ejpmr, 2018,5(7), 333-344

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

FORMULATION AND EVALUATION OF BILAYER TABLETS BEARING FLOATING PMMA MICROSPHERES OF BACLOFEN AND RABEPRAZOLE SODIUM

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Article Received on 29/04/2018 **Article Revised on 19/05/2018** **Article Accepted on 09/06/2018**

ABSTRACT

The present study aims at formulation, optimization and characterization of floating Poly Methyl Methacrylate (PMMA) microspheres of Baclofen and Rabeprazole sodium and to formulate into a bilayer tablet for the management of multiple sclerosis and persistent hiccups. Here, both the drugs are having short biological half-life which limits their therapeutic potential. Gastro Retentive Floating Drug Delivery System is used for increasing gastric residence time of the drugs. Due to its high biocompatibility, PMMA is a widely used polymer. Baclofen and Rabeprazole sodium microspheres were prepared separately by o/w solvent evaporation method. The prepared microspheres of baclofen and rabeprazole sodium were characterized forits percentage entrapment efficiency, percentagebuoyancy, percentage *in-vitro* drug release profile, Scanning electron microscopy study and differential scanning calorimetry. Nine formulations each of baclofen (FB1-FB9) and rabeprazole sodium (FR_1-FR_9) floating microspheres were prepared according to the response surface 3 level factorial design by Design expert 11 trial version. Based on statistical evaluation of Software suggested one optimum batch each for Baclofen and rabeprazole sodium floating PMMA microspheres. The Optimized microspheres of baclofen and rabeprazole sodium were evaluated and formulated into bilayer tablet by direct compression. Sustained release layer of baclofen showed an *in-vitro* drug release of 77.4% and that of rabeprazole sodium exhibited an *in-vitro* drug release of 79.7% in 0.1N HCl at the end of 8^{th} hour *in-vitro* dissolution study. In-vivo floating study of the formulated bilayer tablet was performed in New Zealand rabbit for 6 hours and was well retained in the stomach.

KEYWORDS: Baclofen, Rabeprazole sodium, Floating PMMA microspheres, Bilayer tablet, Multiple sclerosis, Persistent hiccups.

INTRODUCTION

Baclofen is a centrally acting antispastic and muscle relaxant $GABA_B$ agonist, which is indicated for multiple sclerosis and persistenthiccups.[1] Multiple sclerosis is an inflammatory and demyelinating autoimmune disease of the central nervous system. Its characterised by acute attacks and relapse due to new formations of plaque in brain and spinal cord. Baclofen is indicated for the relief of spasm, pain and stiffness. Immunosuppresants and cytokines are used to prevent relapse frequency.[2] Uncontrolled and repetitive muscle contraction of diaphragm is termed as hiccups. Generally hiccups subside after a few minutes of onset. But the hiccups that last for more than 48 hours are considered persistent hiccups.[3]

Multiple sclerosis patients quite often experience a condition called MS reflux or acid reflux. It affects the sphincter muscles at the top of the stomach and cause excess gastric acid to flow back to oesophagus causing severe heartburn and indigestion.[4] One of the major underlying factors of persistent hiccups is the gastritis associated with indigestion. Hence preparation of floating PMMA microspheres of baclofen and rabeprazole sodium[proton pump inhibitor which reduces the excess H^+ concentration in stomach] and formulating it into a bilayer tablet will efficiently reduce the ill effects of acid reflux in multiple sclerosis patients and the gastritis associated with persistent hiccups. Koek, sifrim, lerut et al has emphasized the combination benefits of baclofen and proton pump inhibitors in the management of gastro oesophageal reflux disease.[5]

The present work was aimed to increase the gastric residence time by the incorporation of baclofen and rabeprazole sodium into sustained release floating microspheres and formulation of the floating microspheres into bilayer tablet will reduce the number of dosage forms and improves the patient compliance. Here, both the drugs are having short biological half-life which limits their therapeutic potential. Gastro Retentive Floating Drug Delivery System (GRFDDS) are used for increasing gastric residence time of the drugs. Floating microspheres containing baclofen and rabeprazole

sodium have been prepared to achieve this. The floating microspheres contains gel forming highly swellable cellulosic [ethyl cellulose] and matrix forming polymer [polymethyl methacrylate]. PMMA is a widely explored polymer due to its high biocompatibility. These particulate carriers for oral dosage forms mainly target the GI tract and encapsulation process can enhance GI treatment.[6] Upon contact with gastric fluid, these polymers will form a colloidal gel barrier which controls the fluid penetration into formulation. The density is lowered because of the air entrapped by the swollen polymer and aids the buoyancy.[7] By this process the gastric residence time of both the drugs are increased which improves the bioavailability of the drugs.

