Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Optimization of Gastric Floating Microspheres of Dextromethorphan Hydrobromide Using a Central Composite Design

LIAN-DONG HU*, WEI LIU, JIAN-XUE YANG, LI LI,
College of Pharmaceutical Sciences, Hebei University, Baoding, PR CHINA

ARTICLE DETAILS

Article history:
Received on 12 April 2011
Modified on 12 June 2011
Accepted on 29 June 2011

Keywords:
Floating drug delivery systems,
Floating microspheres,
Emulsion-solvent diffusion method,
Central composite design,
Dextromethorphan hydrobromide

ABSTRACT

The purpose of this study was to prepare and evaluate the gastric floating microspheres of dextromethorphan hydrobromide that would prolong the gastric residence time and continuously release the drug in controlled manner. These microspheres were prepared by emulsion-solvent diffusion technique using ethyl cellulose (as carrier polymer) with drug in a mixture of dichloromethane and ethyl acetate. The physico-chemical properties of microspheres such as floating ability, drug loading, entrapment efficiency and in vitro drug release were investigated. The central composite design was chosen to obtain the optimum formulation. The microspheres prepared with optimal formulation were spherical with a size distribution range between 45 and 200 µm. The floating rate after 24h was (76.59±6.53) %, and the drug loading capacity and entrapment efficiency was found to be (13.79±0.93) % and (80.24±1.12) %.

INTRODUCTION

In recent years, the high cost involved in the development of a new drug molecule has diverted the pharmaceutical industries to investigate various strategies in the development of new drug delivery systems (DDS). [1,2]These methods have been used to retain the dosage form in the stomach as a way of increasing the gastric residence time (GRT) include: floating drug delivery systems (FDDS) [3]; high-density systems [4-6]; magnetic systems; swelling or expanding systems [7,8] and other delayed gastric emptying devices. [9-11]

Dextromethorphan hydrobromide (DMB), a well-known antitussive agent, is generally used as an ingredient in cough and cold remedies. It has no narcotic, analgesic or addictive properties and its potency as an antitussive agent is almost equal to that of codeine. DMB has liabilities with respect to its abuse potential at doses above the therapeutic dose. At recommended therapeutic doses (20–30 mg), it has an excellent safety profile; however, at doses associated with abuse (>100 mg), commonly observed side effects include euphoria, disorientation, hallucinations, visual disturbances, and psychosis. [12]

Author for Correspondence:
Email: pharm126@126.com (L. Hu)

It is unclear whether dex-tromethorphan, dextrorphan, or both are responsible for the effects that contribute to its abuse potential. This drug has a short half-life and the recommended oral dose for adults is three to four times a day. [13] Therefore, sustained release dosage forms were necessarily developed to avoid repeated administration and increase patient compliance.
Conventional oral drug administration does not provide rate-controlled release or target specificity. Accordingly, floating drug delivery systems (FDDS) have been developed to avoid this drawback. The physico-chemical properties of DMB and its shorter half-life make it a suitable candidate for preparation of floating microspheres. The principle of floating preparation offers a simple and practical approach to achieve increased gastric residence time for the dosage form and sustained drug release. Prolonged gastric retention improves bioavailability, reduces drug waste and improves solubility for DMB that are less soluble in a high pH environment. [14-16]

At present there are no DMB-SR floating microspheres available. The DMB-SR floating microspheres can lead to the reduction of the dosage frequency; less of a chance of an overdose, and it is a good dosage form for asthma patients for night time cough. In this study, a central composite design was used to

optimize the preparation of DMB floating microspheres. The concentration of poloxamer 188, the concentration of EC and the theoretical drug content were selected as independent variables while the entrapment efficiency, buoyancy, drug loading, and drug release after 24 hours as the dependent variables. Then the particle size, scanning electron microscopy, drug loading and entrapment efficiency, in vitro buoyancy and release characteristics study of the prepared floating microspheres were investigated.

MATERIALS AND METHODS

Materials

DMB as reference substances were supplied by National Institute for the Control of Pharmaceutical and Biological Products. DMB as the model drug was purchased from Shanghai Dingkang Pharmaceutical Co., Ltd. α-Asarone was provided from National Institute for the Control of Pharmaceutical and Biological Products. Ethyl cellulose (EC, 7 cps) was provided from Shanghai Colorcon Co. Ltd. Poloxamer 188 and cetyl alcohol were provided by Shang Hai Xie Tai Chemical Industry Co. Ltd. Acetonitrile (HPLC grade) was obtained from Tianjin Kermel Chemical Reagent Co. Ltd. All other reagents were of analytical grade.

