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Research paper

Bi-layered self-emulsifying pellets prepared by co-extrusion and spheronization: Influence of formulation variables and preliminary study on the *in vivo* absorption

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Abstract

The aim of this work was to produce by co-extrusion–spheronization pellets with two cohesive layers, one of them containing a self-emulsifying system for vinpocetine, a poorly water soluble model drug. Two layers were prepared: an inert layer of microcrystalline cellulose, lactose and water and a second one wetted with the self-emulsifying system. Different formulations of both layers were tested, evaluating the effects of formulation variables with an experimental design. The screening amongst formulations was performed preparing rod extrudates and using the extrusion profiles to assess their suitability for extrusion and to anticipate quality of the spheronized extrudates. Tubular extrudates and co-extrudates/spheronized pellets were then produced. Two types of bi-layered pellets were prepared: type I with the self-emulsifying system internally and the inert matrix externally, whereas type II vice versa. The pellets were characterized for sizing and shape, density, hardness, *in vitro* dissolution and disintegration and released droplets size and *in vivo* tests. Although both types of pellets demonstrated adequate morphological and technological characteristics, pellets type II revealed an improved drug solubility and *in vivo* bioavailability. These preliminary technological and pharmacokinetic data demonstrated that co-extrusion/spheronization is a viable technology to produce bi-layered cohesive self-emulsifying pellets of good quality and improved *in vivo* bioavailability.

Keywords: Bi-layer extrusion; Spheronization; Solid dosage form; Oral drug delivery

1. Introduction

Self-emulsifying drug delivery systems (SEDDS) should be considered to overcome problems associated with poor water solubility of drugs. These systems have a unique property: they are able to self-emulsify rapidly in the gastro-intestinal fluids, forming under the gentle agitation provided by gastro-intestinal motion fine O/W emulsions. This fine O/W emulsion results in small droplets of oil dispersed in the gastro-intestinal fluids that provide a large interfacial area enhancing the activity of the pancreatic lipase to hydrolyze triglycerides and thereby promote a faster release of the drug and/or formation of mixed micelles of the bile salts containing the drug. Further, in most cases the surfactant used for this kind of formulations promotes an increase on bioavailability of the drug, by activation of different mechanisms: maintaining the drug in solution, and thus avoiding the dissolution step from the crystalline state and enhancing at the same time intestinal epithelial permeability. Moreover, the oil droplets lead to a faster and more uniform distribution of the drug in the gastro-intestinal tract, minimizing the irritation due to the contact between the drug and the gut wall [1–4]. Last but not least

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it must be mentioned the impact of the lipids on the oral bioavailability of the drug compounds that exert their effect through several mechanisms including the protection of the drug from the enzymatic or chemical degradation in the oil droplets and the activation of lipoproteins promoting the lymphatic transport of lipophilic drugs [5].

Such systems are normally prepared as liquid dosage forms that can be administered in soft or hard gelatin capsules [6]. An alternative method which is currently investigated by several authors is the incorporation of liquid self-emulsifying ingredients (oil/surfactant/water mixture) into a powder in order to create a solid dosage form (tablets, capsules). Examples of such solid systems are self-emulsifying tablets [7] and pellets produced by extrusion/spheronization [8–12] or by wet granulation in high shear mixer [13]. Another approach is given by the inclusion of the fluid dispersion in microporous or cross-linked polymeric matrices [14,15].

While a lot of research has been carried out in the conventional extrusion/spheronization process [16,17], the co-extrusion/spheronization, first reported by Pinto et al. [18], is relatively new and scarcely applied. It involves four steps: the preparation of two wet masses (granulation), the shaping of the wet masses into concentric cylinders (co-extrusion in a ram extruder having two concentric barrels), breaking up the extrudate and rounding the particles into spheres (spheronization), and finally drying of the pellets. Amongst the several advantages and the potentials of the co-extruded pellets over the conventional ones, it is worth mentioning the possibility of modulating the release of drugs by loading it in matrices having different composition or position inside the spheroid (a change in the release of a drug can be achieved if the matrix plays the role of the external layer or inner core).

As a model drug, the poorly soluble vinpocetine [19], a vincamine derivative used for the treatment of disorders arising from cerebro-vascular and cerebral degenerative diseases [20], was chosen. This drug is usually marketed in tablets containing 5 mg of active, but its absorption after an oral administration is very poor and limited by the dissolution rate and the remarkable first pass effect [21–23]. Due to its low oral dose and poor solubility, vinpocetine seems a perfect drug candidate to test the solubility and bioavailability enhancement obtainable from such kind of delivery systems. As a further confirmation of this, previous studies demonstrated that it is possible to dramatically increase the solubility of vinpocetine and *in vitro* trans-dermal permeation by formulating the drug in microemulsions [24].

The purpose of the present work was to investigate the feasibility of preparing solid self-emulsifying systems (SES) by the technology of co-extrusion/spheronization. This research represents the first attempt to design a SEDDS pellet by simultaneous processing of two different formulations, one with the SES dispersion in the core and the coat made of an inert matrix (pellet of type I) and vice versa (pellet of type II).

2. Materials and methods

2.1. Materials

Microcrystalline cellulose (MCC, Microcel 101®, Faravelli, Milano, Italy), lactose monohydrate (Granulac 200®, Meggle, Wasserburg, Germany), mono- and di-glycerides (M, Akoline MCM, AarhusKarlshamn, Sweden), polysorbate 80 (P, Montanox 80 VG PHA®, Seppic, Castris, France), peanut oil (O, Galeno, Milano, Italy), croscarmellose sodium (CS, Ac-Di-Sol®, FMC Corp., Philadelphia, USA) and vinpocetine (Polichimica srl, Bologna, Italy) were used as starting materials. All the materials were of reagent grade.

2.2. Experimental design

Since the aim of the work was to produce bi-layered pellets by co-extrusion/spheronization, two different formulations were considered: a matrix made of lactose and MCC loaded with a SES dispersion (formulation A) and an inert matrix only composed of lactose, MCC and water (formulation B). Due to the complexity of the study, reflected on the difficulty of selecting the most suitable formulations for a simultaneous extrusion and spheronization, an experimental design of experiences was considered to optimize each formulation *per se*. Thus, a set of trials were carried out preparing rod extrudates based on formulation A or B, singularly. Based on the data of these trials, the quantitative limitations for each component were defined (Table 1). As regards to formulation A the amount of oil–surfactant mixture in the formulation A has been kept constant at 35%, thus decreasing the number of formulation variables from 4 to 3. It followed that an irregular experimental region was obtained, representing only a restricted part of the study's initial tetrahedral area (Fig. 1). Whereas for formulation B, including three components, the restricted area of the simplex is shown in Fig. 2. In order to explore both restricted regions, an experimental strategy for mixture design was followed [25]. Once the formulation compositions and

Table 1
Lower and upper limits of formulation containing SES (formulation A) and of inert formulation (formulation B) components

	Components	Lower constraints (%) (wt)	Upper constraints (%) (wt)
Formulation A[a]	Water (X_1)	13.0	31.0
	MCC (X_2)	2.0	33.0
	Lactose (X_3)	2.0	32.5
Formulation B (inert)	Water (X_1)	36.0	47.2
	MCC (X_2)	24.3	34.0
	Lactose (X_3)	24.3	34.0

[a] The mixture of mono- and di-glycerides + peanut oil + Polysorbate 80 was kept constant in all formulations at 35%.

