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Dual buoyant/mucoadhesive macroporous polypropylene microparticles for gastric delivery of Repaglinide

Mahmoud Soliman[1]*, Enas Elmowafy[1], Abdulfattah Almogerbi[1], Samar Mansour[1], Abdelhameed El Shamy[1]

*Corresponding author:

Mahmoud Soliman

[1]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

Abstract

Preparation and characterization of dual buoyant/mucoadhesive polypropylene microparticles (MPs) loaded with Repaglinide (REP) for gastric drug delivery in order to augment the weak mucoadhesion in the stomach.

Porous foam powder MPs were prepared using coating polymers with variable permeability (Eudragit L100, Eudragit RSPO) alone or in combination by the soaking method. Thiolated Eudragit L100 (Eudragit L100-SH) was also synthesized and tried in an attempt to enhance the mucoadhesive properties of MPs. All formulae were characterized for their yield, flow properties, particle size, encapsulation efficiency (EE %), morphology, and drug release and its mechanistics. Possible interactions inside MPs matrix were also elucidated using FTIR study. The suitability of the selected formulae for gastroretention was evaluated by in vitro buoyancy and *ex-vivo* mucoadhesion studies.

All REP-loaded MPs demonstrated a passable powder flow, high yield values, promising floatation and mucoadhesion.Encapsulation efficiency % values were nearly tripled upon addition of Eudragit polymers. Compared to the Eudragit free REP loaded foam powder, all formula showed more sustained release features.Eudragit L100-SH was synthesized and confirmed by FTIR. Furthermore, its incorporation, alone or in combination, exhibited a significant increase in mucoadhesion strength compared to the unmodified one.

Dual buoyant/mucoadhesive MPs loaded with REP encourage planning for future *in-vivo* performance studies for the management of diabetes.

Keywords: Dual buoyant/mucoadhesive system, polypropylene foam powder, thiolated-Eudragit L100, Repaglinide

Introduction

Various approaches remain the mainstay of successful gastroretention, increasing the oral residence of delivery systems such as swellable systems, mucoadhesive systems and density controlled systems which can be either, high density systems or low density (floating) systems[1].

Mucoadhesive systems are localized delivery devices in the upper gastrointestinal tract, aiming at enhancing the drug absorption process. Mucoadhesive polymers, either unmodified or thiolated, have the ability to interact with hydrated biological materials, retaining dosage forms for a prolonged period of time[2]. Compared to unmodified ones, thiolated polymers present an effective option, exhibiting strong mucoadhesion and permeation enhancing effects worthy of experimentation in gastroretentive systems[3].In an attempt to develop mucoadhesive system based on thiolated polymers, Bravo-Osuna et al. prepared coated poly(isobutyl cyanoacrylates) nanoparticles witheither chitosan or thiolated chitosan. The authors evaluated their mucoadhesion properties using rat intestinal mucosal surfaces. The abundance of thiol groups on the surface of nanoparticles were found to increase the mucoadhesion of such formulae by forming disulfide linkages with the cysteine residues of mucous membranes[4].

However, effective mucoadhesive systems are challenging since the bond formation between mucoadhesive polymer and mucus is dramatically reduced by the acidic environment present in the stomach[5]. Moreover, high turnover rate of the gastric mucus renders the retention of mucoadhesive system in stomach very difficult [6].

Floating drug delivery systems, as another gastroretention mechanism, receive extensive attention owing to their promising buoyant potential. Inherent low density of floating drug delivery systems can be provided by the incorporation of low density materials, such as fatty substances or oils [7], or by entrapment of air in porous materials which imparts low density for such material and result in their buoyancy. Several research groups have investigated the use of low density macroporous polypropylene foam powder [8, 9]and silicates [10] carriers. Such porous carriers are effective in terms of facilitating the incorporation of drugs inside their pores and the cavities and further optional coating with rate

controlling polymers, in particular, Eudragit® copolymers. Despite of the coating process, the polymers partially cover the pores and trap air within the coated system. The trapped air in the coated formulation is gradually removed from the system leading to extended floating times[11]. In a study conducted by Streubel et al., floating MPs based on low-density foam powder were successfully prepared using oil-in-water solvent extraction/evaporation method and their *in vitro* performance was tested [8]. Eudragit® RS, ethyl cellulose and polymethyl methacrylate (PMMA) were also used as release controlling polymeric coats. The MPs possessed good *in vitro* floating behavior and drug release was found to be affected by drug loading, polymer type and amount.

In this respect, combined floating, based on macroporous foam powder, with mucoadhesion mechanisms were designed in this study to enhance the gastroretentive potential of matrices and avoid the premature loss of the system by mucous turn over. Based on their extensive incorporation in controlled release gastroretentive matrices [12], Eudragit polymers are employed in this work. Eudragit L100 and RSPO polymers are chosen as reservoirs, giving a possible chance for release of drug over a specific period of time at a predetermined and controlled rate. Eudragit L100 is an anionic copolymer based on methacrylic acid and methyl methacrylate with the ratio of the free carboxyl groups to the ester groups is approx. 1:1. Its solubility was revealed above pH 6 medium, resulting in effective controlled release and variable drug release profiles [13]. Eudragit RSPO is a insoluble copolymer of ethyl acrylate, methyl methacrylate and a low content of methacrylic acid ester with quaternary ammonium groups, possessing low permeability and pH independent swelling characteristics [14].

