

Exfoliation Technique of Composing and Depictions of Clopidogrel Bisulphate Afloat Microspheres

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ABSTRACT

BACKGROUND

Gastro retention dosage forms are obligatory for holding the drug in the stomach for area definite drug discharge by afloat technique. Floating microspheres (FM) of Clopidogrel Bisulphate (CB) were fabricated to increase the drug's reach to blood and to show elongated drug release.

METHODS

FM of CB were prepared by the ion gelation (exfoliation) method using ethyl cellulose, carrageenan gum, and sodium alginate. The drug-loaded FM were designed and assessed for their physicochemical characteristics including drug-excipient friendly behaviour by Differential Scanning Calorimetry (DSC) and Fourier Transform Infra-Red (FTIR).

RESULTS

The DSC and FTIR study revealed the compatibility of CB with the excipients used. The percentage yield of FM from all formulations was good and showed satisfactory buoyancy and floating time. % CB release for the formulations was found up to 98.5% (F8) till 10th hour. The release kinetics revealed that CB discharge from the devices was best fitted to Hixson Crowell's model with regression values ranging from 0.887 to 0.989.

CONCLUSIONS

FM with CB can be formulated using sodium alginate, ethyl cellulose and carrageenan gum by exfoliation gelation method.

KEY WORDS

Clopidogrel Bisulphate, Carrageenan, Buoyancy, Discharge

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BACKGROUND

The abundant dosage forms available in the market are of oral as they are said to be safe, expedient, liveness, handling, and wide acceptance by the patients. Retaining the dosage form at the stomach is gaining fascination by the researchers owing to its local action or discharge.

The floating drug delivery system (FDDS) is designed for gastrointestinal retention for a long duration.¹ The FDDS offers a beneficial volume of a drug at the appropriate site and then to preserve the expected drug levels all over the delivery period. A foremost pitfall of oral systems is the uneven absorption of the drug in all the regions of GIT. Multiple doses required for conventional oral systems for maintaining the uniform drug concentration. The attainment of the drug from the delivery system hinges on the degree of absorption through GIT. So, the knowledge of attaining drug absorption demanded in emerging of the Gastro retentive drug delivery system (GRDDS). Gastro retaining of solid dosage forms can be attained by the contrivances of altered density, mucus adhesion, expansion, and unfolding, etc.

The approach of microencapsulation makes all range of drugs like solids/liquids/gases, which can be encircled in a suitable thin coat of polymers. Microspheres are size ranged <1000 m. They are more impressive, as they flow freely, upgraded bioavailability, good entrapment, reduced side effects, and the polymers used in them are of biodegradable behavior. Floating Microspheres (FM) which were prepared with low-density (less than gastric fluids) polymers, which can be retained at the site (stomach) for a sufficiently long time, that makes and becoming interesting for many researchers in these dosage forms.

Clopidogrel Bisulphate (CB), inhibits P2Y₁₂ adenosine 5'-diphosphate (platelet receptors) and shows its anti-platelet activity² used to constrain blood clots in a diversity of circumstances like peripheral vascular issues and clots in coronary artery/cerebral arteries. CB acts on the ADP receptor on the membrane of the platelets. Carboxyl Clopidogrel is activated from Clopidogrel pro-drug by cytochrome P450 (liver) and CYP2C19, leads in diminished cross-linking of the protein fibrin. CB has a t_{1/2} of ~ 8 h and performs by creating a disulfide channel with the platelet receptor ADP. CB is absorbed orally and reaches only to its half owing to deprived water solubility. CB has reported side effects like gastric haemorrhage and CB resistance through continuing therapy. Controlled release of floating CB need is anticipated for a number of reasons viz., better bioavailability and diminished side effects like gastric bleeding, less chance to attaining drug resistance, results in improved patient acquiescence.

