Taste masking of propranolol hydrochloride by microbeads of EUDRAGIT® EPO obtained with prilling technique for paediatric oral administration

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Abstract

The purpose of this study was to develop a new solid paediatric formulation for propranolol hydrochloride (PR). This drug is used to treat various paediatric diseases and recently for haemangioma. However, PR has a bitter salty taste that does not permit a good compliance of young patients especially in liquid formulations. In addition, the solid formulations are aimed for adults and often their dosage is not suitable for children that require a flexible dose based on their weight. Therefore, matrix microbeads of EUDRAGIT® EPO containing PR were manufactured to overcome these limitations. Seven different samples were prepared using the prilling-congealing technique with high yield. Using 2 nozzles, 300 and 450 μm (code n), the diameters obtained of microbeads (from 333 to 699 μm) were homogenous and appropriate to be swallowed by children. In this study, the ratio drug:matrix for the microbeads was also examined in detail: 1:25 (**F_1**), 1:15 (**F_2**) and 1:10 (**F_3**) in aqueous and tert-butanol/aqueous (code t) media. Most of the examined microbeads were characterized by high percentage of encapsulation efficiency (81-100%) and drug loadings (22-77 mg of drug per g of matrix) effective for the administration of low and high doses of PR. SEM analysis revealed a matrix with a radial or a spongy structure, with numerous pores that generated floating microbeads in aqueous solution. Release studies confirmed a low release and dissolution of the drug in artificial saliva, mainly **F_{1n}** >**F_1** >**F_{2nt}**, and a prompt dissolution in simulated gastric media. Finally, electronic tongue measurement revealed the ability of these formulations to mask the bitter drug taste especially for the sample with a ratio 1:25 (**F_{1n}**). This last sample and **F_1** were chemically and physically stable for four months. In conclusion, the projected microbeads **F_1**, and **F_{1n}** reached the goal of the study and could be proposed as new solid oral formulations dedicated to children.

Keywords: paediatric formulation, prilling, microbead, EUDRAGIT, propranolol, taste masking

1. Introduction

Propranolol hydrochloride (PR) (Fig. 1) is a nonselective beta-adrenergic receptor blocking agent with no other autonomic nervous system activity. The chronotropic, inotropic, and vasodilator responses to beta-adrenergic stimulation are decreased proportionately when beta-receptor sites are blocked by PR (Al-Majed et al., 2017). PR is used for the treatment of several diseases: hypertension, pheochromocytoma and different cardiac pathologies. In addition, it is used to control symptoms of sympathetic overactivity in the management of hyperthyroidism, anxiety disorders, and tremor. Other indications cover the prophylaxis of migraine and of upper gastrointestinal bleeding in patients with portal hypertension (Al-Majed et al., 2017). PR has a well-documented safety in paediatric therapy for the treatment of the above mentioned diseases, but in particular, it is the drug of choice for the treatment of supraventricular tachycardia, the most common arrhythmia of infancy (Ensom et al., 2004; Villain et al., 2004; Bidabadi and Mashouf, 2010). Furthermore, a finding of Leaute-Labreze in 2008 (Leaute´-Labreze et al., 2008) and subsequent other case-reports indicated PR for treatment of haemangioma, a disfiguring benign vascular tumour, the most usual tumour of childhood, affecting about 1 in 10 infants (Esterly, 1987; Drolet et al., 1999; Bruckner and Frieden, 2003; Sans et al., 2009; Naouri et al., 2010; Hermans et al., 2011; Marqueling et al., 2013). As a result of these studies, the Food and Drug Administration (FDA) and the European Medicinal Agency (EMA) have approved the use of PR as first-line therapy to treat haemangioma in paediatric patients (Castaneda et al., 2016; EMA report, 2014). The dose of PR to be administered must be established by a doctor, depending on the disease, age and body weight of the child. Daily doses of PR for the treatment of arrhythmias are 0.5 to 5, up to 8 mg/kg (Ensom et al., 2004). Currently, PR formulations as tablets (40-160 mg of PR) and one oral solution (3.75 mg/mL of PR) are available on the European market. Although PR is widely used in the treatment of infantile haemangiomas, tablets are not manufactured in a dosage form suitable for children and often the standard 40 mg tablet needs to be fractioned to obtain 10 mg parts, with even lower doses (i.e., 2-3 mg/kg/day divided into 2-3 daily doses) required in infants. Sometimes the prescribed daily dose of medication for a paediatric patient may be too small to be accurately prepared by cutting a tablet (Ensom et al., 2004).

Recently, an oral liquid formulation named Hemangiol® was proposed with a concentration of drug equal to 3.75 mg/mL (EMA report, 2014). Nevertheless, this good alternative, it is not practical, inconvenient to transport and its stability is only 60 days after opening. Moreover, due to the bitter taste of PR this solution is masked with vanillin and sodium saccharin (EMA report, 2014). However, EMA strongly counsels another way for masking the unpleasant taste of the formulation instead of adding sweeteners or flavours. The use of these excipients should be avoided due to the poor results of this method of taste masking and the possibilities of toxic and allergic reactions of these excipients in paediatric patients (EMA report, 2013.

Paediatric population is extremely sensitive to the taste of bitter or salty substances and vigorously reject the unpalatable formulations (Maniruzzaman and Douroumis, 2014). Hence, there exists a need and attention of global researchers for appropriate development of patient-centric formulations, which are suitable to be administered for paediatric population, whilst also being presented in an acceptable dosage form to ensure safety and compliance (Hanning et al., 2016). Anyway, the oral route is still the most convenient and appropriate way to take PR and the unpleasant taste of the drug needs to be masked only for a relatively short period of residence (from seconds to few minutes) in the mouth and throat. Hence, masking of active pharmaceutical ingredients (APIs) with bad taste is considered an important challenge in pharmaceutical industry (Ley, 2008; Maniruzzaman et al., 2012).

Furthermore, there is a significant competitive pressure among the pharmaceutical companies to ensure that the commercial products do not fall short in patient compliance.

A perfect resolution for this problem should take into account the prevention of interaction between the unpleasant APIs and the taste buds, thus avoiding the use of taste-masking additives. One of the most effective taste masking method is to form a barrier between the drug and the tongue buds by

using polymers. An ideal formulation can be developed by applying an appropriate coating or encapsulating the API or manufacturing solid dispersions (Pein et al., 2014; Joshi et al., 2013) in inert polymeric or lipidic matrices. In particular, the microencapsulation is a technique where an API is embedded in a homogeneous or heterogeneous polymeric matrix. High efficiency and effectiveness of the microencapsulation of APIs is the reason of the development of various technological platforms that take advantage of this process (Singh et al., 2010; Benita, 2005). The obtained multiparticulates must be characterized by small sizes, in order to be directly administered into the mouth or dispersed in an appropriate volume of a preferred vehicle prior of the administration and must be easy to swallow for a paediatric patient.

