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Original Article

PREPARATION AND CHARACTERIZATION OF ENTERIC-COATED DELAYED-RELEASE MICROSPHERE OF PHYTOSOME LOADING ALLICIN-RICH EXTRACT

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ABSTRACT

Objective: Allicin, a natural organosulfur compound, is the main garlic ingredient, which has extensive pharmacological activities. Its unstable under acidic conditions due to alliinase's inactivation causes the need for preparations that delayed-release in the stomach to maximize allicin absorption. This study aimed to prepare and characterize the enteric-coated microsphere of phytosome loading allicin-rich extract to protect it from gastric acid.

Methods: The allicin-rich extract phytosome (ArE-Ps) was prepared and evaluated for characteristics. Microsphere was made in three formulas with different molar ratios of ArE-Ps and Eudragit L30D-55 (1:1; 1:1.5 and 1:2) by spray dry. The three microspheres compared to particle size, entrapment efficiency, and dissolution test in acid and 7.4 pH medium.

Results: Optimized ArE-Ps has a size of 251.6 nm, polydispersity index 0.466, zeta potential 34.11, entrapment efficiency of 62.62 %, and specific gravity of 1,005 g/ml. The surface topography of the three formulas shows an almost spherical shape with concave surfaces. The particle size of the microsphere ranges from 215±6.27 nm to 548.8±10.15 nm. Entrapment efficiency increases with an increasing number of polymers with a maximum value of 65.44 % at F3. The results dissolution test *in vitro* showed no drug release in acidic medium, and drug release occurred at a 7.4 pH medium. Drug release of three microsphere formulations followed the Korsmeyer-Peppas model with a k value of 12.7088±0.1769; 17.9322±1.5621; and 12.958±1.2677; respectively.

Conclusion: Based on these results, the polymer's increase in three microsphere formulations can affect characteristics and retain drug release under acidic conditions.

Keywords: Enteric-coated, Delayed-release, Phytosome, Allicin-rich extract, Characterization

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INTRODUCTION

Garlic (*Allium sativum* L.) is a bulbous plant that is easy to grow at temperatures and tropical conditions globally, including in Indonesia. Generally, garlic is used as a flavoring agent and seasonings [1]. This plant has many organosulfur compounds such as allicin, volatile compounds [2]. That reported having extensive pharmacological activities [3] such as antimicrobial [4], antihypertensive, nephroprotective, cardioprotective, antioxidant [5], anti-carcinogenic, antidiabetic, and cytochrome activity [2]. Allicin is known to be unstable and results from the action of the alliinase enzyme in alliin. Formulations containing allicin are complicated because of their instability. Conventional formulations to form allicin *in vivo* also have a big challenge because alliinase is destroyed by gastric acid [6].

One of the new vesicular complexes is a phytosome made from the encapsulation of phospholipid and extracts into phytoconstituents to improve bioavailability and enhance therapeutic benefits in oral and topical use. Phytosome technology is considered a suitable method for obtaining a better pharmacodynamic and pharmacokinetic profile for active constituents in extracts because the structure of the vesicles protects the herbs from being damaged in an acidic environment. Increased penetration into the biomembrane can be achieved with lipid-containing vesicle complexes [7]. The research conducted by Almajdoub shows that the phosphatidylcholine that forms the phytosome experiences instability in gastric fluid pH simulation [8]. Another study stated that the enzyme alliinase in garlic extract, which converts alliin compounds into allicin, deactivates at the pH of gastric acid. It causes the reduction of allicin compounds to 99 % in the product [3]. Based on this, the phytosomes system was made into an enteric-coated preparation, Eudragit L30 D55. Enteric-coated preparations prevent the drug's release in the stomach and release at a more suitable place, the intestine [9, 10].

Polymeric Eudragit is a series of acrylic and methacrylate polymers available in various forms of ions. Eudragit L30D-55, with a molecular weight of 320,000 g/mol [11], is an anionic copolymer containing free carboxyl groups and esters in a ratio of 1: 1. The carboxyl group ionized in an aqueous medium at a pH of 5.5 and above makes the polymer resistant to the stomach's acidic environment but dissolves at intestinal pH [12]. Spray dryer performed enteric coating—this method is widely used to make microparticles, such as microspheres, with polymers containing hydrophilic, lipophilic, and macromolecular drugs [13]. The main advantages are that the process is only one step, easy to control and improve, and possibly free from organic solvents [13]. This study expected that the formed microparticles could be protected from the acidic environment and released in the intestinal environment.