Thiolated EudragitL100 (Eudragit L100-SH) as a thiomer was also utilized, aiming at optimally achieving improved mucoadhesive features. Eudragit L100-cysteine conjugate was synthesized by forming covalent bonds between the amino groups of the cysteine and the carboxylic groups of the Eudragit L100 moreover, formation of disulfide bridges between the thiolated polymers and cysteine-rich subdomains of glycoproteins secretions in the biosystems mimic the natural anchoring of mucus glycoproteins in the mucus layer[15].

Repaglinide (REP), a carbamoyl methyl benzoic acid derivative which act by enhancing as insulin secretion in a manner similar to glucose stimulation in the human body. The drug is capable of controlling blood glucose level, and minimize macro and microvascular complications associated with diabetes. REP is only available as conventionaloral tablets, however, REP suffers from poor absorption in the upper intestinal tract , low bioavailability (50%) and short plasma half-life (<1hr) [16]. The use of mucoadhesive floating REP delivery system would enhance its gastroretention, and improve drug bioavailability.

The goal of this study was to encapsulate REP in polypropylene foam powder MPs, comprising Eudragit L100, RSPO and thiolated Eudragit L100polymers. All formulations were evaluated by yield, powder flowability, particle size, encapsulation efficiency, drug release and FTIR. Surface characteristics and morphology of MPs were investigated using scanning electron microscopy (SEM). In vitro floating behavior was performed in 0.1N HCl. The MPs mucoadhesive potential was also tested using *ex-vivo* model.

Materials and methods

Materials

Polypropylene foam powder (Accurel® MP1000) was purchased from Membrana GmbH, Obermburg, Germany. Repaglinide (REP) was supplied as a gift from EPICO Pharmaceuticals, Egypt. Eudragit L100 and RSPO were purchased from Evonik Pharma, GmbH, Darmstadt, Germany. Ethanol, hydrochloric acid and sodium hydroxide pellets were of analytical grade and purchased fromEl Nasr pharmaceutical Co., Cairo, Egypt. Dimethyl sulfoxide (DMSO), *N,N*-dicyclohexylcarbodiimide (DCC), *N*-hydroxyl succinimide (NHS), l-cysteine hydrochloride were purchased from Sigma Chemical Co., St. Louis, US.

Preparation of thiolated EudragitL100 (Eudragit L100-SH)

Eudragit L100-SHwas synthesized according to method reported previously [15]. Briefly, Eudragit L100solution in dimethyl sulfoxide (DMSO) was prepared then*N,N*-dicyclohexylcarbodiimide(DCC) and *N*-hydroxyl succinimide (NHS) were added before l-cysteine hydrochloride was dissolved in smallest amount of DMSO and added to reaction mixture. Reaction was left at room temperature under nitrogen environment then it was stopped by the removal of unbound l-cysteine hydrochloride by dialysis against DMSO initially, and then against distilled water. After dialysis, the polymer solution was freeze-dried and the conjugate was stored in air tight containers at 4 °C until further use.

Preparation of MPs (F1-6)

REP-loaded polypropylene foam powder formulations (F1-6) were prepared using soaking technique[9, 17].MPs were prepared by first dissolving REP and polymer(s) (Eudragit L100, Eudragit RSPO or Eudragit L100-SH) in ethanol then polypropylene foam powder (physically separated fraction between 25 and 50 Mesh) was dispersed in the ethanolic solution and the resulting suspension was poured into Teflon trays. The drug was allowed to adsorb into the MPs porous structure and onto their surface as ethanol was let to evaporate at room temperature. MPs were removed from trays, dried in desiccator and free-flowing MPs were obtained. Six formulations were prepared, keeping the amount of the drug, ratio between polymer and foam powder constant (at ratio of 1:1) while varying the polymer type. The compositions of various formulae were given in Table 1.

Table1: Composition of REP-loaded macroporous foam powder MPs.

	Formula code					
	F1	F2	F3	F4	F5	F6
Foam powder	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg
REP	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg
Eudragit L100		100 mg		50 mg		
Eudragit RSPO			100 mg	50 mg		50 mg
Eudragit L100-SH					100 mg	50 mg

MPs characterization

MPs yield

The MPs yield values were calculated by gravimetry, comparing the initial total solid (drug, foam powder and polymer) with the resultant powder amount after the formation MPs

Powder flowability

The fixed height cone method was adopted. In this method, a glass funnel with an internal diameter of 3 mm was tightened at 1 cm height. The spray dried powder was allowed to flow at a constant rate through the funnel orifice till the apex of the formed cone reached the funnel stem. The powder flow was stopped and the diameter of the base of the formed cone was measured. The angle of repose was calculated as follows:

$$\tan \quad \theta \quad = \frac{2\ h}{D} \qquad (1)$$

Where: θ is the angle of repose, h is the height of the cone and D is the diameter of the base of the formed cone. The test was repeated five times for each run and the average value was taken. Values for the angle of repose below 25 indicate excellent flow, 25-30 good flow, 30-40 passable flow while those above 40 denote very poor flow.