The selection of a polymeric excipient is a crucial factor to be considered for the preparation of microbeads. Physical, chemical, and biological polymeric properties, such as film forming capacity, non-toxicity, and biodegradability are important parameters for the final product performance, and a large number of natural and synthetic polymers are available on the market. Derivative polymethacrylates have been widely used in pharmaceutical formulations as film-coating agents and matrix carriers in solid dispersion preparations and in hot-melt extrusion processes (Liu et al., 2009; Schilling et al., 2010; Jeganathan and Prakya, 2015). In particular, EUDRAGIT® EPO (Fig. 1), a cationic polymer prepared by copolymerization of butyl methacrylate, dimethylaminoethyl methacrylate, and methyl methacrylate (molecular mass of about 47,000 Dalton), is very suitable for masking purpose, showing these excellent properties even at low film thickness (Singh et al., 2015). Recently, this polymer has been used also for the preparation of new formulations including microcapsules and nanoparticles to improve the solubility of poorly water soluble drugs (Moustafine et al., 2013; Newa et al., 2007).

Insert Figure 1

Among the different physical methods of microencapsulation, such as the well-known spray drying, fluid bed coating, extrusion, etc., an innovative technique called prilling or laminar jet breakup is able to produce microparticles or beads in a very narrow dimensional range and with high encapsulation efficiency by breaking apart a laminar jet of polymer solution into a row of mono-sized drops by means of a vibrating nozzle device (Del Gaudio et al., 2009). The resultant droplets fall into a consolidation medium in which they are solidified as beads.

The aim of this work was to realize new formulations of the bitter API PR with the masking agent EUDRAGIT® EPO, using the innovative technique prilling in order to improve the palatability of a dosage form and be compatible with other commercial formulations given to paediatric patients. The obtained beads were characterized in terms of drug loading, efficiency of encapsulation, size and morphology. The *in vitro* drug release and electronic tongue (e-tongue) tests of these formulations were also performed in order to evaluate the efficacy of these microparticulate systems in avoiding the bitterness in the mouth after administration and the complete release of the API in the stomach. These new multiple-unit systems could prove to be better than single-unit systems due to their several advantages: a flexible dose, a better patience compliance by masking an unpleasant taste, and less swallowing and stability problem. Furthermore, these flexible modified dosage forms could comply with prescribed dosing regimens due to differences in age and weight of child.

2. Materials and methods

2.1 Materials

The drug PR was a gift of Farmalabor s.r.l. (Canosa di Puglia, Italy). EUDRAGIT® EPO and stearic acid (Parteck® LUB STA 50, Merck KgAa, Germany) were donated by Evonik Nutrition & Care GmbH (Darmstadt, Germany). Dodecyl sulphate 90% USP was obtained from A.C.E.F. S.P.A. Bidistilled water for analysis (obtained from Carlo Erba Reagents S.A.S.) was used for all experiments. Tert-butyl alcohol, other solvents, and buffer agents (technical or HPLC grade) were purchased from Sigma-Aldrich (Milan, Italy)-

2.2 Preparation of feeds for prilling method

The aqueous colloidal feed solution without drug was obtained adding to a suspension of EUDRAGIT® EPO (8.57% w/v), sodium dodecyl sulphate (0.86% w/v), as wetting and dispersing agent, and stearic acid (1.29% w/v), as salt former, under magnetic stirring. The pale yellow colloidal dispersion of the matrix (10.72% w/v) was processed by prilling method to formulate empty microbeads. Various feeds with PR were prepared to formulate microbeads loaded with drug. The selected ratios (w/w) in the feeds between PR and matrix were 1:25, 1:15 and 1:10. In detail, for the feed 1:25, 214.4 mg of PR was weighted and dissolved in a matrix aqueous solution (5.36 g matrix in 50 mL of water).Initially, a precipitate was obtained by mixing PR with the matrix in water that was easily and homogeneously suspended under high-shear mixing using an Ultraturrax (T 25 basic IKA® WERKE) for 5 minutes at 13000 rpm. At the ratio 1:15 of drug (357.3 mg) and matrix (5.36 g) in 50 mL of water a rubbery precipitate was formed that could not be redisperded by mixing with Ultraturrax. Therefore, the precipitate was separated by paper filtration and the filtered aqueous suspension homogenized and processed by prilling. Instead, the precipitate was isolated and lyophilized in order to evaluate the presence of the drug, its relative amount, and ratio with the matrix. In alternative, for the ratio 1:15, a physical mixture of EUDRAGIT® EPO (2.47 g) and PR (357.3 mg) was prepared and stirred magnetically for 1 hour, in 23 mL of tert-butyl alcohol, to obtain a clear solution. Hence, 27 mL of aqueous matrix 10.72 % w/v were added to this organic solution (ratio water/tert-butyl alcohol 1:0.85 v/v) and mixed under magnetic stirring. This final colloidal solution was the other feed for the preparation of the microbeads by prilling method.

It was not possible to process a suspension of drug and matrix at the ratio 1:10 in pure water. As consequence, only the feed with a ratio 1:10 of drug (536 mg) and matrix (5.36 g) in 50 mL of water and tert-butyl alcohol (1:0.85 v/v) was processed as well.

2.2 Preparation of microbeads by prilling method

Microbeads were obtained by prilling technique using the Encapsulator B395 Pro (Büchi Labortechnik AG, Switzerland) equipped with a single nozzle and processing the feeds described above. With the aim to set up optimal parameters for the production of microbeads with desired properties by prilling process, a certain number of preliminary tests were conducted changing the experimental parameters of the feed suspensions (ratio drug:matrix, viscosity etc) and of the instrument, such as, diameter of the nozzle, nozzle vibration frequency, volumetric solution rate,

electrode potential. The goal was to realize spherical matrix beads with homogenous diameters under 1 mm. Those were obtained by using an appropriate nozzle and when a stable cone jet without satellite drops formation was created (Auriemma et al., 2013). Table 1 summarizes the best instrumental parameters used for the preparation of these microbeads. A liquid nitrogen bath was used to consolidate the microdrops of the feed suspension formed during the process. Then, the freeze microbeads were collected and immediately freeze-dried using a Christ Alpha 1-4 LSC for 48 hours under reduced pressure (0.018 mbar) at -51.0˚C.