The concentration of enteric coating polymers influences microparticles characteristics and their drug release in the gastrointestinal tract. A study conducted by Pyar and Peh in drug release tests *in vitro* showed that the concentration of L30D-55 Eudragit above 7.5 % could hold active substances in the stomach environment [14]. The research was carried out on increased Eudragit L30D-55 as an enteric-coated polymer on characterizing the microsphere ArE-Ps to protect it from gastric acid.

MATERIALS AND METHODS

Materials

The materials used include an allicin-rich extract (Lansida), soy lecithin (Lansida), S-allyl 2-propane-1-sulfinothioate (Sigma Aldrich), Eudragit L30D-55 (Evonik). All other reagents used analytical grade.

Methods

Preparation of phytosome

A-4.5 of ArE and soy lecithin weighed, respectively. Then soy lecithin diluted with dichloromethane, while ArE diluted with 96 % ethanol. Both solutions were mixed in a round-bottom flask. The solvent

evaporated using a rotary evaporator (EYELA) at 30 °C with 125 rpm. Then, a thin layer formed stored in a refrigerator for up to 24 h at 7 °C and hydrated with a phosphate buffer solution pH 5.5. Then, suspension sonicated for 60 min.

Evaluation of phytosome

Phytosome evaluated to characterize the vesicle produced. Evaluations include entrapment efficiency, particle morphology, particle size, index polydispersity, potential zeta, and specific gravity.

Preparation of phytosome-loaded microsphere

Phytosome-loaded microsphere formulations seen in table 1. The polymer amount is proportional to the weight of solid phytosome in 1:1; 1:1.5; and 1:2 for F1, F2, and F3, respectively. Eudragit L30D-55 was mixing with triethyl citrate and aqua dest to make a polymer solution. The dispersion of polymer and phytosome suspension dried with 150 °C as inlet temperature and 70 °C as outlet temperatures to obtain microsphere by spray dryer (BUCHI 190).

Table 1: Formula of ArE-Ps microsphere

Materials (Units)	Concentration		
	F1	F2	F3
Phytosome (%)	50	50	50
Eudragit L30 D55 (%)	15	22.5	30
Triethyl citrate (%)	0.9	1.125	1.35
Aqua dest (ml)	ad 100	ad 100	ad 100

Entrapment efficiency determination

The entrapment efficiency determined by 200 mg microcapsule dissolved in phosphate buffer pH 7.4, then centrifuged at 10,000 rpm for 10 min. A-1 ml supernatant taken to measure allicin levels, which are not absorbed in the phytosome vesicles. Furthermore, the volume is sufficient with a mixed solvent phosphate pH 6.8 and 95 % ethanol (2:8), until 10 ml. A-0.5 ml is taken and diluted into a 10 ml volumetric flask, and absorbance is measured using a UV-Vis spectrophotometer (1601-SHIMADZU). Measurement of absorbed-allicin in the phytosome, centrifuge precipitate diluted 0.5 ml dichloromethane, and vortexed. Then, it is diluted in a 10 ml volumetric flask, and the absorbance is measured. The percentage of absorbed-allicin determined with the formula, $\%EE=(M_1-M_2)/M_1\times100$, where M_1 is the total allicin in the microcapsule, and M_2 is allicin concentration in supernatant.

Morphology of microspheres

The morphology of microspheres evaluated using scanning electron microscopy (SEM JEOL JSM-6510LA). The microsphere is attached to the holder then inserted into a vacuum evaporator. At a specific vacuum level, the holder is incandescent so that gold vapor will coat the material attached to the holder. The holder is then inserted into the SEM device and then examined.

Particle size distribution

The particle size distribution and zeta potential were measured using a particle size analyzer (Delsa Max Pro). Some 200 mg of microcapsules dispersed in aqua proinjection in a ratio of 1:19. Then it is directly inserted into a particle size analyzer, and the particle size distribution curve is determined. Zeta potential and polydispersity index measurements were also performed.

