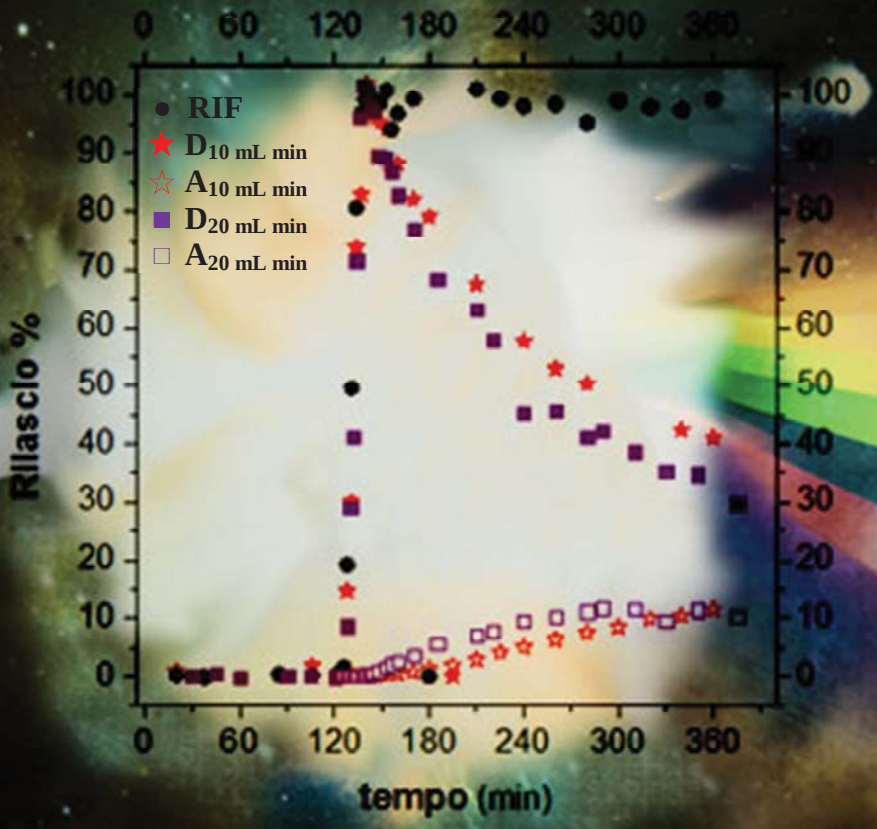
Simulazione del rilascio di farmaci e dello scambio di materia nel tratto gastrointestinale



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Simulazione del rilascio di farmaci e dello scambio di materia nel tratto gastrointestinale Margherita Iannaccone

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Corso di Laurea in Ingegneria Chimica

Simulazione del rilascio di farmaci e dello scambio di materia nel tratto gastrointestinale

Tesi in

Principi di Ingegneria Chimica

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Anno Accademico 2012/2013

“Il silenzio del rumore
delle valvole a pressione

I cilindri del calore
serbatoi di produzione

Anche il tuo spazio è su misura

Non hai forza per tentare
di cambiare il tuo avvenire
per paura di scoprire
libertà che non vuoi avere

Ti sei mai chiesto quale funzione hai?

...quale funzione hai ti sei mai chiesto
per paura di scoprire libertà...”

Franco Battiato, “Il silenzio del rumore”
Pollution (1972)

Questo testo è stato stampato in proprio, in Times New Roman
La data prevista per la discussione della tesi è il 13/06/2013
Fisciano, 07/06/13

Sommario

Indice delle figure

Indice delle tabelle

Abstract

In this work a device simulating the release of drugs within the gastrointestinal tract and the exchange of matter from pharmaceutical formulations through the intestinal wall has been designed and realized.

Three pharmaceutical system of increasing complexity were studied: a controlled release system composed by theophylline 25% and HPMC 75%, a pharmaceutical gastro-resistant system Diclofenac DOC 100 mg and the immediate release gastro-resistant tablets Voltaren ® 50 mg with diclofenac as active principle.

Conventionally the controlled release from pharmaceutical matrices is tested using an USP II apparatus in which during the first two hours the pharmaceutical formulation is placed in a solution at pH 1, physiological pH within the stomach. After the two hours, the solution is neutralized to the pH value of 6.8, the physiological pH of the intestine.