The present objective of this investigation was to develop CB exploring FM was primed by exfoliation technique using sodium alginate, Ethyl Cellulose, and carrageenan gum. The intention of this study to retain the formulation and release of CB in the stomach for a sufficiently long time in the targeted region. The prepared FM were explored for their size, the incorporation of efficiency, the buoyancy. The in-vitro release and kinetics of CB discharges from the microspheres.

METHODS

Material

Clopidogrel Bisulphate was gifted by Apotex, Bangalore. Sodium alginate, Ethyl Cellulose, Carrageenan, and Calcium chloride were of Fischer Chemic Ltd, Hyderabad. Double distilled water was used when desired during the work. The slant of equipment involved were digital balance (Vibra Technologies, Bangalore), magnetic stirrer (Remi, Secunderabad), melting point apparatus (Sisco Ltd. Hyderabad), DSC scanner (Perkin Elmer, USA), FTIR (Bruker Alfa II), UV visible spectrometer (Shimadzu-S1210), membrane filter (millipore), probe Sonicator (power sonic, Mumbai) and dissolution test apparatus (Electro Lab USP TDL-081).

Drug Excipient Compatibility Studies

Before fabricating the formulation, the feasibility of CB with the polymers used can be ascertained by DSC and FTIR studies.

Differential Scanning Calorimetry (DSC)

The DSC analyses of CB and formulation blend were performed with the DSC apparatus. Each sample was positioned in an aluminum pan discretely with heating rates of 10 °C/min from 50 – 300 °C under nitrogen environment, with a flow rate of 50 ml/min.

FTIR Study

Typical peaks of CB and CB with an excipient blend were obtained by an FTIR spectrophotometer.

Preparation of FM

The FM of the CB was prepared by the exfoliation technique. The sodium alginate cross-linking polymers were immersed in water for 24 h. The CB has taken in water (10 ml) dissolved and then assorted with the polymer mixture. Later the solution was poured slowly using a 24-gauge syringe into calcium chloride (15%) solution. The formed FM was left for 30 min in the resulted solution with stirring to complete the reaction and to form spherical MS.³⁻⁵ the prepared MS were strained, splashed with distilled water and finally dried in an oven at 45°C. The dried MS preserved in an airtight container. The contents of the MS were charted in Table 1.

Ingredients	Formulations							
	F1	F2	F3	F4	F5	F6	F7	F8
Clopidogrel Bisulphate	75	75	75	75	75	75	75	75
Sodium alginate	10	20	30	10	20	30	10	20
Ethyl Cellulose	-	-	-	10	20	30	10	20
Carrageenan (%)	5	5	5	10	10	10	15	15
Calcium chloride (%)	3	3	3	3	3	3	3	3

Table 1. Formulations of Various FM

Characterization of FM

Determination of Entrapment Efficiency

To determine the incorporation efficiency, 100 mg of FM were taken, carefully ground and suspended in HCl (0.1 N). Subsequently, the contents suspended in the water were maintained by probe sonication (power sonic 505) for 20 min and stirred with a magnetic agitator (Remi) for the whole extraction of the CB from the FM. The subsequent solution was clarified through a 0.45 μ membrane filter (millipore). The CB was dogged by a UV-visible spectrophotometer (Shimadzu-

S1210) at 254 nm. The entrapping percentage was calculated using the following formula.⁶

$$\text{Drug entrapment efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100$$

Particle Size Distribution

Particle size analyses of the FM were performed by sieve analysis in a sieve shaker⁷ with the help of sieve sets ranged from # 16, #20, #30, #40, #60 and #80.