Preparation of microspheres

These microspheres were prepared by emulsion-solvent diffusion technique. DMB, ethyl cellulose and cetyl alcohol were dissolved in a mixture of dichloromethane and ethyl acetate (7:3, v/v) at room temperature. This was slowly added to 100 ml water containing poloxamer 188 maintained at a temperature of 30–40°C and subsequently stirred at ranging agitation speed for 20 min to allow the volatile solvent to evaporate. The microspheres were collected, washed with distilled water and oven-dried (40°C).

Experiments design

As the basis of preliminary experiment, the central composite design (CCD) was selected for the optimization of the parameters. The chosen independent factors, i.e. the concentration of poloxamer 188(X_1), the concentration of EC (X_2) and the theoretical drug content(X_3), have more significant effects on the property of microspheres. The three independent variables and their levels are listed in Table 1. The entrapment efficiency, drug loading, drug release after 24 hours (F_{24h}), and particle size were kept as dependent variables. A second-order polynomial model was used to generate response surfaces:

$$Y=B_0+B_1X_1+B_2X_2+B_3X_3+B_{12}X_1X_2+B_{13}X_1X_3+B_{23}X_2X_3+B_{11}X_1^2+B_{22}X_2^2+B_{33}X_3^2$$

where Y is the measured response associated with each factor level combination; B_0 is an intercept; B_1 to B_{33} are regression coefficients; and X_1, X_2 and X_3 are the independent variables. The dependent and independent variables selected are presented in Table 1 along with their low, medium and high levels selected for the study.

Table 1: Experimental design of level and factor

Level	X_1	X_2	X_3
- 1.732	0.5	10	10
- 1	1.35	12.11	14.23
0	2.5	15	20
+ 1	3.65	17.89	25.77
+ 1.732	4.5	20	30

Scanning electron microscopy

Scanning electron microscopy (SEM) (KYKY Technology Development LTD. Beijing, China) studies were performed to characterize the surface of formed microspheres. Before scanning, the microspheres were sputtered with gold to make the surface conductive.

Determination of drug loading and entrapment efficiency

The samples were determined using the HPLC method. The HPLC system consisted of a P3000A pump and a UV detector. A Venusil XBP C18 column (5 μm, 250 × 4.6 mm) was used. The mobile phase consisted of 0.5% triethylamine (pH 3.5 adjusted by phosphoric acid)-acetonitrile (75:25, v/v), The flow rate was 1.0 ml·min^{-1}, and the UV detector was set at 278 nm.

A certain amount of microspheres were ground to powder. Then the powder containing approximately 10 mg drug was weighed and added to a 100 ml volumetric flask containing 70 ml of mobile phase. After 20 min of ultrasonic extraction, the sample was diluted with mobile phase to 100 ml and then filtered through a 0.45 μm membrane. 20 μl of the filtered solution was injected for analysis. All samples were analyzed in triplicate. The drug loading (DL) and entrapment efficiency (EE) were calculated as follows:

$$DL\ (\%) = \frac{W_D}{W_T} \times 100$$

W_D: the weight of the drug loaded in the microspheres; W_T: the total weight of the microspheres.

$$EE\ (\%) = \frac{W_A}{W_T} \times 100$$

W_A: actual drug content; W_T: theoretical drug content.

In vitro evaluation of floating ability

The floating test was performed to investigate the floatability of the prepared microspheres. The microspheres (0.1g) were spreaded on 500 ml of 0.1 mol/L HCl containing 0.02% Tween 80 as surfactant to simulate gastric conditions. The mixture was stirred with a paddle at 100 rpm, temperature was maintained at 37±0.5°C.12h later, the floating and the settled portions of microspheres were recovered separately. The microspheres were dried and weighed. The buoyancy was calculated as the following equation:

$$Buoyancy\ (\%) = \frac{Q_f}{Q_f + Q_s} \times 100$$

Q_f: weights of the floating microspheres;
Q_s: weights of the settled microspheres.

In vitro release studies

The in vitro release studies were performed in 250ml of artificial gastric fluid (pH 1.2) thermostatically maintained at 37 ± 0.5°C based on Chinese Pharmacopoeia (2005 Ed.) Method Ⅲ. Paddle rotational speed was set to 50 rpm. 5 ml samples were withdrawn and replaced with an equal volume of the same fresh medium at predetermined time intervals. The sample solutions were filtered through a 0.45 μm membrane and analyzed using a UV spectrophotometer at 278 nm.

