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Formulation and *in-vitro* evaluation of gastroretentive microballoons of riboflavin

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ABSTRACT

The present study involves the preparation and evaluation of floating microballoons of riboflavin that has narrow absorption window in the upper part of small intestine. By retaining the dosage form in the stomach and controlling the rate of release prior to reaching the absorption window, free drug can be continuously supplied to its absorption site in the GIT. Microballoons of riboflavin were formulated by emulsion solvent diffusion method employing hydroxy propyl methyl cellulose (HPMC K4M) and ethyl cellulose (EC) polymers and characterized for floating behaviour, in-vitro drug release study, surface morphology using scanning electron microscopy, FT-IR, and differential scanning calorimetry (DSC). FT-IR and DSC studies confirmed the absence of interaction among the drug and polymers. Scanning electron microscopy showed that the microballoons were smooth and almost spherical with free flowing characteristics. Optical microscopic studies revealed the average particle size of 147.7±2.54µ.m. The microballoons were found to produce the percentage yield of 76.42±0.35%, drug encapsulation efficiency of 75.92±0.82 and buoyancy of 96.24±0.08% . *In-vitro* release showed cumulative drug release of 87.28±0.294% at the end of 12 hours and the release data found to be zero order super case II transport.

Keywords: Gastroretentive system, Hollow microballoons, Riboflavin, HPMC K4M, Emulsion solvent diffusion method.

INTRODUCTION

Gastroretentive drug delivery systems may provide increased bioavailability for drugs that are absorbed in the upper region of the GIT i.e. narrow absorption window (NAW) drugs by retaining the drug in the stomach and controlling the rate of release prior to reaching the absorption site in the upper GIT. To develop an efficient gastroretentive drug delivery system has proven to be challenging since the system should possess in addition to controlled release properties, an ability to withstand physiological adversities such as repeated peristaltic contractions in the stomach whilst achieving extended gastric retention [1]. It is a novel approach for delivery of different types of drugs with various advantages such as effective retention in the stomach, sufficient drug loading

capacity, controlled drug release profile, full degradation and evacuation after the drug release, no effect on gastric motility including empty pattern, no other local effects.

Rationale for using riboflavin as model drug

Riboflavin (Vitamin B2) was selected as the model compound for this study since it demonstrates absorption mainly in the proximal segment of the small intestine; it is classified as a "NAW" drug. It is used for the treatment of Ariboflavinosis associated with weakness, throat soreness/swelling, tongue swelling (glossitis), angular stomatitis/cheilosis (skin or sores at the corners of the mouth), dermatitis (skin irritation), and anemia. This model drug is advantageous because it lacks adverse effects and has no pharmaceutical effect on gastric motility.

MATERIALS AND METHODS

Riboflavin was gifted by Aurobindo Pharma Ltd, Hyderabad. Eudragit S 100, Eudragit RS 100, were obtained from Evonik degussa India pvt.ltd and Hydroxyl propyl methyl cellulose, Dichloromethane, Tween 80, Poly vinyl alcohol, Glyceryl monostearin, Ethyl cellulose were obtained from S.D. Fine Chemicals Pvt. Ltd Mumbai. All other chemicals and reagents were of analytical grade.

Preparation and Evaluation of Riboflavin floating microballoons

Riboflavin microballoons were prepared by emulsion solvent diffusion method [2]. Accurately weighed quantities of drug (riboflavin) and polymers (HPMC K4M, ethyl cellulose) were dissolved in mixture of ethanol and dichloromethane (1:1 solvent ratio) at room temperature. This solution was poured into 200 mL distill water containing 0.01%v/v Tween 80. The resultant emulsion was stirred with a propeller type agitator at 600 rpm for 25 min to allow the volatile solvent to evaporate. The microballoons formed were filtered, washed with water and dried overnight at room temperature.

FORMULATION AND DEVELOPMENT

Various formulations were prepared using different polymers like eudragit S 100, eudragit RS100, HPMC K4M and ethyl cellulose to obtain satisfactory yield and spheracity of placebo microballoons. The optimized formula of placebo microballoons was used for the further development of drug loaded microballoons to obtain satisfactory yield, morphology entrapment efficiency and drug release characteristics. Compositions of microballoon formulations are given in Table 1.

