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Research Article

Preparation and *In-vitro* Evaluation of Metformin Microspheres Using Non-Aqueous Solvent Evaporation Technique

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Abstract

***Purpose**: To prepare and evaluate metformin microspheres for prolonged release.*
***Methods:** Metformin microspheres were prepared by non-aqueous solvent evaporation method using various polymers, including ethylcellulose (EC), hydroxypropyl methylcellulose (HPMC), carbopol 934P (CA) and chitosan (CH). The effect of process variables, viz, drug/polymer ratio, stirring rate and type of polymer on the mean particle size, drug entrapment efficiency, yield, drug content, micromeritic properties and drug release of the microspheres were studied.*
***Results:** It was observed that as the stirring speed increased from 600 to 1800 rpm, microsphere size decreased and hence drug release rate increased. Drug release rate at 1:2 drug: polymer for microspheres produced at a stirring rate of 1200 rpm was in the following order: carbopol 934P > HPMC > ethyl cellulose > chitosan. The formulations containing carbopol 934P (CA3) and HPMC (HPMC3) released drug faster than chitosan microspheres (CH3).*
***Conclusion:** Amongst the developed microspheres, CH3 formulation (with chitosan as the polymer) exhibited maximum prolonged drug release at gastrointestinal pH or at least 15 h. This oral sustained metformin formulation could potentially improve the bioavailability of the drug as well as patient compliance.*

***Keywords:** Metformin, Microspheres, Prolonged release, Solvent evaporation, Ethylcellulose, Hydroxypropyl methylcellulose, Chitosan*

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INTRODUCTION

Microspheres have gained wide acceptance as a means to achieve oral and parenteral controlled drug delivery systems. They often require a polymer as carrier as well as core material [1]. Among the various methods developed for the formulation of microspheres, the solvent evaporation method has gained much attention due to its ease of fabrication without compromising the activity of the drug.

Ethyl cellulose, a non-ionic, inert hydrophobic, non-biodegradable and biocompatible polymer with minimal toxicity. It is one of the extensively studied encapsulating materials for the controlled release of pharmaceuticals [2]. HPMC is a non-ionic, swellable polymer. Hydrophilic polymer gel matrix systems are widely used in controlled drug delivery to obtain a desirable drug release profile and cost effectiveness because of their flexibility. The hydration rate of HPMC increases with increase in the hydroxypropyl content. Carbopol is a polymer consisting of acrylic acid cross-linked with either polyalkenyl ether or divinyl glycol. It readily absorbs water, gets hydrated and swell. In addition to its hydrophilic nature, cross-linked structure and insolubility in water, carbopol is an anionic polymer, and hence a potential candidate for use in controlled release drug delivery [3].

The use of natural polymers is preferred due to their proven biocompatibility and safety. In this respect, chitosan, a cationic polymer, has attracted particular attention. It is biocompatible, biodegradable and bioadhesive at physiological pH and possesses OH and NH_2 groups that can give rise to hydrogen bonding. It is a high molecular weight polysaccharide, comprising of glucosamine and N-acetyl glucosamine obtained by deacetylation of chitin [4]. Because of its low production cost, non-toxic nature, and FDA approval, chitosan has found application in multiparticulate drug delivery [5].

Diabetes mellitus (DM) is the name given to a group of disorders characterized by chronic hyperglycemia, polyurea, polydipsia, polyphagia, emaciation and weakness due to disturbance in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion and/or insulin action. A drug of choice, either alone or in combination with other hypoglycemic agents, particularly in Type II diabetes, metformin, is an effective antidiabetic drug, belonging to the biguanide class [6].

EXPERIMENTAL

Materials

Metformin hydrochloride was received as a gift from Sun Pharmaceuticals, Baroda, India. Acetone and liquid paraffin (Merck Ltd, Mumbai, India), ethylcellulose (EC, S.D. Fine Chemicals, Mumbai, India), hydroxypropyl methylcellulose (HPMC-E15) and carbopol-934P (CA) (Central Drug House, Mumbai, India), chitosan (CH, medium viscosity grade, Central Institute of Fisheries Technology, Cochin, India. All other chemicals and deionised water used were of analytical grade.