Buoyancy Percentage

The FM (0.3 g) was placed in USP XXIV, type II- dissolution apparatus occupied with 900 ml of 0.1 molar HCl with a surfactant-Tween 80 (0.01 %). The medium was enthused at 100 rpm for 12 h. The floating and a stabilized portion of the FM was recovered separately. The FM were withered and pondered. The buoyancy rate was premeditated as the proportion of the bulk of the FM that endured fluctuating and the total mass of the FM⁸. The percent float was assessed by the given formula.

$$\% \text{ Buoyancy} = \frac{\text{Weight of the FM}}{\text{Weight of the settled FM}} \times 100$$

In Vitro Release Studies

CB discharge from prepared FM done by the USP basket dissolution rate test device. FM of the required quantity was suspended in 900 ml of HCl (0.1 M of pH 1.2). The medium was enthused (100 rpm) and upheld at $37 \pm 0.5^\circ\text{C}$ by maintaining sink conditions⁹. After adequate dilution, the samples were analysed for quantification of the CB at 254 nm using the Shimadzu UV-VIS dual-beam spectrophotometer.

Mechanism of Release

The mechanism of CB release was estimated by fitting CB discharge to the various kinetic equations such as zero-order, first-order, Hixson Crowell's and finding the slope (y) and regression (R²) values of the release profile corresponding to each model.¹⁰⁻¹³

RESULTS

In this investigation, CB floating microspheres were made with Sodium alginate, Ethyl Cellulose, and Carrageenan by exfoliation technique.

Drug-Excipient Compatibility

The CB thermo gram was characterized by a single acute endothermic started at 159.21 °C, gave an optimum peak at 157.01 °C and ended at 163.24 °C, on the other hand, the CB mixture presented the initiation of an endothermic peak at 157.95 °C, optimum at 154.53 °C and ended at 157.99 °C. These thermo grams indicated that a small change to the left when combined with excipients, this could be owing to the CB melting or its conversion into an amorphous form. These thermo grams indicate that there are no signs of incompatibility between CB and excipients. The details of the DSC thermo gram are shown in table 2.

DSC Sample	Endothermic Events (°C)			ΔH Fusion	Inference
	T Onset	T Peak	Tend	Enthalpy (J)	
CB	159.21	157.01	163.24	-189.69	An endothermic peak
CB + excipients	157.95	154.53	157.99	-192.48	A shift in peak to left due to interaction between CB with excipients used
CB-Clopidogrel Bisulphate					

Table 2. DSC Data of Drug and Excipient

The FTIR spectra of CB showed characteristic stretches of 'OH' group at 3450 cm⁻¹, 'CH' Stretch at 2954 cm⁻¹, carboxylic (C=O) at 1752 cm⁻¹, etc., which were also seen in FTIR spectrum of CB-excipient blend indicating no possible incompatibility of CB with the excipients used. The FTIR stretches the values of CB and its mixture with excipients (table 3).

Characteristic Peaks and Stretches	Frequency (cm ⁻¹)	
	Clopidogrel Bisulphate	Clopidogrel Bisulphate + Excipients
OH (Carboxylic)	3450	3452
CH Stretch	2954, 3077, 3119	2959, 3079, 3121
CS-C stretch	2464	2480
Co carboxylic	1752	1784
Ring stretch	1474, 1496	1465
Pyridine methylene wag	1439, 1383	1441, 1392
C-o carboxylic acids	1188	1193
Methylene twist	1298, 1275	1297
C-Cl stretch and bend	1221	1222
Pyridine ring stretch	1154	1155
Pyridine-methylene rock	1068, 1028, 1014, 993	1068, 1025, 1014, 998
Spatial bend	749, 724, 697	745, 723, 694

Table 3. FTIR Spectral Data of Drug and Excipients

Drug Entrapment Efficiency (DEE)

It was observed that the DEE was good in all the FM at the rousing of 500 rpm (table 4). The trapping efficiency was ranged from 66.22 ± 0.32 to $73.73 \pm 0.95\%$ for formulation F1 to F8. The DEE was augmented by hike the polymer concentration in FM. This may be owing to the low solubility of CB in water, which facilitates the diffusion of a part of the CB trapped in the surrounding during the preparation of the FM.

