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Taste masking of enalapril maleate by microencapsulation in Eudragit EPO® microparticles

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Microencapsulation is one of the most commonly used taste masking techniques. It can be accomplished by various methods, including coacervation, solvent evaporation, extrusion and spray-drying. Enalapril maleate, a bitter-tasting ACE-inhibitor, is available worldwide in conventional tablet formulations and as oral solution in the USA. The purpose of this study was to develop enalapril-loaded microparticles using spray-drying and to test their taste masking potential. Eudragit EPO® was used as a taste masking polymer for the preparation of a drug-polymer suspension. The suspension was then spray-dried under the following conditions: inlet temperature 65 °C, outlet temperature 30 °C, aspiration 100% and pump rate 10%. The drug-to-polymer ratio was varied and seven different microparticle models were developed. The yield of spray-dried particles ranged from of 51.3 to 85.4%, drug loading varied from 7.75 to 24.69% and encapsulation efficiency ranged from 58.5 to 95.7%. The particle size varied between 5.00 µm and 17.47 µm and the moisture content varied between 7.1% and 10.3%. *In vitro* taste assessment revealed minimal or no ENA release in artificial saliva. *In vivo* studies (with experimental animals and healthy volunteers) were used to evaluate the taste masking potential of spray-dried microparticles of enalapril maleate and Eudragit EPO®.

1. Introduction

Oral administration of pharmaceuticals is considered the most convenient and cost-effective route of administration in humans and is preferred by patients. This is due to several advantages such as easy and precise dosing, painless administration and possible self-administration by the patient. Most medicines are unpleasant in taste, which is a reason for taste masking. The pleasant taste of pediatric oral dosage forms is paramount for patient adherence. Children have specific taste preferences, which is a challenge for manufacturers. Therefore, taste masking is a key problem in the development of pediatric oral forms. According to a survey performed among pediatricians by the American Association of Pediatricians in 2003, unpleasant or bitter taste of drugs is the most severe limitation for successful treatment in pediatric practice (Ayenew et al. 2009). Another study found that children adherence to the therapy ranged from 11 to 93 % with formulation and taste being the most important factors (Walsh et al. 2014). Therefore, different methods of taste masking (e.g. microencapsulation) are applied to increase drug stability to moisture and light, to improve the organoleptic characteristics of the drug, and to increase patient adherence. The most common microencapsulation techniques described in the literature are spray-drying, emulsion solvent evaporation, coacervation and emulsion polymerization (Wagh et al. 2009).

Enalapril maleate is commonly used to treat congestive heart failure and high blood pressure. The drug acts as an inhibitor of angiotensin-converting enzyme (ACE) and belongs to Class III drugs according to the Biopharmaceutics Classification System (BCS) (Verbeeck et al. 2017). It has a bitter taste and so it is suitable for inclusion in taste masking structures.

The purpose of this study was to prepare and characterize microparticles with enalapril maleate and check their taste masking properties.

2. Investigations, results and discussion

Numerous methods for masking the bitter taste of the drugs such as the preparation of drug resin complexes, the usage of flavours and sweeteners, the use of polymeric carriers are used (Bora et al. 2008). Microencapsulation is one of the most preferred taste masking techniques due to the undeniable advantages of carriers – protection against oxidation and drug degradation. Microparticles have proper organoleptic properties, acceptable flow characteristics, compressibility and stability (Al-Omran et al. 2002). A possible microencapsulation technique is the spray-drying method, which usually results in the formation of matrix-type micrometric structures. Our goal was to obtain microparticles that were easily dissolved in the acidic environment of the stomach, providing drug release, but would not be soluble upon contact with saliva. For this purpose, pH-sensitive polymers of the Eudragit E® group (Basic Butylated Methacrylate Copolymers, Ph.Eur.) may be used. This group of polymers is used in oral and topical formulations and is generally considered non-toxic, non-irritant and safe for humans (Rowe et al. 2009).

2.1. Preparation of enalapril-loaded microparticles

Eudragit EPO® is soluble in solvents with a pH < 5 and organic solvents. 0.1 N hydrochloric acid (HCl) was used to prepare the polymer solution. A mini spray-dryer (Buchi B-290, Buchi Labortechnik AG, Switzerland) was used for the preparation of polymeric microparticles. Spray-drying was performed under pre-determined experimental conditions. Seven batches of microparticles were obtained under different drug: polymer ratios, as presented in Table 1. Talc was used as an anti-tacking agent (Felton et al. 2016).

Table 1: Models of polymeric microparticles with enalapril maleate and Eudragit EPO® obtained by spray drying

MODEL	DRUG-POLYMER RATIO	ENALAPRIL MALEATE, g	EUDRAGIT EPO®, g	TALC, g
M2	1:2	1,0	2,0	1,0
M3	1:3	1,0	3,0	1,5
M4	1:4	1,0	4,0	2,0
M5	1:5	1,0	5,0	2,5
M6	1:6	1,0	6,0	3,0
M7	1:7	1,0	7,0	3,5
M10	1:10	1,0	10,0	5,0

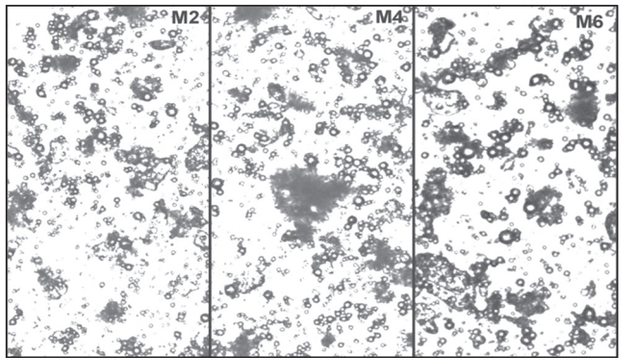


Fig. 1: Micrographs of M2, M4 and M6 microspheres captured with a light microscope at 400x magnification

2.2. Characterization of the microparticles

The microparticles were characterized by structural-morphological and physico-chemical parameters. The results make it possible to find out whether the obtained microparticles are suitable carriers of enalapril maleate with improved organoleptic properties.

2.2.1. Production yield, drug loading and encapsulation efficiency

Yields, drug loading and encapsulation efficiency are listed in Table 2. Yields ranged from 51.29 to 85.41%. Talc was used as an anti-tacking agent. Proportional relationship between the amount of talc and the yield was established. Drug loading varied between 7.75 and 24.69%. In general, ENA loading was reduced when large amounts of polymer and talc were used. Although talc-to-polymer ratio was kept 1:2, Eudragit EPO® capability for ENA incorporation was reduced. We suppose that the polymer holds limited capacity for solid phase entrapment; therefore, talc is expected to compete with the drug for entrapment in the carriers. As a result, the presence of talc in the formulations would diminish ENA loading which was confirmed by the obtained results. The highest ENA loading of 24.69% was observed at drug-to-polymer ratio 1:2. Batch M10 (drug-to-polymer ratio 1:10) exhibited DL of 7.75%. The higher polymer content did not increase the incorporation of a larger amount of drug in the microparticles. Encapsulation efficiency (DEE) was between 58.52 and 95.68%. The highest DEE reached 95.68% in batch M10. As the amount of polymer increases, DEE also increased. This trend has been reported in a number of studies (Dhakar et al. 2010; Huand et al. 2018).
Some of the following tests were performed solely with batches M2, M4 and M6 to account for changes in performance when the amount of polymer was doubled.