RESULTS AND DISCUSSION

Experimental design

Central composite design results

For a three-factor central composite design, 20 experiments are required and the results of entrapment efficiency, drug loading, drug release after 24 hours (F_{24h}), and drug loading are shown in Table 2. Experiments l-8 represent the simple factorial design using two levels of each variable. Experiments 9-14 are the star points and experiments 15-20 represent the central point.

Data evaluation

The entrapment efficiency, drug loading, buoyancy and F_{24h} (drug release after 24 hours) were selected as the dependent variables. The equations by a second-order polynomial model were shown as follow, and then the three-dimensional response surface graphs fitted to the equation were shown in Figure 1. (The concentration of EC (X_2) was held at 15% because of its insignificant influence compared with X_1 and X_3.)

The model equation for DL:

$$DL/\% = -0.541 + 1.038X_1 - 0.305X_2 + 0.991X_3 + 0.029X_1^2 + 0.009X_2^2 - 0.020X_3^2 - 0.076X_1X_2 - 0.029X_1X_3 + 0.023X_2X_3 \quad (P<0.001, r=0.988) \quad (1)$$

As seen in equation (1), the drug loading related to the theoretical drug content significantly ($P<0.05$). Following the Fig. 1A, it can be seen that the theoretical drug content(X_3) showed a negative effect on drug loading and poloxamer 188 concentration (X_1) showed a nearly linear decreasing trend with drug loading. DL would be enhanced by the increment of the theoretical drug content but not the increase of poloxamer 188 proportion. Nevertheless, the influence of theoretical drug content is distinctly far more significant than that of poloxamer 188 concentration. Hence, the higher levels of theoretical drug content have to be complemented with lower levels of poloxamer 188 concentration to maintain drug entrapment at a constant level. The maximum acquired DL could be obtained at poloxamer 188 concentration of 0.5-2% and the theoretical drug content proportion of 10-25%.

The model equation for EE:

$$EE/\% = 86.935 - 0.912X_1 - 2.143X_2 + 1.576X_3 + 0.327X_1^2 + 0.099X_2^2 - 0.079X_3^2 - 0.218X_1X_2 - 0.011X_1X_3 + 0.039X_2X_3 \quad (P<0.01, r=0.907) \quad (2)$$

The equations show that there was a significantly positive relationship between the entrapment efficiency and the theoretical drug content ($P<0.05$). This means that increased theoretical drug content increased the entrapment efficiency of the microspheres. The higher theoretical drug content proportion could be favorable to entrap the drug into the microspheres. At the fixed poloxamer 188 proportion, high theoretical drug content could increase the EE. However, when the theoretical drug content exceeded 12%, the EE began to decrease. In other words, it was not advisable to use higher theoretical drug content

in order to enhance EE. Furthermore, Fig. 1B reveals a decline in the value of drug entrapment with increase in the concentration of poloxamer 188. From the response surface, it can be elucidated that the variation in drug entrapment is a complex function of the concentration of poloxamer 188 and the effect of theoretical drug content. The maximum EE (100%) could be obtained when theoretical drug content ranged from 10% to 12% with fixed poloxamer 188 concentration of 0.5%.

The model equation for Buoyancy:

$$\text{Buoyancy}/\% = 90.351 - 3.850X_1 + 1.100X_2 + 0.650X_3 - 0.281X_1^2 - 0.073X_2^2 - 0.036X_3^2 + 0.139X_1X_2 + 0.123X_1X_3 + 0.007X_2X_3 \quad (3)$$

($P<0.01$, $r=0.911$).

Fig. 1C depicts a quite linear decreasing trend in the values of buoyancy with increased value of poloxamer 188 and nearly linear increasing trend with the theoretical drug content. When poloxamer 188 levels are decreased, the floating ability increases. The higher levels of theoretical drug content result in lower buoyancy.

