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Research Article

FORMULATION OF ENTERIC GRANULES CONTAINING GREEN TEA (*CAMELLIA SINENSIS*) EXTRACT USING EUDRAGIT L100-55 AS A DELAYED RELEASE MATRIX

EFFIONORA ANWAR*, JESSICA RAMADHANTY VALENSYA SHERMAN

Laboratory of Pharmaceutics and Pharmaceutical Technology Development, Faculty of Pharmacy, Universitas Indonesia, Depok, Indonesia. Email: effi.nora@gmail.com

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ABSTRACT

Objective: Epigallocatechin gallate (EGCG) inhibits glucose absorption into the blood by inhibiting small intestinal α-glucosidase but is unstable in gastric fluid. Hence, we formulated EGCG into enteric preparations that prevent release in gastric fluid.

Methods: Granules were prepared using a wet granulation method and were formulated into polyvinylpyrrolidone (PVP)-Eudragit L100-55 (5:1; F1), PVP-Eudragit L100-55 (1:1; F2), and Eudragit L100-55 (F3) preparations using 30% w/w Eudragit L100-55 as a matrix. EGCG contents of granules were evaluated and dissolution tests were performed at pH 1.2 and 6.8.

Results: F1–3 formulas had good flow properties and contained EGCG at 24.05%±0.15%–24.96%±0.28%. Dissolution tests showed that F1 and F2 formulas released EGCG at 50.53%±0.04% and 17.80%±0.55%, respectively, after 2 h in HCl medium at pH 1.2. Cumulative drug release from F1 and F2 formulations after 2 h under these conditions (pH 1.2) and 1 h in phosphate buffer (pH 6.8) was 94.40%±1.58% and 93.70%±1.08%, respectively.

Conclusion: As the optimal formula, F3 granules limited drug release to 7.03%±0.22% in HCl at pH 1.2 over 2 h and cumulative drug release in HCl medium (pH 1.2) followed by phosphate buffer (pH 6.8) of 86.13%±0.20%.

Keywords: Delayed release, Enteric, Epigallocatechin gallate, Eudragit L100-55, Granules, Green tea (*Camellia sinensis*) extract.

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INTRODUCTION

Epigallocatechin gallate (EGCG) is a major active compound in green tea and reportedly inhibits dietary glucose absorption into the blood. The previous studies show that EGCG inhibits intestinal α-glucosidases [1]. Accordingly, *in vivo* experiments showed that 100 mg of EGCG significantly reduced post-prandial blood glucose levels in rats fed on corn starch [2]. EGCG is known to be sensitive to acid degradation and hydrolysis in gastric fluids [3] and hence lacks gastrointestinal stability. EGCG is, however, stable at pH 4–6 [4] and degradation of EGCG in gastric acid can be prevented by enteric formulations.

Enteric coat technology is commonly used to develop delayed release systems for drug preparations. These limit drug release in the stomach and favor rapid release in the intestine [5]. Manufacture of enteric-coated preparations requires several stages and is time-consuming [6], warranting the development of alternative pH-sensitive enteric matrixes.

In this study, the matrix granules Eudragit L100-55 and polyvinylpyrrolidone (PVP) were used in combination. Recent experiments show that Eudragit L100-55 can prohibit drug release to <10% in HCl medium of pH 1.2 at a minimum concentration of 5% [7]. Eudragit L100-55 is a methacrylate copolymer that resists drug release in the stomach and prioritizes release in the intestine [8]. Dissolution profiles at various pH values are strongly correlated with the performance of such enteric polymers. Eudragit L100-55 polymer releases its contents at the small intestinal pH of >5.5, where α-glucosidase enzyme is located. PVP is a hydrophilic polymer that can be used to control and increase drug release, and also acts as a binder [9]. Thus, in this study, PVP-Eudragit L100-55 combinations were expected to increase drug release in the small intestine. With the expectation that polymer controlled drug release systems can regulate the release of EGCG into the small intestine, we generated enteric green tea leaf extract granules containing EGCG using Eudragit L100-55 as a delayed-release matrix polymer.