Insert Table 1

2.3 HPLC analysis

Qualitative and quantitative analysis of PR were conducted by high performance liquid chromatography (HPLC, Agilent 1200 Infinity Series), using a Hyperclone ODS C18 column (250 mm x 4.6 mm, 5 µm particle size) with an isocratic elution mode. The mobile phase was acetonitrile, methanol, and an aqueous solution of 0.01 M sodium phosphate dibasic adjusted at pH 2.7 with phosphoric acid in a ratio of volumes 50:35:15 (% v/v). The flow rate, the injection volume and the temperature of the column were 1 mL/min, 20 µL and 25°C respectively. The spectrophotometric detector was operated at a wavelength of 254 nm. A series of standard solutions of PR across a range of concentrations between 2.12-106 µg/mL were prepared. Each sample was injected in duplicate. The observed retention time of the drug was 5.6 minutes. The peak areas of PR were then plotted against the corresponding concentrations and the calibration curve was described by a linear equation ($y = 5606.5x$, $R^2 = 0.9979$).

2.4 Determination of drug loading, encapsulation efficiency and production yield

To determine PR loading in the microbeads, an accurately weighed amount of each formulation (3 mg) was dissolved in 2 mL of 0.01 M sodium phosphate dibasic adjusted at pH 2.7 with phosphoric acid (complete dissolution was obtained in less than 2 minutes). The solution was filtered with 0.45 µm cellulose acetate filters and the concentration of PR was determined by the HPLC method described above. The results were expressed in terms of mg of PR per gram of sample (drug loading, DL).

The percentage of encapsulation efficiency (EE%) was computed using the equation (1):

$$EE\% = (\text{Actual Loading}/\text{Theoretical Loading}) \times 100 \quad (1)$$

where, actual loading is the determined drug content in the sample evaluated by HPLC and the theoretical loading is the theoretical amount of the drug present in the weighed microbeads. The analysis were conducted for each sample on three different batches and reported as mean ± standard deviation (SD). The percent yields (Y%) of each sample were determined using equation (2):

$$Y\% = (\text{Mass microbeads}/\text{Theoretical Mass}) \times 100 \quad (2)$$

where, mass microbeads is the amount of microbeads obtained from the process and theoretical mass is the amount of PR and matrix used.

2.5 Beads size, morphology and inner structure

The size of each formulation was measured directly by digital caliper (Hi-Tech Diamond) or by optical microscopy. The optical microscope used a light stereomicroscope (LEICA Galen III) equipped with a Panasonic camera (WVCP 230) and interfaced with an image analysis program (Leica Qwin 2.4 software). For each batch produced, a minimum of one hundred bead images were examined to calculate mean diameters and their relative SDs.
Scanning electron microscopy (SEM) was performed using a JEOL JSM-IT-300 microscope with a secondary electron detector SED high vacuum mode (JEOAL Ltd.). Each sample was sprinkled onto an electrically conductive adhesive pad and coated with a gold/palladium layer (sputter coating 15-20 nm). To get an impression of inner structure, cross sections were produced for each sample and coated in the same way. The other parameters set for the analysis were: acceleration voltage 10kv, EI Magnification 20x-5000x, WD about 10 mm.

2.6 Fourier Transform infrared spectroscopy (FT-IR) and Differential Scanning Calorimetry (DSC)

FT-IR spectra were sampled in KBr pellets and were recorded on a Perkin Elmer 1600 FT-IR spectrophotometer (Spectrum One). DSC thermograms of PR, EUDRAGIT® EPO and the prepared microbeads were acquired using Mettler Toledo DSC 822e (Stare 202 System) equipped with a thermal analysis automatic program and using indium as internal standard. The operative conditions for the DSC analysis were: sample weight 2-4 mg, scanning speed 10˚C/min, in a range between 25˚C and 200˚C. The samples were heated in opened aluminium pans.

2.7 Drug release study

Release studies were conducted for each sample using the VanKel system VK 7000, with the aim to evaluate the effect of some properties of the microbeads, such as porosity and size, on the PR release kinetics from the systems. The rotational speed of the paddle was set at 75 rpm and the temperature of the dissolution medium was maintained at 37 °C. For each experiment, a quantity of microbeads equivalent to 30 mg of drug was weighed and subsequently poured into 800 mL of dissolution medium. Under these experimental conditions, the theoretical drug concentration was equal to 0.0375 mg/mL and a sink condition was realized. The dissolution media used in our studies were a simulated saliva at pH 6.8 and 5.5 (table 2) and a simulated gastric fluid at pH 1.2 and 4.0 (HCl 0.1 M and phosphate buffer 0.05 M respectively) as described in the European Pharmacopeia 6.0 Edition (Ph.Eur. 6.0, 2007). Withdrawals of 1 mL from dissolution medium were made at set times and replaced with an equal volume of fresh medium. The amount of the drug released during the time was determined analysing the samples by HPLC, using the method described previously. Each study was conducted in triplicate.

Insert Table 2

2.8 E-tongue measurements

Analyses were performed with the Taste-Sensing System SA 402B (Intelligent Sensor Technology Co. Ltd, Japan), namely, Electronic Tongue (e-tongue). For these measurements, two detecting sensors (AC0; AN0), specific for bitter taste of pharmaceutical products, were applied. The detecting sensors and reference electrodes were first dipped into the reference solution (30 mM potassium chloride and 0.3 mM tartaric acid) and the electric potential, measured for each sensor, was defined as Vr. Then the sensors were dipped for 30 s into the sample solution and the measured potential was

defined as Vs. For each sensor the "relative value" (Rv) was represented by the difference (Vs-Vr) between the potential of the sample and the reference solution. Sensors were then rinsed for 6 s and then dipped into the reference solution again. The new potential of the reference solution was defined as Vr'. From the difference (Vr'-Vr) between the potential of the reference solution before and after sample measurement, the "CPA value" (CPAv) can be detected, where CPA stands for "Change of membrane Potential caused by Absorption". Before a new measurement cycle started, electrodes were rinsed for 90 s with a washing solution and then for 180 s with the reference solution. The "taste values" can be calculated by multiplying the Rv and CPAv of the sensors for appropriate coefficients based on Weber-Fechner law, which gives the intensity of sensation considering the sensor properties for tastes (Kobayashi, et al., 2010). In this work the "bitterness intensity" was estimated as: Bitterness 1 = 1.26 × CPAv (AC0), and Bitterness 2 = 1.80 × CPAv (AN0). In order to evaluate the ability of e-tongue to reveal the bitter taste of PR, two calibration curves were built in two consecutive days (day 1 and day 2) by considering the bitterness 1 and bitterness 2 intensity of API standard solutions ranging from 0.00 mg/100 mL to 0.05 mg/100mL The samples analyses were performed on the following samples: artificial saliva prepared as reported in table 2, PR aqueous solutions (concentration range: from 0.00 mg/100 mL to 0.05 mg/100mL, and three final formulations (**F_{1n}**, **F_{2nt}** and **F_{3nt}**). For the dissolution studies, a proper amount of samples was weighed, dispersed in 100 mL artificial saliva, stirred for fixed times (dissolution times), filtered under vacuum and then analysed by e-tongue. Details about sample preparation and test protocol are given in table 3. All samples were analysed twice in duplicate and the sensor outputs were collected and converted to taste information (i.e. bitterness intensity).

