Garud Navneet et al. / Journal of Pharmacy Research 2011,4(7),

Research Article
ISSN: 0974-6943

Available online through
www.jpronline.info

Formulation design and *in-vitro* evaluation of metformin microspheres using ionotropic gelation technique

Garud Navneet[1*], Garud Akanksha[1], Jain Neetesh[2]
Institute of Professional Studies, College of Pharmacy, Gwalior (M.P.)
Pranav Institute of Pharmaceutical Education and Research, Gwalior (M.P.)

Received on: 12-04-2011; Revised on: 18-05-2011; Accepted on:21-06-2011

ABSTRACT

Metformin hydrochloride microspheres were prepared by ionotropic gelation method using non-ionic (ethylcellulose and HPMC), anionic (Carbopol 934P) and cationic (chitosan) polymers, respectively. The effect of varying calcium chloride concentration and drug-polymer ratios was studied. The prepared microspheres were studied for their morphology, average particle size, % drug content, % drug entrapment, % yield and micromeritic properties. Ethylcellulose microspheres showed least swelling and mucoadhesion property in both simulated gastric pH and simulated intestinal pH medium depicting its non-mucoadhesive nature. The microspheres prepared at 10% calcium chloride concentration and 1:3 drug:polymer ratio showed the most sustained effect.

Key words: Metformin, microspheres, polymers, drug release

INTRODUCTION

Controlled release multiple unit dosage forms have advantages over single unit ones as they can spread out in a more uniform manner over a large surface area in the gastrointestinal tract [1]. This can reduce local irritation of the gastrointestinal tract by some drugs and can provide a large area for absorption. Microspheres form an important part of novel drug delivery system which can precisely control release rates and target drugs to a specific body sites. Bioadhesion is a topic of interest in the design of drug delivery systems to prolong the residence time of the dosage form at the site of application or absorption and thereby facilitating intimate contact of the dosage form thus improving and enhancing bioavailability. [2]

Metformin is an effective antidiabetic drug, belonging to biguanide class particularly in Type II diabetes (Non-insulin dependent diabetes mellitus) [3]. It has half-life of 4-5 hr and is generally given in 500 mg dose 2-3 times a day. To reduce the dosing frequency and adverse effect (mainly gastrointestinal irritation) it is needed to formulate in long acting dosage forms. The purpose of present work was therefore to design, characterize and evaluate the microspheres of metformin by employing various polymers viz. ethylcellulose (non-ionic), hydroxypropylmethyl cellulose (HPMC) [non-ionic], carbopol 934P (anionic) and chitosan (cationic).

MATERIALS AND METHODS

Materials

Metformin hydrochloride was a gift sample from Sun Pharmaceuticals, Baroda. Sodium alginate (CDH, New Delhi), Calcium chloride (Qualigens, Mumbai), Ethylcellulose (EC) was commercially obtained from S.D. Fine Chemicals, Mumbai. Hydroxypropylmethylcellulose (HPMC-E15) and carbopol-934P were procured from Central Drug House, Mumbai. Chitosan (medium viscosity grade) was obtained from Central Institute of Fisheries Technology, Cochin and glutaraldehyde from Spectrochem Pvt. Ltd (Mumbai). All other reagents used in experiment were of analytical grade and purchased from their respective commercial sources.

Methods

Preparation of Microspheres

Metformin microspheres were prepared by ionotropic gelation method[4,5,6] employing sodium alginate in combination with various polymers using EC, HPMC, carbopol 934P and chitosan cross-linked with glutaraldehyde in different ratios (1:1, 1:2, 1:3 and 3:1). Sodium alginate (1.0 g) was dissolved in purified water (50 ml) to form a homogeneous polymer solution with 1.0 g of each polymer. The drug, metformin (2.0 g) was added to the polymer solution and the resulting dispersion was added manually dropwise into concentrations of varying calcium chloride solution (about 40 ml) through a syringe with a needle size no. 18. The added droplets were retained in the calcium chloride solution for 30 min to complete the curing reaction. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with water and dried at 45° C for 12 hrs.