REP-loaded MPs encapsulation efficiency (EE %)

Samples (5 mg) of REP-loaded MPs were soaked in 5mL of ethanol. The obtained solution was stirred for 12 hr then filtered. Drug content was determined by UV spectrophotometry(UV-1601,Shimadzu) at predetermined λ_{max}= 240nmand the drug EE% was calculated as follows:

$$EE\% = \frac{Actual\ \ REP\ amount}{theoritical\ \ \ REP\ amount} \times 100 \qquad (2)$$

Morphological examination

The morphological characteristics of REP- loaded MPs were examined by scanning electron microscopy (SEM) (JEOL 5500, Tokyo, Japan). A small amount of powder was spread on an aluminum stub and, after gold sputtering, was visualized at 20 kV acceleration voltages under argon atmosphere and images were obtained.The porous morphology of foam powder and F5 particles were also visualized using optical microscope(Carl Zeiss, Berlin, Germany) and photographed at a magnification of 100X by means of a fitted camera (Panasonic, Japan) for morphological evaluation, as a confirmatory tool for the partial coating process.

Particle size (PS) determination

The size of REP-loaded polypropylene microparticles (F1-6) was estimated using optical microscope images taken by binocular optical microscope Ziess Axiolab A. Images were then analyzed using Image J software package version 1.49 to measure the Feret`s diameter of microparticles and mean values(n>40) were calculated (mean Feret's diameter is the distance between two parallel and opposite tangent lines to the particle profile in different directions).

In-vitro drug release

A suitable amount of MPs equivalent to 1mg REP were added to 10 mL of 0.1 N HCl (pH 1.2) in closed vials shaken at 75 rpm/ min in an incubator (GFL Shaker, LABOTEC, Germany) maintained at 37 ± 0.5 C. At specified time intervals (15, 30, 60, 120, 180, 240, 300, 360, 420, 480 minutes), 0.5mLsamples were removed and replaced with fresh 0.1 N HCl. The samples were centrifuged, properly diluted if required and analyzed spectrophotometrically at predetermined λ_{max}=240 nm to determine drug concentration.
In-vitro release profiles were analyzed using different kinetic models(zero order, first order, and Higuchi's model), and the release mechanism was determined using Korsmeyer-Peppas model[18].

Fourier transform Infrared (FTIR) spectroscopy

FTIR spectra of REP, foam powder, EudragitL100, Eudragit RSPO or Eudragit L100-SHand REP loaded MPs were recorded with an FTIR spectrometer using KBr disc method. Each sample was gently triturated with KBr powder in a weight ratio of 1:100 and then pressed using a hydrostatic press at a pressure of 10 tons for 5 min. The disc was then placed in the sample holder and scanned from 4000 to 400 cm^{-1}. All spectra were recorded at ambient temperature under vacuum to remove air humidity contribution at a resolution of 4 cm^{-1} and 16 times scanning for each measurement to obtain an adequate signal to-noise ratio.

Percentage buoyancy of MPs

Floating behavior studies were performed by placing 60 particles into beakers containing preheated (at 37 C)30 mL 0.1 N HCl pH 1.2, containing 0.02% w/v Tween 20 to exclude floating due to non-wetted surfaces, followed by horizontal shaking (37 C, 75 rpm). At predetermined time intervals, the beakers were allowed to stand for 5 min without agitation and the number of settled particles was counted then subtracted from the total number of particles and buoyancy was expressed as percentage particles remain floating at certain time interval [8].The floating behavior of polypropylene foam powder as received and some representative examples of formulae was inspected visually as well.

Ex-vivo mucoadhesive strength determination

Mucoadhesion studies of formulations were carried out using Texture Analyzer CT3 (Brookfield Engineering Laboratories, Inc., Middleboro, US). Freshly excised rat intestinal mucosa was used. The mucosal tissue was cut in small pieces and attached to the probe (stainless steel cylindrical probe with 10 mm diameter). The moistened formulations were held on the lower plate. The probe was lowered at a speed of 3 mm/s until the formulation made contact with mucosal tissue. A constant force of 3 N was applied for 3 min[19], after which the probe was withdrawn at a speed of 3 mm/s to a distance of 20 mm. Work of adhesion (mJ) was calculated from force-distance plot using Texture Exponent software package of the instrument. Each experiment was carried out in triplicate. Gross morphological evaluation of mucoadhesive potential of representative F1 and F5 was assessed as well. The freshly excised rat intestines were examined macroscopically for the ability of MPs to adhere to the mucosal surface.

Statistical analysis

Data are expressed as mean of three determinations ± standard deviation. Comparison of the mean values was performed using either Student's *t*-test or ANOVA (analysis of variance) using GraphPad In Stat software program. Statistical significance was set at *P*-value 0.05.

Results and discussion

In the present investigation, REP-loaded macroporous polypropylene foam powder MPs were prepared by soaking technique. EudragitL100, EudragitRSPOandEudragitL100-SHwere used as release retarding polymeric materials. Polymers were used separately or in combination to prepare different formulae using ethanol as solvent for dissolving controlled release polymers. The MPs prepared were further evaluated for various physical parameters such as yield, angle of repose, particle size, and encapsulation efficiency. *In-vitro* dissolution studies were carried out on all the formulae employing 0.1N HCl (pH 1.2) as dissolution medium. *In-vitro* floating behavior and *ex-vivo* mucoadhesion studies were also performed to assess tested formulation gastroretentive potential.

