

INVITRO EVALUATION AND OPTIMIZATION OF CONTROLLED RELEASE FLOATING DRUG DELIVERY SYSTEM OF METFORMIN HYDROCHLORIDE

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ABSTRACT

The floating microspheres have been utilized to obtain prolonged and uniform release in the stomach for development of a once daily formulation. The major advantage of the preparation technique includes short processing time, the lack of exposure of the ingredients to high temperature, and high encapsulation efficiencies. In the present study, preparation of metformin hydrochloride floating microspheres, evaluation of Floating Drug Delivery System (FDDS) *in vitro*, prediction of the release, and optimization of floatation and drug release pattern to match target release profile was investigated. Floating microspheres were prepared by non-aqueous emulsification solvent evaporation technique using Ethylcellulose as the rate controlling polymer and 250 mg of metformin hydrochloride per batch and its *in vitro* performance was evaluated by the usual pharmacopoeial and other tests such as drug-polymer compatibility (FTIR scan), yield (%), particle size analysis, drug entrapment efficiency, surface topography, and *in vitro* floatation and release studies. Results showed that the mixing ratio of components in the organic phase affected the size, size distribution (250-1000 μm), drug content (61 – 134% of theoretical load), yield (58 – 87%) and drug release of microspheres (47 – 87% after 8 h), floating time (> 8 hr) and the best results were obtained at the ratio of drug: polymer: solvent (250:750:12 and 250:146.45:9 [mg: mg: ml]), when both the batches were mixed in equal proportions. In most cases good *in vitro* floating behavior was observed and a broad variety of drug release pattern could be achieved by variation of the polymer and solvent ratio, which was optimized to match target release profile. The developed floating microspheres of metformin hydrochloride may be used in clinic for prolonged drug release in stomach for at least 8 hrs, thereby improving the bioavailability and patient compliance.

INTRODUCTION

Diabetes is one of the major causes of death and disability in the world. The latest WHO estimate for the number of people with diabetes worldwide, in 2000, is 171 million, which is likely to be at least 366 million by 2030. The focus of medical community is on the prevention and treatment of the disease, as is evident from the rising number of research papers every year on the subject.

A plethora of antidiabetic drugs are used in clinic, of which metformin hydrochloride is a very widely accepted drug. Unlike other antidiabetics, metformin hydrochloride does not induce hypoglycemia at any reasonable dose, and hence it is usually called an Antihyperglycemic (or Euglycemic) rather than a hypoglycemic drug (1). In spite of its favorable clinical response and lack of significant drawbacks, chronic therapy with metformin hydrochloride suffers from certain specific problems of which, the most prominent being the high dose (1.5-2.0 g/day), low bioavailability (60%) and high incidence of GI side effects (30% cases). Therefore, there are

continued efforts to improve the pharmaceutical formulation of metformin hydrochloride in order to achieve an optimal therapy. These efforts mainly focus on controlled/slow release of the drug including the sophisticated gastroretentive systems. Formulation development has also accelerated with this drug after its patent expiry in 2001 (2-7). The situation is complicated further with decrease in absorption of the drug with food that delays t_{max} by up to 35 mins (8). The rationality, therefore, exists for formulation of metformin hydrochloride as a CR/SR formulation of it has been reported (2).

However, bioavailability of the drug has been found to be reduced further with CR dosage forms, probably due to the fact that passage of the CR single unit dosage forms from absorption region of the drug is faster than its release and most of the drug released at the colon where metformin hydrochloride is poorly absorbed (9-10). CR formulation suitable for metformin hydrochloride, therefore, should be a gastro-retentive dosage form (2), which releases the drug slowly in the stomach for gradual

absorption in the intestines. The slow but complete drug release in the stomach is expected to increase bioavailability of the drug as well its complete utilization which may result to, lower dose and GI side effects. Multi unit dosage forms are considered to release the drug at a controlled rate and remain in the stomach for a prolonged period with much less chance of dose dumping. Furthermore they are supposed to cause less gastric adverse reactions and are insensitive to concomitant food intake, thereby reducing inter- and intra-patient variability and increasing the predictability of the dosage form (11-18).

