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FORMULATION AND EVALUATION OF ORAL SUSTAINED RELEASE DRY SUSPENSION OF METFORMIN HYDROCHLORIDE

Vivek V. Nalawade[*a,b], Dr. Ashok L. Peepliwal[b], Dr. Supriya S. Shidhaye[a,b], Anuradha Mhadgut[a], Swasti Pandey[a]

[a]VES College of Pharmacy, Hashu Advani Memorial Complex, Behind Collector Colony, Chembur (E), Mumbai - 400 074, Maharashtra, India.

[b]NIMS University Jaipur, Rajasthan, India, 303121.

***Author for Correspondence: Vivek V. Nalawade**

VES College of Pharmacy, Hashu Advani Memorial Complex, Behind Collector Colony, Chembur (E), Mumbai - 400 074, Maharashtra, India.

Mail Id: vickypharmahere@gmail.com, anuradha.mhadgut@gmail.com

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ABSTRACT

The developed formulation aim was to overcome the problem with Metformin HCl sustained release tablets like difficulty in swallowing due to large tablet size and elimination of intact tablet in the stools resulting in patient incompliance. Hence multiparticulate sustained release drug delivery system to be converted to a liquid oral formulation was developed for patients of Type 2 diabetes. The developed formulation will also be beneficial for patients of Type 2 diabetes suffering from dysphagia, stroke, difficulty in swallowing, geriatric patients etc. These patients may consume it easily and long term therapy may be maintained. A sustained release dosage form of Metformin HCl in the form of microspheres was prepared by solvent evaporation method using polymers Ethyl Cellulose and *EUDRAGIT® S 100*. Optimization of the factors like Drug to Polymer and the Polymer to Polymer ratio was done using a 2^2 factorial design. The trials were designed using Drug to polymer ratio and Polymer to Polymer ratio as the two factors at 2 levels. The resulting microspheres were evaluated for entrapment efficiency, drug loading and percent yield of the process and dissolution profile. The solutions obtained by this optimization were converted in to a dry sustained release ready to use suspension and also compared with the marketed formulation.

KEYWORDS: Metformin HCl, Ethyl cellulose, *EUDRAGIT® S 100*, Sustained release, Solvent evaporation, Factorial design, Dry sustained release suspension.

INTRODUCTION

In the development of novel drug delivery multiunit formulations such as micro particles have advantages over single unit dosage forms. Multiparticulate systems could also be formulated as liquid suspensions allowing ease of swallowing and flexibility in the dose adjustment for paediatric and geriatric patients.[1] Many techniques for the preparation of micro capsules have been developed and reviewed.[2] Metformin is an oral anti hyperglycemic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function.[3,4,5] It has an absolute oral bioavailability of 40 to 60% and gastrointestinal absorption is apparently complete within 6 h of ingestion. An inverse relationship is observed between the dose ingested and relative absorption with therapeutic doses (0.5 to 1.5 g), suggesting the involvement of active, saturable absorption process. Suitable dosage regimens of the drug include unit doses of 500 mg two to three times daily and can even be built up to five times daily or 850 mg once or twice daily. Such multiple dosing regimens are not preferred since they lead to patient noncompliance, potential side effects and danger of overdosing. There is, therefore, the need to provide formulations and processes that would deliver extended release of Metformin HCI from the dosage form when consumed.[6] It is observed in the commercial Metformin tablets that the tablet size is huge and the ghost matrix is excreted in the faeces in intact form this reduces patient's confidence in the formulation leading to reduced compatibility towards such tablets. The aim is to develop a formulation which is available in the microcapsules form which can be easily converted in to liquid suspensions, it would help patients suffering from dysphagia, unconscious patients and geriatric patients would find such formulation useful.

In the previous study microcapsules of Metformin HCl were prepared by using different Polymers at different Drug to Polymer ratios. Ethyl Cellulose & *EUDRAGIT®* S 100 in the ratio of 1:1 shows a drug release profile which is comparable to marketed preparation and have shown the F2 value 57.[7]

Thus, in this study, in order to develop a successful formulation, the formula for microparticles were further optimized using factorial design. An *experimental design* is the statistical strategy for organizing the experiments in such a manner that the required information is obtained as efficiently and precisely as possible.[8,9,10,11] *Runs* or *trials* are the experiments conducted according to the selected experimental design.[12,13]

Such DoE trials are arranged in the design space so that the reliable and consistent information is attainable with minimum experimentation. The resultant formula from these trials was further evaluated for its feasibility of conversion into an oral sustained release formulation.

MATERIALS AND METHODS

Drug

Metformin Hydrochloride was obtained as a gift sample from Aarti Drugs Ltd, Mumbai.

Excipients

Ethyl cellulose was purchased from Rajesh Chemicals, EUDRAGIT® S 100 was received as gift sample from Evonik Industries.

As the drug was highly water soluble, a non-aqueous method was selected for preparation of the microcapsules.

For the efficient multiparticulate formulation, such as gastro retentive sustained release floating microspheres, selection of appropriate encapsulation material is very important.

Ethyl cellulose (EC) is a water insoluble polymer, and widely used in pharmaceutics as a coating material for sustained-release microcapsules. This is due to its high safety, good stability, easy fabrication and cost efficiency.[14]

Therefore ethyl cellulose was selected as a model encapsulation material for preparation of microspheres.

