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Research paper

Melt extruded helical waxy matrices as a new sustained drug delivery system

Dritan Hasa [a], Beatrice Perissutti [a], Mario Grassi [b], Marina Zacchigna [a], Milva Pagotto [c], Davide Lenaz [d], Peter Kleinebudde [e], Dario Voinovich [a,*]

[a] Department of Chemical and Pharmaceutical Sciences, University of Trieste, Trieste, Italy
[b] Department of Industrial Engineering and Information Technology, University of Trieste, Trieste, Italy
[c] Centro Clinico Studi Fegato, AOUTS, Trieste, Italy
[d] Department of Geosciences, University of Trieste, Trieste, Italy
[e] Institute of Pharmaceutics and Biopharmaceutics, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany

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ABSTRACT

The aim of this research was to prepare helical and cylindrical extrudates by melt extrusion and to evaluate their potential as sustained release dosage form. The systems contained theophylline as water-soluble model drug and microcrystalline wax as thermoplastic binder. The temperature suitable to ensure a successful extrusion process of formulations containing the wax in three different percentages was found to be below the melting point of the excipient. After the production of the extrudates in three different helical shapes (having 2, 3 and 4 blades) and a classical cylindrical shape, the systems were studied by means of X-ray powder diffraction and differential scanning calorimetry to check possible variations of the solid state of the drug during the thermal process. The morphology and chemical composition of the surface of the extrudates were examined by Scanning Electron Microscopy/Energy Dispersive X-ray Microanalysis to evaluate the presence of the drug on the surface of the extrudates and to monitor changes on the aspect of the waxy matrix during dissolution. Then, the different systems were analysed from the *in vitro* dissolution point of view to study the influence of the shape and of the composition on the drug release. An *in vivo* pilot study on the best performing system (helix with 3 blades) was carried out on five healthy volunteers and monitoring the intestinal transit by X-ray images. The resulting plasma profiles were analysed by means of a suitable pharmacokinetic analysis. Finally, an *ad hoc* mathematical model was developed to perform an accurate description of the *in vitro* release and *in vivo* performance of the 3-blades helical system.

1. Introduction

In the latest years, the request for formulations having modified release of active pharmaceutical ingredient (API) increased significantly, due to the possibility of reducing frequency of dosing. In this way, side effects can be reduced, increasing safety of patients. Melt extrusion is a recent pharmaceutical processing technique that has demonstrated to be applicable to immediate release [1] and sustained release dosage forms [2,3]. During the process, the mixture of binder, excipients and APIs are fed into the heated barrel and extruded through the die attached at the end of the barrel. The molten thermoplastic polymer, functioning as binder during the process, rapidly solidifies when the extrudate exits the machine through the die. The geometrical design of the die will control the physical shape of the final product, giving, for example, cylinders, pipes or films. Melt extrusion offers different advantages over traditional processing techniques such as the absence of water and solvents, shorter and more efficient processing times, high reproducibility in selected conditions, and safety and compliance of the patient [4]. Thus, the potential exists of producing a solid dosage form directly by extrusion.

Non-bioerodible excipients are commonly used for sustained release dosage forms. Waxes or synthetic triglycerides (e.g. carnauba wax [5] or monoacid triglycerides [6,7] or microcrystalline wax [8]) are common inert matrix forming excipients, which use may have particular advantages due to their relatively low melting point and to their chemical inertness against other materials. However, due to their marked lipophilic properties, the diffusion process of API from these inert matrix excipients can be very slow. As a consequence, the amount of API released in such systems often is not satisfactory [6]. To overcome these drawbacks, an approach to increase the drug release, both in terms of rate and amount of solubilised API, is the addition of hydrophilic excipients. These substances, mainly PEGs and their derivatives, are expected to dissolve and hence to favour the API release by forming inside

* Corresponding author. Department of Chemical and Pharmaceutical Sciences, University of Trieste, Piazzale Europa 1, 34127 Trieste, Italy. Tel.: +39 040 5583106; fax: +39 040 52572.
E-mail address: vojnovic@units.it (D. Voinovich).

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the lipophilic inert matrix a porous network (hollow channels) where the diffusion is more rapid [7].

Another approach to speed up the diffusion process is to modify the shape of the extrudates. In fact, the low drug diffusion from the inert lipophilic matrix can be due to the reduced surface area in contact with the dissolution medium of the cylindrical form of most common extrudates. Hence, some studies are aimed to improve dissolution performances by changing the length of the cylindrical extrudates or by using hollow cylinders [8,9]. To obtain a higher specific surface area and a faster dissolution, it is also possible to decrease the diameter of the extrudate or to use a certain sieve fraction of milled extrudates [2].

In this context, the goal of the present study is a further in depth examination upon the influence of the shape on the release from melt extrudates. In particular, in this research, helical extrudates (with 2, 3 or 4 blades) and conventional cylindrical extrudates were produced by means of a hot-stage process, by a suitable design of the die. It is hence expected that increasing the surface area in contact to the medium, the release is speeded out. The choice of the helical extrudates was due to the technological reasons (following the natural tendency of the masses to bend). This shape has also a more convenient mass-to-encumbrance ratio to be fit in a hard gelatine capsules.

After the production of the extrudates in three different helical shapes and in a classical cylindrical one, the physico-chemical characterisations will be carried out, followed by the *in vitro* release and *in vivo* studies. Finally, an *ad hoc* mathematical model will be developed to perform an accurate description of the *in vitro* release and *in vivo* performance of the best release system (helical 3 blades).

2. Materials and methods

2.1. Materials

Anhydrous theophylline (theo) E.P. grade was purchased by Polichimica s.r.l. (Bologna, Italy), and microcrystalline wax (or paraffinic wax, complex blends of mineral hydrocarbon waxes marketed as Paracera P (melting range 58–62 °C) (wax) was kindly donated from Paramelt BV (Heerhugowaard, Netherlands). The mean diameter (±SD) was 56 (±18) and 204 (±95) μm for theophylline and microcrystalline wax, respectively. Solvents of HPLC grade were purchased by Carlo Erba (Milan, Italy).

2.2. Methods

2.2.1. Preparation of helical and cylindrical extrudates

Prior to extrusion, the powdered materials (binary mixtures of theo and wax) were mixed in 2.81 one step mixer (Rotolab, Zanchetta®, Lucca, Italy) for 10 min at 120 rpm. After a screening between several formulations, different compositions were selected on the basis of their feasibility. The formulations considered for each type of extrudates (helical and cylindrical form) are reported in Table 1.

Table 1
Formulations (expressed as weight percentage) considered for the production of the extrudates.