2.2.2. Shape and surface morphology of the obtained polymeric microparticles

Figure 1 presents photographs of microparticles of batches M2, M4 and M6 obtained with a light microscope. Rounded shape was found in all the models, which was expected in view of the preparation technique. A tendency for particle aggregation was noted. The aggregates were probably the result of a higher moisture content, which was > 7%. The SEM photomicrograph of batch M6 is shown in Fig. 2. Distinctive features of M6 microparticles were the smooth surface and the rounded shape. Aggregation was clearly visible. The ideal spherical shape is a prerequisite for drug incorporation into the inner space of the particles. It can be assumed that there was no drug deposition on the surface but mainly in the polymer matrix and no release of ENA into the saliva is expected.

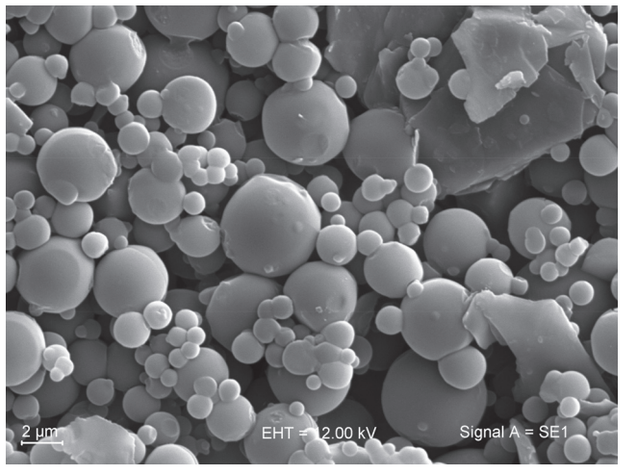


Fig. 2: Micrograph of batch M6 at 10000x magnification captured by Scanning Electron Microscopy (SEM)

Table 2: Yield%, Drug loading, (DL%), Encapsulation efficiency (DEE%), Mean particle size (d_{50}, µm) and Moisture content % of the polymeric microparticles

MODEL	YIELD, % ± SD	DL, % ± SD	DEE, % ± SD	Mean particle size, d_{50}, µm ± SD	Moisture content, % ± SD
M2	62,88 ± 0,52	24,69 ± 1,42	62,09 ± 3,57	13,89 ± 0,02	10,32 ± 1,2
M3	51,29 ± 0,26	20,74 ± 0,13	58,52 ± 0,35	15,01 ± 0,52	10,09 ± 0,9
M4	76,60 ± 0,32	17,79 ± 0,44	95,37 ± 2,36	17,47 ± 0,26	8,89 ± 0,25
M5	85,41 ± 0,58	11,24 ± 1,03	81,61 ± 7,46	16,95 ± 0,85	7,69 ± 1,3
M6	71,25 ± 0,65	13,39 ± 0,56	95,41 ± 3,97	9,10 ± 0,02	8,93 ± 0,65
M7	83,48 ± 0,01	9,74 ± 0,60	93,53 ± 5,78	6,89 ± 0,36	7,12 ± 0,45
M10	77,13 ± 0,08	7,75 ± 0,52	95,68 ± 6,36	5,00 ± 0,78	9,96 ± 0,98

(n=3 ± SD)

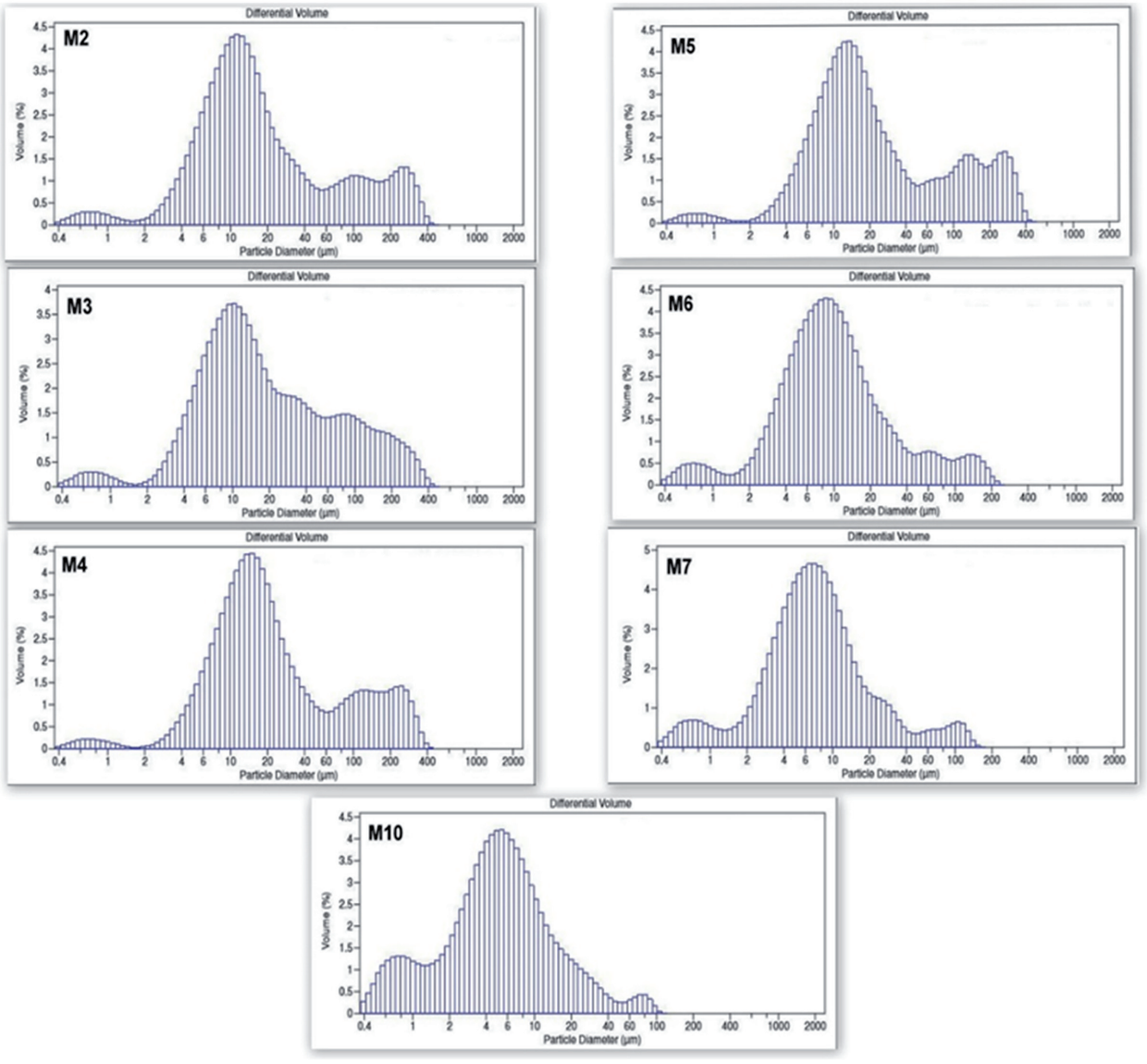


Fig. 3: Particle size distribution of the microparticles obtained with a Beckman coulter particle size analyzer