EUDRAGIT® S100 can be used for granulation of drug substances in powder form for controlled release. It is effective and forms a stable enteric coating with a fast dissolution in the upper Bowel. The polymer provides site specific drug delivery in intestine by combination with EUDRAGIT® S grades. The polymer can show variable release profiles.

METHOD

1.1 Experimental

Aim of the formulation development was to develop sustained release microspheres of Metformin HCl, with optimum yield, encapsulation efficiency. The criterion for drug release was set as a sustained release of drug from the microspheres for about 8 hr and comparable with the marketed formulation. Various formulation trials were carried out aiming to produce desired formulation.

1.1.1 Formula Optimization

From the previous study, it was established that Ethyl cellulose & *EUDRAGIT*® S 100 in the ratio of 1:1 shows a drug release profile which is comparable to marketed preparation and have shown the F2 value 57.

For optimization of the formula it is important to consider the various factors that dictate the overall release profile of the desired SR formulation. In the present Metformin Microcapsules coated with a combination of the polymers: Ethyl Cellulose and Eudragit S100, the Drug to Polymer Ratio and the Polymer to Polymer ratio of the two polymers used are considered as the two significant independent factors. The responses would be the percentage of drug release at different time points of 1hr, 2hrs, 3 hrs, 5hrs and 8hrs. The 2 independent factors were considered at 2 levels i.e. high and low levels. Thus a 2^2 factorial design was used to optimize the formula. Experimental trials were performed for all 4 possible combinations. The drug to polymer and polymer to polymer ratios were selected as independent variables in 2^2 full factorial design, while % drug release values of 1hr, 2 hrs, 3 hrs, 5hrs, 8hrs were taken as dependent variables. A center point was also added to the design. All trials were run in triplicate. The formulation layout for the factorial design batches (Run 1 to 15) and their responses are shown in Table 1.

Table 1: Layout of factors and responses of the factorial design.

Select	Std	Run	Factor 1 A:Drug Poly...	Factor 2 B:Polymer R...	Response 1 Disso 1HR %	Response 2 Disso 2HR %	Response 3 Disso 3HR %	Response 4 Disso 5HR %	Response 5 Disso 8HR %
	14	1	0.00	0.00	22.5954	39.771	56.9466	72.0611	77.8626
	13	2	0.00	0.00	33.5294	58.056	74.8824	84.593	100.322
	8	3	-1.00	1.00	54.1176	55.1251	55.8062	52.9583	54.2919
	7	4	-1.00	1.00	132.288	137.779	140.07	143.146	141.322
	12	5	1.00	1.00	27.5817	37.9876	42.3857	45.7402	51.8415
	4	6	1.00	-1.00	2.15686	13.3409	25.1058	32.3732	64.2196
	3	7	-1.00	-1.00	75.2941	79.7762	79.0971	81.0974	83.1641
	6	8	1.00	-1.00	17.2549	41.7733	60.8138	85.0272	111.802
	10	9	1.00	1.00	10.7843	22.6851	40.605	71.0828	106.086
	5	10	1.00	-1.00	10.0654	33.0769	45.5118	71.0901	98.3805
	1	11	-1.00	-1.00	89.8693	124.947	127.82	130.041	137.23
	15	12	0.00	0.00	50.9804	83.6203	94.1849	100.964	112.191
	11	13	1.00	1.00	1.48036	2.83763	4.75654	9.81418	18.8382
	9	14	-1.00	1.00	60	89.2457	102.231	104.113	110.112
	2	15	-1.00	-1.00	80.2614	99.3212	109.436	109.491	116.408

1.1.2 Experimental Domain

The dimensional space defined by the coded variables is known as *factor space.* Figure 1 illustrates the factor space for two factors on a bidimensional (2-D) plane during the formulation of controlled release microspheres. The part of the factor space, investigated experimentally for optimization, is the *experimental domain.*

Also known as the *region of interest,* it is enclosed by the upper and lower levels of the variables. The factor space covers the entire figure area and extends even beyond it, whereas the design space of the experimental domain is the square enclosed by *Q1, Q2, Q3, Q4.*

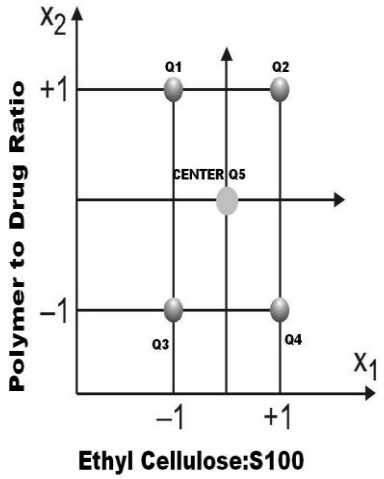

Fig. 1: Quantitative factors and factor space. The axes for the natural variables ethyl cellulose: s100 and polymer: drug ratio are labelled *x*1 and *x*2.

1.1.3 Coding

The process of transforming a natural variable into a nondimensional coded variable, X1, so that the central value of experimental domain is zero is known as coding (or normalization).[10,15,16] Generally, the various levels of a factor are designated as –1, 0, and +1, representing the lowest, intermediate (central), and highest factor levels investigated, respectively.[12,15,17]

Table 2: Denotation of trials according to their factors.