Formulations	Theophylline (%)	Microcrystalline wax (%)	$BaSO_4$ (%)
A	50	50	
B	60	40	
C	70	30	
Radio-opaque	35	30	35

The extrudates were prepared using a lab scale ram extruder (Thalassia®, Trieste, Italy), described into details in a previous work [3]. The cylindrical barrel, acting as the materials reservoir, is made of nickel-plated brass, with a capacity of 66 cm^3 and an internal diameter of 2.5 cm. It can be thermo-stated with an electrically heated jacket (maximum temperature 120 °C ± 2 °C). The movement of the stainless steel ram is promoted by a hydraulic cylinder which is alimented by an electric pump. An interchangeable die made of nickel-plated brass can be attached at the end of the barrel, this die having a cylindrical shape (with a conical entry (120°), an internal diameter of 1 mm and a length of 0.837 cm) or a helical shape (with a flat entry, a length of 0.837 cm and 2, 3 or 4 blades, as depicted in Fig. 1A, B, C, respectively). On the basis of the preliminary trials, the temperature of 50 °C was selected which is below the melting point of the excipient. Batches of 50 g of mixture were packed to a constant volume, by applying hand pressure, into the barrel. After an equilibration time of 60 min, also selected during preliminary trials, the mass was extruded through the suitable die (see Fig. 1), using a ram velocity of 20 mm/min and an extrusion pressure ranging from 100 to 140 bars. Once the samples have been cooled at ambient temperature, the extrudates were sliced up by use of a hot cutter in extrudates of length suitable to have a drug content for dissolution studies in sink conditions (see Section 2.2.5) and for subsequent *in vivo* studies (see Section 2.2.6.).

2.2.2. Assay of drug content

The analysis of theo content was carried out by dissolving a weighed amount of extrudate (ranging about 50 mg) in 250 ml of freshly distilled water. The amount of drug was then assayed spectrophotometrically (UV2 Spectrometer, Unicam, Madison, WI) at 271 nm. The fat material did not interfere with the UV analysis. Each type of extrudate was analysed in triplicate.

2.2.3. Solid state analyses

Differential scanning calorimetry (DSC) was recorded with a differential scanning calorimeter (Mod. STARe Software version 9.20, equipped with a measuring cell DSC 1 Mettler-Toledo, Giessen, Germany). Slices of extrudates, containing approximately 5 mg of theophylline, were weighed in pierced aluminium pans (nominal capacity of 40 μl). The heating range was from 0 °C to 300 °C at 10 °C min^{-1} under nitrogen purge gas (gas flow 70 ml min^{-1}). The same procedure was followed for the pure drug and the

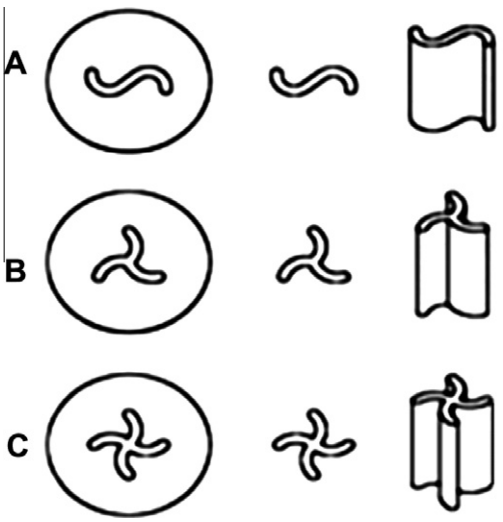

Fig. 1. Schematic representation of the three types of dies, leading to a two-blades (A) three-blades (B) four-blades extrudate (C) (middle: upper view, right: longitudinal view).

physical mixtures of the components. For the pure microcrystalline wax, the heating range was 0–150 °C.

Samples of extrudates (gently ground in an agate mortar), physical mixtures and raw materials were then studied by means of X-ray powder diffraction technique (XRD) using a D500 (Siemens, Munich, Germany) diffractometer with CuK radiation (λ = 1.5418 Å), monochromatised by a secondary flat graphite crystal. The current used was 20 mA and the voltage 40 kV. The scanning angle ranged from 5 to 35° of 2θ. The step size was 0.05° of 2θ, and the counting time was of 1 s per step.

2.2.4. Scanning Electron Microscopy/Energy Dispersive X-ray Microanalysis

For SEM analysis, samples were sputter-coated with Au/Pd using a vacuum evaporator (Edwards) and analysed using a scanning electron microscope (Philips XL-30, Eindhoven, NH) at 25 kV accelerating voltage.

During SEM analysis, the constituent elements of the extrudates were investigated by EDS microanalysis by a X-EDS detector (Oxford INCA350, Oxon, UK).

2.2.5. In vitro dissolution studies

In *vitro* dissolution tests (conducted in triplicate) were performed using the USP rotating basket apparatus (Pharmatest, Steinheim, Germany), with a stirring rate of 100 rpm. Since theo dissolution is independent from the pH of the medium [10], 900 ml of purified water containing 0.1% polysorbate 20 was used as dissolution medium at a temperature of 37 ± 0.5 °C. In order to guarantee sink conditions and the same drug amount (25 mg) to be released in each experiment, extrudates length was different depending on shape and composition. In particular, in the case of cylinders, the target dose (25 mg) was achieved by considering, respectively, 14, 11 and 9 extrudates (length 4 mm) when theo composition was 50%, 60% and 70%. In the case of helical extrudates, regardless of composition and shape (2, 3 and 4 blades), only one extrudate was considered and its length varied between 2.5 and 7.5 mm depending on composition and shape.

The solution was filtered and continuously pumped (12.5 ml/min) to a flow cell in a spectrophotometer (UV2 Spectrometer, Unicam, Madison, WI). The amount of drug dissolved was analysed at the maximum absorbance wavelength of the drug (271 nm). Each dissolution test was the average of three-replicated experiments, and standard deviations were within 5% of mean value.

2.2.6. In vivo studies

For this experiment, five healthy volunteers, aged between 25 and 50 years and weighing on average 70 kg, were chosen. Before the analysis, a written informed consent was signed by each subject. The research followed the tenets of the Declaration of Helsinki promulgated in 1964 and was approved by the institutional human experimentation committee. All the volunteers had normal hepatic and renal function. All subjects were asked not to take any drug before and to fast from 12 h before drug administration until lunch on the treatment day. They were also not allowed to smoke, not to take coffee or alcoholic beverages 12 h before and 24 h after the drug administration. The subjects were all given a standard lunch 6 h after the dosing and were allowed to drink water during the treatment period.