***In vitro* drug release studies**

The procedure on this study refers to Anwar *et al.* [15]. The allicin releases from microcapsule carried out in two types of mediums, hydrochloric acid pH 1.2 and phosphate buffer pH 7.4. Drug release testing was carried out on a glass beaker placed on a magnetic stirrer at 37±0.5 °C and 100 rpm; the medium used was 100 ml. Some microcapsules are inserted into the cellophane membrane and dipped in the dissolution medium. The liquid sample is taken as much as 10 ml at a particular minute. On the acidic medium, samplings carried out at minutes of 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 420. While on the pH 7.4 medium, samplings carried out at minutes of 5, 10, 15, 30, 45, 60, 90, 120, and 180. Every 10 ml sample liquid was taken, then 10 ml of the medium solution was added to the dissolution flask, in triplicate. Then allicin levels were determined by UV-Vis spectrophotometer.

Release kinetic models [16, 17]

The mechanism of releasing allicin from three microspheres studied from the calculation with four different kinetic models was performed based on experimental data. Model 1 given by the Ritger-Peppas and Korsmeyer Peppas equations, $f_1=M_i/M_\infty=K.\ t^n$, where f_1 is the amount of drug released, M_∞ and M_i is the amount of drug at the equilibrium state and over time t, respectively; K is the constant of incorporation of structural modifications and geometrical characteristics of the system, and n is the exponent of release in the function of time t. When n = 0.43, the drug release mechanism is the Fickian diffusion for the spherical delivery system. When n = 0.85, the drug release mechanism is ultimately Case II transport. When 0.43>n>0.85, anomalous transport was observed.

Model 2 is the Higuchi model. Although this model is frequently used to describe the drug transport mechanism of thin-film hydrogels, this model can also be used to analyze spherical hydrogel systems' transport mechanism —the equation, $f_1=K_H\sqrt{t}$, where K_H is the release constant of Higuchi.

Model 3 is based on the zero-order drug delivery and expressed when n = 1 in Korsmeyer Peppas equation, $f_i=K_0.\ t$, where $f_i=1-(W_i/W_0)$ represents the fraction of active agent dissolved during the time t, and K_0 is a constant of the apparent velocity of dissolution.

Model 4 represents first-order drug delivery and is expressed by the following equation, $\log Q_1=\log Q_0+(k_1.\ t/2.303)$, where Q_1 is the amount of active agent released on time t, Q_0 is the initial amount of drug dissolved, and K_1 is the first-order constant.

Statistical analysis

One-way Analysis of Variance (ANOVA) analyzed dissolution rate data with a confidence level of 95 % ($\alpha = 0.05$) to determine any differences in all formulas. Then, further test by Tukey HSD to determine significant differences in each formula.

RESULTS AND DISCUSSION

Table 2: Characteristics of ArE

Parameters (Units)	Result	Requirement
Organoleptic		
Form	Sticky	Sticky
Odor	Strong aromatic	Strong aromatic
Color	Brown	Brown
Taste	Bitter	Bitter
Water content (%)	1.21	No more 12
Total ash content (%)	3.43	No more 2.7
Acid-insoluble ash (%)	0.15	No more 0.7

Before use, ArE preformulated by various evaluations parameter presented in table 2. Organoleptic observation gives the physical form, color, smell, and taste of the extract. The water content and acid-insoluble ash of ArE was a meet requirement. While total ash content showed exceed form requirement. These results are the same as those obtained by Phan *et al.* [18], the total-ash value of 3.5-3.7 % by gravimetric method, wherein mineral content associated with raw garlic condition used and planting grounds. Allicin (S-Allyl-2-propene-1-sulfinothioate) is a volatile compound; allicin in extracts identified using gas chromatography with a mass spectrometer detector. The chromatogram (fig. 1) showed a similar peak between extract sample and standard allicin at a retention time of 7.72 min. Allicin calibration curve equation is y =-0.0497+0.0520x with a correlation coefficient (R) of 0.9981 by spectrophotometric. Based on the curve, allicin concentration in extract was 11.29 %; it becomes a reference in preparing phytosome.

