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FORMULATION DEVELOPMENT, CHARACTERIZATION AND INVITRO EVALUATION OF ANTI DIABETIC DRUG FLOATING DRUG DELIVERY SYSTEM

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ABSTRACT

In the present work, the cellulose polymers EC was used to develop floating drug delivery systems which will modify the release of rosiglitazone. Owing to prolonged residence time at absorption site, enhanced bioavailability can be attained. Optimized formulations show satisfactory drug release upto 12 hours. The observed release mechanism from microspheres was diffusion and erosion controlled. The optimized formulation during stability studies does not show any variation in physicochemical properties of microspheres. Thus, successful development of controlled release floating microspheres of RG was achieved during the research work, which signifies the possibility to increase the systemic effect of drug via gastroretentive system on the basis of floatation.

KEYWORD: Floating drug delivery system**,** cellulose polymers, stability, bioavailability.

INTRODUCTION

Oral route is the most preferred route of drug delivery due to ease of administration and greater patient compliance[1], although studies revealed that this route is subject to two physiological influences, a short gastric residence time (GRT) and variable gastric emptying time (GET), which may lead to unpredictable bioavailability and times to achieve peak plasma levels. Furthermore, the brief GET in humans, which normally averages 2-3 h through the major absorption zone (stomach and upper part of the intestine), can result in incomplete drug release from the drug delivery system leading to diminished efficacy of the administered dose. Thus, control of placement of a drug delivery system in a specific region of the gastro intestine (GI) tract offers numerous advantages like improved bioavailability and therapeutic efficacy, local delivery of drug and possible reduction of dose size. All these considerations have led to the development of oral controlled release (CR) dosage forms possessing gastric retention capabilities.

Gastroretentive systems can remain in the gastric region for several hours and significantly prolong the gastric residence of the drugs. Prolonged gastric retention improves bioavailability, reduces drug waste, improve solubility of drugs that are less soluble in a high pH environment. It has application also for local drug delivery to the stomach and proximal small intestine.[2–4]

Repaglinide (Prandin) a member of meglitinide class used orally for secretion of insulin was selected for the present investigation. Repaglinide is indicated only in type II DM as an alternative to sulfonylurea's, or to supplement metformin/long acting insulin. It should be avoided in the liver disease. Repaglinide is the first derivative of meglitinide group developed to normalize the elevated glucose level after meals. It represents fast onset of action with short lasting insulin release.

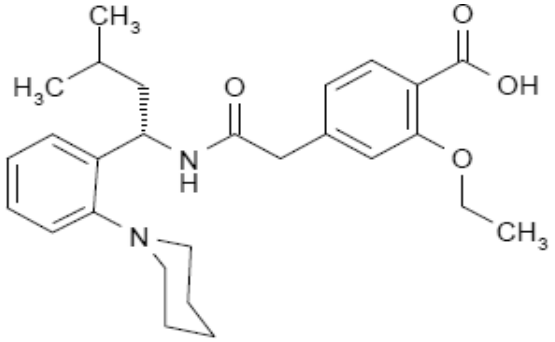


Figure 1: chemical structure of Repaglinide.

EXPERIMENTAL WORK

Materials: Repaglinide was gift sample from Gift sample from Torrent Pharmaceuticals, Ahmadabad, India., Ethylcellulose, Hydroxypropyl methyl cellulose, Polyvinyl alcohol (PVA), Ethanol, Dichloromethane, Distilled water, Polyethylene glycol, Calcium chloride, Concentrated Hydrochloric Acid, Chloroform, Methanol, Acetonitrile, Benzene, Sodium Hydroxide pellets, Octanol, Calcium chloride, Petroleum ether, Potassium dihydrogen, orthophosphate, Di-potassium hydrogen phosphate anhydrous was purchased from CDH Pvt Ltd., New Delhi. And equipment and instruments like Electronic Weighing Balance from Shimadzu Corporation Tokyo, Japan, UV-Vis Spectrophotometer (T60) from PG Instrument, FTIR Spectrophotometer from Shimadzu Corporation Tokyo, Japan, Dissolution Apparatus from LAB India, Magnetic stirrer from Remi industries, Kerala.

METHODOLOGY

Physicochemical Properties (Aulton 1996, Sahitya et al., 2013)

The physical characterization of procured drug sample of repaglinide and polymers was determined as follows:

Organoleptic properties

Drug is characterized for its colour, odour and taste results were reported utilizing descriptive terminology.

Melting Point

Melting point apparatus (Lab-Hosp Corporation, Mumbai) was used to determine the melting point of drug by open capillary method.

Solubility

The solubility of drug and polymers were determined in different polar and non- polar solvents. Fixed quantity of drug and polymers subsequently were added separately to a series of 10 ml solvents in test tubes at room temperature till particles get solubilises. These test tubes containing solutions were vortexed and kept for 24 hours. The observations are recorded as per I.P. 1985.

