Drug Development and Industrial Pharmacy

ISSN: 0363-9045 (Print) 1520-5762 (Online) Journal homepage: http://www.tandfonline.com/loi/iddi20

In vitro and in vivo investigation of taste-masking effectiveness of Eudragit® E PO as drug particle coating agent in orally disintegrating tablets

Milica Drašković, Djordje Medarević, Ivana Aleksić & Jelena Parojčić

To cite this article: Milica Drašković, Djordje Medarević, Ivana Aleksić & Jelena Parojčić (2016): In vitro and in vivo investigation of taste-masking effectiveness of Eudragit® E PO as drug particle coating agent in orally disintegrating tablets, Drug Development and Industrial Pharmacy, DOI: 10.1080/03639045.2016.1220572

To link to this article: http://dx.doi.org/10.1080/03639045.2016.1220572

Accepted author version posted online: 05 Aug 2016.
Published online: 05 Aug 2016.

In vitro and *in vivo* investigation of taste-masking effectiveness of Eudragit® E PO as drug particle coating agent in orally disintegrating tablets

Milica Drašković*, Djordje Medarević, Ivana Aleksić, Jelena Parojčić

Department of Pharmaceutical Technology and Cosmetology, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade, Serbia

*Corresponding author

Milica Drašković

Department of Pharmaceutical Technology and Cosmetology,

Faculty of Pharmacy, University of Belgrade,

Vojvode Stepe 450, 11221 Belgrade, Serbia

Telephone number: +381 11 3951367

Fax number: + 381 11 3972840

e-mail: milicam@pharmacy.bg.ac.rs

Abstract

Context: Considering that bitter taste of drugs incorporated in orally disintegrating tablets (ODTs) can be the main reason for avoiding drug therapy, it is of the utmost importance to achieve successful taste-masking. The evaluation of taste-masking effectiveness is still a major challenge.

Objective: The objective of this study was to mask bitter taste of the selected model drugs by drug particle coating with Eudragit® E PO, as well as to evaluate taste-masking effectiveness of prepared ODTs using compendial dissolution testing, dissolution in the small-volume shake-flask assembly and trained human taste panel.

Materials and methods: Model drugs were coated in fluidized bed. Disintequik™ ODT was used as a novel co-processed excipient for ODT preparation. Selected formulations were investigated *in vitro* and *in vivo* using techniques for taste-masking assessment.

Results and discussion: Significantly slower drug dissolution was observed from tablets with coated drug particles during the first three minutes of investigation. Results of *in vivo* taste-masking assessment demonstrated significant improvement in drug bitterness suppression in formulations with coated drug. Strong correlation between the results of drug dissolution in the small-volume shake-flask assembly and *in vivo* evaluation data was established ($R \geq 0.970$).

Conclusion: Drug particle coating with Eudragit® E PO can be a suitable approach for bitter taste-masking. Strong correlation between *in vivo* and *in vitro* results implicate that small-volume dissolution method may be used as surrogate for human panel taste-masking assessment, in the case of physical taste-masking approach application.

Keywords: taste-masking, small-volume shake-flask assembly, trained human taste panel, *in vitro-in vivo* correlation, drug particle coating

Introduction

Orally disintegrating tablets (ODTs) emerged as convenient alternative to conventional solid dosage forms. According to European Pharmacopoeia, ODTs should disintegrate in less than 3 minutes into a suspension or solution in the oral cavity, without applying additional water[1]. Orally disintegrating dosage forms offer numerous advantages, such as: i) facilitated swallowing[2] and, thus, increased compliance in the pediatric and geriatric populations and possibility of use "anytime and anywhere"[3], ii) faster achievement of desired therapeutic drug effect[4] and iii) increase in the bioavailability of poorly soluble drugs[5,6]. One of their main drawbacks is the need for masking the taste of incorporated, bitter, highly soluble, active pharmaceutical ingredients (APIs). After ODT disintegration, highly soluble drugs will release, dissolve and interact with receptors of the taste buds, predominantly distributed over the surface of the tongue[7,8]. Considering that bitter taste of APIs can be the main reason for avoiding drug therapy, it is of the utmost importance to achieve successful taste-masking[9].

Different taste-masking approaches include: i) sensory (gustatory), ii) chemical and iii) physical approach. Sensory method is usually combined with more complex chemical or physical methods. It includes flavors or sweeteners addition[10-14]. The aim of physical and chemical approaches is to slow down dissolution of the unpleasant tasting drug in the oral cavity. Physical taste-masking approaches include granulation, extrusion or coating the drug with different polymers[10,14-17]; or preparation of microspheres by solvent evaporation method[18]. Coating in fluidized bed can be used for masking the unpleasant taste of drug[11,15,19]. However, it is necessary to demonstrate that coating does not violate critical ODT characteristics, such as tablet hardness, disintegration time, as well as dissolution of the incorporated drug[20]. Generally, particles larger than 350 μm can lead to gritty taste in mouth, which can impact patient compliance[11]. In order to

maintain the small particle size, direct drug particle coating can be applied. Guhmann and coworkers investigated effectiveness of drug particle coating, in the case of diclofenac, low solubility model substance[11]. In the present study, paracetamol and caffeine anhydrous were selected as highly soluble bitter drug substances, which could dissolve in the limited saliva volume, taking into consideration the reported aqueous solubility at 25° C of 14.3 mg/ml and 21.9 mg/ml for paracetamol and caffeine anhydrous, respectively[21,22].

Despite the fact that numerous techniques for masking the taste of drug substances have been developed, the evaluation of taste-masking effectiveness is still a major challenge. Standardized guidance for the *in vitro* taste-masking assessment is lacking. Therefore, many authors attempt to develop specific methods, which are in correlation with the *in vivo* data[23]. There are several *in vitro* techniques used for evaluation of taste-masking effectiveness, such as: i) compendial dissolution testing[10,11,24,25], ii) shake-flask method[26,27] and iii) electronic taste-sensing systems[11,25,28,29]. Although application of electronic taste-sensing system recently gained in significance, there are still certain difficulties considering evaluation of taste-masked ODTs, such as sample preparation[23]. Insoluble particles can cause damage of the taste-sensing system sensors[23]. Hence, before investigation ODTs should be dispersed in adequate medium and, filtrated immediately in order to remove insoluble particles and avoid ongoing drug dissolution. For proper evaluation, solutions in the volume range from 25 up to 100 ml have to be investigated[30]. Therefore, the crucial step is fast filtration of large solution volumes. There is a limitation related to establishment of the relationship between sensor responses and different drug concentration, in the case of neutral substances (such as paracetamol and caffeine)[30]. Trained human taste panel is still the gold standard in taste-masking effectiveness evaluation[15,23,28,29,31], irrespective of being expensive and subject to ethical considerations and

inter-subject variability[20]. Correlation between *in vitro* and *in vivo* methods for taste-masking evaluation is difficult to establish considering higher sensitivity of the proposed *in vitro* methods[28].

The aim of the present study was to mask bitter taste of the selected model drugs by drug particle coating, evaluate taste-masking effectiveness of prepared orally disintegrating tablets and establish correlation between *in vitro* and *in vivo* data. Eudragit® E PO, amino methacrylate copolymer insoluble above pH 5, was used for particle coating[32]. Disintequik™ ODT, novel co-processed excipient consisting of lactose monohydrate, spray-dried mannitol, crospovidone and dextrose monohydrate, was used as diluent for direct compression. Taste-masking effectiveness was evaluated *in vitro* and *in vivo*, using compendial dissolution testing, dissolution in small-volume shake-flask assembly and trained human taste panel.

Materials and methods

Materials

Paracetamol - PAR (Acros Organics, Geel, Belgium) and caffeine anhydrous - CA (BASF, Ludwigshafen, Germany) were used as model drugs; Eudragit® E PO, (Evonik, Nordrhein-Westfalen, Germany) was used as coating polymer; sodium lauryl sulfate (Ph. Eur. 8.0), stearic acid (Ph. Eur. 8.0), and talc (Ph. Eur. 8.0) were used, respectively, as wetting agent, plasticizer and glidant in the polymer dispersion; Disintequik™ ODT (Kerry, Beloit, WI USA), co-processed excipient, was used for direct compression of ODTs; sodium stearyl fumarate (JRS Pharma, Rosenberg, Germany) was used as a lubricant; raspberry flavor was kindly gifted by Firmenich (Geneva, Switzerland); sodium chloride (Sigma–Aldrich Chemie GmbH, Steinheim, Germany), potassium phosphate monobasic (Sigma–Aldrich Chemie GmbH, Steinheim, Germany), disodium hydrogen phosphate (Ph. Eur. 8.0), and hydrochloric acid (Sigma–Aldrich

Chemie GmbH, Steinheim, Germany) were used for preparation of simulated saliva. Commercial bottled water (Aqua viva™, Knjaz Miloš, A.D. Aranđelovac, Serbia) was used for standard solutions preparation and mouth rinse for *in vivo* taste-masking assessment.