MATERIALS AND METHODS

Materials used

Baclofen, Rabeprazole sodium, Ethyl cellulose, Microcrystalline cellulose, Sunset yellow (Yarrow chem. products, Mumbai). PMMA (chemdyes corporation, Gujarat). Talc and magnesium stearate (Nice chemicals, Pvt.Ld, Kochi). All other chemicals/solvents used were of AR grade. The invivo floating study of bilayer tablet in New Zealand rabbit was approved by IAEC. [CPCSEA No and date of registration: CPCSEA No: 499/GO/RE/S/01/CPCSEA, 02/01/2017].

Methods

Pre formulation studies of drug

Determination of melting point

The melting point of baclofen and rabeprazole sodium was found out individually by open capillary tube method using Thiele's apparatus.[8,11]

Determination of solubility

Solubility of baclofen and rabeprazole sodium was performed in Dichloromethane, water, ethanol.[8,11]

Compatibility studies by IR spectroscopy

The FTIR spectrum of baclofen and rabeprazole sodium were analysed to check for any potential physical or chemical interactions. FTIR spectrum of drugs alone, combination of two drugs, and combination of drug-polymer mixtures were taken. The absorption maxima in spectrum were compared with the reference spectrum of baclofen and rabeprazole sodium.[8,11]

Preparation of Floating PMMA microspheres

Nine formulations of floating PMMA microspheres of baclofen FB_1-FB_9 and nine formulations of floating PMMA microspheres of rabeprazole sodium (FR1-FR9) were prepared according to the experimental design suggested by design expert 11-trial version. The microspheres were prepared by oil in water solvent evaporation method. Accurately weighed 200 mg of drugs (Baclofen/Rabeprazole sodium) and ethyl cellulose[EC] were dissolved in 20 ml of ethanol.500 mg of polymethyl methacrylate [PMMA] was dissolved in 20 ml of dichloromethane [DCM] in another beaker. The two resultant solutions were mixed together to form the internal phase. This drug-polymer [EC-PMMA] solution was added drop by drop into aqueous solution containing 1% v/v tween 80 as an emulsifier. The resultant mixture was stirred constantly with the aid of a magnetic stirrer upto 3 hours until the organic solvent DCM evaporated completely. The microspheres obtained were collected after filtration rinsing thrice with water and then dried overnight at room temperature.[12,16]

Evaluation of floating PMMA microspheres of baclofen and rabeprazole sodium

Evaluations of floating PMMA microspheres of Baclofen and Rabeprazole sodium included percentage drug entrapment efficiency, percentageinvitrobuoyancy, in-*vitro* drug release, scanning electron microscopy study [SEM], and differential scanning calorimetry [DSC], optimization studies, pre compression parameters of optimized baclofen and Rabeprazole sodium microspheres.[12,16]

Drug entrapment efficiency

Accurately weighed 50 mg of drug loaded microspheres from each formulation were taken in 2 separate beakers for evaluation. The weighed microspheres were dissolved and made upto 100 ml using 0.1 N HCl. The drug was repeatedly extracted using aliquots of 0.1N HCl upto 8 hours by means of mechanical shaker. The resultant solution was filtered. From the filtered solution 2ml was pipetted and made upto 10 ml using 0.1N HCl. The absorbance were measured at 219.5nm for baclofen microspheres and at 259 nm for rabeprazole sodium microspheres.[17] Percentage entrapment efficiency was calculated by following formula,

Percentage entrapment efficiency= (actual drug loading/theoretical drug loading) ×100

In-vitro buoyancy studies

100 mg of accurately weighed microspheres were spread over the dissolution medium (0.1N HCl) surface which was agitated by a paddle rotated at 100 rpm. The microspheres that remained floated and settled microspheres at the bottom were recovered and dried separately. After drying each fraction was weighed and Percentage *in-vitro* buoyancy was calculated by following formula,

Percentage buoyancy= [Q f /(Q f + Q s)] × 100

Where,

Q fand Q s *are* the weight of floating and settled microspheres respectively.[17]

In-vitro drug release study

Microspheres equivalent to 20 mg of baclofen and rabeprazole sodium 20 mg were taken for the study.

A USP type 2 (paddle) dissolution apparatus was used to study *in-vitro* drug release from the prepared floating microspheres. Accordingly an amount of the microspheres equivalent to 20 mg of drug(s) were filled in a muslin cloth and were placed in the dissolution medium containing 900 ml of 0.1N HCl maintained at

37±0.5º with paddle rotating at 100rpm. Samples of 10 ml were withdrawn at 1,2,3,4,5,6,7 and 8 hours and filtered the solution. Equal volume of plain dissolution medium was replaced for maintaining sink condition. Each of the samples were analysed spectrophotometrically at their respective wavelength against 0.1N HCl as blank.[18] (Baclofen -219.5 nm and rabeprazole sodium-259 nm).