Trial	Polymer to Drug Ratio		Ethyl Cellulose: S100	
		Factor Denotation		Factor Denotation
Q1	4:1	1.00	1:2	-1.00
Q2	4:1	1.00	3:1	1.00
Q3	1:1	-1.00	1:2	-1.00
Q4	1:1	-1.00	3:1	1.00
Q5	2:1	0.00	1:1	0.00

1.1.4 Procedure: For Microencapsulation of all the above batches the following procedure was followed

- For the preparation of microspheres by solvent diffusion-evaporation method the drug and polymers were added to the solvent mixer and dissolved to form homogenous polymer solution containing drug.
- Liquid Paraffin (continuous phase) was kept under mechanical overhead stirrer equipped with a four bladed with required stirring speed.
- The polymer solution was slowly introduced into Liquid Paraffin oil dispersion with the help of pipette by dipping it into the dispersion media. Then the emulsion formed was stirred for about 3 hrs, so that the organic solvents were diffused in to continuous phase and were evaporated.
- The microspheres formed were collected by filtration of continuous phase. The collected microspheres were washed with n-Hexane to remove non-encapsulated drug and then with warm distilled water to remove traces of n-Hexane and Liquid Paraffin oil.
- The collected microspheres were air dried for 24 hrs.

Method of introducing polymer solution

Polymeric solution was introduced into the dispersion phase by using a pipette. Polymer solution was sucked in dry pipette. Then tip of pipette was inserted deep in dispersion phase and polymer solution blown out slowly inside. The contact of the polymer solution at the

interface of dispersion phase and outer environment was avoided. As soon as polymer solution contacts at the interface, the organic solvents like ethanol diffuses to water and air phase, causing the precipitation of polymer at the surface of dispersion phase forming film.

All the batches were evaluated for various properties.

1.2 Studies on the trial batches

1.2.1 Percent Yield[18]

Percent yield of microspheres of all the 15 trial runs was calculated by the formula,

$$\% \text{ Yield} = \frac{\text{Total weight of microspheres}}{\text{Total weight of drug, polymer and other excipients added}} \times 100$$

This parameter was helpful in choosing the preparation method of microsphere giving minimum losses and highest yield.

1.2.2 Encapsulation efficiency[18]

The drug content of drug loaded microspheres was determined by dispersing 200 mg of microspheres in 80ml of phosphate buffer pH 6.8 followed by agitation with a magnetic stirrer to extract the drug for 24 hrs following which the volume was made up to 100ml. After filtration through Whatman (0.45micron) filter paper, the drug concentration in the aqueous phase was determined by taking the absorbance of this solution spectrophotometrically at 233.2 nm. EUDRAGIT® polymers and Ethyl cellulose did not interfere under these conditions. Each determination was made in triplicate. The concentration of Metformin HCl in solution was calculated from the formula.

Concentration= (Absorbance-0.011)/0.0786

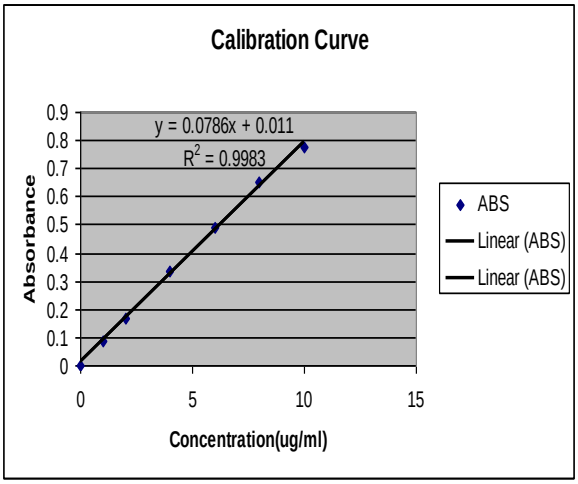

Fig. 2: Calibration curve of Metformin.

Calibration curve of Metformin from standard plot of Metformin HCl in phosphate buffer

Thus, the drug entrapped in 100 mg of microspheres was calculated, which is referred as "Percent drug loading" and further, total drug encapsulated in total recovered microspheres from the procedure is calculated. It was expressed in percentage called as "Percent drug encapsulation". The experiment was done in triplicate and the average ± S.D was calculated.

Table 3: Comparative studies of % Yield and % Entrapment of different batches.

	Q1			Q2			Q3			Q4			Q5		
% Yield	90	93.3	92.6	90.6	94.6	90.7	85	81.7	80	81.3	85.3	79.83	88.8	87.8	93.3
% Entrapment	15	14.4	14.7	15.7	18	14.3	38.4	37.9	37.4	34.4	31.4	34.5	24.3	25.1	26.8

1.2.3 Release kinetics

A USP basket apparatus has been used to study *in-vitro* drug release from microspheres from all the trial batches. A weighed amount of microspheres equivalent to 500 mg drug was placed in the basket. Dissolution medium used was phosphate buffer (pH 6.8, 1000 ml) and maintained at 37±0.5°C at a rotation speed of 100 rpm. Prefect sink conditions prevailed during the drug release studies. Five ml of sample was withdrawn at each 1, 2, 3, 5 & 8hrs interval. Sample was then passed through a Whatman filter paper (0.45micron), and analyzed spectrophotometrically at 233.2 nm to determine the concentration of drug present in the dissolution medium. The volume was replenished with the same amount of fresh dissolution fluid each time to maintain the sink condition. Linear regression was used to analyze the *in-vitro* release mechanism. The experiment was conducted in triplicate and the average ± S.D was calculated.