A vast number of studies, reviews and books have been written on microspheres of which the interested readers are referred to compilations by Deasy (19) and Benita (20) for a broad overview of the dosage form for further information. In the recent literature, the Ethylcellulose micro-capsules have been reported by several authors for encapsulation of a variety of drugs such as zidovudine (21), cimetidine (22), potassium chloride (23), isosorbide dinitrate (24), theophylline (25), Isoniazid (26), etc for a variety of reasons. The floating microspheres are relatively new compared to non-floating multiple unit systems. There are reports for such as repaglinide (27), atenolol (28), diclofenac (13), terfenadine (17), riboflavine (18) etc., which have been incorporated in floating multiple unit systems. Various novel excipients such as chitosan (12, 29), calcium silicate (27), low density foam powder (15,16) besides the conventional polymers such as acrylic resins (30) and polycarbonate (14) have been used to achieve floatation. There are several excellent reviews on the gastro-retentive systems including floating dosage forms to which the interested readers are referred (31, 32, 33). However, no floating microsphere of metformin has been reported. A non-floating multi-particulate metformin containing system has been reported in literature (34), though the intention of the work was to optimize the pellets for extrusion-spherization purpose rather than to extend the drug release.

There have been contradictory reports on the utilization of metformin in gastro-retentive dosage forms (2, 35). However the investigated systems were single-unit type. Therefore, it seemed reasonable to improve the earlier studies by formulating metformin in a multiparticulate floating (gastro-retentive) system in order to optimize the pharmacokinetics and pharmacodynamics of the drug. Hence, to achieve the ultimate goal of formulating a clinically effective FDDS of metformin hydrochloride for effective control of Non Insulin Dependent Diabetes

Mellitus (NIDDM), the present work was designed to address the following objectives- preparation of micro-particles, evaluation of FDDS in vitro, predicting the release, and optimization of floatation and drug release pattern to match target release profile.

MATERIALS AND METHODS

Metformin hydrochloride was a gift sample from Lupin Laboratories Ltd., Aurangabad, India. Ethyl cellulose (18-22 cps) and Acetone LR were commercially obtained from S.D. Fine Chem. Ltd., Mumbai, India. Liquid paraffin LR used was commercial grade available from Qualigens Fine Chemicals, Mumbai, India. Petroleum ether (40°-60°C) was obtained from Nice Chem. Pvt. Ltd., Cochin, India. All other chemicals were of analytical grade and were used as procured.

Preparation of Microspheres

Microspheres containing anti-diabetic drug as a core material were prepared by a Non-aqueous Solvent Evaporation method (19). Briefly, drug and ethyl cellulose were mixed in acetone at various ratios. The slurry was slowly introduced into 30ml of liquid paraffin while being stirred at 1200 rpm by a mechanical stirrer equipped with a three bladed propeller at room temperature. The solution was stirred for 2 h to allow the solvent to evaporate completely and the microspheres were collected by filtration. The microspheres were washed repeatedly with petroleum ether (40°-60°C) until free from oil. The collected microspheres were dried for 1 h at room temperature and subsequently stored in a desiccator over fused Calcium chloride.

IR Spectra

FTIR spectra of pure drug, polymer (EC), 1:1 and 2:1 microspheres were obtained in KBr pellets at moderate scanning speed between 4000-200cm⁻¹ in a Perkin- Elmer FTIR Spectroscope.

Yield of Microspheres

The prepared microspheres with a size range of 251-µm were collected and weighed. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres.

$\% \text{ Yield} = (\text{Actual weight of product} / \text{Total weight of excipient and drug}) \times 100$

Particle size analysis

Size distribution was determined by sieving the microparticles using a nest of standard BSS sieves (36) as well as by optical microscopy

using stage micrometer slide and calibrated eyepiece by counting at least 100 microspheres.