MPs characterization

The physical parameters evaluated for various MPs were given in Table 2.The % yield values of MPs prepared by soaking technique was found to be in the range of 71.39-88.23%.All formulations showed passable flowability as expressed in terms of angle of repose ranging from 30.25 – 38.52 deg. This suggests that such MPs are ideal candidates for direct compression into tablets and that the floating microparticles produced are non-aggregating.
The mean Feret`s diameter measured by microscopy was found to be in the range of412.73- 564.62 µm with no significant difference between the tested non coated formula F1 and Eudragit coated formulae (F2-6). This means that using coating polymers did not affect particle size and this may be due to the small thickness of drug/polymeric coats on foam powder surface as seen in Figure 1.

REP-loaded MPs encapsulation efficiency (EE %)

The values of encapsulation efficiency % of entrapped REP inside the porous compartment of prepared MPs was found to be in the range of 39.75-58.29%. REP-loaded foam powder(F1) showed high EE% reaching 54.91%. Addition of polymers did not significantly ($p<0.05$) affect the proportions of REP incorporated. The interaction between the positively charged Eudragit RSPO and the acid carboxyl group in REP might have occurred, forming molecular complexes that will increase REP solubility[20]. This increase in drug solubility will result in hindering the drug to be incorporated into the macroporous microparticles during solvent evaporation step leading to the slight decrease in EE% values that was noticed with foam powder formulae containing Eudragit RSPO (F3, F4and F6)(Table 2).

Table 2: Characterization of REP-loaded macroporous foam powder MPs.

Formula code	% yield ± SD	Angle of repose ± SD	*FD** ± SD	*EE%* ± SD	n value	Release mechanism
F1	77.40 ± 0.32	38.52± 1.23	564.62± 111.52	54.91 ± 10.53	0.411	Fickian
F2	74.58 ± 0.31	36.61± 1.07	484.67± 104.37	58.29 ± 19.21	0.548	Non-Fickian
F3	72.48 ± 0.36	38.17± 0.82	412.73±106.39	39.75 ± 11.2	0.419	Fickian
F4	88.23 ± 0.24	32.60± 0.81	471.54± 142.93	48.24 ± 6.96	0.886	Non-Fickian
F5	71.39 ± 0.45	30.25± 0.65	501± 118.07	53.68 ± 4.01	0.507	Non-Fickian
F6	77.32 ± 0.19	30.35± 2.49	419.32± 116.23	50.55 ± 7.98	0.519	Non-Fickian

**FD* is Feret diameter measured from optical microscope images*

Morphological examination

Accurel MP1000 is a polypropylene microparticles which have low density due to the macroporous nature of such microparticles with pore size in the range of 5 to 20 μm[21].

SEM imaging of F1-6 showed that all dry formulae were irregular in shape and non-fused[8, 17] and they also possess highly porous structure with pores of almost similar size to those recorded on foam powder substrate surface. As shown in Figure 1, the porous system of polypropylene foam powder was mostly in the macroporous range and pores can be seen to be either totally or partially covered with either drug (Figure 1A) or drug/ polymer (Figure 1B-F). The deposits formed can be on both molecular level and as precipitates. During the preparation of different formulae and as the solvent evaporate, the drug initially adsorbed on substrate surface then precipitated within its porous system[22].

Optical microscope imaging was also consistent with SEM data where the porous nature of F1 was confirmed, showing an outer layer with lighter color and a distinct dark interior of the MP marked by the drawn red line in its perimeter (Figure1G). Although the inner core is porous; the high opacity shown in the image is due to the strong light scattering in the core which has higher thickness compared to the outer layers. Moreover, the coating of foam powder particles with drug/polymer deposits adhering to foam powder surface as thin sheets can be occasionally seen as denoted by the white arrow in both SEM image of F2 (Figure 1B) and optical micrographs of F5 (Figure 1H).