Particle Size Analysis

The size of the FM varied slightly batch to batch owing to the variation in the formula. The size of the FM is shown in table 4. The F1 formulation showed a relatively large percentage of large size (294.31 ± 8.88) and the F8 formulation showed fluctuating FM of relatively small size ($218.84 \pm 5.29 \mu$) as the viscidness of the medium elevated to a higher polymer concentration, resulting in improved interfacial tension. The particle size diminutions were observed by aggressive stirring of calcium chloride solution. The FM were globular with no significant visible irregularity on the surface. The buoyancy percentage was shown in table 4.

The Buoyancy Percentage

The buoyancy rate for all the lots was almost over 70% (for 10 h). The buoyancy average as a percentage has gone from $71.04 \pm 0.64\%$ to $89.36 \pm 5.52\%$. The uppermost % was acquired with the formulation of F8.

Formulation	Yield (%)	Particle Size (µ)	Drug Entrapment (%)	Buoyancy Percentage (%)	Floating Time (h)
F1	54.25±1.20	294.31±8.88	72.21±1.20	71.04±0.64	4.1±0.07
F2	62.53±1.36	272.15±6.35	67.95±0.35	75.26±1.37	4.5±0.06
F3	74.51±2.25	279.35±6.35	70.19±0.68	79.28±1.69	3.5±0.05
F4	79.85±5.21	270.23±7.84	72.25±1.35	85.51±1.27	5.2±0.01
F5	62.52±1.02	284.71±9.67	76.47±0.69	84.22±0.63	9.3±0.15
F6	78.13±2.09	285.91±8.45	68.55±0.54	86.84±0.92	7.5±0.11
F7	68.71±0.98	287.33±4.85	66.22±0.32	72.52±1.35	8.0±0.35
F8	65.65±2.35	218.84±5.29	71.06±0.64	89.36±5.52	9.9±0.29

Values are in mean± SD; Trials made=3

Table 4. Efficiency of FM of Clopidogrel FM

Calibration Curve

The CB concentrations were estimated by the regression equation which produced a slope (y) 0.0131 x - 0.0049 and a regression (R²) value of 0.9899 for the calibration curve of CB in 0.1 N HCl (pH 1.2). The calibration curve was found to be linear. This calibration curve was exploited for the determination of CB discharge from the microspheres. The calibration curve of CB was shown in figure 1.

In-Vitro Dissolution Study

The FM showed a prolonged emission of the CB in an acidic medium and the release of the CB was approximately linear (about 40% of CB was initially released). The CB release was observed to be slow and systematically extended for a prolonged period of time. Furthermore, the release of the CB from the FM matrix was regulated by the discharge retardant polymer used in the formulations. The CB discharge was detected to be unrelenting in a dawdling and unvarying pattern in the presence of ethyl cellulose. This can reduce the total DR from the polymer matrix. Moreover, the smaller FM are designed at a minor polymer level has a wider surface opened for the dissolution medium, which results in a faster DR (figure 2).

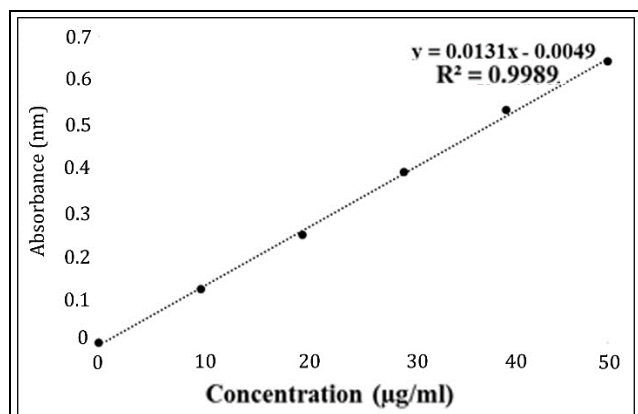


Figure 1. Standard Calibration Curve of CB

Type	Kinetic Data					
	Zero Order		First Order		Hixson Crowell's	
	K (min ⁻¹)	R ²	K (min ⁻¹)	R ²	K (min ⁻¹)	R ²
F1	1.283	0.679	0.033	0.948	0.052	0.989
F2	1.160	0.567	0.019	0.968	0.045	0.957
F3	1.085	0.482	0.011	0.993	0.041	0.972
F4	1.056	0.471	0.017	0.979	0.033	0.978
F5	1.099	0.445	0.033	0.964	0.046	0.887
F6	1.095	0.459	0.035	0.967	0.043	0.966
F7	1.060	0.364	0.017	0.978	0.033	0.978
F8	1.049	0.356	0.013	0.918	0.046	0.889

