Probing the effect of various lipids and polymer blends on clopidogrel encapsulated floating microcarriers

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Abstract

Background Clopidogrel (CLOP) is an antiplatelet drug with poor solubility in intestinal fluid, which limits its bioavailability after oral administration.

Objectives Current study focuses on developing site-specific floating microcarriers of CLOP using solvent diffusion evaporation method (SDEM) for retaining the drug in the stomach, thus improving the solubility of drug for better absorption.

Methods SDEM was employed to formulate floating microcarriers using lipidic excipients, namely Gelucires (GL) to impart floating properties, in combination with ethyl cellulose as release retarding polymer.

Results Prepared particles were $169 \pm 6 \mu\text{m}$ to $375 \pm 13 \mu\text{m}$ in size, whilst encapsulation efficiency was ranged from $39.6 \pm 0.60\%$ to $96.50 \pm 3.50\%$. Electron micrographs depicted discrete spherical microcarriers with porous structure, which amplified with increasing HLB value of GL and concentration of Eudragit E100. FTIR study confirmed absence of major drug polymer interactions while DSC and XRD studies revealed the presence of non-crystalline nature of drug in all formulations. Drug release at pH 1.2 enhanced more than 2-folds with increasing HLB value with 32% cumulative drug release for GL 43/01 and 69% for GL 50/13. More interestingly, adding various proportions of Eudragit E100 to GL 43/01 based formulations resulted in increased drug release as high as 71%. In all formulations, the drug release followed diffusion dependent process.

Conclusion It is envisaged that this formulation strategy for CLOP is promising and could possibly be tested in future for its in vivo performance.

Keywords Gastroretentive floating microcarriers · Solvent diffusion evaporation method · CLOP · Gastric retention time · Gelucire

Introduction

A number of hydrodynamically balanced single-unit floating drug delivery systems (FDDS) have been reported in literature [1]. “All or none phenomenon” associated with single-unit drug delivery system is responsible for variable gastric retention time (GRT) and poor bioavailability of drug [2]. In comparison multi-unit dosage forms such as microparticles are beneficial as they can prolong GRT which leads to improved bioavailability [3] and avoids dose dumping, which is often associated with the single-unit delivery systems such as tablets, capsules etc. [4]. Microparticles are small sized multi-unit dosage forms as compared to single-unit systems; the presence of food in gastrointestinal tract (GIT) and GIT motility pose fewer effects on transport of these particles. This obviates the early emptying of FDDS from stomach and an adjustable drug

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release can be obtained. Additionally, the inter-subject variation in absorption of drug and risk of local irritation to gastric mucosa can also be reduced [5]. A variety of multi-unit FDDS such as microparticles, microcapsules, hydrogel beads etc. (5 to 5000 μm) have been developed by microencapsulation technique based on different principles such as, a) porous carrier based microparticles [6], b) oil encapsulating gel beads (prepared by gelation method), c) multiple unit systems with air compartment [7], and d) low density systems [8]. A number of marketed products based on FDDS containing different drugs such as levodopa, diazepam, aluminum magnesium antacid are available [9].

CLOP is used to inhibit the clot formation in cerebrovascular, peripheral vascular and coronary artery diseases [10]. CLOP bisulphate is freely soluble in water but its solubility is strongly dependent on pH of the solubilizing medium. Therefore, oral absorption is maximum in the stomach or upper GIT, however poor water solubility at high intestinal pH decreases its bioavailability to only about 50 % [11]. This warrants a delivery system that can enable CLOP to stay in the upper GIT for longer duration of time. Additionally, 5 to 40% of CLOP treated patients show resistance, which may be due to insufficient absorption or impaired metabolic activation of the drug. Furthermore, its chronic use could lead to gastric bleeding. Surprisingly, in one study, the plasma half-life of active metabolite of CLOP (after oral administration of 300 mg CLOP tablets) was 0.520 h [12]. CLOP irreversibly bound to the receptor on the platelet throughout its life span of 7–10 days. However, approximately 10% of circulating platelets are replaced with newly born platelets every day. Thus, sustained or multiple doses are required for the effective treatment [13]. CLOP exhibits higher cardiovascular safety [14] and is highly recommended for the treatment of secondary cardiovascular events in patients who are either allergic to aspirin or have experienced aspirin induced GIT adverse effects [15]. To the best of our knowledge, tablet dosage forms are available but no study has reported gastroretentive particles of CLOP, therefore, an alternative delivery systems is plausible to enhance the solubility by retaining it into stomach, which may lead to improved bioavailability, reduced side effects of the drug and eventually, increased patient compliance [16].