2.2.3. Particle size and size distribution of the microparticles

Particle size and size distribution of the microparticles were determined using a laser diffraction apparatus for dry and wet dispersions. The particle size distribution (Fig. 3) was monomodal for M3, M6 and M7. Batches M2, M5, M4 and M10 showed bimodal distribution with a small fraction of larger particles, probably due to aggregation. The average particle size ranged from 5.00 µm to 17.47 µm (Table 2). The results were a prerequisite for good flowability. A tendency for gradual decrease in the particle size was registered from M4 to M10. Batches M2, M3, M4 and M5 showed minimal differences in particle size. Microparticles prepared using larger polymer and talc amounts were of smaller sizes, whereas in batch M10 (formulated at high polymer concentration) the average particle size was around 5.00 µm. Increasing the amount of talc affected the particle size which is significantly reduced from batch M5 to batch M10. As the amount of talc increased, DEE also increased, whereas DL and particle size were reduced (Table 2). The relationship between the three parameters evaluated – particle size, drug-to-polymer ratio and drug loading – may be clarified, but this needs further investigation to fully explain the exact dependence.

2.2.4. Moisture content

Determination of moisture content is crucial for further processing of the powder material in a solid dosage form for oral administration. Moisture content of the particles was determined and the results are shown in Table 2. The moisture content of the batches ranged from 7.12% M7 to 10.32% M2. These percentages are relatively low, so they would not affect the consolidation of the particles into larger agglomerates. No further drying will be necessary in the subsequent processing.

2.3. In vitro ENA release in artificial gastric fluid

This test was performed to determine the release of enalapril maleate from polymeric microparticles produced with pH-sensitive Eudragit EPO®. Because the polymer is soluble in acidic pH, the microparticles were expected to dissolve in gastric juice. They must release ENA in intact form to produce the desired therapeutic effect. The release profiles of M2, M4 and M6 in artificial gastric fluid (pH 1.2) are shown in Fig. 4. It is evident that batch M2 released 93.45% of the drug involved over a period of 120 min, while in batch M4 the percentage was 83.35%, respectively. In batch M6, 78.33% of the encapsulated enalapril was released

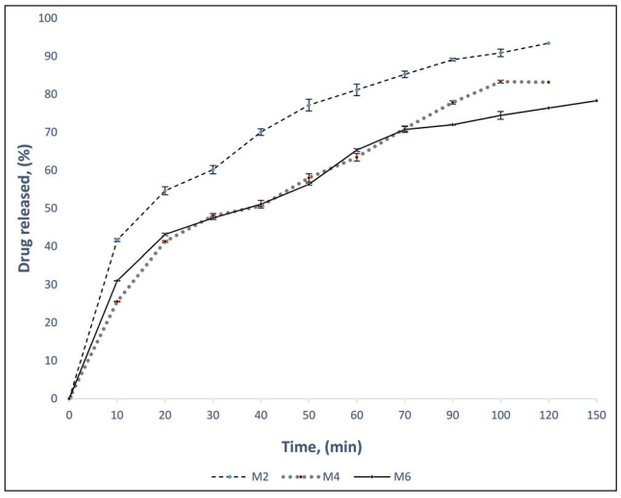


Fig. 4: In vitro release of ENA in artificial gastric fluid from batches M2, M4 and M6 (n=3, ± SD)

within the first 150 min. It is very likely that a larger amount of polymer will delay the release of the drug. The amount of released enalapril in artificial gastric juice was found to decrease from batch M2 to batch M6. Release profiles showed an initial burst effect during the first 30 min, followed by a sustained release that lasted up to 120-150 min. The rapid release of a larger amount of enalapril at the start of the test may be due to the high solubility of the polymer and the drug in the acidic medium.

2.4 Study of compatibility between ENA and the polymer

2.4.1. Infrared spectroscopy

Infrared spectroscopy was used to assess potential chemical interactions between ENA and the polymer during the spray-drying process. The spectra of pure substances (enalapril maleate, Eudragit EPO® and talc), of physical mixture of the components, and of batch M6 are displayed in Fig. 5.

In the spectrum of enalapril maleate (Fig. 5-1), vibrations at 1750 cm^{-1} and 1725 cm^{-1} corresponding to two carbonyl groups (C = O) were observed. The methyl groups were visualized with peaks at 1450 cm^{-1} and 1376 cm^{-1}. At 1226 cm^{-1}, a characteristic peak was observed, corresponding to a C-N bond (C-NH-C). Three vibrations of C = C bonds (at 1646, 875 and 668 cm^{-1}) were detected. Other characteristic peaks were seen at 1360 cm^{-1} (O-H bond) and 700 cm^{-1} (C-H bond) of monosubstituted benzene.

The distinctive emission peaks of Eudragit EPO® (Fig. 5-2) were identified as follows: 1) at 1725 cm^{-1}, corresponding to the C = O bond of the ester group; 2) at 1455 cm^{-1}, to C-H bond (methyl group); 3) at 1150 cm^{-1}, to a C-O ester bond.

In the spectrum of talc, conventional peaks (Fig. 5-3) at 1072 cm^{-1}, 1000 cm^{-1}, and 667 cm^{-1} were attributed to Si-O bonds. Characteristic peaks for ENA, Eudragit EPO® and talc appeared in the spectra of the physical mixture (Fig. 5-4) the. The spectrum of batch M6 (figure 5-5) showed the distinctive peaks of enalapril maleate at 875 (C = C), 700 (O-H) and 668 cm^{-1} (C = C). The vibrations at 1750 and 1725 cm^{-1} due to the carbonyl group were also well defined. The characteristic peaks of Eudragit EPO® at 1725 cm^{-1} and at 1455 cm^{-1} corresponding to the C = O bond and the C-H bond respectively were also detected. Talc was identified by a peak at 1000 cm^{-1} as a result of the Si-O bond. No new peaks were observed, suggesting that no new chemical bonds were formed between enalapril maleate, Eudragit EPO® and talc during the spray-drying process.