Optimization studies

Response surface methodology using three level factorial design was chosen for the optimization of the prepared floating PMMA microspheres of Baclofen and Rabeprazole sodium. The independent factors were amount of polymer X1, stirring speed X_2. The response variables were buoyancy percent Y_1, drug entrapment efficiency Y_2, % cumulative drug release Y_3. The responses obtained from the design matrix were statistically evaluated using design expert 11, statistical software trial package stat-ease 11.0.5.0.[19] The concentration of polymer and the stirring speed are the independent factors that could affect the percentage drug entrapment; percentage buoyancy and % cumulative drug release are used for the process of optimization.[19]

Development of optimum batch

Based on the statistical evaluations, the software suggested one optimum batch each for Baclofen floating microsphere formulation and rabeprazole sodium floating PMMA microsphere formulation.[19]

Evaluation of the optimized baclofen and rabeprazole sodium floating PMMA microspheres

Drug entrapment efficiency

Percentage entrapment efficiency= (actual drug loading/theoretical drug loading) ×100.[17]

***In-vitro* buoyancy studies**

Percentage buoyancy= [Q f / (Q f + Q s)] × 100

Where,

Q fand Q s *are* the weight of floating and settled microspheres respectively.[17]

***In-vitro* drug release study**

Microspheres equivalent to 20 mg of baclofen and rabeprazole sodium 20 mg were taken for the study.[18]

Scanning electron microscopy (SEM) study

The size of microspheres were recorded under SEM at a magnification ranging from 500x to 10000x and operated at an accelerating voltage of 10 kV.[12,17]

Differential scanning calorimetry (DSC)

Differential scanning calorimetry was performed for baclofen pure drug, rabeprazole sodium pure drug, optimized baclofen microspheres, optimized rabeprazole sodium microspheres.[20]

Pre compression parameters of optimized microspheres

Angle of repose

A funnel is placed 2 cm above from a base and it's held by a stand. Adequate amount of optimized microspheres were transferred to the funnel carefully. The procedure is carried on until the pile of powder touches the funnel tip which is 2 cm above the base.[21] **Angle of repose, tan Θ = h/r.[21]**

Bulk density

3 g of optimized microsphere formulations were weighed and transferred to a 25 ml graduated/measuring cylinder. The volume occupied by the powder mass was noted, **Bulk density=Mass of optimized microspheres / Bulk volume.[21,22]**

Tapped density

3g of optimized microsphere formulation was transferred to a 25 ml graduated/measuring cylinder. The graduated cylinder was placed above a flat surface and performed 500 taps. The volume occupied by the microsphere powder after 500 tapping's were noted, **Tapped density=Mass of optimized microspheres / Tapped volume** (500 taps).[21,22]

Compressibility index

Compressibility index=100 (Tapped density-Bulk density/Tapped volume).[21-23]

Hausner ratio

Hausner ratio, HR = Tapped density / Bulk density.[21,22]

Preparation of sustained release layer of optimized baclofen and Rabeprazole floating PMMA microspheres

Optimized floating PMMA microspheres equivalent to 20 mg of drugs (baclofen/rabeprazole sodium) were taken. This 5 mg of talc and 5 mg of magnesium stearate were added. The total compressible weight was made up to 250 mg by adding sufficient quantity of microcrystalline cellulose, which act as an excellent diluent. Sufficient quantity of sunset yellow FCF (FD and C) was added to the sustained layer of rabeprazole sodium microspheres.[20]

Formulation of Bilayered tablets

Bilayered tablets were prepared by direct compression method.one of the two layers was composed of sustained release layer of floating microspheres of baclofen and the other layer was composed of sustained release layer of rabeprazole floating microspheres. All other ingredients including microcrystalline cellulose, talc and magnesium stearate were mixed with the Baclofen and Rabeprazole loaded microspheres prior to compression. Sunset yellow colour was added to the rabeprazole loaded microspheres. The tablets were compressed using 12.7 mm diameter flat circular punch in multi station compression machine (Karnavati Minipress, India). The lower layer, baclofen loaded microspheres was introduced first and a slight compression was made so

that the layer will be uniformly distributed. After that the second layer rabeprazole sodium loaded microspheres was added and the final compression was made with complete force.[20]

Evaluation of bilayer tablet

The prepared bilayer tablets were evaluated for its thickness and diameter, hardness, friability, weightvariation, in-*vitro* drug release, kinetics of drug release, *in-vivo* floating study in New Zealand rabbit, and stability study.[23]

Thickness and diameter

Thickness and diameter of the bilayer tablet was performed by using micrometer. Least count of the micrometer was found out as

Least count (mm) =Pitch / Head scale reading

Corrected reading (mm) =Main scale reading+ (Head scale reading× Least count)

