DEVELOPMENT OF A BUOYANCY-BASED MULTIPLE-UNIT GASTRO RETENTIVE SYSTEM CONTAINING METFORMIN HYDROCHLORIDE TO IMPROVE ANTIDIABETIC ACTIVITY

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ABSTRACT

Diabetic disease is one of top ten threathening diseases requires effective therapy with high effectivenes and safety. As a chronic disease, diabetes may need long term treatment. Metformin is one of standard therapies for diabetic patients. It has serious pharmacokinetic problems: incomplete and slow absorption as well as plasma concentration fluctuation as a consequence of its narrow absorption window. The aim of this study was to develop a multiple-unit gastro retentive sustained release system of metformin hydrochloride to improve its efficacy. This system consisted of a drug core pellet prepared by extrusion-spheronization method, coated subsequently with release controller polymer (Eudragit RS PO), gas generating substance (sodium bicarbonate), and gas entrapping matrix (Eudragit RS PO and Eudragit RL PO). Coating process was performed with fluidization method. A complex combination of formula resulted in product with long resident time in the gastric via buoyancy mechanism. This formula contained 18.68 mg metformin HCl per 100 mg coated pellet, with a desirable floating lag time (<30 s), and 81.10% pellet remained floating until 8 hs. The *in vitro* drug release study also revealed that this formula showed the best sustained release system, with 68.16% of metformin HCl released in the gastric compartment after 8 hs.

KEY WORDS: Multiple-unit, gastro Retentive system, Sustained release, Metformin hydrochloride, Narrow absorption window, Buoyancy.

INTRODUCTION

Type II diabetes mellitus (T2DM) is still a worldwide health challenge and problem, which causes the increase of mortality and morbidity, also the decrease of social and economic status of human society. According to World Health Organization, in 2013, diabetes mellitus is rank as the 9th disease with highest mortality rate in the world [1].

Metformin hydrochloride (HCl) is the first line drug administered orally for T2DM therapy [2]. However, there are a lot of challenges faced to formulate it into an optimal and effective oral dosage form. Metformin HCl has a narrow absorption window (NAW) characteristic that leads to its incomplete absorption in the gastrointestinal tract [3]. It has a very low membrane permeability (log P - 1,43) and exists very largely as hydrophilic cationic species at physiological pH (pKa 2,8 and 11,5). Therefore,

absorption of metformin HCl into the bloodstream is mostly via active transport mechanism. Active transporters for metformin HCl (PMAT and OCT 1) are localized on the stomach and upper small intestine [4]. Its NAW characteristic combined with short gastric residence time and unpredictable gastric emptying in the stomach (approximately 30 min – 3 hrs depends on many factors), lead to a very low oral bioavailability (50-60%) of metformin HCl [5].

Prolonging the gastric resident of drug with NAW characteristic in the stomach and upper small intestine like metformin HCl will help optimizing their efficacy. Gastro retentive drug delivery system (GRDDS) seems to be the only target of therapeutical approach. GRDDS is designed to be retained in the gastric region for prolonged period of time and sustained the release of incorporated drug in this compartement thus ensuring improved bioavailability [6].

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Metformin HCl also has a short elimination half life i.e 0.9-2.6 h after administration [4]. To maintain its effective plasma concentration, frequent daily dosing schedules are therefore needed. But, this scheme leads to the increase of the side effect on the gastrointestinal tract [7]. On the other hand, sustained release system can be developed to achieve therapeutically effective concentration of drug in the systemic circulation over an extended period of time, while reduction of the adverse side effect is obtained [8].

In present study, metformin HCl was formulated into a gastro retentive multiple-unit system with demostrating a sustained release property. The multiple-unit in the form of pellets provide several advantages. It has a larger surface area for drug absorption, freely disperse in gastrointestinal fluids due to their small size, reduce the accumulation of drugs on the gastrointestinal tract, and also less susceptible to dose dumping thus lowering the risk of adverse effects [9].

The buoyancy pellet system was chosen for this purpose. Prolong gastric resident was achieved by floating of the pellet on the gastric fluid, due to its lower density over the gastric medium [10]. The floating system was generated by effervescent reaction between basic component of the coating formula and acidic gastric liquid. The reaction produced CO_2 gas which was subsequently captured by second layer leading to the pellets were lifted up [11].