2.4.2. Powder X-ray diffraction

The diffraction patterns of the physical mixture of enalapril maleate, Eudragit EPO® and talc, and batch M6 are presented in

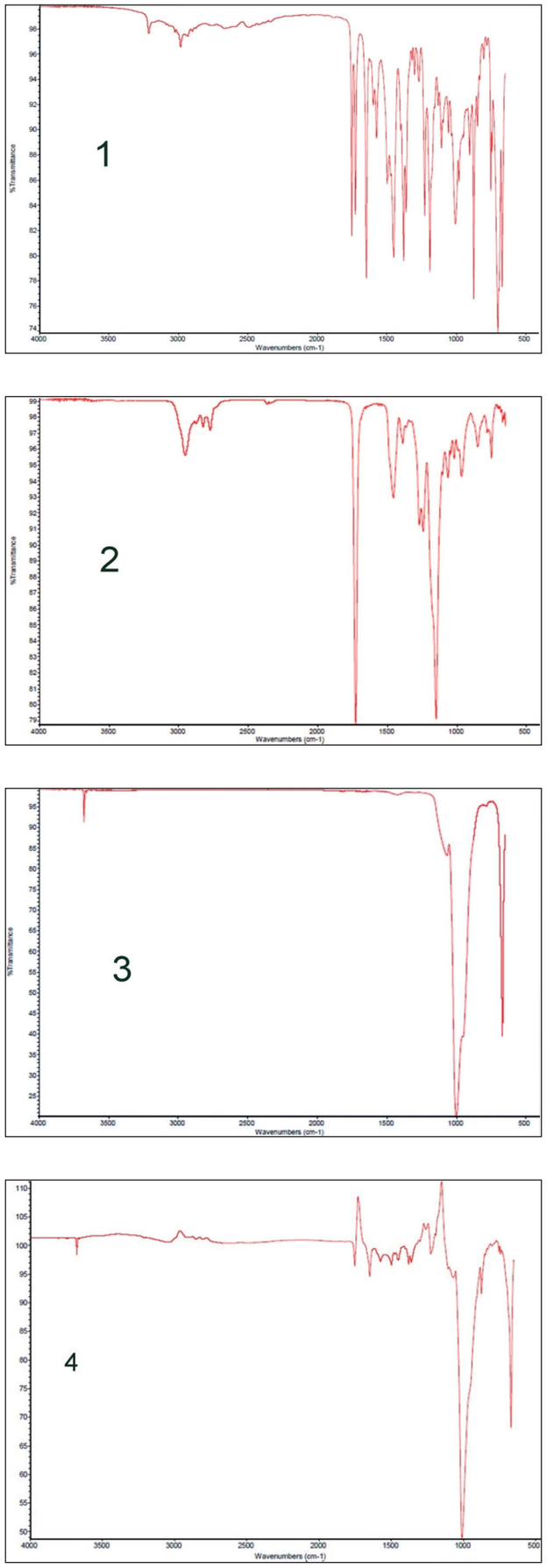


Fig. 5: FTIR-ATR spectra of 1-enalapril maleate, 2-EUDRAGIT EPO®, 3-talc, 4-physical mixture of enalapril maleate, Eudragit EPO® and talc

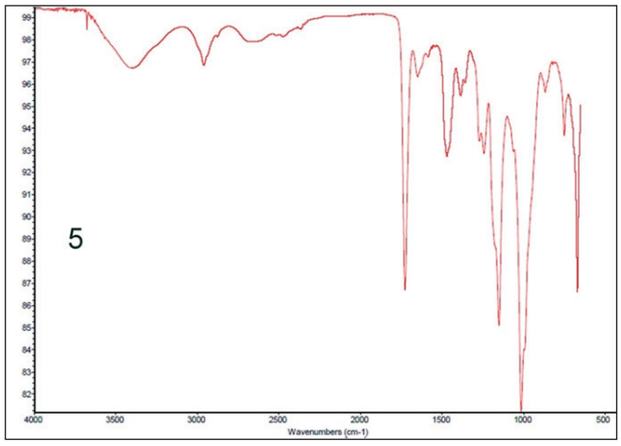
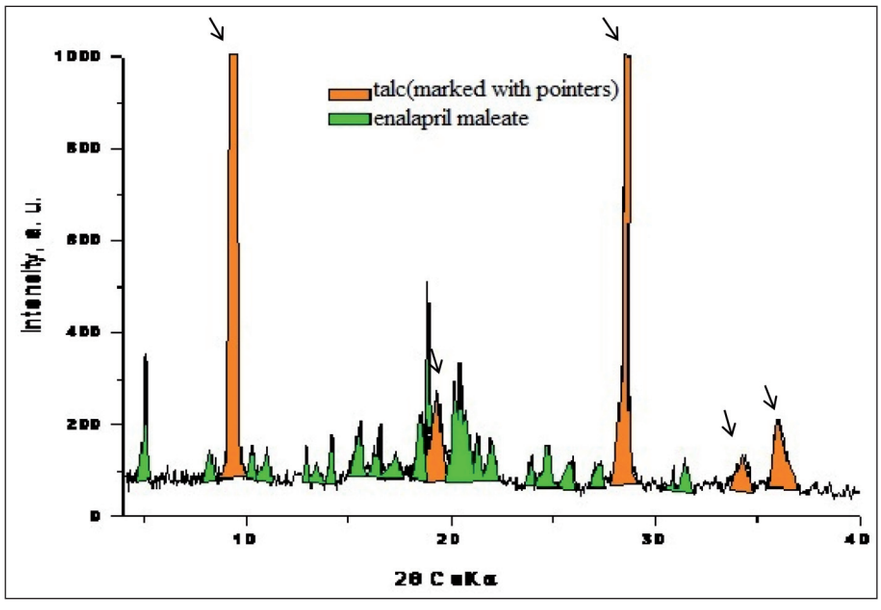


Fig. 5: FTIR-ATR spectra of 5 - batch M6

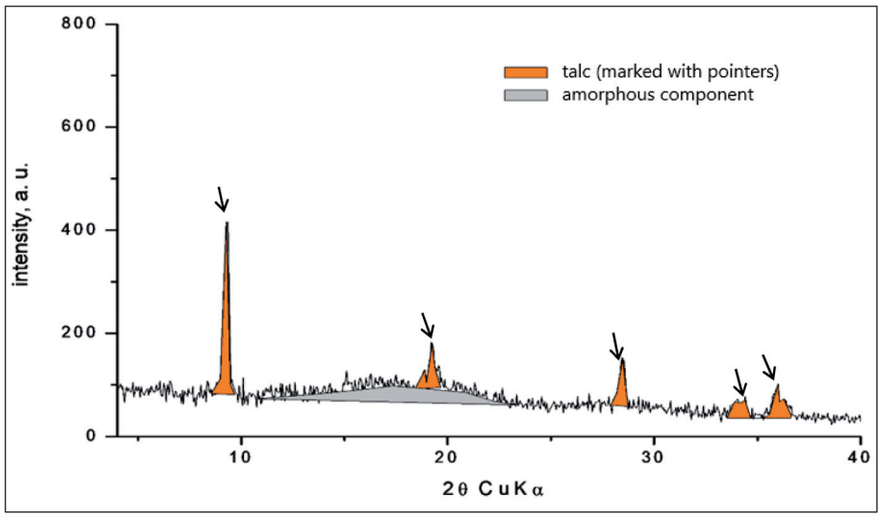
Fig. 6. The diffractogram of enalapril maleate revealed crystalline state, while Eudragit EPO® was amorphous (data not presented). Talc was detected in the physical mixture and in the formulated microparticles of batch M6. However, no changes in its state and no chemical effect on the microparticles were observed. Figure 6.2 reveals that talc remained chemically inert and did not interact with the drug or the polymer. In the physical mixture Enalapril maleate was in a crystalline state, as evidenced by the many characteristic peaks at 5°, 7°, 10°, 13°, 15°, 20°, 25° and 32° (Fig. 6.1). In batch M6, the drug was in amorphous state (Fig. 6.2), with no characteristic peaks for the crystalline form. The amorphous component corresponds of enalapril maleate and Eudragit EPO®.