Partition Coefficient

Partition coefficient of repaglinide was examined in n-Octanol: water system. 5 mg of drug was placed in a separating funnel having 10 ml of octanol and distilled water each. The apparatus was shaked for 2-3 hours on rotatory shaker for equilibration. The concentration of drug in octanol was estimated spectrophotometrically by preparing calibration curve in octanol. The partition coefficient of drug in phases was calculated as:

The Partition Coefficient K=

$$\frac{\text{Amount of drug in organic layer}}{\text{Amount of drug in aqueous layer}} \quad (1)$$

Determaintion of wave length

10 mg of repaglinide was weighed accurately and placed in 10 ml volumetric flask to which 10 ml of methanol was added. 1 ml of the solution from the above stock was taken into a separate volumetric flask to which 10 ml of methanol was added. Same procedure was again followed with this sub-stock to prepare a solution of 10 µg/ml. This solution was then scanned between 200-400 nm in UV-Visible spectrophotometer to determine the absorption maximum Result of UV scan is shown in Fig. 5.1.

Fourier Transform Infra-red spectral analysis

To generate FTIR spectra base line correction of FTIR spectrophotometer (Shimadzu 8400, Japan) was done by taking IR grade potassium bromide previously dried at 40 -50°C. A known amount of drug was thoroughly mixed with potassium bromide and compressed in a hydraulic press under 10-ton pressure to form pellet and was scanned from 4000-400 cm^{-1}. The spectra of other polymers were also obtained using same procedure. Band frequencies obtained are reported in Table 5.4 to 5.7.

Compatibility studies of drug and polymer

Compatibility of drug with different polymers was studied by the following ways:

Physical observation

Using a glass mortar 100 mg of drug is Ethyl cellulose polymer and was triturated for 15 minutes separately. The mixture was packed in closed vials using butter paper and placed in accelerated environmental conditions (40°C/75% RH). Any physical change was observed visually after every week for 4 weeks. The results are tabulated in Table 5.8.

Using FTIR

Drug polymer compatibility was studied by FTIR spectroscopy. Equal amount of drug with polymer separately were thoroughly mixed with potassium bromide and compressed in a hydraulic press under 10 ton pressures to form pellet and was scanned from 4000-400 cm^{-1}. The FTIR spectra's of combination of both were compared with individual spectra of pure drug and polymers.

RESULTS AND DISCUSSION

Organoleptic properties

By visual observation RG is white or almost off white, crystalline, odorless powder. EC is white odorless powder.

Melting Point

RG is having melting point in the range of 128-131° C.

Solubility

The solubility of drug and excipients was determined and reported below in Tabulated in 5.2.

FTIR spectral analysis

FTIR spectra of RG and EC polymer was obtained and shown in Fig. 5.2-5.6.

The observed peaks were identified and were similar to reported reference FTIR spectra (Prajapati et al., 2011). Characteristic peaks are summarized in Table 5.4.

The FTIR spectra were identified and were similar to reported reference spectra (Lakshmi et al., 2013, Patel et al., 2011) for different polymers (EC, HPMC, HPMC K100 and HPMC K4 M) are shown in Table 5.5-5.7.

Drug polymer compatibility studies

Physical observation

Any change in color, formation of lumps or gas and liquefaction was observed during the study. Results of physical observations are shown in Table 5.8. During the studyno mark able variation in the physical properties of drug and polymer were seen. Thus no physical interaction between the drug and polymer resulted.

Using FTIR

Physicochemical compatibility was also studied by FTIR analysis. The characteristic peaks observed in spectra of physical admixture of drug and polymer were almost similar to the peaks of drug alone. There was no major shift in the peaks of repaglinide along with appearance of any additional peaks was observed. Thus the probabilities of chemical interaction were nullified. The FTIR spectra are shown in Fig. 5.7-5.9. The comparative data was reported in Table 5.9.

Determination of λ max

UV-Visible Spectrophotometer (Shimadzu-1700) having 2 nm spectral bandwidth, wavelength accuracy of ±0.5nm and a pair of 1.00 cm matched quartz cells was used for analytical determinations.

Preparation of stock and sub-stock solutions

10 mg of repaglinide was weighed and dissolved in 10 ml of 0.1 N HCl to prepare stock solution of 1000 µg/ml. 1ml of above solution was diluted with 9 ml solvent to form sub-stock of 100 µg/ml.

Preparation of calibration curves

From the prepared sub-stock solution different aliquots were withdrawn using calibrated graduated pipette into a series of volumetric flasks and diluted upto10 ml with 0.1 N HCl resulting in solutions of concentration ranging from 2 - 20 µg/ml respectively.

The calibration standards were analyzed at λ_{max} 247nm by spectrophotometer (Table 5.10) taking 0.1 N HCl as blank. Above procedure was repeated three times. Standard curve was plotted between absorbance and concentration as shown in Fig. 5.10. Slope, intercept and coefficient of regression were calculated.