Table 5. Regression Values and Rate Constants of Different Kinetic Models

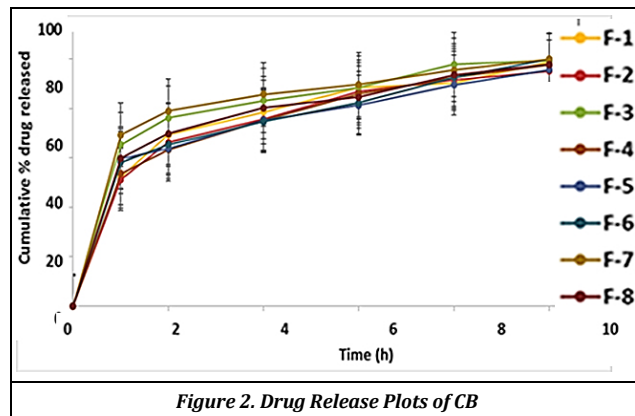


Figure 2. Drug Release Plots of CB

Mechanism of Release

The data gained for in-vitro discharge were snug into equalities for the zero-order, first-order and Hixson Crowell's release models to know the best fit model. The in-vitro DR showed the uppermost recession coefficient reading for Hixson Crowell's model (with the regression values ranged from 0.887 to 0.989), representing diffusion to be the predominant mechanism of DR from the prepared microspheres (table 5).

DISCUSSION

In this research work CB floating microspheres which were made by exfoliation technique and characterized for morphology and drug release profiles. The CB thermo gram produced a single acute endothermic started at 159.21 °C, gave an optimum peak at 157.01 °C and ended at 163.24 °C, on the other hand, the CB mixture presented the initiation of an endothermic peak at 157.95 °C, optimum at 154.53 °C and ended at 157.99 °C. These thermo grams indicated that a small change to the left when combined with excipients, this could be owing to the CB melting or its conversion into an amorphous form. These thermo grams indicate that there are no signs of incompatibility between CB and excipients.

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The FM were globular with no significant visible irregularity on the surface. The buoyancy rate for all the lots

was almost over 70% (for 10 h). The buoyancy average as a percentage has gone from $71.04 \pm 0.64\%$ to $89.36 \pm 5.52\%$. The uppermost % was acquired with the formulation of F8. The CB concentrations were estimated by the regression equation which produced a slope (y) $0.0131x - 0.0049$ and a regression (R^2) value of 0.9899 for the calibration curve of CB in 0.1 N HCl (pH 1.2). The calibration curve was found to be linear. This calibration curve was exploited for the determination of CB discharge from the microspheres.

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The in-vitro DR showed the uppermost recession coefficient reading for Hixson Crowell's model (with the regression values ranged from 0.887 to 0.989), representing diffusion to be the predominant mechanism of DR from the prepared microspheres.

CONCLUSIONS

The study summarizes and confirms the compatibility of clopidogrel bisulphate (CB) with the polymers used. The in-vitro data obtained from the prepared floating microspheres (FM) of CB displayed good incorporation effectiveness, appreciable buoyancy, and lengthy drug release in a controlled manner. The microspheres of different sizes and CB content could be attained by fluctuating the ingredients. The in-vitro DR from the prepared from the CB microspheres revealed that the Hixson Crowell's model is best fitted to the CB release from the prepared microspheres. This experiment concludes that CB can be formulated as FM using sodium alginate, ethyl cellulose and carrageenan gum by ionotropic gelation method. The FM disclosed a better suitable balance amid buoyancy and CB discharge.

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