Preparation of microspheres

The microspheres containing the anti-diabetic drug, metformin, as the core material were prepared by a non-aqueous solvent evaporation method [7]. Here, the drug (250 mg) and the polymers (250, 500, or 750 mg) were mixed in acetone (8 ml) at various ratios. The slurry was introduced into 30 ml of liquid paraffin while stirring (600, 1200 or 1800 rpm) with a mechanical stirrer equipped with a three-blade propeller at room temperature. The solution was stirred for 4 h to allow the solvent to evaporate and the microspheres were collected by filtration by Whatman filter paper no. 1. The microspheres were washed repeatedly with petroleum ether (40 - 60 °C) until free from oil. The microspheres were collected and dried for 3 h at room temperature and

subsequently stored in a desiccator over fused calcium chloride.

The effect of process variables including drug/polymer ratio, stirring rate and polymer type on mean microsphere size, drug entrapment efficiency, yield, drug content, micromeritic properties and drug release was studied. Metformin microspheres were prepared in varying drug/polymer ratio (see Table 1) while keeping stirring speed (1200 rpm) constant. Similarly, microspheres were prepared at various stirring rates (i.e., 600, 1200 and 1800 rpm) while keeping drug/polymer ratio constant at 1:2.

Evaluation of micromeritic properties of microspheres

The microspheres were characterized for their micromeritic properties - angle of repose, bulk density, tapped density, Carr's index, and Hausner's ratio [7,8]. Angle of repose (θ) of different formulations was measured according to the fixed funnel standing method (n = 3) and calculated using Eq 1.

$\theta = \tan^{-1} h/r$ …… (1)

where *r* is the radius of the cone base, and *h* the height of the base.

Bulk and tapped densities were measured using a 10 ml graduated cylinder. The sample placed in the cylinder and the volume (bulk) noted. The cylinder was then,tapped 100 times and the volume (tapped) again noted. The bulk and tapped densities were calculated were calculated from the ratio of the weight and volume as appropriate. Compressibility (Carr's) index (Ci) of microparticles was computed according to Eq 2.

Ci = (Dt – Db)/ Dt × 100 ……..... (2)

where Dt is tapped density and Db the bulk density.

Hausner's ratio of the microparticles was determined as in Eq 3.

Hausner's ratio = Dt/Db …… (3)

Morphology and particle size

The shape and surface characteristics of the microspheres were studied using scanning electron microscope (SEM, LEO – 430, Leo Electron Microscopy Ltd., Cambridge, England). The microparticles were coated uniformly with gold-palladium by using a sputter coater (Polaron SC-76430) after fixing the sample in individual stubs. The operating parameters were an acceleration voltage of 20kV and chamber pressure of 0.6 mm Hg (n = 3). Size distribution based on mean diameter of the microspheres was determined by optical microscopy method [8,9]. Approximately, 100 microspheres were counted using a calibrated optical microscope (Magnus MLX-DX) and mean particle size was calculated.

Evaluation of entrapment efficiency, drug loading and yield

Microspheres (50 mg) 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 ºC. At the end of 2 h, it was filtered and the filtrate analyzed spectrophotometrically (n = 3) at 232 nm (Shimadzu, Pharmspec UV-1700 series, Japan). Drug entrapment efficiency, drug loading and yield were were calculated using Eqs 4 - 6 [10].

Drug entrapment (%) = (A/T)100 ….. (4)

where A is actual drug concentration and T is the theoretical drug concentration.

Drug loading (%) = (Wd/Wm)100 …… (5)

where Wd is the weight of drug and Wm the weight of the microspheres**.**

Yield (%) = (Wm/Wdp)100 ……... (6)

where Wdp is the expected total weight of drug and polymer.

