Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 67 (2007) 196–201
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Formulation and development of hydrodynamically balanced system for metformin: *In vitro* and *in vivo* evaluation

Javed Ali [a,*], Shweta Arora [a], Alka Ahuja [a], Anil K. Babbar [b], Rakesh K. Sharma [b], Roop K. Khar [a], Sanjula Baboota [a]

[a] *Department of Pharmaceutics, Jamia Hamdard, New Delhi, India*
[b] *Department of Radiation Biology and Radiopharmaceuticals, Institute of Nuclear Medicine and Allied Sciences, Delhi, India*

Received 1 October 2006; accepted in revised form 14 December 2006
Available online 28 December 2006

Abstract

The objective of the present study was to develop a hydrodynamically balanced system of metformin as a single unit floating capsule. Various grades of low-density polymers were used for the formulation of this system. They were prepared by physical blending of metformin and the polymers in varying ratios. The formulation was optimized on the basis of *in vitro* buoyancy and *in vitro* release in simulated fed state gastric fluid (citrate phosphate buffer pH 3.0). Effect of various release modifiers was studied to ensure the delivery of drug from the HBS capsules over a prolonged period. Capsules prepared with HPMC K4M and ethyl cellulose gave the best *in vitro* percentage release and were taken as the optimized formulation. By fitting the data into zero order, first order and Higuchi model it was concluded that the release followed zero order release, as the correlation coefficient (R^2 value) was higher for zero order release. It was concluded from R^2 values for Higuchi model that drug release followed fickian diffusion mechanism. *In vivo* studies were carried out in rabbits to assess the buoyancy, as well as the pharmacokinetic parameters of the formulation using gamma scintigraphy. The formulation remained buoyant during 5 h of study in rabbits. The comparative pharmacokinetic study was performed by administration of the optimized HBS capsules and immediate release capsules, both with radiolabeled metformin, using gamma counter. There was an increase in AUC in optimized HBS capsules of metformin when compared with immediate release formulation.

Keywords: Metformin; Hydrodynamically balanced system; Gastroretention; Gamma scintigraphy

1. Introduction

Drug absorption from gastrointestinal tract is a complex procedure and is subject to many variables. It has been reported that the extent of gastrointestinal tract drug absorption is related to contact time with the small intestinal mucosa [1]. Gastroretentive systems can remain in the gastric region for several hours and therefore significantly prolong the gastric residence time of drugs. Many approaches have been reported in the literature for the formulation of HBS systems viz. mucoadhesion [2], floatation [3], sedimentation [4], expansion [5,6], modified shape systems [7,8] or by the simultaneous administration of pharmacological agents [9,10] which delay gastric emptying. Both single unit systems (tablets or capsules) [3] and multiple unit systems (multi particulate systems) have been reported in the literature [11,12]. Floating drug delivery offers a number of applications for drugs having poor bioavailability because of narrow absorption window in the upper part of gastrointestinal tract. It retains the dosage form at the site of absorption and thus enhances the bioavailability [13,14].

Metformin is an anti-hyperglycemic agent, which improves glucose tolerance in type II diabetes. It has been

* Corresponding author. Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi 110 062, India. Tel.: +91 9811312247; fax: +91 11 2605 9663.
E-mail addresses: javedaali@yahoo.com, jali@jamiahamdard.ac.in (J. Ali).

0939-6411/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2006.12.015

reported that the absolute bioavailability of metformin when given orally is 50–60%. Biological half-life of metformin is 1.5–1.6 h and the main site of its absorption is proximal small intestines. A HBS system was planned for metformin as such a system when administered would remain buoyant on the gastric fluids for a prolonged period of time and the drug would be available in the dissolved form at the main site of its absorption i.e., proximal small intestines [15]. This would lead to improvement in the bioavailability of the drug. In this way it stands an advantage over conventional dosage form, which needs to be administered twice or thrice a day.

2. Experimental

2.1. Materials

Metformin was obtained as a gift sample from M/s Novartis, Mumbai, India. Different grades of the poly ethylene oxide (PEO) (grades–WSR 1105, WSR 301, WSR 303, WSR 60 K, and WSR N80), HPMC K4 M were obtained as gift samples from Ranbaxy Research Laboratories, Gurgaon, India.