Table 1: Compositions of various microballoon formulations

Ingredients	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9*	F-10**	F-11***
Riboflavin (g)	-	-	-	-	-	-	-	-	-	-	0.1
Eudragit S 100(g)	1	0.5	0.3	-	0.3	-	-	-	-	-	-
Eudragit RS 100 (g)	-	-	-	0.3	0.3	-	-	-	-	-	-
HPMCK4M (g)	-	-	-	-	-	0.1	0.2	0.3	0.3	0.3	0.3
Ethyl cellulose (g)	-	-	-	-	-	0.1	0.2	0.3	0.3	0.3	0.3
PVA %w/v	0.75	0.5	0.2	0.2	0.2	-	-	-	-	-	-
Glyceryl monostearin (g)	0.5	0.5	0.5	0.5	0.5	-	-	-	-	-	-
Tween 80 %w/v	-	-		-	-	0.01	0.01	0.01	0.01	0.01	0.01
Ethanol:dichloromethane (ml)	8:8	8:8	8:8	8:8	8:8	5:5	5:5	5:5	5:5	5:5	5:5

*r.p.m = 200, stirring time = 5min
**r.p.m = 400, stirring time = 15 min
*** r.p.m = 600, stirring time = 25 min

OPTIMIZATION OF THE FORMULA

The present study is focused on the development of placebo hollow microballoons. Hollow microballoons were prepared using Eudragit S100 as polymer which has been developed earlier [5]. Riboflavin hollow microballoons with satisfactory yield and morphology were prepared using HPMC K4M and ethyl cellulose as polymers. In the present study investigations were made to optimize the preparation of hollow microballoons.Overal 11 Trials were carried out to optimize the formulation.Trial 11 was optimized and the formula was given in the Table 2.

Table 2: Formula for Trial-11 (F-11)

S.no	Ingredient	Use	Percentage used(% w/w)	Actual quantity
1	Riboflavin	Drug	0.87%	0.1g
2	HPMC K4M: ethyl cellulose(1:1)	Polymer	5.24%	(1:1) (0.3g:0.3g)
3	Ethanol	Solvent	35.8%	5ml
4	Dichloromethane	Solvent	57.8%	5ml
5	Tween 80 (0.01%v/v)	Emulsifying agent	0.175%	0.02ml
6	Distilled water	Dispersion medium	-	200ml

RESULTS AND DISCUSSION

Percentage yield of microspheres

The prepared microspheres were collected and accurately weighed. The percentage yields of formulations from F-6 to F-11 were calculated and the yield was found to be in the range of 43-76.4%. The loss of material during preparation of microspheres may be due to process parameters as well as during filtration of microspheres. The percentage yield of riboflavin microballoons was found to be 76.42±0.35% is shown in Table 3.

Table 3: Percentage yield of Formulations

S.no	Formulation code	%yield
1.	F-6(placebo)	43.67±0.42%
2.	F-7(placebo)	56.43±0.38%
3	F-8(placebo)	78.29±0.27%
4.	F-9(placebo)	78.95±0.43%
5.	F-10 (Riboflavin microballoons)	79.31±0.21%
6.	F-11(optimized formula)	76.42±0.35%

Particle size analysis

The mean particle size of formulations from F-6 to F-11 are shown in Table 4 and the range is found to be 126.8±2.26µ.m 163.4±2.52µ.m.The average particle size of riboflavin microballoons was found to be 147.7±2.54µ.m.