Methods

Preparation of Eudragit® E PO aqueous dispersion

Preparation of Eudragit® E PO dispersion in distilled water was carried out in three steps: sodium lauryl sulfate was stirred in 70% of the total water amount for 5 min, using magnetic stirrer, heated at 50 °C; then, stearic acid and Eudragit® E PO were added and the suspension was stirred for 1 h; talc was dispersed in the remaining water, using rotor–stator homogenizer (IKA Ultra-Turrax® T25 digital, IKA®-Werke GmbH, Staufen, Germany). Two suspensions were mixed together and stirred with Ultra-Turrax® T25, for 15 min, reducing the speed of mixing over time (from 10 000 to 6 000 rpm). Suspension was heated to facilitate formation of the colloidal dispersion and to prevent foaming[33]. Proportion of solids in the coating dispersion was 12%. Eudragit® E PO load was 30% relative to the amount of drug while the contents of sodium lauryl sulfate, talc and stearic acid, were, respectively, 10, 15 and 10% relative to the polymer weight.

Fluidized bed coating

Paracetamol and caffeine were sieved in order to remove fines and particles agglomerates. Drug particles fraction in the range from 125 to 355 μm was coated in Mycrolab fluid bed processor (OYSTAR Hüttlin, Schopfheim, Germany), in the top-spray configuration, using the 0.8 mm nozzle. Fluid bed processor was connected to a personal computer allowing the process parameters to be monitored and recorded. The batch size was 140 g. Processing chamber was preheated to 55-60 °C, whereupon drug powder was filled into the processing chamber and fluidized. Eudragit® E PO aqueous dispersion was sprayed onto the fluidized particles, at feed

rate of 2.2-4.3 g/min. The inlet air flow rate was 15-20 m^3/h, and the inlet air temperature was set to 45 °C. The microclimate and spray air pressure were 0.5 and 0.8 bar, respectively. Filters were shaken for 0.2 s in alternating mode during the whole process.

Characterization of coated particles

Size distribution

Size distribution of coated particles (sample size 100 g) was evaluated by sieve analysis using the vibrating shaker (Erweka AR400, Heusenstamm, Germany) and five standard sieves in the range 63-355 µm. The amount of material remained on each sieve was accurately weighted to determine the particle size distribution.

Drug content

Drug content was determined spectrophotometrically (Cary 50, Varian, Santa Clara, USA), after dispersion of 100 mg of coated particles in 100 ml of 0.1 M hydrochloric acid, using the shaker (KS 260 basic, IKA®-Werke GmbH, Staufen, Germany) at 400 rpm. Dispersion was filtered through a 0.45 µm Millipore filter (Millipore, Bedford, MA, UK), diluted and analyzed at 245 nm in the case of PAR and 272 nm in the case of CA. Each sample was analyzed in triplicate and results are shown as the mean with standard deviation.

Flowability

Flowability of uncoated and coated drug particles was determined using Flow meter (Erweka GDT, Heusenstamm, Germany). Results are expressed as the mean value of three replicates.

Moisture content

Residual moisture content of the prepared coated particles was determined gravimetrically using a halogen moisture analyzer (Chyo IB-30, Sun Scientific Co Ltd, Japan).

Scanning electron micrographs

Morphology of uncoated and coated drug particles was examined using high-resolution desktop scanning electron microscope (Phenom G2 Pro, Phenom-World, Eindhoven, Netherlands). Coated particles were cut with a scalpel and a surface was de-dusted by compressed air to obtain a clear cross section. Samples were placed in the microscope holder and images were taken at a suitable magnification.

Tablet preparation

Orally disintegrating tablets were prepared by mixing appropriate amounts of drug with Disintequik™ ODT, sodium stearyl fumarate and flavor and compressed on a single-punch tablet press (EKO Korsch, Berlin, Germany), using flat faced punches with a diameter of 8 mm. All samples were compressed under the same compression force and filling volume. Tablet weight was set to 200 mg, while the drug content was 50 mg per tablet (25% of total tablet weight). Composition of the investigated samples (F1-F8) is presented in Table 1. Reference drug-free formulation consists of Disintequik™ ODT, sodium stearyl fumarate and coating dispersion components.

Characterization of tablets

Tensile strength

Tablet hardness was evaluated using tablet hardness tester (Erweka TBH 125D, Heusenstamm, Germany). Tensile strength (T, MPa) was calculated using the formula devised by Fell and Newton[34]:

$$T = (2 \times P)/(\pi \times D \times t) \quad (1)$$

Where P (N) is the force applied for tablet breaking, D (mm) is the tablet diameter and t (mm) is the tablet thickness. Thickness of the tablet was determined using caliper. Six tablets of each formulation were subjected to tensile strength determination.

Friability

Tablet friability was evaluated using friabilator (Erweka AR400, Heusenstamm, Germany) at 25 rpm/min for 4 minutes. Ten tablets of each formulation were tested. Friability was reported as a loss in tablet weight (%).

Disintegration testing

In vitro ODT disintegration time was determined using the compendial disintegration apparatus (Erweka ZT52, Heusenstamm, Germany). 800 ml of simulated salivary fluid (SSF), pH 6.75, heated to 37 ± 0.5 °C, was used as immersion medium. SSF consists of 2.38 g disodium hydrogen phosphate, 0.19 g potassium phosphate monobasic and 8.00 g sodium chloride per liter of distilled water adjusted with hydrochloric acid to pH 6.75[35]. Six tablets from each sample were investigated and the values are reported as mean ± standard deviation.

Dissolution testing

Drug dissolution testing was performed using the standard rotating paddle apparatus, as well as the small-volume shake-flask assembly. Drug release studies for all the investigated samples (F1-F8) were carried out in 800 ml of degassed SSF using the rotating paddle apparatus (Erweka DT600, Heusenstamm, Germany) at 50 rpm and 37 ± 0.5 °C. Content of dissolved drug was measured *in-situ,* every 10 s up to complete tablet disintegration, using the UV fiber optic probe (Cary 50, Varian, Santa Clara, USA) operating at 270 nm in the case of PAR and 245 nm in the case of CA. Results are expressed as the mean value of six replicates.

The selected ODT formulations containing uncoated drug and flavor (F2 and F6), or coated drug particles (F3 and F7) were investigated using the small-volume shake-flask assembly simulating drug dissolution in oral cavity. ODT samples were shaken in 10 ml of SSF, in 25-ml Erlenmeyer flask, on the laboratory shaker (KS 260 basic, IKA®-Werke GmbH, Staufen, Germany) at 50 rpm, in order to simulate agitation in the oral cavity. In the predefined time points (10, 20, 30, 45, 60, 90 and 120 s) samples were filtered through a 0.45 μm Millipore filter (Millipore, Bedford, MA) and the amount of drug dissolved was determined spectrophotometrically (Cary 50, Varian, Santa Clara, USA). Results are expressed as the mean value of six replicates.

In vivo taste-masking assessment

In vivo taste-masking assessment was performed in a panel of ten healthy, non-smoking, trained, adult, human volunteers, of either sex. The ages of panelists were from 20-29. Prior to the *in vivo* study, volunteers were informed in detail on the purpose of the test and gave informed consent. The study was approved by the Ethics Committee of the Faculty of Pharmacy, University of Belgrade. *In vivo* study was conducted in three phases: i) determination of drug bitterness value, ii) training of panelists and iii) evaluation of taste-masking effectiveness. First phase was performed on Day 1 of the study, while the second and third phases were carried out on Day 2. The panelists were instructed to abstain from food and beverage intake for two hours before samples administration and during the study.