MATERIALS AND METHODS
Materials

Metformin hydrochloride was received as a gift sample from PT. Narda Tita Indonesia, Avicel PH 101 and Polyvinylpyrrolidone (PVP) K-25 were gift of PT Combiphar, Bandung, Indonesia, Eudragit RS PO and Eudragit RL PO were obtained commercially from Degussa-Germany, cetyl alcohol, PEG 6000, sodium bicarbonate, were purchaed from PT Bratachem Indonesia, Methocel® E-15 was commercially obtained from Dow Chemical company USA. All solvents used were analytical grade.

Core Pellet Preparation

Core pellet was prepared by extrusion-spheronization method. Drug, Avicel PH 101, and PVP K-25 were granulated with a combination of water-ethanol (1:1), further on extruded with 1 mm pore size extruder and agitated at speed of 100 rpm. The extrudate was spheronized for 2 min with speed of 900 rpm. Finally, the core pellets was dried in oven for 2 hs at 55°C.

Core Pellet Evaluation
Surface morphology

Surface morphology of the core pellets was observed visually and with a JSM-6510LV Jeol (USA) Scanning Electron Microscope (SEM). For SEM analysis, the core pellet was put on a sample holder. This sample stub was then coated with a thin layer of gold. Sample was subsequently scanned by SEM and photographed under various magnifications with direct data capture of the images onto a computer.

Size distribution analysis

Size distribution analysis of pellets was determined using the sieve analysis method. To do so, pellets were sieved for 15 min using sifter (Retsch) fixed at an amplitude of 10 x 0.1 mm with a set of calibrated sieves (0.6-1.18 mm). After sieving, the fraction remained on each sieve was weighed. Pellets with diameter between 0.6-1.18 were then used as core pellets for further coating process.

Determination of drug content

The metformin HCl content in core pellets were determined by mean of crushing 250 mg of pellets in mortar with pestle. The powder was subsequently dissolved in 100 mL of HCl solution pH 1.2 and sonicated for 30 min in a bath ultrasonicator. This solution was filtered through Whatman filter paper, diluted (250x), and analyzed using spectrophotometer Beckman DU 650i at a maximum wave length of 233 nm. Drug content was calculated from metformin HCl calibration curve. The content of metformin HCl was expressed as the amount of metformin HCl in 100 mg of pellets.

In vitro release study

The *in vitro* release study of the core pellets was performed in a gastric simulation fluid (HCl solution pH 1.2) using United States Pharmacopoeia (USP) type II apparatus, 900 mL of the dissolution medium at 37 ± 0.5°C, 100 rpm, for 2 hs. All experiments were done in triplicate. An aliquot of 5 mL was withdrawn from the dissolution apparatus and then replaced with equivalent volume of fresh dissolution medium. The samples were filtered through Whatman filter paper and analyzed using spectrophotometer Beckman DU 650i at a maximum wavelength of 233 nm.

Coating Process of Core Pellet

Coating process was applied on core metformin HCl pellet. Process optimization of the core pellets coating was controlled by weight gain value. There were 3 different coating processes to obtain buoyancy pellet.

First coating (I) was aimed to control the drug release. The main component for this coating was Eudragit RS PO as a polymer controlling the drug release. Required quantity of Eudragit RS PO (Table 2) was dissolved in acetone:water (9:1) and homogenized by stirring at 250 rpm for 45 min. Eudragit RS PO content of this solution was 8% w/v. Required quantity of cetyl alcohol was dispersed in the solution by continuous stirring for 15 min. The coating was applied on core pellet using fluid bed dryer apparatus (XC-FBD-2 Yen Chen Machinery) with 7.5 mL/min spraying speed and 3 second interval of spray-dry cycle. Atomization pressure was 1.8 barr, the inlet

temperature was 47°C, and the bed temperature was 37°C.

Subsequent second coating (II) was aimed to generate CO_2 gas. Required quantity of sodium bicarbonate (Table 2) was dissolved in specified volume of water and stirred for 45 minutes, 250 rpm. Required quantity of Methocel® E15 was dissolved separately in water and homogenized using a stirrer. Both solutions were mixed and homogenized by 250 rpm speed stirring. During this homogenization process, PEG-6000 as a plastisizer was added. The coating process was similar as the first coating.