The thickness and diameter of five tablets were determined.[23]

Hardness

Five formulated bilayer tablets were subjected to hardness test by employing Pfizer hardness tester. The tablets were placed between the anvils of Pfizer tester and pressure was applied. the pressure required to break the tablet is noted. Hardness is given in kg/cm^2.[23]

Friability

Friability test was performed using Roche friabilator. Initial weights of 20 tablets were found out. Then the 20 tablets were placed into the plastic chamber of Roche friabilator and closed the lid. The apparatus was operated at 25 rpm for a total of 100 revolutions. Then the final weights of 20 tablets were determined.[23]

Percentage friability= (initial weight-final weight/initial weight) ×100

Normal range should be less than 0.5-1%

Weight variation test

Randomly selected 20 bilayer tablets for the weight variation study. Weights of individual tablets were found out and the average weights of 20 tablets were calculated.[23]

Percentage weight variation= (difference in weight/average weight) ×100

***In-vitro* drug release study**

The in-vitro dissolution study of bilayer tablet of floating PMMA microspheres of baclofen and rabeprazole sodium were carried out in USP type II dissolution test apparatus(paddle). The drug release study was carried out in 900 ml 0.1 N HCl as the dissolution medium with agitation speed of 100 rpm at 37±0.5º. Samples of 10 ml were withdrawn at1, 2, 3, 4,5,6,7 and 8 hours and filtered the solution. Equal volume of plain dissolution medium was replaced to maintain the sink condition. The samples were analysed for drug release at 219.5 nm and 259 nm in UV spectrophotometer. The amount of drug present in the sample was calculated with the help of simultaneous equation method.[18,24] (Vierodt's method).

$$C_x = A_2 ay_1 - A_1 ay_2 / ax_2 ay_1 - ax_1 ay_2$$

$$C_y = A_1 ax_2 - A_2 ax_1 / ax_2 ay_1 - ax_1 ay_2$$

Kinetics of invitro drug release

In-vitro release data of the two sustained layers of baclofen and rabeprazole sodium were plotted into different kinetic models such as zero order (% CDR Vs time), first order (log % drug retained Vs time), higuchi model(% CDR Vs root time), Korsmeyer-Peppas(log % CDR Vs log time).[25,26]

***In-vivo* floating study**

The *in-vivo* floating study of bilayer tablet in Newzealand rabbit was approved by IAEC. (CPCSEA No and date of registration: CPCSEA No: 499/GO/RE/S/01/CPCSEA, 02/01/2017) 25% of drug loaded microspheres were replaced with $BaSO_4$ for clear visualization of the tablet inside the abdomen of the rabbit. The rabbit was fasted overnight with free access of water. Prior to the tablet administration x-ray image of empty stomach of the rabbit was taken to ensure there weren't any radio opaque substance inside. Tablet was administered orally by natural swallowing with water. X-ray images were taken at 2-hour intervals upto 6 hours.[27,28]

Stability studies

Inthe present study prepared bilayer tablets of baclofen and rabeprazole sodium were monitored upto 60 days at room temperature. After 60 days, the tablets were evaluated for hardness, thickness and diameter, *in-vitro* drug release studies.

RESULTS AND DISCUSSION

Preformulation studies

Table 1: Determination of melting point.

Drug	Melting point
Baclofen	204º
Rabeprazole sodium	136º

Here all the melting point values are in accordance within accepted range. The numerical values are given in table 1.

Table 2: Determination of solubility.

Drug	DCM	Ether	Water	Ethanol
Baclofen	Soluble	Insoluble	Soluble	Soluble
Rabeprazole sodium	Soluble	Insoluble	Soluble	Soluble