The model equation for F_{24h}:

$$F_{24h}/\% = 7.943 + 9.388X_1 + 1.040X_2 + 5.690X_3 - 0.236X_1^2 - 0.044X_2^2 - 0.060X_3^2 - 0.193X_1X_2 - 0.483X_1X_3 - 0.074X_2X_3 \quad (4)$$

($P<0.01$, $r=0.950$),

As seen in equation (4), the drug release after 24 hours related to the theoretical drug content significantly ($P<0.05$). As shown in Fig. 1D, it indicates that there was a significant interaction between poloxamer 188 concentration and the theoretical drug content for F_{24h}. The higher poloxamer 188 concentration and the higher theoretical drug content lead to increase the drug release in 24h. In addition, increased theoretical drug content increased the initial burst release.

Optimization of the formulation of microspheres

After the effects of entrapment efficiency, drug loading, drug release after 24 hours (F_{24h}), and drug loading on the formulation of microspheres were investigated, the optimum ranges for each independent variable were found to generate microspheres with high drug loading, entrapment efficiency, buoyancy and F_{24h}.The optimum formulation conditions were as follows: the concentration of poloxamer 188 (X_1) =0.5%, the concentration of EC(X_2) =15%, the theoretical drug content (X_3) =10%. Then, the floating microspheres were prepared according to the optimized prescription. The characteristics of these microspheres were investigated, the results were shown as below.

Buoyancy, drug loading and entrapment efficiency

As the result revealed, these microspheres showed good floating ability. More than 80% of the particles kept floating for at least 12 h. It was also observed that the microspheres of larger size, showed the longer floating time. The floating properties of hollow microspheres may be attributed to the low bulk density and the porosity of the microspheres; implying that they will have the propensity to exhibit an excellent buoyancy effect *in vivo*.

According to the method introduced above, the results indicated a high quality of the floating microspheres with DL and EE was (13.79±0.93) % and (80.24±1.12) % respectively. The high entrapment efficiency of DMB in microspheres may be attributed to its poor aqueous solubility. Furthermore, results demonstrated that an increase in the concentration of EC increased the entrapment efficiency of the drug. This may be explained on the basis that an increase in viscosity of the EC solution with increase in concentration prevents drug crystals from leaving the droplet.

Scanning electron microscopy (SEM)

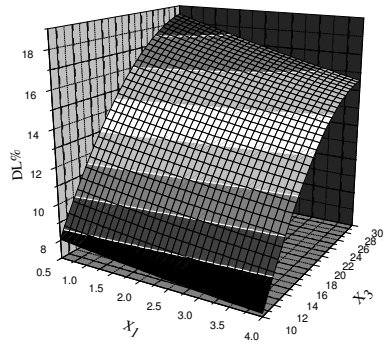
The surface morphology and internal structure of floating microspheres were investigated by scanning electron microscopy (SEM). As it was shown in Fig.2, the surface of the microsphere was uneven and there are some micropores and voids scattered on the surface. It may be caused by the evaporation of solvent entrapped within the shell of microspheres after forming a dense skin layer. And these micropores and voids help the drug release from inner part of the microspheres.

***In vitro* drug release**

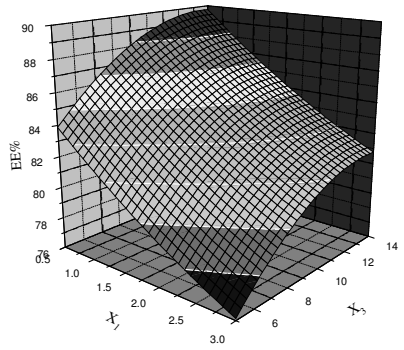
The in vitro drug release of the optimized floating microspheres formulation by the central composite design was illustrated in Fig.3. The accumulated amount of drug released in 12 h was about 82.2%. From the figure, it was clear that the effect of burst release was not obvious, and these microspheres have significant sustained-release characteristics. It was obvious that DMB was released from the microspheres in a steady and sustained fashion. These results suggest that the floating microspheres can be used as a drug-delivery system for treating gastric diseases.

Table 2: Experimental design of microsphere preparation according to central composite design