Subsequent third coating (III) was aimed to entrap the generated gas produced by second coating after contact with gastric fluid. Eudragit RL PO was used as an entrapping matrix. Required quantity of Eudragit RL PO (Table 2) was dissolved in acetone:water (9:1) and homogenized by stirring at 250 rpm for 45 min. Eudragit RL PO content of this solution was 8% w/v. Required quantity of PEG-6000 was also dissolved in acetone:water (9:1) and added to Eudragit RL PO solution. This mixture was then homogenized by continuous stirring for 15 min. Similar coating process was also applied for this third layer (C).

Evaluation of the Coated Pellet
Surface morphology
The surface morphology study of coated pellets was done visually and using scanning electron microscope (SEM) as previously described.

Size distribution
As mentioned previously, the size distribution of coated pellets was determined using a sieve analysis method. The coated pellets were sieved for 15 min with sifter (Retsch) fixed at an amplitude of 10 x 0.1 mm with a set of calibrated sieves (0.6-1.68 mm). After sieving, the fractions remained on each sieve were weighed.

Weight gain
Weight gain (WG) is the increase of weight after coating process of the pellets, which was measured by calculating the deviation of drug concentration in the pellet before and after coating process. The WG study was done using 300 mg crushed pellets. This experiment was done in triplicate. The metformin HCl content was measured by spectrophotometry method. The weight gains were calculated using following formulas:

The total WG $= \frac{A-D}{A} \; x \; 100\%$

The WG of first coating $= \frac{A-B}{A} \; x \; 100\%$

The WG of second coating $=$ $total \; WG - WG \; for \; first \; coating$

A = metformin HCl content in core pellet
B = metformin HCl content in pellet coated I

C = metformin HCl content in pellet coated II
D = metformin HCl content in pellet coated III

Floating characteristics
Floating characteristics of coated pellet included floating lag time, floating percentage, and floating time. All tests were done in a 250 mL beaker glass containing 50 mL of HCl pH 1.2. The tests were subjected to 30 coated pellets. All experiments were done in triplicate. Floating lag time of the coated pellets was performed by measuring the average time required by pellets to float on the test medium. Floating percentage study was done by counting the amount of coated pellet floated after 8 hs (n2) of observation and calculated using following formula:

% floating $= \frac{n2-n}{n} \; x \; 100\%$

Floating time analysis was performed by counting the average amount of coated pellets that keep on floating after 8 hs of experiment.

In vitro *release study*
The *in vitro* release study for the coated pellet was performed in HCl solution pH 1.2 by using United States Pharmacopoeia (USP) type II apparatus, 900 mL of the dissolution medium at 37 ± 0.5 ^{o}C, 100 rpm, for 8 hs. This study was subjected for both pellet coated I and pellet coated II. All experiments were done in triplicate. An 5 mL of aliquot was withdrawn and replaced with equivalent volume. The sampling intervals were 0, 0.5, 1, 2, 4, 6, and 8 hs. The samples were filtered through Whatman filter paper and analyzed using spectrophotometer Beckman DU 650i at a maximum wavelenght of 233 nm.

RESULTS AND DISCUSSION
Core Pellet Preparation and Evaluation
Core pellet was prepared using extrusion-spheronization method. Extrusion-spheronization is a technique that incorporates multiple processes involving extrusion followed by spheronization to produce uniform size and free flowing spherical particles [9]. In this study, Avicel PH 101 was used as a pellet matrix due to its ability to produce spherical pellets with good flow properties, especially when combined with PVP as a binder [12]. The use of 50% of ethanol resulted best extrudate property suitable for maintaining the pellets moisture content during spheronization process which is important parameter to produce good physical property of the pellets [13].

Surface morphology
The visual appearance of the core pellets were found to be spheric (Figure 1). Surface morphology of the pellets shows porous characteristic both at the surface (figure 2a) and in the inner part of the pellets (figure 2b).

Size and size distribution

The core pellets were found to have a good size distribution, with dominant size was in the range of 0. 6-1. 18 mm as shown in Figure 3.