Tc_{99m}-pertechnetate was obtained from Regional Center for Radiopharmaceuticals, Board of Radiation and Isotope Technology (BRIT), Department of Atomic Energy, Delhi.

2.2. Preparation of capsules

Single unit capsules were formulated with the help of different low-density floating polymers, which upon administration would attain a density of less than that of the gastric fluids and therefore would float. Five hundred milligrams of metformin and different ratios of the polymer were accurately weighed and the drug and polymer blend was filled into the empty capsule shells manually. The polymer and drug mixture were blended for 10 min in a Double Cone Blender (lab scale Kalweka apparatus of 4 kg capacity). The composition of the HBS capsules is given in Table 1.

2.3. In vitro buoyancy studies

The capsules were immersed in 900 ml of citrate phosphate buffer pH 3 (simulating the pH of gastric contents of stomach in fed state) in USP paddle type apparatus at 50 rpm.

The time for which the capsules remained buoyant was observed and was taken as the floating time. The polymer that showed the best floating behavior was taken for *in vitro* release studies.

2.4. In vitro release studies

Based on the *in vitro* buoyancy studies, formulations showing good floatation were subjected to *in vitro* release studies. *In vitro* release studies were carried out by carrying out the dissolution studies in USP paddle type apparatus at 50 rpm using 900 ml of citrate phosphate buffer pH 3. Five milliliters of sample was withdrawn at regular intervals and replaced with the buffer. The samples were diluted up to 30 times with buffer and evaluated spectrophotometrically at 237 nm (λ_{max}).

2.5. Effect of release modifiers

Ethyl cellulose (EC), cellulose acetate phthalate (CAP), and liquid paraffin (LP) were used at different concentrations as shown in Table 1. For addition of ethyl cellulose (5%) to the formulation a 5% solution of ethyl cellulose was made in acetone and granulated with weighed drug content and these granules were then passed through ASTM # 25 mesh and granules equivalent to 500 mg of metformin were filled into capsules. CAP was added in dry form and liquid paraffin 1% was added by making a solution in isopropyl alcohol. The granules were dried in a lab scale fluid bed drier at 60 °C, were passed through ASTM # 25 mesh and filled into capsules.

2.6. Analysis of in vitro drug release

To analyze the mechanism of drug release from the capsules the *in vitro* dissolution data were fitted to zero order [16], first order [17], Higuchi release model [18], Hixson and Crowell powder dissolution method [19] and Korsmeyer and Peppas model [20]. The equations for the said models are given in Table 2.

2.7. In vivo studies for metformin hydrochloride (gamma scintigraphy studies)

In vivo buoyancy of the formulation was evaluated by gamma scintigraphy using rabbits. Permission was obtained from Institutional Ethical Review Board.

2.8. Radiolabeling of metformin

Metformin was radiolabeled with technetium (Tc_{99m}) as it offers several advantages in terms of its short half-life of 6 h and as it allows very less amount of electron emission. It can be administered in milli curie amounts of Tc_{99m}, resulting in very low radiation dose to the patient. Moreover, Tc_{99m} is readily available in sterile, pyrogen-free, and carrier-free state. A 1 mg/ml solution of metformin hydrochloride was prepared in distilled water. Similarly a 1 mg/ml solution of stannous chloride ($SnCl_2$) was prepared in 0.1 N HCl. To 1 ml of drug solution, 2 mci of Tc-99m was added and different concentrations of $SnCl_2$ were added to this solution. pH was adjusted to 6.5 with the help of 0.5 M sodium bicarbonate.

2.9. Radiochemical purity

The radiochemical purity of Tc_{99m} labeled metformin was assessed by using ascending instant thin layer

Table 1
Composition of HBS capsules containing metformin along with and without release modifiers

	Quantity (in mg)									
	Without release modifiers					With release modifiers				
	I	II	III	IV	V	VI	VII	VIII	IX	X
Metformin	500	500	500	500	500	500	500	500	500	500
HPMC K4 M	150	–	–	–	150	150	150	–	–	–
PEO 60 K		150	–	–						
PEO WSR 303			150	–	–	–	–	150	150	150
PEO WSR 301				150						
EC					5%	–	–	5%	–	–
CAP					–	5%	–	–	5%	–
LP					–	–	1%	–	–	1%