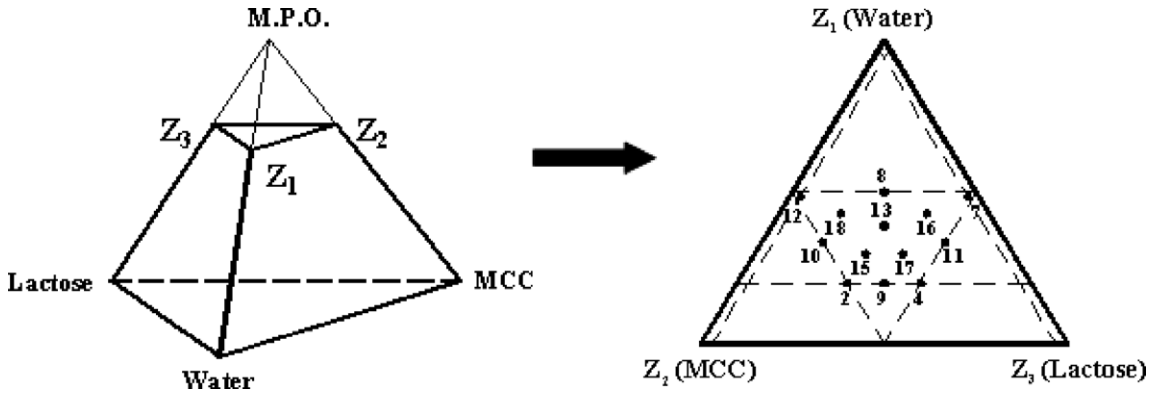

Fig. 1. Transformation steps of the experimental design from the tetrahedral region to the simplex applied for the optimization of formulation A.

the operating conditions were selected, the production of tubular and co-extrudates started up.

2.3. Preparation of "formulation A" wet masses

2.3.1. Preparation of the fluid dispersions

The oil–surfactant mixture was prepared in batch of 175 g each following the M:P:O:vinpocetine weight proportions: 20.0:49.4:30.0:0.6 by magnetic stirring for 15 min in a glass beaker at room temperature (25 °C). The self-emulsifying dispersions were prepared by slowly adding distilled water to the oil–surfactant mixture under constant gentle stirring, in selected relative proportions according the experimental design (Table 2). The mixtures were used on the same day as they were prepared.

2.3.2. Loading on a solid carrier

Formulation A was prepared as follows: the above-mentioned SES fluid dispersion was loaded into a powder mixture composed of MCC and lactose using a planetary mixer (Kenwood Chef, Kenwood Ltd., UK) for 20 min. One hundred and fifty grams of each of the 13 different mixtures was prepared according to the component proportions reported in Table 1. The experiments were carried out following the experimental design (Table 2) in a random order. The mass was stored for 12 h in a sealed plastic bag prior to extrusion, in order to allow fluid dispersion to equilibrate throughout.

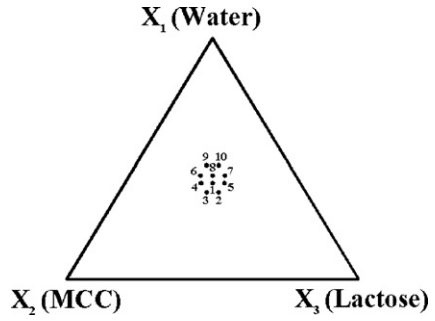

Fig. 2. Restricted experimental area inside the simplex used for the optimization of formulation B.

2.4. Preparation of (inert) "formulation B" wet masses

Ten masses based on formulation B were prepared by simply wetting the binary mixes of MCC and lactose (plus 2% w/w of croscarmellose sodium only in the case of below-mentioned "pellets type I plus 2% CS") with distilled water in the appropriate proportions using the component proportions shown in Table 2 and applying the same operating conditions as the preparation of formulation A wet masses.

2.5. Extrusion procedure

The extrusion process was carried out in a ram co-extruder equipped by a mechanical press (Lloyd Instruments LR 50K, UK) with a 50 kN load cell, which allowed the production of extrusion profiles. The co-extruder was built in-house and designed with two concentric stainless steel chambers, an internal cylindrical one and an external toroidal one, as described in a previous paper by Pinto et al. (see Fig. 3) [18]. The cross-section of the internal and external barrels is 122 and 1047 mm^2, respectively. At the bottom of the barrels two concentric dies are fitted to the chambers: the external die with an external diameter of 5 mm and an internal hollow of 4 mm, whereas the internal die has a diameter of 1 mm. The length to diameter ratio was 1 and 20 for the external and internal dies, respectively. The chambers were fed manually and the two coupled rams pushed both the wet masses at the same ram speed toward the end of the barrels. The internal die is fed directly from the ram, whilst the external die is fed with the mass passing through a converging chamber, that was, itself, filled with the mass of the external chamber after crossing 6 holes (diameter of 1 mm). Extrusion of the material contained in the internal and external dies occurs simultaneously to produce a co-extrudate, due to the narrowing of the tip of the external die, to 2 mm.

Approximately 150 g batches of wet masses were manually fed into the chambers, feeding 110 g in the outer chamber and 40 g in the inner chamber. Rod extrudates were produced by feeding only the inner chamber with all types

Table 2
Composition (% by weight) of the formulations tested and the corresponding extrusion characteristics

No. Exp.	Water X_1 (%)	MCC X_2 (%)	Lactose X_3 (%)	Extrusion pressure (kN)[a]	Extrusion profile[b]
Formulation A (active)					
1	13.0	32.5	19.5		No extrusion
2	13.0	19.5	32.5		IF
3	31.0	2.0	32.0		No extrusion
4	32.5	16.0	16.5		No extrusion
5	13.0	26.0	26.0	12	SS
6	22.0	33.0	10.0	<10	SS
7	22.0	11.0	32.0	<10	SS
8	31.5	31.5	2.0		No extrusion
9	25	20	10.0		No extrusion
10	19	27	19.0	<10	SS
11	28	11	26.0		No extrusion
12	19	19	27.0	<10	SS
13	28	26	11.0		No extrusion
Formulation B (inert)					
1	40.0	30.0	30.0	10	SS/IF
2	36.0	30.0	34.0		FF
3	36.0	34.0	30.0		FF
4	40.0	34.0	26.0		FF
5	40.0	26.0	34.0		FF
6	43.0	24.3	32.7		FF
7	43.0	32.7	24.3		FF
8	43.0	28.5	28.5	10	SS/IF
9	47.2	28.5	24.3		FF
10	47.2	24.3	28.5		FF

[a] At the steady state.
[b] IR, irregular flow; FF, forced flow; SS, steady state flow.

of formulation A or B (see Table 2); tubular extrudates were prepared by feeding only the external chamber with the optimized formulation A or B. Finally, bi-layered co-extrudates were produced by feeding the inner chamber with optimized formulation A and the outer chamber with optimized formulation B (co-extrudates type I), and vice versa (co-extrudates type II).