Uniformity of drug content

The content of drug in pellets is 38.64/100 mg pellet and considered for further dissolution studies.

In vitro release study

In vitro drug release was performed in order to study the release profile of metformin HCl from the core pellets. As presented in figure 4, immediate release with more than 80% of metformin HCl was released nearly complete at 30 min and was slowed the release up to 100% after 2 hs.

Coated Pellet Evaluation

The development of gastric floating pellets was aimed to maintain the release of the drug in the gastric compartment for a prolonged period of time [14]. This was obtained by coating the pellets by several layers. The first layer was a combination of Eudragit RS PO polymer and cetyl alcohol, functioned to sustain the release of drug. Eudragit RS PO is a type of Methacrylic resins that widely known to have a good properties for controlling drug delivery due to their high chemical stability and compatibility. Eudragit RS PO served as a matrix that mediated the release of drug by diffusion mechanism. It has a low permeability to water, so the rate of water out from the system carrying the drug was also low. While, cetyl alcohol served as a drug release controlling agent from the Eudragit matrix [15].

The second layer was aimed for CO_2 gas generation, consisted of sodium bicarbonate as gas generating substance that dispersed in Methocel® E-15 polymer. Sodium bicarbonate generated gas through an effervescent reaction with the acid presence in the gastric fluid. PEG-6000 was used as a plasticizer and functioned to prevent the brittleness of the coating layer. It also provided the mechanical strength of the layer when it was in contact with gastric fluid [16].

The third (outer) layer was aimed to capture and entrap the CO_2 gas generated upon effervescent reaction, consisted of Eudragit RL PO or the combination of RL PO and RS PO. These polymers functioned as the matrix that entrapped the CO_2 gas generated by second layer. Eudragit RL PO has a good water permeability, enabling water comes in and out easily from and to the surrounding environment. A best combination of Eudragit RL PO and RS PO was used in formula F3 to study the effect of Eudragit RS PO on the floating ability of the system [16].

Surface morphology of coated pellet

The visual appearance of the coated pellets from different formulas is presented in figure 5. As compared to core pellet, the coated pellets have better surface morphology but similar porosity especially in the inner part of the pellet (figure 6).

Size distribution

Table 3 shows that 100% of pellets from all formulas (F1, F2, F3) passed through 1.68 mm sifter and remained in 0.6 mm sifter. Coated pellets of these three different formulas exhibited a good size distribution.

Weight gain study

The weight gain of the coated pellets using different formulas is presented in table 4. As seen in the tablet, there was a significantly different increase of the weight gain of F3 as compared to F1 and F2 in all coating processes, as well as the total weight gain. The effect of this coating thickness will be clearly shown on their floating characteristics (table 5) as well as in the release profile as depicted in figure 8 and 9.

Floating characteristics of coated pellet

The floating characteristic of pellets is a set parameter determining the successful formulation of the coating system. This consists of lag time floating, floating time and floating percentage of the pellet. Lag time floating was obtained by measuring the time required for the pellet system to completely float in the gastric fluid immediately after the pellets contacted with gatric fluid. Lag time floating is influenced by the permeability of both gas entrapping layer (III) and gas generating layer (II). The floating time and floating percentage are directly influenced by the ability of gas entrapping layer to prevent the loss of gas generated after effervescent reaction [17].

Table 5 presents the floating characteristic of the pellets of formula F1, F2, and F3. These characteristics were determined by the coating thickness indicated by weight gain value. As shown, F3 suggested as the most appropiate formula mainly based on the floating percentage both after 4 and 8 hours observations. However, the main contribution layer on this floating percentage is the third layer. This means that the longer the matrix entrapped the generated CO_2 gas, the longer the pellets were maintaining low pellet density hence remaining on the surface of the gatric fluid.

The profile of floating percentage of three formulas was shown in figure 7. As previously described, the thickness of layer III contributed dominantly in controlling the floating percentage as a function of time. Formula F1 and F2 which have not significantly different weight gain value shows similar floating percentage profile. The number of floated pellets decrease gradually due to lost of generated CO_2 gas leading to the increment of pellets density higher than gastric fluid density. In contrast to formula F3, the weight gain value of nearly 50% enable maintaining the lower pellet density.