The experimental tests using the device have been structuring in two steps. In the first two-hour step, an USP II apparatus containing a pH 1 solution simulates the controlled release of drug from the pharmaceutical system in the human stomach. After the two hours, the average residence time within the stomach, the hollow fiber filter simulating the mass exchange through the intestinal wall from the intestine content to the circulatory system is activated and the pH of the dissolution medium is increased rapidly to 6.8. From the donor compartment, simulating the intestinal lumen, rich in active principle, the fluid passes through the hollow fiber membrane of the filter into the acceptor compartment simulating blood vessels. Thus the acceptor compartment enriches of drug during the experiment.

The experimental tests were conducted by varying an operating parameter at time to assess the effect on the mass exchange. The

results show that for both the pharmaceutical systems with theophylline and diclofenac, the configuration of the mass exchange, co-current or counter-current, does not affect the exchange itself. Instead the volumetric flow rate of fluids affects the mass exchange. In particular increasing the volumetric flow rate, the mass exchange is faster. The same result has been obtained for both the pharmaceutical systems with theophylline and HPMC that for the pharmaceutical system Voltaren ® 50 mg.

Finally, it has been developed a mathematical model to predict the controlled release throughout the gastrointestinal tract for the overall duration of the experimental tests. The mathematical model consists of the mass balance equations that describe the physical phenomena involved. The parameters in the mathematical model are obtained by fitting the experimental data obtained from exchange tests characterized by fixed concentrations of drug within the compartment donor, for both drugs. Once calculated the parameters needed for the model, its validity was tested using experimental tests examined. The mathematical model developed predicts accurately the evolution of the masses for both the pharmaceutical systems with theophylline and diclofenac. Regarding the pharmaceutical system Voltaren ® 50 mg, the model does not predict accurately the experimental tests probably because other aspects have to be taken into account: the presence of excipients could affect the exchange parameters.

The future goal will be to optimize the mathematical model so that it can predict also more complex controlled release pharmaceutical forms. Besides in the thesis has not been taken into account that in real physiology, some drugs is subtracted from the circulatory system to be metabolized and cleared by the liver. Thus it should be considered a subtractive term from acceptor compartment. The full aim of the research being to find valid correlations between in vitro and in silico models to limit the amount of in vivo experiments.

Appendice

Pubblicazioni

International Journal of Pharmaceutics 439 (2012) 165–168

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Rapid communication

In vitro simulation of drug intestinal absorption

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ARTICLE INFO

Article history:
Received 15 August 2012
Received in revised form
26 September 2012
Accepted 8 October 2012
Available online 16 October 2012

Keywords:
Theophylline
Intestinal absorption
Oral administration
Mass balance
Controlled drug release

ABSTRACT

In this work, a simple set-up was designed, realized and tested to evaluate the effect of intestinal absorption on the in vitro drug release studies. The conventional USP-approved dissolution apparatus 2 was equipped with an hollow fibers filter, along with the necessary tubing and pumps, to simulate the two-fluids real behavior (the gastro intestinal lumen and the gastro intestinal circulatory system). The realized set-up was characterized in term of mass exchange characteristic, using the theophylline as the model drug, also with the aid of a simple mathematical model: then the release kinetics of a controlled release tablet was evaluated in the conventional test as well as in the novel simulator. The concentration of drug in the release compartment (which simulates the gastric lumen) was found lower in the novel simulator than in the traditional one.