2.4.3. Thermal analysis

The results of the thermal analysis are presented in Fig. 7. A peak at 152.34 °C corresponding to the melting temperature of enalapril maleate was observed in the thermogram of the physical mixture (Fig. 7.1). This endotherm was present in the physical mixture but disappeared in batches M4 (Fig. 7.2) and M6 (Fig. 7.3). The lack of the melting endotherm during heating indicated that enalapril maleate was not crystalline in these batches and there were no drug crystals on the particle surface (Ramírez-Rigo et al. 2014). The results of the thermal analysis confirmed the results of the powder X-ray diffraction. The polymer melting point varied from 250 to 450 °C and two inflection points were recorded in the physical mixture (Fig. 7.1) and in batches M4 (Fig. 7.2) and M6 (Fig. 7.3). The thermograms of the batches presented the glass transition behavior of the polymer in the range between 42 and 46 °C (Ramírez-Rigo et al. 2014). This result indicated that the amorphous form of polymer remained unchanged.



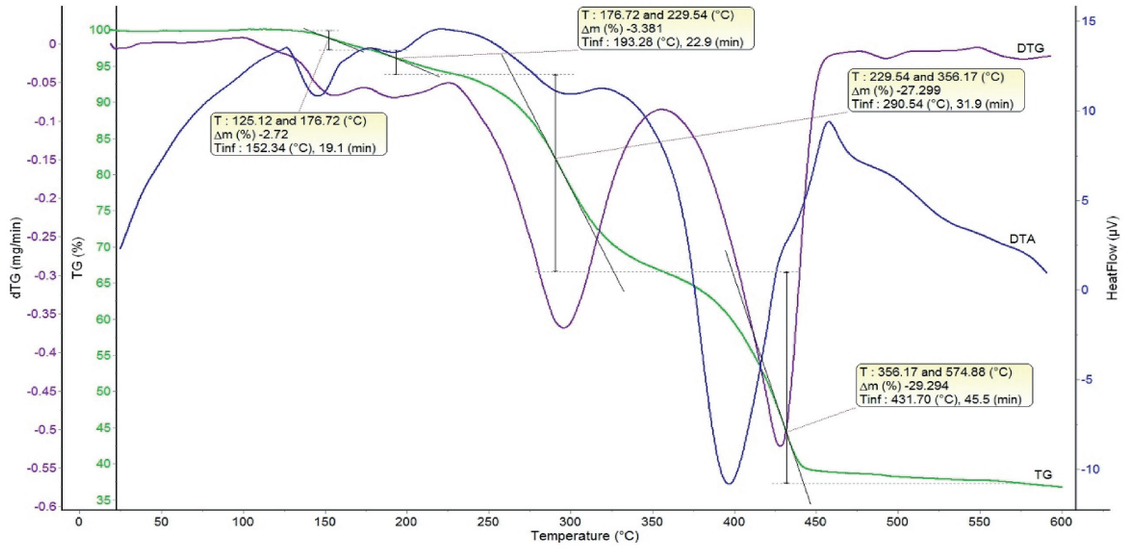
6.1 Powder X-ray diffractogram of a physical mixture of enalapril maleate, Eudragit EPO® and talc



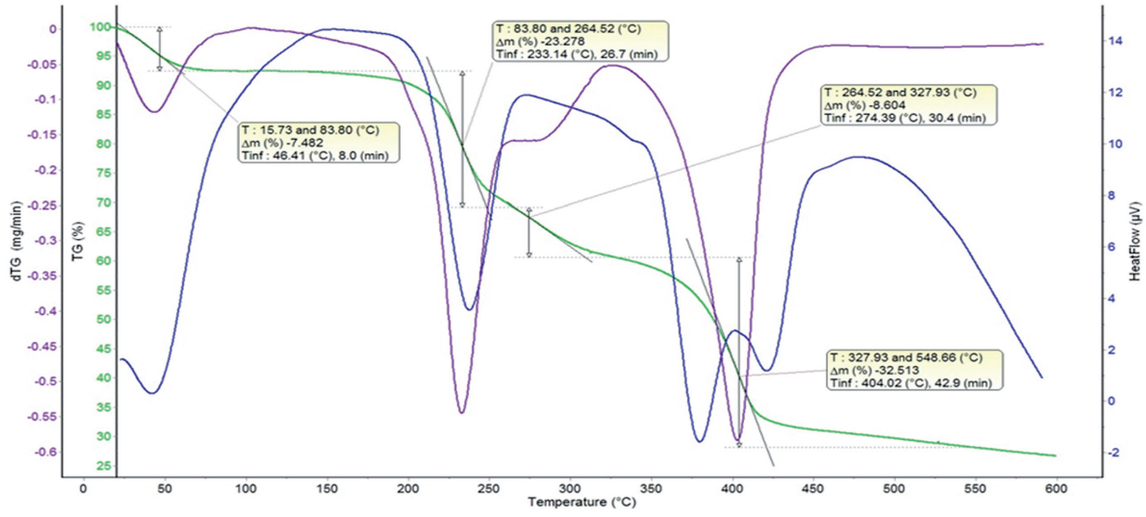
6.2 Powder X-ray diffractogram of batch M6

Fig. 6: Powder X-ray diffractograms of a physical mixture of enalapril maleate, Eudragit EPO®, talc -5.1 and batch M6-5.2