In vitro release study

The *in vitro* drug release study for coated pellet was done only for pellet coated with polymer controlling release (layer I) and pellet coated with polymer entrapping the generated gas (layer III) for F1, F2, and F3. The release of metformin HCl from coated pellet is depicted in figure 8, accounted as 83.23% (F1), 80.13% (F2), and 75.04% (F3) after 8 hs of observation. As shown in figure 4 and 8, first coating which was aimed to control the drug release was achieved. Uncoated pellet showed immediate release profile (figure 4) and reached 100% after 2 hours. The presence of Eudragit delayed the release of the drug at more than 8 hours. In line with weight gain value, F3 showed most delay release profile as revealed both in figure 8 and 9. The lower release profile of drug after third coating (figure 9) is obviously explained by the weight gain value as well. The presence of more layers retained the diffusion rate of medium into the pellet. This means that the layers also functioned as diffusion barriers.

Table 1. **The formula of core pellet containing metformin HCl**

| Material	Percentage (%)
Metformin HCl	40
Avicel PH 101	56
PVP K-25	4

Table 2. **The composition of soating solution of each layer of three different formulas**

| Coating	Material	F1 (%)	F2 (%)	F3 (%)
I	Eudragit RS PO	10	12	15
	Cetyl alcohol	1.2	1.8	1.8
II	Sodium bicarbonate	10	12	12
	HPMC E-15	2.5	3.0	3.0
	PEG-6000	1.25	1.5	1.5
III	Eudragit RL PO	8	10	-
	Eudragit RL PO : RS PO (75:25)	-	-	15
	PEG-6000	0.96	1.5	1.5

Figure 1. Visual appearance of core pellets
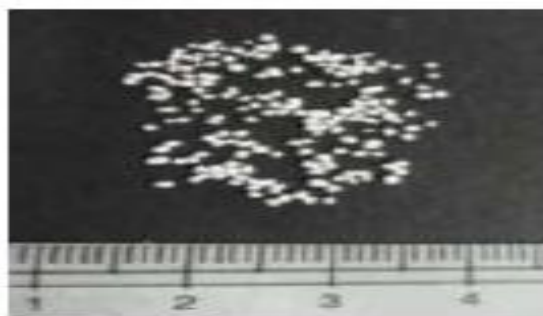
Figure 2a. Scanning Electron Microscopic presentation of core pellet. Magnification 80x
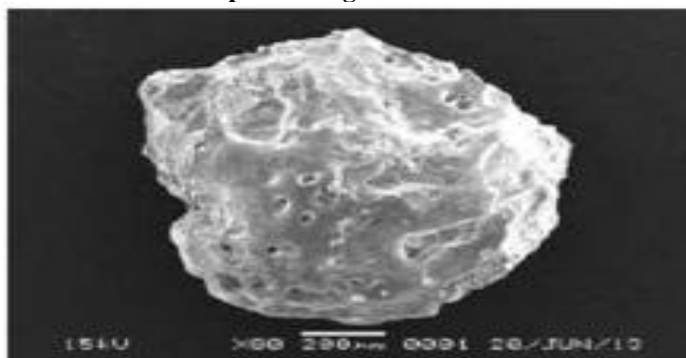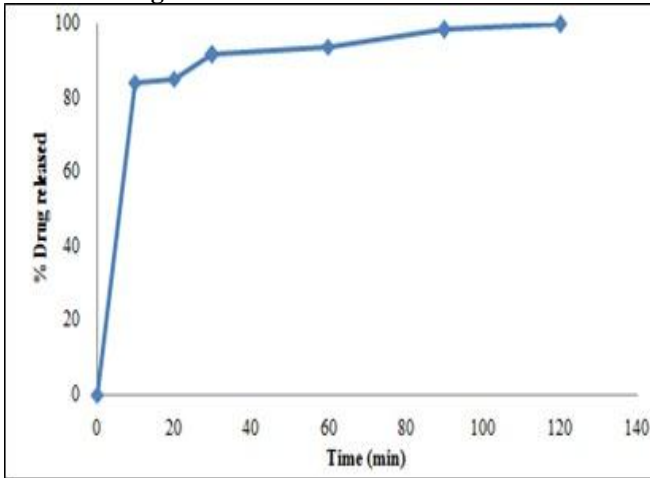