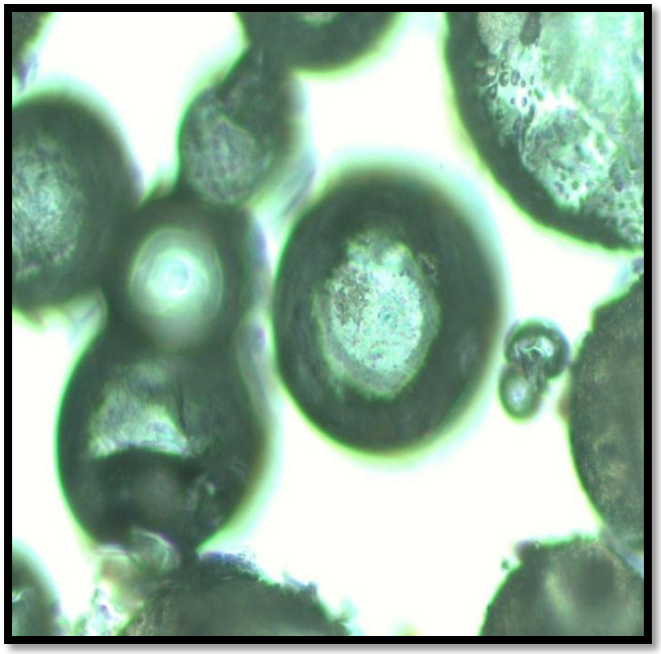

Figure 1: Microscopic image of riboflavin microballoons

Table 4: Results of particle size analysis

S.no	Formulation code	Average particle size(µm)
1.	F-6(placebo)	126.8±2.26
2.	F-7(placebo)	142.3±2.15
3.	F-8(placebo)	163.4±2.52
4.	F-9(placebo)	152.6±2.32
5.	F-10(Riboflavin microballoons)	145.2±2.14
6.	(F-11)(optimized formula)	147.7±2.54

Micromeritic properties

The formulations from F-6 to F-11 were evaluated for micromeritic parameters such as bulk density, tapped density, Carr's compressibility index, Hausner's ratio and angle of repose.

Bulk densities of the formulations were found to be in the range of 0.109±0.014-0.261±0.008.True density was found to be in the range of 0.152±0.015 to 0.293±0.012.

The value of Hausner's ratio for the optimized formulations F-10 and F-11 was below 1.25 which indicates good flow property.

The Carr's compressibility index for optimized formulations (F-10 and F-11) was found be less than 15 which indicates excellent flow property and the formulations from F-6 to F-9 does not show good flow characteristics.

The values of angle of repose for F-11 and F-10 were found to be below 20.16^0C and 18ºC respectively which indicates excellent flow properties of microballoons. The formulation from F6-F-9 showed between 25ºC-30ºC which indicated good flow property. The improvement in flow property for the optimized formulation suggested that the microballoons can be handled easily during processing. The following table shows the micromeritic properties of the formulations

Table 5: Micromeritic properties

Formulation	Bulk density(g/ml)	True density(g/ml)	Hausner's ratio	Carr's index	Angle of repose(^{0}C)
F-6	0.213±0.011	0.287±0.013	1.347±0.012	25.7±0.5	26.23±0.042
F-7	0.182±0.013	0.226±0.012	1.241±0.017	19.4±0.7	26.29±0.049
F-8	0.109±0.014	0.152±0.015	1.394±0.013	28.2±0.5	26.32±0.061
F-9	0.162±0.012	0.205±0.011	1.265±0.012	20.9±0.4	25.24±0.054
F-10	0.215±0.006	0.242±0.017	1.125±0.015	11.1±0.8	20.16±0.043
F-11	0.261±0.008	0.293±0.012	1.122±0.011	10.9±0.6	18±0.056

Scanning electron microscopy (SEM)

Morphology of microballoons was examined by scanning electron microscopy. The view of microspheres showed a hollow spherical structure. The outer surface was smooth and dense, while the internal surface was porous.The SEM images of the optimized riboflavin microballoons are shown in fig 2.

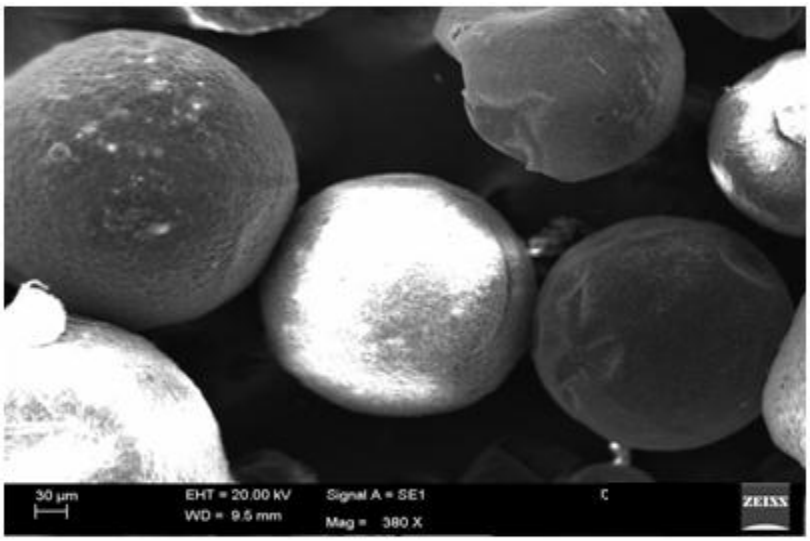

Figure 2: SEM images of riboflavin microballoons

Differential scanning calorimetry (DSC)