Compatibility studies by IR spectroscopy

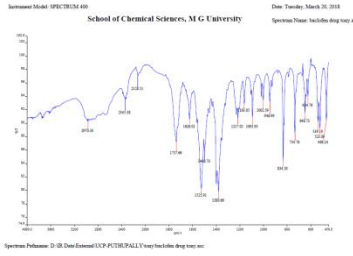

Fig 1

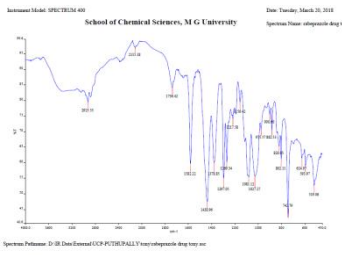

Fig 2

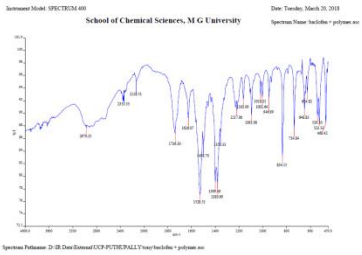

Fig 3

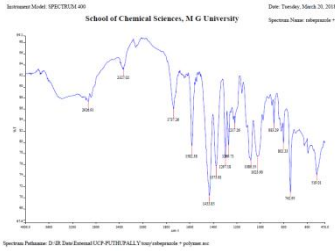

Fig 4

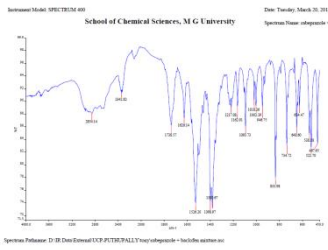

Fig 5

Fig 1 IR spectrum of Baclofen.
Fig 2 IR spectrum of Rabeprazole sodium.
Fig 3 IR spectrum of baclofen-polymer mixture.
Fig 4 IR spectrum of rabeprazole sodium-polymer mixture.
Fig 5 IR spectrum of baclofen-rabeprazole sodium mixture.

All the characteristic peaks of baclofen and rabeprazole sodium were compared with the standard reference spectrum of the respective drugs. Baclofen and rabeprazole sodium showed characteristic peaks which were present in the reference spectrum which ascertains the authenticity of the drugs used in the study. All the characteristic peaks of baclofen were present in baclofen-polymer mixture and that of rabeprazole sodium was present in the rabeprazole sodium-polymer mixture, hence no drug-polymer interactions were observed. The IR spectrum of baclofen-rabeprazole sodium mixture was performed to analyse the presence of any drug-drug interactions. All the characteristic peaks of baclofen and rabeprazole sodium were present in the baclofen-rabeprazole sodium mixture which leads to the inference that no drug-drug interactions were involved.

Preparation of floating PMMA microspheres of Baclofen and Rabeprazole sodium

Table 3: Formulation code for the preparation of Baclofen microspheres.

	FB_1	FB_2	FB_3	FB_4	FB_5	FB_6	FB_7	FB_8	FB_9
Baclofen	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg
EC	400 mg	400 mg	400 mg	500 mg	500 mg	500 mg	600 mg	600 mg	600 mg
PMMA	500mg	500mg	500mg	500mg	500mg	500mg	500mg	500mg	500mg
DCM	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml
Ethanol	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml
Tween 80	1 % v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v
Stirring speed	500 rpm	700 rpm	900 rpm	700 rpm	500 rpm	900 rpm	500 rpm	700 rpm	900 rpm

Table 4: Formulation code for the preparation of rabeprazole sodium microspheres.

	FR_1	FR_2	FR_3	FR_4	FR_5	FR_6	FR_7	FR_8	FR_9
Rabeprazole sodium	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg
EC	400 mg	400 mg	400 mg	500 mg	500 mg	500 mg	600 mg	600 mg	600 mg
PMMA	500 mg	500 mg	500 mg	500 mg	500 mg	500 mg	500 mg	500 mg	500 mg
DCM	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml
Ethanol	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml
Tween 80	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v
Stirring speed	500 rpm	700 rpm	900 rpm	700 rpm	500 rpm	900 rpm	500 rpm	700 rpm	900 rpm

Table 5: Percentage drug entrapment and Percentage buoyancy of baclofen microspheres.

Formulation code	Percentage buoyancy	Percentage drug entrapment efficiency (% DEE)
FB_1	58.5	87.4
FB_2	56.4	86.2
FB_3	54.7	85.1
FB_4	63	91.5
FB_5	65	92.46
FB_6	61	90.6
FB_7	76.5	95.12
FB_8	73	93.62
FB_9	70.5	92.71

Table 6: *In-vitro* drug release of baclofen microspheres.

Time (in hrs)	FB_1 % Drug release	FB_2 % Drug release	FB_3 % Drug release	FB_4 % Drug release	FB_5 % Drug release	FB_6 % Drug release	FB_7 % Drug release	FB_8 % Drug release	FB_9 % Drug release
0	0	0	0	0	0	0	0	0	0
1	17.80	17.43	18.91	17.06	18.73	20.86	22.16	19.93	19.10
2	23.01	22.83	22.36	23.57	25.33	28.03	29.23	23.50	22.92
3	29.25	28.88	28.42	28.23	32.69	31.95	32.60	28.98	27.96
4	36.52	36.06	33.55	36.15	39.59	35.98	37.65	36.52	34.94
5	48.90	44.72	42.96	40.64	49.00	39.87	50.49	49.83	43.33
6	51.09	50.34	46.53	48.94	58.71	48.68	56.67	60.65	54.42
7	56.43	54.20	53.36	54.66	63.59	54.02	66.66	66.28	63.39
8	64.60	62.40	60.10	66.69	68.80	64.30	78.10	77.72	76.33

Table 7: Percentage drug entrapment and Percentage buoyancy of Rabeprazole sodium microspheres.