MATERIALS AND METHODS

Materials

Green tea leaf (*Camellia sinensis*) extract (AndyBiotech, China), EGCG (Sigma Aldrich, Singapore), Eudragit L 100-55 (Evonik, Germany), PVP (Nanhang Industrial Co., China), Avicel pH 101 (AIC, USA), acetonitrile (Merck, Germany), HCl (Brand, Germany), glacial acetic acid (Merck, Germany), potassium dihydrogen phosphate (Merck, Germany), methanol for LC (Merck, Germany), 96% ethanol (Brataco, Indonesia), aqua pro injection (IKAPHARMINDO, Indonesia), and aqua demineralisata (Brataco, Indonesia) were purchased from the parenthetic manufacturers.

Methods

Optimization of total polymer concentrations

Total polymer concentrations were optimized to withstand drug release in acidic medium. Polymer concentrations of 5%, 15%, and 30% were used with reference to previous research [7,10]. Granule formulas are listed in Table 1.

Granules were prepared using a wet granulation method. Briefly, green tea leaf extract, Avicel pH 101, and Eudragit L100-55 were combined to a total of 100 g and were mixed until a homogenous color was obtained. Wet granulation was then performed by adding up to 40 mL of 96% ethanol to obtain wet masses that could be clenched. Wet masses were then formed into granules using an eight mesh sieve. Moist granules were dried in an oven at 40±0.5°C for 2 h and were then reduced to desired sizes using an 18 mesh sieve.

Optimization of polymer concentrations

Visual tests

Granule samples of 300 mg with polymer concentrations of 5% (fumaric acid), 15% (FB), or 30% (FC) were placed in 150 mL glass beakers containing 100 mL of HCl media at pH 1.2 for 2 h. Intact granules were then tested for dissolution.

Dissolution tests

Dissolution tests were performed as described previously [11,12] with various modifications. Representative gastric and duodenal fluids were produced in 100-mL aliquots using HCl (pH 1.2) and phosphate buffer (pH 6.8), respectively. After preparation in 150-mL glass beakers, HCl media at pH 1.2 were added to 1000-mL glass beakers containing 200 mL of water and were then heated on a hot plate to 37±0.5°C. Granule samples of 260 mg containing 65 mg of EGCG were placed in 2 cm×2-cm bags made of filter paper sewn with mattress thread. Bags were then placed an acid medium at 37±0.5°C and were stirred at 100 rpm using a magnetic stirrer. After 2 h, 2-mL aliquots were transferred to 100 mL of phosphate buffer (pH 6.8) at 37±0.5°C and were stirred at 100 rpm. After 15, 30, 45, and 60 min, 2-mL aliquots of buffer media were taken and replaced with phosphate buffer pH 6.8. Dissolved EGCG contents of phosphate buffer samples were then determined using high-performance liquid chromatography (HPLC).

Formulation of granules

Enteric granule formulae of green tea leaf extracts are listed in Table 2. Granules containing green tea leaf extract, Avicel pH 101 (as a filler), Eudragit L100-55, and PVP in the relative quantities listed in Table 2 were synthesized using the wet granulation method as described above.

Evaluation of enteric granules of green tea leaf extract

Organoleptic tests

Organoleptic tests included observations of shape, color, odor, and taste of the produced granules [13].

Granule size distribution tests

Size distributions of the granules were evaluated using sieve shakers. Briefly, series of five sieves with numbers 16, 25, 35, 45, and 60 were arranged in descending order from the largest sieve hole. Subsequently, 25-g granule samples were placed in the top sieve and the sieving machine was run for 10 min. Weights of fractions remaining in the sieves were recorded as described previously [14].