7.1 DTA, DTG and TG curves of the physical mixture of enalapril maleate, Eudragit EPO® and talc



7.2 DTA, DTG and TG curves of batch M4



7.3 DTA, DTG and TG curves of batch M6

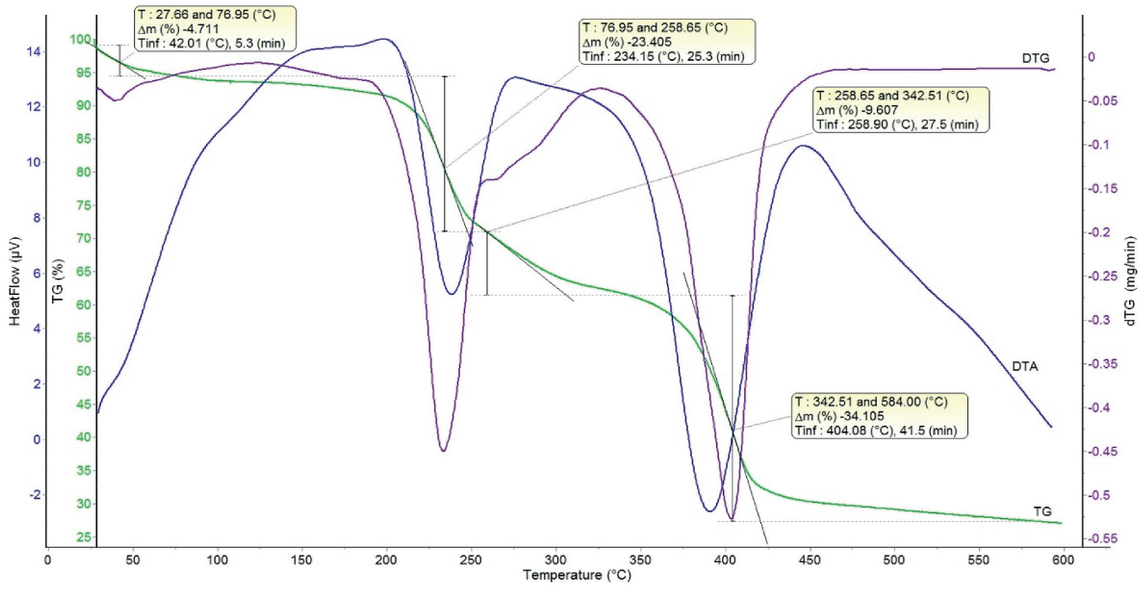


Fig. 7: Thermal analysis of a physical mixture of enalapril maleate, Eudragit EPO® and talc -6.1, batch M4 - 6.2 and batch M6 - 6.3

2.5. Taste evaluation by in vitro drug release in simulated salivary fluid (pH=6.8)

The release of ENA in artificial saliva was evaluated to predict the release of the drug into true salivary fluid in humans. The test is based on the released amount of ENA in simulated salivary fluid (phosphate buffer with pH=6.8) and the results are shown in Fig. 8. The higher release rate of the drug into the artificial saliva resulted in a greater amount of ENA in the saliva and in bitter sensation in the mouth, respectively. In batch M2 (drug-to-polymer ratio 1:2) the released drug amount after 60 s in artificial saliva was 20.11% and a tendency of decrease to 0% in batch M10 (drug-to-polymer ratio 1:10) was observed. A ratio of 1:3 (ENA:Eudragit EPO®) resulted in the release of 19.19% in simulated saliva and decreased to 10% in batch M4 (drug-to-polymer ratio 1:4). A satisfactory result for the purposes of this study would be one in which no ENA was released in simulated saliva, as observed for batch M10. Batch M10 contained the largest amount of polymer. Because of patient safety, batch M6 (drug-to-polymer ratio 1:6) was selected for further studies, which released less than 2% of ENA in the saliva but contained significantly less amount of polymer.

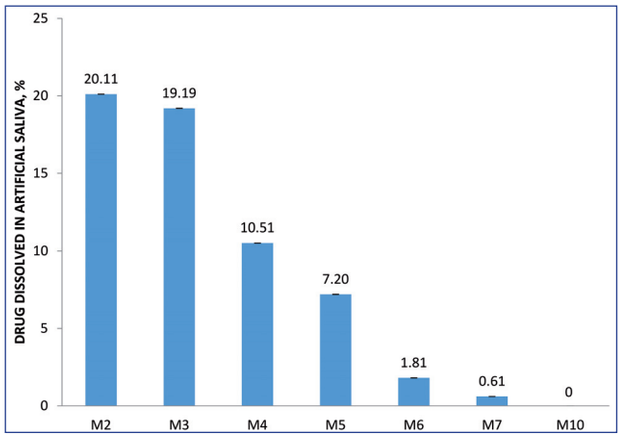


Fig. 8: Percent ENA dissolved in artificial saliva (pH= 6.8) for 1 minute at 37 ± 0,5 °C. (n= 3 ± SD)

2.6. In vivo taste evaluation

2.6.1. In vivo taste evaluation in experimental animals

Rats have inherent licking behaviors and reactions to pleasant or aversive taste stimuli that are comparable to those of humans (Noorjahan et al. 2014). The test is based on the licking frequency of aqueous dispersion of ENA-loaded microparticles from experimental animals (rats). The results are presented in Fig. 9.

The licking frequency of distilled water in the control group was taken as a control and was 99.3±8.44%. When presented with enalapril solution at a concentration of 13 mg/ml, a significant decrease in the number of lickings was registered (50.1±11.1% , p <0.001). The licking frequency for the selected batches was over 80% (82.8±1.9% (M2), 88.9±2.79% (M4) and 91.5±2.72% (M6). The experimental animals showed a satisfactory licking frequency in batch M2. For batch M4 the licking frequency increased to almost 89%. Batch M6 was associated with licking rate similar to distilled water. This result was evidence that batch M6 was preferred because of the significant taste masking effect.

2.6.2. In vivo taste evaluation in humans

The taste evaluation by healthy volunteers aimed to determine the degree of bitterness of the drug and some of the batches. The volunteers tried ENA and models and evaluated them using a scale (see Experimental). The results were similar to those observed in the animal study.

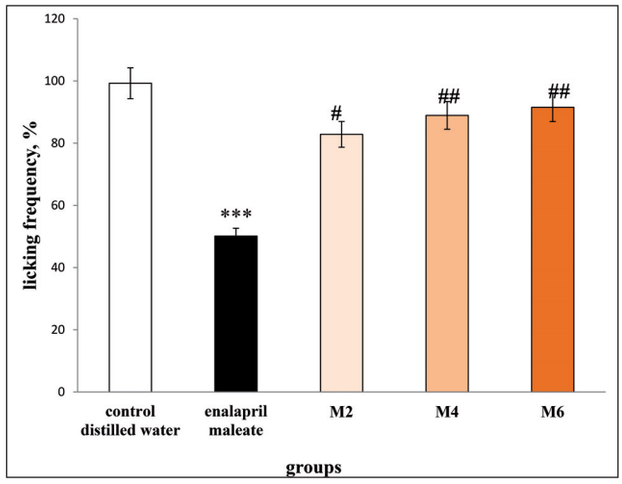


Fig. 9: In vivo taste evaluation by rats - The test indicator was the licking frequency of models of polymeric microspheres M2, M4 and M6; *** p≤0.001 against the control; # p≤0.05 vs. enalapril maleate; ## p <0.01 versus enalapril maleate; ### p <0.001 versus enalapril maleate. (n=8)

Eighteen healthy volunteers (5 male and 13 female) aged 25-28 participated in the study. Fourteen of the volunteers found the taste of enalapril maleate to be moderately bitter, and four of them described it as very bitter on a four-stage scale (see Experimental). Group 1 that evaluated batch M2 rated it as mild to moderately bitter. In group 2 (evaluating batch M4) there was one volunteer who did not consider any bitter taste of test batch M4. For the rest of the group batch M4 had a mild to moderately bitter taste. Group 3 (received batch M6) found the taste of the test samples slightly bitter or without bitterness. According to the volunteers, batch M6 was the most palatable, which confirmed the results obtained in the previous tests.