1.3 Effects and response surfaces

The magnitude of the change in response caused by varying the factor level(s) is termed as an *effect.*[10,15]

The *main effect* is the effect of a factor averaged over all the levels of other factors. Such data from the main effects can be suitably modelled to generate mathematical relationships between the independent variables and the dependent variables. Graphical depiction of the mathematical relationship is known as a *response surface.*[21, 22, 23]

A *response surface plot* is a 3-D graphical representation of a response plotted between two independent variables and one response variable. The use of 3-D response surface plots allows us to understand the behaviour of the system by demonstrating the contribution of the independent variables.

Table 4: Release profile of the trial batches.

Time (hrs)	1	2	3	5	8
Q1	9.16	28.34	43.1	63.37	91.22
	10.25	30.77	43.81	65.03	90.8
	10.06	29.08	44.51	60.09	92.38
Mean	9.82333333	29.3966667	43.8066667	62.83	91.4666667
Q2	12.58	20.98	27.38	42.74	57.84
	12.78	22.68	30.6	41.08	58.83
	14.48	19.83	29.76	42.81	60.08
Mean	13.28	21.1633333	29.2466667	42.21	58.9166667
Q3	82.29	101.78	106.1	101.1	110.16
	82.87	102.95	100.82	107.82	110.23
	80.26	99.32	109.44	107.44	116.41
Mean	81.8066667	101.35	105.453333	105.453333	112.266667
Q4	84.12	95.12	100.1	104.1	101.3
	82.29	97.78	95.8	95.9	94.29
	80	89.24	102.2	100.2	110.11
Mean	82.1366667	94.0466667	99.3666667	100.066667	101.9
Q5	33.52	58.05	74.88	84.59	97.3
	35.98	63.62	76.94	87.06	97.19
	37.49	59.77	74.18	85.96	95.86
Mean	35.6633333	60.48	75.3333333	85.87	96.7833333

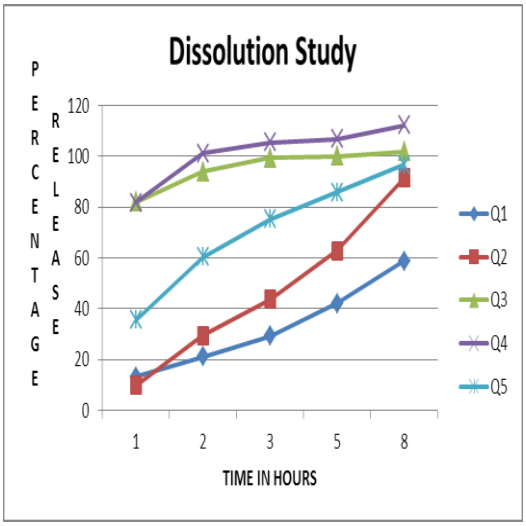

Fig. 3: Average release profile of different trial batches.

1.4 Optimization of microcapsules

The data obtained from these trails was computed using the Statease software Design Expert Version 8. A suitable mathematical model for the objective(s) under exploration is proposed, the experimental data thus obtained are analysed accordingly, and the statistical significance of the proposed model discerned. Optimal formulation compositions are searched within the experimental domain, employing graphical or numerical techniques. This entire exercise is invariably executed with the help of pertinent computer software as Statease Design of Experiments Version 8 used here. The mathematical model, simply referred to as the *model*, is an algebraic expression defining the dependence of a response variable on the independent variable(s).[19,20]

The mathematical model or the algebraic expression obtained are listed below:

The parameters considered in the optimization of formula for Microencapsulation of Metformin HCl were the Polymer to Drug Ratio (A) and the Polymer to Polymer ratio of Ethyl cellulose to S100 (B).

Drug Release at 1hr =+44.55-35.21* A+0.95* B+0.78* A*B Eq. 1

Drug Release at 2 hrs=+61.29-36.21* A-3.88* B-0.23*A*B Eq. 2

Drug Release at 3hrs=+70.64-32.94*A-5.16*B-2.12 *A*B Eq. 3

Drug Release at 5hrs=+79.57-25.48*A-6.86*B-3.45 *A*B Eq. 4

Drug Release at 8hrs=+92.27-15.95*A-10.73* B-5.55*A*B Eq. 5

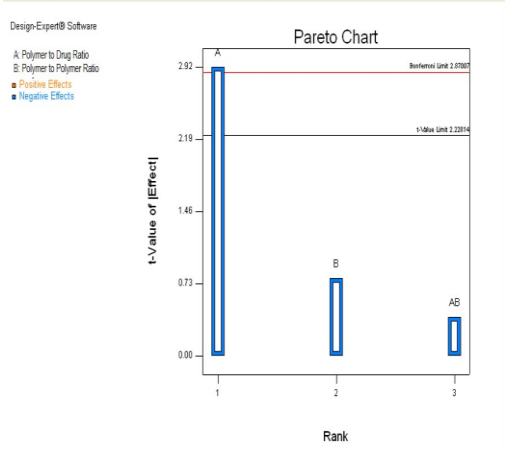

Fig. 4: Pareto chart showing positive and negative effect of factors.