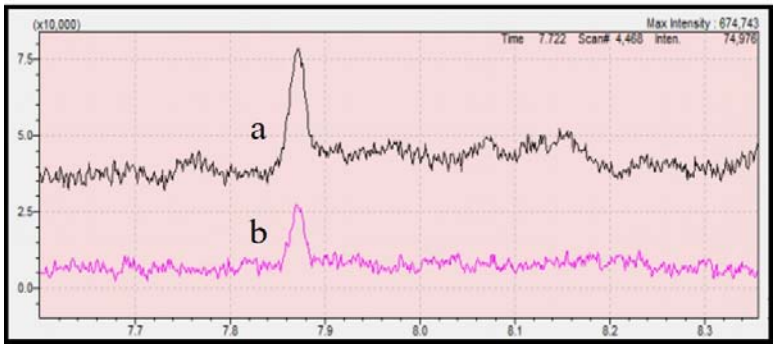

Fig. 1: Chromatogram of S-Allyl-2-propene-1-sulfinothioate from ArE (a) and standard allicin (b)

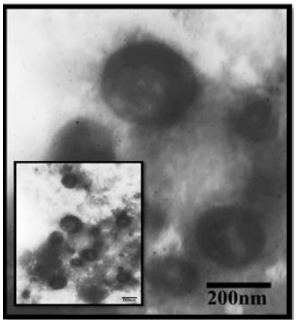

Fig. 2: Morphology of ArE-Ps using TEM

The optimal conditions for preparing phytosomes are using 4.5 g ArE, 4.5 g soy lecithin rotated with a temperature of 30 ˚C and speed of 125 rpm. Soy lecithin is a phospholipids source with a higher proportion of around 76 % phosphatidylcholine with a high content of polyunsaturated fatty acids, such as linoleic acid, about 70 %, linolenic acid and oleic acid [19]. Based on the TEM result in fig. 2, phytosome vesicles have a round shape with varying sizes. This shape is formed after the choline head of the molecule binds with phytoconstituents, and the fat-soluble part of phosphatidylcholine then encloses the material bound to choline. It produces small cells such as micelles in the water environment. The vesicle size from the TEM is not far from the PSA, around 200 nm.

Table 3: Characterization of ArE-Ps optimum formulation

Parameters (Units)	Result
Entrapment efficiency (%)	62.62
D_{mean} (nm)	251.605
Polydispersity index (PDI)	0.466
Zeta potential	34.1088
Specific density (g/ml)	1.0051

Microsphere, made in three formulas with different molar ratios of ArE-Ps and Eudragit L30D-55 (1:1; 1:1.5 and 1:2) by spray dry, produces different sizes. Based on the result, the microsphere size was 548.8 nm, 215.0 nm, and 335.3 nm, respectively, for F1, F2, and F3. Particle size from spray drying can be influenced by the size of the nozzle, viscosity of the polymer solution, dispersion of the active substance in the polymer solution, and surface tension [20]. There are significant differences in the three formulas in the particle size values from the results of data analysis with differences in the concentration of Eudragit L30D-55 polymers. The michrosphere of F1 has zeta potential below-30 mV to allow agglomeration between particles to make the particle size larger. Nanoparticles with zeta potential below-30 mV and above+30 mV have good suspension stability because the surface charge prevents aggregation between particles [15]. Zeta potential values F1, F2, and F3 respectively; -28.88±0.81;-32.15±1.29; and-30.20±0.61. The results show that only F2 and F3 can prevent aggregation between particles because they have a negative potential zeta value below-30 mV.