Firstly, the rod extrudates were produced using the ram velocity of 100 and 150 mm/min and setting maximum extrusion force of 20 and 25 kN, for extruding formulations A and B, respectively.

The extrusion experiments were characterized by analyzing the extrusion profiles allowing for the identification of the different extrusion stages (compression, steady state flow and forced flow) and to collect some data (e.g. kind of flow curve, mean extrusion force or force at the steady state and duration of this stage), enabling the characterization of each extrusion cycle and to anticipate the quality of the pellets to be produced by spheronization. From the preliminary trials it was observed that wet masses giving a steady state extrusion force smaller than 10 kN were overwetted and hence were not expected to give rise to agglomerates during the subsequent process of spheronization [26]. On the contrary, when force required for extrusion exceeded 35 kN, the wet masses resulted in a product too dry and hence impossible to be molded into spheres. As for the duration of the steady state, good quality extrudates usually resulted from processes characterized by long static flow phases and short compression and forced flow phases [17].

Due to the differences in wet mass capacity of the outer (110 g) and inner (40 g) barrels, a ram velocity of 150 mm/min and maximum extrusion force fixed at 35 kN were chosen for the preparation of tubular extrudates with the two optimized formulation A or B.

Finally, type I co-extrudates were made using a piston speed of 100 mm/min and setting a force of 25 kN, whilst for type II, containing the formulation A in the outer cylinder, the speed was 150 mm/min and the maximum force 35 kN.

2.6. Spheronization and drying procedure

The extrudates were spheronized in a radial plate spheronizer (model 230 Caleva Process Solution Ltd., Dorset, UK) at 1000 rpm, for 20 min. Drying of spheroids was carried out in a fluidized bed dryer (Aromatic Fielder AG, Niro Inc., USA) for 45 min at 35 °C.

2.7. Pellet characterization

2.7.1. Size and size distribution of the pellets

A vibrating apparatus (Octagon 200, Endecotts, London, UK) and a set of sieves (2000, 1400, 1000, 710, 500 μm) were used for size distribution determinations.

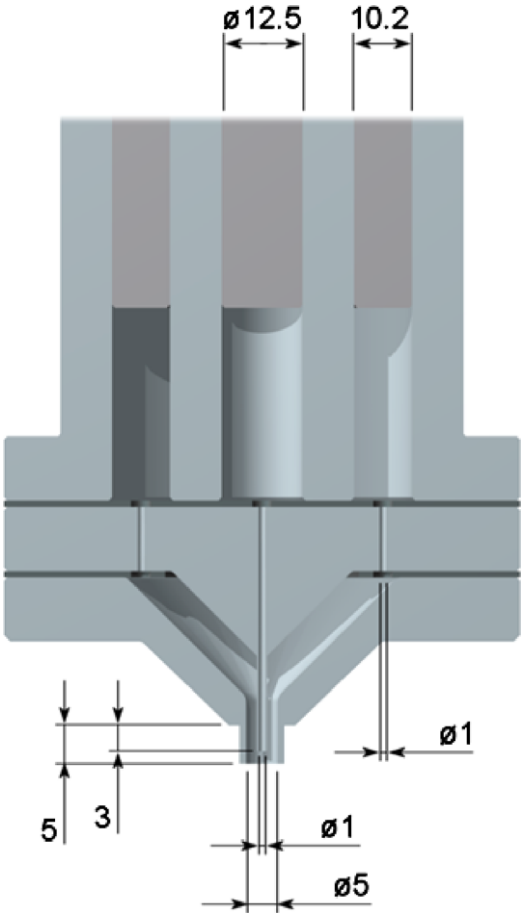

Fig. 3. Schematic drawing of the ram co-extruder (side view) including dimensions (mm).

The subsequent characterizations were carried out on the modal size fraction.

2.7.2. Disintegration time

The mean disintegration time of pellets was studied in deionized water at 37 °C using a disintegration test apparatus (Model ZT 3, Erweka, Eusenstamm, Germany) modified including a 30 μm sieve at the bottom of the disintegration tube. Six pellets taken of each formulation were evaluated and the end point was taken as the time for disintegration of the pellets.

2.7.3. Crushing test

The crushing strength was determined on 10 pellets by compression at a platen speed of 1 mm/min with a CT5 tablet strength tester (Engineering System, Nottingham, UK) fitted with a 5 kg load cell.

2.7.4. True density determinations

The true density has been determined using a helium pycnometer (Multi-pycnometer, Quantachrome corporation, Bayton Beach, USA) in a 35 cm^3 cell calibrated with a steel sphere of known volume. For each formulation, three measurements were recorded and the average and standard deviation value were calculated.

2.7.5. Scanning electron microscopy

Pellet morphology was evaluated by scanning electron microscopy (SEM). Samples were sputter-coated with Au/Pd using a vacuum evaporator (Mod. 306 Edwards, Milano, Italy) and examined using a scanning electron microscope (Leica Stereoscan 430 i, Leica Instruments Ltd, Cambridge, UK) at 10 kV accelerating voltage using the secondary electron technique.

2.7.6. Size determination of the droplets

The size of the droplets released from the pellets was determined in water ($T = 37$ °C) to check possible diameter variations during the *in vitro* dissolution test. Samples of 5 ml were collected from the aqueous environment and filtered through cellulose regenerated syringe filters (Sartorius 0.80 μm, Goettingen, Germany). The size of the submicron dispersed liquid phase was determined using a Laser Light Scattering Technique (Nano ZS – Zetasizer, Malvern, United Kingdom). The experiments were carried out in replicates (SD < 1%).

2.7.7. In vitro dissolution studies

The USP 26 rotating basket apparatus (Model DT-1, Erweka) with a stirring rate of 100 rpm and a temperature of 37 °C was used. The composition of the dissolution medium was 0.2 M KH_2PO_4/0.2 M NaOH (pH 7.4) according to USP 26. Samples of 150 mg of pellets, containing a suitable amount of pellets to observe sink conditions ($C \ll Cs$), were placed in the basket, which was put in place, i.e. deepen in 500 ml of dissolution medium. Two ml samples were withdrawn at 5, 10, 30, 60 min and centrifuged for 10 min in Eppendorf tubes at high speed (13,000 rpm) using a bench top centrifuge (MicroCentaur, Sanyo Ltd, Loughborough, UK). The analysis of vinpocetine in the supernatant was performed by HPLC. The results were averaged from at least triplicate dissolution experiments and the standard deviations were within 4% of mean value. As a reference, a physical mixture of drug, lactose and MCC was tested in identical conditions to understand the effect of processing on the drug availability.