Insert Table 3

2.9 Stability study

The stability study was performed on the selected samples **F_1** and **F_{1n}**. The microbeads containing an equivalent of 30 mg of drug were taken and stored in amber colour vials at room temperature. After 1 months, microbeads containing equivalent to 5 mg of drug were taken placed and shaken in the digestive medium phosphate buffer pH 2.7, for 15 minutes. After making proper dilutions, the samples were analysed for drug content. In the similar way, the formulation was again analysed for the drug content after being stored for 2, 3, and 4 months. Visual inspections were also conducted to evaluate physical stability of the samples.

2.10 Statistical analysis

The experimental data were expressed as mean ± SD. Statistical analysis was carried out using GraphPad Prism version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Groups of data were compared with one-way analysis of variance, followed by the Bonferoni's test. The values were considered statistically significant when P value was <0.05.

3.0 Result and discussion

This study explored the feasibility of developing a new swallowable solid formulation, PR-loaded microbeads, made with EUDRAGIT® EPO, a pharmaceutical excipient with a pH-dependent solubility, useful for masking the unpleasant organoleptic properties of the drug in the mouth. In added, this formulation should enhance the paediatric patient compliance with a flexible dosage.

Indeed, PR is a bitter API and require different dosage as function of the disease, the weight and age of the patient (Ensom et al., 2013; EMA report 2014).

3.1 Production of microbeads by prilling method

In this work, for the first time, prilling technology was used to formulate microbeads made with the polymer EUDRAGIT® EPO, which can entrap the drug PR in a matrix system. A processable colloidal aqueous solution made with the polymer and adequate concentrations of selected excipients (table 4) was used for the preparation of empty and PR-loaded microbeads. The feasibility to prill and consolidate the matrix colloidal solution was studied and the best operative conditions were applied to realize microbeads with and without drug. Several attempts were made in order to obtain a favourable consolidation bath for the production of our microbeads (medium of the bath, its composition, pH or temperature). The best option chosen was a consolidation bath made with liquid nitrogen. The colloidal solution dropping in liquid nitrogen froze immediately and then could be recovered and lyophilized. We called this technique prilling/congealing and to our knowledge no studies has been published using this particular approach with EUDRAGIT® EPO.

However, in the presence of PR some noncovalent interactions between drug and polymer took place during the preparation of the colloidal aqueous feed, leading to the formation of a precipitate that clogged the spray nozzles preventing the prilling process. As shown in table 1 three different ratios drug/matrix were considered (1:25, 1:15 and 1:10). Higher and lower ratios were avoided in order to contain the dilution of PR in the polymeric matrix and stick the nozzle.
In all these cases, we observed that mixing the drug with the matrix colloidal solution a precipitate was obtained. Only the obtained suspension at a ratio of 1:25 could be processed by prilling after homogenization of the precipitate formed. Two nozzles were adopted (300 μm and 450 μm) to process the feeding suspension with the drug and two formulations, called **F_1** and **F_{1n}**, were obtained respectively.
At the ratio 1:15, two kinds of feeds were considered: an aqueous- and a solvent/aqueous-based feed. It was possible to process the aqueous-based suspension only with the 450 μm nozzle after elimination of a consistent precipitate (4.67 g) (**F_{2n}**). The drug content in the precipitate was determined by HPLC analysis. Thus, it allowed us to determine the new ratio drug/polymeric matrix in the prilled suspension, that was 1:10.
The solvent/aqueous feed was made by adding with an opportune procedure, drug and matrix in 50 mL of a mixture of tert-butyl alcohol (23 mL) and water (27 mL). The presence of the organic solvent avoided the interaction among the drug and the other components of the matrix and was determinant for the preparation of the feeding colloidal solution and subsequent prilling procedure.
The organic/aqueous colloidal solution was dropped without problems with the two selected nozzles obtaining the samples **F_{2t}** and **F_{2nt}**. Finally, at the ratio 1:10, it was possible to process the feeding suspension using the solvent/aqueous mixture and two formulations were obtained **F_{3t}**, **F_{3nt}**.

Insert Table 4

3.2 Determination of EE%, PR loading, and production yield

The EE%, drug loading and the yield of production of the prepared microbeads are reported in table 5. According to this table, the EE% improves when the ratio of drug-matrix increases from 1:10 to 1:25 and is in a range between 81% and 100%. It is possible that less drug was exposed on surface of the native microbeads when the feed was in the ratio 1:25 and that reduced the leakage of drug during the consolidation and recovery of the microbeads. Conversely, the EE% for the formulation **F_{2n}** is about 22%. This result is not in contrast with the other values reported in table 5. In fact, quantitative

HPLC analysis of the drug performed on the precipitate and aqueous suspension, demonstrated that approximately 75% of the PR was eliminated from the feed suspension as a rubbery precipitate. This precipitate was hard to reduce to a suspension processable also with nozzles larger than 450 μm and therefore discarded for the prilling process.
As expected, the DL of the formulations (except **F_{2n}**) increased when the ratio of drug/matrix decreased from 1:25 to 1:10, with values ranging from 38 to 76 mg of PR per g of microbeads. We should highlight that an oral dose of 10 mg of PR could be administered to a child (10-20 Kg body weight) with only 131-263 mg of these formulations.
The yield of process was high, as shown in table 5, demonstrating the effectiveness of the new adopted method. As explained above, the sample **F_{2n}** reduced considerably its yield due to the elimination of the rubbery precipitate before prilling process.

Insert Table 5

Insert Figure 2

3.3 Beads size, morphology, inner structure

The mean diameters of the microbeads are listed in table 5. The diameters ranged from about 327 to 357 μm and from 648 to 669 μm using a 300 or 450 μm nozzle respectively. These sizes are desired for a paediatric formulation because small particulates may be easier swallowed and thus more acceptable than single-unit formulations. Spomer and coworkers found that very young children (6–12 months) were fully capable of swallowing mini-tablets of 2 mm of diameter, often accepting them in preference to sweet liquid formulations (Spomer et al., 2012).
However, there is a lack of evidence on the size and amount of multiparticulates that is acceptable to patients, although a recent FDA guidance suggests a maximum targeted size of 2.5 mm (Kobayashi et al., 2010; Lopez et al., 2015).