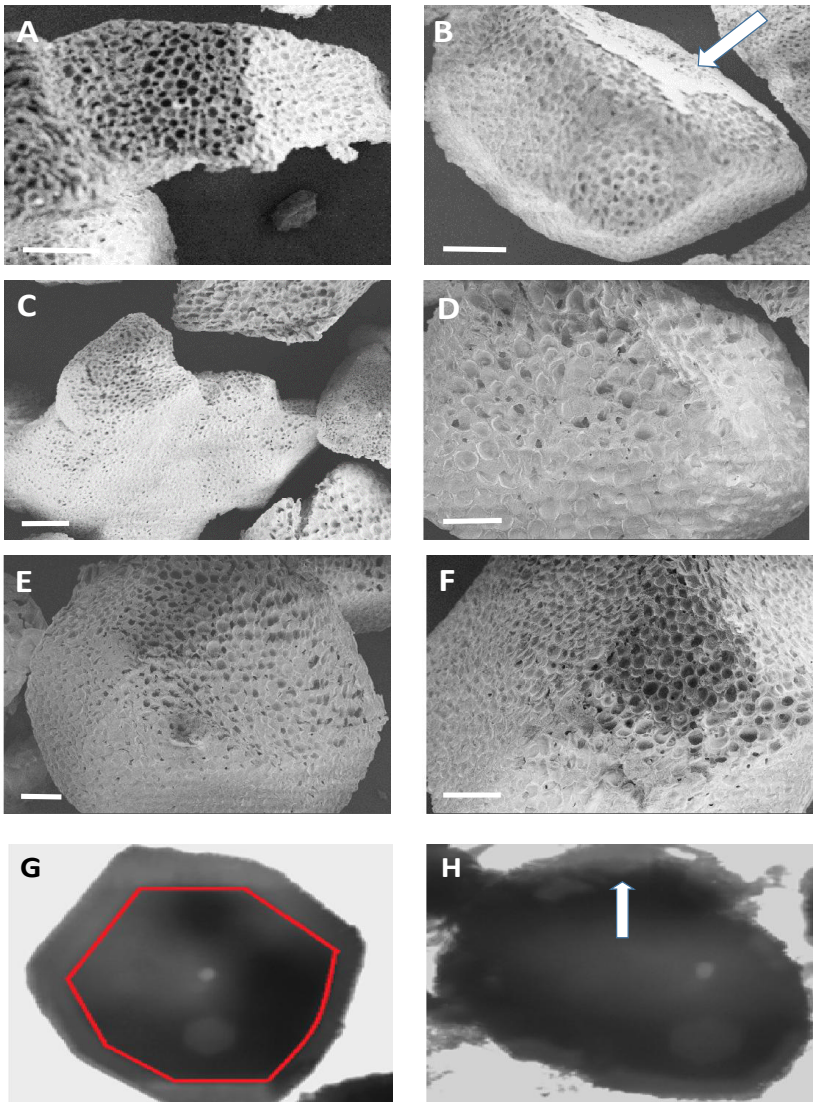

Figure 1: SEM images of REP-loaded MPs of (A) F1 (white arrow show drug/polymer coat), (B) F2, (C) F3, (D) F4, (E) F5 and (F) F6 (white bar 100 μm). Below photomicrograph of (G) Foam powder, (H) F5 (white arrow show drug/polymer coat).

In-vitro drug release and release kinetics

In-vitro release profiles of REP from the prepared REP-loaded MPs were determined at various times intervals are shown inFigure2. The amount of REP released in 8 h from different MPs (Q_{8h}) was used for comparison. F1 which is the product of solvent deposition of REP on foam powder showed the highest release rate when compared to F2-6 ($p<0.001$) with a value of 58.31% REP being dissolved after8 hrs. This would confirm that mixing REP with polyacrylate polymers would sustain its release. Moreover, depositing REP with coating polymers into the pore system and on the surface foam powder MPs led to a sustained pattern of drug release over 480 min depending on the polymer type and composition and with no observed burst release in any of these formulae (F2-6). It is worth noting that MPs retained their integrity during release study, owing to their composition of polypropylene foam powder[8] and acrylic polymers [23] which are insoluble in acidic release medium. It was also found thatQ_{8h}values of the MPs were in the range of 13.87-40.27%. On one hand, F2 and F4 exhibited the lowest release profile among all the formulae with no significant difference between Q8h values of the two formulae ($p>0.05$). This suggests the negative influence of the addition of Eudragit L100 alone or in combination with Eudragit RSPO to foam powder particles on REP release. Such markedly suppressed release of REP might be attributed to the pH dependent solubility of Eudragit L100 which do not allow for REP molecules to escape to release medium until MPs are exposed to higher pH values. At high pH only, the carboxylic groups of Eudragit L100 become progressively ionized and hydrated, causing particles to swell as an initial prerequisite for drug release. This finding was in agreement with that reported previously for similar systems coated with pH sensitive Eudragit and its derivatives [3, 24, 25]. The slight solubility of REP in low pH render coating polymer solubility to be the limiting factor for drug release [10]. As for F4, decreasing the amount of Eudragit L100 by blending with the swellable Eudragit RSPO fails in increasing the drug release a little higher ($p>0.05$). On the other hand, significantly higher Q_{8h} values were noticed with F3 (MPs containing Eudragit RSPO), F5 (MPs containing Eudragit L100-SH) and F6 (MPs containing blend of Eudragit L100-SH and Eudragit RSPO) amounting to 40.27, 33.36 and 25.57 % respectively ($p<0.05$). This could be probably due to the higher permeability of these MPs to the surrounding dissolution medium owing to their content of Eudragit RSPO and mucoadhesive Eudragit L100-SH.However, statistical analysis revealed non significant difference in Q_{8h} forF3 and F5 ($p> 0.05$), confirming the superiority of these two polymers in modulating drug release.

A Higuchi model was found to describe REP-loaded MPs release kinetics, showing the highest R^2 values (0.9882-0.9986) amongst zero and first order release kinetics. This indicates that diffusion through matrices was the main factor controlling REP release.

The dissolution data were fitted according to the exponential Korsemeyer-Peppas Equation [26]and the calculated exponent n values were listed in Table 2. Peppas model gave a good fit to most of the dissolution data of the formulae as shown by the R values (0.9939 - 0.9993). While the majority of formulae exhibited Fickian or near Fickian release behavior with n value around 0.5, non-Fickian release mechanism (0.886) was seen with F4 (containing Eudragit L100-Eudragit RSPO combination)[27].