Formulation code	Percentage buoyancy	Percentage drug entrapment efficiency (% DEE)
FR_1	60	92.71
FR_2	57	93.82
FR_3	54.96	91.5
FR_4	70	94.14
FR_5	71	92.3
FR_6	69.5	93.2
FR_7	73.4	93.7
FR_8	73.8	95.65
FR_9	73	94.5

Table 8: *In-vitro* drug release of Rabeprazole sodium microspheres.

Time (in hrs)	FR_1 % Drug release	FR_2 % Drug release	FR_3 % Drug release	FR_4 % Drug release	FR_5 % Drug release	FR_6 % Drug release	FR_7 % Drug release	FR_8 % Drug release	FR_9 % Drug release
0	0	0	0	0	0	0	0	0	0
1	16.64	19.16	16.10	20.96	18.08	15.56	23.30	27.62	20.06
2	19	25.3	17.74	25.49	22.96	18.10	26.57	29.09	28.19
3	25.32	32.89	22.08	30.37	28.21	20.28	31.64	32.90	39.19
4	35.97	37.97	26.24	39.23	38.68	23.90	40.13	41.76	45.18
5	49.87	50.79	40.49	53.17	51.68	35.99	56.19	56.92	51.53
6	59.10	60.92	53.31	63.62	61.27	50.42	66.15	65.99	60.94
7	69.06	71.43	66.87	72.33	70.34	64.70	73.27	74.16	71.99
8	73	75.3	77.1	76.4	73.4	70.34	78.1	80.72	75.3

Development of optimum batch

Based on the above data, the design expert software suggested an optimum batch for both baclofen floating PMMA microspheres and rabeprazole sodium floating PMMA microspheres.

Table 9: Optimum batch of floating PMMA baclofen microspheres.

Polymer concentration	Stirring speed	% buoyancy	% drug entrapment release(%DEE)	% cumulative drug release (% CDR)	Desirability
600 mg	500 rpm	76.261	94.981	78.419	1.000

Table 10: Optimum batch of floating PMMA rabeprazole sodium microspheres.

Polymer concentration	Stirring speed	% buoyancy	% drug entrapment release(%DEE)	% cumulative drug release (% CDR)	Desirability
600 mg	700	73.371	95.651	80.662	1.000

The optimum batch of floating PMMA baclofen microspheres(Optimized.FB) and optimized Rabeprazole sodium floating microspheres were further evaluated by SEM analysis, DSC analysis, % buoyancy,% DEE,% CDR, Pre compression parameters of optimized microspheres.

Scanning electron microscopy analysis (SEM)

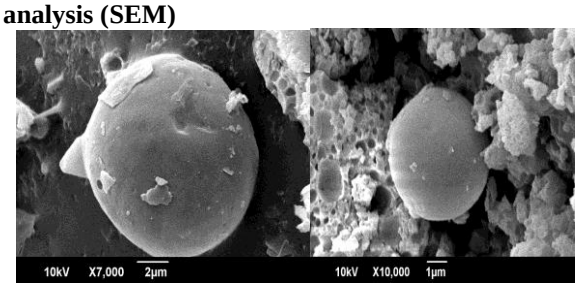

Fig 6 **Fig 7**

Fig 6 SEM image of optimized.baclofen floating PMMA microspheres.
Fig 7 SEM imageoptimized.rabeprazole sodium floating PMMA microspheres.

The surface morphology of both the floating microspheres of baclofen and rabeprazole sodium were studied. The microspheres were spherical in shape and having smooth surface.

Differential scanning calorimetry analysis

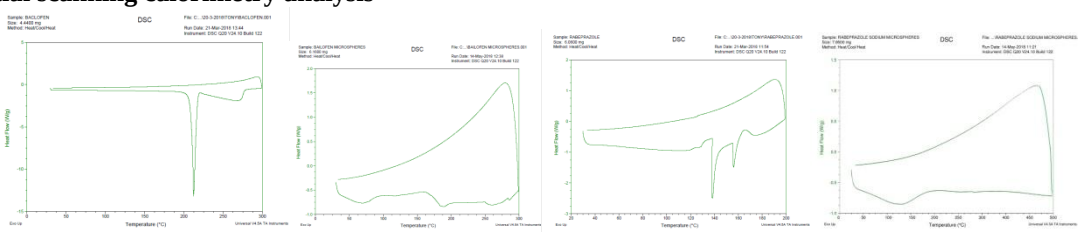

Fig 8 **Fig 9** **Fig 10** **Fig 11**

Fig 8 DSC analysis of baclofen pure drug.
Fig 9 DSC analysis of optimized.baclofen floating PMMA microspheres.
Fig 10 DSC analysis of Rabeprazole sodium pure drug.
Fig 11 DSC anlaysis of optimized.rabeprazole floating PMMA microspheres.