Panelists were asked to taste five aqueous standard solutions of each model drug, by keeping 10 ml of solution for 30 s in oral cavity. The range of concentrations used was 0.5-2.1 mg/ml for PAR and 0.1-0.3 mg/ml for CA, as determined in the preliminary trials. The washout period between each solution tasting was 10 min. After each sample tasting, volunteers rinsed the mouth with water and reported if the solution was bitter or not. Concentration of the most diluted drug

solution, for which a bitter taste was reported at least by one of the panelists, was defined as the drug bitterness value[36].

Second phase of the study included volunteers training, in order to standardize their responses in the third phase[23]. According to preliminary trials and results from the first phase, five standard solutions of each drug were prepared (Table 2). After tasting 10 ml of each solution during 30 s, panelists were informed about numerical value and taste description of the tasted solution. Training was replicated.

The third phase consisted of ODTs tasting with the aim to evaluate effectiveness of masking the drug bitterness. Panelists were asked to taste randomly ODT samples (samples F2, F3 containing PAR, and samples F6, F7 containing CA), as well as the reference drug-free formulation, and assign the bitterness score for each tablet after 10 s, 30 s, at the time of tablet disintegration and 10 s after that. The ODT was placed on the tongue and panelists moved the tablet against the upper part of the mouth, to produce tumbling. The moment when no lumps remained in oral cavity, was considered as point of tablet disintegration[37]. *In vivo* disintegration time was recorded. After tasting each tablet, the mouth was rinsed with water, without swallowing the disintegrated material, and panelists waited for 30 min before tasting the next sample.

Statistical analysis was carried out using IBM SPSS Statistics software package, version 20.0 (SPSS Inc., Chicago, USA). One-way analysis of variance (one-way ANOVA) with post hoc analysis using Tukey's HSD test, was applied. Statistical significance was estimated based on the p-values ($p < 0.05$). Regression analysis was applied in order to estimate degree of correlation between obtained data (Microsoft Office Excel 2007, Microsoft, Redmond, USA).

Results and discussion

Coated particles characteristics

Particle size distribution and flow properties of coated paracetamol and caffeine samples are shown in Table 3. Particle size analysis revealed that fractions of fine (< 63 µm) and very large (> 355 µm) particles were negligible for both PAR and CA samples. The largest particle fractions in the case of both drugs were in the size range from 125 to 180 µm. Because particles greater than 350 µm can lead to unpleasant taste in mouth[11], coated drug particles in the size range from 125 up to 355 µm were selected for ODT manufacture. Coated drug powder demonstrated significantly improved flow properties, compared to uncoated drug. The results obtained in this study indicate that flowability of both model drugs can be significantly improved by coating the particles with Eudragit® E PO dispersion. This can be visualised by representative scanning electron micrographs of caffeine uncoated and coated particles (Figure 1), that depicted smoother surface of coated particles compared to uncoated caffeine particles. From the cross section area of coated particles difference between coated layer and caffeine crystals, as well as consistency of Eudragit® E PO dispersion layer, can be observed. Moisture content was 0.5% for coated CA particles and 0.3% for coated PAR. Content of PAR and CA in coated drug samples was, respectively, 70.9 ± 0.3% and 69.3 ± 0.5% (which is close to theoretical value of 71.2%). The uniform distribution and high drug load in coated material indicate consistent coating procedure. High drug load in coated particles facilitates incorporation of greater amount of bitter drug in ODTs. To our best knowledge there are no available data in the literature about direct particle coating of drugs with relatively high solubility. Guhmann and coworkers applied diclofenac particle coating in fluidized bed as the taste-masking approach, however, additional

information about process performance and coated particles characteristics has not been provided[11].

Tablet preparation

ODTs were prepared with novel co-processed excipent Disintequik™ ODT. Published data on Disintequik™ ODT are still lacking, but according to manufacturer, Disintequik™ ODT is suitable for direct compression and production of tablets with short disintegration time and high breaking strength[39].

Tablet characteristics

Tablet mechanical properties

Tablet thickness, hardness, friability as well as calculated values of tablet tensile strength are shown in Table 4. Despite the fact that tensile strength of the investigated ODT samples showed high variability (1.19 up to 2.21 MPa), all values were higher than 1 MPa, which is generally accepted as appropriate[40]. Friability values were less than 1% for samples containing coated drug particles, while somewhat higher friability was observed for samples which did not contain Eudragit® E PO.

Disintegration testing

Results of ODT disintegration testing are presented in Table 4. It can be observed that coating with Eudragit® E PO affected tablet disintegration time, as tablets containing coated drug particles (F3 and F7) disintegrated slower in comparison to tablets containing uncoated drug (F1 and F5) (158.1 s and 73.3 s for ODTs with coated PAR or CA, compared to 22.9 s and 30.2 s for ODTs with uncoated PAR or CA). Interestingly, ODTs containing coated drug particles and flavor (F4 and F8) exhibited markedly prolonged disintegration time and did not fulfill relevant pharmacopoeial requirement. Samples containing coated drug particles, as well as reference

formulation (in which equivalent amount of Eudragit® E PO was added) exhibited higher tensile strength, lower friability and longer disintegration time. Such data may result from the Eudragit® E PO binding effect.

Dissolution testing

Dissolution profiles of paracetamol (samples F1-F4) and caffeine (samples F5-F8), during the first 3 minutes, are presented in Figure 2. PAR was not detected during the first 30 s. Considerably slower PAR dissolution was observed from tablets containing coated drug, compared to tablets with uncoated drug. After three minutes, 9.5% of PAR was dissolved from tablets with coated drug particles (F3), compared to 92.5% of PAR dissolved from tablets containing uncoated drug particles (F1). In the case of tablet samples with coated drug particles, more than 80% of PAR was dissolved during 11 minutes from formulation F3, while for the same time only 19.9% of PAR was dissolved from formulation F4 which contained flavor (inset in Figure 2a). Slower dissolution of PAR from sample F4 can be explained by its considerably longer disintegration time.

Significant differences were observed between caffeine dissolution profiles from samples containing uncoated drug (F5 and F6) in comparison to samples containing coated drug (F7 and F8). Similar to PAR release, in the first 30 s, CA concentration in the dissolution medium was not detectable. Caffeine dissolution from tablets containing coated particles (F7), in the first three minutes was three times slower compared to caffeine dissolution from tablets with uncoated drug particles (F5) (22.8%, compared to 66.5% of dissolved drug). 80% of caffeine was dissolved in less than 15 min from samples with coated drug particles (F7, F8) (inset in Figure 2b). In the case of both model drugs, addition of flavor to the samples with uncoated drug particles, did not affect drug dissolution. Because of long disintegration time and slower drug

dissolution, ODTs containing coated drug particles and flavor (F4 and F8) were excluded from further *in vitro* and *in vivo* investigation. Considering that more than 85% of drug was released from formulations with coated drug particles (F3 and F7) in less than 15 min, it can be assumed that coating with Eudragit® E PO would not affect bioavailability and efficacy of the formulations.

Drug dissolution in the small-volume shake-flask assembly was used as modified *in vitro* technique for taste-masking assessment which is supposed to simulate disintegration and drug release from ODTs in the oral cavity. Drug dissolution in small-volume shake-flask assembly was performed for samples F2, F3, F6 and F7, which were also selected for the *in vivo* study. The results obtained are presented in Figure 3. The profiles obtained revealed slower drug dissolution from formulations with coated drug particles, which is in accordance with the results of the compendial dissolution testing.

In vivo study

In the first phase of the *in vivo* study, bitterness values of paracetamol and caffeine were determined. Bitterness value obtained for PAR in individual panelist ranged from 0.9-1.3 mg/ml. This is in accordance with mean PAR bitterness value, determined by Albertini and coworkers which was 1.08 mg/ml[10]. Caffeine demonstrated higher bitterness in the individual panelist, since the range for bitterness value was from 0.2 to 0.3 mg/ml. Dsamou and coworkers reported 0.35 mg/ml, as the mean bitterness value for caffeine, within the studied range from 0.07 up to 1.17 mg/ml, including subjects hypersensitive and hyposensitive to caffeine[41]. As mentioned earlier, in the present study drug solution concentration which resulted in bitter taste sensation at least in one panelist was selected as a threshold, bitterness value. Defined values in the panelist were 0.9 mg/ml for PAR and 0.2 mg/ml for CA. Pein and coworkers considered that taste-masking can be

assumed only if the released amount of drug (*in vitro*), within the predefined dissolution time, does not exceed human perception threshold bitterness value[20]. In the present study, concentrations of PAR dissolved from tablets prepared with coated drug particles (F3), in the small-volume, shake-flask assembly, were below 0.9 mg/ml, while concentrations of PAR dissolved from tablets with uncoated drug particles (F2), in all the time points (excluding the 10 s), were above 0.9 mg/ml. Dissolved amounts of CA from tablets containing uncoated drug (F6), in all the investigated time points, were significantly higher than defined bitterness value (0.2 mg/ml). Concentrations of CA dissolved from tablets with coated drug (F7) were lower than 0.2 mg/ml, except at 90 and 120 s where they were, respectively, 0.22 mg/ml and 0.25 mg/ml. Those values were below upper bitterness value defined in the individual panelist (0.3 mg/ml), based on which taste-masking can be considered successful.