RESULTS AND DISCUSSIONS

The equations 1, 2, 3, 4 and 5 obtained from the ANOVA of the various responses, helped us to draw inferences about the effect of the factors on the responses and their interaction. The factorial model equation can be expressed in general form as:

$Y=\beta_0+ \beta_1 X_{1+} \beta_2 X_{2+} \beta_{12} X_1 X_2$

The value for the intercept ($\beta_{0)}$ of 44.55, 61.29, 70.64, 79.57, and 92.27 in equations 1, 2, 3, 4 and 5 respectively represent the average of all actual responses. The coefficients can be directly compared to assess the relative impact of the factors.

In case of Equation 1, we can see that factor A (coefficient -35.21) causes a bigger negative effect than factor B (coefficient + 0.95) which has a small value of positive effect on the response.

In case of equations 2, 3, 4 and 5, we can see that factor A causes a bigger negative effect than factor B. Thus it can be concluded that factor A which is the Polymer to Drug ratio, has a major negative effect which causes retardation of drug release. The Factor B which is the Polymer to Polymer ratio plays a very small role in retardation of drug release.[24]

Optimized solutions were suggested by the software for achieving the desired release profile. 35 solutions were obtained.

A random solution of Polymer to Drug Ratio of 0.32 and Ethyl Cellulose to S100 ratio of 0.77 which statistically showed a desirability of 1 with respect to the criteria which was set for achieving the desired release profile. The chosen solution formula was formulated and tested for its Release Profile.

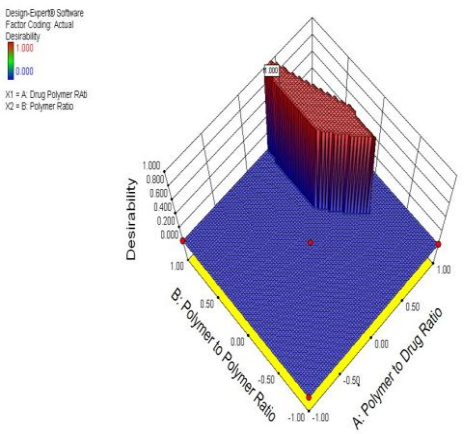

Fig. 5: 3D surface plot showing desirability of selected solution.

1.5 Optimized solution

The optimized solutions suggested by the software were obtained on the basis of the criteria which was input inorder to achieve a desired release profile for the development of Sustained Release Metformin HCl microcapsules.

One solution was randomly chosen from the 35 solutions yielded by the software. The statistical desirability of the chosen solution was 1.

Formula

		Polymer to Drug Ratio	Ethyl Cellulose to S100 Ratio
High	**1**	4:1	3:1
Low	**-1**	1:1	1:2
Suggested by Solution:	**0.52**	3.04:1	
	0.23		1.13:1

According to the calculated ratios, batches of the Solution were formulated, henceforth designated as 'S'.

Procedure

For Microencapsulation Oil Phase was used as Manufacturing Vehicle. The procedure followed was same as described above under 1.1.4.

1.6 Evaluation of microspheres

1.6.1 Particle Size and morphology evaluation

Optical Microscope was used to evaluate both the morphology and surface characteristics of the microcapsules.

1.6.2 Percent Yield[18]

Percent yield of microspheres was calculated by the formula,

$$\% \text{ Yield} = \frac{\text{Total weight of microspheres}}{\text{Total weight of drug, polymer and other excipients added}} \times 100$$

This parameter was helpful in choosing the preparation method of microsphere giving minimum losses and highest yield.

1.6.3 Encapsulation efficiency[18]

The drug content of drug loaded microspheres was determined by dispersing 200 mg of microspheres in 80ml of phosphate buffer pH 6.8 followed by agitation with a magnetic stirrer to extract the drug for 24 hrs. After filtration through Whatman (0.45micron) filter paper, the drug concentration in the aqueous phase was determined by taking the absorbance of this solution

spectrophotometrically at 233.2 nm. EUDRAGIT® polymers and Ethyl cellulose did not interfere under these conditions. Each determination was made in triplicate. The concentration of Metformin HCl in solution was calculated from the formula;

Concentration= (Absorbance-0.011)/0.0786

Fig 2: Calibration Curve of Metformin from standard plot of Metformin HCl in phosphate buffer pH 6.8.

Thus, the drug entrapped in 100 mg of microspheres was calculated, which is referred as "Percent drug loading" and further, total drug encapsulated in total recovered microspheres from the procedure is calculated. It was expressed in percentage called as "Percent drug encapsulation". The experiment was done in triplicate and the average ± S.D was calculated.