2.8. In vivo absorption experiments

2.8.1. Animal procedures

Intestinal permeation was assessed with re-circulating perfusion using a modified Schurgers and de Blaey method [27], whilst bioavailability was tested after administration with a gavage according to a previously published method, by taking blood samples from the abdominal aorta of the rats [15].

Wistar rats, 250–300 g, were fasted overnight prior to the experiments. Water was available *ad libitum*. The experiment was run on group of four animals for each time level.

After anesthesia with ethyl urethane (1.5 mg/kg body weight), a midline abdominal incision was made and a suit-

able segment of gut, about 105 cm long, from pylorus to outlet coecum-ileum was placed outside the abdomen on a heating platform (37 °C). The rectal temperature of the animal was kept by a second heating platform. After rinsing the segment with warm isotonic solution until the effluent was clear, teflon *cannulae* were tied into the proximal and the distal end of the segment. The entering cannula was then connected with a peristaltic pump (Reglo digital, Ismatec, Glattbrugg, Germany) which ended in a reservoir containing 25 ml of warm saline solution (37 °C). The reservoir was then filled with the aqueous dispersion to be tested containing the drug in the form of pellets or physical mixtures (drug, lactose and MCC, as a reference) diluted in saline solution. Two different media were used: saline drug aqueous dispersion (initial drug concentration = 4.6 μg/ml) and pellet dispersion in saline solution yielding an initial drug concentration equal to 19.8 μg/ml, which was re-circulated with a flow rate of 1 ml/min, forming a close loop between gut, pump and reservoir. The drug concentration was measured at 0, 0.5, 1, 1.5, 2, 2.5 h, by testing the decrease of drug concentration in the reservoir. At the end of the experiment the volume of the formulation present in the reservoir was measured. The length of the intestinal segment of each rat was measured after stretching the segment for 60 s applying a weight of 2 g. Apparent permeability P was determined by fitting to experimental data (decreasing drug concentration in the reservoir $C_r(t)$) a previously developed mathematical model [28]:

$$t < t_1 + t_2 + t_{int}, \frac{dm_r(t)}{dt} = -q_i \frac{m_r(t)}{V_r(t)} + (q_i + q_w) C_{r0} \left(\frac{q_i + q_w}{q_i} \right)^{-\left(\frac{E_p PL}{q_w}+1\right)} \quad (1)$$

$$t > t_1 + t_2 + t_{int}, \frac{dm_r(t)}{dt} = -q_i \frac{m_r(t)}{V_r(t)} + (q_i + q_w) \frac{m_r(t - t_{int} - t_1 - t_2)}{V_r(t - t_{int} - t_1 - t_2)} \left(\frac{q_i + q_w}{q_i} \right)^{-\left(\frac{E_p PL}{q_w}+1\right)} \quad (2)$$

where m_r is the drug amount in the reservoir at time t, V_r is the reservoir volume, q_w is the re-circulating flow rate (1 ml/min), E_p is the internal intestine perimeter (1.08 cm), L is the length of the cannulated intestine portion (46 cm), q_w is the flow rate relative to intestinal water absorption (evaluated as the ratio between difference ($V_{r\ initial} - V_{r\ final}$) and the experimental time (0.013 ml/min)), C_{r0} is the initial reservoir drug concentration (aqueous dispersion = 4.6 μg/ml; pellet dispersion 19.8 μg/ml), while t_{int}, t_1 and t_2 are, respectively, the time needed for dispersion to flow through the cannulated intestine portion and the two (in and out) connecting tubes making possible the re-circulating loop (t_{int} = 3.8 min; t_1 = 0.088 min, t_2 = 0.088 min).

After administration with a gavage of both the physical mixture and the pellets in aqueous dispersion (in a single dose of 40 mg/kg for each animal), the blood samples, 2–3 ml each, were collected at different time intervals (0, 0.25, 0.5, 1, 1.5, 2, 2.5, 4 h) in heparinized tubes from abdominal aorta.

This study was approved by the Italian Ministry of Health (D. LVO 116/92) in accordance with the "Principle of Laboratory Animal Care".

2.8.2. Quantification of vinpocetine

HPLC analyses were carried out with a Perkin–Elmer Series 4-high-performance liquid chromatograph, equipped with a Rheodyne Model 7125 injector with a 100 μl loop and connected to a Perkin–Elmer Model LC 75 variable-wavelength UV detector. A Lichrosorb 10-RP-18 (Perkin–Elmer, Norwalk, USA) 250 mm × 4.6 mm ID column packed with 10 μm particle size was used.

2.8.3. Sample preparation

One ml of nitril-acetate was added to 0.5 ml of plasma samples. After mixing for 10 s in a vortex mixer and centrifugation for 10 min at 10,000 rpm, a 100 μl aliquot of supernatant was injected into the column.

2.8.4. HPLC analysis of vinpocetine

A reverse phase HPLC C18 column was used. A mixture of nitril-acetate and water (90:10 v/v%) was employed as a mobile phase at a flow rate of 1.2 ml/min and the detection wavelength was 230 nm. The retention time of vinpocetine was 8.5 min. The vinpocetine in the plasma samples was quantified by HPLC using a validated method: the limit of quantification was approximately 1 μg/ml; the coefficient of variation calculated for the six identical samples analyzed did not exceed 5%. The precision of the assay was calculated by determining the relative standard deviations of peak height ratios obtained from six replicate assays within a concentration interval of 1–100 μg/ml. The relative standard deviation ranged from 1% to 5% for intra-day analysis and from 2% to 6% for inter-day analysis. The absolute recovery of vinpocetine in plasma was determined by comparing the slopes of the processed human plasma standard curves and the standard curves prepared in methanol. The recovery of vinpocetine was 88.4 ± 4%.

3. Results and discussion

3.1. Preparation of rod extrudates

Rod extrudates based on the 13 formulations A and on 10 formulations B, whose compositions are listed in Table 2, were produced by feeding only the inner barrel of the co-extruder. As above-mentioned previously, the screening between the formulations was based on the analysis of the extrusion profiles characteristics as indicators to assess the suitability of a particular formulation for extrusion and to anticipate the quality of the final spheronized product.