Table 2
Kinetics of optimized formulation of metformin hydrochloride

S. No.	Model	Equation	R^2	k
1	Zero order	$F = k \times t$ (where F is the fraction of drug release, k is the release constant and t is the time)	0.9688	8.9049
2	First order	$\ln F = k \times t$ (where F is the fraction of drug release, k is the release constant and t is the time)	0.8847	−2.197
3	Higuchi	$F = k\sqrt{t}$	0.9773	25.7616
4	Hixson and Crowell powder dissolution method	$F = 100(1 - (1 - kt)^3)$	0.9660	17.2918
5	Korsmeyer and Peppas model[a]	$F = kt^n$	0.9900	−0.0503

[a] n (diffusional coefficient) = 0.6927.

chromatographic plates (ITLC) using silica gel (SG)-coated fiber glass sheets (Gelman Sciences Inc., Ann Arbor, MI, USA) and dual solvent systems viz., 100% acetone and a solvent mixture of pyridine:acetic acid:water (PAW: 5:3:1.5 v/v). The radioactive contaminants were identified as reduced/hydrolyzed (R/H) Tc_{99m} and free Tc_{99m}-pertechnetate [21].

2.10. Effect of pH and stannous chloride concentration

Effect of varying pH of the reaction mixture on the labeling efficiency was studied and pH was optimized on this basis. In another experiment, the pH of the reaction mixture was kept constant and the quantity of stannous chloride was varied from 50 to 200 μL. The labeling efficiency of metformin was measured using ascending ITLC plates (Tables 3 and 4).

Table 3
Effect of $SnCl_2$ concentration on radiolabeling efficiency of metformin hydrochloride

$SnCl_2$ concentration (μg)	%free Tc_{99m}	%R/H Tc_{99m}	%Tc_{99m} metformin
100	10.90	5.80	83.30
150	10.00	6.30	83.70
250	3.10	6.90	90.00
500	2.10	23.00	74.90
1000	1.80	35.00	63.20

Optimization of $SnCl_2$ concentration.

Table 4
Effect of pH on radiolabeling efficiency of metformin hydrochloride (optimization of pH)

pH	%free Tc_{99m}	%R/H Tc_{99m}	%Tc_{99m} metformin
5.0	12.90	5.90	81.20
6.5	3.90	5.50	90.60
7.5	15.80	7.00	77.20

2.11. In vitro stability of radiolabeled metformin

The *in vitro* stability of the radiolabeled complex was assessed by ascending ITLC plates. One hundred micrograms of the radiolabeled complex was mixed with 2 ml of physiological saline i.e., 0.9% NaCl. ITLC was used to examine the labeling efficiency after incubation at 37 °C at different time intervals (Table 5).

2.12. Comparative dissolution studies

A comparative dissolution study was carried out with optimized capsule with and without radioactivity to ensure that there is no leaching out of the activity from the capsule and to correlate the drug release between the HBS capsule with radiolabeled drug and without radiolabeled drug. Tc_{99m} (0.2 ml) labeled metformin solution was added to the contents of the optimized capsule. The capsules were subjected to *in vitro* dissolution studies in fed state simulated gastric fluid (pH 3.0) and the release was compared to the release from the capsule without radioactivity as shown in Fig. 3.

Table 5
In vitro stability studies on radiolabeled metformin hydrochloride

Time (h)	%free Tc_{99m}	%R/H Tc_{99m}	%Tc_{99m} metformin
0	3.20	5.70	91.10
1	3.90	5.20	90.90
2	3.50	6.30	90.20
3	3.80	6.60	89.60
4	4.20	6.70	89.10
5	4.10	6.10	89.60
24	5.10	7.20	87.70

2.13. Gamma imaging in rabbits

Before starting the study permission was taken from the Institutional Ethical Board. The scintigraphy was performed in six healthy male New Zealand Albino rabbits weighing 2.5–4 kg of either sex. The oral dose was decided based upon the weight of the individual rabbit and the capsule was orally administered (with 0.2 ml radiolabeled metformin). The animal was anaesthetized by calmpose injection, each 2 ml ampoule was composed of 10 mg diazepam, 1.5% v/v benzyl alcohol, 0.035% benzoic acid and 0.38% sodium benzoate (Batch No. 9063100, M/s Ranbaxy) 10 min before imaging to sedate the animal. The animal was fixed on a board in posterior position and imaging was performed using a gamma camera (Siemens Private Ltd., Japan). The scans obtained at successive intervals are shown in Fig. 4.

