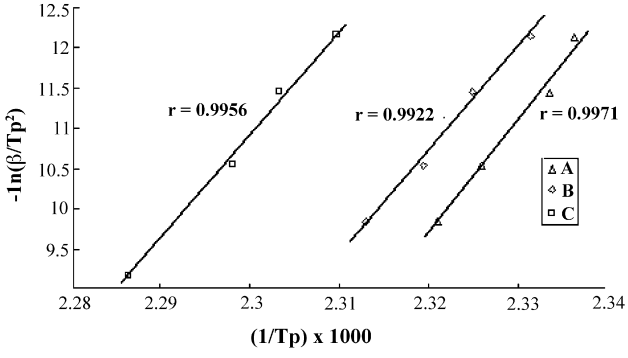

Fig. 3. Plot of $-\ln(\beta/T_p^2)$ vs. $1/T_p$ of tablets A (A), tablets B (B) and tablets C (C) (r standing for the correlation coefficient of the straight line).

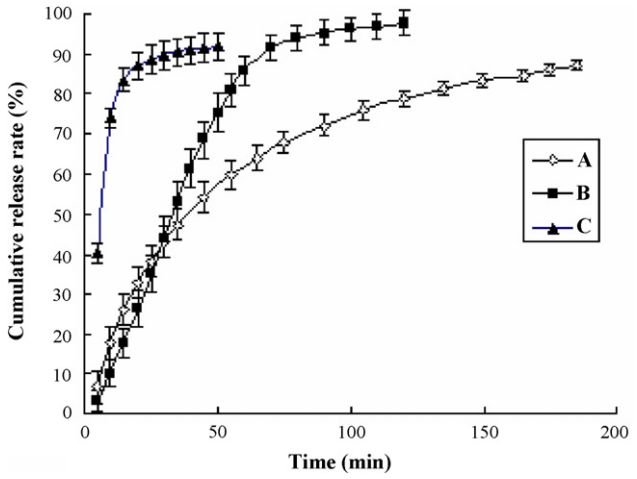

Fig. 4. Cumulative release rate of tablets A (A), tablets B (B) and tablets C (C) in pH 7.4 PBS.

was calculated to be congruent with Higuchi equation:

$$Q_A = 9.48t^{1/2} - 10.94, \quad r = 0.994 \quad (n = 18)$$

The cumulative release rate of tablet B conformed with zero-order kinetics (for initial 60 min)

$$Q_B = 1.54t - 2.91, \quad r = 0.997 \quad (n = 12)$$

with a release constant $k_r = 1.54\%$/min and a delay time of 1.9 min (2.91/1.54) for release. For comparison, the cumulative release rate of tablet C was also calculated to be congruent with first-order kinetics

$$\ln(1 - Q\%) = -0.192t + 0.516, \quad r = 0.986 \quad (n = 8).$$

3.6. Irritation to rat stomach

Irritation to stomach usually happens within 1–3 h after oral administration (Chen et al., 2003), so we sacrificed the rats 5 h after po administration for irritation examination.

LD_{50} of IDM administered orally to rats was reported to be 12 mg/kg (Specification Sheet of entric-coated indomethacin, Ke Rui Pharmaceutical Company of Chongqing, Ltd., China). Preliminary tests showed that a single dose ≤20 mg/kg would not give a significant result of ulcer formation in rat stomach, so we used 25 mg/kg to obtain comparable results. The rat stomach irritation results are shown in Table 3 and Fig. 5. Addition to the number of ulcers, index number was also calculated. The definition for the index number was: 0 for normal stomach, 1 for small spots of bleeding in one stomach, 2 for 1–5 small ulcers (<3 mm) in one stomach, 3 for 6–10 small ulcers, 4 for over 10 small ulcers or 1 big ulcer (≥3 mm), 5 for 2 or more big ulcers, 6 for stomach perforation. As shown in Table 3, for tablets C, not only the number of ulcers was the largest, but the index number was also the largest.

The results of Student's *t*-test for number of ulcers in rat stomach showed that tablets A and B significantly reduced the gastric irritation compared with tablet C ($P < 0.01$), and that there existed insignificant difference between tablets A and tablets B ($P > 0.1$).