1.6.4 Release kinetics[25]

A USP basket apparatus has been used to study *in-vitro* drug release from microspheres. A weighed amount of microspheres equivalent to 500 mg drug was placed in the basket. Dissolution medium used was phosphate buffer (pH 6.8, 1000 ml) and maintained at 37±0.5°C at a rotation speed of 100 rpm. Prefect sink conditions prevailed during the drug release studies. Five ml of sample was withdrawn at each 1,2,3,5 & 8 hrs interval. Sample was then passed through a Whatman filter paper (0.45micron), and analyzed spectrophotometrically at 233.2 nm to determine the concentration of drug present in the dissolution medium. The volume was replenished with the same amount of fresh dissolution fluid each time to maintain the sink condition. Linear regression was used to analyze the *in-vitro* release mechanism. The experiment was conducted in triplicate and the average ± S.D was calculated.

1.6.5 SEM[26]

The microcapsules were scanned using scanning electron microscope. The scanning electron micrograph (SEM) of microcapsules showed that microcapsules were spherical in shape with the presence of rough porous polymeric film.

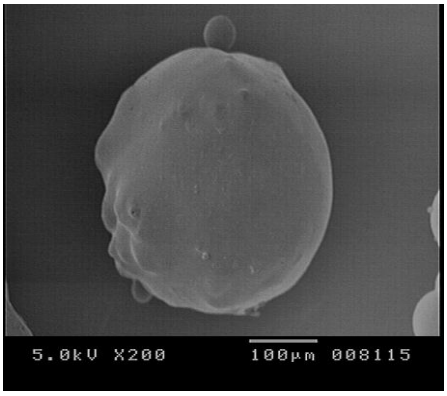

Fig. 6: An intact microcapsule.

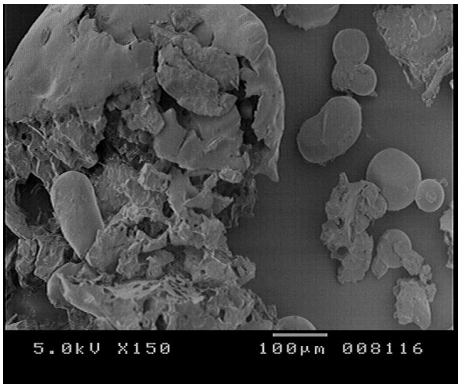

Fig. 7: An empty microcapsule after release of drug contents.

Different batches of sustained release suspension were developed by using microcapsules of Metformin HCl. The concentration of sweetener was kept constant. The formulation was optimized for amount of xanthan gum and talc. The amount of microcapsules equivalent to 500 mg of metformin HCl was used to prepare the dry suspension.

Table 5: Various formulation for optimized microcapsules.

Sr. no	Ingredients	D1	D2	D3	D4	D5	D6
1.	Microcapsules equivalent to Metformin HCl	500mg	500mg	500mg	500mg	500mg	500mg
2.	Sucralose	0.004g	0.004g	0.004g	0.004g	0.004g	0.004g
3.	Xanthan Gum	2%	2%	4%	4%	6%	6%
4.	Talc	5%	10%	5%	10%	5%	5%

1.6.6.1 Evaluation of Formulation

1.6.6.1.1 Angle of repose

Angle of repose (θ) = $\tan^{-1}(2h/d)$.

Where, h is the height of the pile and d is the diameter of the pile. The experiment was done in triplicate.

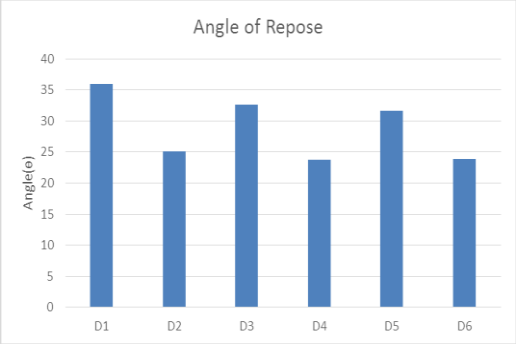

Fig. 8: Angle of repose of all the formulations.

1.6.6.1.2 Sedimentation value

$V_s = H_u / H_o$

H_u = ultimate height of the sediment

H_o = initial height of the total suspension.

The larger this fraction, the better is the suspendability.

The height of the sediment was noted at particular time intervals. The H_u / H_o ratios were obtained and plotted as ordinates with time as the abscissa. The plot just described will at time zero start at 1.0, with the curve then being either horizontal or gradually sloping downward to the right as time goes on. One can compare different formulation and choose the best by observing the lines, the better formulations obviously producing lines that are more horizontal and/or less steep.

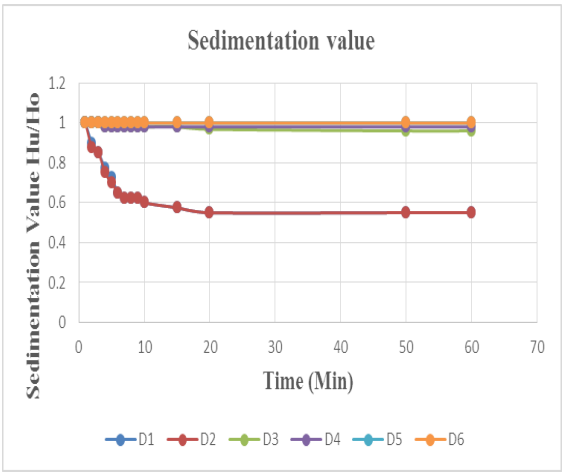

Fig. 9: Sedimentation value for all the formulations.