2.14. Pharmacokinetic studies of optimized HBS capsules of metformin by gamma counter

For comparing pharmacokinetic parameters of immediate release and HBS optimized formulation of metformin a comparative study was performed. To determine the pharmacokinetic parameters of the optimized formulation, metformin was labeled with Tc_{99m}. Radiolabeled metformin was added to the optimized HBS formulation and administered to three healthy rabbits of either sex (weighing 2.5–4 kg). Three milliliters of blood was collected from the right ear vein of the rabbit at regular intervals and counts/ml were measured using gamma counter. A control study was also performed, by administering an immediate release capsule to the rabbits.

The pharmacokinetic parameters viz., AUC, T_{max}, C_{max}, K_a were calculated. The % increase in the AUC was calculated as per the following formula

$$\%\ \text{Increase in } AUC_{0-t} = \frac{AUC_{HBS} - AUC_{IR}}{AUC_{IR}} \times 100$$

3. Results and discussion

3.1. In vitro buoyancy studies

From the *in vitro* buoyancy studies it was observed that PEO WSR 60K and PEO WSR 303 and HPMC K4 M containing formulations showed good buoyancy with floatation time up to 12 h in citrate phosphate buffer pH 3.0.

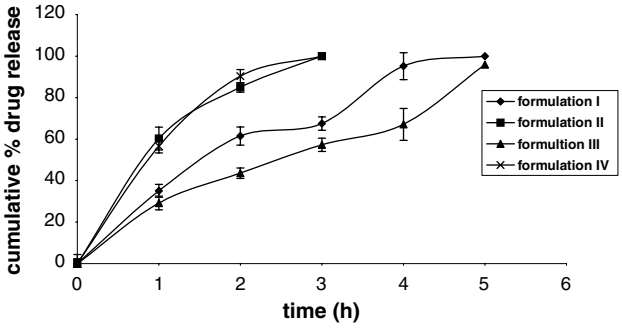

Fig. 1. *In vitro* drug release from formulations (set I).

3.2. In vitro drug release studies

The *in vitro* release studies revealed that the formulations remained buoyant but probably because of the high aqueous solubility of metformin, the release could not be sustained. Only formulation I containing HPMC K4M and formulation III containing PEO WSR 303 could sustain the drug release up to 5 h (Fig. 1), and therefore it was decided to add release modifiers to these formulations (as shown in Table 1) so as to aid the sustained release of the drug from the formulation.

3.3. Effect of release modifiers

It was observed that the best results were obtained with formulation V containing HPMC K4M, metformin and ethyl cellulose at 5% level as the drug release could be sustained for 12 h. But the formulation VIII containing PEO WSR 303 with the same level of ethyl cellulose could sustain the drug release for up to 8 h only.

Dissolution of formulation VI containing HPMC with CAP at 5% level could sustain the drug release for 9 h and the formulation IX with PEO and CAP at the same level could not sustain the release for more than 7 h.

Formulation VII containing liquid paraffin at 1% level gave good results with 100% drug release after 9 h. Formulation X with liquid paraffin at the same level could sustain the drug release for 8 h. Formulation V containing HPMC K4 M gave better results as it formed a firm matrix and could control the drug release for a longer duration of time. Among the release modifiers used to control the release of metformin from the formulation, formulation containing ethyl cellulose gave the best *in vitro* release as it could control the release for 12 h and hence it was taken as the optimized formulation (Fig. 2).

3.4. Analysis of in vitro drug release

The *in vitro* release pattern of the optimized formulation was analyzed by fitting the dissolution data into various

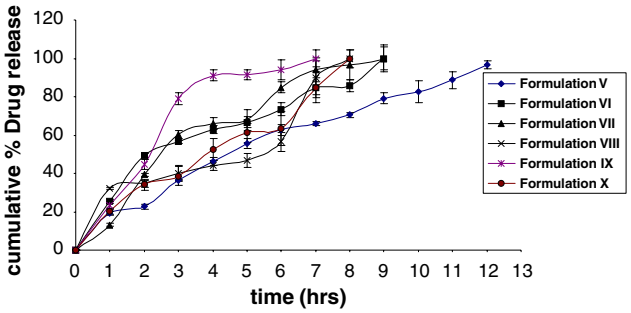

Fig. 2. Comparative *in vitro* drug releases from HBS capsules of metformin with various release modifiers.