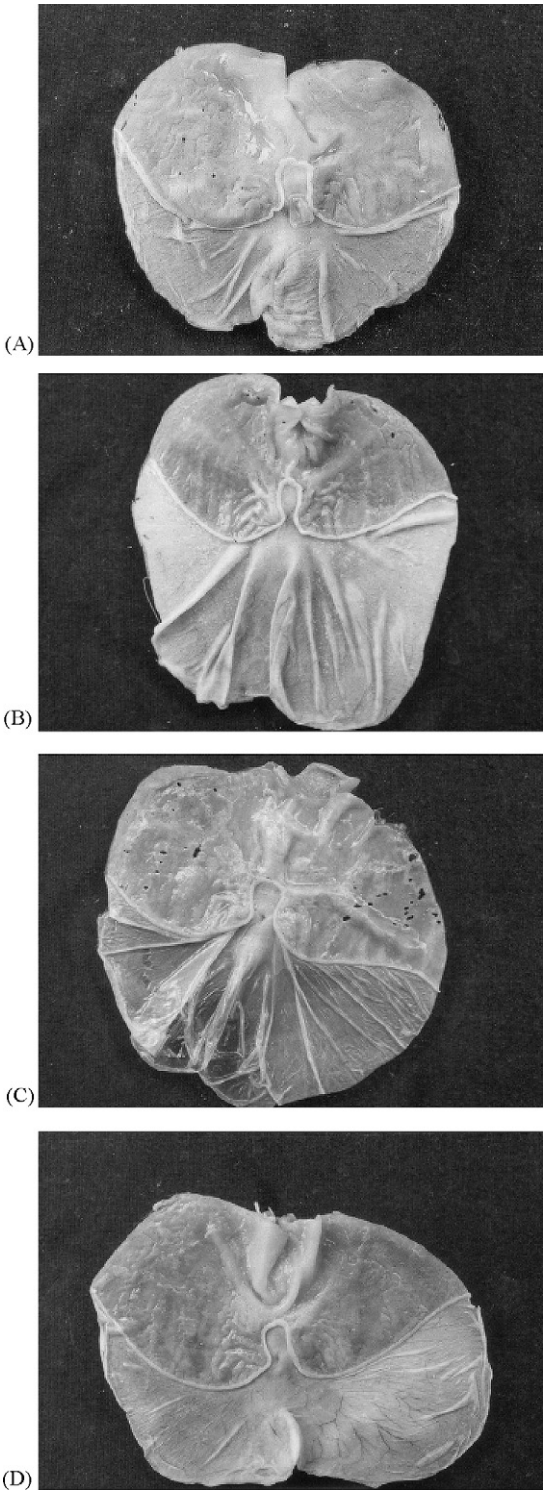

Fig. 5. Photos of rat stomach showing irritation results (black spots being ulcers) (A) tablets A, (B) tablets B, (C) tablets C and (D) physiological saline (plain stomach).

3.7. Bioavailability of the tablets in rabbits

Toxic blood concentration of IDM for human adults was reported to be 75 μg/ml and efficient concentration was 0.3–3.0 μg/ml (Luo et al., 2001). We used a pretty high dose (100 mg/kg) for rabbits for fearing that the blood concentration

Table 3
Number of ulcers in rat stomach of different groups

Groups	No. of rats	Weight of rats (g), mean ± S.D.	No. of ulcers, mean ± S.D.	P	Index no. of ulcers, mean ± S.D.
Tablets A	8	195.4 ± 19.6	12.0 ± 3.9	$P_1{}^* < 0.01$, $P_2{}^* > 0.01$	3.60 ± 0.5
Tablets B	8	188.1 ± 30.1	10.9 ± 7.2	$P_1{}^* < 0.01$	3.25 ± 0.7
Tablets C	8	195.9 ± 24.3	34.6 ± 5.8		4.50 ± 0.5
Blank	8	195.4 ± 23.7	0		0

* P_1: ulcer number compared with tablets C; P_2: compared with tablets B.

Table 4
Main pharmacokinetic parameters of IDM tablets in rabbits ($n = 3$)

Samples	t_{max} (h)		C_{max} (μg/ml)		$AUC_{0-24\,h}$ [mg/(ml h)]	Bioavailability (%)
	1	2	1	2		
Table A	10.0	16.0	20.32	6.19	189.94 ± 82.13	93.2 ± 40.3
Table B	6.0	14.0	18.56	15.45	206.99 ± 94.83	101.6 ± 46.5
Table C	4.5	10.0	32.07	14.63	203.77 ± 28.47	100.0 ± 14.0

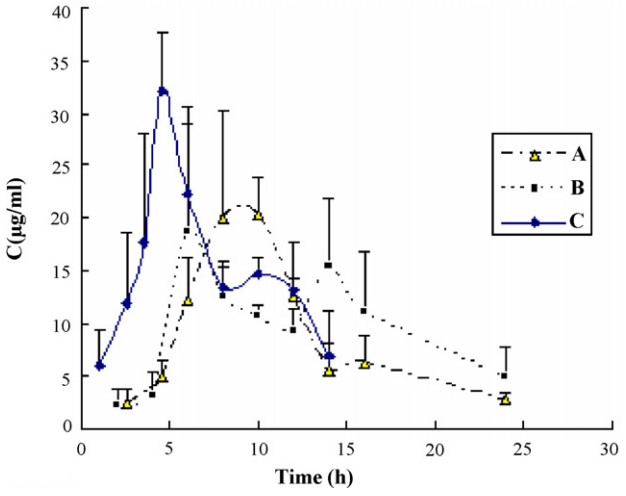

Fig. 6. Blood concentrations of IDM vs. time curves after po administration in rabbits (A) tablets A, (B) tablets B and (C) tablets C.

would be too low to be detected. But the blood concentration of IDM (even C_{max}) was still tolerable for rabbits.