Flow properties

Angle of repose

Test samples of 100 mL were introduced into dry funnels with nozzles of 10 mm in diameter. The angle of repose was determined according to the United States pharmacopeia 35. Specifically, an integrated driven laser of the Flow Tester was directed to the sidewall of the built-up cone and actual angles were calculated [14].

Table 1: Granule formulas for optimization of enteric polymer concentrations

Material	FA (%)	FB (%)	FC (%)
Green tea leaf extract	50	50	50
Avicel pH 101	45	35	20
Eudragit L 100-55	5	15	30
Total	100	100	100

Table 2: Formula for preparation of granules

Material	Concentrations (% b/b)		
	F1	F2	F3
Green tea leaf extract (contains 50% EGCG)	50	50	50
Avicel pH	20	20	20
Eudragit L100-55	-	-	30
PVP-Eudragit L 100-55 (1:1)	-	30	-
PVP-Eudragit L 100-55 (5:1)	30	-	-

EGCG: Epigallocatechin gallate, PVP: Polyvinylpyrrolidone

Calculation of compressibility indexes and Hausner ratios

Compressibility index values and Hausner ratios were calculated according to the United States pharmacopeia 35 equations using apparent density data and tapped density tests. The results are shown in Table 3.

Determination of EGCG levels in granules

Instrumentation and operation conditions for HPLC

EGCG concentrations were determined using a Shimadzu HPLC column model LC6A with a SPD-6AV UV-visible detector and a Kromasil column 100–5 C18 of 250 mm×4.6 mm. Samples of 20 µL were injected and eluted using a mobile phase comprising 0.05% acetic acid-acetonitrile (87:13 v/v) with a final pH of 3.5–4 and with a column temperature of 20±3°C and a flow velocity of 1 mL/min. Elutes were detected at a wavelength of 280 nm [15].

Determination of EGCG levels in granules

Granule samples of 1 g were grinded to obtain powder, of which 50-mg aliquots were diluted to 25 mL in volumetric flasks. Solutions were sonicated for 5 min, were filtered through Millipore filters to remove particles, and were then injected into the HPLC instrument. Tests were performed in triplicate and EGCG concentrations were calculated by entering measured areas under the curve into linear regression equations.

Dissolution tests

Dissolution tests were performed as described for optimization of total polymer concentrations in granule formulations.

RESULTS AND DISCUSSION

Optimization of total polymer concentrations in formulas

After treating granules in acid medium for 2 h, visual optimization observations showed that enteric granule matrixes containing polymer at 5% formed unfavorable soft masses at the bottoms of the containers (Fig. 1). Yet at polymer concentrations of 15% and 30%, granule shapes remained and did not become soft.

Formulas B (Eudragit L100-55 15%) and C (Eudragit L100-5 30%) were then tested for dissolution in HCl at pH 1.2 over 2 h. Formula B released 24.03%±2.28% of the active substance under these conditions, whereas formula C (30% Eudragit L100-55) released only 7.03%±0.22% of active substance. Based on the results of these preliminary tests, the optimal polymer concentration for resisting the release of active substance into acid medium was 30%.

To improve on 30% Eudragit L100-55 matrixes, we produced F1–3 formulas with PVP–Eudragit L100-55 ratios of 5:1, 1:1, and 0:1, respectively. The best of these formulas was used as the enteric preparation in dissolution tests.

Table 3: Flow evaluation results of granule properties

Formula	Flowability (g/s)	Angle of repose (°)	Compressibility index (%)	Hausner ratio
1	4.37±0.07	32.52±0.41	12.25±0.07	1.14±0.00
2	4.12±0.03	33.00±0.37	12.70±1.37	1.14±0.01
3	4.20±0.02	32.43±0.16	12.09±0.05	1.13±0.00

Table 4: Cumulative epigallocatechin gallate release

Formula	Cumulative drug release in HCl medium at pH 1.2; mean±SD (%)	Cumulative drug release in HCl medium at pH 1.2 and then at pH 6.8; mean±SD (%)
1	50.53±0.04	94.40±1.58
2	17.80±0.55	93.70±1.08
3	7.03±0.22	86.13±0.20

Formulation of granules

Following manufacture with 100-g mixtures, F1–3 granules were yielded at 92.82, 91.52, and 90.45 g, respectively. The 8%–10% losses in mass reflect residues left in containers and sieves during manufacture.