The dose (400 mg) of theophylline (corresponding to 13 pieces of 4 mm long 3-blades extrudates) was administered in 4 hard gelatine capsule type "0". Blood samples (5 ml) were drawn at 1, 2, 4, 8, 12 and 24 h following capsule administration. Each sample was collected in a heparinised tube, and plasma was immediately separated by centrifugation (at 1845 g for 15 min), subsequently frozen and stored at −20 °C until assayed.

2.2.7. Analytical procedure

The concentrations of theophylline in the plasma samples were measured using a previously described HPLC method [11].

2.2.8. Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using WinNonlin Version 2.1 (Pharsight Corporation, Mountain View, CA) software. The program calculates the area under the plasma concentration–time curve from time zero to the last sampling time ($AUC_{0\to24}$) using a linear trapezoidal method. Terminal half-life ($t_{1/2}$) was calculated with the formula $t_{1/2} = \ln 2/\lambda_z$, where λ_z represent the terminal slope of the semi-log plot calculated by linear regression. Time and value of maximum concentration (t_{max} and C_{max}, respectively) and the time to obtain 75% of C_{max} ($t_{75\%Cmax}$) were reported as observed.

2.2.9. In vitro/in vivo mathematical modelling

As the proposed mathematical model is aimed to yield the drug plasma concentration profile following extrudates oral administration, it must account for four fundamental phenomena: drug release from matrices, drug absorption by the gastro-intestinal (GI) mucosa, drug metabolism in the blood stream and drug spreading inside tissues. Assuming that (1) drug pharmacokinetics can be represented by a two compartments model with first order elimination in the blood that (2) drug release kinetics in the GI fluids is not affected by the absorption, metabolism and spreading phenomena, and it can be replaced by the *in vitro* release kinetics and that (3) the drug concentration in the blood compartment (C_b) can be always retained negligible in comparison with the drug concentration (C_r) in the release environment (GI fluids), model equations read:

$$\frac{dC_r}{dt} \approx R(t) - k_{ab}\frac{V_b}{V_r}C_r \quad k_{ab} = \frac{AP}{V_b} \tag{1}$$

$$\frac{dC_b}{dt} = k_{ab}C_r - (k_{el} + k_{12})C_b + k_{21}C_T \tag{2}$$

$$\frac{dC_T}{dt} = k_{12}fC_b - k_{21}fC_T \quad f = \frac{V_b}{V_T} \tag{3}$$

$$\frac{dm_{el}}{dt} = V_b k_{el} C_b \tag{4}$$

where t is time, $R(t)$ represents the drug release kinetics coincident with that measured *in vitro*, V_b and V_r are, respectively, the volume of the blood (distribution volume) and GI tract compartments, k_{el} and k_{ab} are, respectively, the elimination and the absorption constants, A is the GI tract geometrical surface (i.e. that not accounting for the presence of villi and microvilli that is considerable higher), P is GI apparent permeability, k_{12} and k_{21} are, respectively, the forward and reverse kinetics constants ruling drug exchange between blood and tissues, f is the ratio between blood (V_b) and tissues (V_T) volumes, C_T indicates drug concentration inside tissues, while m_{el} is the drug amount metabolised.

Eqs. (1)–(4) differ from the usual expression of pharmacokinetic equations [12] for the assumption that drug distribution among blood and tissues is ruled by drug concentration instead of by drug mass. This leads to the introduction of parameter f that renders the model more physiologically oriented as it allows differentiating the extension of blood and tissue volumes. It is worth noticing that when f = 1, this approach and the traditional one [12] coincide. In addition, when k_{12} is set to zero, Eqs. (1)–(4) represent the simpler one compartment pharmacokinetics model. Although many other choices can be considered [13], $R(t)$ is determined assuming that *in vitro* release kinetics can be properly fitted by a sum of m exponential functions:

$$\frac{M_t}{M_\infty} = 1 - \sum_{i=1}^{i=m} A_i e^{-k_i t} \quad \sum_{i=1}^{i=m} A_i = 1 \tag{5}$$

$$R(t) = \frac{M_0}{V_r}\frac{d}{dt}\left(1 - \sum_{i=1}^{i=m} A_i e^{-k_i t}\right) = C_{r0}\sum_{i=1}^{i=m} A_i k_i e^{-k_i t} \tag{6}$$

where m, A_i and k_i are parameters to be determined by Eq. (5) fitting to *in vitro* data, and M_0 is drug dose. The reasons for choosing Eq. (5) relied on the high mathematical power of this equation for what concerns *in vitro* data fitting and on the fact that it allows getting an analytical solution of the Eqs. (1)–(4) system. Inserting Eq. (6) into Eq. (1) and assuming that $C_r = C_b = C_T = 0$ for $t = 0$, model solutions reads:

$$C_b = C_{r0}k_{ab}\left(G_1 e^{z_1 t} + G_2 e^{z_2 t} + G_3 e^{-\alpha t} + \sum_{i=1}^{i=m} \frac{A_i k_i}{\alpha - k_i}\left(\frac{k_{21}f - k_i}{(k_i + z_1)(k_i + z_2)}\right)e^{-k_i t}\right) \tag{7}$$

$$G_1 = \sum_{i=1}^{i=m} \frac{A_i k_i}{(\alpha - k_i)(z_1 - z_2)}\left(\frac{\alpha - k_{21}f}{(\alpha + z_1)} + \frac{k_{21}f - k_i}{(k_i + z_1)}\right) \tag{8}$$

$$G_2 = \sum_{i=1}^{i=m} \frac{A_i k_i}{(\alpha - k_i)(z_2 - z_1)}\left(\frac{\alpha - k_{21}f}{(\alpha + z_2)} + \frac{k_{21}f - k_i}{(k_i + z_2)}\right) \tag{9}$$

$$G_3 = \sum_{i=1}^{i=m} \frac{A_i k_i}{(\alpha - k_i)} \frac{\alpha - k_{21}f}{(\alpha + z_2)(\alpha + z_1)} \tag{10}$$

$$\alpha = k_{ab}\frac{V_b}{V_r} \quad p = k_{el} + k_{12} + fk_{21}$$
$$q = k_{el}k_{21}f \quad z_{1,2} = \frac{-p \pm \sqrt{p^2 - 4q}}{2} \tag{11}$$

It is easy to verify that, when all k_i become very large, Eq. (7) degenerates into the equation describing C_b trend in the case of an instantaneous release from the delivery system, i.e., the situation occurring after the oral administration of a drug solution.