DSC curves of the riboflavin, and floating microballoons of riboflavin were recorded using modulated differential scanning calorimeter to find out possible interaction between drug and polymer. The DSC thermo gram of floating microballoons of riboflavin are shown in figure 3.

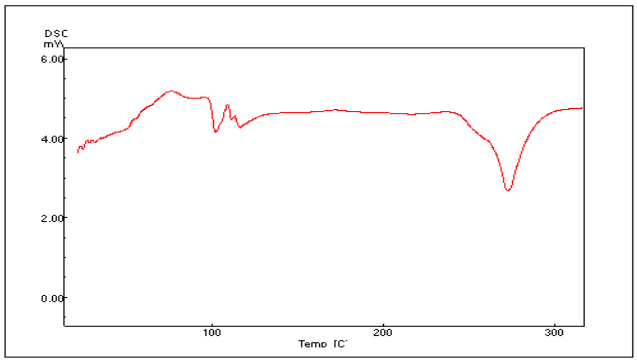

Figure 3: DSC thermogram of floating microballoons of riboflavin

DSC thermogram of pure riboflavin shows sharp peak at 290^0C corresponding to its melting point. The thermogram of riboflavin loaded floating microballoons containing ethyl cellulose and HPMC K4M showed a similar endothermic peak at 290^0C. This confirms there was no polymer drug interaction.

Drug entrapment efficiency/drug loading

Microballoons weighing 30mg were taken for evaluation. The amount of drug entrapped was estimated by crushing the microballoons and extracting with aliquots of simulated gastric fluid (pH 1.2) repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using (SGF). The solution was filtered and the absorbance was measured spectrometrically against appropriate blank. The amount of drug entrapped in the microballoons was calculated by the following formula.

$$\text{Drug entrapment efficiency} = \frac{\text{Amount of drug actually present X 100}}{\text{Theoretical drug load}}$$

$$\text{Drug loading} = \text{Weight } \frac{\text{of drug presnt in microballoons X100}}{\text{Total weight of microballoons}}$$

Drug entrapment efficiency and drug loading of the optimized formulation of riboflavin microballoons were shown in the table 6.

Table 6: Drug loading and Entrapment efficiency

Formulation code	Drug loading	Drug encapsulation
F-11	14.19±0.08%	75.92±0.82

In-vitro buoyancy

The purpose of preparing floating microspheres was to extend the gastric resident time of a drug. Buoyancy test was carried out to investigate the floatability of the prepared microspheres. The microspheres were spread over the surface of a stimulated gastric fluid and the fraction of microspheres buoyant and settled down as function of time was quantified.

Table 7: *In-vitro* buoyancy

S.no	Formulation code	%Buoyancy
1.	F -6(placebo)	71.38± 0.14%
2.	F -7(placebo)	82.64±0.12%
3.	F -8(placebo)	91.27±0.15%
4.	F - 9(placebo)	94.32±0.09%
5.	F -10(riboflavin microballoons)	95.73±0.06%
6.	F 11(optimized formula)	96.24±0.08%

The buoyancy of the formulations from F-6 to F-8 is found to be in the range of (71.32±0.14% to 91.27±0.15%).Increase in the buoyancy of the formulation may be due to increase in particle size of the formulation. Larger the particle size longer the floating time. Buoyancy also increased from F-9 to F-10. *In-vitro* buoyancy of the optimized riboflavin microballoons were found to be 96.24±0.08%.

[13]Reported floating microspheres of ketorolac trometamol highest buoyancy (72%). Further it was observed that in case of ethyl cellulose and HPMC K4M based microspheres, buoyancy was high, as compared with only ethyl cellulose based microspheres.