Evaluation of granule masses

Organoleptic tests

The three granule formulas described herein had single large granular solid forms, were brownish-orange in color and had a green tea flavor with a slightly bitter taste.

Particle size distribution tests

Particle size distributions of F1–F3 granules were measured using multilevel sieves covering the range 0–1180 µm. The results of tests with 710–1180-µm sieves are shown in Fig. 2.

Particle size distribution tests ensure uniformity of contact surface areas with dissolution medium during dissolution testing, thus facilitating comparisons of granules.

Tests of flow properties

As shown in Table 3, flowabilities of granules were tested using various methods and properties of the formulations were characterized as precisely as possible. To identify differences in flow characteristics of the granules, we measured of angles of repose, performed flowability tests, and determined compressibility indexes and Hausner ratios.

The values for angles of repose, compressibility index, and Hausner ratios were similar for all granules (Table 3). Moreover, all prepared formulations had good flow properties, with angles of repose within 31°–35°, compressibility indexes of 11%–15%, and Hausner ratios of 1.12–1.18.

Determination of EGCG levels in granules

EGCG levels were determined by grinding 1 g of granules and then diluting 50-mg samples (equivalent to 12.5 mg EGCG) in 25 mL of mobile phase. EGCG contents of F1–3 preparations were 24.05%±0.15%, 24.78%±0.14%, and 24.96%±0.28%, respectively.

Dissolution test

To evaluate drug release profiles from granules, we set up calibration curves of EGCG standard solutions in acid (pH 1.2) and phosphate buffer (pH 6.8). These gave linear concentration curves following the equations y=21626x+103950 (r=0.9998) and y=20691x–128135 (r=0.9999), respectively.

Dissolution profiles of granules from F1–3 preparations in acid for 2 h followed by phosphate buffer for up to 60 min are shown in Fig. 3.

The dissolution profiles of EGCG in Fig. 3 show that F1 and F2 granules containing green tea leaf extracts failed to prevent release of the drug in acidic medium, releasing 50.53%±0.04% and 17.80%±0.55% of active compound, respectively. In contrast, the F3 formula comprising Eudragit L100-55 at 30% cumulatively released only 7.03%±0.22% of the active compound over 2 h in an acid medium.

Addition of PVP at 5 times, the concentration of Eudragit L100-55 (5:1) led to greater release of the drug in acid medium over 2 h. These observations can be explained by the predominance of PVP over Eudragit L100-55 as the matrix constituent polymer. Under these conditions, the matrix was eroded in acidic medium because PVP polymers are pH-sensitive, although PVP can act as a binder [9]. In F2 preparations, the percentage of drug release in acid media was smaller than from F1 preparations. Thus, additional Eudragit L100-55 concentrations in the matrix can reduce drug release in acid medium. F2 was better able to withstand drug release in acidic medium than F1, although it still released >10% of the active compound. These results show that 5:1 and 1:1 combinations of PVP and Eudragit L100-55 in granule matrixes are not optimal for preventing drug release in acid medium over 2 h. The granules with a matrix consisting of 30% Eudragit L100-55 are at least released from the granules at ph 2.