For chitosan polymer, the microspheres were prepared by using 40 ml of 2.5% w/v chitosan-drug solution, to which varying concentration of calcium chloride was added and the solution was stirred continuously at 1000 rpm for 10 min. Glutaraldehyde (5% solution), a cross-linking agent was added dropwise while stirring at the same speed. The dried microspheres were sieved and kept in desiccator until used for further studies.

FT-IR Studies

FTIR study of Metformin and polymers was carried out to find out any possible interaction between the drug and the polymers used in the formulations[7]. FTIR spectra of blank and drug-polymer loaded microspheres were obtained in KBr pellets using a Perkin-Elmer model spectrum BX-FTIR spectrometer in the ranges, 4000- 400 cm^{-1}.

Morphology and particle size

The shape and surface characteristics were studied using scanning electron microscope (SEM, LIO - 430). The size distribution in terms of average diameter of the microspheres was determined using optical microscopy method[8].

Micromeritic Properties of Microspheres

The microspheres were characterized by their micromeritic properties such as particle size, tapped density, Carr's index, and flow property. [8,9]

Drug loading

50 mg of microspheres were treated with 50 ml. of phosphate buffer (pH 6.8), in 100 ml. amber coloured vial with stirring at 250 rpm. The temperature was maintained at 37 ± 0.2° C. At the end of two hours it was filtered, and the filtrate was analyzed photometrically at 232 nm using U.V. Visible spectrophotometer (Shimadzu, Pharmspec UV-1700 series, Japan). Drug loading efficiency[10] was calculated as:

Drug entrapment (%) = (Actual drug concentration/Theoretical drug concentration) × 100 Drug loading (%) = (Weight of drug / Weight of microspheres) × 100

Yield (%) = (Weight of microspheres/ Total expected weight of drug and polymer) × 100

Swelling behaviour

About 200 mg of dry microspheres were placed in dissolution solution for at least 10 h. The wet weight of the swollen microspheres was determined by first

***Corresponding author.**
Navneet Garud
M-213, Madhav Nagar
Gwalior-474002
Tel.: *+91- 9425117028*
E-mail:*navneetgarud@gmail.com*

blotting the microspheres with filter paper to remove surface water and then weighing them immediately on an electronic balance. The % swelling of the prepared microspheres was calculated using following formula[11]:

S = (We – Wo) / Wo x 100 %

We = weight of the gel microspheres at equilibrium swelling,
Wo = initial weight of the microspheres.

In-vitro Wash-Off Studies[12]

Freshly excised pieces of intestinal mucosa (5 x 2 cm) from sheep were mounted onto glass slides (3 x 1 inch) with adhesive material. About 50 microspheres were spread onto each wet rinsed tissue specimen, and immediately thereafter the support was hung onto the arm of a U.S.P. tablet disintegrating test machine. By operating the disintegrating test machine the tissue specimen was given a slow, regular up and down movement in the test fluid at 37ºC contained in a 1 l vessel of the machine. At the end of one hr, and at hourly intervals up to 12 hrs, the machine was stopped and the number of microspheres still adhering to the tissue was counted. The test was performed at both simulated gastric fluid (0.1 N HCl, pH 1.2) and simulated intestinal fluid (phosphate buffer, pH 6.8). The mucoadhesiveness of the microspheres were compared with microspheres of a nonbioadhesive material, ethylene vinyl acetate (EVA).

In-vitro drug release of microspheres[13]

The in-vitro drug release profiles from various formulations of microspheres were studied in 900 ml of buffer with gastrointestinal simulated pH conditions, viz. simulated gastric fluid (0.1N HCl, pH 1.2) for first 2 hours followed by next 4 hours in simulated intestinal fluid (phosphate buffer solution, PBS, pH 6.8) and finally up to 15 h in simulated intestinal fluid (phosphate buffer solution, PBS, pH 7.4). Withdrawn sample was filtered and estimated for metformin concentration at 232 nm using Shimadzu, Pharmspec UV-1700 series, Japan.