3. Results

In the first part of the research, helical extrudates were prepared in the vertical ram extruder. In order to check the range of processability of various theophylline/melting binder formulations depending on the temperature, preliminary experiments have been carried out using different temperatures (from 40 to 60 °C). The temperature of 50 °C was chosen as it was the lowest operating temperature for such kind of mixtures and allowed a wide range of binary extrudable mixtures. As the temperature increased, the minimum level of binder allowing extrusion slightly decreased, but there was a dramatic drop in the limiting upper range where the system became fluid in nature and exited the die without pressure. Conversely, the choice of a lower temperature would mean reducing dramatically the range of extrudable mixtures, since most of them would be too hard and could not be processed.

Comparison of the selected operating temperatures with the DSC results showed that the extrusion took place at least 10 °C below the melting point of the systems. Hence, the binder material was "softened" but not molten. The extrusion pressure ranged from 100 to 140 bars, depending on the composition of the mass to be extruded. As previously noticed [1], also in this case a decrease in binder content in the mixtures promotes a progressive increase in extrusion pressure.

The previously mentioned operating conditions permitted to collect extrudates with the desired helical uniform shape (blade thickness 0.9 mm, helical diameter: approximately 6 mm) and smooth surface, as they appeared in the pictures (Fig. 2), with the 70/30, 60/40 and 50/50 wt theo:wax formulations and with all the three types of cylindrical dies.

The same three formulations (70/30, 60/40 and 50/50 wt theo:wax) were processed at the same temperature of 50 °C using a cylindrical die. Also in this case, a satisfactory regularity in shape and a smooth surface was obtained in most extrudates, as shown in the example of Fig. 2D. Furthermore, the drug content was found to be in all cases very similar to the theoretical one.

Subsequently, the solid state characterisation was performed by means of DSC and XRD analyses (Figs. 3 and 4, respectively) to detect possible modification of the physico-chemical properties of theo or wax and to study possible interactions between the drug and the carrier underwent during the thermal process of preparation. A comparison with the corresponding physical mixtures of the components indicated that solid structure of extrudates was substantially identical to that of physical mixtures, as one would expect if it is the microcrystalline wax that deforms and not the drug crystals. Furthermore, there were no differences in thermal and X-ray parameters between the samples prepared with different extrusion dies or different compositions. It can be concluded that DSC and XRD analyses indicated the presence of the drug in the original crystalline structure in all the extrudates. For brevity, only the DSC curves (Fig. 3) and the diffractograms (Fig. 4) of the 50/50 theo:wax system (the system with maximum drug dilution) are reported.

To test the influence of shape and composition on the dissolution properties, extrudates differing for shape (2, 3, 4 blades helical systems or cylinder) and amount of waxy material (30%, 40% and 50% by wt) were subjected to dissolution test. As in each dissolution test, the amount of theophylline to be released was the same (25 mg), extrudates length and number varied as specified in Section 2.2.5.

In Fig. 5, the dissolution profiles of the system having different composition but identical shape are compared, allowing evaluation of the effect of the amount of microcrystalline wax on the dissolution. The greater the amount of waxy materials, the slower the drug release. In fact, only the 70:30 theo:wax systems were able to reach the 90% of drug release in 12 h of testing, whereas for 50:50 and 60:40 theo:wax systems, the maximum amount of released drug was 65%. Formulation 70/30 theo:wax was hence selected for further considerations. It is interesting to underline that, regardless of the matrix shape (cylindrical or helical), when theophylline mass fraction is equal to 70%, the release kinetics is considerably increased (see, in particular Fig. 5B–D). We believe that, in this condition, we are very close to the percolation threshold that implies the existence of a continuum theophylline path pervading the entire matrix body. Accordingly, the number of dead ends (i.e. clusters of theophylline particles that are completely sealed inside the waxy matrix and that can never dissolve) should be very small. The reasonability of this argumentation is supported by the fact that theophylline volume fraction, in the 70/30 system, is around 0.57 (it differs from the mass fraction value of 0.7 due to higher theophylline density (1.55 g/cm^3) with respect to the wax one (0.9 g/cm^3)) which is close to the theoretical percolation threshold value of 0.59, typical of a square lattice [14].

In Fig. 6A, the amount of drug released per unit area (mass/area (M_t/S) vs. time (t)) from differently shaped extrudates based on this formulation is compared. It appears that all these systems, after an initial rapid release, behaved as a sustained release formulation over a period of 12 h. Interestingly, the introduction of blades considerably increases the drug release per unit area (M_t/S), and this increment is maximum for the 3 blades system. The 4 blades sys-

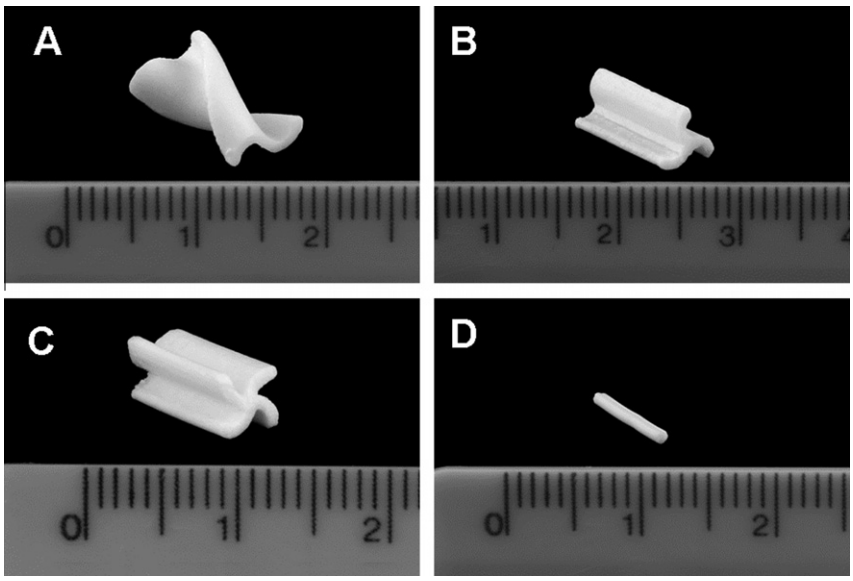

Fig. 2. Images of helical extrudates with 2 blades (A), 3 blades (B), 4 blades (C) and of cylindrical extrudates (D).