In fact, an extrudate must have a smooth surface finish because surface irregularities cause breakage into irregular lengths on subsequent processing and can give rise to problems on conversion to spherical pellets. In spheronization,

these defects result in less round pellets and a wide size distribution. The main problem is known as shark-skinning which consists in a characteristic ridge-like spiral structure, running transversally to the flow direction. The dominant factor ruling surface characteristics of pharmaceutical extrudates is formulation variables: wet masses containing components of large particle size, for example, are more prone to shark-skinning. Defects can be hence eliminated by using other excipients or varying their concentration [17]. Another key factor affecting the extrusion process is the moisture content. The right amount of water to be added needs to be optimized: it can range between a lower and an upper limit and still produce pellets of acceptable quality [29,30]. If the moisture content is less than the lower limit, the mixtures do not flow satisfactorily inside the barrel and often the extrusion procedure needs to be stopped before completion when reaching the maximum acceptable force. Further, during the spheronization, a lot of dust will be generated resulting in a large yield of fines. Conversely, an increase of water content facilitates the process of extrusion by reducing particle–particle interactions [31], thus reducing the viscosity of the mass. Hence, the wetter mass becomes softer and less force is needed for extrusion [32]. However, there is also an upper limit for the moisture content: in fact, overwetted mixtures often extrude well but form large agglomerates on spheronization [16].

Not all the 13 formulations A were suitable for extrusion, and amongst the extrudable ones different surface finishes of the cylinders and different extrusion profiles were obtained. In Table 2 the performances during extrusion of the tested formulations are summarized. Amongst the 13 processed formulations A, it was therefore possible to choose formulation no. 5, a binary mix in equal parts of MCC (26%) and lactose (26%), wetted by 13% of water and 35% of oil–surfactant mixture. This formulation was chosen in the light of its smooth surface after extrusion (Fig. 4a) and its promising extrusion profile, in terms of magnitude of force (about 12 kN) and duration at the steady state (SS) (about 120 mm) (Fig. 5). As for the other formulations, as reported in Table 2, formulations 1, 3, 4, 8, 9, 11, 13 reached the force safety limit of the apparatus and hence extrusion process was not possible, while formulation 2 was characterized by a very intense irregular flow (IF), consisting in an intense waving of the profile during extrusion, and finally formulations 6, 7, 10, 12 showed a steady state profile but required a very low extrusion pressure, smaller than the above-mentioned 10 kN required for a good quality extrudate (see "Section 2.5").

The next step consisted of optimizing inert formulation B, which was processed with a ram speed of 150 mm/min. The reason why different ram speeds were chosen during the preliminary trials for processing formulation type A and B can be explained by their remarkably different water content. In both cases to achieve a homogeneous water distribution through the barrel, as suggested by several authors [32,33], formulation B, containing more water than formulation A, was processed with a faster speed. In fact,

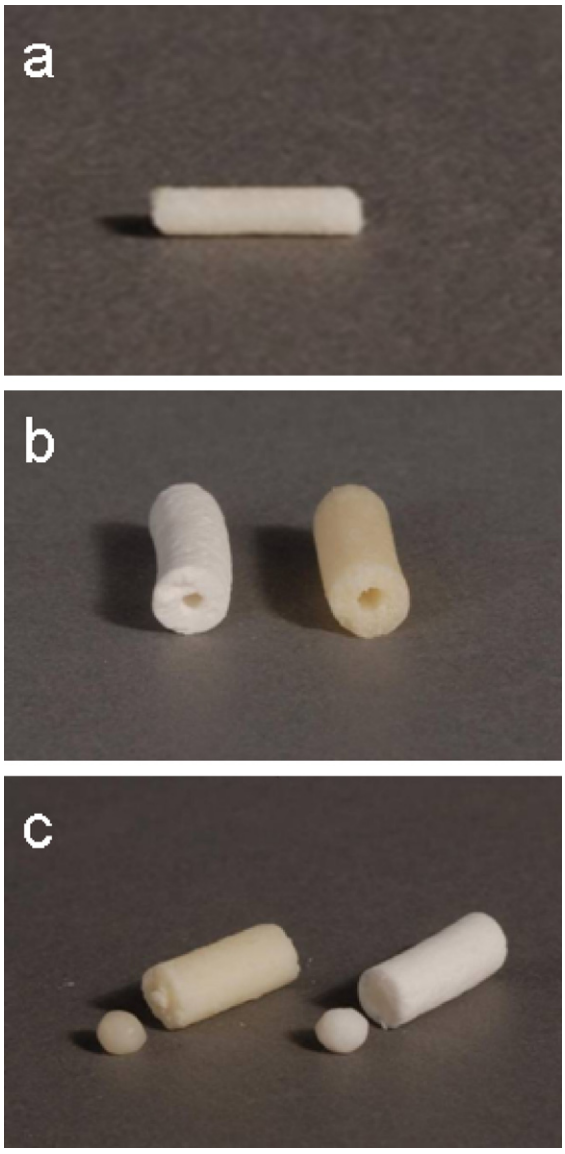

Fig. 4. Images of rod extrudates (a), tubular extrudates based on formulations A (right) and B (left) (b), co-extrudates type I (right) and II (left) (c).

lower the extrusion speed, higher the water movement in the barrel [32], which should be avoided. Amongst the 10 possible different formulations B (Table 2), following the same criteria adopted in the screening of Formulation A, formulation no. 1 and formulation no. 8 were selected. These formulations, very similar in composition (see Table 2), had similar extrusion performances. As for formulations 2–7 and 9–10, these mixtures were extruded under increasing forces for the same displacement of the ram, generating typical "forced flow" profiles (FF) (see Table 2). Hence it would be expected that, as previously reported [34], difficulties in spheronizing the extrudate would occur, resulting in irregular pellets with a wide size distribution. Conversely, both formulations 1 and 8 showed promising extrusion profiles characterized by a satisfactory long period of steady state (SS) with a small irregular flow (IR) (recognizable by the waving around the steady state pres-

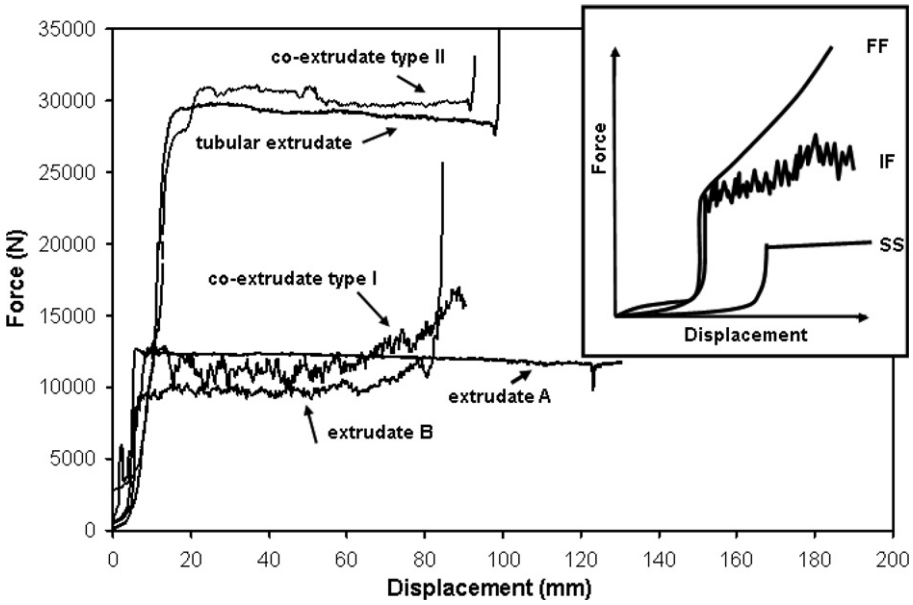

Fig. 5. Extrusion profiles of the selected rod extrudates, tubular extrudates and co-extrudates; in the frame: examples of typical extrusion profiles in the case of steady state (SS), forced flow (FF) and irregular flow (IF).