In-vitro drug release studies

The *in-vitro* drug release profiles of the various microsphere formulations were studied in 900 ml of buffer in simulated gastrointestinal pH conditions, viz, simulated gastric fluid (0.1M HCl, pH 1.2) for the first 2 h followed by 4 h in simulated intestinal fluid (phosphate buffer solution, PBS, pH 6.8) and finally for 9 h in simulated intestinal fluid (phosphate buffer solution, PBS, pH 7.4) [11]. Samples (5 ml) were withdrawn at various time intervals, and replaced with the same volume of test medium to maintain sink conditions. The withdrawn samples were diluted, where necessary, filtered through a 0.45µ membrane filter and analyzed for metformin content at 232 nm using Shimadzu spectrophotometer (Pharmspec UV-1700 series, Japan).

Statistical analysis

Experimental results (n = 3) were expressed as mean ± S.D. One-way ANOVA was performed using Sigma Stat software (SPSS Inc, USA) for Windows, version 2.30. Differences were considered significant at $p < 0.05$.

RESULTS

Particle characteristics

Scanning electron microphotographs showed that the microspheres were spherical with a smooth to rough surface (Fig 1). Pores were observed on the microsphere surface. Increasing polymer concentrations (i.e., drug-polymer ratio from 1:1 to 1:3) increased microsphere size from 352.1 ± 7.5 to 374.2 ± 7.8 µm for ethylcellulose, 359.3 ± 4.1 to 388.4 ± 8.8 µm for HPMC, 424.5 ± 8.6 to 450.3 ± 8.8 µm for carbopol 934P, and 408.6 ± 10.6 to 426.8±8.2 µm for chitosan. However, in all cases, the increase was not significant ($p > 0.05$).

Increase in stirring rate from 600 rpm to 1800 rpm also seemed to have decreased microsphere size but the decrease was not significant ($p > 0.05$).

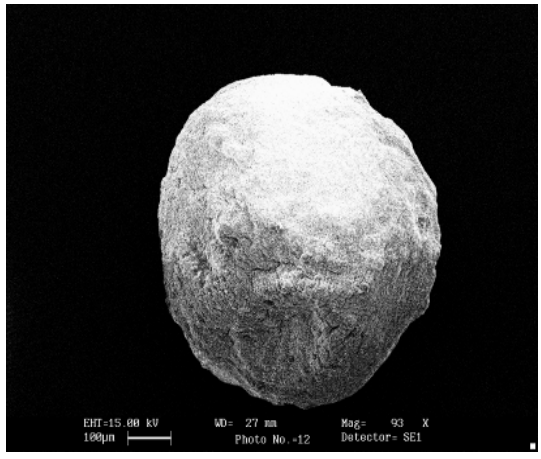

Fig 1: Scanning electron microphotographs (SEM) of metformin microspheres prepared with HPMC using solvent evaporation method

Microsphere yield was > 56.8 % for all the formulations while entrapment efficiency (%) ranged 56.4 ± 2.4 to 85.4 ± 3.4 for EC microspheres, 68.1 ± 4.2 to 85.5 ± 3.9 for HPMC microspheres, 64.1 ±4.8 to 85.2 ± 5.3 for CA microspheres and 61.2 ± 3.3 to 83.5 ± 5.1 for CH microspheres (Table 1).

Micromeritic properties of microspheres

All the formulations showed angle of repose in the range of 25° to 34°. Compressibility, i.e., Carr's index, was between 11.0 and 15.5 % and Hausner's ratio < 1.2, all the parameters indicating good flow property. Of particular note are formulations EC5 and CA5 which showed the best flow properties (Table 2).

In-vitro drug release of microspheres

Drug release (for 1:3 drug/polymer ratio) after 15 h was in the rank order: carbopol 934P > HPMC > ethyl cellulose > chitosan (Fig 2), with the formulation prepared with carbopol 934P releasing approximately 94.8 % of drug after 15 h.