For the second phase of the *in vivo* study, five standard solutions with different drug concentrations were prepared for each model drug. The solution with lowest concentration used in the bitterness value determination was selected as no bitter taste solution, considering that none of the panelists described it as bitter. Solution concentration corresponding to the upper limit of the range of determined bitterness values (1.3 mg/ml for PAR and 0.3 mg/ml for CA) was defined as slightly bitter. In this way it was ensured that each of the panelists will recognize the bitter taste of that solution. The increasing concentrations of other standard solutions were defined in accordance with the results of preliminary trials.

After training, panelists tasted three different formulations of each model drug, in order to estimate effectiveness of applied taste-masking approach. Results of the *in vivo* taste-masking assessment are shown in Table 5. Statistical analysis revealed significant difference in bitterness between formulations with coated drug particles (F3 and F7), compared to formulations with

uncoated drug and flavor (F2 and F6), in every investigated time point ($p < 0.05$). According to lower values of the numerical scores obtained for PAR compared to those obtained for CA, it may be concluded that more efficient taste-masking was achieved in the case of PAR. This indicates that optimal amount of Eudragit® E PO to achieve successful taste-masking should be determined for each drug specifically, taking into account drug bitterness and solubility. Statistical analysis revealed no significant difference in bitterness between ODTs with coated drug and reference drug-free ODT, 10 s after administration, while formulations with uncoated drug and flavor added exhibited bitter taste (numerical score ≈ 3), even at the early time point. The results obtained in human taste panel evaluation, indicate that addition of flavor is not a sufficient taste-masking approach in the case of paracetamol and caffeine ODTs.

Modified method of standard dissolution testing can be applied as a tool for taste-masking effectiveness evaluation. But, correlation between *in vitro* dissolution data and results of *in vivo* taste assessment is still lacking. In order to estimate correlation between the *in vivo* and *in vitro* data, *in vivo* obtained numerical scores of all the investigated samples (F2, F3, F6 and F7) were converted to PAR or CA concentrations based on the values given in Table 2 and compared to *in vitro* drug dissolution observed in the small-volume shake-flask method. The results obtained are presented in Figure 4. Logarithmic relationship between *in vivo* PAR and CA concentration and *in vitro* drug dissolution results was observed. After logarithmic transformation of the *in vitro* determined drug concentrations, linear correlation between the *in vivo* and *in vitro* data was estimated, for both model drugs. Calculated values of Pearson's coefficients were 0.997 for PAR and 0.970 for CA, respectively. Values for Pearson's coefficient greater than 0.97 indicate strong positive correlation between *in vivo* and log transformed *in vitro* data, for both model drugs. Such a correlation can be used for taste-masking prediction based on *in vitro* drug dissolution

study. The obtained results revealed that small-volume, shake-flask dissolution method, may be used as a surrogate for human panel taste-masking evaluation in the case of physical taste-masking approach application. This is of great importance considering generally high variability of the human sense of taste, as well as the ethical and safety concerns associated with human *in vivo* studies.

The most important characteristic of ODTs is their fast disintegration in the small amount of saliva. Compendial tablet disintegration test is not considered suitable for ODTs, due to large volumes and strong agitation[42]. Therefore, the alternative *in vitro* methods, which reflect the disintegration process in the oral cavity, have been proposed[43,44]. Mean values of *in vivo* ODTs disintegration time, reported by each volunteer, are shown in Table 4. *In vivo* disintegration times are plotted against the *in vitro* data obtained in compendial disintegration test, and relevant correlation plot is shown in Figure 5. High value of Pearson's coefficient (r = 0.997) indicate strong positive linear correlation between analyzed data. Kim and coworkers obtained similar results, although slightly longer disintegration time of ODTs, in their case, was noticed during the *in vivo* study[45]. According to this, *in vivo* ODT disintegration time may be predicted based on the results of compendial disintegration test.

Conclusions

The results obtained *in vitro* and *in vivo* indicate that drug particle coating with Eudragit® E PO can be a suitable approach for bitter taste-masking of paracetamol and caffeine anhydrous. Significantly slower drug dissolution from tablets with coated drug particles was observed during the first three minutes (which is important to prevent contact between dissolved drug and taste receptors and reduce bitter taste perception), while tablet disintegration and drug release from prepared ODTs have not been significantly impaired. Disintequik™ ODT proved to be a useful

co-processed excipient in preparation of ODTs with good mechanical characteristics and excellent disintegration profile. Results of taste-masking assessment in a trained human taste panel demonstrated significant improvement in drug bitterness suppression in formulations with coated drug particles. Strong correlation between *in vitro* drug dissolution and hypothetical drug dissolution in oral cavity, in the predefined time points was established for both model drugs (Pearson's coefficient greater than 0.97). The results obtained indicate that, in the case of physical taste-masking approach, small-volume, shake-flask dissolution method may be used as surrogate for human panel taste-masking assessment.

Acknowledgements

The authors gratefully acknowledge Kerry (USA) and Evonik (Germany) for the kind donation of Disintequik™ ODT and Eudragit® E PO.

Declaration of interest

This work was supported by the project No. TR 34007, funded by the Ministry of Education, Science and Technological Development, Republic of Serbia.

References

1. Council of Europe. Strasbourg: European Pharmacopoeia; 2014. 8th ed.
2. Abed KK, Hussein AA, Ghareeb MM, Abdulrasool AA. Formulation and optimization of orodispersible tablets of diazepam. AAPS PharmSciTech 2010;11:356–61.
3. Hirani JJ, Rathod DA, Vadalia KR. Orally disintegrating tablets: a review. Trop J Pharm Res 2009;8:161–72.
4. Varia I, Venkataraman S, Hellegers C, et al. Effect of mirtazapine orally disintegrating tablets on health-related quality of life in elderly depressed patients with comorbid medical disorders: a pilot study. Psychopharmacol Bull 2007;40:47–56.
5. Ahmed IS, Aboul-Einien MH. In vitro and in vivo evaluation of a fast-disintegrating lyophilized dry emulsion tablet containing griseofulvin. Eur J Pharm Sci 2007;32:58-68.
6. Clarke A, Brewer F, Johnson ES, et al. A new formulation of selegiline: improved bioavailability and selectivity for MAO-B inhibition. J Neural Transm 2003;110:1241–55.
7. Bandari S, Mittapalli RK, Gannu R, Rao YM. Orodispersible tablets: An overview. Asian J Pharm 2008;2:2–11.
8. Fu Y, Yang S, Jeong SH, et al. Orally fast disintegrating tablets: developments, technologies, taste-masking and clinical studies. Crit Rev Ther Drug 2004;21:433-76.
9. Breitkreutz J, Boos J. Paediatric and geriatric drug delivery. Expert Opin Drug Del 2007;4:37–45.
10. Albertini B, Cavallari C, Passerini N, et al. Characterization and taste-masking evaluation of acetaminophen granules: comparison between different preparation methods in a high-shear mixer. Eur J Pharm Sci 2004;21:295–303.