Figure 2b. Scanning Electron Microscopic presentation of core pellet. Magnification 500x
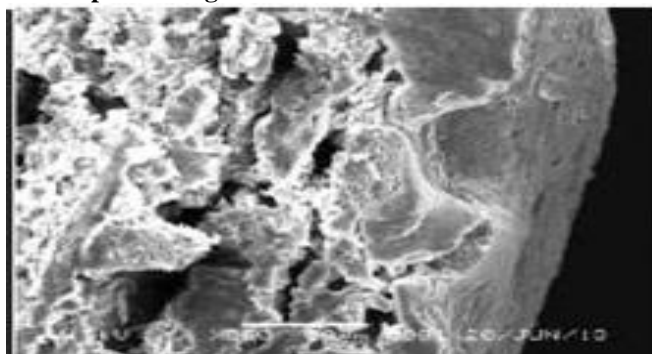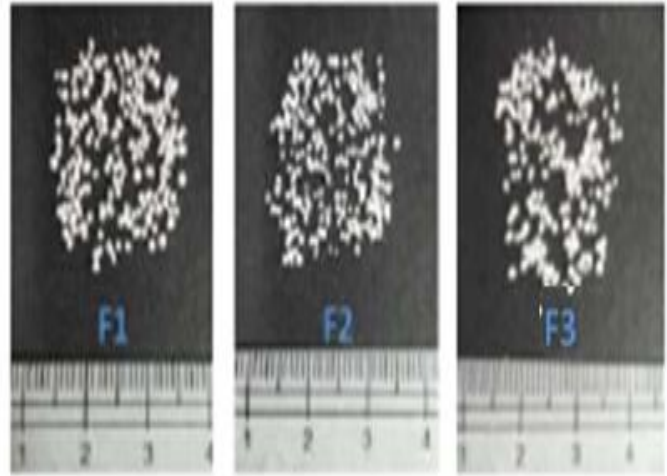

Figure 3 . Size distribution curve of core pellet: (A) x>1.68 mm (B) 1.18<x<1.68 mm (C) 0.6<x<1.18 mm (D) x<0.6 mm
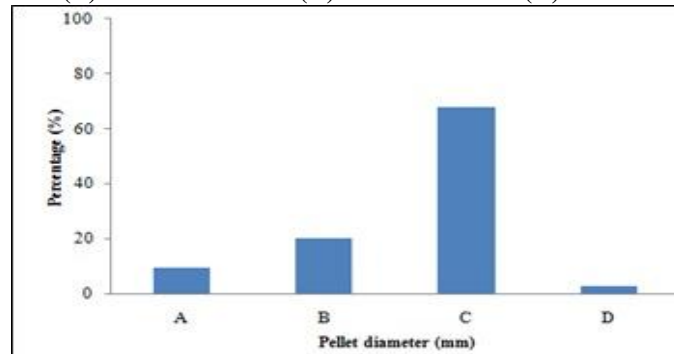
Figure 4. *In vitro* drug release profile of the core pellets in simulated gastric fluid