Table 1: Characteristics of metformin microspheres prepared using solvent evaporation method (mean ± SD, n = 3)

Batch	Drug:polymer ratio	Stirring speed (rpm)	% Drug content	Entrapment efficiency (%)	Yield (%)
EC1	1:1	1200	42.1	67.85±6.1	56.8
EC2	1:2	1200	34.6	78.68±4.3	59.8
EC3	1:3	1200	26.5	85.42± 3.4	65.3
EC4	1:2	600	28.2	56.36± 2.4	62.5
EC5	1:2	1800	29.5	72.21±1.3	58.8
HPMC1	1:1	1200	43.7	69.23± 2.8	64.9
HPMC2	1:2	1200	45.3	73.92± 4.8	71.8
HPMC3	1:3	1200	25.8	85.54± 3.9	78.3
HPMC4	1:2	600	27.5	68.13± 4.2	72.8
HPMC5	1:2	1800	28.6	71.8±2.2	66.1
CA1	1:1	1200	44.8	70.23± 3.9	68.7
CA2	1:2	1200	47.1	76.92± 6.0	72.2
CA3	1:3	1200	26.2	85.22± 5.3	83.2
CA4	1:2	600	28.4	64.12± 4.8	76.5
CA5	1:2	1800	29.8	67.87±2.4	70.2
CH1	1:1	1200	37.6	62.23± 3.3	59.7
CH2	1:2	1200	40.2	78.29± 4.8	71.4
CH3	1:3	1200	23.6	83.51± 5.1	82.3
CH4	1:2	600	28.7	69.23± 3.3	73.8
CH5	1:2	1800	30.6	61.23± 3.3	68.9

Key: EC = ethylcellulose, HPMC = hydroxypropyl methylcellulose, CA = carbopol 934P, and CH = chitosan, microspheres

Table 2: Some micromeritic properties of the formulations

Batch	Average particle size (µm)	Tapped density (g/cm^3)	Bulk density (g/cm^3)	Carr's index (%)	Hausner's ratio	Angle of repose
EC1	352.1 ± 7.5	0.720	0.625	13.19	1.15	31°12´
EC2	361.4 ± 6.2	0.663	0.582	12.22	1.14	29°18´
EC3	374.2 ± 7.8	0.659	0.568	13.81	1.16	27°34´
EC4	396.5 ± 7.6	0.608	0.514	15.46	1.18	25°43´
EC5	338.1±4.5	0.627	0.556	11.35	1.13	33°52´
HPMC1	359.3 ± 4.1	0.675	0.588	12.89	1.15	32°14´
HPMC2	375.6 ± 6.2	0.647	0.563	12.98	1.15	29°26´
HPMC3	388.4 ± 8.8	0.625	0.548	12.32	1.14	27°42´
HPMC4	410.2 ± 6.4	0.566	0.486	14.13	1.17	26°27´
HPMC5	352.6±5.2	0.583	0.516	11.49	1.13	33°88´
CA1	424.5 ± 8.6	0.617	0.535	13.29	1.15	30°20´
CA2	432.1 ± 9.2	0.584	0.512	12.33	1.14	29°12´
CA3	450.3 ± 8.8	0.556	0.489	12.05	1.14	27°24´
CA4	484.7 ± 4.2	0.497	0.424	14.69	1.17	26°45´
CA5	412.5 ±5.6	0.516	0.458	11.36	1.13	31°81´
CH1	408.6 ± 10.6	0.562	0.482	14.23	1.17	33°18´
CH2	417.2 ± 5.8	0.550	0.474	13.82	1.16	31°24´
CH3	426.8 ± 8.2	0.539	0.466	13.54	1.16	29°82´
CH4	458.1 ± 5.7	0.490	0.416	15.28	1.18	27°65´
CH5	385.7 ± 6.8	0.505	0.441	12.74	1.15	33°91´