Preparation of RG Loaded EC Floating Microspheres

Floating microspheres were prepared by slightly modifying solvent diffusion- evaporation method (Kawashima et al., 1992). EC and 0.1% of PEG (as surfactant) both were dissolved in 1:1 mixture of ethanol and dichloromethane at room temperature. Drug was dispersed to this polymeric solution. The slurry was slowly introduced into 80 ml of water containing polyvinyl alcohol emulsifier (0.46% w/v). The system was stirred using propeller agitator for about 1 hour to evaporate the organic solvent. Microspheres prepared were washed properly 3-4 times with distilled water, dried at room temperature for about 1 hour and finally kept in desiccators containing fused calcium chloride. Compositions of different formulations prepared are shown in Table 6.1.

Characterization of EC Floating Microspheres

Micromeritics properties (Aulton, 2002, Jain et al., 2006, Hanna 1990)

The prepared microspheres were characterized for micromeritics properties like particle size, bulk density, tapped density and angle of repose.

Particle size: Optical microscope was used to determine the particle size of prepared microspheres. In distilled water dried microspheres were dispersed and suitably placed on a glass slide. Using stage micrometer the number of divisions of the eye piece was counted. 200 microspheres were randomly selected and there mean particle diameter was measured using calibrated ocular micrometer. Using Edmundson's equation (Rawat et al., 2007) average particle size was determined.

$$D_{mean} = \frac{\sum nd}{\sum n}$$

Morphological study using SEM

Scanning electron microscope was used to study the morphology of prepared microspheres which helps in correlating characteristics features at surface of the samples. SEM is better than light microscope as higher resolution maximum upto 10-20 nm was obtained as compared to 200-300 nm from light microscope. SEM studies were carried out using Jeol JSM-1600, Tokyo, Japan. Prepared microspheres were lightly sprinkled on a double adhesive tape which is fixed to aluminum stubs. A thin layer of gold about 300ºA was vacuum coated using a sputter coater and samples were randomly scanned and photographs were taken. The SEM images obtained were shown in Fig. 6.1.

FTIR spectral analysis

Investigation of any sample by FTIR confirms the chemical integrity between drug and polymer used in formulation. Bruker (Lab India), Germany FTIR Spectrometer was used to obtained the spectra. Scanning range of 400 to 4000 cm^{-1} with resolution of 1 cm^{-1} was selected. Sample was prepared by mixing 1 mg of formulation with 300 mg of dried powder of potassium bromide (FTIR grade) which is then uniformly spread in the die and compressed under vacuum at a pressure under 10 ton. Prepared disc was mounted in the holder in the FTIR spectrophotometer and spectra were recorded. The spectrum is shown in Fig. 6.2. In the

spectra positions and relative intensities of the absorption bands obtained for the pure drug, place bo and RG loaded microspheres were compared and reported in Table 6.5

Percentage Yield (Singhal et al., 2011)
The prepared microspheres of all the batches were accurately weighed. The percentage yield of floating formulations was calculated using following formula:

$$\% \text{ Yield } = \frac{\text{Actual weight of product}}{\text{Total weight of polymer and drug}} \times 100$$

Drug entrapment efficiency
The floating microspheres containing 50 mg of drug from each batch was weighed accurately and crushed. The powdered microspheres were placed in ethanol (10 ml). After 12 hours solution was filtered using whatmann filter paper no. 44. After proper dilution the absorbance of the sample was recorded at 247 nm using UV spectrophotometer and entrapment of drug was estimated by using the formula given below.

$$\% \text{ Drug entrapment} = \frac{\text{Calculated drug content}}{\text{Theoretical drug content}} \times 100 \quad (9)$$

***In-vitro* buoyancy study**
In-vitro buoyancy was determined by placing 50 mg of formulation in 100 ml of SGF (pH 1.2) containing Tween 20 (0.02 w/v %) stirred at 100 rpm using a magnetic stirrer. Layer of floating microspheres were separated from the microspheres which were settled down by filtration after 12 hours. Both the obtained particles were dried and separately weighed. Using the formula given below buoyancy of microspheres was determined.

$$\text{Buoyancy (\%)} = W_f / (W_f + W_S) \text{ X } 100 \quad (10)$$

Where Wf and Ws are the respective weights of the floated and settled microparticles.

The results of percent yield, drug entrapment efficiency and percent buoyancy forall the batches were reported in Table 6.4

***In-vitro* drug release study**
Paddle type dissolution apparatus having six stations (Veego, VDA-6DR, USP Std) was used to determine release of drug from formulation. Floating microspheres equivalent to 16 mg of drug was kept in 0.1N HCl containing Tween 20 (0.02 w/v %). Temperature was maintained at 37±0.5º C with 100 rpm speed of rotation. During the study sink condition was maintained. 1 ml sample was withdrawn at 30 minutes time interval, passed through 5µm membrane filter and analyzed spectrophotometrically at 247nm. The cumulative percent drug release was calculated using standard calibration curve.