Based on *in-vitro* drug release results, controlled REP release pattern might provide us an idea about the effect of REP interaction inside MPs matrices on its release pattern. FTIR has always been proven a good confirmatory tool in this respect.

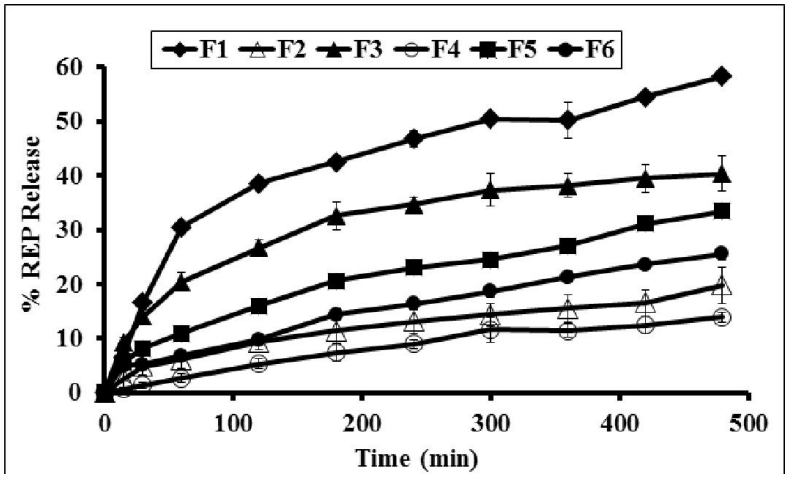

Figure2: Release profiles of REPloaded foam powder F1-F6 in 0.1 N HCl at 37 C.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the starting materials and MPs formulae are presented in Figure3.REP spectrum, illustrated in Figure3, shows: a strong absorption peaks at 3307.5 cm^{-1} assigned to N-H

stretching vibration, a C-H stretching band at 2984.1 cm^{-1}, carbonyl group band at 1687.6 cm^{-1} and bands at 1445.0 and 1215.5 cm^{-1} resulting from C-O stretching and O-H bending vibrations [28].
The Eudragit L100 polymer contains both carboxylic acid and ester groups. In Eudragit L100 FTIR spectrum, several characteristic absorbance peaks could be identified: strongly associated OH vibrations were recorded at 3509.4 and 3233.9 cm^{-1}, peaks at 2997.8 and 2953.6 cm^{-1} assigned to CH vibrations, and characteristic peaks at 1726.1and 1449.5 $cm^{1}$was attributed to carbonyl vibrations of the ester group and C–O bond stretching respectively[29].Similar FTIR spectrum of Eudragit L100-SHwas observed, however, anew absorption band at 1549.8 cm^{-1} was noticed in Eudragit L100-SHFTIR spectrum indicative of the sulphydryl groups confirming the formation of Eudragit L-100–cysteine conjugate [30].Moreover, a shift to a higher wave number (+25 cm^{-1}) was observed for the hydroxyl part (OH) peaks of carboxylic group in Eudragit L100-SH. This O-H shift might predict the possible formation of intramolecular hydrogen bonding between hydroxyl groups of the polymer and sulfhydryl group[15].

Eudragit® RSPO spectrum shows the characteristic bands of the ester groups at 1147.0 cm^{-1} and 1242.0cm^{-1}as well as the C=O ester vibration at 1729.2cm^{-1}[31]. In addition, absorption peak at 3439.2 cm^{-1} assigned to N-H stretching vibration and a C-H stretching bands at 2992.4 – 2954.0 cm^{-1} were recorded too.
In polypropylene foam powder FTIR spectrum, The CH_3 bands were recorded at 2958.1 cm^{-1}, 2872.7 $cm^{-1}$and 1377.1 $cm^{-1}$and peaks at 2839.0 $cm^{-1}$and 1459.0 cm^{-1}assigned to the CH_2 bands [32].
FTIR spectrum of all formulae F1-6 showed that the characteristic N-H stretching vibration peak of REP at 3,307.5 cm^{-1}disappeared together with many other drug characteristic peaks in REP-loaded MPs, indicating the presence of a strong interaction between REP and MPs matrix. Changing the foam powder MPs matrix by incorporation of polymers with variable permeability and their blends resulted in formation of molecular interactions which may have implications on drug release characteristics. This might be at the base of the retardation of REP release and change in its mechanistic.