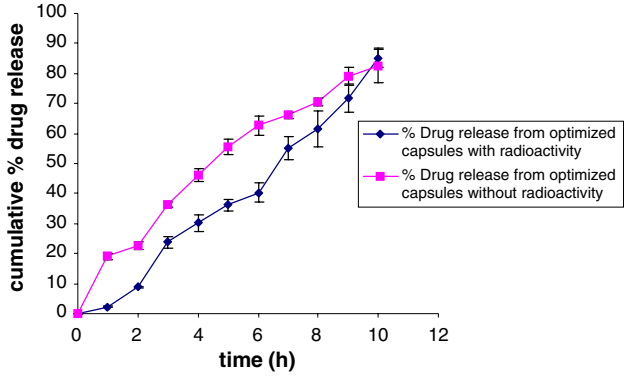

Fig. 3. Comparative *in vitro* dissolution profile of optimized HBS capsules with and without radioactivity.

kinetic models. It was observed that R^2 value was higher when fitted to zero order equation as compared to first order equation, which indicated a zero order release from the optimized HBS capsule of metformin hydrochloride (Table 2).

3.5. Optimization of radiolabeling method

For the optimization of radiolabeling method, the best labeling efficiency could be attained with 250 μg $SnCl_2$. The radiolabeled complex was most stable at pH 6.5. The radiolabeled complex was subjected to *in vitro* stability test for 24 h and it was observed that the complex was stable up to 24 h (Tables 3–5).

In the comparative dissolution studies, a good correlation existed between the drug release from the capsule with plain and radioactive drug ($R^2 = 0.9634$) and hence there was no leaching of the radioactivity from the capsule and it could be administered to rabbit for the *in vivo* buoyancy test (Fig. 3).

3.6. Gamma scintigraphy studies

Gamma scintigraphic studies revealed the location of capsule in six healthy rabbits. Posterior whole body images at time intervals (0.25, 0.5, 1, 2, 3, 4, and 5 h) showed the retention of capsules in stomach for more than 5 h as shown in *in vitro* studies (Fig. 4).

3.7. Pharmacokinetic parameters

The pharmacokinetic parameters viz. AUC_{0-t}, T_{max}, C_{max}, and K_a were calculated and compared with the control i.e., immediate release (IR) formulation. It was observed from the study that the drug release from the optimized HBS capsules could be sustained for a prolonged period, with C_{max} and T_{max} being 76.97% in 7 h, whereas in immediate release capsules the drug release could not be sustained with C_{max} and T_{max} being 97.21% in 3 h. Hence, it was concluded that our formulation was able to sustain the drug release and remained buoyant in the stomach as