Drug release kinetics
Nearly first order drug release profile was observed for swollen hydrophilic matrix systems. For such systems the dissolution of the drug available at the surface shows high release rate initially followed by rapid decline in rate owing to swelling and consequent increasing of the dissolution path-length of the matrix (Narasimhan et al., 1997). Optimized formulation's release data was fitted to various mathematical models to reveal the release mechanism from the microspheres (Martin et al., 2001). All curve fitting, simulation and plotting were performed using commercially available Microsoft excel solver and regression coefficient (r^2) values were calculated. The mathematical models utilized for the study includes:

Zero order release kinetics
In a zero-order process, the rate of diffusion is constant. The differential rate law would take the form Rate= k. This characteristic indicates that the process progresses at the same speed regardless of the concentration of the substance present until the substance is completely consumed. It is represented by following equation:

$$Q_t = Q_o + K_o t \quad (11)$$

Where
Q_t = amount of drug dissolved in time t Q_o = initial amount of drug in solution K_o = zero order release constant.

Zero order graph is the plot of % cumulative drug release versus time.

First order release kinetics
In a first-order process, the rate of diffusion is directly proportional to concentration of drug. The rate law follows Rate = k [A], where k is a rate constant whose units vary depending on the rate order and [A] is the concentration of substance "A." The first- order law shows the progression of physical process and consumption of contained concentration, the diffusion rate decreases with the drop in molecular concentration. It is represented by following equation:

$$\text{Log } Q_t = \log Q_o + K_1 t / 2.303 \quad (12)$$

Where Qt = amount of drug released in time t Q_o = initial amount of drug in solution K_1 = first order release constant.

First order graph is the plot of log % drug release versus time.

Higuchi model
Higuchi developed several theoretical models to study the release of water soluble and low soluble drugs incorporated inside solid or semisolid support. Thus, equation obtained for drug particles dispersed in a uniform matrix behaving as the diffusion medium. The higuchi equation is:

$$Q_t = K_H \text{ X } t_{1/2} \quad (13)$$

Where Qt = amount of drug released in time t K_H= Higuchi diffusion constant.

Higuchi graph is the plot of % cumulative drug release versus square root of time.

Peppas exponential equation

To study this model release data is fitted to the following equation:

$M_t / M = K. t^n$ (14)

M_t / M = Fraction of drug release K = release constant t= drug release time and n = diffusional exponent for the drug release that is dependent on the shape of the matrix dosage form.

It is represented as the plot of log % drug release versus log time (Costa et al., 2001).

Preparation and optimization of EC microspheres

Solvent diffusion evaporation technique was successfully used to prepare floating microspheres of EC. During optimization different ratios of drug and polymer were studied by varying stirring speed, concentration of drug and emulsifier for determination of qualitative and quantitative characteristics of floating microspheres. Drug and EC were dissolved in solution containing equal volume of ethanol and dichloromethane forming organic phase and was finally dispersed into polyvinyl alcohol containing aqueous phase. As the organic phase is added to external aqueous phase it gets partitioned into the two phases and results in complete precipitation of polymer around the drug particle. Continuous stirring of the aqueous phase helps in proper evaporation of solvent and formation of microspheres.

SEM analysis

SEM images shown below indicated that the microparticles prepared were discrete, perfectly good sphere having smooth and dense outer surface. Number of pores and inter- granular spaces are present in the surface of microspheres. The ruptured surface showing hollow nature of microspheres from the interior which helps them to remain buoyant on the GIT fluid (Fig. 2 B).

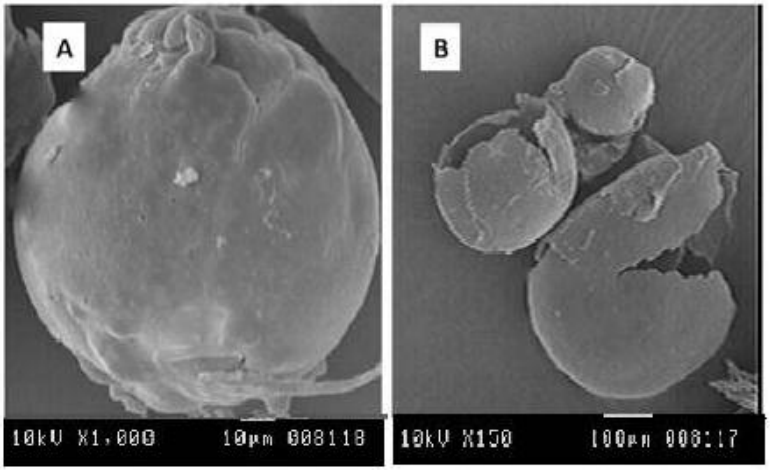


Fig. 2: SEM images: A) Spherical shaped EC microsphere and B) Ruptured surfaceshowing hollow nature of microspheres.

FTIR analysis

The FTIR spectra of drug loaded EC microsphere clearly reveal the presence of characteristic peaks which were not present in the spectra of placebo microsphere (without drug). The results show that neither ethylcellulose nor the process of formulation affects the stability of drug (Fig. 3).