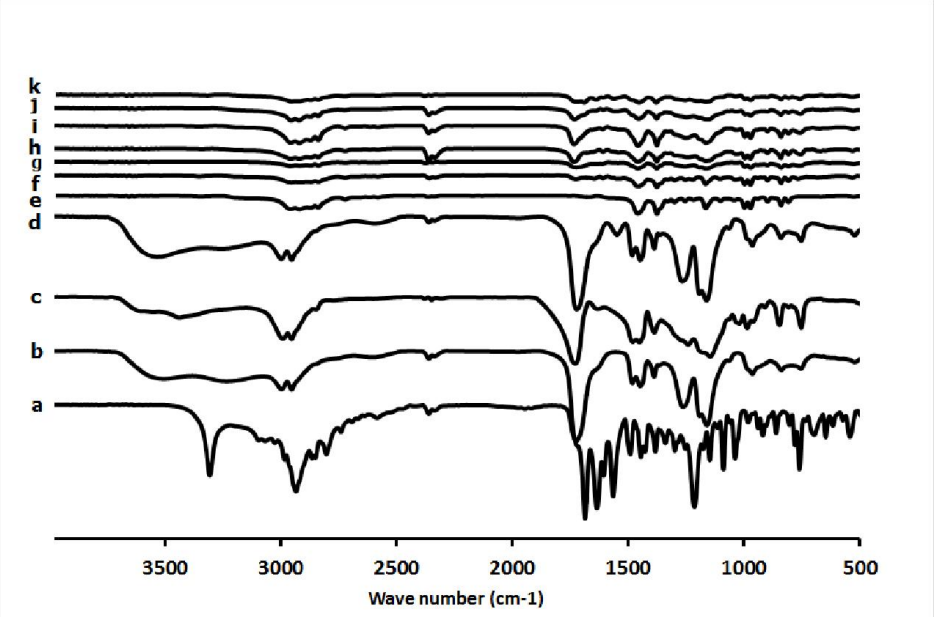

Figure3: FTIR spectra of a) REP, b) Eudragit L100, c) Eudragit RSPO, d) Eudragit L100-SH, e) foam powder and f-k)F1-6 respectively.

Percentage Buoyancy of MPs

Figure4 shows the percentage of floating MPs versus time. Immediate *in-vitro* floatation and extended floating times (up to at least 8 hr) were observed in all formulae. This is due to the highly porous structure as noticed in SEM micrographs. Thus, the porous nature of MPs could produce an upward motion of the dosage form to float on the gastric contents[8].
Buoyancy % values varied considerably among formulae, ranging from 54.44 to 95.55% and were dependent on formulae composition.

Uncoated REP-loaded foam powder (F1), as a control, showed the highest floatability (95.55%). The foam powder particles have a highly porous structure as noticed in SEM micrographs. The addition of polymers to foam powder particles significantly affected the floating behavior of the MPs. As said previously, the partial coverage of foam powder surfaces by the polymers might be at the base of the observed lower buoyancy values. However, the entrapped air within the system guaranteed their instantaneous floating. In addition, upon exposure to aqueous media, the entrapped air is slowly removed from the system leading to extended floating times (Figure5).

Moreover, the nature of the polymer influenced the floating behavior of the MPs. Buoyancy percentage of MPs containing Eudragit L100 (F2) was significantly higher than that of MPs containing Eudragit RSPO (F3) and their combination (F4). It was evident that Eudragit RSPO increased the permeability of MPs to the surrounding dissolution medium and hence, their hydration due to the swelling nature of the polymer. Consequently, their hydration replaced the air inside the floating MPs, thus rendering them less buoyant.

Moreover, MPs containing Eudragit L100-SH alone or in combination with Eudragit RSPO were least buoyant (formulae F5 and F6). It is likely that the thiolated polymers incorporated in the formulations would have increased their wettability and increased amount of absorbed liquid medium as compared to unmodified polymer. Despite of these findings, satisfactory and acceptable floatation was achieved in all formulae when compared to what was reported earlier[23, 33, 34].Figure 5 shows the floating behavior of foam powder, F1 and F5 as observed visually, however only F5 containing Eudragit L100-SH show not only floatation but also adhesivety to container wall.

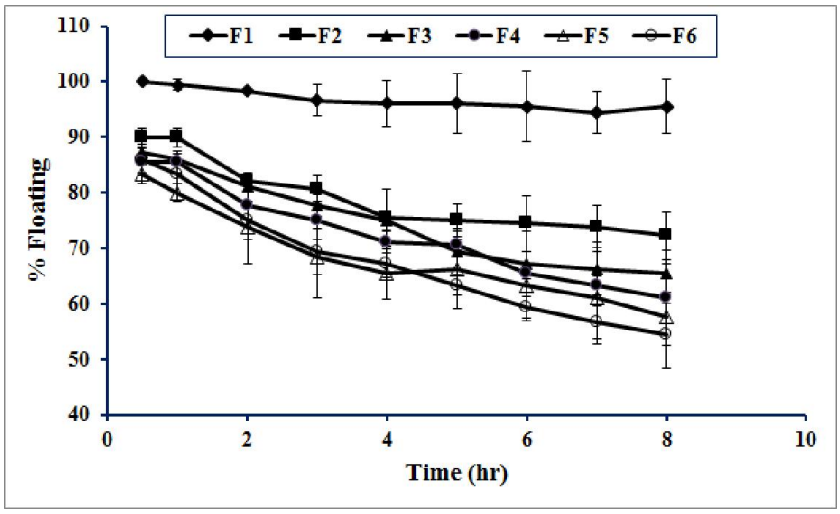

Figure4: Percentage buoyancy of REP-loaded foam powder MPs F1-F6.