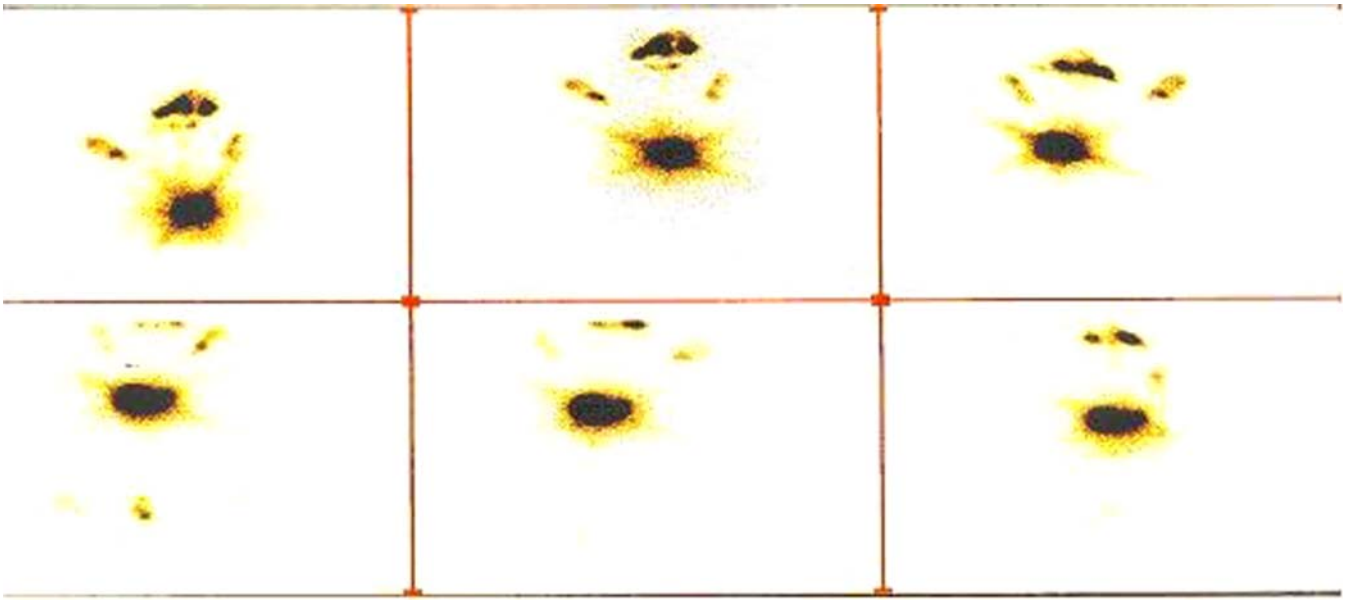

Fig. 4. Whole body gamma images of rabbit after administration of HBS metformin capsule at successive intervals.

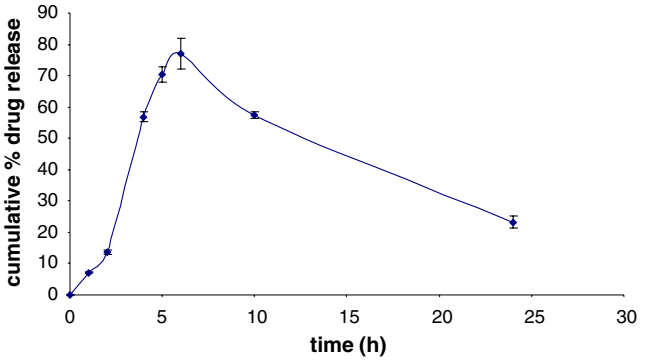

Fig. 5. *In vivo* drug release from optimized HBS capsules of metformin.

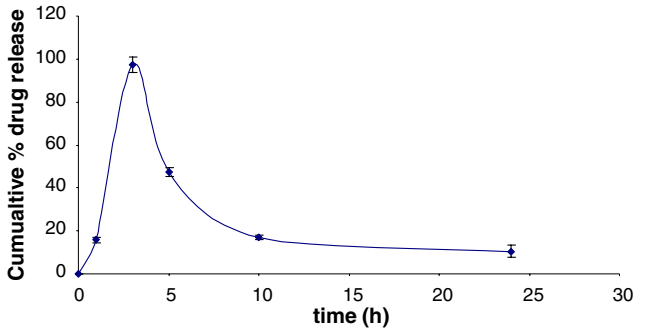

Fig. 6. *In vivo* drug release from immediate release capsules of metformin.

revealed by gamma scintigraphic images and the pharmacokinetic parameters. There was a 136% increase in extent of absorption i.e., AUC between the optimized HBS capsules and the Immediate Release capsules of metformin (Figs. 5 and 6). The absorption rate constant (K_a) was calculated to be 1.109 h^{-1} for the immediate release capsules and 0.389 h^{-1} for the optimized formulation.

Hence, it was concluded that the optimized HBS formulation of metformin could sustain the drug release in addition to remaining buoyant in the stomach as revealed by the gamma scintigraphic images.

4. Discussion

It was concluded on the basis of buoyancy and *in vitro* release kinetics that optimized formulation containing 500 mg of metformin granulated with 5% of ethyl cellulose, and 150 mg of HPMC K4M (extragranular) gave the best *in vitro* release of 97% in 12 h in simulated gastric fluid at pH 3. The release of metformin from the matrix formulation followed zero order release kinetics.

Gamma Scintigraphic studies revealed that the optimized HBS capsule was retained in the gastric region (stomach) for a prolonged period and the study of pharmacokinetic studies showed an increase in AUC as compared to immediate release capsules of metformin.