In the DSC analysis of optimized Baclofen floating PMMA microspheres, the sharp endothermic peak at 210ºC has been shifted towards the left side at 190ºC with a broadening of the sharp peak. It indicates the amorphization of the drug and microsphere formation. The sharp endothermic peak at 139ºC indicates the crystalline nature of Rabeprazole sodium pure drug, In the optimized Rabeprazole sodium floating PMMA microspheres, the sharp peak at 139 has been shifted to 135ºC with a decrease in intensity of the peak indicating amorphization of the drug and microsphere is developed.

Table 11: Evaluation of % buoyancy, % drug entrapment efficiency of the optimized floating PMMA microsphere.

Drug	Percentage buoyancy	% DEE=A.L/T.L×100				
		Absorbance nm	Concentration µg/ml	Actual drug loading(A.L)	Theoretical drug loading(T.L)	% Drug Entrapment Efficiency(% DEE)
Optimized.baclofen floating microspheres	76.413 %	0.721 nm	14.66µg/ml	7.33	7.69	95.31 %
Optimized.rabeprazole sodium floating microspheres	73.354 %	0.390 nm	14.58 µg/ml	7.29	7.69	94.80 %

Table 12: *In-vitro* drug release of optimizedfloating PMMA microspheres of baclofen (Optimized.FB) and rabeprazole sodium (Optimized.FR).

Time (in hrs)	Optimized.FB % Cumulative Drug Release. (% CDR)	Optimized.FR % Cumulative Drug Release (% CDR)
0	0	0
1	22.05	27
2	28.82	28.83
3	32	31.56
4	37.89	42.39
5	50.08	57.74
6	56.43	66.80
7	66.40	75.43
8	78.15	81.10

Table 13: Evaluation of pre compression parameters of optimized microspheres of baclofen(Optimized.FB) and rabeprazole sodium(Optimized.FR) including angle of repose, tappeddensity, bulkdensity, carr'sindex, Hausner ratio.

Formulation code	Angle of repose,Θ(˚)	Bulk density(g/cm^3)	Tapped density(g/cm^3)	Carr's index	Hausner ratio
Optimized.FB	**34.82**	**0.2142**	**0.25**	**14.32**	**1.167**
Optimized.FR	**31.1**	**0.333**	**0.4**	**16.75**	**1.201**

Angle of repose of both microspheres comes in the range of 31-35, hence the microsphere formulation is having good flowability. Carrs index value is below 25 for both indicating good flowability.since the Hausner ratio is between 1.19-1.25, the microspheres exhibit good flowability.

Formulation code for bilayer tablet preparation

Table 14: Preparation of sustained release layer using Optimized.baclofen floating microspheres (Optimized.FB) and Optimized.rabeprazole sodium floating microspheres (Optimized.FR).

Formulation code	Microspheres(mg)	MCC(mg)	Talc(mg)	Magnesium stearate(mg)	Sunset yellow
(Optimized.FB)	**136**	**104**	**5**	**5**	**----------**
(Optimized.FR)	**133**	**107**	**5**	**5**	**q.s**
***MCC-Microcrystalline cellulose Total compressible weight=500 mg**					

Fig. 12: Bilayer tablet containing floating PMMA microspheres of baclofen and rabeprazole sodium.

Evaluation of Bilayer tablets

Table 15: Thickness and diameter, Hardness.

Sl no	Thickness (mm)	Diameter (mm)	Hardness (kg/cm^2)
1	4.70	11.82	5.6
2	4.10	11.86	7.2
3	4.90	11.88	6.4
4	4.10	11.80	6.6
5	4.99	11.89	6.2
Mean values	4.558 mm	11.85 mm	6.4 kg/cm^2

Friability test

Initial weight of 20 tablets = 10.250 g, Final weight of 20 tablets after friability test=10.186 g, Percentage friability=0.6243%, the percentage friability was within the permitted limit. Hence, the tablets passed friability test.

Weight variation test

Table 16: Determination of weight variation.

Sl no	Individual weight (g)	Difference in weight from average(g)	% weight variation
1	0.520	0.016	3.174
2	0.502	0.002	0.396
3	0.504	0.000	0.000
4	0.510	0.006	1.190
5	0.488	0.016	3.174
6	0.512	0.008	1.587
7	0.487	0.017	3.373
8	0.516	0.012	2.380
9	0.512	0.008	1.587
10	0.524	0.020	3.968
11	0.506	0.002	0.396
12	0.486	0.018	3.571
13	0.515	0.011	2.182
14	0.510	0.006	1.190
15	0.494	0.010	1.984
16	0.489	0.015	2.976
17	0.511	0.007	1.388
18	0.508	0.004	0.793
19	0.515	0.011	2.182
20	0.484	0.020	3.968
Average weight=10.093/20=0.504 g			

Since the weight of bilayer, tablets were more than 324 mg 5% limit were permitted. All the tablets were within the accepted limit. Hence the tablets passes weight variation test**.**

***In-vitro* drug release study of bilayer tablet**

In-vitro release study of bilayer tablet was performed with the help of simultaneous equation method (Vierodt's method). The percentage cumulative drug release of sustained layer containing optimized baclofen floating microspheres(Optimized.FB) and sustained layer containing optimized rabeprazole sodium floating microspheres(Optimized.FR) were calculated and given in table 17.