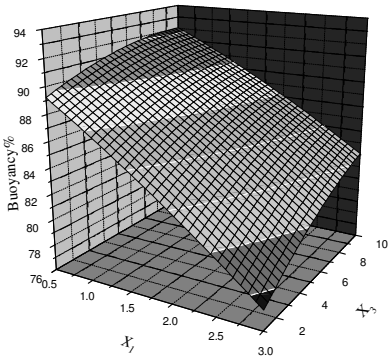
No.	Drug loading (%)	Entrapment efficiency (%)	Buoyancy (%)	F_{24h} (%)
1(-1,-1,-1)	11.20	88.3	94.56	74.17
2(-1,+1,-1)	12.32	96.2	88.96	61.15
3(-1,-1,+1)	15.21	70.5	89.25	89.60
4(-1,+1,+1)	18.19	79.9	86.96	78.36
5(+1,-1,-1)	10.46	82.9	89.02	68.88
6(+1,+1,-1)	10.88	86.8	88.09	60.04
7(+1,-1,+1)	14.01	63.7	89.81	78.24
8(+1,+1,+1)	15.66	71.3	86.54	57.70
9(-a,0,0)	14.78	83.4	91.45	76.74
10(0,-a,0)	13.09	76.2	92.86	78.40
11(0,+a,0)	15.01	84.5	85.67	56.53
12(0,0,-a)	6.55	73.9	93.66	51.78
13(0,0,+a)	17.03	66.1	81.38	73.32
14(+a,0,0)	13.11	75.0	88.50	58.59
15(0,0,0)	14.05	81.0	91.38	65.68
16(0,0,0)	14.02	78.9	90.98	74.43
17(0,0,0)	13.86	79.8	91.59	73.47
18(0,0,0)	14.26	80.8	91.46	71.50
19(0,0,0)	13.87	79.6	89.90	72.51
20(0,0,0)	13.69	78.9	92.05	71.45



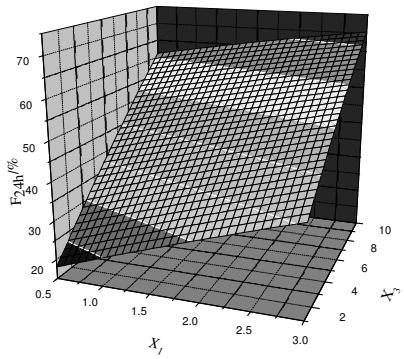
(A)



(B)



(C)



(D)

Figure 1: Response surface plots diagrams

A - drug loading as a function of poloxamer 188 concentration (X_1) and theoretical drug content (X_3) ; B - drug entrapment efficiency as a function of poloxamer 188 concentration (X_1) and theoretical drug content (X_3) ; C - Buoyancy as a function of poloxamer 188 concentration (X_1) and theoretical drug content (X_3) ; D - F_{24h} as a function of poloxamer 188 concentration (X_1) and theoretical drug content (X_3) , with EC concentration (X_2) equal to 15%.

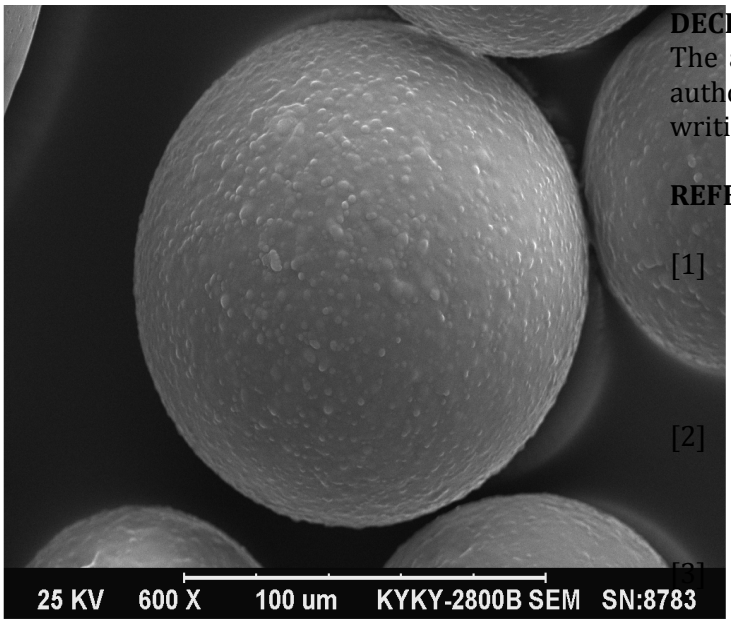


Figure 2: SEM of DMB floating microspheres.