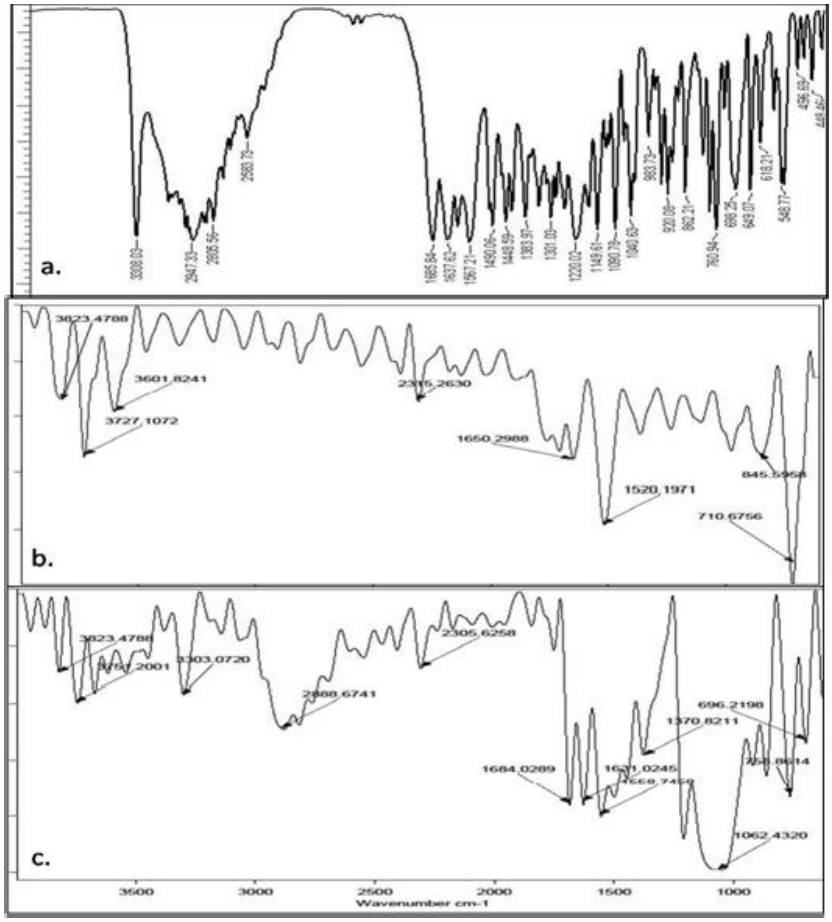


Fig. 3: FTIR spectrum of (a) Repaglinide, (b) Placebo EC microspheres and (c)Drug loaded microspheres.
Effect of experimental variables.

Micromeritics properties

Results show that the mean particle size of the microspheres significantly increases (187±7.2 - 234±10.2 µm) with increase in EC concentration. On increasing the concentration of polymer the viscosity of the medium increases thereby increases the interfacial tension. Larger particles were formed due to increased shearing efficiency at higher viscosities. Increase in stirring speed from 600 to 1200 rpm decreases particle size. Carr's index for all the formulation was found to be in range of 11.25 - 17.97% which indicates the flow to be excellent to good. While the results for Hausner ratio followed the same trend ranging between 1.12 – 1.21 indicating good flowability of microspheres. The angle of repose was observed to be less than 40º implies non-aggregated nature of microspheres and hence excellent flow property (Table 6.3).

Table 6.3: Results of micromeritics properties of EC microspheres.

Batch code	Mean particle size (µm)	Bulk density	Tapped density	Carr's index (%)	Hausner ratio	Angle of repose
E1	187.12± 7.2	0.71± 0.01	0.80± 0.05	11.25	1.12	29.6±5.2º
E2	200.01± 6.1	0.73± 0.09	0.85± 0.03	14.11	1.16	31.2±6.6º
E3	234.21± 10.2	0.74± 0.04	0.87± 0.04	14.94	1.17	33.9±4.4º
E4	243.41± 9.1	0.75± 0.03	0.89± 0.06	15.73	1.18	32.2±4.8º
E5	188.17± 4.6	0.70±0.02	0.82± 0.02	14.63	1.17	29.4±8.7º
E6	185.29± 9.0	0.72±0.02	0.84± 0.01	14.28	1.66	27.2±7.9º
E7	174.41± 11.4	0.71±0.02	0.81± 0.05	12.34	1.48	26.4±0.1 º
E8	235.32± 5.1	0.73± 0.14	0.89± 0.04	17.97	1.21	31.5±8.4º
E9	240.42± 3.9	0.77± 0.21	0.88± 0.05	12.50	1.42	32.2±4.7º

All values are represented as mean±SD (n=3).

Percent buoyancy, yield and entrapment efficiency

Determination of buoyancy is the most important parameter during optimization of formulation. Change in concentration of drug, EC, emulsifier and speed of rotation shows prominent effect in parameters characterized during the study.

The buoyancy of microspheres slightly decreases with increase in concentration of EC (81.3-78.6%). This may be due to increase in particle density as concentration of polymer increases resulting in reduction in the porosity; thus decreasing the buoyancy. As the stirring speed is increased from 600 to 1200 the buoyancy of microparticles decreases sharply from 84.1 to 71.8%.