sure on the profile). Between them, formulation 8 was chosen in the light of the satisfactory appearance of the extrudate and of the better profile, with only a very slight waving around the long plateau for a mean steady state force of about 10 kN (Fig. 5).

As a consequence, formulation 5, type A and formulation 8, type B were selected and used for subsequent productions of tubular extrudates and co-extrudates.

3.2. Preparation of tubular extrudates

The next step consisted in the preparation of tubular extrudates to check the extrudability of both optimized formulations A and B when feeding only the external chamber of the extruder. Both formulations were successfully extruded, providing extrudates with a smooth finish (Fig. 4b). Interestingly, optimized formulations A and B gave almost superimposable extrusion curves, and the magnitude of the force was greater than that observed for rod extrudates (approximately 30 kN). The extrusion of the material in the external chamber was more complex than in the internal chamber, as previously noticed by Pinto et al. [18]. This fact was probably due to the higher amount of mass fitted in the external cylinder and the complex pathway for the mass to move before reaching the exit of the die. It follows that a higher force form is required for the extrusion because in the tubular extrudate shear between the extruding materials and the barrel is double (internal and external surfaces of the tube), whereas in the rod extrudate shear only took place at the external surface of the material [18]. It is assumed that the shear within the masses was equivalent.

For the sake of brevity, due to the superimposable profiles of formulations A and B, only one extrusion profile is reported in Fig. 5.

3.3. Preparation of co-extrudates

Two batches of co-extrudates were prepared: co-extrudates type I having formulation A in the inner part and formulation B in the outer, co-extrudates type II having formulation B in the inner core and formulation A externally. As mentioned, type I co-extrudates were made using a piston speed of 100 mm/min for a maximum force (safety reasons) of 25 kN, whilst for type II the speed was 150 mm/min and the force 35 kN. These velocities were chosen in consideration of several preliminary trials since they allowed for a successful co-extrusion process, that is, the production of a bi-layered coherent compact.

The extrusion profile, in this case, describes a co-extrusion process: thus, after the initial phase of compression, two steady state occurred simultaneously: one in the inner and the other in the outer cylinder. In fact, the two concentric rams had to simultaneously compress two different masses.

Due to the design of the apparatus and to the larger amount of mass fitted in the outer barrel, the extrusion undergoing in the toroidal external barrel represents the step controlling the pressure of the entire co-extrusion process. In other words, the sector governing the extrusion profile and the required force is the component of the barrel that, when loaded with material, creates more resistance to the movement of the ram during the extrusion process.

As a confirmation of this, when a highly wetted formulation is contained in the outer cylinder, as in the case of co-extrudates type I, the co-extrusion profile is typical of "irregular flow" (Fig. 5) (for a better comprehension, see also explanatory frame depicted in the same figure). This behavior is probably due to the presence of large amount of water in the mass, when it is therefore reasonable to accept that water movement occurs during the extrusion process causing a non-homogeneous mass flow.

On the other hand, when the toroidal external barrel is fed with formulation A (co-extrudates II) the process provided an ideal steady state profile, characterized by a long period of constant force (Fig. 5). The lipidic components, prevalent in the formulation A, acted as lubricant and allowed the ram to flow easily in the outer cylinder. Further a lubricant role can be played also by surfactants with high HLB such as Polysorbate 80, as previously reported [35]. The mass flow toward the barrel is then favored, the shear at the die wall is reduced and consequently less resistance is opposed to the movement of the piston during the extrusion process.

The subsequent step involved the spheronization of the co-extruded cylinders. Both type I and II were successfully spheronized, giving pellets with suitable morphological and technological characteristics (see Fig. 4c, Tables 3 and 4).

The SEM micrographs revealed that in both cases round pellets were obtained, though having different surface characteristics. Pellets type I (Fig. 6a and b) were characterized by a smooth finish, whilst a rough (orange peel) surface was observed in pellets type II (Fig. 6c and d), probably due to the presence of lactose and MCC suspended on the underlying smooth structure of the lipidic mixture.

Equatorial cuts of the spheroids have shown that the inner layer was round and centered in the pellet and completely covered by the outer layer (Fig. 6g).

From Tables 3 and 4 it appears that the modal size fraction of both types of pellets was the same (2000–1400 μm), whilst the two kinds of pellets differed remarkably in terms of disintegration time and crushing strength. This diversity was probably due to the presence in the outer layer of pellets type I of lactose and MCC which can form, in mechanical stress conditions, a very hard cover around the pellets that improves their resistance. Further, several authors have reported that the higher the water level of the formulation, the longer the disintegration time [36] or the higher the hardness of the pellets [37].

Table 3
Percentage of pellets in each size (μm) range

Size range (μm)	Percentage of pellets		
	Type I	Type I + 2% wt CS	Type II
>2000	31.99	0	6.94
2000–1400	66.33	13.81	69.98
1400–1000	1.59	69.43	20.73
1000–715	0.08	14.19	2.38
715–500	0	2.57	0

Table 4
Properties of the modal size fraction pellets

	Type I[a]	Type I + 2% wt CS[b]	Type II[a]
Density (g/cm^3) ($n = 3$)	1.34	1.48	1.28
Crushing force (kg) ($n = 10$)	0.719	0.220	0.043
Disintegration time (min) ($n = 6$)	37	14	5

[a] 2000–1400 μm size fraction.
[b] 1400–1000 μm size fraction.

Therefore, to shorten disintegration time of the pellets type I, the introduction of a little quantity of disintegrant agent was considered, in particular adding 2% w/w of croscarmellose sodium (CS) in the outer formulation mixture B. It is well known that croscarmellose possesses wicking and swelling abilities and hence favors the water ingress inside the pellets [38]. In fact this strategy resulted in a dramatic reduction of disintegration time from 37 to 14 min. As for the other technological characteristics, when the 2% wt of CS had been added, the pellets modal size fraction was the 1400–1000 μm size although the crushing strength slightly decreased. Details of the percentage of pellets in each size range are listed in Table 4, while the technological properties of modal size fraction of these pellets (1400–1000 μm) are reported in Table 4. As regards to morphological and surface properties of these pellets (Fig. 6e and f), the addition of the superdisintegrant did not reduce roundness but changed remarkably the surface of the pellets resulting, as it appears from the SEM images, in an increase of its porosity.