Table 17: *In-vitro* drug release study of bilayer tablet.

Time (in hrs)	% cumulative drug release	
	Sustained release layer of Optimized.FB	Sustained release layer of Optimized.FR
0	0	0
1	21.20	25.17
2	28.72	27.91
3	32.98	33.87
4	37.38	38.73
5	50.96	57.86
6	57.71	68.51
7	67.95	76.97
8	77.40	79.70

Kinetics of *in-vitro* drug release

Table 18: Kinetics of *in-vitro* drug release.

Formulations	Zero order	First order	Higuchi	Kosmeyer-Peppas	
	R^2	R^2	R^2	R^2	n
Sustained layer of optimized baclofen floating microspheres(Optimized.FB)	0.9745	0.9414	0.9415	0.946	0.6302
Sustained layer of optimized rabeprazole sodium floating microspheres(Optimized.FR)	0.9619	0.9476	0.9253	0.8815	0.6261

From the table it is evident that the drugs baclofen and rabeprazole sodium are released in a sustained manner over a period of time and shows zero order model. The release exponent n values in Peppas model for both sustained layers were >0.5, but<1 indicating an anomalous or Non-Fickian release, suggesting a coupled erosion-diffusion transport mechanism.

***In-vivo* floating study**

The *in-vivo* floating study of bilayer tablet was done in new Zealand rabbit after getting approval from the IAEC.(CPCSEA No and date of registration:CPCSEA No:499/GO/RE/S/01/CPCSEA,02/01/2017).

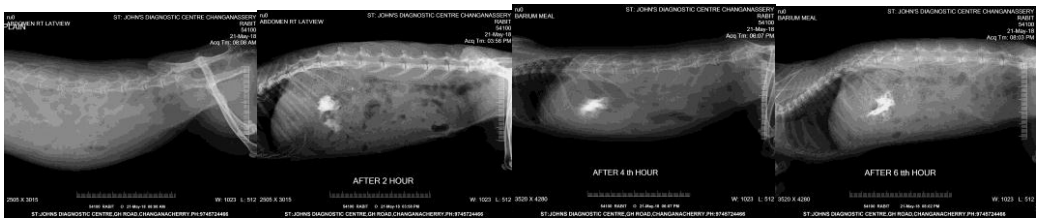

Fig 13 **Fig 14** **Fig 15** **Fig 16**

Fig 13 x-ray image of rabbit's abdomen prior to tablet administration.
Fig 14 x-ray image after 2nd hour of tablet administration.
Fig 15 x-ray image after 4th hour of tablet administration.
Fig 16 x-ray image after 6th hour of tablet administration.

From the x-ray images, the prepared bilayer tablet of floating PMMA microspheres of baclofen and rabeprazole sodium were well retained in the stomach for over a prolonged period providing enhanced gastric retention of drugs.

Stability studies

Table 19: Stability study of bilayer tablet.

No of days stability studies were performed	Physical appearance	Hardness (kg/cm^2)	% cumulative drug release	
			Sustained layer of optimized baclofen floating microspheres(Optimized.FB)	Sustained layer of optimized rabeprazole sodium floating microspheres(Optimized.FR)
0	No change	6.47	77.40	79.7
60	No change	6.21	76.04	77.17

CONCLUSIONS

The combination of baclofen and Rabeprazole sodium will be highly beneficial for the effective management of multiple sclerosis and persistent hiccups. Both drugs are limited to their therapeutic potential due to their short half life. Based on the present study sustained release of both drugs were achieved by developing gastro retentive floating microspheres using polymethyl methacrylate and ethyl cellulose polymers. Enhanced gastro retention of the prepared formulation of floating microspheres will improve the bioavailability.

The drug release from bilayer tablet followed zero order kinetics which ascertains the sustained release of baclofen and rabeprazole sodium. All the prepared bilayer tablets had sufficient mechanical strength. The *in-vivo* floating study in the New Zealand rabbit points out the formulated bilayer tablet was well retained in GI tract for more than 6 hours.

In conclusion, combination of baclofen and rabeprazole sodium floating PMMA microspheres into a single bilayer tablet will be very effective in the management of both multiple sclerosis and persistent hiccups.

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