Increase in concentration of emulsifier does not have much effect on floating property (decreases to lesser extent). On increasing the concentration of drug during formulation from 10 to 30 mg decreases buoyancy from 80.4-73.8%. This may be due to decrease porosity and hydrophobicity of the system as the concentration of EC is reduced.

Entrapment efficiency was good for all the prepared formulations (58.6-73.1%). The high entrapment was obtained owing to poor water solubility of RG. Drug loading have noteworthy effect on particle size distribution of formulations. Larger particles were formed due to high loading of drug. On increasing concentration of polymer resulted in increasing drug loading capacity (63.2-72.3%). The entrapment efficiency was increased when stirring speed was increased from 600 to 900 rpm. However on further increase in stirring speed to 1200 rpm, the entrapment was observed to decrease. This can be attributed to reduced particle size at higher stirring speed.

As the concentration of EC and stirring speed (600 - 900) was increased the yield of microspheres also increases from 68.5 – 79.8% and 72.4 – 76.1% respectively. However further increase in speed to 1200 rpm results in decrease in yield. Increase in the concentration of emulsifier (0.46 - 0.86%) shows no noteworthy effect on yield of microspheres. However slight increase was observed with increasing concentration of drug from 10 to 30 mg. The effect of stirring rate and emulsifier concentration on various parameters are shown in Fig. 6.3 and 6.4 respectively.

Thus on the basis of obtained results it is concluded that formulation E2 with 80.2% buoyancy, 68.0% drug entrapment and 76% yield is the most satisfactory among all the formulations.

Table 6.4: Percent buoyancy, entrapment efficiency and yield of EC microspheres.

Batch code	Buoyancy (%)	Drug Entrapment (%)	Yield (%)
E1	81.31±1.1	63.28±2.4	68.54±4.6
E2	80.41±2.4	68.03±2.2	76.15±2.5
E3	78.67±2.2	72.32±4.1	79.81±3.6
E4	84.31±3.2	65.47±2.2	72.42±2.3
E5	71.81±2.4	60.21±5.1	68.47±5.4
E6	77.12±3.3	62.45±1.5	74.12±2.3
E7	76.53±1.1	58.63±1.7	72.46±1.2
E8	76.24±3.2	71.47±2.2	76.94±3.2
E9	73.48±4.0	73.16±3.3	78.43±2.5

All values are represented as mean±SD (n=3).

Analysis of drug release

An analysis of drug release studies reveals "no initial burst effect" in EC formulations; indicates homogenous distribution of drug (Table 6.6). The release rate of RG decreases from 68.2 – 60.2% as the EC concentration was increased in formulation E1 to E3. As the presence of drug closer to the surface for release is decreased owing to in polymer concentration. The release of drug is not very high, only 70.2% RG was the maximum release, owing to hydrophobic characteristics of EC. Drug release is also less during initial hours of study as the solubility of EC in gastric fluid is poor. Fig. 6.5 shows the controlled release of drug from all the formulations.

Slight increase in rate of release of RG was observed with increase in stirring speed from 600 to 1200 rpm. This may be due to reduced size of the particle with increasing stirring speed, thus exposing large surface area in the medium for drug release. A reduction in particle size is also observed with increasing concentration of emulsifier from 0.46-0.86%. There by release of drug is increased from 65.1-69.6%. Increase in drug concentration does not significantly influence the release of drug from the formulations.

Table 6.6: Results of *in-vitro* drug release from EC microspheres.

Time (h)	Mean % drug released								
	E1	E2	E3	E4	E5	E6	E7	E8	E9
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	4.1±0.2	3.7±0.2	3.7±0.8	3.7±0.5	3.9±0.8	3.8±0.9	4.4±0.8	4.3±0.8	4.6±0.2
2	7.9±0.9	7.4±0.8	9.4±1.9	7.4±0.9	7.6±2.9	7.8±0.4	7.9±0.3	7.8±0.9	7.9±0.5
3	14.8±0.7	14.1±1.2	17.1±2.6	14.1±1.6	14.5±3.0	14.5±0.3	15.8±0.6	14.8±2.1	15.9±0.5
4	21.4±1.5	20.3±0.4	24.1±1.8	20.1±2.8	20.7±2.4	20.7±0.7	21.4±1.3	21.4±1.1	21.4±2.6