The analysis of a pharmaceutical system's fate after its oral administration is a widely investigated field of study. A number of apparatuses have been developed to simulate what happens to a tablet once swallowed; several of them have been approved and codified by pharmacopoeias, some of them are still under investigation and their use is not widespread (Cascone et al., 2011; Grassi et al., 2011). Thermal, chemical, biological, and fluid-dynamic histories have to be carefully reproduced, and, to our knowledge, an apparatus able to do this is still lacking in the laboratories all over the world. The USP-approved apparatuses are limited to analyze what happens during the dissolution step, but they are also limited in the kind of data produced, since they give only a measure of how much drug is released in the time. For example, experimental protocols to clarify what happens during the hydration of tablets made of hydrogels have been developed (Barba et al., 2009b,d), even based on previous work done on different systems (Acierno et al., 2004; Barba, 2005; Barba and Lamberti, 2003), and they were further corroborated by theoretical works (Barba et al., 2009c; Lamberti et al., 2011). The most interesting alternatives to traditional apparatuses were developed in recent years, the gastro intestinal model (TIM) by TNO in the Netherlands (Minekus et al., 1995, 1999), and the model gut in the UK (Wickham and Faulks, 2007), even if these studies starts long time ago, with the Sartorius absorption model (Stricker, 1973). In particular, the gastro intestinal model and the Sartorius absorption model focused on the relevance of the absorption process after the drug oral administration. Indeed, after swallowing and dissolution/release, the absorption is a key point for the drug effect, and several studies are devoted to clarify the mechanisms of absorption and to relate them with the characteristics of the pharmaceutical systems (Kim et al., 2008; Sarma et al., 2011). There is a strong need for a test able to mimic the physiological conditions at the maximum extent. For example, novel enteric materials (Barba et al., 2009a) and formulations (Dalmoro et al., 2012a,b, 2010) cannot to be efficiently tested in conventional systems, thus *ad-hoc* protocols were developed each time they needs, and this process is not a good scientific approach. Working with non-enteric pharmaceutical/biomedical systems has similar drawbacks (Barba et al., 2009e). Most of these problems were circumvented using in vivo tests. However, while FDA clearly stated that "The basic principle in an in vivo bioavailability study is that no unnecessary human research should be done." (21CFR320.25, a concept which could be easily extended to studies different than bioavailability), the large use of animal and human tests in industry is still increasing (Thomas, 2009). In principle, in vitro and in silico tests should substitute in vivo tests (Carmichael et al., 2009).

Aim of this work is to propose and to characterize a simple system to be used in connection with a traditional USP-approved dissolution apparatus 2, to simulate the absorption process. Such a device should be able to improve the simulation of the real behavior of gastro-intestinal animal tract.

Theophylline (TP, Sigma–Aldrich, Milan, Italy) was the selected drug model. Hydroxypropyl methylcellulose (HPMC, Methocel K15M, Colorcon, Varese, Italy) was used as the controlled release excipient. Distilled water and buffer solutions, prepared in agreement with USP recommendations, were used as dissolution media.

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0378-5173/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2012.10.012

The controlled release tablets were obtained by powders mixing and compression of TP and HPMC. Both HPMC and drug powders were used as provided and shaped in cylindrical matrices (tablets, radius 6.5 mm and thickness 2.1 mm) through powders mixing (25% TP, 75% HPMC) and compression steps, using, for this latter operation, a tableting machine (Specac PN3000, equipped with flat-faced punches, diameter 13 mm and with a Carver Press), implementing a loading force of 50 kN kept for 5 min.

The dissolution tests were performed using an USP-approved dissolution apparatus 2 (AT7Smart, Sotax, Allschwil, Switzerland). The vessels of the apparatus, thermostated at 37 °C, were used for the dissolution of the tablets without any absorption simulation (conventional test), as well as to reproduce the two compartments in the simulation of dissolution/absorption: one vessel was intended to reproduce the gastro intestinal lumen (called "donor"), another vessel was intended to reproduce the gastro intestinal circulatory system (called "acceptor"). The physiological counterparts of the donor compartment are the stomach and the intestine; for the acceptor compartment they are the mesenteric artery and the portal vein. The fluid in the donor compartment was pumped through the "dialysis fluid compartment" of an hollow fibers filter (Phylther LF 17 SD, Bellco, Mirandola (MO), Italy). The filter exchange area is of 1.7 m^2, the fibers, made of polyphenylene (PPE), have an inside diameter of 200 μm and a wall thickness of 35 μm. The filter has been selected with the purpose of mimicking not a single organ, but a part of the intestinal wall. Permeability and exchange area, unknown before the experimentation reported in the present work, will be used to establish a comparison with real physiological tissues. In principle, the set-up proposed could be used in the mimicking of other organs like kidneys, too, the key point being the estimation of transport properties, possible using the model and the technique proposed in this work. The fluid in the acceptor compartment was also pumped through the filter ("blood side"). A schematic of the set-up is drawn in Fig. 1, graph (a). At given times, small amount (1 mL) of both fluids were sampled and assayed by HPLC for theophylline.