To this end, we aimed to develop CLOP laden sustained release gastroretentive floating microcarriers to retain the drug in the stomach for longer period of time, which is its major site of absorption and thus may possibly lead to improved bioavailability. To achieve this, hydrophobic polymer (ethylcellulose) and low-density lipids were used to prepare sustained release floating microcarriers. In selected formulation showing limited drug release, pH sensitive polymer (Eudragit E100) was incorporated to modulate drug release

by creating channels. The prepared particles were investigated for drug loading, entrapment efficiency, percentage floating, flow properties, FTIR, DSC, XRD, SEM studies and drug release profile.

Experimental

Materials

Saffron pharmaceuticals (Faisalabad, Pakistan) generously gifted CLOP bisulphate with a minimum purity of 98%. Four types of GL, namely GL 43/01; HLB 1, GL 44/14; HLB 11, GL 48/16; HLB 12, and GL 50/13; HLB 11, acquired from Gattefosse, France were used as low-density lipids. Ethyl cellulose was purchased from Daejung, Korea. Dichloromethane and ethanol were acquired from Merck, and were used without further purification. Polysorbate 80 was purchased from Croda Pvt. Ltd., Singapore. Eudragit E100 was procured from Evonik, Germany. Deionized/distilled water produced at in-house facility was used throughout the experiments. All other chemicals used in research were of standard pharmaceutical grade.

Fabrication of floating microcarriers

CLOP bisulphate loaded floating microcarriers were prepared by solvent diffusion evaporation (SDEM) method (Fig. 1) with minor modifications. Briefly, lipid, polymers and drug were dissolved in 16 mL mixture of organic solvents (dichloromethane and ethanol; 1:1 v/v) and later on introduced into a surfactant solution (0.5% w/v aqueous solution of polysorbate 80) with continuous mixing over a magnetic stirrer at 1000 RPM and room temperature for 1 h. Formed microcarriers were collected by filtration, washed with distilled water and then dried at room temperature overnight. Compositions of floating microcarriers are given in the Table 1.

Fig. 1 Schematic representation of solvent diffusion evaporation process to fabricate microcarriers

Table 1 Composition of gastroretentive floating microcarriers

Ingredients	Formulations						
	CM1	CM2	CM3	CM4	CM5	CM6	CM7
Clopidogrel (mg)	100	100	100	100	100	100	100
Gelucire 43/01 (mg)	450	–	–	–	437.5	425	400
Gelucire 44/14 (mg)	–	450	–	–	–	–	–
Gelucire 48/16 (mg)	–	–	450	–	–	–	–
Gelucire 50/13 (mg)	–	–	–	450	–	–	–
Eudragit E 100 (mg)	–	–	–	–	25	50	100
Ethylcellulose (mg)	450	450	450	450	437.5	425	400

Percent recovery

While preparing microcarriers, a number of mechanical and formulation variables may lead to loss of charge material i.e. active ingredient and matrix forming agent. Hence, it is not possible to get 100% yield. Type of polymer, stickiness of polymer to the container wall or blades, drug content, drug miscibility with the polymer and the external phase, and rate of precipitation of the dispersed phase are some of the critical factors that could affect the % yield [17]. The percent yield of floating microparticles was determined as [18].

%yield =

$$\frac{\text{Weight of dried microparticles}}{\text{Weight of non-volatile ingredients used to prepare microparticles}} \times 100$$

Particle size analysis

The particle size was determined by using ocular micrometer fitted on eyepiece of optical microscope (XSZ-107B (N) China) and calibrated with stage micrometer before use. Fifty microparticles from each formulation were used to determine average particle size.