As shown in Figure 2 all the microbeads formulated are quite spherical and homogeneous. This characteristic together with the softness of prepared microbeads could help to reduce the grittiness and mouthfeel, disadvantages often associated to multiparticulates (Kobayashi et al., 2010). Furthermore, spherical size particulates have good flow property that is important during the packaging. The microbeads obtained using the feed with the organic solvent tert-butyl alcohol (**F_{2t}**, **F_{2nt}**, **F_{3t}** and **F_{3nt}**) appeared to be a little more spherical than the formulations produced using the aqueous feed. The presence of the organic solvent tert-butyl alcohol in the feed suspension could be extremely important in dissolving the matrix and the drug, in reducing the interaction between them and forming a homogeneous suspension that affects the morphology of the microbeads due to more regular prilled drops.

Figure 2 shows the surface and inner morphology of the microbeads. All the microbeads appear to be cracked on the surface with the presence of many pores, more evident for those samples obtained using an aqueous/organic feed and lower ratio drug/matrix (see Fig.1 A-E II and III). The observed higher porosity of the formulations prepared with the aqueous/organic mixture is probably due to the ability of tert-butyl alcohol to enhance the ice sublimation rate, ascribed to its own high vapor pressure and its ability to modify the ice crystal habit (Oesterle et al., 1998). No other particles imputable to PR were identified on the surface also at higher magnification. Information about the inner polymeric texture of the microbeads were obtained when they were cryo-fractured and successively analysed by SEM (Fig. 2A-E, IV). The crossection of beads shows a well-organized matrix microstructure resembling a radial capillary structure for the samples obtained using an aqueous feed (Lakehal et al., 2019; Sereni et al., 2017) and a sponge for those formulations made from the aqueous/organic solvent

feed. These particular structures of the inner, characterized by the presence of pores and channels, generate water floating microbeads. These particles potentially could ease the swallowing process in paediatric patients using small volumes of water or another vehicle.

3.4 DSC and FT-IR

Thermograms of the PR and EUDRAGIT® EPO alone, the freeze-dried microbeads and the physical mixture of the polymer and the drug with the excipients described in table 4 are shown in figure 3A a-g. A sharp endothermic peak of the pure drug is observed at 164.52°C, corresponding to its melting point. This signal tends to shrink or disappear in the physical mixture and the analysed samples. This behaviour could be explained by the presence of drug-polymer interactions due to hydrogen bonds between the hydrogens of the hydroxyl and secondary amine groups of the drug (donor) and the lone pair of electrons of oxygen on the carboxylate group and nitrogen tertiary amine of the polymer (acceptor) (Bruce and McGinity, 2008). It is interesting to observe that the thermogram g (sample **F_{3nt}**) in figure 3A shows three major endothermic peaks at around 65, 125 and 165˚C. The first and the third could be attributed to the endothermic peaks of the polymer and the drug respectively. The peak at 125˚C could be explained by the formation of a drug-polymer interactions due to hydrogen bonds between the drug and the polymer. A thermogram of the precipitate isolated from the aqueous suspension of drug-polymer at the ratio 1:15 showed the same behaviour of the formulation g (data not shown), confirming that similar interactions are present also in the feed processed by prilling.
The quantitative HPLC analysis of the precipitate isolated from the aqueous suspension drug/matrix 1:15 confirmed that the solid was mainly constituted by drug and polymer in a ratio 1:20.
The interactions between PR and the polymer were also confirmed comparing the IR spectra of the pure drug and EUDRAGIT® EPO with the spectra of the microbeads (Fig. 3B a-g). As shown in figure 3B the characteristic peaks of PR at 3283 cm^{-1} (hydroxyl group), 2965 cm^{-1} (secondary amine group), 1267.27 cm^{-1} (aryl alkyl ether) and the peak at 798 cm^{-1} (substituted naphthalene) shift or disappear in the spectra of the microbeads. The peak of the aryl alkyl ether group is absent in our samples where we have revealed the presence of a peak due to the ester group of the EUDRAGIT® EPO. The peak of PR at 3283 cm^{-1} (hydroxyl group) shifts at around 3300 cm^{-1} (spectra d-g) and the presence of new absorption bands at around 1550 cm^{-1} (spectrum g) could be the result of the formation of drug-polymer interactions (Lee et al., 1991; Bruce and McGinity, 2008).

Insert Figure 3

3.5 Release studies in artificial saliva at pH 5.5 and 6.8

A first aim of this work was to manufacture microbeads able to cover the salty and bitter taste of PR. Release studies in artificial saliva gave us the preliminary information about the ability of hitting the target. In this study, we evaluated the drug release from our samples in artificial saliva at pH 6.8 and pH 5.5, since the oral cavity could be constantly subjected to changes in the pH values. Figure 4 shows the release profiles of the samples compared with PR alone. As expected, PR immediately dissolved in artificial saliva at pH 6.8 with 89.1% of drug released after 2.5 min. The higher is the concentration of drug in the oral cavity the greater is the perception of the unpleasant taste, even if the drug stays briefly in the mouth. The release rates of the drug and its dissolution from manufactured microbeads are much slower than that of the drug alone and statistically significant ($P<0.05$). Especially the samples **F_1**, **F_{1n}** and **F_{2n}** dissolved and released 5.67%, 3.28% and 7.02% of drug

respectively after 2.5 min at pH 6.8 that was 15.71-, 27.2-, and 12.7-fold lower than the percentage of PR alone dissolved at the same time and pH value. As consequence, the unpleasant taste of drug in these samples should be reduced considerably. Even though after 15 minutes, the amount of drug released from these samples was much less than the PR powder dissolved alone. Finally, no significant differences were observed between the $\mathbf{F_{1n}}$ and $\mathbf{F_{2n}}$ at the respective ratio of 1:25 and 1:15(effective ratio 1:10) drug:matrix.