Cumulative drug release from F1 and F2 preparations was higher than from F3, as shown in Table 4. Similarly, previous research [7,10] showed that higher Eudragit L100-55 concentrations in the formula reduce dissolution rates. Accordingly, at low pH, Eudragit L100-55 does not dissolve and drug release is regulated by diffusion of the active substance from the matrix. At higher pH, drug release is affected

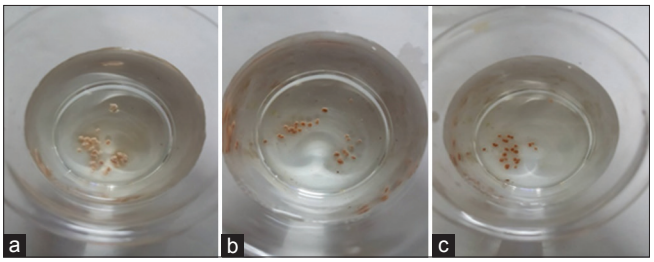

Fig. 1: (a) Fumaric acid FA, granules with 5% Eudragit; (b) FB, granules with 15% Eudragit; (c) FC, granules with 30% Eudragit; after incubation in acid medium for 2 h

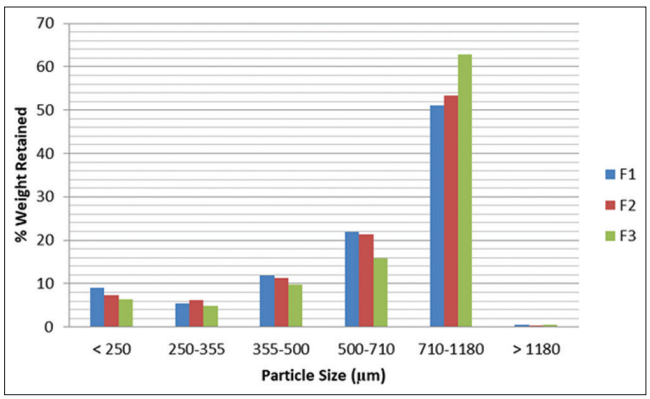

Fig. 2: Particle size distribution curves of granule formulas containing green tea leaf extract; Data are presented as means±standard deviations (n=3)

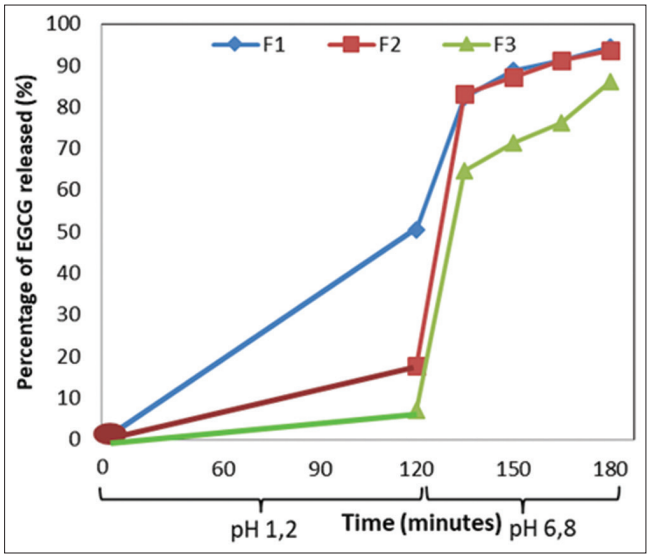

Fig. 3: Epigallocatechin gallate dissolution profiles of F1–3 formulations containing green tea leaf extract; tests were performed in HCl (pH 1.2) for 2 h followed by phosphate buffer (pH 6.8) for up to 60 min. Data are presented as means±SD; n=3

by Eudragit L100-55 polymer solubilization and degradation of the matrix [10]. Therefore, higher Eudragit L100-55 concentrations in F3 likely require longer dissolution times to achieve the same cumulative release as from the Eudragit L100-55 formulas F1 and F2.

CONCLUSION

Enteric granules formulated with 30% w/w Eudragit L100-55 as a matrix polymer released only 7.03%±0.22% of their drug contents in acidic medium over 2 h but cumulatively released 86.13%±0.20% of drug contents after a further hour in phosphate buffer at pH 6.8.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

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