Floating properties

Floating properties of CLOP loaded microcarriers were estimated by using USP dissolution apparatus II (Rc- 8DS Dissolution tester) [19]. Briefly, 100 mg of particles were dispersed in three different vessels containing 0.1 N HCl solutions (pH 1.2) with surfactant polysorbate 80 (0.02% w/w). The polysorbate 80 was used to get wetting property as provided by phospholipids in GIT. Particles were agitated at 50 rpm and 37 ± 0.5 °C for 12 h. Later both floating and settled microparticles were filtered and dried overnight. The weight of dried particles was determined and percent floating calculated for each formulation with following formula;

$$\% \text{floating} = \frac{Q_f}{Q_f + Q_s} \times 100$$

where,

Q_f weight of dried floating particles

Q_s weight of dried settled particles [20].

Drug loading (DL) and encapsulation efficiency (EE)

Twenty milligram of particles were dissolved in 50 mL of methanol. Afterwards, it was diluted with 50 mL (3:2 v/v) of acetonitrile (ACN) and 0.1% triethylamine (TAE) mixture, sonicated, filtered and examined by HPLC at 225 nm. Amount of drug was calculated by previously constructed standard calibration curve in 0.1 N HCl solution having pH 1.2 [21].

DL was determined by;

$$DL = \frac{\text{Actual contents of drug}}{\text{Particles weight}} \times 100$$

EE was calculated as;

$$EE = \frac{\text{Actual quantity of drug entrapped in particles}}{\text{Theoretical quantity of drug}} \times 100$$

Flow properties

Flow properties of all formulations were analyzed by measuring repose angles, compressibility index and hausner's ratio.

Angle of repose

Repose angles were determined by fixed base cone method [22] and quantified as given below;

$$\tan \theta = \frac{h}{r}$$

where, 'h' and 'r' denote the height and radius of base of pile, respectively [23].

Compressibility index

It is also referred as Carr's index and denoted by "Ci". It was given by following formula;

$$\text{Car's index} = \left(1 - \frac{\text{Bulk density}}{\text{Tapped density}} \right) \times 100$$

Whereas bulk and tapped density were measured by

$$\text{Bulk density} = \frac{\text{Weight of microcarriers}}{\text{Bulk volume}}$$

$$\text{Tapped density} = \frac{\text{Weight of microcarriers}}{\text{Tapped volume}}$$

Ci value <15% indicates good flow properties while >25% indicates poor flow properties [23].

Hausner's ratio

It was determined by formula given below;

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

For free flow properties, the value of hausner's ratio should be preferably <1.2, however value close to 1 also represents good flow properties [22].

Scanning electron microscopy (SEM)

SEM was used to determine the shape and surface morphology of microcarriers. These particles were sputter coated with palladium and examined at accelerated voltage of 10 KV under vacuum (Quanta 250 FEI 500) [24].

Fourier transform infrared spectroscopy (FTIR)

Spectral variations reveal differences in specific bonds or chemicals groups. Drug polymer interaction was studied by comparing FTIR spectra of CLOP bisulphate with drug containing floating microcarriers obtained over a scanning range of 4000–400 cm^{-1} [25].

Differential scanning calorimetry (DSC)

DSC was performed by using Perkin Elmer, Pyris 6 DSC system to further confirm drug polymer interaction and physical state of drug after encapsulation. DSC analysis was performed on drug and prepared formulations sealed in an aluminum pan against reference pan at 10 $^{\circ}\text{C}/\text{min}$ heating rate in an inert environment over a temperature range of 30 to 250 $^{\circ}\text{C}$.

X-ray diffraction analysis (XRD)

The XRD pattern of drug and all formulations were determined by subjecting them in an X-ray diffractometer (XD-D1 Shimadzu Japan), at 2 $^{\circ}$ min^{-1} within the range of 6–60 $^{\circ}$ of 2 θ . The working conditions were CuK α radiation 20KV, 20 mA with a slit of 1–0.3 mm.

Drug release study

Drug release study was carried out by incubating 100 mg of each formulation in USP Apparatus II (RC-8DS Dissolution tester) at 50 rpm. 500 mL of 0.1 N HCl solution (pH 1.2) containing 0.02% w/w polysorbate 80 at 37 $\pm$ 0.5 $^{\circ}\text{C}$ was used as dissolution media. Samples (3 mL) were collected from dissolution vessel at predetermined time intervals by using syringe filter to avoid extraction of particles. Each extracted media was replaced with equal volume of fresh media pre-warmed at 37 $\pm$ 0.5 $^{\circ}\text{C}$. The collected samples were analyzed by HPLC (Auto sampler Shimadzu Isocratic) at 225 nm. Drug concentration was determined by using previously constructed calibration curve. CLOP release studies were performed in triplicate ($n = 3$) for all formulations in an identical manner.