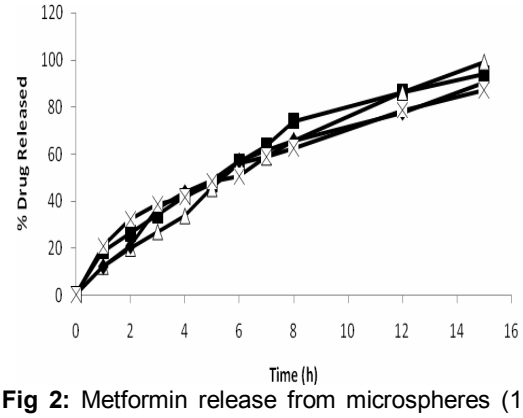

Fig 2: Metformin release from microspheres (1:3 drug:polymer) ratio. ***Key:*** ■ = EC, Δ = HPMC, ♦ = CA, × = CH

Increase in stirring speed from 600 rpm to 1800 rpm enhanced drug release rate. At 1800 rpm, complete drug release was observed except for chitosan microspheres. Fig 3 shows that the difference in drug release pattern among the various polymer types was not significant *(p* > 0.05).

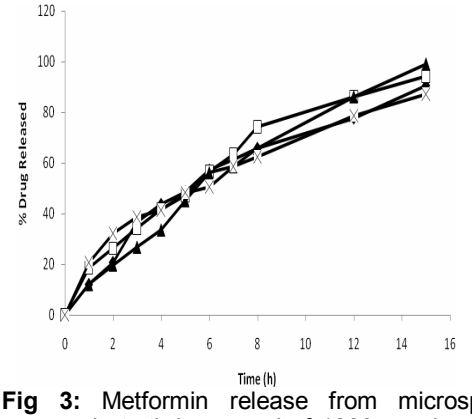

Fig 3: Metformin release from microspheres prepared at stirring speed of 1200 rpm by solvent evaporation method (***Key:*** ♦ = EC, = HPMC, ▲= CA, × = CH).

DISCUSSION

Increase in polymer concentration from 1:1 to 1:3 (drug:polymer) increased yield and entrapment efficiency probably because with increasing polymer content, more particles of metformin would be coated leading to higher encapsulation efficiency [12]. However, this increase was not significant.

The pores on the microsphere surface of some formulations indicate leaching of the drug during dissolution prior to gelation of the polymeric surface. It was observed that as the drug-polymer concentration increased, the percentage entrapment efficiency increased whereas the percentage yield decreased.

Increase in the particle size of the microspheres can be attributed to increase in viscosity with increasing polymer concentrations, which resulted in large emulsion droplets [13,14]. Shearing efficiency during stirring also diminishes at higher viscosities, thus resulting in the formation of larger droplets and hence bigger particles [15].

As drug:polymer ratio was varied from 1:1 to 1:3, with the consequent increase in the polymer content, drug release rate gradually decreased. The increased density of the polymer matrix at higher polymer concentrations results in increased diffusional pathway decreasing overall drug release from the polymer matrix [17]. Furthermore, smaller microspheres formed at lower polymer concentrations have a larger surface area exposed to the dissolution medium, thus leading faster drug release. The microspheres prepared with EC, HPMC and CA completely released the drug after 12 h. However, sustained drug release was observed for microspheres formulated with chitosan polymer extending to 15 h.

The drug release was slower at acid pH than at alkaline pH. However, when chitosan was used as the polymer, more drug was released (34 %) at acid pH in 2 h at a stirring speed of 1800 rpm. This is due to the increased solubility of chitosan at acid pH.

CONCLUSION

Varying degrees of sustained metformin release were obtained for microspheres formulated EC, HPMC, CA and CH with CH microspheres being the most drug sustaining of them all. Therefore, the developed formulations, prepared by non-aqueous solvent evaporation method, are promising for the sustained oral delivery of metformin hydrochloride.

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