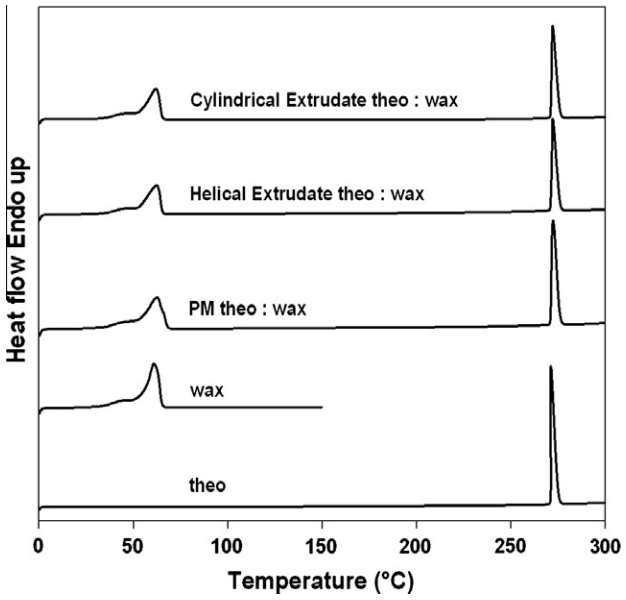

Fig. 3. DSC curve of the 50:50 wt theo:wax systems compared with raw materials.

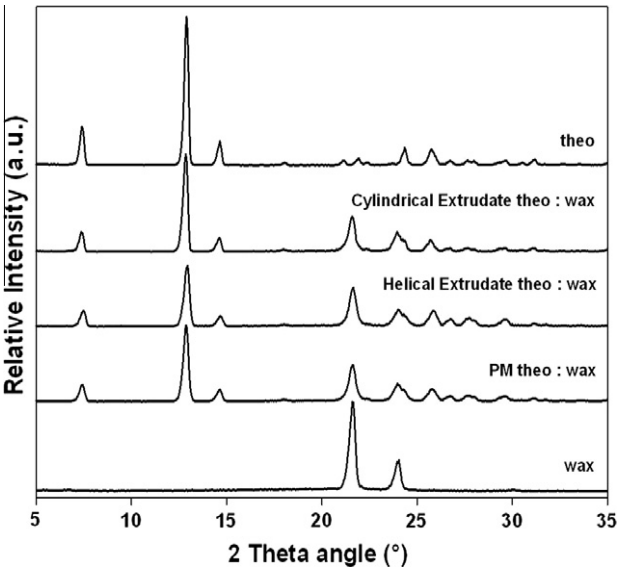

Fig. 4. XRD patterns of the 50:50 wt theo:wax systems compared with raw materials.

tem shows a small reduction in M_t/S with respect to the 3 blades system. This is probably the result of two counteracting phenomena: (1) the increase in the release area per unit volume of extrudate with the increase in the blades number, and (2) the increase in shape complexity (i.e. blades number) makes more and more pronounced the release resistance exerted by the stagnant layer surrounding the extrudate surface (it is reasonable that in the 4 blades case, the majority of the fluid comprised between blades is stagnant (i.e. not stirred) while this should be not the case in the 2 and 3 blades system). In conclusion, Fig. 6A enlightens the superiority of the helical 3 blades system, whose release per unit surface was the highest amongst all the systems considered. Fig. 6B, on the other hand, reporting the log(per cent) drug release vs. log(time), makes clear that, for all systems, the Korsmeier–Peppas equation [15] provides a good data fitting (R^2 always greater than 0.99) assuming the release exponents (n) very close to 0.5. This proves that the release mechanism is essentially diffusive and fickian.

From literature data [16], the ideal theophylline sustained release is characterised by an *in vitro* release of a quote of 30–40% during the first hour, 50–60% between the second and the fourth hour and 70–80% before the eighth hour of analysis. Helical extrudates having 3 and 4 blades perfectly comply with these requirements, but, in consideration of its higher specific release (M_t/S), the extrudate having 3 blades and 70/30 theo:wax composition was selected for subsequent characterisations and *in vivo* pilot study.

First of all, the following key point must be burned in mind. The visual observation, by naked eyes, of these extrudates during the dissolution test revealed that the waxy matrix remained substantially unchanged in terms of geometrical shape and dimensions, this suggesting that the main mechanism ruling the release of the drug was the diffusion from the hydrophobic matrix. In the case of the extrudates having 3 or 4 blades, sometimes the detachment of blades from the extrudates was observed at the end of the dissolution test (after about 12 h), since at this stage the drug is al-