The drug release profiles for the samples $\mathbf{F_{2t}}$, $\mathbf{F_{2nt}}$, $\mathbf{F3_t}$, and $\mathbf{F_{3nt}}$ obtained from aqueous/organic feed show a slightly higher value of PR released after 2.5 min (about 25%) and substantially different after 15 min (until to 60%). This behaviour could be due to the use of the organic solvent tert-butyl alcohol for the preparation of the feed drug/matrix suspension. As one can see in the images obtained by SEM (Fig. 2) these beads are more porous on the surface and their inner appears more like a spongy matrix. This particular architecture of the polymeric matrix could allow an easier water permeability and its retention inside the beads, determining a quicker solubilization and diffusion of PR into the bulk solution. Nevertheless, the perception of bitter taste of the oral formulation could be reduced during a fast transit through the mouth (generally 30-60 s). However, for a better conclusion about the taste masking the best samples ($\mathbf{F_{1n}}$) and two worst case ($\mathbf{F_{2nt}}$ and $\mathbf{F_{3nt}}$), as comparison, were analysed by e-tongue test and the results discussed in the next section.
Analogue profiles were obtained with artificial saliva at pH 5.5. The calculated drug release values were slightly greater than the percentages observed at pH 6.8 for a higher acid environment that solubilize minimally the matrix. The release of PR from the samples realized with a 300 μm nozzle ($\mathbf{F_1}$, $\mathbf{F_{2t}}$ and $\mathbf{F_{3t}}$) are also presented in Fig. 4. As expected, decreasing the size of the microbeads (samples $\mathbf{F_1}$, $\mathbf{F_{2t}}$ and $\mathbf{F_{3t}}$, see table 6), a higher percentage of drug was released and dissolved during the time of the analysis (for instance 5.67% for $\mathbf{F_1}$ and 3.28% for $\mathbf{F_{1n}}$ after 2.5 min). These findings could be justified by the higher specific surface area of the samples $\mathbf{F_1}$, $\mathbf{F_{2t}}$ and $\mathbf{F_{3t}}$ per unit of mass of microbeads.

Insert Figure 4

3.6 Release studies in simulated gastric environment

As observed in figure 5, a complete drug-release from the matrix at pH 1.2 takes place in less than 3 min for all samples processed with a 300 μm nozzle size. This behaviour is similar for the microbeads obtained with the nozzle 450 μm ($\mathbf{F_{1n}}$, $\mathbf{F_{2n}}$, $\mathbf{F_{2nt}}$ and $\mathbf{F_{3nt}}$). As shown in figure 5, the drug-release profiles at pH 4.0 of the microbeads processed takes place more slowly than drug-release at pH 1.2. This finding can be explained by the easier solubilization process of the matrix at lower pH value. Microbeads $\mathbf{F_1}$ show a percent of drug released of 53.15% after 1 min, 87.92% after 5 min and 98.88% after 20 min, similarly for the other samples.

Insert Figure 5

3.7 E-tongue measurement

The capacity of e-tongue to individuate the bitter taste of PR is shown in figure 6. As it is possible to notice, there is a clear dependence between the PR concentration and the intensity of bitterness 1

and bitterness 2, thus demonstrating that the two selected sensors (AC0 and AN0) are suitable for detecting the bitter taste of the active ingredient.

Insert Figure 6

In figure 7 are reported the bar graphs representing the bitterness intensity (bitterness 1 and bitterness 2) and bitterness score values of artificial saliva, PR (API) and the samples **F_{1n}**, **F_{2nt}** and **F_{3nt}**.
As it is possible to notice during the dissolution test of formulation **F_{1n}** (A), the bitterness intensity increase very slightly compared to saliva, moreover the increase is significant only for bitterness 1, while for bitterness 2 there are not significant differences between saliva (base value) and the final formulation samples during dissolution. Considering the bar graphs of **F_{2nt}** (B) and **F_{3nt}** (C), a progressive increase of bitterness intensity can be observed during dissolution, with statistically significant values especially with regard to bitterness 1. Finally, the scores were calculated for bitterness 1, bitterness 2 and total bitterness (bitterness 1 + bitterness 2) by setting to 0 the bitter intensity of saliva and to 100 the bitter intensity of API (Pimparade et al., 2015). Considering the formulation **F_{1n}**, the total bitter taste scores are equal to 9 at 2.5 min of dissolution and to 20 after 15 min of dissolution, corresponding to a bitterness reduction percentage of 91% and 80% when compared to PR alone. After 15 min of dissolution the scores of bitterness 1 and bitterness 2 are 25 and 15, respectively. For the samples **F_{2nt}** and **F_{3nt}** the total bitterness scores, evaluated at 2.5 min and 15 min of dissolution is about 25 and 50, respectively. In particular, for the formulation **F_{2nt}** (ratio 1:15) a bitterness 1 score of 60 and a bitterness 2 score of 46% has been reached after 15 min of dissolution; for the formulation **F_{3nt}** (ratio 1:10) the bitterness 1 and the bitterness 2 scores are equal to 56 and 46, respectively. These finding are in agreement with the proposed assumptions present in the section describing the release of PR in artificial saliva, where the sample **F_{1n}** is recognized as the best to cover the bitter taste, but also the samples **F_{2nt}** and **F_{3nt}** can cover partially the bitterness of PR.

Insert Figure 7

3.8 Stability study

Based on the results obtained from the release and e-tongue analysis, stability studies were conducted only on the sample F1 and F1n. The results obtained from the analysis conducted over 4 months revealed that the drug and the microbeads were chemically and physically stable respectively (no degradation product in the HPLC chromatograms, no sign of shrinking of the volume of the microbeads, changing of the colour on their surface or sticky phenomenon were observed over 4 months).

4. Conclusion

A solid oral formulation dedicated to paediatric population was developed with the advantages to have a flexible dose, mask bitter taste of the API PR and obtain a stable handy system to carry. For the first time, matrix microbeads loading PR based on excipient EUDRAGIT® EPO were developed using an innovative prilling/congealing technique. Among the several prepared microbeads, the samples **F_1** and **F_{1n}** could be considered robust formulations able to mask the bitter taste of PR and appropriate for the aim of this study. These systems could be taken in consideration as alternative dosage forms of PR to the commercial formulations present on the market. This new patient-centric formulation could be useful not only for paediatric patients but also geriatric patients or adults with reduced capability to take conventional solid dosage forms, providing several advantages.

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Reference

Al-Majed, A.A., Bakheit, A.H., Aziz, H.A.A., Alajmi, F.M., AlRabiah, H., 2017. Propranolol. In *Profiles of Drug Substances, Excipients and Related Methodology* 42, pp. 287-338. Academic Press.

Auriemma, G., Mencherini, T., Russo, P., Stigliani, M., Aquino, R.P., Del Gaudio, P., 2013. Prilling for the development of multi-particulate colon drug delivery systems: Pectin vs. pectin–alginate beads. *Carbohyd. Polym.*, 92(1), 367-373.

Benita, S., (Ed.). 2005. Microencapsulation: methods and industrial applications. Crc Press.

Bidabadi, E., Mashouf, M., 2010. A randomized trial of propranolol versus sodium valproate for the prophylaxis of migraine in pediatric patients. *Pediatric Drugs*, *12*(4), 269-275.

Bruce, L.D., McGinity, J.W., 2008. Polymer interactions with drugs and excipients. *DRUGS AND THE PHARMACEUTICAL SCIENCES*, 176, 369.

Bruckner, A.L., Frieden, I.J., 2003. Hemangiomas of infancy. *J. Am. Acad. Dermatol.*, *48*(4), 477-496.