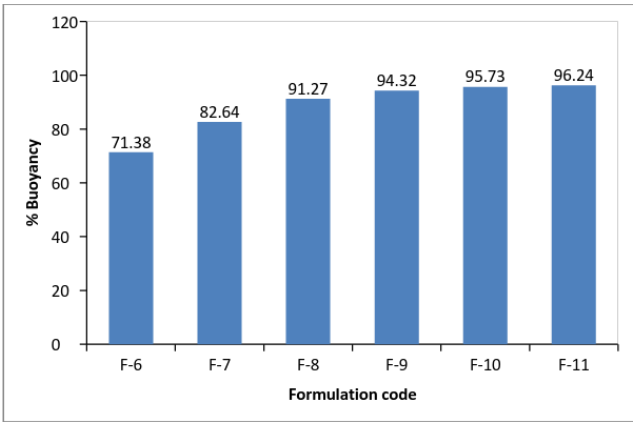

Figure 4: Graph of % *in-vitro* buoyancy

Invitro drug release

Dissolution studies of riboflavin floating microballoons were carried out using a USP paddle type apparatus. *In-vitro* cumulative drug release of optimized riboflavin microballoons is shown in table 8. The cumulative drug release at the end of 12 hours was found to be 87.28±0.294%.

Table 8: In-vitro drug release data of riboflavin from microballoons (F-11)

Time (hrs)	Log T	Cumulative (%) drug Released	Cumulative (%) drug Retained	Log cumulative % drug release	Log cumulative % drug retained	(Cumulative %drug Retained)$^{1/3}$
1	0	7.24	92.76	0.8597	1.9673	4.5267
2.	0.3010	15.83	84.17	1.1994	1.9251	4.3824
3.	0.4771	20.36	79.64	1.3087	1.9011	4.3023
4.	0.6020	29.89	70.11	1.4755	1.8457	4.1234
5.	0.6989	34.76	65.24	1.5410	1.8145	4.0256
6.	0.7781	41.32	58.68	1.6161	1.7684	3.8859
7.	0.8450	48.24	51.76	1.6834	1.7139	3.7267
8	0.9030	55.64	44.36	1.7453	1.6469	3.5399
9.	0.9542	64.31	35.69	1.8082	1.5525	3.2924
10.	1	71.65	28.35	1.8552	1.4525	3.0491
11.	1.0413	76.24	23.76	1.8821	1.3758	2.8748
12.	1.0791	87.28	12.72	1.9409	1.1044	2.3343

Graph of cube root of cumulative % of drug retained versus time resulted in straight line with R^2= 0.957 and the correlation coefficients obtained for Hixson-crowell plot was found to be superior on comparison with R^2 values of Higuchi plot indicating that drug release of riboflavin from microballoons followed anomalous diffusion.