RESULTS AND DISCUSSION

FT-IR Studies

The FTIR spectrum of pure metformin hydrochloride showed two typical bands at 3372 and 3296 cm^{-1} relative to the N-H primary stretching vibration and a band at 3174 cm^{-1} due to the N-H secondary stretching, and the characteristic bands at 1627 and 1568 cm^{-1} assigned to C=N stretching. The spectra obtained from the drug-polymer combination presented band assignments at the same wavelength ranges depicting no interaction between the drug and polymer combinations (Data not shown).

Physical characterization of microspheres

The microspheres were found to be discrete, spherical, free-flowing, and of the monolithic matrix type. The effects of alginate concentrations and polymer ratios on the average particle size, % drug content, % drug entrapment and % yield of microspheres are shown in Table 1 and 2. On the basis of surface morphology and percentage drug entrapment of the microspheres, the 10% calcium concentration was taken as optimum for further study and evaluation purpose. Increase of mean particle size with increase in polymer concentration may have occurred due to the fact that as polymer concentration increases it produces a significant increase in the viscosity, leading to an increase of the emulsion droplet size and finally a higher microsphere size[14].

Table 1-Characteristics of Prepared Microspheres using Ionic gelation method

Batches	Coat Composition	% Drug Content	% Entrapment Efficiency	% Yield
A1	Alginate:ethylcellulose (1:1)	26.3 ± 0.55	54.4 ± 6.12	70.86
A2	Alginate:ethylcellulose (1:2)	25.1 ± 0.64	63.2 ± 4.28	69.56
A3	Alginate:ethylcellulose (1:3)	24.5 ± 0.54	65.8 ± 3.36	64.45
A4	Alginate:ethylcellulose (3:1)	26.5 ± 0.46	66.3 ± 2.42	62.3
B1	Alginate:HPMC (1:1)	33.2 ± 0.66	62.1 ± 2.78	74.6
B2	Alginate:HPMC (1:2)	31.3 ± 0.39	66.2 ± 4.79	71.2
B3	Alginate:HPMC (1:3)	29.2 ± 0.55	68.2 ± 3.89	63.7
B4	Alginate:HPMC (3:1)	35.3 ± 0.49	68.9 ± 4.24	58.7
C1	Alginate:carbopol934P (1:1)	36.1 ± 0.62	64.5 ± 3.86	75.3
C2	Alginate:carbopol934P (1:2)	34.5 ± 0.52	70.3 ± 6.01	73.7
C3	Alginate:carbopol934P (1:3)	32.2 ± 0.24	75.3 ± 5.32	71.1
C4	Alginate:carbopol934P (3:1)	36.8 ± 0.32	78.8 ± 4.79	65.7
D1	Alginate:chitosan (1:1)	35.3 ± 0.49	68.3 ± 3.25	69.2
D2	Alginate:chitosan (1:2)	33.6 ± 0.55	72.5 ± 4.79	65.4
D3	Alginate:chitosan (1:3)	32.3 ± 0.39	76.2 ± 5.13	62.9
D4	Alginate:chitosan (3:1)	36.2 ± 0.48	78.4 ± 5.14	59.3

Values are given as Mean ± S.D.