Castaneda, S., Melendez-Lopez, S., Garcia, E., De la Cruz, H., Sanchez-Palacio, J., 2016. The role of the pharmacist in the treatment of patients with infantile hemangioma using propranolol. *Adv. Ther.*, 33(10), 1831-1839.

Cerciello, A., Del Gaudio, P., Granata, V., Sala, M., Aquino, R.P., Russo, P., 2017. Synergistic effect of divalent cations in improving technological properties of cross-linked alginate beads. *Int. J. Biol. Macromol.*, 101, 100-106.

Del Gaudio, P., Russo, P., Lauro, M.R., Colombo, P., Aquino, R.P., 2009. Encapsulation of ketoprofen and ketoprofen lysinate by prilling for controlled drug release. *AAPS PharmSciTech.*, 10(4), 1178.

Drolet, B.A., 1999. Esterly NB Frieden IJ Hemangiomas of Infancy. *New Engl. J. Med.*, *341*, 173-80.

EMA report 2013. Guideline on pharmaceutical development of medicines for paediatric use. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-pharmaceutical development-medicines-paediatric-use_en.pdf.

EMA report 2014. Hemangiol Propranololo. https://www.ema.europa.eu/en/documents/overview/hemangiol-epar-summary-public_it.pdf.

Ensom, M.H., Kendrick, J., Rudolph, S., Decarie, D., 2013. Stability of propranolol in extemporaneously compounded suspensions. *Can. J. Hosp. Pharm.*, *66*(2), 118.

Esterly, N.B., Northwestern University Medical School, H., 1987. Cutaneous hemangiomas, vascular stains, and associated syndromes. *Curr. Probl. Pediatr.*, *17*(1), 7-69.

Hanning, S.M., Lopez, F.L., Wong, I.C., Ernest, T.B., Tuleu, C., Gul, M.O., 2016. Patient centric formulations for paediatrics and geriatrics: Similarities and differences. *Int. J. Pharm.*, 512(2), 355-359.

Hermans, D.J.J., van Beynum, I.M., Kool, L.J.S., van de Kerkhof, P.C.M., Wijnen, M.H.W.A., van der Vleuten, C.J.M., 2011. Propranolol, a very promising treatment for ulceration in infantile hemangiomas: a study of 20 cases with matched historical controls. *J. Am. Acad. Dermatol.*, 64(5), 833-838.

Jeganathan, B., Prakya, V., 2015. Interpolyelectrolyte complexes of Eudragit® EPO with hypromellose acetate succinate and Eudragit® EPO with hypromellose phthalate as potential carriers for oral controlled drug delivery. *AAPS PharmSciTech*., 16(4), 878-888.

Joshi, S., Petereit, H.U., 2013. Film coatings for taste masking and moisture protection. *Int. J. Pharm.*, 457(2), 395-406.

Kobayashi, Y., Habara, M., Ikezazki, H., Chen, R., Naito, Y., Toko, K., 2010. Advanced taste sensors based on artificial lipids with global selectivity to basic taste qualities and high correlation to sensory scores. *Sensors*, 10(4), 3411-3443.

Lakehal, I., Montembault, A., David, L., Perrier, A., Vibert, R., Duclaux, L., Reinert, L., 2019. Prilling and characterization of hydrogels and derived porous spheres from chitosan solutions with various organic acids. *Int. J. Biol. Macromol.*, 129, 68-77.

Léauté-Labrèze, C., De La Roque, E.D., Hubiche, T., Boralevi, F., Thambo, J. B., Taïeb, A., 2008. Propranolol for severe hemangiomas of infancy. *New Engl. J. Med.*, 358(24), 2649-2651.

Lee, H.K., Hajdu, J., McGoff, P., 1991. Propranolol: methacrylic acid copolymer binding interaction. *J. Pharm. Sci.*, 80(2), 178-180.

Ley, J.P., 2008. Masking bitter taste by molecules. *Chemosens. Percept.*, 1(1), 58-77.

Liu, F., Lizio, R., Meier, C., Petereit, H.U., Blakey, P., Basit, A.W., 2009. A novel concept in enteric coating: a double-coating system providing rapid drug release in the proximal small intestine. *J. Control. Release*, 133(2), 119-124.

Lopez, F.L., Ernest, T.B., Tuleu, C., Gul, M.O., 2015. Formulation approaches to pediatric oral drug delivery: benefits and limitations of current platforms. *Expert Opin. Drug Del.*, 12(11), 1727-1740.

Maniruzzaman, M., Boateng, J.S., Bonnefille, M., Aranyos, A., Mitchell, J.C., Douroumis, D., 2012. Taste masking of paracetamol by hot-melt extrusion: an in vitro and in vivo evaluation. *Eur. J. Pharm. Biopharm.*, 80(2), 433-442.

Maniruzzaman, M., Douroumis, D., 2015. An in-vitro–in-vivo taste assessment of bitter drug: comparative electronic tongues study. *J. Pharm. Pharmacol.*, 67(1), 43-55.

Marqueling, A.L., Oza, V., Frieden, I.J., Puttgen, K.B., 2013. Propranolol and infantile hemangiomas four years later: a systematic review. *Pediatr. Dermatol.*, *30*(2), 182-191.

Moustafine, R.I., Bukhovets, A.V., Sitenkov, A.Y., Kemenova, V.A., Rombaut, P., Van den Mooter, G., 2013. Eudragit E PO as a complementary material for designing oral drug delivery systems with controlled release properties: comparative evaluation of new interpolyelectrolyte complexes with countercharged eudragit L100 copolymers. *Mol. Pharm.*, 10(7), 2630-2641.

Naouri, M., Schill, T., Maruani, A., Bross, F., Lorette, G., Rossler, J., 2010. Successful treatment of ulcerated haemangioma with propranolol. *J. Eur. Acad. Dermatol. Venereol.*, *24*(9), 1109-1112.

Newa, M., Bhandari, K.H., Li, D.X., Kwon, T.H., Kim, J.A., Yoo, B.K., Woo J.S., Lyoo, W.S., Yong, W.S., Choi, H.G., 2007. Preparation, characterization and in vivo evaluation of ibuprofen binary solid dispersions with poloxamer 188. *Int. J. Pharm.*, 343(1-2), 228-237.

Oesterle, J., Franks, F., Auffret T., 1998. The influence of tertiary butyl alcohol and volatile salts on the sublimation of ice from frozen sucrose solutions: implications for freeze-drying, *Pharm. Dev. Technol.*, 3(2), 175-183.

Pein, M., Preis, M., Eckert, C., Kiene, F.E., 2014. Taste-masking assessment of solid oral dosage forms–a critical review. *Int. J. Pharm.*, *465*(1-2), 239-254.