11. Guhmann M, Preis M, Gerber F, et al. Design, development and in-vitro evaluation of diclofenac taste-masked orodispersible tablet formulations. Drug Dev Ind Pharm 2015;41:540–51.
12. Nakano Y, Maeda A, Uchida S, Namiki N. Preparation and evaluation of unpleasant taste-masked pioglitazone orally disintegrating tablets. Int J Pharm 2013;446:160–5.
13. Sheshala R, Khan N, Chitneni M, Darwis Y. Formulation and in vivo evaluation of ondansetron orally disintegrating tablets using different superdisintegrants. Arch Pharm Res 2011;34:1945–56.
14. Sugiura T, Uchida S, Namiki N. Taste-masking effect of physical and organoleptic methods on peppermint-scented orally disintegrating tablet of famotidine based on suspension spray-coating method. Chem Pharm Bull 2012;60:315–9.
15. Douroumis DD, Gryczke A, Schminke S. Development and evaluation of cetirizine HCl taste-masked oral disintegrating tablets. AAPS PharmSciTech 2011;12:141–51.
16. Haware RV, Chaudhari PD, Parakh SR, Bauer-Brandl A. Development of a melting tablet containing promethazine HCl against motion sickness. AAPS PharmSciTech 2008;9:1006–15.
17. Walsh J, Cram A, Woertz K, Breitkreutz J, Winzenburg G, Turner R, European Formulation Initiative. Playing hide and seek with poorly tasting paediatric medicines: do not forget the excipients. Adv Drug Deliv Rev. 2014;73:14-33.
18. Shah S, Madan S, Agrawal SS. Formulation and evaluation of microsphere based oro dispersible tablets of itopride hcl. DARU J Pharm Sci 2012;20:24.

19. Hu X, Li Y, Zhang E, Wang X, et al. Preparation and evaluation of orally disintegrating tablets containing taste-masked microcapsules of berberine hydrochloride. AAPS PharmSciTech 2013;14:29–37.
20. Gittings S, Turnbull N, Roberts CJ, Gershkovich P. Dissolution methodology for taste masked oral dosage forms. J Control Release 2014;173:32–42.
21. Kalantzi L, Reppas C, Dressman JB et al. Biowaiver monographs for immediate release solid oral dosage forms: Acetaminophen (paracetamol). J Pharm Sci 2006;95(1):4-14.
22. Cesaro A, Russo E, Crescenzi V. Thermodynamics of Caffeine Aqueous Solutions. J. Phys Chem 1976;80:335–9.
23. Pein M, Preis M, Eckert C, Kiene FE. Taste-masking assessment of solid oral dosage forms–A critical review. Int J Pharm 2014;465:239–54.
24. Bora D, Borude P, Bhise K. Taste masking by spray-drying technique. AAPS PharmSciTech 2008;9:1159–64.
25. Pimparade MB, Morott JT, Park JB, et al. Development of taste masked caffeine citrate formulations utilizing hot melt extrusion technology and in vitro–in vivo evaluations. Int J Pharm 2015;487:167–76.
26. Cantor SL, Khan MA, Gupta A. Development and optimization of taste-masked orally disintegrating tablets (ODTs) of clindamycin hydrochloride. Drug Dev Ind Pharm 2015;41:1156–64.
27. Yan YD, Woo JS, Kang JH, et al. Preparation and evaluation of taste-masked donepezil hydrochloride orally disintegrating tablets. Biol Pharm Bull 2010;33:1364–70.

28. Eckert C, Pein M, Reimann J, Breitkreutz J. Taste evaluation of multicomponent mixtures using a human taste panel, electronic taste sensing systems and HPLC. Sensor Actuat B-Chem 2013;182:294–9.
29. Harada T, Uchida T, Yoshida M, et al. A new method for evaluating the bitterness of medicines in development using a taste sensor and a disintegration testing apparatus. Chem Pharm Bull 2010;58:1009–14.
30. Woertz K, Tissen C, Kleinebudde P, Breitkreutz J. A comparative study on two electronic tongues for pharmaceutical formulation development. J Pharm Biomed Anal 2011;55:272–81
31. Suzuki H, Onishi H, Takahashi Y. Development of oral acetaminophen chewable tablets with inhibited bitter taste. Int J Pharm 2003;251:123–32.
32. Eudragit® E PO technical information, Evonik Industries [Online]. Available from: http://eudragit.evonik.com/product/eudragit/en/products-services/eudragit-products/protective-formulations/e-po/pages/default.aspx. Accessed on 23 March 2016.
33. Joshi SS, Guha AS, Jain V. Process for preparing aqueous dispersion. US patent 20,150,250,888. 2015.
34. Fell JT, Newton JM. Determination of tablet strength by the diametral-compression test. J Pharm Sci 1970;59:688-91.
35. Peh KK, Wong CF. Polymeric films as vehicle for buccal delivery: swelling, mechanical, and bioadhesive properties. J Pharm Pharm Sci 1999;2:53–61.
36. Gao Y, Guan Y, Yang L, et al. Preparation of roxithromycin-polymeric microspheres by the emulsion solvent diffusion method for taste masking. Int J Pharm 2006;318:62–9.

37. Mady FM, Abou-Taleb AE, Khaled KA, et al. Evaluation of carboxymethyl-β-cyclodextrin with acid function: improvement of chemical stability, oral bioavailability and bitter taste of famotidine. Int J Pharm 2010;397:1-8.
38. Wu CY, Benet LZ. Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. Pharm Res 2005;22:11–23.
39. Disintequik™ ODT technical information, Kerry [Online]. Available from: http://www.sheffieldbioscience.com/Content.aspx?id=134&terms=disintequik%20odt, Accessed on: 23 March 2016.
40. Amidon GE, Secreast PJ, Mudie D. Particle, powder and compact characterization, in: Qiu Y, Chen Y, Zhang GG, Liu L, Porter W, editors. Developing solid oral dosage forms: pharmaceutical theory and practice. San Diego: Academic press; 2009:177.
41. Dsamou M, Palicki O, Septier C, et al. Salivary protein profiles and sensitivity to the bitter taste of caffeine. Chem Senses 2012;37:87–95.
42. Watanabe Y, Koizumi K, Zama Y, et al. New compressed tablet rapidly disintegrating in saliva in the mouth using crystalline cellulose and a disintegrant. Biol Pharm Bull 1995;18:1308–10.
43. Bi Y, Sunada H, Yonezawa Y, et al. Preparation and evaluation of a compressed tablet rapidly disintegrating in the oral cavity. Chem Pharm Bull 1996;44:2121-7.
44. Brniak W, Jachowicz R, Krupa A, et al. Evaluation of co-processed excipients used for direct compression of orally disintegrating tablets (ODT) using novel disintegration apparatus. Pharm Dev Technol 2013;18:464–74.

45. Kim JI, Cho SM, Cui JH, et al. In vitro and in vivo correlation of disintegration and bitter taste masking using orally disintegrating tablet containing ion exchange resin-drug complex. Int J Pharm 2013;455:31–9.