DEE (Drug Entrapment Efficiency)

Microspheres equivalent to 50 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1N HCl repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using 0.1N HCl. The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically (UV 1700, Shimadzu, Japan) at 233 nm against appropriate blank. The amount of drug entrapped in the microspheres was calculated by the following formula:

$$\text{DEE} = \left(\frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \right) \times 100$$

Surface Topography (SEM)

The surface morphology of the microspheres was examined by scanning electron microscopy in a Cambridge Instruments, Stereo Scan 360.

In vitro Evaluation of Floating Ability (12,37)

An in vitro floating study was carried out using simulated gastric fluid USP containing 1% Tween 80 as a dispersing medium. Microspheres were spread over the surface of 500ml of dispersing medium at $37 \pm 0.5^\circ\text{C}$. A paddle rotating at 100 rpm agitated the medium. Each fraction of microspheres floating on the surface and those settled down were collected at a pre-determined time point. The collected samples were weighed after drying.

$$\% \text{Floating microspheres} = \left(\frac{\text{weight of floating microspheres}}{\text{initial weight of floating microspheres}} \right) \times 100$$

In vitro Drug Release Study (30)

In vitro drug release studies were carried out for all products and for the pure drug in USP type II (38) [DISSO 2000, Labindia, Chennai, India] dissolution test apparatus. One hundred mg of pure drug was used for the dissolution studies and microspheres equivalent to 100mg of the pure drug were used. Two ml of the aliquot was withdrawn at predetermined intervals and filtered. The required dilutions were made with 0.1N HCl and the solution was analyzed for the drug content spectrophotometrically (UV 1700, Shimadzu, Japan) at 233nm against suitable blank. Equal volume of the dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. Three trials were carried out for all formulations. From this percentage drug release was calculated and

plotted against function of time to study the pattern of drug release.

The similarity of dissolution profile of the prepared formulations was compared with that of the marketed formulations to arrive at the optimum profile. There are several methods which are recommended for comparison of dissolution in literature (39-42), though there seems to be no general agreement regarding the best method in a particular situation. However, in view of the wide regulatory acceptance of the "similarity factor" (f_2) method (mentioned in SUPAC-MR document of FDA (43)) in comparison to others, the former was used as a model-independent statistical tool for comparison of dissolution profile in this study.

The closeness of drug release profile to that of target profile (market product) was calculated using FDA recommended similarity factor (f_2 value), that must be within 50-100 for similarity was calculated as follows:

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum (R_i - T_i)^2 \right]^{-0.5} \times 100 \right\}$$

Where, R_i and T_i are percent of drug which was dissolved at each time point for the test and reference products respectively, n is the number of time points considered.

Statistical Optimization of the formulations (44)

Modern formulator needs to conserve time and resources to deliver the best formulation and hence optimization following statistical experimental design. In the present study, a two step design was followed. In the first step, a central composite design (CCD; Table 1) (44) was followed to study the effect of formulation variables on product characteristics. In the second step, a simplex lattice mixture design (45) was followed to optimize the desired release profile using minimum number of experiments. For optimization of the formulation the desired target release profile (equivalent to marketed products) was achieved by initially building empirical polynomial models for the set response parameters of the study. In the present study the following response parameters were selected for optimization – similarity factor (f_2) and cumulative % of the drug released at different hours (CR1, CR2, CR3, CR5, CR8) (Table 2). The polynomial models (Table 3) which were developed were simultaneously solved with the target values for the response parameters as objective functions to arrive at the predicted optimum formulations. The predicted formulation was then prepared and evaluated through all physicochemical tests and the percentage of error in prediction was calculated to validate the quality of prediction.

RESULTS AND DISCUSSION

Several preformulation trials were undertaken for various proportions of drug and polymer by variation of the liquid paraffin and acetone volumes for qualitative and quantitative determination of microsphere characteristics. It was found that Ethylcellulose microspheres show desirable high drug content, yield, floatation and adequate release characteristics and hence were suitable for development of a CR system. The 25# BSS sieve has highest yield. No drug polymer incompatibility was noted in their FTIR spectra (Data are not shown). The surface morphology and internal texture of microspheres were determined by scanning

electron microscopy (SEM) as shown in Figure 1. Presence of pores were detected on the microsphere surface which increased in size and number after dissolution indicating leaching of the drug through these channels. The microspheres, however, did not change in shape or size after dissolution as is expected for a hydrophobic water insoluble polymer ethylcellulose.