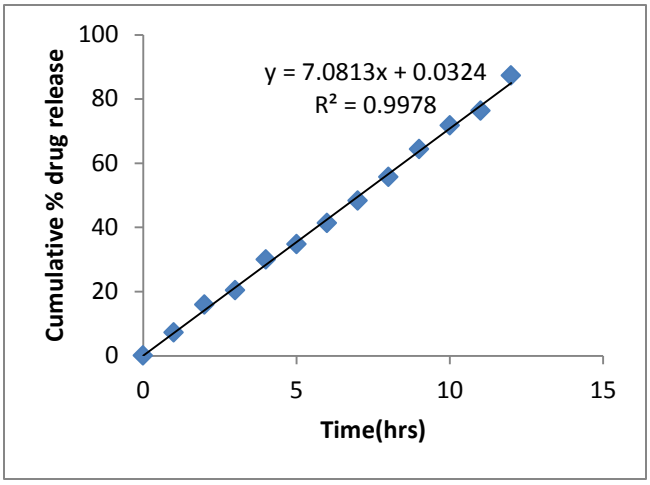

Fig 5: Zero order plot of optimized formulation

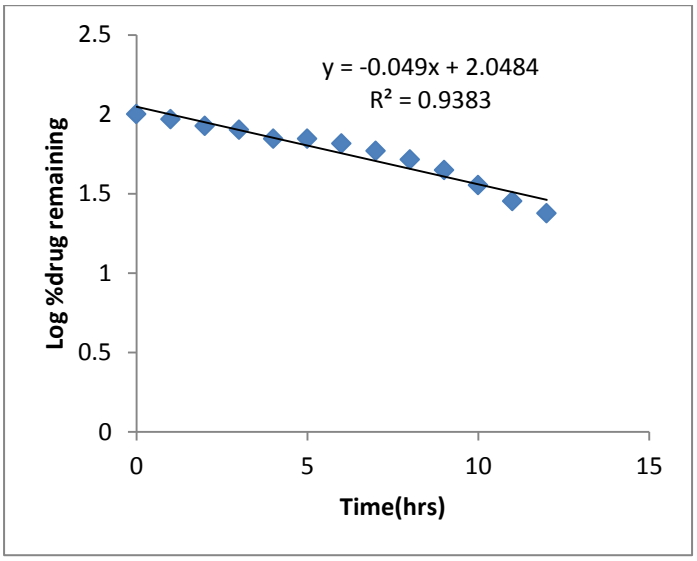

Fig 6: First order plot of (F-11)

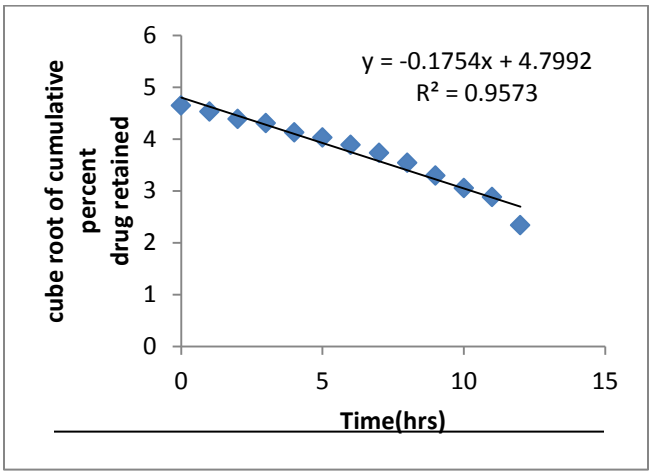

Fig 7: Hixson crowell plot of (F-11)

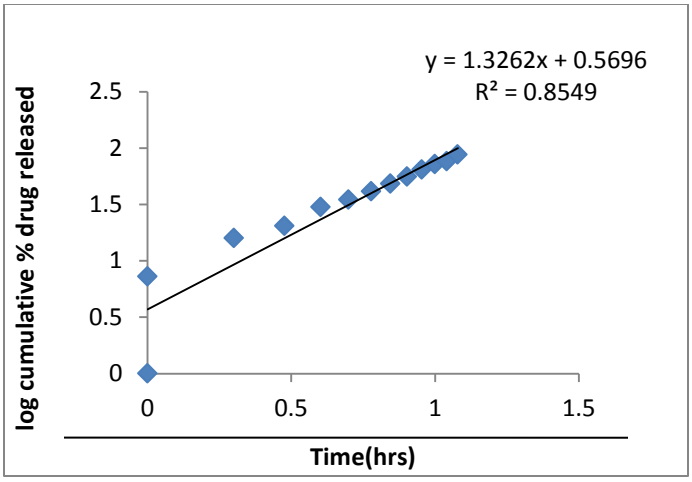

Fig 8: Korsmeyer Peppas plot of (F-11)

To confirm the diffusion mechanism, the data fit into Korsmeyer-Peppas equation, the n value and correlation coefficient obtained for the respective model are 1.326 and 0.854.

[16] Reported the *in-vitro* drug release showed the highest regression coefficient values for peppas model, indicating swelling diffusion to be the predominant mechanism of drug release.

Generally n value < 0.45 indicates fickian release, > 0.89 indicates case II transport and between 0.45-0.89 indicates anomalous or non-fickian drug. In the present study the value of n for release of riboflavin from microballoons followed super case II transport mechanism controlled by swelling of the polymer.

Melting point

The melting point of obtained drug sample was found to be 281ºC, which complies with the one specified in Merck Index, 280ºC.

Compatibility study

Compatibility studies were performed using FT-IR spectrophotometer

Fourier transforms infrared spectroscopy (FT-IR)

The IR spectrum of pure drug, polymers and hollow microballoons of riboflavin were studied making a KBr disc. Spectral measurements were performed using thermo electron FTIR spectrometer at wavelengths 4000cm^{-1} to 400 cm^{-1} to confirm the absence of interaction between drug (riboflavin) and polymers (HPMCK4M, ethyl cellulose). FTIR data and spectrum of riboflavin miroballoons is shown in table 9 and fig 9.