5	30.2±1.8	30.5±0.4	29.8±0.5	30±0.7	28.4±0.8	28.2±1.3	31.2±2.4	30.2±1.6	31.2±1.8
6	37.8±2.1	36.2±0.9	35.2±0.7	36.2±0.6	37.1±0.4	37.2±2.4	38.2±1.8	37.8±1.5	38.2±2.4
7	45.4±1.9	45.1±1.2	41±0.1	44.9±0.2	44.5±0.2	44.5±2.3	45.9±1.9	45.2±0.7	46.4±2.6
8	50.9±0.3	50.3±0.7	45.3±0.6	49.3±0.9	49.5±0.6	49.5±1.4	51.5±1.4	50.5±1.6	52±1.5
9	56.1±0.8	56.1±0.6	50.1±0.8	55.1±1.8	53.4±0.1	55.4±0.2	57.1±2.9	56.1±1.8	57.1±2.0
10	61.3±0.7	60.3±0.3	56.3±0.2	60.3±2.6	60.7±0.8	60.3±0.7	63.3±2.6	61.3±2.5	64.3±4.0
11	66.4±0.3	64.4±1.1	58.1±1.9	62.4±3.5	64±0.4	64±0.8	67.4±2.4	66.4±2.6	67.4±0.3
12	68.2±0.8	65.1±1.2	60.2±1.4	63.1±2.6	67.5±0.9	67.1±2.8	69.6±0.5	68.3±2.0	70.2±0.6

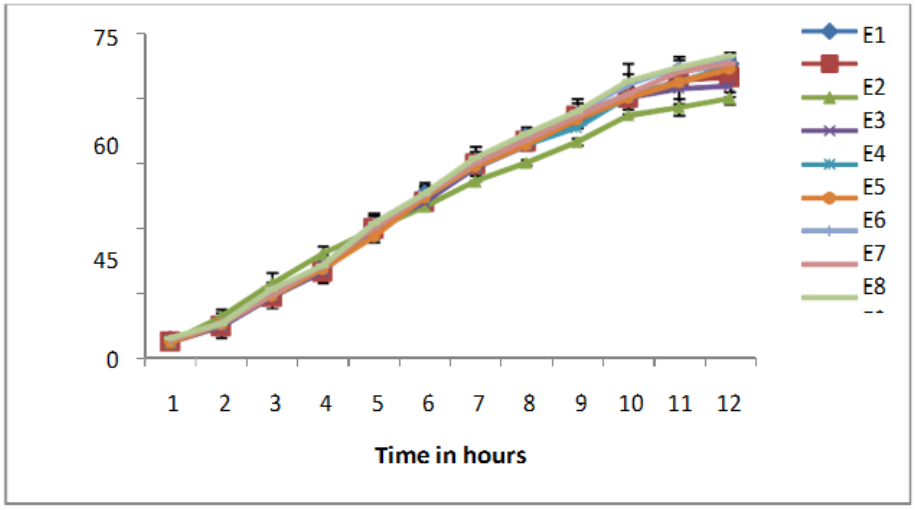


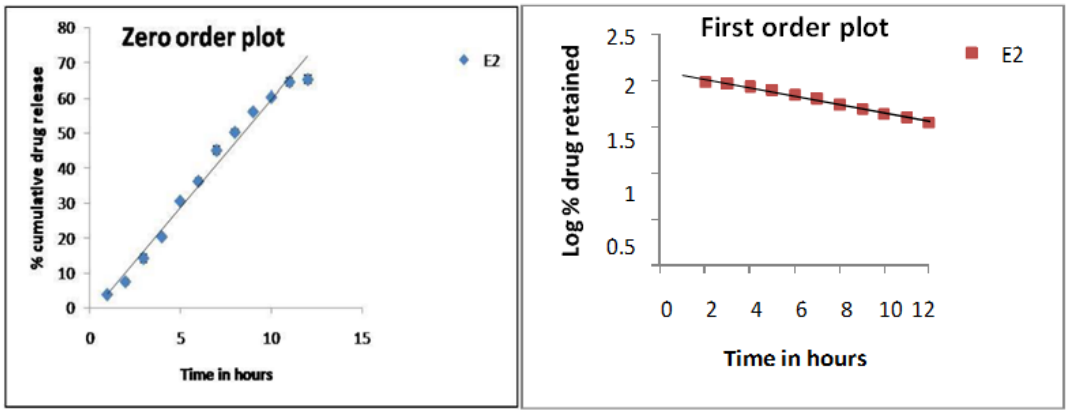
Fig. 6.5: Percent cumulative drug release of EC microspheres.

Kinetic analysis of release

Release data produced were substituted in different equations of zero, first order, Higuchi and Peppas model. The results were interpreted based on the values of regression coefficients obtained. The result in Table 6.7 shows that i*n-vitro* release follows first order kinetics followed by Peppas model. Study by Peppas equation helps to explain the release mechanism. Value of slope (n) was calculated and was found to be ≤ 0.89, suggesting anomalous diffusion which is the coupling of diffusion and erosion mechanism, indicating that rate of drug release is governed by more than one process. Thus, it is concluded that drug release follows first order, diffusion and erosion mechanism.

Table 6.7: r^2 of release of E2 formulation using different kinetic models.