To precisely understand and quantify the effect of drug-polymer ratio and the effect of process variables such as volume of solvent and manufacturing vehicles a Central Composite Design (CCD) was devised in which the polymer and solvent were used as the variables

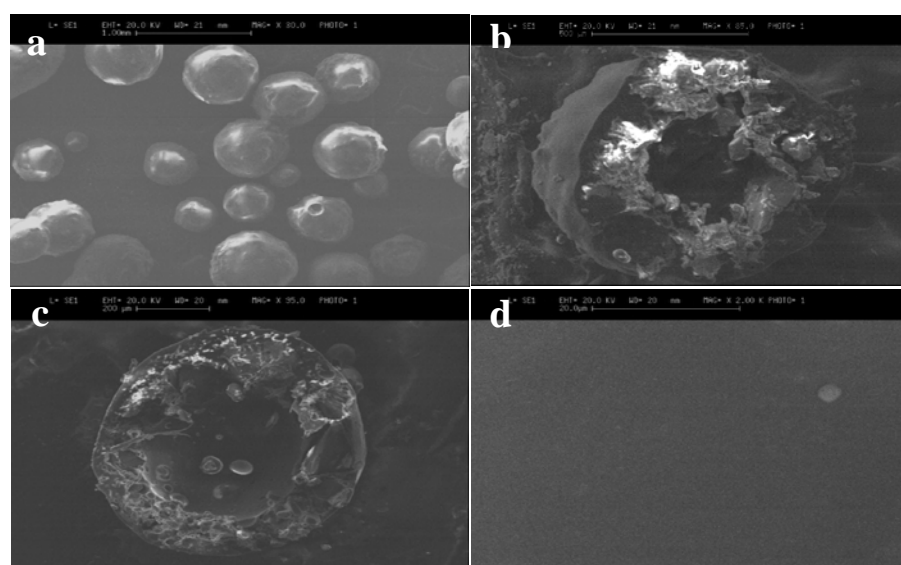


Figure 1. SEM of metformin floating microspheres **a**; Original microspheres, **b**; Transverse section of microspheres, **c**; Transverse section of microspheres after dissolution, **d**; Surface of microsphere before dissolution.

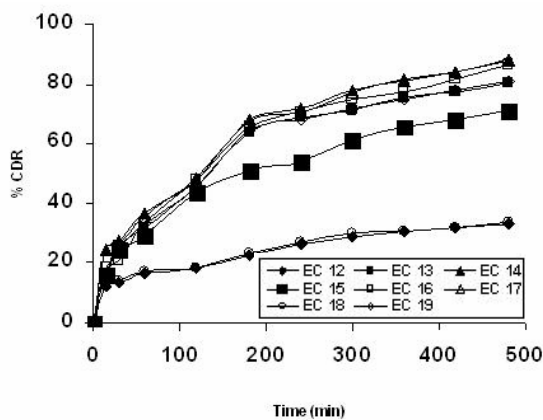


Figure 2. Release profile of microsphere blends. EC = Ethylcellulose microspheres of different batches (refer to Table 2)

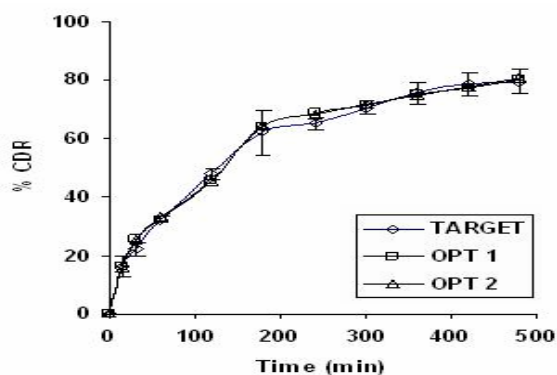


Figure 3. Comparison of optimized formulations with target. Target = Target release profile; OPT1 = Optimum formulation # 1; OPT2 = Optimum formulation # 2

Table 1. Characteristics of the microspheres obtained following CCD.