Table 2-Micromeritic study

Batches	Average particle size (µm)	Tapped density (g/cm^3)	Bulk density (g/cm^3)	Carr's index	Hausner's ratio	Angle of repose (é)
A1	463.0 ± 17.3	0.766	0.658	14.1	1.16	40°18´
A2	469.0 ± 11.5	0.745	0.653	12.3	1.14	39°16´
A3	480.0 ± 9.8	0.736	0.639	13.2	1.15	38°21´
A4	484.0 ± 8.6	0.738	0.625	15.3	1.18	39°78´
B1	460.5 ± 4.85	0.783	0.691	11.7	1.13	42°28´
B2	483.0 ± 11.2	0.771	0.689	10.6	1.12	41°16´
B3	498.0 ± 8.4	0.762	0.667	12.5	1.14	40°21´
B4	503.0 ± 11.5	0.754	0.661	12.3	1.14	40°78´
C1	530 .0 ± 8.6	0.683	0.594	13.0	1.15	41°28´
C2	541.0 ± 15.2	0.671	0.586	12.7	1.14	39°26´
C3	563.0 ± 8.8	0.662	0.553	16.4	1.19	39°21´
C4	602.4 ± 3.6	0.654	0.566	13.4	1.15	39°38´
D1	515.0 ± 8.6	0.716	0.612	14.5	1.17	44°18´
D2	523.0 ± 9.3	0.689	0.581	15.6	1.18	40°28´
D3	529.0 ± 7.8	0.675	0.596	11.7	1.13	39°72´
D4	548.0 ± 8.2	0.664	0.573	13.7	1.16	40°16´

Values are given as Mean ± S.D.

The size of the microspheres increased with increase in the alginate concentration which may be due to the increase in viscosity, resulting in increase in droplet size during addition of the polymer dispersion to the harvesting medium[6]. The polymer surface of the microcapsules as observed by SEM; was heterogeneous and porous (Figure 1). These findings were similar with those of other researchers[15] who reported rough surface morphologies for chitosan microspheres prepared by the ionotropic gelation technique. The surface morphology characteristics have an impact on bioadhesion. It has been earlier reported that microspheres with a coarser and more porous surface may offer enhanced bioadhesivity as compared to those with a smooth texture[16]. The surface morphologies of whole microspheres after exposure to the dissolution medium are shown in Figure 2. Formation of "cracks"/channels on the surface of the microspheres were observed which may be due to the penetration of the dissolution medium into the microspheres and the subsequent dissolution of the drug and hence its diffusion through the polymer matrix.

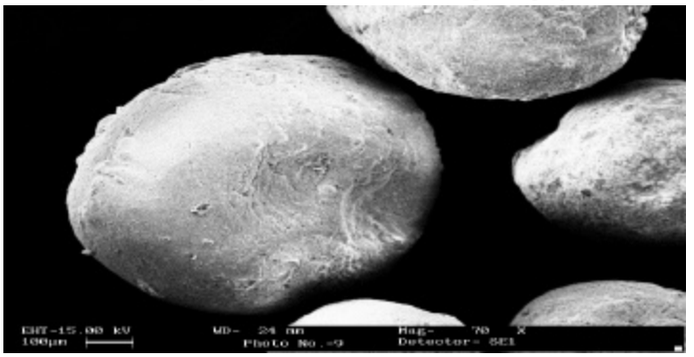

***Figure 1**: Surface morphology of metformin microspheres using chitosan polymer by ionotropic gelation method*

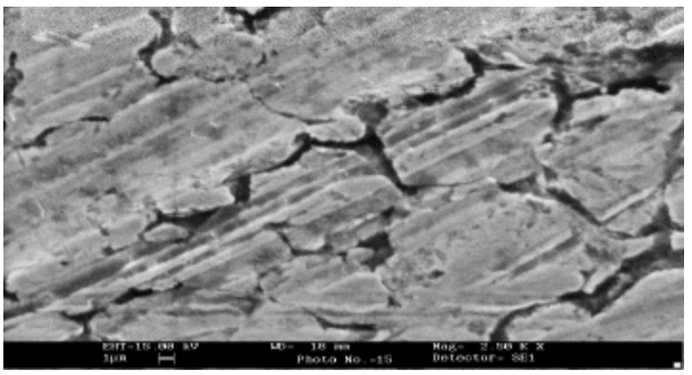

***Figure 2:** Surface morphology of metformin microspheres using hydroxy propyl methyl cellulose polymer by ionotropic gelation method at 8 h post dissolution.*

Micromeritic properties of microspheres

The rheological parameters like angle of repose, tapped density, bulk density and packing properties (Table-3) confirms better flow and packing properties of the prepared microspheres. All the formulation showed angle of repose value within the range of 30° to 45° (n=3), which is an appreciable limit for microspheres to show flow property while formulating in the dosage form.