Figure 5. Visual appearance of coated pellets

Figure 6. Scanning Electron Microscopic presentation of pellet coated with polymer controlling the drug release, magnification 80X (a) and 500X (b), gas generating layer, magnification 80X (c) and 500X (d), and gas entrapping layer, magnification 80X (e) and 500X (f)
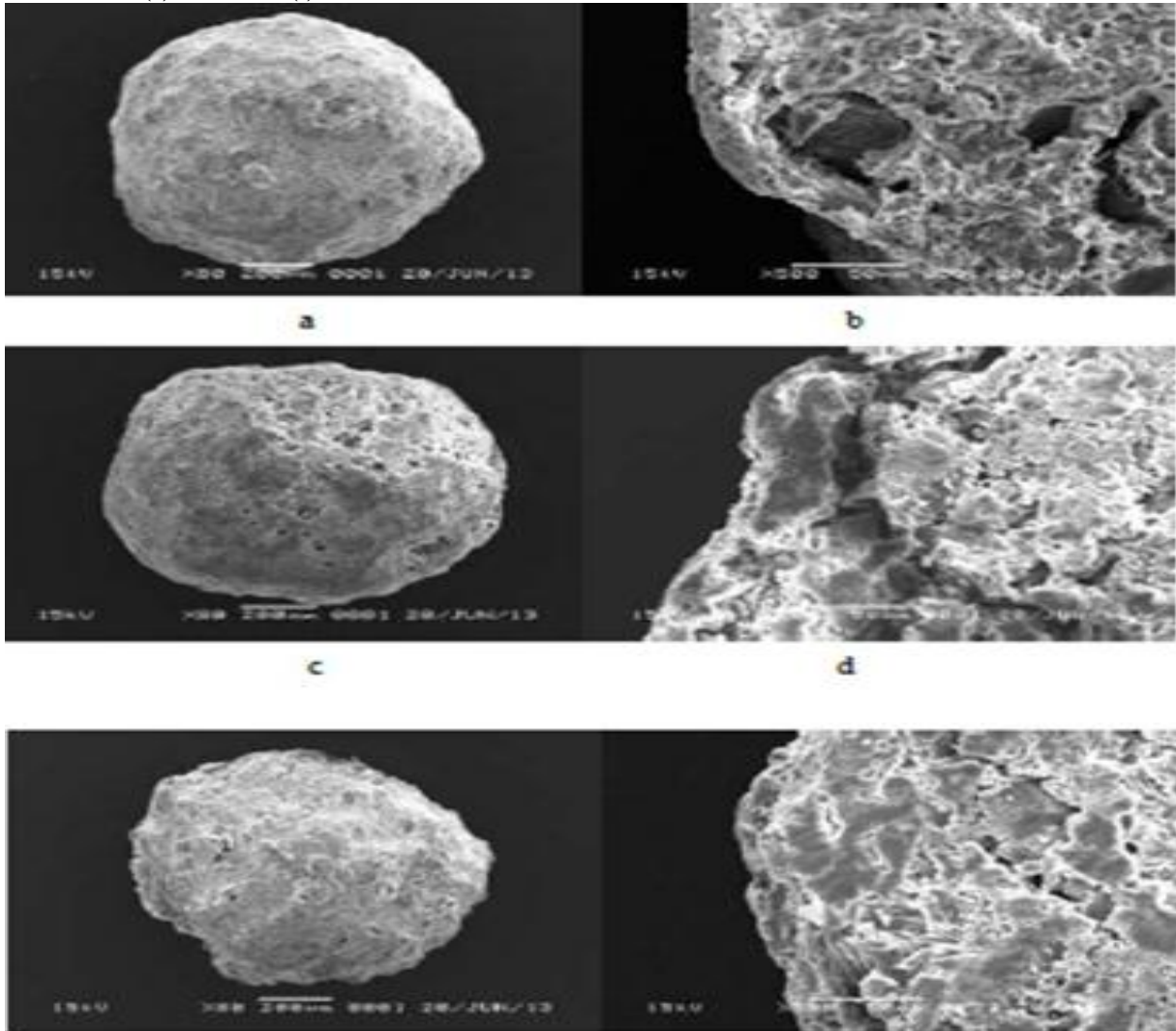
Figure 7. Floating percentage profile of the coated pellet for 8 hours of observation
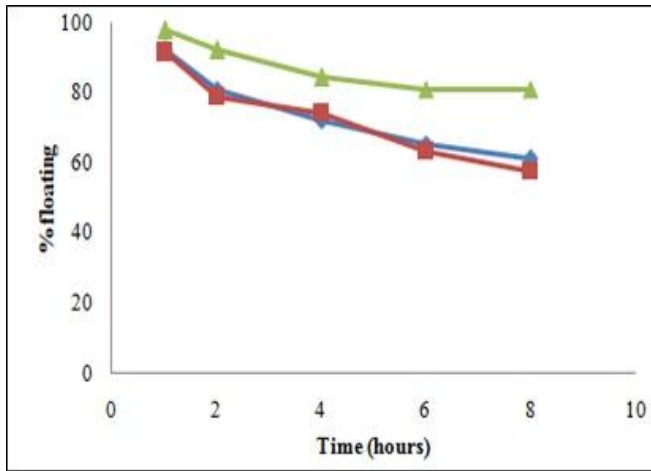