The data obtained from in-vitro drug release studies were analyzed by Korsmeyer-Peppas model [26–28] to assess the drug release mechanism. Korsmeyer-Peppas model is given as [22];

$$Q_t/Q_o = kt^n$$

Where “ Q_t ” is the quantity of drug release at time “ t ”. “ Q_o ” is the initial quantity of drug release, “ k ” is the rate constant. In Korsmeyer-Peppas model, when $n \sim 0.5$, release mechanism is fickian/diffusion controlled, where $0.5 < n < 1.0$ the release mechanism is non-fickian, if $n = 1$, then zero order (case II transport) and if “ n ” is greater than 1.0, it indicates super case II transport drug release mechanism.

Results and discussion

Preparation of floating microcarriers

We successfully employed SDEM technique to prepare lipid/polymer floating microcarriers containing CLOP so as to target its absorption window. This method uses good solvent, bridging solvent and poor solvent such as ethanol, dichloromethane and water, respectively. In brief, the polymer (ethyl cellulose and Eudragit E100), lipid (Gelucire) and drug (CLOP) were dissolved in a mixture of good and bridging solvents. This organic solution was dispersed in an aqueous media containing surfactant (polysorbate 80) with continuous stirring to obtain discrete emulsion droplets. On continuous mixing, the good solvent diffuses rapidly due to its aqueous solubility while bridging solvent remains in core and evaporates slowly. This leads to co-precipitation of drug, lipid and polymers in the form of microcarriers having central hollow cavity surrounded by lipid/polymer blend wall [29]. Organic solvents (dichloromethane and ethanol) used in dispersed phase were selected owing to their low toxicity [30].

Percent recovery

The percent recovery of microcarriers rests in the range of 72.7–82.1% (Table 2). Percentage yield of microencapsulation process depends on method of preparation as well as type of lipid and polymer used. For all the formulations, yield was in order of CM1 > CM3 > CM4 > CM5 > CM6 > CM7 > CM2. This could be related to HLB values of lipids employed. GL having higher HLB values exhibit better aqueous solubility thus resulting in loss of lipid and could also lead to irregular shaped particles. That is why CM1 showed greater yield than the other formulations containing lipids with higher HLB. However, CM2 was found to display lower yield than the CM3 and CM4, despite the fact that these have similar HLB values. This could be attributed to the structural differences among the GL 44/14, GL 48/16, and GL 50/13 which are also referred as lauroyl PEG-32 glycerides, PEG-32 stearate and PEG-32 hydrogenated palm glycerides respectively. Ammar et al., have reported the formation of irregularly shaped shells of microparticles by using GL of high HLB value such as GL 44/14. They ascribed it to increased miscibility of GL with water. In their study lipids of low HLB value formed more compact and regular shaped microparticles with percent recovery [20].

A general trend of slightly decreased percentage yield of microparticles was observed as the ratio of Eudragit E100 (hydrophilic polymer) increased from CM5 to CM7, however, keeping in view the standard deviations of percentage yield, this trend seems insignificantly different. Eudragit E100 is miscible with water and can diffuse to aqueous continuous phase during hardening of microcarriers to decrease their yield. Decrease in percentage yield of microspheres by gradually increasing concentrations of Eudragit E100 was also reported by other authors [31]. Nepal et al. reported an increased percentage yield of microparticles by increasing polymer concentration up to an optimum level after which the yield decreased [32] due to slow hardening of particles during which they stick to vessel walls and also form aggregates.

Particle size analysis

Maximum particle size was obtained for CM7 as shown in Table 2. It may be due to higher viscosity of dispersed phase

with increasing concentration of Eudragit E100, which led to an enhanced interfacial tension. Secondly, shearing efficiency also decreases at higher viscosities [20, 31, 33] and we believe both of these factors were involved in the development of large sized particles. Among the particles prepared without Eudragit E100 with various GL, GL 44/14 resulted in largest particle size (290 μm). This could possibly be related to HLB value of GL 44/14 whose affinity with continuous phase favored loose structure. On the other hand, due to low affinity with the continuous phase, hydrophobic GL 43/01 having lowest HLB resulted in more compacted structure with smaller particle size (169 μm). Furthermore, we assume that with increasing hydrophilicity of GL there is a need of a bigger cavity to encapsulate hydrophobic drug.