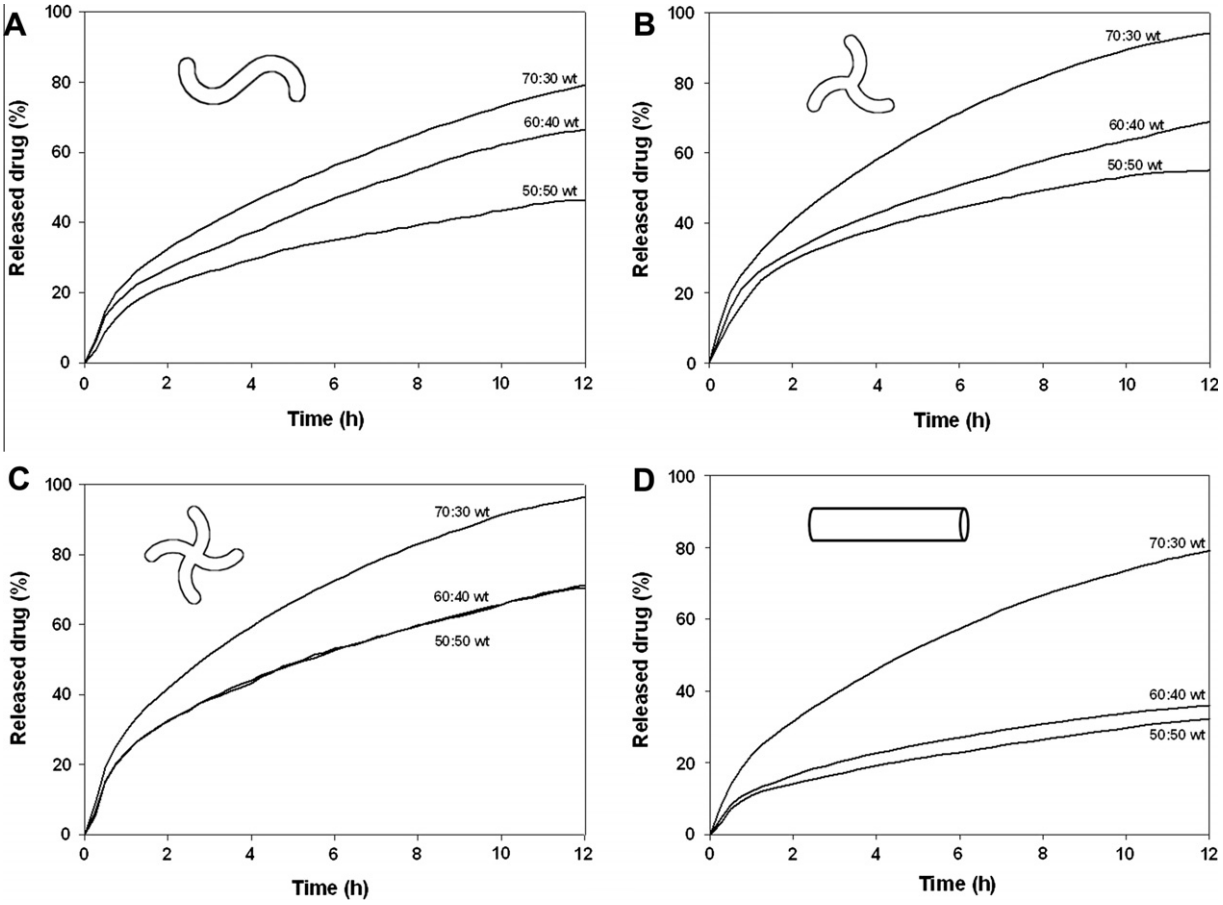

Fig. 5. *In vitro* theophylline dissolution profiles of helical extrudates having (A) 2 blades, (B) 3 blades and (C) 4 blades compared to cylindrical extrudates (D).

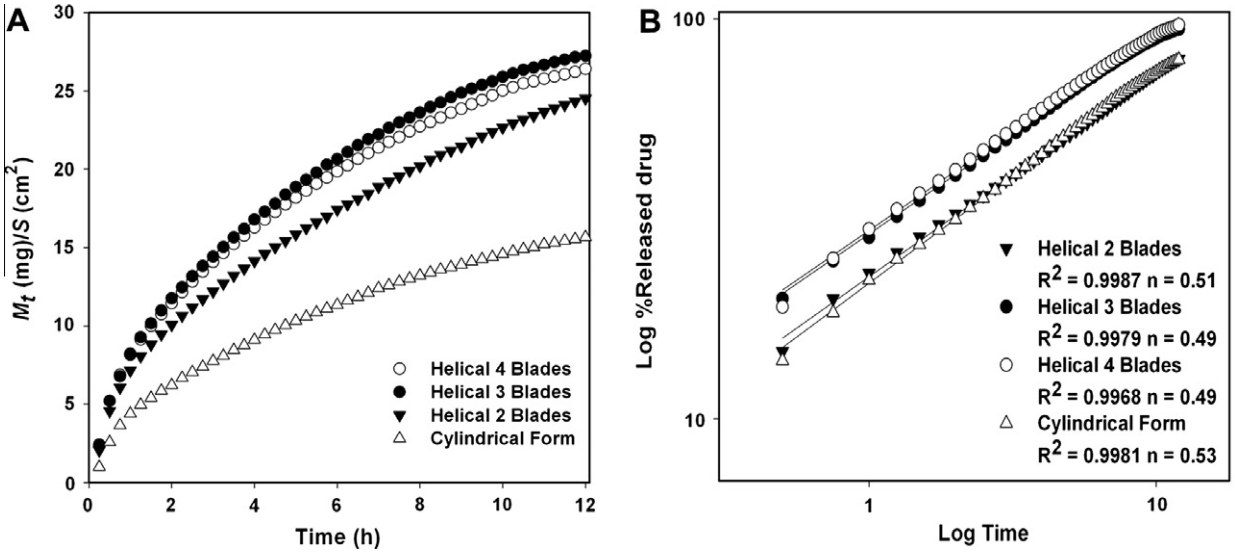

Fig. 6. Comparison of *in vitro* dissolution profiles (drug amount released per unit surface M_t/S (mg/cm^2) (A) and Korsmeier–Peppas [13] (B) plots (log(per cent) drug release vs. log(time)) of cylindrical extrudates and helical extrudates (2, 3, and 4 blades) containing 70/30 theo:wax.

most completely released and hence the matrix is very porous (see Fig. 9B) and has a low mechanical resistance. This hypothesis was further confirmed by the fact that the 3-blade extrudates, as they appeared on X-ray images of the intestine of the tested healthy volunteers, did not disintegrate, even after 12 h (Fig. 7B) from the administration of hard gelatine capsule containing the extrudates and once again only the separation of singular blades from the core was occasionally noticed. These extrudates were prepared replacing in the formulation half amount of theophylline with barium sulphate, radio-opaque (Table 1).

The *in vitro* dissolution behaviour of the extrudates was considered also in the light of the physical characterisation performed

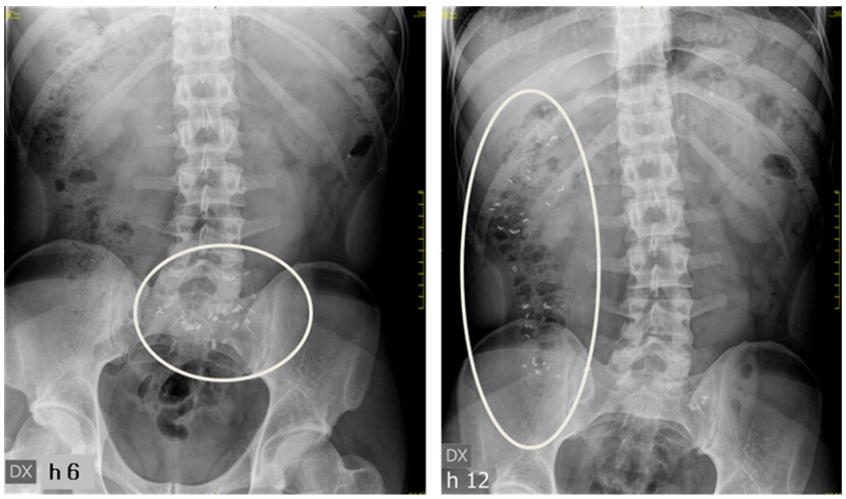

Fig. 7. X-Rays image of the intestine of a healthy volunteer 6 h (left) and 12 h (right) after the administration of samples of 3-blade extrudates containing barium sulphate as radio-opaque agent (see Table 1).