In a first set of tests (experiment "a"), the donor compartment was filled with a solution of theophylline at known concentration, the acceptor compartment being filled with a 7.4 pH buffer solution to simulate the blood. In a second set of tests (experiment "b"), the donor compartment and a reference vessel initially contains 750 ml. of an acid solution, to mimic the gastric environment, in which at the time zero one controlled release tablet (25% TP, 75% HPMC) for each vessel was added. After 2 h, mimicking the passage from the stomach to the intestine, the pH was raised adding 250 mL of a phosphate salt solution to both the vessels (donor and reference), and the pumps were switched on, allowing the start of the mass transfer within the filter (this protocol assumes that no absorption take place in the stomach, during the first two hours). The acceptor compartment was filled with 1 L of pH 7.4 buffer solution, initially drug free.

The first set of experiments (type "a", detailed in the previous section) were carried out to characterize the system. The donor compartment was filled with a theophylline solution of known concentration, and the acceptor compartment with a fluid initially drug free. Then, the system was operated monitoring the evolution of drug concentrations in the two compartments, to investigate the transport phenomena which take place during the test. Prior of any analysis of the experimental results, the system has to be described mathematically, i.e. a model of the system has to be proposed.

The experimental system could be modeled by a simple compartmental approach, depicted in Fig. 1, graph (b). The two sides are the intestinal lumen, called donor compartment, and the gastrointestinal blood system, called acceptor compartment. The two compartments are separated by the filter membrane, which constitutes another compartment itself. The fluxes of drug which take place are indicated by arrows in the graph. The drug balances within the three compartments can be written as:

$$\begin{aligned}
\frac{dm_D}{dt} &= \frac{d(V_D C_D)}{dt} = -S(j_{DA} + j_{DM}) + G \\
\frac{dm_A}{dt} &= \frac{d(V_A C_A)}{dt} = S(j_{DA} - j_{AM}) \\
\frac{dm_M}{dt} &= S(j_{DM} + j_{AM})
\end{aligned} \tag{1}$$

In Eq. (1), C_D and C_A are the concentration in donor and acceptor compartments, S is the surface exchange area, and j_{ik} are the three fluxes (i, k being two of the three compartments) also shown in Fig. 1, graph (b). V_D and V_A are the fluid volumes present in donor and acceptor compartments. G is the generation term (mass of drug liberated in the donor compartment for unit of time), which could be due to the release from a tablet. The expressions for the three fluxes are:

$$\begin{aligned}
j_{DA} &= P(C_D - C_A) = P\left(\frac{m_D}{V_D} - \frac{m_A}{V_A}\right) \\
j_{DM} &= K\left(C_D - K_p\frac{m_M}{V_M}\right) \\
j_{AM} &= K\left(C_A - K_p\frac{m_M}{V_M}\right)
\end{aligned} \tag{2}$$

In Eq. (2) P is the permeability of the drug (i.e. an overall transport coefficient which takes into account the convection in donor and acceptor compartment, and the diffusion through the membrane), K is an overall transport coefficient from the donor or from the acceptor toward the membrane (it should be higher than P, since P takes into account one resistance more than K), K_p is the partition coefficient of the drug between the liquid compartments and the membrane, and V_M is the volume of the membrane. These parameters can be used as three optimization variables: P, K, and K_p/V_M.

The tests of type "a" were performed working with an initial concentration of theophylline in the donor compartment roughly equal to 1000 mg/L, and they were carried out varying the "blood" flow rate, the values investigated being 10, 20 and 30 mL/min. The evolutions of donor and acceptor TP concentration were summarized in Fig. 2 (graphs (a), (b), and (c)). During the mass exchange, some of the drug was embedded into the membrane, its value being easily obtainable from a mass balance. Therefore, the evolutions of the TP mass embedded in the membrane were also reproduced in the graphs of Fig. 2. In particular, the TP concentration in the donor compartment are drawn as full squares, the concentration in the acceptor compartment as open circles, and the mass in the membrane as full upward triangles. As expected, the amount of drug which enters in the membrane is flow-independent (roughly 200 mg), and the kinetics of the transport phenomena increase as the flow rate increases. I.e. the concentrations of the donor and acceptor compartments tend to the common stationary value of about 400 mg/L, and the difference between them decreases below 50 mg/L in less than 90 min working with a "blood" flow rate of 30 mL/min, in about 135 min working with 20 mL/min, and in more than 210 min working with 10 mL/min. Similarly, the saturation of the membrane (i.e. the embedding of about 200 mg of drug) occurs in a time shorter working with the higher flow rate.