Floating properties

Floating studies in simulated gastric fluid revealed that the percent floating values were highest for GL 43/01 (CM1 and CM5) containing formulations as shown in Fig. 2 and Table 2.

The floating behavior could be linked to capsular nature and then to HLB values of GL. In general, % floating decreases with increase in HLB value [20]. GL 43/01 has low HLB (1) and is extremely hydrophobic lipid with a melting point of 43 °C. The highest hydrophobicity of GL 43/01 along with low density (true density 0.0856 g/cm³) is responsible for good floating behavior [34] in comparison to GL 44/14, GL 50/13 and GL 48/16 with HLB values of 11, 11 and 12, respectively. Chauhan et al., prepared residronate sodium floating microparticles by using GL 43/01 and other lipids having higher HLB value. The microparticles prepared by GL 43/01 showed highest floating ability [35].

The hydrophilic property of GL 48/16, GL 50/13 and GL 44/14 is due to the presence of polyethylene glycol chain in their structure and tends to decrease the floating ability of microparticles. The percent floating of microparticles with GL 44/14 was the lowest indicating its structural unsuitability for the formation of floating microparticles. Ammar et al., presented similar observations where microparticles prepared by GL 43/01 showed highest percent floating as compared to one prepared by GL 44/14 and GL 53/01 [20].

Table 2 Particle size, % yield, % floating, drug loading & % encapsulation efficiency of microcarriers. Mean $\pm$ S.D.; $n = 3$

Formulation	Particle size (μm)	Yield (%)	Floating (%)	DL (%)	EE
CM1	169 $\pm$ 6	82.1 $\pm$ 5.2	93.37 $\pm$ 0.07	5.37 $\pm$ 1.90	53.71 $\pm$ 1.50
CM2	290 $\pm$ 9	72.7 $\pm$ 2.4	49.94 $\pm$ 3.85	3.96 $\pm$ 1.10	39.60 $\pm$ 0.60
CM3	230 $\pm$ 8	81.0 $\pm$ 3.7	60.52 $\pm$ 2.07	7.29 $\pm$ 0.50	72.94 $\pm$ 1.90
CM4	275 $\pm$ 17	80.5 $\pm$ 5.7	59.71 $\pm$ 1.42	7.99 $\pm$ 1.60	79.94 $\pm$ 0.90
CM5	313 $\pm$ 11	77.3 $\pm$ 8.1	89.80 $\pm$ 3.85	9.09 $\pm$ 1.30	90.87 $\pm$ 2.60
CM6	356 $\pm$ 7	75.8 $\pm$ 7.9	56.64 $\pm$ 4.12	9.32 $\pm$ 1.50	93.23 $\pm$ 1.80
CM7	375 $\pm$ 13	74.0 $\pm$ 9.1	49.10 $\pm$ 5.00	9.65 $\pm$ 1.70	96.50 $\pm$ 3.50

Fig. 2 % floating of prepared formulations. Error bar represents standard deviation, $n = 3$

By adding Eudragit E100 in microparticles containing GL 43/01 (CM5 to CM7), floating tendency decreased. This develops higher affinity of particles with aqueous media [36] and ingress more liquid and sank. In addition to hydrophilicity, density also plays an important role in floating properties [37]. By increasing the concentration of Eudragit E100 in these formulations, the density of particles increased, which led to decreased floating time. Similarly, Singh et al., witnessed decrease in floating ability of ranitidine containing microparticles with increasing concentration of Eudragit E100 [31].

DI & EE

DL and EE of particles depend on the process variables, physicochemical properties of excipients, drug molecule and nature of matrix material employed. DL and EE were ranged from 3.96 to 9.65% and 39.6% to 96.50%, respectively (Table 2). Here, we observed variation in DL & EE as a function of GL nature and size of particles. Higher EE indicates suitability and efficiency of SDEM to formulate floating microparticles of CLOP.