Formulation code	Zero order	First order	Higuchi's	Peppa's
E2	0.9797	0.9899	0.9812	0.9888



SUMMARY

The aim of present investigation is to develop, characterize and evaluate floating microspheres of RG an antidiabetic drug used for treating type II Diabetes Mellitus. The plasma half life of RG is about one hour. Due to short half-life and prime absorption from the gastrointestinal tract (GIT) it is quickly eliminated from blood circulation and thus required frequent dosing. Several side effects emerge due to repeat dosing of RG. Thus, the research work was mainly emphasis on development of floating microspheres to overcome such problems. EC was employed as control release and

swellable polymers for effective release of drug. An attempt is thus, made to microencapsulate RG by solvent evaporation technique with a view to prevent the gastric side effects and to achieve control release of drug. Floating microspheres of EC have been developed which shows good in-vitro buoyancy and release in gastric fluid.

Drug was stable, slightly water soluble whereas soluble in acetonitrile, methanol, dichloromethane and chloroform. Several UV spectrophotometric, colorimetric, chromatographic methods were reviewed in the literature for identification and determination of RG in biological fluids and in various dosage forms. In the present study RG was estimated by UV spectroscopy owing to easiness and feasibility to study number of samples. For in-vitro assessment in 0.1 N HCl 247 nm was selected where maximum absorption of RG was observed. Beer-Lambert's law was obeyed from 0.2 - 2µg/ml concentration range. The value of r2 was calculated to as 0.9990 which indicates positive correlation. Low RSD (< 0.44) values ensured reproducibility of the method during validation of the method.

During preformulation studies organoleptic properties, melting point, solubility, partition coefficient, UV and IR analysis of drug and polymers were performed. The drug and polymers solubility were determined in various solvents as per IP. FTIR spectra of RG, EC was having peaks almost similar to their reported reference FTIR peaks. Compatibility of drug and polymer was studies by visible physical observations for any change in colour, formation of lump or gas and liquefaction. Physical properties of drug and polymer show no significant variation indicating no interaction. FTIR analysis is also utilized to study the physical and chemical compatibility of drug with polymer. The chief characteristic peaks of drug were mostly similar to the peaks obtained in the mixture of drug with EC. No interaction revealed as no significant change and major shifts in the characteristic peaks of drug was observed. Similarly no chemical interaction between RG and polymer was revealed as no additional peaks appeared in any of the spectra studied.

The placebo and drug loaded EC floating microspheres were formulated by solvent diffusion-evaporation method with slight change (Kawashima et al., 1992). The RG loaded microspheres (E1-E9) of EC were prepared using various process variables like polymer ratio, stirring speed, drug and emulsifier concentration. The effect of process variables was observed for different characterized parameters of formulations such as flow behaviords, SEM, FTIR, in-vitro buoyancy, percent yield, drug entrapment and drug release studies.

Microscopic data of SEM indicated that the RG loaded microspheres of different polymers were discrete, spherical shaped, free flowing and uniform. The ruptured surface showing hollow nature of EC microspheres from the interior which helps them to remain buoyant over gastric fluid.

FTIR spectra of drug, placebo and drug loaded microspheres prepared from EC polymer was studied and any change in the functional group frequency of microspheres were compared with frequency obtained in spectra of drug. Obtained results shows that the stability of drug is not affected by the polymers or method of preparation as FTIR spectra of individual batch of microspheres did not show any band shift, broadening or appreance of additional peaks.

The effect of process variables selected was studied and its effect on parameters used for characterization of floating microspheres were observed. The size of particles increases on increasing concentration of EC viscosity at the same ratio. The outcomes of micromeritics properties studied shows excellent flow ability and non- aggregated nature of all the formulations prepared from different polymers. Yield and drug entrapment increases with increase in EC concentration, whereas increase in speed of rotation first increases than decrease the same. Increasing speed of rotation, polymer and drug concentrations resulted in decreased buoyancy in all the categories of prepared microspheres.

The experimental condition of release study was quite similar to the physiological requirement. Release was observed in 0.1 N HCl for 12 hours. Release rate of drug decreases with increasing EC concentration. Drug release increases for microspheres of all the four batches on increasing the speed of rotation from 600 to 1200 rpm.

The in-vitro release data of optimized formulation (E2) of all the batches were fitted to various mathematical models to study the release kinetics. Selected optimized formulation showed highest correlation coefficient for first rather than zero order release equation. Thus, all the RG microspheres were found to follow first order kinetics for drug release. But this model fails to explain drug release mechanism.

Therefore, Krosmeyer- Peppas equation was also studied and value of 'n' was obtained which best describe the release mechanism. Optimized EC microspheres find to follow first order kinetics having r2 = 0.989 followed by Peppas equation whose r2 = 0.988. Finally it was concluded that drug is released by first order, diffusion and erosion mechanism.

CONCLUSION

The cellulose polymers EC was used to develop systems which will modify the release of RG. Owing to prolonged residence time at absorption site, enhanced bioavailability can be attained. Optimized formulations show satisfactory drug release upto 12 hours. The observed release mechanism from microspheres was diffusion and erosion controlled. The optimized formulation during stability studies does not show any

variation in physicochemical properties of microspheres. Thus, successful development of controlled release floating microspheres of RG was achieved during the research work, which signifies the possibility to increase the systemic effect of drug via gastroretentive system on the basis of floatation.

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