Ph.Eur. 6.0. Guidance on dissolution testing European Pharmacopoeia (2007), pp. 273-275.

Pimparade, M.B., Morott, J.T., Park, J.B., Kulkarni, V.I., Majumdar, S., Murthy, S.N., Lian, Z., Pinto, E., Bi, V., During, T., Murthy, R., Shivakumar, H.N., Vanaja, K., Kumar, P.C., Repka, M.A., 2015. Development of taste masked caffeine citrate formulations utilizing hot melt extrusion technology and in vitro–in vivo evaluations. *Int. J. Pharm.*, *487*(1-2), 167-176.

Sans, V., de la Roque, E.D., Berge, J., Grenier, N., Boralevi, F., Mazereeuw-Hautier, J., Lipsker, D., Dupuis, E., Ezzedine, K., Vergnes, P., Taïeb, A., Léauté-Labrèze, C., 2009. Propranolol for severe infantile hemangiomas: follow-up report. *Pediatrics*, 124(3), e423-e431.

Schilling, S.U., Shah, N.H., Malick, A.W., McGinity, J.W., 2010. Properties of melt extruded enteric matrix pellets. *Eur. J. Pharm. Biopharm.*, *74*(2), 352-361.

Sereni, N., Enache, A., Sudre, G., Montembault, A., Rochas, C., Durand, P., Perrad, M.H., Bozga, G., Puaux, J.P., Delair, T., David, L., 2017. Dynamic structuration of physical chitosan hydrogels. *Langmuir*, *33*(44), 12697-12707.

Skalsky, B. Eudragit® Application Guidelines, 13th Edition, chapter 7, pp 3.

Singh, S., Neelam, S.A., Singla, Y.P., 2015. An overview of multifaceted significance of eudragit polymers in drug delivery systems. *Asian J. Pharm. Clin. Res.*, *8*(5), 1-6.

Singh, M.N., Hemant, K.S.Y., Ram, M., Shivakumar, H.G., 2010. Microencapsulation: A promising technique for controlled drug delivery. *Res. Pharm. Sci.*, 5(2), 65.

Spomer, N., Klingmann, V., Stoltenberg, I., Lerch, C., Meissner, T., Breitkreutz, J., 2012. Acceptance of uncoated mini-tablets in young children: results from a prospective exploratory cross-over study. *Arch. Dis. Child*, *97*(3), 283-286.

Villain, E., Denjoy, I., Lupoglazoff, J.M., Guicheney, P., Hainque, B., Lucet, V., Bonnet, D., 2004. Low incidence of cardiac events with β-blocking therapy in children with long QT syndrome. *Eur. Heart J.*, *25*(16), 1405-1411.

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Table 1. Parameters used for the preparation of the microbeads by prilling.

Sample code*	Ratio PR/matrix (w/w)	PR (mg)	Volume processed (mL)	Nozzle size (μm)	Frequency (Hz)	Flow rate (mL/min)	Electrode potential (V)
F_1	1:25	214.4	50	300	1500	23	2500
F_{1n}	1:25	214.4	50	450	1500	20	2500
F_{2n}	1:15**	357.3	50	450	1500	21	2500
F_{2t}	1:15	357.3	50	300	1000	14	1500
F_{2nt}	1:15	357.3	50	450	1000	12	1500
F_{3t}	1:10	536.0	50	300	1000	12	1500
F_{3nt}	1:10	536.0	50	450	1000	10	1500
A	-	-	50	300	1500	17	2500
A_n	-	-	50	450	1500	14	2500

* The codes (**n**) and (**t**) indicate a nozzle diameter of 450 □m and a feed in the vehicle water:tert-butyl alcohol (1:0.85 v/v) respectively. ** Theoretical ratio.

Table 2. Components for preparation of artificial saliva according to Pimparade et al., 2015.

Component*	**Amount**
$CaCl_2 \cdot 2H_2O$	0.228 g
$MgCl_2 \cdot 6H_2O$	0.061 g
NaCl	1.017 g
$K_2CO_3 \cdot 1.5H_2O$	0.603 g
$Na_2HPO_4 \cdot 7H_2O$	0.204 g
$NaH_2PO_4 \cdot H_2O$	0.273 g
Water	q.b, 1L

* The pH 6.8 or 5.5 was adjusted with HCl.

Table 3. Samples preparation and protocol of analysis by e-tongue.

Samples	Sample preparation	Dissolution Time (min)					
		0	**2.5**	**5**	**7.5**	**10**	**15**
Artificial saliva	As reported in table 2	ν					
PR	3.75 mg/100mL of saliva	ν					
F_{1n}	98.33 mg*/100mL of saliva		ν	ν	ν	ν	ν
F_{2nt}	59.12 mg*/100mL of saliva		ν	ν	ν	ν	ν
F_{3nt}	46.17 mg*/100 mL of saliva		ν	ν	ν	ν	ν

* Equivalent to 3.75 mg of PR

Table 4. Composition of the aqueous and aqueous/ organic solvent feeds.

Function	Ingredient	(% w/w)* in water	(% w/w) in water/tert-butanol
Taste masking	EUDRAGIT® EPO	8.57	8.57
Emulsifier	Sodium dodecyl sulphate	0.86	0.46
Salt former	Stearic acid	1.29	0.70
	Total matrix°°	10.72	9.73

* Skalsky; °° Amount based on the dry component.

Table 5. DL, EE % and percent of the yield of production, size of microbeads**.** Data are reported as mean of three results ± SD.

Sample	Ratio PR/matrix	DL*	EE %	Yield %	Size (μm)
F_1	1:25	38.4 ± 1.8	100.0 ± 1.6	87.0 ± 1.7	356.8 ± 19
F_{1n}	1:25	38.4 ± 1.5	100.0 ± 1.2	89.0 ± 1.5	663.5 ± 38
F_{2n}	1:15**	22.0 ± 1.6	22.4 ± 1.8	18.0 ± 0.8	699.8 ± 25
F_{2t}	1:15	60.0 ± 2.6	96.0 ± 2.2	71.6 ± 2.4	333.5 ± 13
F_{2nt}	1:15	59.4 ± 4.0	95.1 ± 2.6	84.3 ± 1.2	654.3 ± 37
F_{3t}	1:10	76.5 ± 1.9	84.0 ± 1.8	90.2 ± 2.0	354.2 ± 22
F_{3nt}	1:10	74.3 ± 5.6	81.6 ± 4.4	80.2 ± 2.4	648.6 ± 24
A	-	-	-	98.2 ± 2.1	327.5 ± 29
A_n	-	-	-	99.1 ± 1.8	663.6 ± 38

* DL is expressed as mg of PR per g of microbeads; ** Theoretical ratio, the effective ratio drug/matrix was 1:10.