Figure captions

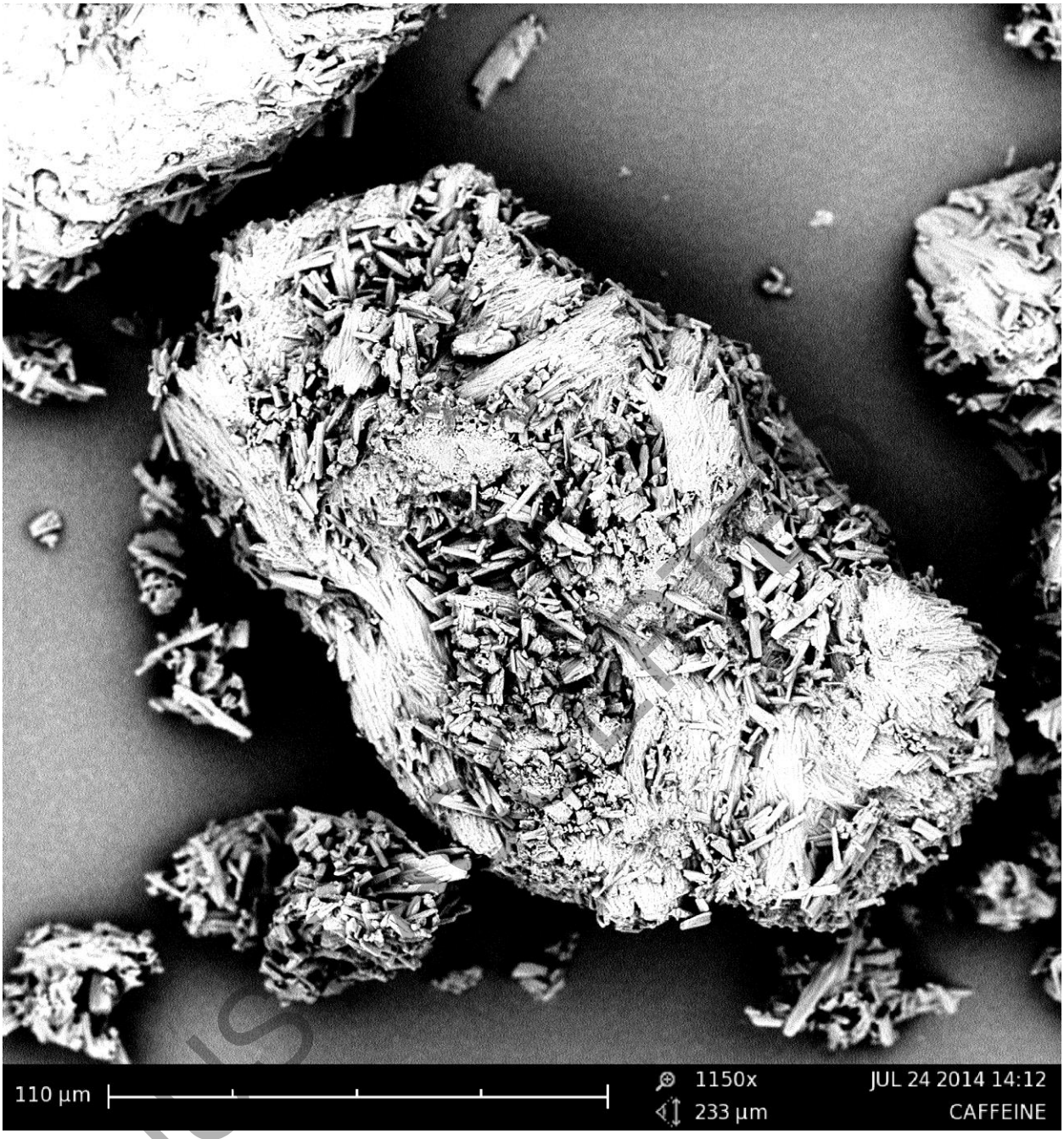
Figure 1. Scanning electron micrographs of: a) uncoated caffeine particles; b) coated caffeine particles; c) cross-section of coated caffeine particles.

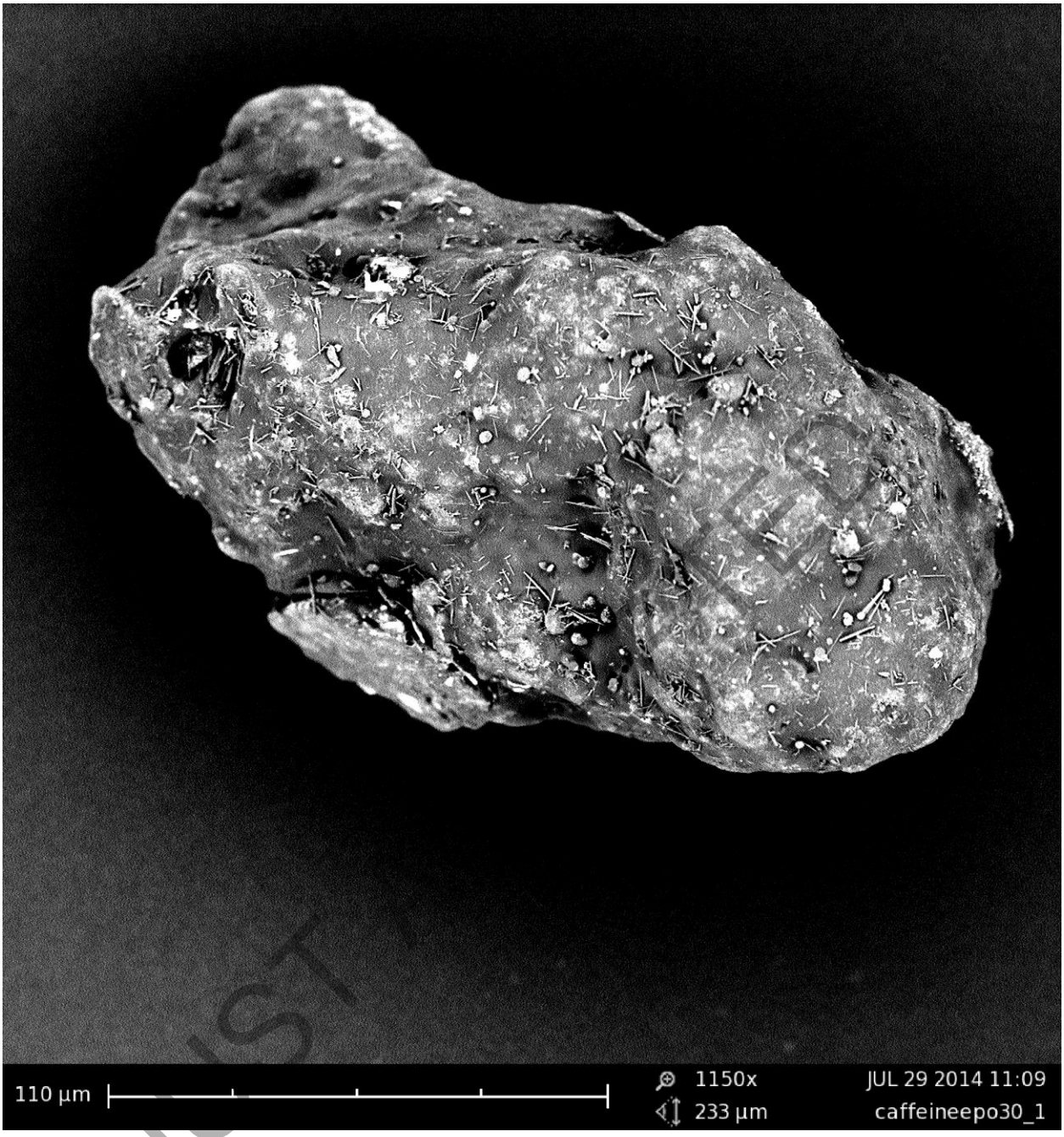
Figure 2. Dissolution profiles of: a) paracetamol, F1-F4 formulations; b) caffeine, F5-F8 formulations.

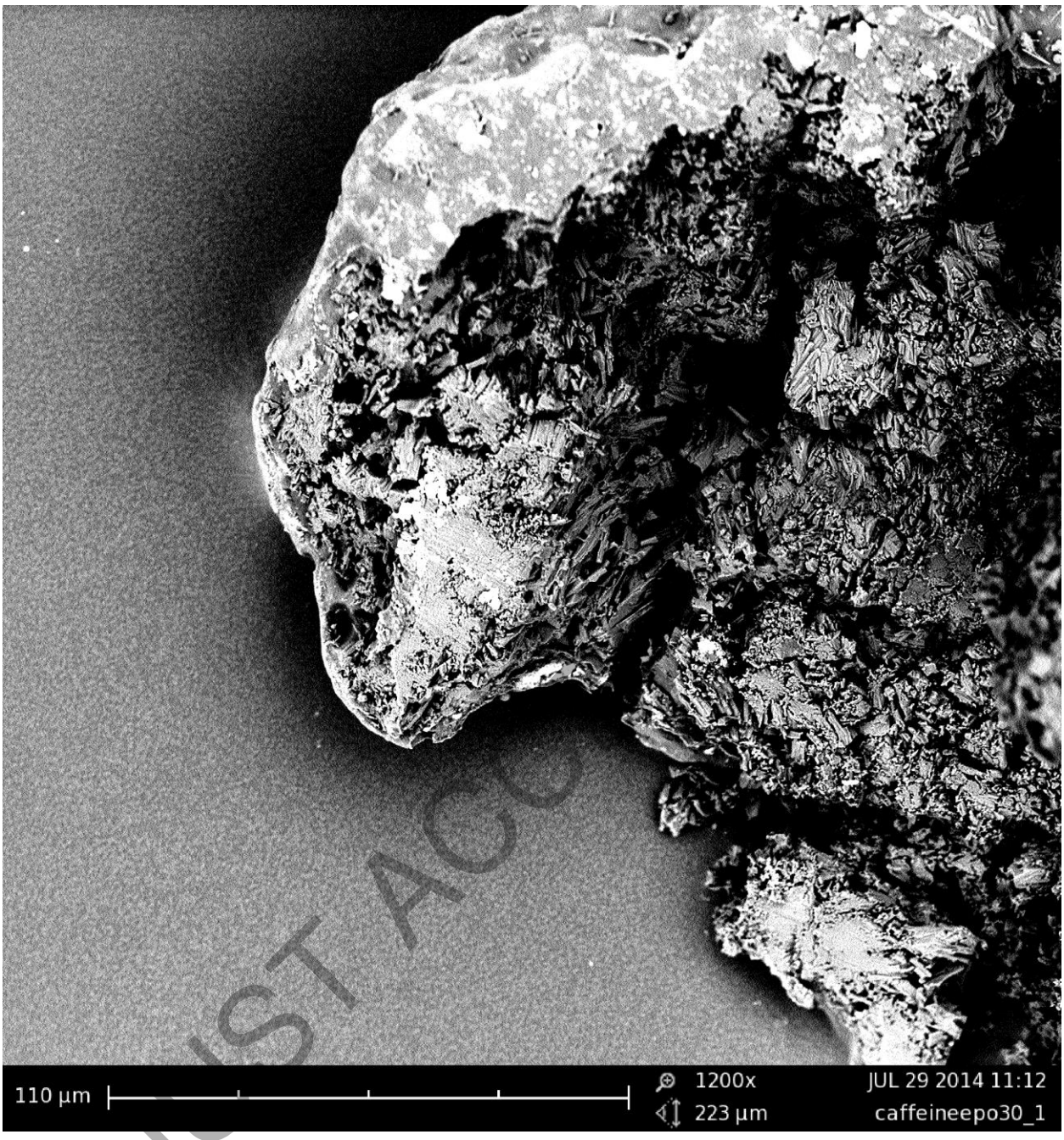
Figure 3. Dissolution profiles of paracetamol (F2, F3) and caffeine ODTs (F6, F7) observed in the small-volume, shake-flask assembly.