After the technological characterization, the *in vitro* dissolution studies were performed to compare the dissolution profiles of the modal size fraction of both pellets type II and type I plus 2% wt CS with the dissolution profile of a physical mixture of lactose, MCC and drug.

As evident form Fig. 7, pellets type I plus 2% of CS released 90% of vinpocetine in 30 min and pellets type II in 20 min. Conversely, the release from the physical mixture was slow: only 25% of drug was released after 60 min. Since the beginning of the test both formulations showed very different dissolution kinetics, mainly related to their different composition in outer layer. Even though the superdisintegrant agent added in the outer layer of pellets I had favored their disintegration and hence their dissolution, the presence of the drug in the self-emulsifying system in the outer layer is prevalent and determines the highest dissolution enhancement. To go into more details, observing the dissolution profile of pellets type I plus 2% wt CS a slope change is clear: initially, the drug is released slowly from the hard external layer, then after its disintegration the inner self-emulsifying core is in contact with the dissolution medium and the release rate is very fast.

At the end of technological and *in vitro* dissolution analyses, pellets type II were preferred to pellets type I, these pellets were shown to enhance *in vivo* bioavailability in rats.

Firstly, the intestinal permeation with re-circulating perfusion was assayed and compared to that of a saline drug solution. Fig. 8 shows the satisfactory agreement between the mathematical model (Eqs. (1), (2)) (solid line) and experimental data (symbols) representing the drug concentration variation in the reservoir with time. The amount of drug absorbed by intestinal mucosa revealed that the presence of pellets II system did not increase vinpocetine permeation through the intestinal mucosa. While in the case of aqueous vinpocetine dispersion, apparent permeability results to be equal to $(1.10 \pm 0.1) \times 10^{-4}$ cm/s, in the case of pellets II, vinpocetine permeability turns out to be $(1.6 \pm 0.4) \times 10^{-4}$ cm/s.

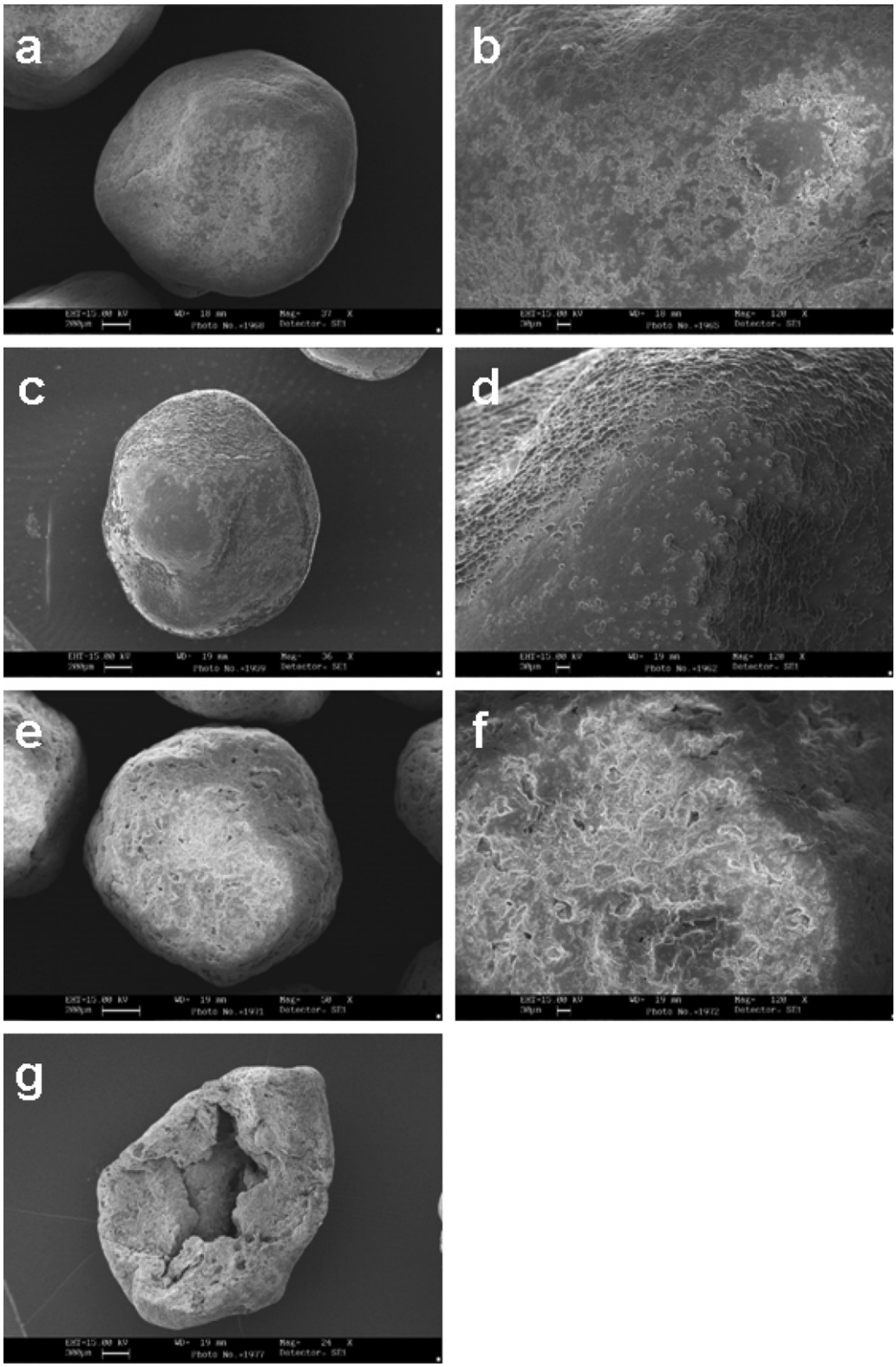

Fig. 6. SEM micrograph of pellets type I (a and b), pellets type II (c and d), pellets type I + 2% CS (e and f), equatorial cut of a pellet (g).