Table 3- Results of in-vitro wash-off test to assess mucoadhesive property of the prepared microspheres

Batches	Percent of microspheres adhering to tissue after 12 h duration											
	0.1 N HCl, pH 1.2						Phosphate buffer, pH 6.8					
	1	2	4	6	8	12	1	2	4	6	8	12
A1	62±2.5	51±0.6	33±1.1	20±2.2	-	-	-	-	-	-	-	-
A2	74±1.2	64±0.8	37±1.5	18±2.5	-	-	-	-	-	-	-	-
A3	82±0.4	51±0.61	11±0.57	-	-	-	-	-	-	-	-	-
A4	86±0.8	71±0.5	48±1.2	25±0.7	-	-	78±0.2	64±1.1	37±0.8	08±1.3	-	-
B1	78±0.5	75±0.6	70±0.8	66±1.2	58±1.4	52±1.8	70±0.5	48±2.2	34±2.1	21±0.9	-	-
B2	82±2.1	78±1.1	73±0.6	68±0.4	70±1.1	64±1.6	72±1.7	55±1.5	46±1.2	26±0.5	03±0.2	-
B3	85±2.0	82±2.2	78±1.8	76±1.2	74±0.8	70±1.5	76±1.8	62±2.1	55±1.2	38±1.7	16±0.8	05±1.7
B4	82±1.2	71±0.8	66±0.4	38±1.1	28±0.4	18±1.2	70±1.2	58±1.7	32±2.1	18±1.5	06±0.9	-
C1	80±2.2	76±1.1	70±1.4	60±2.2	55±1.1	49±0.6	72±1.4	58±0.8	62±1.1	51±2.1	32±2.1	08±2.5
C2	84±1.8	79±1.4	74±1.5	62±0.8	67±0.6	62±1.2	78±1.8	72±1.4	68±1.5	55±1.2	41±2.2	14±1.7
C3	88±1.5	85±1.0	81±2.1	77±1.5	72±1.0	68±1.2	82±2.0	76±0.8	72±1.1	64±0.8	53±1.5	21±1.8
C4	75±2.1	62±0.8	56±0.5	42±0.6	35±1.4	20±1.2	72±1.2	57±2.1	48±1.4	37±0.8	22±1.7	11±2.1
D1	58±2.1	46±2.2	38±1.8	25±0.8	19±1.1	11±0.4	74±1.5	53±0.8	46±1.1	30±2.1	19±2.1	-
D2	67±1.5	40±1.2	32±0.4	28±0.6	22±2.2	18±0.8	78±1.2	66±1.4	51±1.5	37±1.2	28±2.2	07±1.1
D3	84±1.8	76±1.1	56±1.0	42±0.8	28±2.1	20±1.1	82±2.5	74±0.8	65±1.1	44±0.8	31±1.5	18±1.5
D4	78±2.1	66±1.4	48±0.8	35±0.5	15±1.2	-	72±1.7	57±2.1	48±1.4	32±0.8	18±1.7	-
EVA	56±1.5	42±1.2	10±1.6	-	-	-	54±2.0	38±2.1	08±2.5	-	-	-

EVA indicates ethylene vinyl acetate (non-mucoadhesive control), Values are given as Mean ± S.D.