Figure 8. The release profile of metformin HCl release from pellet coated with polymer controlling drug release (I) in the simulated gastric fluid
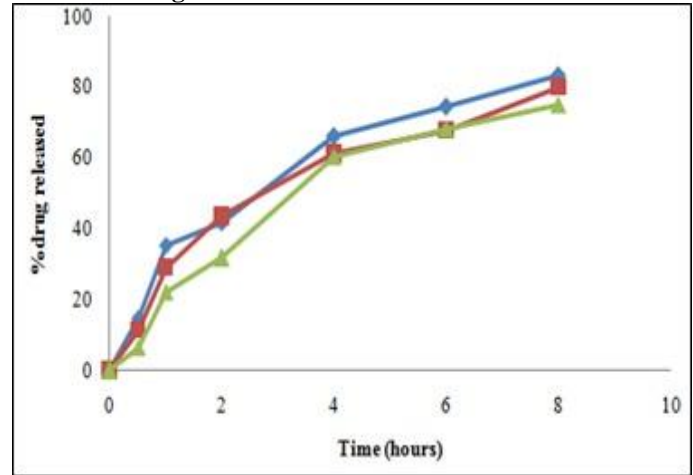

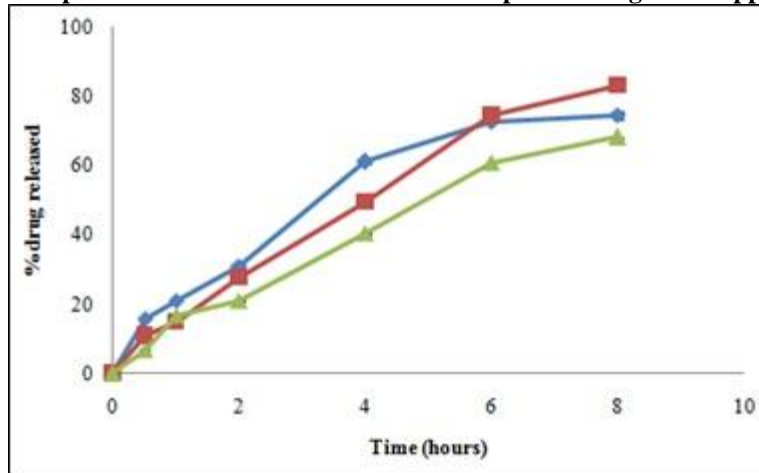**Figure 9. Release profile of metformin HCl release from pellet with gas entrapping layer (III)**

Table 3. Size distribution of coated pellets form three different formulas

Coated pellet	F1 (%)	F2(%)	F3(%)
Remained at 0.6 mm	100	100	100
Remained at 1.68 mm	<1	<1	<1

Table 4. The weight gain of the coated pellets of three different formulas

Layer	WG	F1 (%)	F2 (%)	F3 (%)
I	WG 1	9.89 ± 0.39	11.46 ± 0.46	17.71 ± 0.92
II	WG 2	10.79 ± 0.02	13.85 ± 0.32	12.78 ± 0.80
III	WG 3	9.89 ± 0.11	11.47 ± 0.96	17.72 ± 0.76
Total WG		30.58 ± 0.26	36.79 ± 0.99	48.22 ± 0.66

Table 5. Floating characteristic of the coated pellet from three different formulas

Formula	Lag time (s)	Floating Percentage (%)	
		4 hs	8 hs
F1	5.33 ± 0.57	72.20 ± 5.13	61.10 ± 1.90
F2	10.33 ± 1.52	74.43 ± 1.96	57.75 ± 3.86
F3	17.67 ± 1.57	84.43 ± 1.92	81.10 ± 1.95

CONCLUSION

Multiple-unit gastro retentive system of metformin HCl developed through buoyancy system is able to prolong the drug release up to 8 hs. The layer contributing to the maintenance of the presence of the generated gas on the pellet seems to be the most important part influencing the successful approach. This was shown by formula F3 which was composed by a combination of Eudragit RS PO-RL PO (25:75). In addition, the thickness of the coating layer indicated by the weight gain value entirely determined the characteristic of the pellet targeted for gastric retentive delivery of metformin HCl.

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