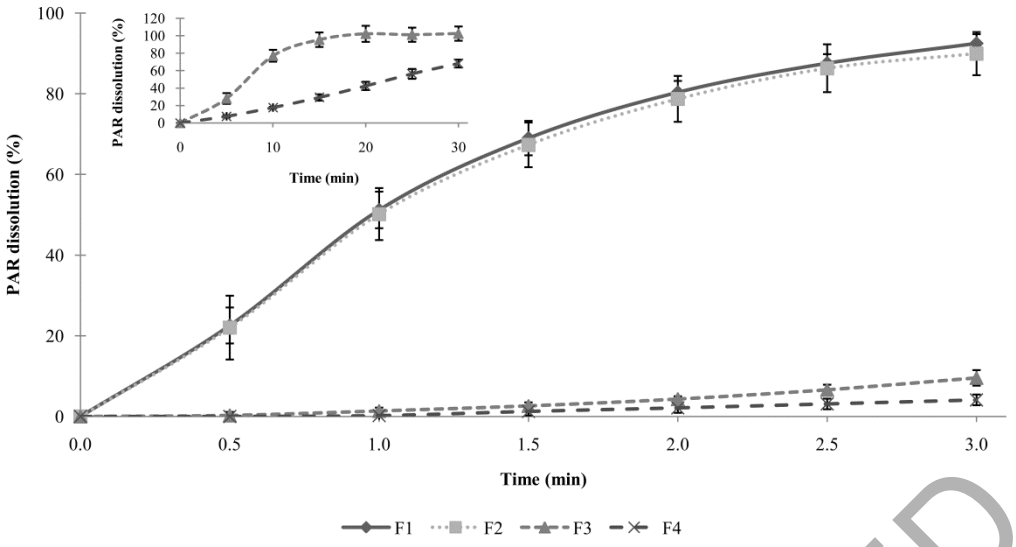
Figure 4. Correlation between the *in vitro* and *in vivo* dissolved amount of a) paracetamol; b) caffeine; (with estimated regression equation and coefficient of determination).

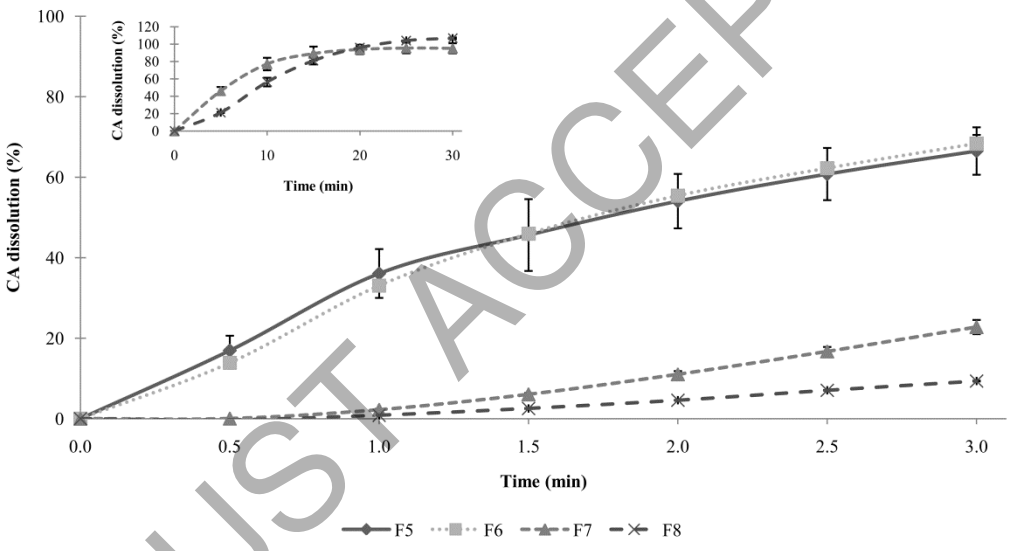
Figure 5. Correlation between *in vitro* and *in vivo* paracetamol and caffeine ODT disintegration time (with estimated regression equation and coefficient of determination).