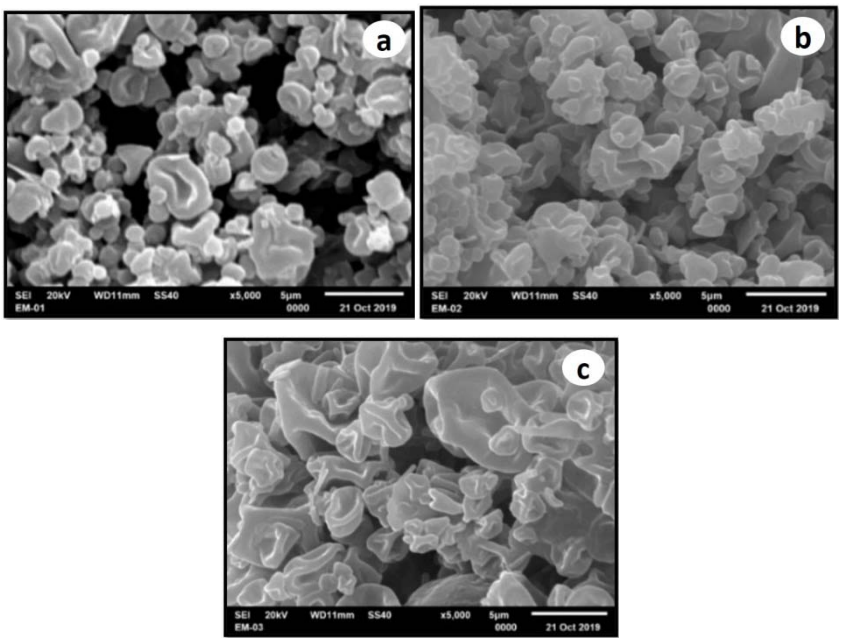

Fig. 3: Morphological structure of 5000x magnification microcapsules (a) ratio 1:1 (b) ratio 1:1.5 (c) ratio 1:2

Table 4: Microsphere evaluation results

Ratio (ArE-Ps: Eud)	Result			
	Entrapment efficiency (%)	D_{mean} (nm)	Zeta potential	Polydispersity index
1:1	58,93	548,8±10,15	-28,88±0,81	0
1:1,5	62,31	215,0±6,27	-32,15±1,29	0,571
1:2	65,44	335,3±11,06	-30,20±0,61	0,571

The morphological structure presented in fig. 3, microsphere appears as an irregular shape with many deflections, no uniform sizes, and no porous surface. Nijdam and Langrish estimate that giving a high temperature causes water to evaporate faster and gives a non-uniform structure [21]. During the high-temperature drying process, the evaporation of water solvent molecules in the droplet occurs so rapidly that it causes "emptiness" in the droplet whose surface has formed a film layer and results in a form of rounded particles that deflate [22].

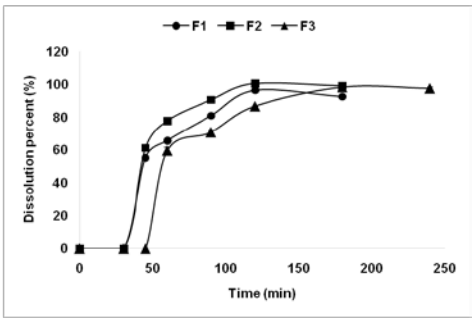

Fig. 4: Percent dissolution of the microsphere in a buffer medium pH of 7.4

The *in vitro* dissolution profile of microsphere from all formulations in acidic medium (pH 1.2) and phosphate buffer medium (pH 7.4) shown in fig. 4. In acidic medium for 480 min, all formulation shows no allicin release, marked with the absence of absorbance in the sample taken. It proves that Eudragit from all three formulations can retain the release of allicin in an acidic medium. The microsphere of F1 and F2 in alkaline pH 7.4, began to release allicin at 45 min by 55.23±0.00 % and 61.26±0,66 %. At the same time, F3 microcapsules release allicin at 60 min by 59.50±1.85 %. The F3 microsphere retains allicin release longer because it contains more polymers than F1 and F2. Release of allicin caused by salt formation between the polymers Eudragit with alkali at pH 7.4 so the polymer layer can dissolve. The microsphere of F1 and F2 dissolve entirely in the 120 min, while F3 in the 180 min. The different rates of dissolving active substances released from the microsphere influenced by the thickness of the coating wall and surface pores. Fewer constituent polymers and thinner coating walls allow active substances to release faster in phosphate buffer pH 7.4.

Furthermore, dissolution percent of microsphere from all formulation analyzed by fitting them to four model drug releases kinetics equations such as kinetics of zero-order, first-order, Higuchi and Korsmeyer-Peppas. From each equation, we get the drug release constant (k), correlation coefficient (r), and exponent dissolution of Peppas (n).