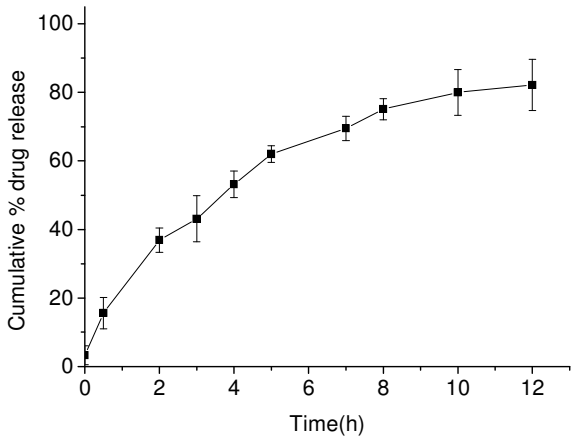


Figure 3: *In vitro* drug release profile of DMB from microspheres (n=3).

CONCLUSIONS

The optimized formulation for dextromethorphan hydrobromide microspheres was obtained with cetyl alcohol, poloxamer 188 and EC using response surface methodology based on a central composite design. It was found that the optimized formulation was achieved with 0.5% poloxamer 188 (X_1), 15% EC (X_2) and 10% theoretical drug content (X_3) and the observed responses were close to the predicted values for the optimized formulation. The drug release from microspheres coated with the optimized formulation showed a controlled-release pattern, in comparison with a commercial product.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCE

[1] Patel A, Modasiya M, Shah D, Patel V. Development and In Vivo Floating Behavior of Verapamil HCl Intragastric Floating Tablets. AAPS PharmSciTech. 2009;10:310-315.

[2] Streobel A, Siepmann J, Bodmeier R. Gastroretentive drug delivery systems. Expert Opinion on Drug Delivery. 2006;3(2):217-233

[3] Deshpande AA, Shah NH, Rhodes CT, Malick W. Development of a novel controlled-release system for gastric retention. Pharm. Res. 1997;14:815-819.

[4] Rednick AB, Tucker SJ. Sustained release bolus for animal husbandry, US Patent. 1970;3:507-520.

[5] Bechgaard H, Ladefoged K. Distribution of pellets in the gastrointestinal tract. J. Pharm. Pharmacol. 1978;30:690-692.

[6] Davis SS, Stockwell AF, Taylor MJ, Hardy JG, Whalley DR, Wilson CG, Bechgaard H, Christensen FN. The effect of density on the gastric emptying of single-and multiple-unit dosage forms, Pharm. Res. 1986;3:20-13.

[7] Urquhart J, Theeuwes F. Drug delivery system comprising a reservoir containing a plurality of tiny pills. US Patent. 1984;4:434-439.

[8] Mamajek RC, Moyer ES. Drug-dispensing device and method. US Patent. 1980;4:207-215.

[9] Rao MR, Borate SG, Thanki KC, Ranpise AA, Parikh GN. Development and in vitro evaluation of floating Rosiglitazone maleate microspheres. Drug Dev Ind Pharm. 2009;35:834-842.

[10] Arora S, Ali J, Ahuja A, Khar RK, Baboota S. Floating drug delivery systems: A review. AAPS PharmSciTech. 2005;6(3):372-390.

[11] Sato Y, Kawashima Y, Takeuchi H, Yamamoto H. In vitro evaluation of floating and drug releasing behaviors of hollow microspheres (microballoons) prepared by the emulsion solvent diffusion method. Eur. J. Pharm. Biopharm. 2004;57:235-243.

[12] Hendrickson HP, Gurley BJ, Wessinger WD. Determination of dextromethorphan and its metabolites in rat serum by liquid–

liquid extraction and liquid chromatography with fluorescence detection. 2003;788:261-268.

[13] Silvasti M, Karttunen P, Tukiainen H, Kokkonen P, Hanninen U, Nykanen K.. Pharmacokinetics of dextromethorphan and dextrorphan: a single dose comparison of three preparations in human volunteers. Int. J. Clin. Pharmacol. Ther. Toxicol. 1987;25:493-497.

[14] Joseph NJ, Lakshmi S, Jayakrishnan A. A floating-type oral dosage form for piroxicam based on hollow polycarbonate microspheres: in vitro and in vivo evaluation in rabbits. J Control Release. 2002;79:71-79.

[15] Ma NN, Xu L, Wang QF, Zhang XR, Zhang WJ, Li Y, Jin LY, Li SM. Development and evaluation of new sustained-release floating microspheres. Int. J. Pharm. 2008;358: 82-90.

[16] Kawashima Y, Niwa T, Takeuchi H, Hino T, Ito Y. Preparation of multiple unit hollow microspheres (microballoons) with acrylic resins containing tranilast and their drug release characteristics (in vivo). J Control Release. 1991;16:279-290.