In addition, preliminary studies upon the bioavailability after oral administration by gavage of pellets type II and a physical mixture (composed of lactose, MCC and drug in the same weight proportions as the pellets type II) were conducted. The plasma curves depicted in Fig. 9 and the pharmacokinetic parameters listed in Table 5 demonstrated that pellets type II resulted in plasma levels 2.4-fold higher than the physical mixture. The increased absorption from the pellets II system could be related to several factors, such as the efficient dispersion of the formulation due to the small size

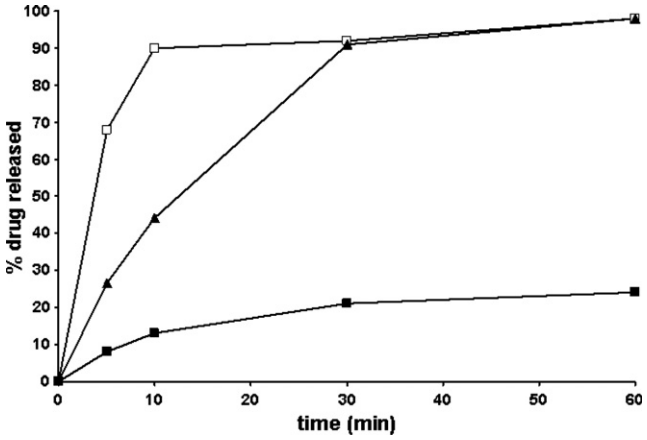

Fig. 7. *In vitro* dissolution profiles of pellets type II (□), type I + 2% of CS (▲) and physical mixture (■).

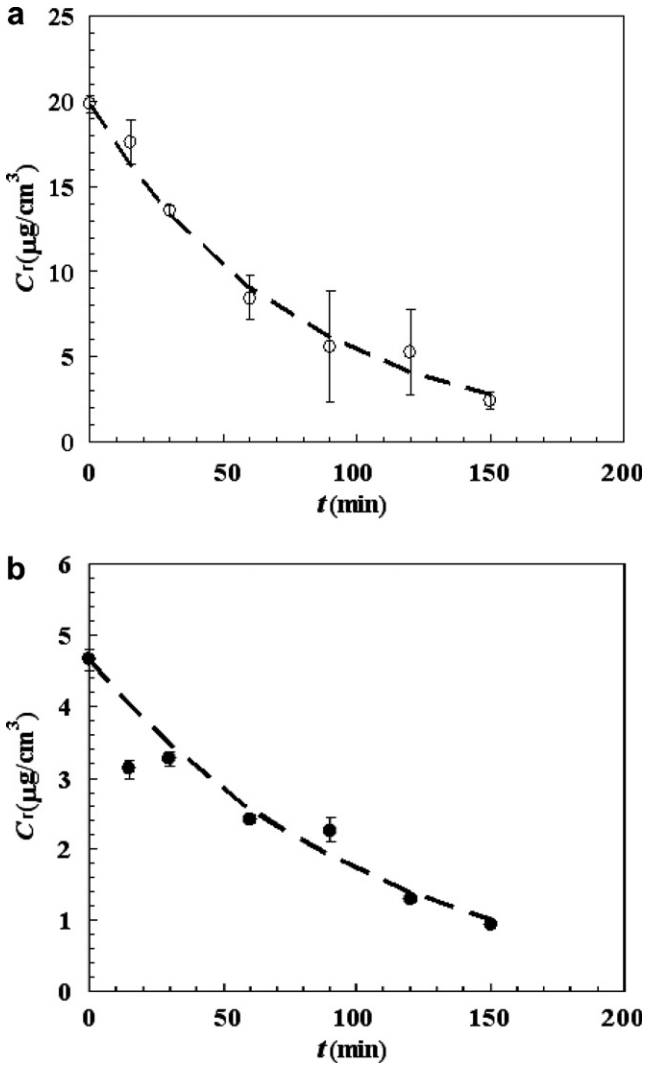

Fig. 8. Comparison of permeation rate of pellets type II (a) and physical mixture (b).

of the released droplets, to the well known favorable impact of the surfactants and of the oil–surfactant mixture [3–6]. In particular, in our case the diameter of the droplets released from the pellets during *in vitro* dissolution tests was determined to be 133 nm at the initial times and subsequently remained almost stable over the whole time of analysis, determined to be 147 nm after 1 h and 138.5 nm after for 2 h.

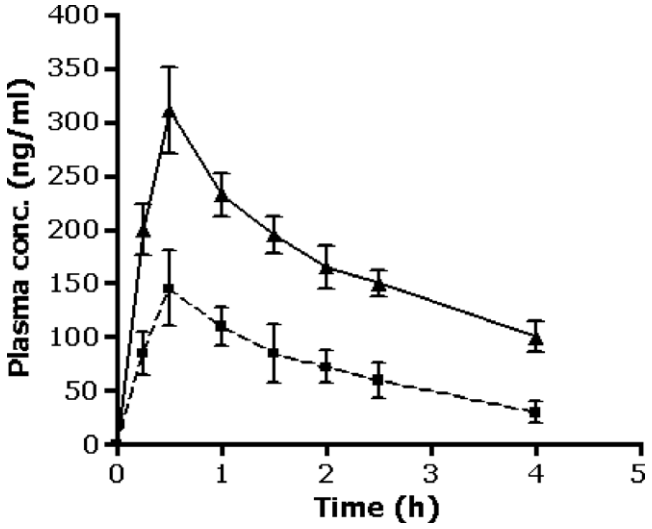

Fig. 9. Comparison of plasma concentration profiles of pellets type II (triangles) and physical mixture (squares).

Table 5
Comparison of bioavailability parameters of pellets type II and a physical mixture (mean ± SD, $n = 4$)

	Pellets type II	Physical mixture
AUC (ng h/ml)	687.0 ± 71.8	291.6 ± 71.7
t_{max} (h)	0.5	0.5
C_{max} (ng/ml)	311.0 ± 40	145.0 ± 35

4. Conclusions

These preliminary investigations proved that the development by co-extrusion/spheronization of a solid dosage form containing a self-emulsifying system is a promising approach for the formulation of drug compounds with poor aqueous solubility, such as vinpocetine. The bi-layered pellets composed by two cohesive layers (a self-emulsifying system containing the drug and an inert matrix of microcrystalline cellulose and lactose) were successfully prepared. The characterization of the pellets through sieve analysis, SEM, density, hardness, *in vitro* dissolution and disintegration and size of the droplet released from the pellets demonstrated the good quality of the pellets. As for the relative position of the two layers inside the pellets, it appears that the greatest enhancement of the biopharmaceutical properties has been obtained when the self-emulsifying system was located on the external layer of the pellets. From these preliminary data, the oral delivery of vinpocetine through these pellets has been shown to enhance its *in vivo* bioavailability in animals. The ongoing studies are aimed on the one hand on testing of the scalability of this preparation method, and on the other hand on the evaluation through *in vivo* studies on animals of

the contribution of the lymphatic transport on the enhanced absorption from such kind of systems. Future studies will be addressed toward the exploration of the various potentials of co-extruded spheroids as carrier systems, such as for example for the simultaneous administration of two drugs in different layers, for the modulation of the release using polymeric materials and for the loading in one or both layers of different types of emulsions, e.g. o/w emulsion or multiple emulsion w/o/w, paying constant attention to the optimization of the formulation and production process.

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