Table 5: Kinetics of allicin release in microsphere

Model	Formulation	Parameter		
		R^2	K	n
Korsmeyer-Peppas	F1	0.8805	12.60	0.40
	F2	0.8461	17.93	0.35
	F3	0.9247	12.96	0.38

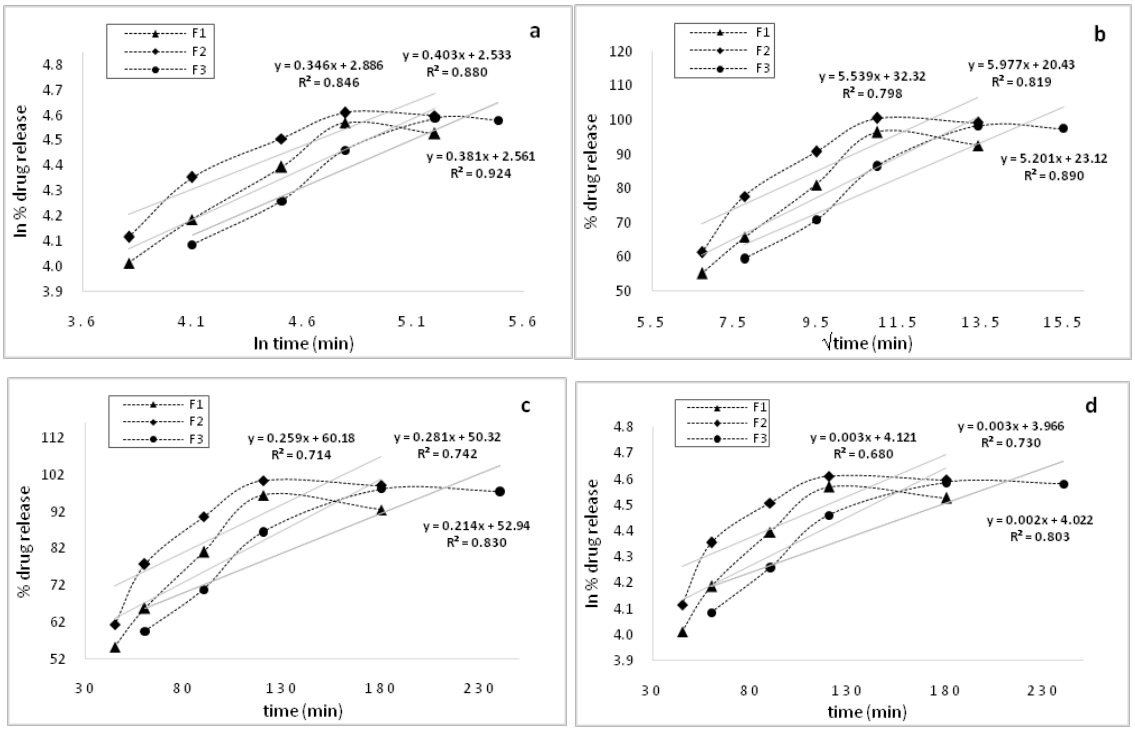

Fig. 5: Drug release kinetics plots for all formulations (a) Korsmeyer-Peppas (b) Higuchi (c) Zero-order (d) First-order

Based on table 5, allicin release from the microsphere follows the Korsmeyer-Peppas kinetics. The Korsmeyer-Peppas equation explains the mechanism of drug release from preparations based on the Fickian model mechanism is the same as Higuchi. In the Korsmeyer-Peppas equation, the release mechanism depends on the value 'n' when n = 0.43; the Fickian diffusion governs the drug release mechanism when 0.43<n<0.85 it is anomalous (non-Fickian) transport, and when n = 0.85 it is case II transport [16]. The n values of all formulation<0.45 indicate the release mechanism follows the Fickian model. According to Fickian, the dissolution rate of solid form is determined by the dissolution rate of a thin layer of a solution formed around the solid. The drug dissolved in a saturated solution diffuses into the solvent from high concentration to an area of low drug concentration.

Data on the dissolution rate analyzed statistically to see the differences between the three formulas. The normality test resulted in sig values of 0.247; 0.429; dan 0.075 (sig>0.05); then, the homogeneity test resulted in sig values of 0.056 (sig>0.05); it concluded the data typically distributed and homogeneous. Furthermore, a one-way ANOVA test resulted in sig values of 0.002 (sig<0.05); it concluded a significant difference in the dissolution rate of microsphere between all formulations.

Based on these results, the polymer's increase in three microsphere formulations can affect characteristics and retain drug release under acidic conditions. The three formulations follow Korsmeyer-Peppas kinetics release with k values 12.71±0.18; 17.93±1.56; and 12.96±1.27, for F1, F2, and F3, respectively.

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Nil

AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICT OF INTERESTS

Declared none

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