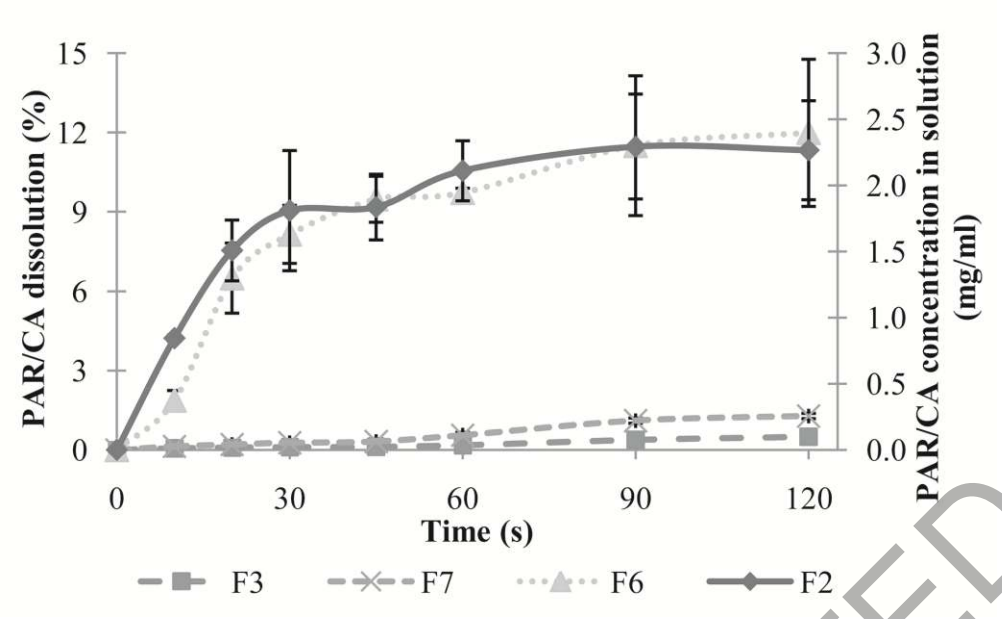

110 µm
1150x
233 µm
JUL 24 2014 14:12
CAFFEINE

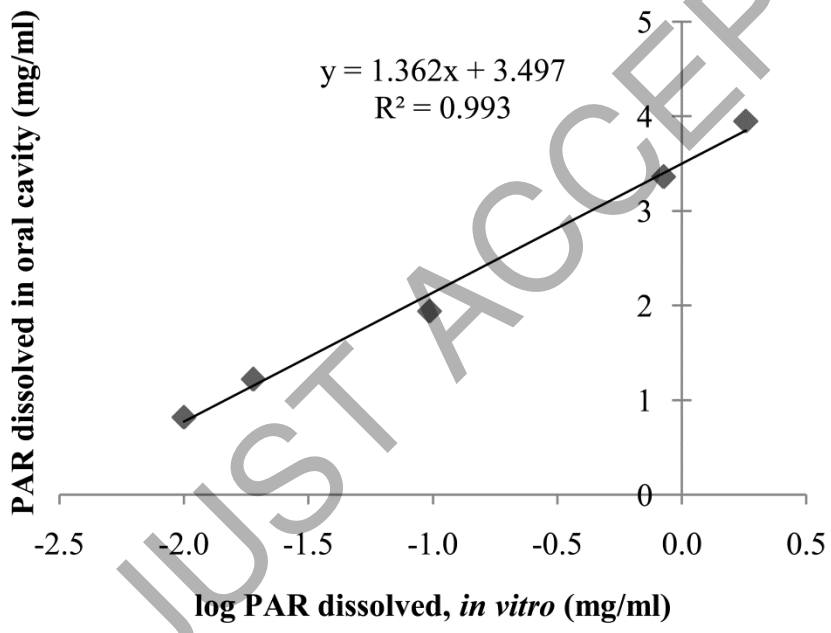

110 µm
1150x
233 µm
JUL 29 2014 11:09
caffeineepo30_1

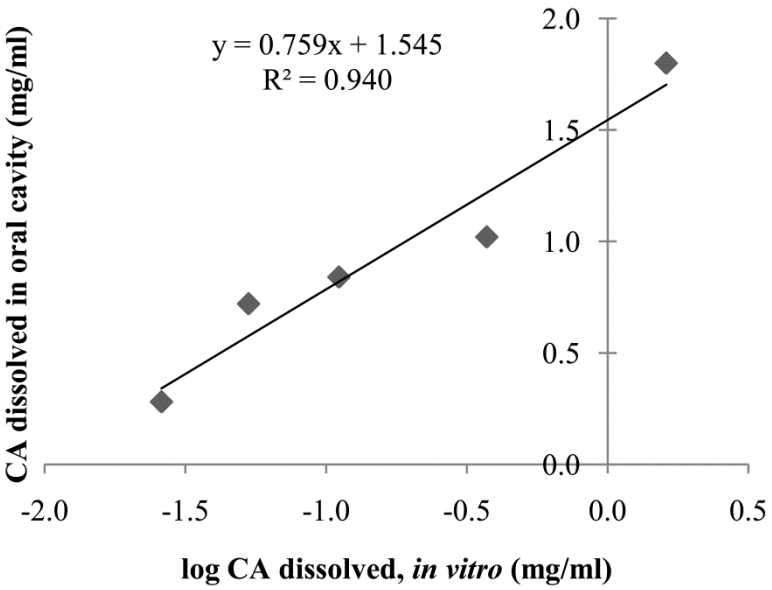












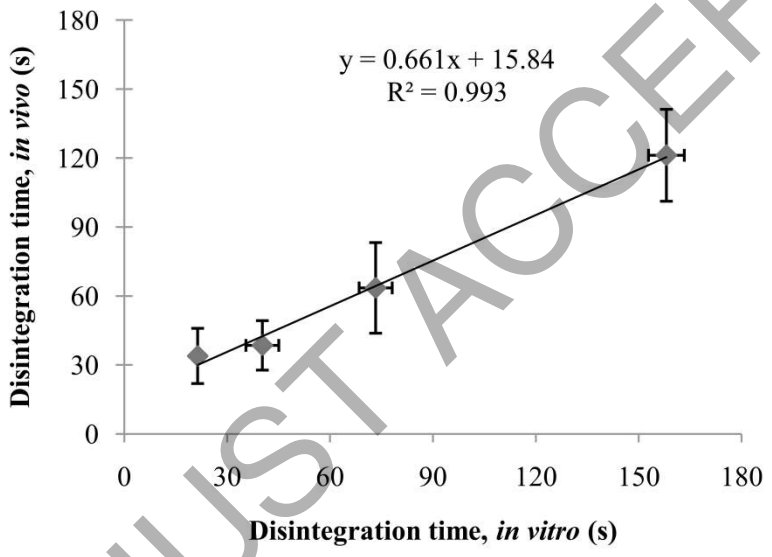


Table 1. Composition of the investigated ODT formulations. Samples F1-F4 contained paracetamol, while samples F5-F8 contained caffeine.

Component (%)	F1	F2	F3	F4	F5	F6	F7	F8
Paracetamol/Caffeine	25.00	25.00	-	-	25.00	25.00	-	-
Coated paracetamol/caffeine	-	-	35.24	35.24	-	-	36.06	36.06
Flavour	-	5.00	-	5.00	-	5.00	-	5.00
Disintequik™ ODT	74.50	69.50	64.26	59.26	74.50	69.50	63.44	58.44
Sodium stearyl fumarate	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

Table 2. Numerical score system and description of drug aqueous solutions used for volunteers training.

Numerical score/Description	Concentration of aqueous standard solutions (mg/ml)	
	Paracetamol	Caffeine
0/no bitter taste	0.5	0.1
1/slightly bitter	1.3	0.3
2/moderately bitter	2.1	0.6
3/bitter	3.5	1.2
4/very bitter	5.0	2.4

Table 3. Drug particle characterization.

	PAR	Coated PAR	CA	Coated CA
Flowability* (g/s)	0.99 ± 0.16	8.31 ± 0.56	1.48 ± 0.01	9.44 ± 0.40
Particle size range		%		%
< 63 µm	-	1.61	-	0.23
63 – 125 µm	-	14.65	-	2.19
125 – 180 µm	-	15.64	-	30.24
180 – 250 µm	-	57.04	-	49.62
250 – 355 µm	-	10.26	-	14.46
> 355 µm	-	0.8	-	3.26

*Mean ± standard deviation (n = 3)

Table 4. ODT characterization.

Tablet properties	F1	F2	F3	F4	F5	F6	F7	F8	Reference formulation
Tablet thickness (mm)	3.15	3.20	3.20	3.30	3.10	3.15	3.22	3.20	3.20
Tablet hardness* (N)	57.67 ± 5.77	48.00 ± 5.00	61.00 ± 1.41	78.33 ± 3.05	62.33 ± 3.06	54.33 ± 1.53	57.67 ± 1.53	77.33 ± 0.58	89.33 ± 3.06
Tensile strength* (MPa)	1.46 ± 0.15	1.19 ± 0.12	1.50 ± 0.01	1.89 ± 0.07	1.62 ± 0.08	1.47 ± 0.05	1.43 ± 0.05	1.93 ± 0.02	2.21 ± 0.07
Friability (%)	2.85	2.41	0.93	0.86	2.16	1.93	1.01	0.69	1.16
DT†, *in vitro** (s)	22.89 ± 1.03	21.40 ± 0.88	158.10 ± 5.22	993.37 ± 10.69	30.18 ± 1.02	40.27 ± 4.79	73.31 ± 4.82	472.51 ± 63.51	172.61 ± 6.08
DT†, *in vivo*‡ (s)	-	33.90 ± 12.01	121.20 ± 38.11	-	-	38.50 ± 10.76	63.50 ± 19.72	-	-

*Mean ± standard deviation (n = 6)

†Disintegration time

‡Mean ± standard deviation (n = 10)

Table 5. Results of taste-masking evaluation.

Mean value of bitterness score determined in volunteers (mean ± SD, n = 10)				
	10 s	30 s	DT*	DT* + 10 s
F2	2.9 ± 1.3	3.3 ± 0.7	3.6 ± 0.5	3.2 ± 0.6
F3	0.4 ± 0.7	0.9 ± 0.7	1.8 ± 0.9	1.9 ± 1
F6	2.7 ± 1.2	3.5 ± 0.5	3.6 ± 0.5	3.5 ± 0.5
F7	0.9 ± 1.3	2.2 ± 1.5	2.4 ± 0.8	2.5 ± 0.8
Reference formulation	0	0	0	0

*Disintegration time