Maximum DL was observed in formulation CM7. DL was increased by increasing the amount of polymer (Eudragit E100) and could possibly be due to following two reasons. Firstly, by adding Eudragit E100, the drug to polymer ratio is increased due to its diffusion to aqueous continuous phase as compared to formulation prepared without Eudragit E100. Ranjha et al. prepared flurbiprofen loaded microspheres of poly(epsilon-caprolactone)–poly(vinylpyrrolidone) and found increase in DL with increasing concentration of hydrophilic poly(vinylpyrrolidone) [22]. Secondly, by increasing the viscosity of dispersed phase, restricts the movement of drug molecules from emulsion droplet to surrounding aqueous phase and hence, DL increases [20]. Similar results were reported by

Sing et al., where DL of microparticles was enhanced by increasing the concentration of Eudragit E100 [31]. Furthermore, among all formulation containing different types of lipid, the formulation containing GL 44/14 (CM2) showed minimum DL whereas GL 48/16 and GL 50/13 displayed quite reasonable but similar drug loading profile. These results imply that regardless of the hydrophobic nature of the floating GL 43/01 microspheres, its encapsulation capacity for the drug was poor. So, inclusion of the Eudragit E100 helped to improve the CLOP encapsulation. On the other hand, except for the GL 44/14, the other high HLB value lipids caused greater packing of the drug payload. The poor yield, floating capacity, DL and EE of the GL 44/14 shows its meager performance as a matrix former for the microspheres.

Flow properties

The obtained results are presented in Table 3. The values of repose angles ranged from 21.22 to 32.49°, which were in the normal acceptable range (20–40°). Thus, floating microparticles showed reasonably good flow properties. This is further substantiated by the values of compressibility index and hausner's ratio which ranged from 11.63 to 14.71 and 1.132–1.172, respectively. Compressibility index of all formulation was <15% and hausner's ratio of all formulations was <1.2, which indicate free flowing particles.

SEM

The SEM photographs of CLOP encapsulated microparticles depicted hard, discrete, spherical, porous surface and capsular structure (Figs. 3a–h and 4a–d).

The porosity of microparticles can be attributed to the presence of ethyl cellulose and majorly to GL. Although, ethyl cellulose is a release retarding hydrophobic polymer yet it is reported to take up limited amount of water. This is attributed to its hydrogen bond forming ability with water. This hydrogen bond is formed due to differences in polarity between ethyl and oxygen atom of the polymer [38, 39].

Table 3 Rheological parameters of all floating formulations. Mean $\pm$ S.D.; $n = 3$

Formulation	Angle of repose	Compressibility Index	Hausner's ratio
CM1	22.78 $\pm$ 0.013	12.12 $\pm$ 0.015	1.137 $\pm$ 0.031
CM2	24.38 $\pm$ 0.031	12.50 $\pm$ 0.013	1.143 $\pm$ 0.012
CM3	25.45 $\pm$ 0.015	12.90 $\pm$ 0.005	1.148 $\pm$ 0.017
CM4	21.22 $\pm$ 0.022	11.63 $\pm$ 0.021	1.132 $\pm$ 0.005
CM5	25.34 $\pm$ 0.038	13.29 $\pm$ 0.078	1.161 $\pm$ 0.048
CM6	29.46 $\pm$ 0.043	14.29 $\pm$ 0.024	1.167 $\pm$ 0.038
CM7	32.49 $\pm$ 0.045	14.71 $\pm$ 0.031	1.172 $\pm$ 0.051

Similar observation was reported by Saravanan, et al., who prepared ethylcellulose containing floating porous microparticles of ranitidine HCl [40]. GL are unique surfactants having mixtures of mono-, di-, and triglycerides and polyethylene glycol fatty acid esters in their structure. Polyethylene glycol section can orient towards continuous aqueous phase during solidification and promote ingress of water to develop porous surface. This assumption is further supported by HLB values of GL, where increased porosity was observed with GL having high HLB in comparison to GL 43/01, which has low HLB value as can be seen in Fig. 3a–h. It was further observed that by adding Eudragit E 100 to CM 1, particles surface became irregular and their size also increased with increasing concentration of pH sensitive polymers as observed in Fig. 4a–d. We can correlate this to tacky nature of polymer, it is soluble below pH 5.5 (continuous phase pH was also around 5.5) and lastly, its addition increases the viscosity of dispersed phase. Thus, leading to bigger particles with uneven surface. Other authors also reported the similar results [20, 31]. For example, Singh et al., prepared ranitidine microspheres by using Eudragit E100 and concluded that by increasing concentration of polymer results in rough, spherical hollow surfaced microparticles.