1.6.6.1.3 Determination of redispersibility

The suspension was allowed to settle in a measuring cylinder. The mouth of the cylinder was closed and was inverted through 180° and number of inversions necessary to restore a homogeneous suspension was determined. If the homogeneity of the suspension was attained in one inversion, then the suspension was considered 100% easily redispersible. Every additional inversion decreases the percentage of ease of redispersibility by 5%.

Table 6: Redispersibility of all the formulation

Formulation	D1	D2	D3	D4	D5	D6
Redispersibility in %	90	90	100	100	100	100

1.6.6.1.4 Rheological study

A Brookfield digital rotational viscometer (Brookfield Engineering Laboratories, Model DV-E) equipped with spindle (No. LV 62) was used to measure the viscosities in cps of the suspension prepared. The measurement was done at ambient temperature. Viscosities were determined in triplicate. Suspensions were poured in to a 100ml glass beaker and the spindle was lowered perpendicularly. The spindle was rotated in the suspension at increasing shear rates 10, 20, 30, 50 and 60 rpm. At each speed, the corresponding dial reading was noted. The reverse readings were also noted and average was taken for these two readings.

RESULT

This suspension exhibits pseudoplastic viscosity of the permanent suspension (Fig 10 to 15) where a decrease in viscosity was observed with increase in shear rate (rpm). The upcurve and the downcurve overlap each other showing no immediate thixotropic nature.

Table 7: Rheological study D1.

D1		
RPM	**Decreasing**	**Increasing**
2.5	**875**	**880**
5	**465**	**478**
10	**298**	**310**
20	**208**	**220**
30	**175**	**198**
50	**133**	**133**
60	**123**	**125**
100	**93**	**99**

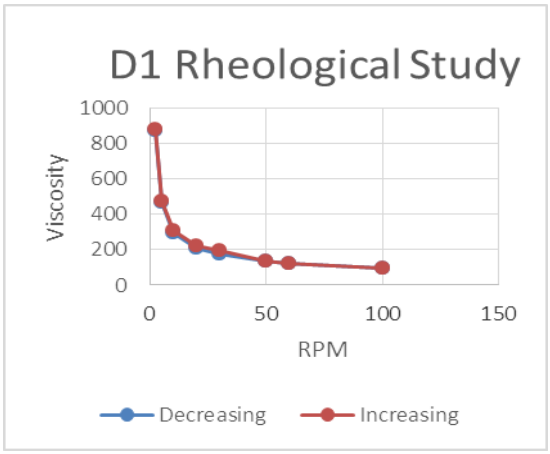

Fig. 10: Rheological study D1.

Table 8:- Rheological study D2.

D2		
RPM	Decreasing	Increasing
2.5	860	910
5	460	460
10	300	290
20	210	198
30	180	168
50	134	132
60	120	120
100	95	96

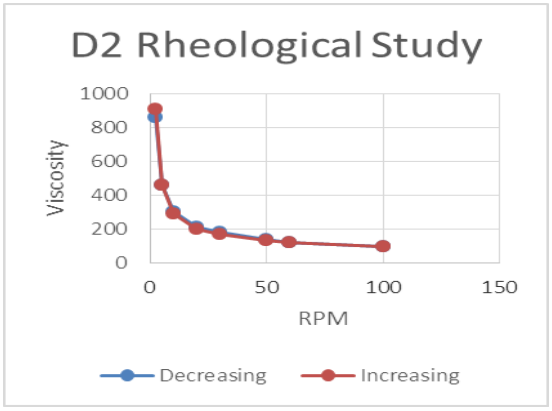

Fig. 11:- Rheological study D2.

Table 9:- Rheological study D3.

D3		
RPM	Decreasing	Increasing
2.5	6000	6000
5	3530	3460
10	2280	2140
20	1506	1380
30	1212	1136
50	875	860
60	782	806
100	564	592

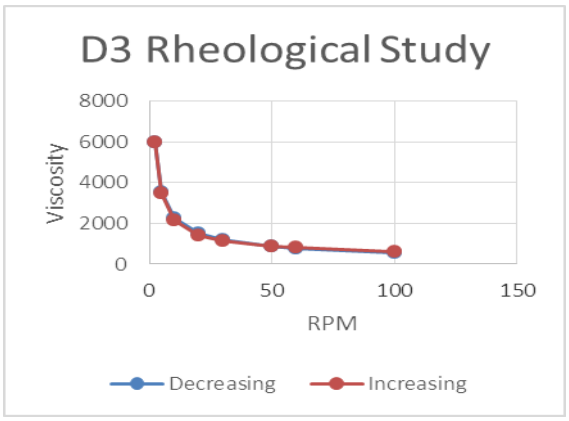

Fig. 12:- Rheological study D3.

Table 10:- Rheological study D4.

D4		
RPM	Decreasing	Increasing
2.5	5950	6050
5	3480	3410
10	2280	2090
20	1488	1386
30	1180	1106
50	866	823
60	790	780
100	577	594

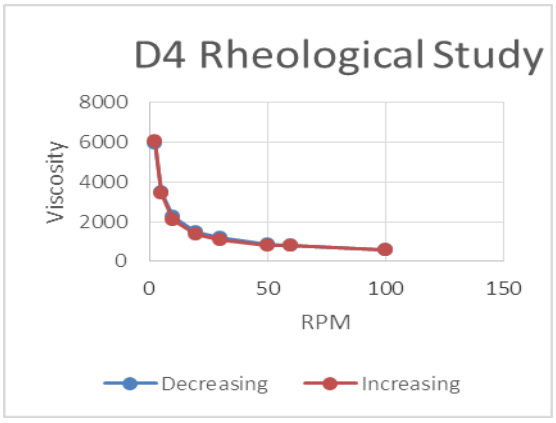

Fig. 13:- Rheological study D4.