Swelling property of microspheres

The high swelling property of carbopol 934P and chitosan microspheres could be attributed to their ionized ability to uncoil the polymer into an extended structure. Higher swelling of carbopol 934P microspheres than chitosan microspheres at pH 6.8 was likely due to its higher molecular weight. In an acidic environment, the amine groups on chitosan molecules become protonated and consequently the molecules become densely positively charged. The high concentration of like charges causes molecular repulsion and thereby swelling of a chitosan matrix[17]. Low percentage swelling of HPMC and ethyl cellulose microspheres at acidic pH can be attributed to their non-ionic nature. The study of ethyl cellulose polymer revealed no volume expansion at all in the medium due to its hydrophobic nature. Slight swelling of the ethyl cellulose microspheres may be due to the alginate content of the microspheres (Figure 3 and 4).

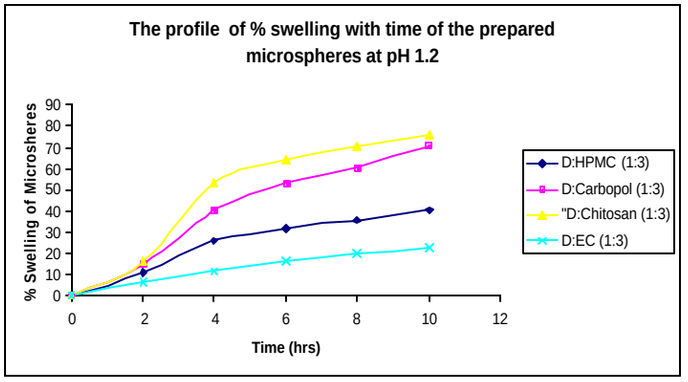

Figure 3: % Swelling vs time of the prepared microspheres at pH 1.2

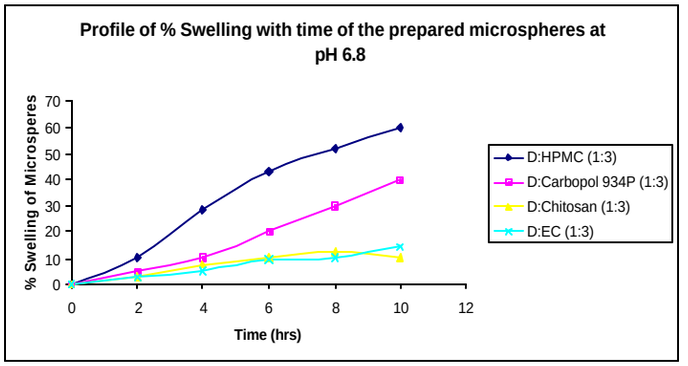

Figure 4: % Swelling vs time of the prepared microspheres at pH 6.8

In-vitro Wash-Off Studies

The mucoadhesion test was performed on both simulated gastric pH (0.1N HCl, pH 1.2) and simulated intestinal pH (phosphate buffer solution, PBS, pH 6.8) for 12h. The wash-off was found to be faster at intestinal pH than at gastric pH in case of Carbopol 934P and HPMC polymers as compared to cross linked chitosan and ethyl cellulose polymers. Ch'ng et al[18] observed that the pH of the medium was critical for the degree of hydration, swelling and mucoadhesion of the polymers. The rapid wash-off observed at simulated intestinal pH 6.8 is due to ionization of carboxyl acid group and other functional groups in the polymers at this pH, which increases their solubility and reduces adhesive strength. The results indicated that with increase in ethyl cellulose concentration, there is a constant decrease in mucoadhesion when compared to EVA, indicating the non-mucoadhesive nature of ethyl cellulose. Slight increase in mucoadhesion time might be due to an increase in alginate concentration (Formulation A4; Table-3). Microspheres with a coat of mucoadhesive polymer alone (except ethyl cellulose, which showed non-mucoadhesive property) could not be prepared by this process due to their water-soluble nature.