FTIR

The drug-polymer or lipid interaction was analyzed by comparing the FTIR spectra of pure drug with that of drug encapsulated microparticles as shown in Fig. 5a–f. In CLOP spectrum, stretching vibrations were observed at $3021/\text{cm}^{-1}$ for CH bond, $2500\text{--}2550/\text{cm}^{-1}$ for NH, $1750/\text{cm}^{-1}$ for C=O and $1151/\text{cm}^{-1}$ for C-O bond [41, 42]. In all prepared formulations, most of functional group frequencies and distinctive absorption band of drug stayed intact. Hence, indicating absence of major drug excipient interaction.

Fig. 3 Scanning electron micrograph of formulation **a** CM1, **b** CM2, **c** CM3, **d** CM4. Porous and capsular nature of **e–f** CM2, **g** CM3 and **h** surface openings of CM4

Fig. 4 Scanning electron micrograph of irregular porous surface of formulation **a** CM5, **b** CM6, **c–d** CM7

DSC

In order to develop a new or improved existing drug delivery system, thermal properties provide important information. Thermal behavior was observed by DSC which gives information about polymers/lipids glass transition temperature, melting profile and also existence of any incompatibilities, purity and physical state of drug in matrix [43]. In our research, DSC of pure CLOP bisulphate showed a sharp melting endothermic peak at $185.03\text{ }^{\circ}\text{C}$ (Fig. 6), which corresponds to its melting point and crystalline nature. In thermograms of formulations CM1, CM2, CM3, and CM4, no endothermic peak of drug was seen suggesting either the CLOP is solubilized completely in lipid-polymer blend forming a single phase or the crystalline nature of drug is lost during encapsulation (Fig. 6a–h). Singh et al., reported

Fig. 5 FTIR spectra of **a** Clopidogrel bisulphate **b** CM1 **c** CM2 **d** CM3 **e** CM4 **f** CM7

the development of solid dispersion of CLOP with PEG 6000 and did not observe endothermic peaks of drug in DSC thermogram [44]. Similarly, Ammar et al., reported the disappearance of risperidone endothermic peak in DSC thermogram of risperidone floating microparticles [20]. Thus, our results suggest the dispersion of drug at molecular

level in formulation carriers and conversion of crystalline nature of drug to amorphous form.

The DSC curves showed endothermic peaks of GL at 48.83 °C, 46.83 °C, 50.12, 49.62 °C, 49.72 °C, and 49.99 °C, which corresponds to the melting point of GL 43/01 (CM1), GL 48/16 (CM3), GL 50/13 (CM4), and GL 43/01 (CM5, CM6 and

Fig. 6 DSC thermogram of **a** Clopidogrel **b** CM1 **c** CM2 **d** CM3 **e** CM4 **f** CM5 **g** CM6 **h** CM7. Dash lines indicate melting point of Gelucires

CM7), respectively. These findings confirm FTIR findings, such as absence of drug excipients interaction. It is noteworthy that the amorphous form of drug is usually desirable as it significantly improves aqueous solubility and bioavailability. The solid state of CLOP in microparticles was further confirmed by XRD.

XRD

XRD is an important, rapid, non-destructive technique to produce distinctive patterns for crystalline materials. The XRD pattern of CLOP depicted its sharp characteristic diffraction peaks (Fig. 7a) indicating crystalline nature. In all the drug-loaded formulations, we observed reduction in intensity of drug characteristic peaks (Fig. 7c–h). The decrease in intensity of diffraction peaks endorses the decrease in crystallinity, enhanced amorphous nature and dispersion of drug at molecular level in developed particles.

In vitro drug release

In an attempt to analyze the impact of various formulation ingredients (GL, ethyl cellulose and Eudragit E 100) on drug release, prepared formulations were subjected to in vitro drug release in acidic environment.

The initial burst release of drug could be due to microporous structure of particles as well as surface associated drug. The microporous structure helps to provide a pathway for movement of water and drug molecule [45]. Hence, augmenting drug release from particles [46].