Table 11:- Rheological study D5.

D5		
RPM	Decreasing	Increasing
2.5	12190	12190
5	7250	7100
10	4510	4240
20	2886	2640
30	2108	2004
50	1543	1459
60	1332	1276
100	950	1039

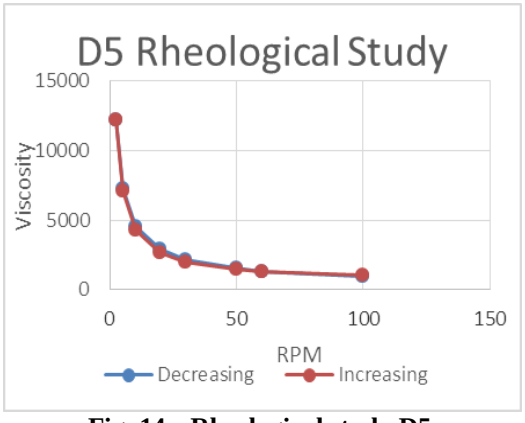

Fig. 14:- Rheological study D5.

Table 12:- Rheological study D6.

D6		
RPM	Decreasing	Increasing
2.5	12220	12210
5	7280	7260
10	4510	4350
20	2690	2580
30	2016	1984
50	1468	1444
60	1280	1216
100	954	980

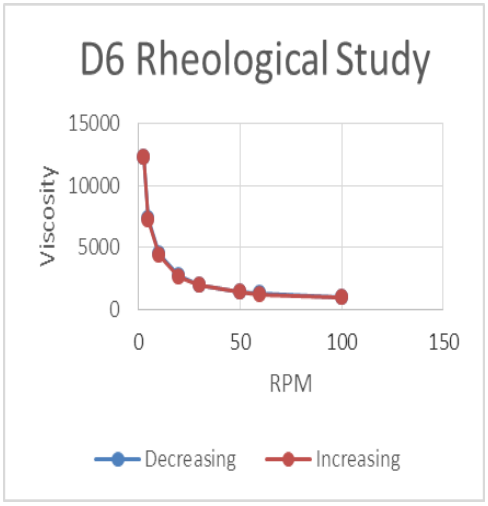

Fig. 15:- Rheological study D6.

1.6.6.1.5 Invitro release study

A USP basket apparatus has been used to study *in-vitro* drug release from microspheres. A weighed amount of microspheres equivalent to 500 mg drug was placed in the basket. Dissolution medium used was phosphate buffer (pH 6.8, 1000 ml) and maintained at 37±0.5°C at a rotation speed of 100 rpm. Prefect sink conditions prevailed during the drug release studies. Five ml of sample was withdrawn at each 1,2,3,4,6,8 & 10 hrs interval. Sample was then passed through a 5 µm membrane filter, and analyzed spectrophotometrically at 233.2 nm to determine the concentration of drug present in the dissolution medium. The volume was replenished with the same amount of fresh dissolution fluid each time to maintain the sink condition. Linear regression was used to analyze the *in-vitro* release mechanism. The experiment was conducted in triplicate and the average ± S.D was calculated. The f2 value for the developed formulation was observed to be 81.

Table 13:- Dissolution study.

Time(hrs)	STD	VESCOP
1	27.60465	23.35216
2	39.10738	35.87076
3	48.40893	47.76146
4	60.33396	60.94267
6	72.3856	73.36752
8	85.3612	85.62403
10	90.33274	91.79528

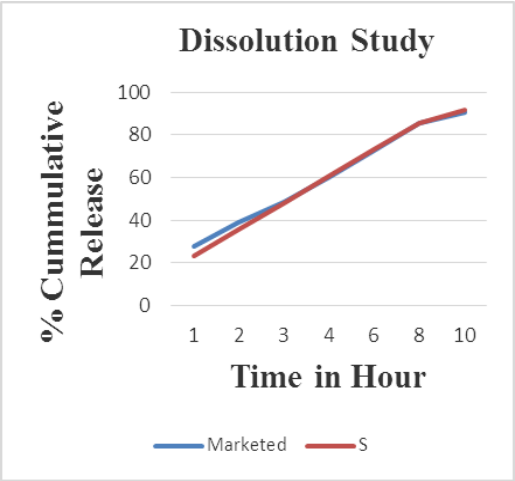

Fig. 16:- Dissolution study.

CONCLUSION

In the present study, a sustained release dosage form of Metformin HCl in the form of microspheres was prepared by solvent evaporation method using polymers, Ethyl cellulose and *EUDRAGIT® S 100*.

Optimization of the factors like Drug to Polymer and the Polymer to Polymer ratio was done using a 2^2 factorial design.

Different batches of sustained release ready to use suspension were developed using microcapsules of Metformin HCl and the developed formulation was found comparable with the marketed formulation.

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