References

[1] J. Hirtz, The git absorption of drugs in man: a review of current concepts and methods of investigation, Br. J. Clin. Pharmacol. 19 (1985) 77S–83S.
[2] G. Ponchel, J.M. Irache, Specific and non-specific bioadhesive particulate system for oral delivery to the gastrointestinal tract, Adv. Drug Deliv. Rev. 34 (1998) 191–219.
[3] A.A. Deshpande, N.H. Shah, C.T. Rhodes, W. Malick, Development of a novel controlled-release system for gastric retention, Pharm. Res. 14 (1997) 815–819.
[4] B. Rednick, S.J. Tucker, Sustained release bolus for animal husbandry, US patent 3 507 952, April 22 (1970).
[5] R.C. Mamajek, E.S. Moyer, Drug dispensing device and method, US Patent 4, 207, 890 (1980).
[6] J. Urguhart, F. Theeuwes, Drug Delivery System comprising a reservoir containing a plurality of tiny pills, US patent. 4,434,153 (1994).
[7] J.A. Fix, R. Cargill, K. Engle, Controlled gastric emptying III: gastric residence time of a non-disintegrating geometric shape in human volunteers, Pharm. Res. 10 (1993) 1087–1089.
[8] F. Fedzierewicz, P. Thouvenot, J. Lemut, A. Etinine, M. Hoffonan, P. Maincene, Evaluation of peroral silicone dosage forms in humans by gamma-scintigraphy, J. Control Release 58 (1999) 195–205.
[9] R. Groning, G. Heun, Oral dosage forms with controlled gastrointestinal transit, Drug Dev. Ind. Pharm. 10 (1984) 527–539.
[10] R. Groning, G. Heun, Dosage forms with controlled gastrointestinal passage-studies on the absorption of nitrofurantion, Int. J. Pharm. 56 (1989) 111–116.
[11] S. Arora, J. Ali, A. Ahuja, R.K. Khar, S. Baboota, Floating drug delivery systems: an updated review, AAPS PharmSciTech. 6 (2005) E372–E390.
[12] K.S. Soppinath, A.R. Kulkarni, E.R. Walter, M.A. Tejraj, Microspheres as floating drug delivery system to increase the gastric residence of drugs, Drug Metab. Rev. 32 (2001) 149–160.
[13] J. Ali, S. Hasan, M. Ali, Formulation and development of Gastroretentive drug delivery system for ofloxacin, Methods Find. Exp. Clin. Pharmacol. 28 (2006) 1–7.
[14] J. Ali, S. Arora, A. Ahuja, R.K. Khar, A.K. Babbar, R.K. Sharma, formulation and development of floating capsules of celecoxib: *in vitro* and in vivo evaluation, AAPS PharmSciTech. (accepted for publication).
[15] P. Marathe, Y. Wen, J. Norton, D.S. Greene, K.H. Barbhaiya, I.R. Wilding, Effect of altered gastric emptying and gastrointestinal motility on metformin absorption, Br. J. Clin. Pharmacol. 50 (2000) 325–332.
[16] G.L. Chen, W.H. Hao, In vitro performance of floating sustained release capsules of verapamil, Drug Dev. Ind. Pharm. 24 (1998) 1067–1072.
[17] M.V. Shah, M.D. De Gennaro, H. Suryakarma, An evaluation of albumin microcapsules prepared using a multiple emulsion technique, J. Microencapsul. 4 (1987) 223–238.
[18] T. Higuchi, Rate of release of medicaments from ointment bases containing drugs in suspension, J. Pharm. Sci. 50 (1961) 874–875.
[19] A.W. Hixson, J.H. Crowell, Dependence of reaction velocity upon surface and agitation: theoretical considerations, Ind. Eng. Chem. 23 (1931) 923–931.
[20] R.W. Korsmeyer, G.E.P. Deolkar, N.A. Peppas, Mechanisms of potassium chloride from compressed, hydrophilic, polymeric matrices: effect of entrapped air, J. Pharm. Sci. 72 (1983) 1189–1191.
[21] A.K. Babbar, T. Singh, U.P.S. Chauhan, A critical evaluation of radiolabeling of glucaric acid with Tc-99m as a potential myocardial infarct imaging radiopharmaceutical, Ind. J. Nucl. Med. 11 (1996) 98–103.