Ethylcellulose is a hydrophobic polymer, which retards the drug release (Fig. 8) and develops spherical microcarriers with smooth surface (Fig. 3a). Incorporation of hydrophilic ingredients to ethyl cellulose produces porous particles to enhance drug release as can be seen in Figs. 3 and 4. Literature shows Saravanan, et al., prepared porous ethylcellulose floating

Fig. 7 XRD pattern of **a** Clopidogrel **b** CM1 **c** CM2 **d** CM3 **e** CM4 **f** CM5 **g** CM6 **h** CM7

microparticles of ranitidine HCl by using polyethylene glycol 4000 as pore forming agent to modulate drug release [40].

Cumulative percent drug release of floating particles containing GL 43/01 (CM1) was found to be less than those containing GL 44/14 (CM2), GL 48/16 (CM3) and GL 50/13 (CM4) (Fig. 8). This is due to its lowest HLB value and extremely hydrophobic nature. It not only retards the drug release but also keeps them in floating condition. Similar observation was given by Chauhan et al., who described the release retarding property of GL 43/01 in floating matrices of risedronate sodium [35]. Furthermore, presence of hydrophobic ethyl cellulose also contributes to delayed drug release [30].

Drug release from microparticles containing GL 50/13 (CM4) was more than particles containing GL 44/14 (CM2). This can be linked to particle size of formulation where CM2 particles are larger than CM4. Smaller particles have larger surface area as compared with the larger particles, thus a faster release of drug is expected. Similar observations were reported by other authors in literature [24, 47].

Fig. 8 Percent cumulative drug release profile of formulation CM1, CM2, CM3, CM4. Error bar represents standard deviation, $n = 3$

Fig. 9 Percent cumulative drug Release profile of formulation CM1, CM5, CM6, and CM7. Error bar represents standard deviation, $n = 3$

As CM1 had shown lowest drug release, we further modified its release pattern by adding different concentrations of pH sensitive Eudragit E100 from formulation CM5 to CM7. It was found that pH sensitive polymer boosted the CLOP release as a function its concentration (Fig. 9). It's because Eudragit E100 is based on dimethyl amino ethyl methacrylate and neutral methacrylic acid ester. It is soluble in gastric fluid and weakly acidic buffer having pH up to 5. [48, 49]. Jung et al., presented the similar idea and used Eudragit E100 to enhance the solubility and dissolution of itraconazole by employing solid dispersion technique (Jung et al., 1999). Addition of Eudragit E100 is responsible for producing channels in microparticles that facilitated the water diffusion and increased the rate of drug diffusion to the surrounding dissolution media [50].

In order to characterize the release mechanism of CLOP bisulphate from prepared microcarriers (CM1–CM7), Korsmeyer-Peppas model was applied by using DD-solver to data obtained at pH 1.2. The value of “ n ” obtained from Korsmeyer-Peppas model ($n < 0.5$) suggested that release of drug follows quasi diffusion mechanism (Table 4) [51].

Table 4 Drug release kinetics of various formulations at pH 1.2

Formulations	Korsmeyer-Peppas model		
	R^2	n - value	kKp
CM1	0.959	0.109	15.91
CM2	0.997	0.048	30.765
CM3	0.927	0.175	16.482
CM4	0.961	0.168	22.863
CM5	0.968	0.102	22.398
CM6	0.993	0.036	41.065
CM7	0.968	0.106	36.347

Conclusion

CLOP containing microcarriers were successfully prepared via solvent diffusion evaporation method by employing various GL types with ethyl cellulose. Later on different concentrations of Eudragit E100 were tested in GL 43/01 containing microcarriers. Nature of GL and presence of Eudragit E 100 affected the size, surface morphology, floating, DL, EE and release profile of microcarriers. Free flowing formulations with highest % floating were observed for GL 43/01 based microcarriers due to its hydrophobicity. This study implies that lipidic materials with low HLB values are suitable for development of floating preparations. However, addition of a Eudragit E100 improves the drug loading and release behavior of the drug. Hence, formulation CM7 showed best release pattern and could be considered as a suitable floating system to achieve the control drug release in gastric media. This particular formulation (CM7) should further be tested for in vivo performance as an alternative anticoagulant therapy with reduced side effects, which is indeed a limitation of this study.

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Compliance with ethical standards

Conflict of interest Authors declare no competing conflict of interest.

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