3. Experimental

3.1. Materials

Enalapril maleate was purchased from Alfa Aesar, Germany. Eudragit EPO® was kindly donated by Evonik GmbH, Germany. Hydrochloric acid (HCl) and talc were obtained from Sigma Aldrich, USA. NaCl, Na_2HPO_4 and KH_2PO_4 were used to prepare the simulated salivary fluid without enzymes (SSF) and simulated gastric fluid without enzymes (SGF) and were purchased from Sigma Aldrich, USA.

3.2. Preparation of models of polymeric microparticles

3.2.1. Preparation of suspensions for spray-drying

A saturated solution of Eudragit EPO® in 0.1 N HCl were prepared using an electromagnetic stirrer (500 rpm) until complete dissolution. Enalapril maleate was added and dissolved in the 0.1 HCL solution with Eudragit EPO®. The compound was transferred to a high-speed homogenizer (MICCRA D-9 Homogenizer – Disperser, Miccra, Germany) and talc was added to 50% of the polymer amount used. The dispersion was stirred at 11,000 rpm for 30 min until a stable, ready-to-spray suspension was obtained.

3.2.2. Preparation of microparticles

Spray-drying technique was applied to prepare polymeric microparticles using Buchi B-290 Mini Spray-Dryer (Buchi Labortechnik AG, Flawil, Switzerland). The procedure was carried out under the following conditions: inlet temperature 65 °C, outlet temperature 30 °C, aspiration 100%, pump rate 10%. According to Thakral et al. (2013), the polymer acquires sticky and elastic consistency at higher outlet temperature (>45 °C), and the glass transition temperature could be achieved (Guzmán et al. 2012). Therefore, such conditions were chosen, in which this temperature was not exceeded. Seven models of microparticles with enalapril maleate and Eudragit EPO® were developed by varying drug: polymer ratio, as presented in Table 1.

3.3. Characterization of the obtained particles

3.3.1. Particle yield, drug loading, and drug encapsulation efficiency

The yield of the particles is presented as a percentage and was calculated based on the following equation:

$$\text{Yield (\%)} = W3 / (W1 + W2) * 100,$$

where W1 is the weight of drug used, W2 is the weight of the polymer, and W3 was the weight of the resulting particles.
The ENA loading percentage (DL%) of the microparticles was calculated: 30 mg particles of each batch were dissolved in 300 ml artificial gastric fluid. A 2 ml sample of the resulting solution was filtered through a Whatman filter with a pore size of 0.45 µm. The sample was analyzed spectrophotometrically using a UV/VIS Spectrophotometer Thermo Evolution 300 (Thermo Fisher Scientific, USA) by measuring the absorption at 206 nm. The concentration of ENA was determined using the calibration curve equation and the DL was expressed in percentages.
The ENA encapsulation efficiency (DEE) is presented in percentage and was calculated according to the following equation:

$$DEE\% = A / A1 * 100,$$

where A is the amount of drug included in the yield, and A1 is the initial amount of drug used to obtain the microparticles.
Three studies of each variable viz. particle yield, drug loading, and drug encapsulation efficiency were performed and the standard deviation calculated using Microsoft Excel.

3.3.2. Shape and surface morphology of the particles

Optical and scanning electron microscopy were used for examination of the particles. The shape of the particles was revealed using an optical microscope (Leica DM2000 LED, Leica Microsystems, Germany) equipped with a digital camera (Leica DMC 2900) and photo processing software (Leica Application Suite, LAS).
Surface morphology of polymeric microparticles was determined by scanning electron microscopy. The analysis was performed using a ZEISS EVO LS25 (Carl Zeiss NTS GmbH, Germany) scanning electron microscope at an acceleration voltage of 20 kV at 5000x magnification.

3.3.3. Particle size and size distribution

To determine the size of the particles and their size distribution, an LS 13 320 Laser Diffraction Particle Size Analyzer (Beckman Coulter, USA) with a tornado powder system (Tornado Dry Powder System, DPS) was used. For each measurement, a sample of 200 mg was assayed.

3.3.4. Moisture content

The moisture content was measured gravimetrically by a moisture analyzer (Kern MLB 09/2004, Kern & Sohn GmbH, Germany) and the results were presented in percentage.

3.4. In vitro ENA release in artificial gastric fluid

In vitro drug release testing of enalapril maleate from polymeric microparticles (M2, M4, and M6) was performed using AT7 Sotax Dissolution Tester Apparatus 1 – rotating basket (Allschwil, Switzerland). The experiment was conducted at the following conditions: acceptor medium – artificial gastric fluid pH 1.2, 500 ml, 37±0.5 °C; rotating speed 50 rpm. Dialysis bags with dimensions 6 cm x 2.5 cm (Sigma, MWCO 12000 Da) were prepared and soaked for 24 h in artificial gastric fluid. A sample of microparticles corresponding to 5 mg enalapril maleate polymeric was placed in the dialysis bag. Aliquots of 2 ml were taken at fixed time intervals at 10, 20, 30, 40, 50, 60, 70, 90, 100, 120 and 150 min and analyzed spectrophotometrically. The absorption was measured at a wavelength of 206 nm. The release of ENA from each batch was assessed in replicate (n=3) and is reported as the mean±SD.

3.5. Study of compatibility between ENA and the polymer

3.5.1. Infrared spectroscopy

To determine potential chemical interactions between ENA and the polymer, infrared spectrophotometry was used. The study was performed under the following conditions: 64 scans, 4 nm resolution, and 4000 – 400 cm^{-1} spectral range. The spectra of the pure substances, their physical mixture and the obtained microparticles were recorded using a Fourier-transform infrared spectrophotometer (Nicolet iS10, Thermo Fisher Scientific, USA), equipped with an ATR (Attenuated Total Reflection) accessory.

3.5.2. Powder X-ray diffraction

X-ray powder diffraction was employed to determine the physical state of the drug in the particles, The spectra were captured on an X-ray powder diffractometer (D2 Phaser Bruker AXS, Cu radiation, 2009) using Ni-filtered Cu radiation in the range of 4-60 2-theta at 30 kV and 10 mA.

3.5.3. Thermal analysis

Differential thermal analysis (DTA) and thermogravimetric analysis (TGA) were used to detect changes of ENA and the polymer during thermal exposure of the samples in comparison with thermograms of pure drug and polymer. Stanton Redcroft STA 1500 thermal imaging apparatus provided with a Plus-V software was used. The samples were analyzed in argon atmosphere at a temperature range of 25 to 600 °C, heating rate of 10 °C min^{-1} and flow rate of 50 mL/min.