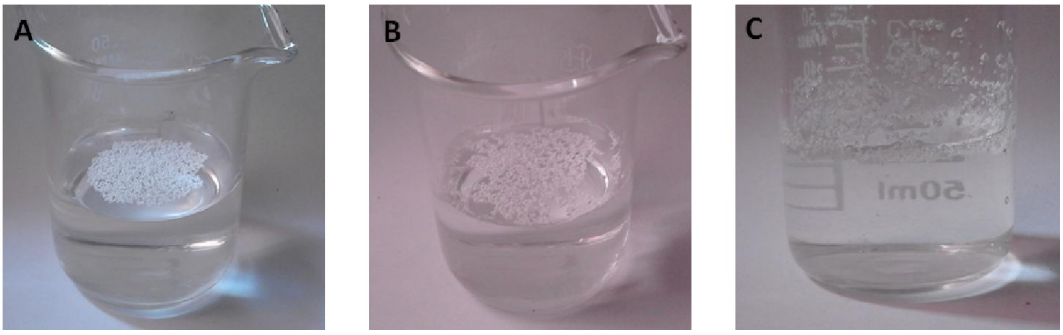

Figure 5: Floating behavior of (A):foam powder, (B): F1 and (C) F5.

Ex-vivo mucoadhesive Strength Determination

When considering gastroretention, it is highly desirable to formulate the MPs with mucoadhesive materials to enhance their retention time in the gastric cavity, thus, improving drug bioavailability[3]. Mucoadhesion test was performed to measure the MPs adhesive strength using a texture analyzer. The measured values for work of mucoadhesion of all formulae as presented in Figure6. MP scan be ranked according to their mucoadhesiveness as follows: F5 > F6 > F4 > F2 > F3. F5 containing Eudragit L100-SHwas superior in terms of mucoadhesive strength, exhibiting a significant 1.56 to 3.56 fold increase in work of adhesion values compared to F2 and F6 respectively($p< 0.05$).Statistical analysis also revealed no significant difference between F2, F3 and F4 ($p> 0.05$). The introduction of sulphydryl groups to Eudragit L100-SH might be at the base for such improvement in Eudragit L100-SH containing MPs. The sulphydryl group can form a disulphide bonds with the mucus glycoprotein, which is supposed to be responsible for the enhanced mucoadhesive properties [35]. Interestingly, the adhesive nature of F5 was also evident while performing buoyancy test Figure 5 (C) and in the sectioned intestine (see Figure6).Upon comparing thiolated polymer with unmodified Eudragit L100 (F2) or

decreasing its content by combining it with Eudragit RSPO (F6) resulted in significantly lower values of the work of adhesion. Moreover, although values of work of adhesion were higher for MPs containing Eudragit L100-Eudragit RSPO combination (F4) as compared to F2 and F3, however no significant difference was found between these formulae.

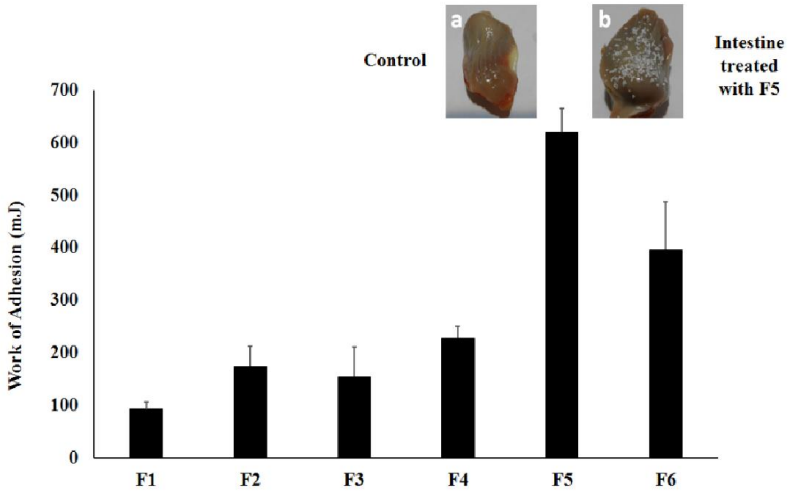

Figure6: Work of adhesion of REP-loaded foam powder MPs F1-F6. In the top photographs for gross appearance of sectioned intestine a) control b) with mucoadhesive formula F5.

Conclusion

REP-loaded macroporous matrices were successfully prepared and their suitability for gastric retention was demonstrated in terms of *in-vitro* buoyancy and *ex-vivo* mucoadhesion. Due to its interaction with positively charged Eudragit RSPO to form molecular complexes highly soluble in ethanol, REP was moderately entrapped inside pores of foam powder formulae coated with such polymer. Type of coating polymers and their blends greatly influenced the drug release, kinetics of release data, floatation and mucoadhesion. All Formulae were able to sustain drug release over 8hr.The *in-vitro* and *ex-vivo* experiments assessed their gastroretentive potential, proving their usefulness as controlled delivery systems. Such promising findings encourage planning for future studies to give evidence of *in-vivo* performance of these matrices for the management of diabetes.

Authors Contributions

Mahmoud Soliman, Enas Elmowafy and Abdulfattah Almogerbihave carried out the research work and compiled the data for the study. This research work has been carried out under the supervision of Prof. Samar Mansour and Prof. Abdel hameed El Shamy. All authors have approved the final manuscript.

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