The adhesion time of microspheres at pH 1.2 was ranked, HPMC > carbopol 934P > chitosan > ethyl cellulose, whereas the adhesion time for the microspheres at pH 6.8 was ranked, carbopol 934P > chitosan > HPMC > ethyl cellulose. Poor mucoadhesion of HPMC microspheres at alkaline pH may be due to its non-ionic nature possessing low hydrogen bonding capability with mucus glycoproteins[19], while excellent mucoadhesion of chitosan microspheres was from the electrostatic attraction between chitosan (cationic in nature) and mucin (anionic in nature). Although carbopol 934P microspheres had negative charge repulsion with mucus, numerous hydrophilic functional groups such as carboxyl groups in carbopol molecules could form hydrogen bonds with mucus molecules, thus producing some adhesive force of this polymer.

In-vitro drug release of microspheres

It was observed that the release of drug, metformin hydrochloride varies as the pH was changed from acidic to alkaline medium. The drug in control microspheres (without any polymer) was rapidly dissolved and released within 30 minutes. This was because metformin hydrochloride (pK$_a$ 9.5) could ionize and completely dissolve in this medium. Alderman[20] reported that the release kinetics of water-soluble drugs is mainly governed by diffusion from matrix system. The release of drug was prolonged when incorporated within mucoadhesive polymers. Higher amount of drug; metformin hydrochloride was released in acidic pH in case of ethyl cellulose and chitosan polymers while the release of drug gradually decreased in case of HPMC and carbopol 934P polymers. At 5% calcium chloride concentration, the microspheres formed were not properly hardened leading to fast release of the drug and at 15%, pores might have developed in the surface leading to faster dissolution and thereby greater drug release. A comparative release profile for 1:3 alginate: polymer concentration (Figure 5) showed that the formulation containing alginate: carbopol 934P (1:3) gave the most sustained effect.

The experimental design supported product development and optimization procedure yielded the desired microspheres by ionotropic gelation technique which gave sustained drug release up to 15 h duration for prolonged slow release. The optimized multi-unit metformin hydrochloride microsphere delivery system is expected to provide clinicians with a new choice of an economical, safe and more bioavailable formulation in the management of type II diabetes mellitus.

Statistical analysis

Experimental results were performed in triplicate and expressed as mean±S.D. One-way analysis of variance (ANOVA) was applied to check significant

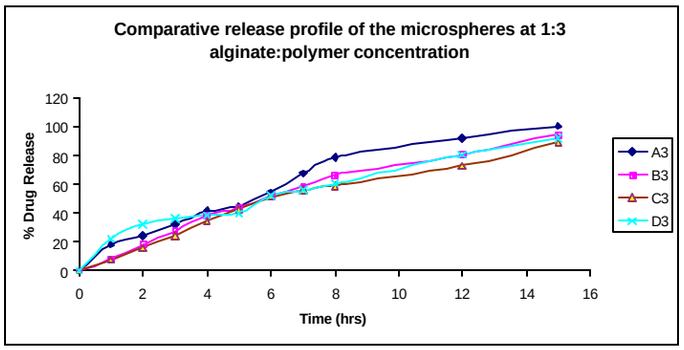

Figure 5: Comparative release profile of the microspheres at 1:3 alginate:polymer concentration

difference in drug release from different formulations. Differences were considered to be significant at $P<0.05$.

ACKNOWLEDGEMENT

Authors are thankful to Sun Pharmaceuticals, Baroda for providing gift sample of the drug Metformin hydrochloride.