3.6. Taste evaluation by in vitro drug release in simulated salivary fluid (SSF)

A sample weighing 10 mg of each batch was placed in 20 ml artificial saliva (pH 6.8 buffer consisting of Na_2HPO_4, KH_2PO_4, NaCl and the required amount of H_3PO_4 to obtain the desired pH) and stirred at 37±0.5 °C at 200 rpm on electromagnetic stirrer for 60 s. The supernatant was isolated via filtration (Whatman filter, pore size 0.45 µm) and the absorption was measured spectrophotometrically at 206 nm. The concentration of enalapril maleate in each sample was calculated using a calibration curve. The release of ENA from each batch was assessed in replicate (n=3) and is reported as the mean±SD.

3.7. Taste evaluation

3.7.1. In vivo taste evaluation in experimental animals

The experiment was approved by the Bulgarian Food Safety Agency and the Ethics Committee of the Medical University-Plovdiv. The study was conducted in accordance with the requirements of the International Council for Ethical Guidelines for Animal Breeding Labs for Researchers, ARRIVE guidelines and the EU Directive 2010/62/EU for animal experiments.
Male Wistar rats with an initial weight of 170-240 g were used. The rats were grown and kept under standard laboratory conditions: a 12-hour light/dark cycle, 50% relative humidity, temperature 24±2 °C, and free access to food and water.
The animals were separated in groups of eight and deprived of water for a period of 24 h as shown in Table 3. After 24 h, all animals were given free access to distilled water. The licking frequency for each animal was recorded. The animals were deprived of water for the next 24 h. After this period each group of animals got the test solution of enalapril maleate and the test suspensions of the batches as given in Table 3. The licking frequency of each animal was observed and calculated as follows (Noorjahan et al. 2014):

$$\text{Licking frequency (\%)} = \left(\frac{\text{mean number of lickings of test substance or formulation}}{\text{mean number of lickings of water}} \right) \times 100$$

Table 3: Groups experimental animals, used for in vivo taste evaluation

Group	Treatment
1 (control)	Distilled water
2 (negative control)	ENA solution 13 mg/ml
3 (test)	M2
4 (test)	M4
5 (test)	M6

The statistical analysis of the results of the experiment was performed with SPSS 17.0. Data are provided as mean±SEM. A non-parametric One-sample Kolmogorov-Smirnov test was employed to determine the distribution of the obtained results. After a normal distribution was confirmed, a one-way ANOVA and Bonferroni's Multiple Comparison Test were used.
Results were considered statistically significant at $p<0.05$.

3.7.2. In vivo taste evaluation with healthy volunteers

The test was carried out according to the requirements of the Ethics Commission of Medical University of Plovdiv, permission of the Ethics committee – protocol 1/22.02.2018. The experiment was performed in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) after informed consent was obtained from the participants. Eighteen healthy volunteers participated in this experiment. The volunteers were divided at random into groups of six and each group was required to assess the taste of a different batch (Table 4). Enalapril maleate was used as a control. Five milligrams of ENA were placed on the tongue of each volunteer for 10 ss, after which the oral cavity was rinsed with water. The same procedure was carried out for the test batches (Chaudhari et al. 2016). The participants assessed the degree of bitterness according to the following scale: 0 = no bitter taste; 1 = slightly bitter; 2 = moderately bitter; 3 = very bitter. The study was performed as a double-blind trial. Due to the degree of subjectivity in the perception of taste, this study was conducted for confirmatory purposes only.

Table 4: Groups of healthy volunteers, included in the taste evaluation test

Group	ENA powder (control)	ENA loaded batch of microparticles (equivalent to 5 mg ENA)
1	5 mg	M2
2	5 mg	M4
3	5 mg	M6

Conflicts of interest: None declared.

References

Al-Omran MF, Al-Suwayeh SA, El-Helw AM, Saleh SI (2002), Taste masking of diclofenac sodium using microencapsulation. J Microencaps 19: 45–52.

Ayenew Z, Puri V, Kumar L, Bansal AK (2009) Trends in pharmaceutical taste masking technologies: a patent review. Recent Patents on Drug Delivery & Formulation 3: 26–39.

Bora D, Borude P, Bhise K (2008) Taste masking by spray-drying technique. AAPS PharmSciTech 9: 1159–1164.

Chaudhari S, Tadavi SA, Rane BR, Pawar SP (2016) A research on formulation & evaluation of mouth dissolving tablet of azithromycine. Int J Adv Res Sci Managem Technol 2(7).

Dhakar RC, Maurya SD, Sagar BP, Bhagat S, Prajapati SK Jain CP (2010) Variables influencing the drug entrapment efficiency of microspheres: A pharmaceutical review. Der Pharmacia Lettre 2: 102–116.

Felton LA (2016). Aqueous polymeric coatings for pharmaceutical dosage forms. CRC Press; Guzmán ML, Manzo RH, Olivera ME (2012). Eudragit E100 as a drug carrier: the remarkable affinity of phosphate ester for dimethylamine. Mol Pharm 9: 2424–2433.

Huang W, Tsui CP, Tang CY, Gu L (2018) Effects of compositional tailoring on drug delivery behaviours of silica xerogel/polymer core-shell composite nanoparticles. Sci Rep 8: 13002.

Noorjahan A, Amrita B, Kavita S (2014) In vivo evaluation of taste masking for developed chewable and orodispersible tablets in humans and rats. Pharm Devel Technol 19: 290–295.

Ramírez-Rigo MV, Olivera ME, Rubio M, Manzo RH (2014) Enhanced intestinal permeability and oral bioavailability of enalapril maleate upon complexation with the cationic polymethacrylate Eudragit E100. Eur J Pharm Sci 55: 1-11.

Rowe RC, Sheskey P, Quinn M (2009). Handbook of Pharmaceutical Excipients. Libros Digitales-Pharmaceutical Press;

Thakral S, Thakral NK, Majumdar DK (2013) Eudragit®: a technology evaluation. Exp Opin Drug Deliv 10: 131–149.

Verbeeck RK, Kanfer I, Löbenberg R, Abrahamsson B, Cristofoletti R, Groot DW, Langguth P, Polli JE, Parr A, Shah VP, Mehta M (2017) Biowaiver monographs for immediate-release solid oral dosage forms: enalapril. J Pharm Sci 106: 1933-1943.

Wagh VD, Ghadlinge SV (2009) Taste masking methods and techniques in oral pharmaceuticals: current perspectives. J Pharm Res 2: 1049–1054.

Walsh J, Cram A, Woertz K, Breitkreutz J, Winzenburg G, Turner R, Tuleu C (2014) European Formulation Initiative. Playing hide and seek with poorly tasting paediatric medicines: do not forget the excipients. Adv Drug Deliv Rev 73: 14–33.