Product Code	Drug: polymer: acetone	Percentage Yield (%)*	Drug Entrapment Efficiency (%)*	Release after 8 h (%)*
EC-1	250:250:6	58.6	78.32	73.13
EC-2	250:250:12	98.4	89.06	78.48
EC-3	250:750:6	44.93	93.72	47.64
EC-4	250:750:12	84.83	134.76	47.64
EC-5	250:500:9	77.11	124.51	55.76
EC-6	250:500:9	75.05	96.54	64.02
EC-7	250:500:6	73.59	96.66	63.62
EC-8	250:146.45:9	81.97	61.17	87.59
EC-9	250:853.55:9	87.06	113.81	86.46
EC-10	250:500:4.76	83.6	95.19	70.18
EC-11	250:500:43.24	84.04	90.53	77.38

* All values are the average of the three determinations

Table 2. Blended formulations of mixture design. (EC = Ethylcellulose formulation; CR= cumulative release)

Code*	EC 4	EC 8	F2	Cumulative % drug release at different hours				
				CR1	CR2	CR3	CR5	CR8
EC 12	1	0	21.78	16.87	18.24	22.68	29.09	33.22
EC 13	0.5	0.5	91.59	32.06	45.59	63.68	71.09	80.21
EC 14	0	1	62.8	36.59	47.58	68.06	77.83	87.59
EC 15	0.75	0.25	53.15	29.33	43.62	50.65	61.54	70.87
EC 16	0.25	0.75	72.09	33.45	48.05	65.55	74.58	86.33
EC 17	0	1	63.41	35.74	48.43	67.47	77.34	88.23
EC 18	1	0	33.9	17.4	18.48	23.21	29.76	33.46
EC 19	0.5	0.5	88.94	33.17	45.62	64.35	71.75	80.93

*Total – 1 or 100%

Table 3. Polynomial models and ANOVA table for the response parameters

Parameter	Model	F	P<0.05	P (LOF)	R ² adj.	R ² pred.
F2	$f_2 = +26.3908 \times F_4 + 61.6752 \times F_8 + 154.04078 \times F_4 \times F_8$	22.63	0.0031	6.60	0.8607	0.7796
CR1	$(CR1 - 16.00)^3 = +188.24862 \times F_4 + 8139.45533 \times F_8$	197.22	0.0001	1.25	0.9659	0.9508
CR2	$(CR2 - 18.00)^3 = +576.38308 \times F_4 + 26895.81704 \times F_8 + 34508.21259 \times F_4 \times F_8$	130.82	0.0001	8.26	0.9737	0.9592
CR3	$\text{Sqrt}(CR3 + 22.00) = +6.70110 \times F_4 + 9.47145 \times F_8 + 4.68109 \times F_4 \times F_8 + 2.94324 \times F_4 \times F_8 \times (F_4 - F_8)$	3650.81	0.0001	2.83	0.9994	0.9981
CR5	$(CR5)^3 = +24634.99428 \times F_4 + 4.66180 \times 10^5 \times F_8 + 4.55218 \times 10^5 \times F_4 \times F_8 + 2.08041 \times 10^5 \times F_4 \times F_8 \times (F_4 - F_8)$	1290.96	0.0001	4.85	0.9982	0.9942
CR8	$(CR8 - 10.00)^3 = +14777.45294 \times F_4 + 4.72570 \times 10^5 \times F_8 + 4.51560 \times 10^5 \times F_4 \times F_8$	1966.11	0.0001	2.38	0.9982	0.9971

(F= Fisher statistics, P = probability of error; LOF = lack of fit; R² = coefficient of determination; adj. = adjusted; pred. = predicted)

Table 4. Deviation of optimum formulation from target drug release (%).