Curves of IDM concentration in rabbit blood versus time are shown in Fig. 6, and the main pharmacokinetic parameters of IDM tablets shown in Table 4. Double peaks (two values for both t_{max} and C_{max}) were found in all blood concentration versus time curves, which conformed with that reported in the literature (Takeda et al., 1981). Compared with tablet C, the time to reach blood peak concentration (t_{max}) was delayed and the values of peak concentration (C_{max}) lowered for tablets A and B, but tablets A, B and C were close in values of $AUC_{0-24\,h}$.

4. Conclusions

Two kinds of tablets (A and B) were prepared from two kinds of microcapsules (A and B). Both kinds of tablets showed sustained-release feature and reduced irritation to stomach, and could be prepared with simple procedures. Since microcapsules A were solidified with toxic formaldehyde as the linking agent (although thoroughly washing off) and microcapsules B with acetic acid, tablets B were safer than tablets A. And tablets B showed a bit less irritation and larger bioavailability compared to tablets A, though without significant difference. Tablets B also gave much higher IDM concentration in blood at 24 h than tablets A (about two times larger, see Fig. 6, both within the efficient concentration). Provided that a patient is given the sustained-release tablets once a day, tablets B with one half dose would be comparable with tablets A. It could be concluded that, while tablets A and B were both satisfactory, tablets B (made of microcapsules B with simple coacervation) was superior to tablets A.

IDM plain tablets are not used clinically at present, and entric-coated tablets (25 mg, tid) or capsules (25 mg, tid), etc. are used to reduce the irritation to stomach. Our sustained-release tablets could not only reduce the irritation, but might also be given less frequently. Of course, more research is required to develop the sustained-release tablets for clinical use.

All the above results showed that the sustained-release IDM tablets A and B prepared in this lab were successful, so we might conclude that they provided a sound basis for developing a long-acting and sustained-release delivery system for oral administration of IDM.

References

Aggarwal, A., Kaur, S., Tiwary, A.K., Gupta, S., 2001. Chitosan microspheres prepared by an aqueous process: release of indomethacin. J. Microencapsul. 18, 819–823.

Chen, X.Q., Jin, Y.Y., Tang, G. (Eds.), 2003. New Pharmacology, 15th ed. People Health Publishing House, Beijing, pp. 186–187.

Chen, Y., Lin, X., 2005. Studies on the drug release properties of nano-encapsulated indomethacin microparticles. J. Microencapsul. 22, 47–55.

Daniels, R., Mittermaier, E.M., 1995. Influence of pH adjustment on microcapsules obtained from complex coacervation of gelatin and acacia. J. Microencapsul. 12, 591–599.

Guterres, S.S., Weiss, V., de Lucca Freitas, L., Pohlmann, A.R., 2000. Influence of benzyl benzoate as oil core on the physicochemical properties of spray-

dried powders from polymeric nanocapsules containing indomethacin. Drug Deliv. 7, 195–199.

Lu, B., Zhang, L., Deng, Y., 1986. Preparation of capsules of microencapsulated indomethacin and their bioavailability in rabbits. J. West China Univ. Med. Sci. 17, 328–331.

Luo, M.S., Gao, T.H., Lao, J.H. (Eds.), 2001. Modern Monograph of Clinical Drugs. Publishing House of Science and Technology of Sichuan, pp. 254–255.

Mason, L., Edward, J., Moore, R.A., McQuay, H.J., 2004. Single dose oral indomethacin for the treatment of acute postoperative pain. Cochrane Database Syst. Rev. 4, CD004308.

O'Brien, W.M., 1968. Indomethacin: a survey of clinical trials. Clin. Pharmacol. Ther. 9, 94–107.

Rowe, J.S., Carless, J.E., 1981. Comparison of the in vitro dissolution behaviour of various indomethacin formulations with their in vivo bioavailability. J. Pharm. Pharmacol. 33, 561–564.

Takeda, Y., Nambu, N., Nagai, T., 1981. Microencapsulation and bioavailability in beagle dogs of indomethacin. Chem. Pharm. Bull. 29, 264–267.

Uzunkaya, G., Bergisadi, N., 2003. In vitro drug liberation and kinetics of sustained release indomethacin suppository. Farmaco 58, 509–512.