REFERENCES

1. Lee JW, Park JH, Robinson JR, Bioadhesive-based dosage forms: the next generation, J Pharm Sci, 2000, 89:850-866
2. Nagda CD, Chotai NP, Patel SB, Soni TJ, Patel UL, Preparation and in vitro evaluation of bioadhesive microparticulate system, International J Pharm Sci and Nanotechnology, 2008, 1(3):257-266
3. Patel A, Ray S, Thakur RS, In vitro evaluation and optimization of controlled release floating drug delivery system of Metformin hydrochloride, DARU 2006;14(2):57-64
4. Khazaeli P, Pardakhty A, Hassanzadeh F, Formulation of ibuprofen beads by ionotropic gelation, Iranian J Pharm Res, 2008, 7(3):163-170
5. Dandagi PM, Manvi FV, Gadad AP, Mastiholimath VS, Patil MP, Balamuralidhar, Microencapsulation of verapamil hydrochloride by ionotropic gelation technique, Indian J Pharm Sci, 2004, 66(5):631-635
6. Das MK, Maurya DP, Evaluation of diltiazem hydrochloride-loaded mucoadhesive microspheres prepared by emulsification-internal gelation technique, Acta Poloniae Pharmaceutica- Drug Research, 2008, 65(2):249-259
7. Kumar RP, Shankar NB. Formulation design, preparation of losartan potassium microspheres by solvent evaporation method and its in vitro characterization. Arch Pharm Sci and Res. 2009;1(1):166-170
8. Lachman L, Lieberman HA, Kanig JL, The theory and practice of industrial pharmacy,1991, 4th Edition, Varghese publishing house, Darar, Bombay:183-184
9. Martin A, Bustamante P, Chun AHC, Physical Pharmacy: Physical chemical principles in the pharmaceutical sciences, 2005, 4th Edition, B.I. Publications Pvt. Ltd.:423-448
10. Sengel CT, Hascicek C, Gonul N, Development and in-vitro evaluation of modified release tablets including ethylcellulose microspheres loaded with diltiazem hydrochloride. J. Microencapsulation 2006;23(2):135-152
11. Shankar NB, Kumar NU, Balakrishna PK, Formulation design, preparation and in vitro evaluation of Mucoadhesive microcapsule employing control release polymers to enhance gastroretention for oral delivery of Famotidine, Int J Pharm Sci Tech,2009,2(1):22-29
12. Chowdary KPR, Rao YS, Mucoadhesive microcapsules of glipizide: characterization, in vitro and in vivo evaluation, Indian J Pharm Sci, 2003, 65(3):279-284
13. Nayak BS, Ghosh SK, Patro KTB, Preparation and characterization of famotidine microcapsule employing Mucoadhesive polymers in combination to enhance gastroretention for oral delivery, IJPPS, 2009,1(2):112-120
14. Behera BC, Sahoo SK, Dhal S, Barik BB, Gupta BK. Characterization of glipizide-loaded polymethacrylate microspheres prepared by an emulsion solvent evaporation method, Tropical Journal of Pharmaceutical Research 2008;7(1):879-885
15. Govender S, Pillay V, Chetty DJ, Essack SY, Dangor CM, Govender T, Optimisation and characterization of bioadhesive controlled release tetracycline microsphere, Int. J. Pharm.,2005, 306(1-2):24-40
16. Vasir JK, Tambwekar K, Garg S, Bioadhesive microspheres as a controlled drug delivery system, Int. J. Pharm., 2003, 255:13-32
17. Miller DA, Fukuda M, McGinity JW, The properties of chitosan as a retardant binder in matrix tablets for sustained drug release, Drug Delivery Technology, 2006, 6(9):44-45
18. Ch'ng HS, Park H, Kelly P, Robinson JR, For oral controlled delivery, II: synthesis and evaluation of some swelling, water-insoluble bioadhesive polymers, J.Pharm. Sci., 1985, 74:399-405
19. El-Hameed MDA, Kellaway IW, Preparation and in vitro characterization of mucoadhesive polymeric microspheres as intra-nasal delivery systems, European Journal of Pharmaceutics and Biopharmaceutics, 1997, 44:53-60
20. Alderman DA, A review of cellulose ethers in hydrophilic matrices for controlled release dosage forms, Int. J. Pharm. Tech. Prod. Mfr., 1984, 5(3):1-9

Source of support: Nil, Conflict of interest: None Declared