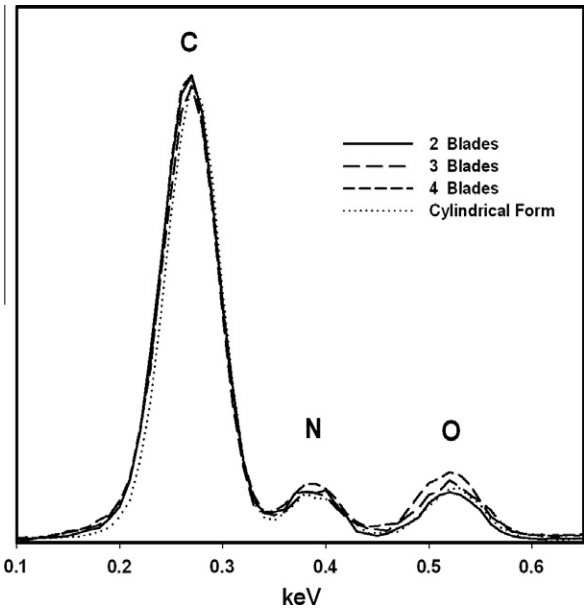

Fig. 8. EDS spectra of the four extrudates containing 70/30 theo:wax (for the sake of brevity only the 0.1–0.6 keV range is reported).

with DSC and XRD analyses. The fact that the drug is unchanged in all samples suggested that any change in dissolution rate was associated with a different amount of wax and a different shape of the extrudates rather than to a change in theophylline solid form or to an interaction with matrix excipients.

Furthermore, the analysis of surface compositions (EDS) demonstrated the presence of theophylline onto the surface of the extrudates in all the samples, independently from the shape of the extrudates (Fig. 8). This fact can give reason of the relatively rapid onset of dissolution followed by a slower process as the surface extrudates become depleted of drug.

This fact is also in agreement with the images of the differences noticed in the aspect of the surface of the extrudates before dissolution and after 8 h of dissolution test. These micrographs (depicted in Fig. 9) show a pronounced morphological variation, from the starting compact structure to a porous matrix, characterised by tortuous holes were the drug has been already released. The released drug particles left their marks in the form of pores and holes in the inert lipid matrix, similarly to what previously noticed with glycerol trimyristate and palmitostearate [17].

The theophylline plasma concentration time profiles, obtained after the administration of capsules containing a theophylline dose of 400 mg, are shown in Fig. 10, while the pharmacokinetic parameters are listed in Table 2. On the basis of the *in vitro* release kinetics, the three blades system was selected for *in vivo* test. The results shown in Fig. 10 demonstrated that this matrix

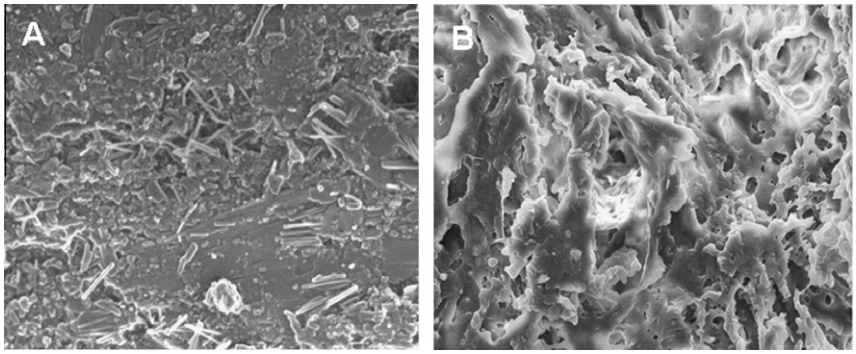

Fig. 9. SEM micrograph of the surface of 70:30 theo:wax 3-blades helical extrudates before (A) and after (magnification: 2000×) (B) *in vitro* dissolution (magnification: 1190×).

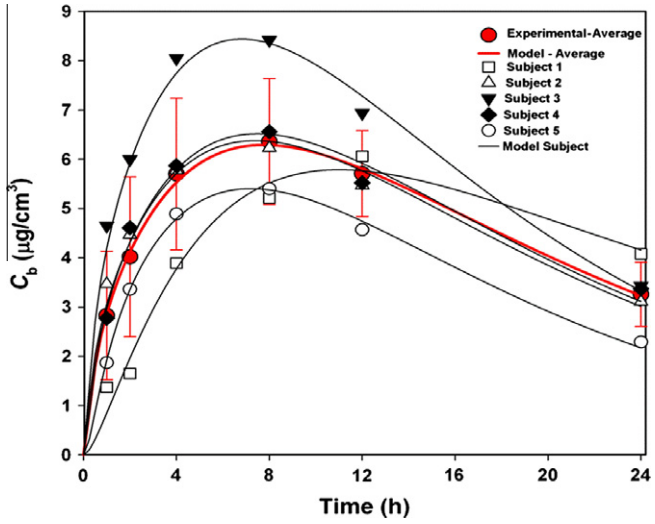

Fig. 10. Individual plasma profiles obtained after oral administration of a dose of 400 mg theo in 3-blades extrudates (black and white symbols) compared to model best fitting for each subject (black lines) and mean plasma profile (red circles) compared to general model best fitting (red line). Vertical bars indicate standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Pharmacokinetic parameters calculated by means of a non-compartmental approach after oral administration of 400 mg of theophylline for every single subject and mean values (±SD; $n = 5$).

Subject	t_{max} (h)	C_{max} (mg l^{-1})	$AUC_{0\to24}$ (h mg l^{-1})	$t_{1/2}$ (h)	$t_{75\%Cmax}$ (h)
S1	8.00	8.42	147.62	12.21	19.2
S2	8.00	6.56	117.88	16.68	12.3
S3	12.00	6.06	109.35	21.08	11.0
S4	8.00	6.23	114.78	15.65	12.8
S5	8.00	5.40	93.49	12.69	12.5
Mean	8.80 ± 1.79	6.53 ± 1.14	116.62 ± 19.71	15.66 ± 3.58	13.60 ± 3.20

behaved as a sustained release formulation, as indicated by the high levels of t_{max} and the $t_{75\%Cmax}$ comparable to that reported in literature for oral sustained release formulation for theophylline [18].

Further, the GI transit of the extrudates containing a radio-opaque agent was monitored by taking X-ray images of the intestine at predetermined time after administration. Looking at the radiographs reported in Fig. 7, it can be noticed that intact extrudates are visible in the small intestine 6 h after the administration (Fig. 7, left). Interestingly, 12 h after the administration (Fig. 7, right), the extrudates are still visible and are located in the ascending colonic segment, that is the last absorption window of our model drug and that is the place where theophylline is reported to be mainly absorbed (40%) [19]. It must, however, burned in mind that the density of the extrudates changes by the introduction of barium sulphate, thus their transit would probably be slightly different with respect to that of extrudates without barium sulphate.