Time (h)	P (Target value)*	OPT1 R1 (EC 13)	OPT 2 R2 (EC 19)	%Error 1**	%Error 2**
1	32.05	32.06	33.17	0.0312	3.494
2	47.7	45.59	45.62	4.421	4.36
3	64.17	63.68	64.35	0.763	0.28.
5	70.07	71.09	71.75	1.455	2.397
8	80.33	80.21	80.93	0.149	0.746
	f_2	91.59	88.94	-	-

(Target = Target release profile; OPT1 = Optimum formulation # 1; OPT2 = Optimum formulation # 2)

*Target value is market products value; ** (P - Ri) x100/P (i = 1,2)

while drug and liquid paraffin (vehicle) were kept constant. Therefore, drug-polymer ratio and also the acetone-liquid paraffin phase-volume ratio were varied.

All formulations floated for more than 8 hr on the simulated gastric fluid USP (Data are not shown). The microsphere, having lower densities (having a hollow core; Figure 1b,c), exhibited buoyancy and are expected to be retained in gastric environment for more than 8 h. The results were in agreement with earlier reports (30) which help to improve the bioavailability of the basic drugs like metformin hydrochloride. *In vitro* release studies of the floating microspheres were carried out in 0.1N hydrochloric acid (pH 1.2) for a maximum period of 8 h. As expected, EC level was found to influence the drug release, particle size and drug entrapment characteristics of the microsphere. Higher level of the EC yielded microsphere with high drug content probably due to polymer loss at high viscosity level. The release did not show any burst effect or lag time, which is indicative of a homogeneous drug distribution. It was found that most of the formulations followed Higuchi square root kinetics indicating a diffusion dependent release as expected from a matrix system like the microspheres which have been developed. This indicated that the smooth walled microspheres had adequate pores and channels to allow smooth, controlled drug release and that there was no polymer dissolution or chain relaxation due to non-swelling insoluble nature of the polymer which was used. This in turn, ensures high reproducibility of the developed systems. The release data was further supported by surface morphology obtained by SEM study (Figure 1a-d). Similar observations have been (25-26) reported on ethylcellulose based multiparticulate systems prepared essentially by the same technique with relatively water soluble drugs. Hence the results obtained w.r.t size distribution, yield, drug entrapment and release were in conformity with earlier published reports.

The formulations in CCD had wide range of release both over and below the target profile

(Table 1). Therefore, two formulations from the investigated range of formulae, namely, EC 4 and EC 8, were selected on the basis of their favorable values for drug content, particle size, yield and release characteristics (Table 1). A simplex lattice mixture design was selected to optimize the release (Table 2). The response parameters used for optimization were the %CDR at 1st, 2nd, 3rd, 5th and 8th hr of dissolution and the target values were obtained from the dissolution study of the marketed products. Regression analysis to build polynomial models (Table 3) followed by numerical optimization of the release data showed that a 1:1 blend formulations of EC 4 and EC 8 provide the desired release. This translates into either formulation blend EC 13 or EC 19 as the optimized formulations. It was found that the observed values of each parameter optimized was very close to the target and predicted values with less than 5% error (Table 4). Hence, the release was finally optimized. A further indication of the success of the optimization procedure could be obtained by a look at the f_2 values. Hence, through the 50:50 (by weight) blend of formulations EC 4 and EC 8, the same release profile was achieved as that of the target marketed products (GluforminXL 500, Gluconorm SR, Metlong 500 – Brands marketed in India). We are not aware of any public literature report utilizing this simple and straightforward methodology to optimize differential release profiles of microsphere blends. Patents on multiparticulates, especially on Spansules™, do refer to optimum blends, but the method of arriving at such blends is rather crude and empirical. In contrast, the method which was followed in this study relies on sound mathematical foundations and sure-shot pathway of achieving any desired release profile within the experimental domain.

CONCLUSION

The experimental design supported product development and optimization procedure yielded the desired microspheres with drug release equivalent to those of the marketed single unit

dosage forms with the added advantage of floatability in gastric juice for prolonged slow release. The optimized multi-unit floating metformin HCl 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. Therefore, it may be concluded that drug loaded floating microspheres are a suitable delivery system for metformin

hydrochloride, and may be used for effective management of NIDDM.

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