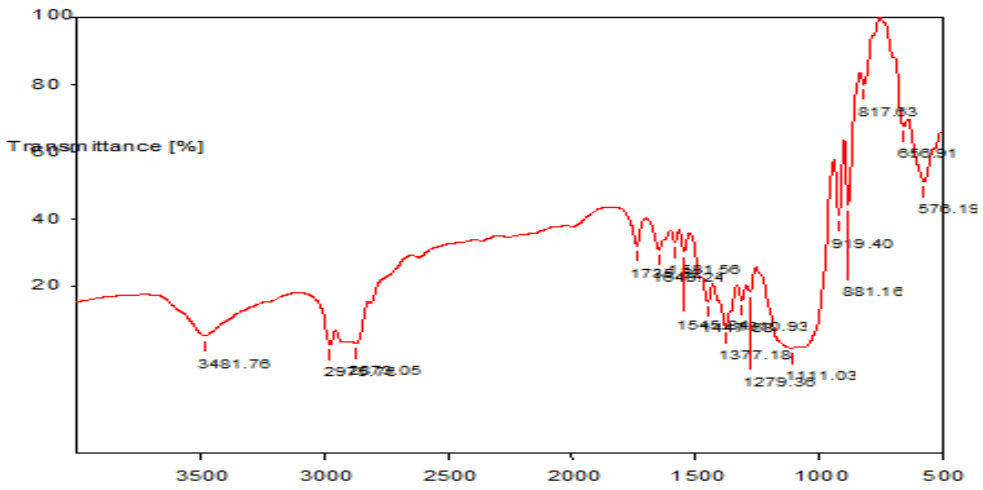

Figure 9: FT-IR spectrum of riboflavin microballoons

Table 9: FT-IR data of riboflavin and riboflavin microballoons

Frequency (in cm^{-1})	**Group**	**Frequency of riboflavin (in cm^{-1})**	**Frequency of riboflavin in microballoons(cm^{-1})**
3100-3500	N-H stretching ,O-H stretching mostly intra molecular H-bonded	3495.95	3481.78
2850-3000	Alkane C-H stretching	2936.08	2975.76
1680-1760	C=O stretching	1732.47	1735.52
1620-1690	C=N	1649.35	1648.24
1400-1600	Aromatic C=C	1580.08	1581.56
1180-1360	C-N stretching	1274.74	1279.36
1000-1300	Alcoholic C-O stretch	1154.65	1111.03
817	Meta-di-substituted aromatic ring ortho-di-sudstituted aromatic ring due to –H moving out of plane of the benzene ring	818.72	817.63

FT-IR spectra of riboflavin microballoons characterized by 3481.78 cm^{-1} due to N-H stretching ,at 2975.76 cm^{-1} corresponding to C-H stretching, at 1735.52 cm^{-1} owing to C=O stretching ,at 1648.24 cm^{-1} due to C=N stretching ,at 1111.03 cm^{-1} corresponding to aromatic C-O stretch and at 817.62 cm^{-1} owing to meta-di-substituted aromatic ring ortho-di-substituted aromatic ring due to –H moving out of plane of the benzene ring. Appearance of characteristic peaks of riboflavin in the formulation i.e riboflavin microballoons indicates no chemical interaction between drug and polymers.

CONCLUSION

Riboflavin loaded novel gastro retentive microballoons were successfully formulated using HPMC K4M and ethyl cellulose as polymers by emulsion solvent diffusion method with satisfactory drug encapsulation efficiency and practical yield. FT-IR spectra and DSC studies of riboflavin gastroretentive microballoons confirmed that the drug is compatible with the polymer used. The obtained microballoons were spherical with hollow cavity and smooth surface morphology.

The flow properties of the microballoons were satisfactory and the particle size analysis revealed that the particles were of the size range of 120-150 µ.m. Further the microballoons exhibited

satisfactory practical yield, drug entrapment, and good floating ability. The release of the drug from the microballoons found to be controlled over a period of 12 hrs and followed zero order and super case II transport.

Therefore the present study has been a satisfactory attempt to formulate gastroretentive microballoons of riboflavin, giving prolonged release of vitamin to upper part of small intestine by retaining the dosage form in the stomach.

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