Eq. (5) fitting to *in vitro* release data (see Fig. 5B (helical 3 blades, containing 70/30 theo:wax)) proved to be statistically very good ($F(2, 45, 0.95) < 10{,}991$), and the following constants values were found: $A_1 = (0.87 \pm 0.02)$, $k_1 = (0.195 \pm 0.005)$ h^{-1}, $A_2 = 1 - A_1$, $k_2 = (1.84 \pm 0.7)$ h^{-1}. Thus, six model parameters (V_b, V_T, k_{12}, k_{21}, k_{el}, k_{ab}) were still unknowns. As their simultaneous determination by means of data fitting to *in vivo* data was not possible due to their high correlation, a different strategy was undertaken. In particular, the work of Mitenko and co-workers [20] was chosen as starting point. By means of the intravenous administration of a drug solution to 16 volunteers, these authors demonstrated that theophylline pharmacokinetics need to be described by a two compartments model and the PK constants are $V_{bM} = (0.3 \pm 0.15)$ l kg^{-1} (blood volume per subject unit mass), $k_{el} = (0.31 \pm 0.2)$ h^{-1}, $k_{12} = (2.7 \pm 2.5)$ h^{-1} and $k_{21} = (2.9 \pm 2.7)$ h^{-1}. In the light of the mathematical model adopted to fit their experimental data, the authors implicitly assumed $V_b = V_T$, i.e. $f = 1$. On the basis of the k_{12} and k_{21} found by Mitenko, Eq. (7) was fitted to *in vivo* data (see Fig. 10) assuming $V_r = 300$ cm^3, knowing that $M_0 = 400$ mg and letting k_{el}, k_{ab} and V_{bM} ($=V_b/M_{vw}$; M_{vw} = mean volunteers weight = 70 kg) to freely vary. The inspection of Fig. 10 reveals the very good agreement between model best fitting (red line) and *in vivo* average data (red circles) as also witnessed by the F test result ($F(2, 3, 0.95) < 441$). Model fitting parameters red $k_{ab} = (0.13 \pm 0.09)$ h^{-1}, $k_{el} = (0.12 \pm 0.004)$ h^{-1} and $V_{bM} = (0.27 \pm 0.006)$ l kg^{-1}. Fig. 10 also shows Eq. (7) best fitting to the experimental data referring to each volunteer. As witnessed by the F test analysis ($F_{S1}(2, 3, 0.95) < 45$, $F_{S2}(2, 3, 0.95) < 60$, $F_{S3}(2, 3, 0.95) < 122$, $F_{S4}(2, 3, 0.95) < 44$, $F_{S5}(2, 3, 0.95) < 280$), the agreement is always good. The average values of k_{ab}, k_{el} and V_{bM}, coming from Eq. (7) best fitting to each volunteer, are $k_{ab} = (0.11 \pm 0.09)$ h^{-1}, $k_{el} = (0.14 \pm 0.04)$ h^{-1} and $V_{bM} = (0.24 \pm 0.06)$ l kg^{-1}. These values, although characterised by a higher standard deviation, do not substantially detach from those determined by Eq. (7) best fitting to average *in vivo* data. It is interesting noticing that these results agree with those deducible by fitting Eq. (7) to the experimental data shown by de Leede et al. [21] assuming an instantaneous theophylline release ($k_i \to \infty \; \forall i$), $V_r = 300$ cm^3, $C_{r0} = 500$ μg/cm^3, $M_{vw} = 75$ kg and the Mitenko and Ogilvie [20] values for k_{12} and k_{21} ($k_{ab} = (0.075 \pm 0.007)$ h^{-1}, $k_{el} = (0.23 \pm 0.011)$ h^{-1}, and $V_{bM} = (0.265 \pm 0.003)$ l kg^{-1}; $F(2, 8, 0.95) < 2001$). Again, similar results ($k_{ab} = (0.2 \pm 0.08)$ h^{-1}, $k_{el} = (0.20 \pm 0.01)$ h^{-1} and $V_{bM} = (0.093 \pm 0.003)$ l kg^{-1}; $F(2, 3, 0.95) < 166$) can be found by Eq. (7) fitting to the data reported by Quintavalle et al. [8] knowing that $V_r = 300$ cm^3, $C_{r0} = 1166.6$ μg/cm^3, k_{12} and k_{21} are those found by Mitenko and Ogilvie [20], and Eq. (5) parameters are: $A_1 = (0.58 \pm 0.02)$, $A_2 = (0.40 \pm 0.02)$, $A_3 = 1 - A_1 - A_2$, $k_1 = (0.08 \pm 0.005)$ h^{-1}, $k_2 = (0.75 \pm 0.03)$ h^{-1}, $k_3 = (28.5 \pm 8.6)$ h^{-1} ($F(4, 13, 0.95) < 18{,}988$).

Finally, assuming a geometrical intestinal area $A = 1316$ cm^2 [14], the definition of the absorption constant k_{ab} (see Eq. (1)) allowed to conclude that the theophylline apparent permeability P evaluated on the basis of this work is, respectively, $(5.4 \pm 3.6) \times 10^{-5}$ cm s^{-1} and $(4.0 \pm 3) \times 10^{-5}$ cm s^{-1} when Eq. (7) is fitted on averaged *in vivo* data or when Eq. (7) is fitted on each volunteer data and then averaged on all volunteers. In the case of de Leede et al. [21] and Quintavalle et al. [8], P turned out to be similar, being, respectively, equal to $(3.2 \pm 0.3) \times 10^{-5}$ cm s^{-1} and $(2.9 \pm 1.2) \times 10^{-5}$ cm s^{-1}.

4. Conclusions

Helical and cylindrical extrudates were successfully obtained by a single step extrusion process using microcrystalline wax as a thermoplastic binder at a temperature below its melting point. The extrudates simply consisted of binary mixtures of drug and microcrystalline wax and permitted the loading of a very high amount of drug, up to 70% (w/w). By the appropriate selection of the shape of the extrudates, the release of the model drug theophylline was tailored. In particular, the system having 3 blades and the composition of 70:30 theo:wax exhibited the desired *in vitro* theophylline release. The shape of the matrix appeared unchanged during dissolution tests, and on the surface of the system, the number of pores increased with time.

The *in vivo* study conducted on healthy volunteers confirmed that the selected helical extrudate behaved as a sustained release formulation and remained intact even after 12 h from the ingestion (as it appeared from X-ray GI tract images). The mathematical model shown in this paper represented a successful attempt of establishing a theoretical connection between *in vitro* and *in vivo* data. In addition, it had the considerable advantage of yielding to an analytical solution, allowing an easy and fast data analysis. Then, the results found in this paper were in line with the results coming from different works. Finally, this model allowed a simple estimation of GI apparent permeability resorting to the oral administration of a delivery system.

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