In the case of type "a" experiments, from the modeling point of view the generation term is zero and the initial concentrations are known, $C_D = C_{D0}$, $C_A = 0$ and $m_M = 0$. Therefore, the model equations can be solved once suitable parameter's values were provided. One way to get such values is an optimization procedure. On the basis of the observed results, the best optimization strategy was to make the parameter K_p/V_M independent from the "blood" flow rate, and to make the transport coefficients, P and K, directly dependent from

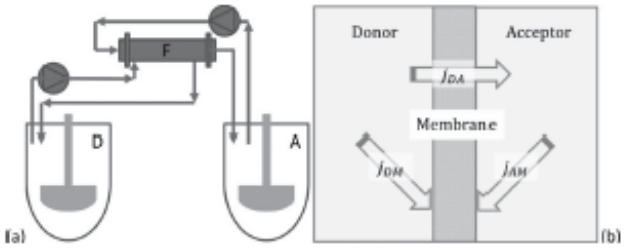

Fig. 1. (a) Schematic of the experimental set-up. D: donor vessel, A: acceptor vessel, F: filter; (b) schematic of the model proposed: the three compartments (donor, acceptor, membrane) and the three main fluxes (J_{DA}, donor–acceptor; J_{DM}, donor–membrane; and J_{AM}, acceptor membrane).

the mass flow rate, i.e. $P = \alpha_1 \dot{V}_B, K = \alpha_2 \dot{V}_B$, in which $\dot{V}_B$ is the "blood" flow rate. The optimization gave the following results: $K_P/V_M = 1.81$ 1/L, $\alpha_1 = 1.92 \times 10^{-4}$ L/(mL m^2), and $\alpha_2 = 7.05 \times 10^{-4}$ L/(mL m^2). As expected, $K > P$. The results of the model calculations were reported in Fig. 2 as curves (continuous for donor TP concentration, dashed for acceptor TP concentration, and dotted for TP mass embedded in the membrane). The agreement between experimental data and fitted model was nice, confirming that the main phenomena were correctly identified and quantified.

During experiments of type "b" the volume of fluid in the vessel changes, therefore the best way to present the results is to report in a graph the masses (not the concentrations) of TP in each compartment. Graph (d) in Fig. 2 shows the evolution of TP masses in the donor (full squares), in the acceptor (open circles), and in the reference (full stars) compartments. It is worth to note that the data from the reference compartment is the only response that one obtain using the conventional USP 2 apparatus. In the novel apparatus, which is closer to the real physiology than the conventional one, the concentration in the donor compartment (which mimics the gastro intestinal tract) does not increase further, after the first two hours, i.e. after the passage in the intestine. On the other side, the concentration in the acceptor compartment (which mimic the gastro intestinal circulatory system), increases from zero, as soon as the mass transfer was allowed (in the intestine). It has to be emphasized that the real behavior is further influenced by phenomena and processes not yet simulated in the present version of the device: mainly the metabolism due to the liver and the clearance due to plasma degradation. These phenomena would cause a decrease in the blood TP concentration.

In the case of type "b" experiments, from the modeling point of view the generation term is the derivative of the evolution observed in the reference vessel (fitted by a suitable equation and then differentiated), and the initial concentrations are all zero, $C_D = 0$, $C_A = 0$ and $m_M = 0$. In Fig. 2, graph (d), also the model calculations (using the parameter's values obtained previously) are reported as curves (continuous line for the TP mass in donor compartment, dashed line for the TP mass in the acceptor compartment, dash-dot line for the TP mass in the reference compartment). Even if the prediction is not very accurate, the agreement between model and data is satisfactory, since no further optimization parameter was used at this stage. This confirms once more that the main phenomena have been correctly identified and quantified.

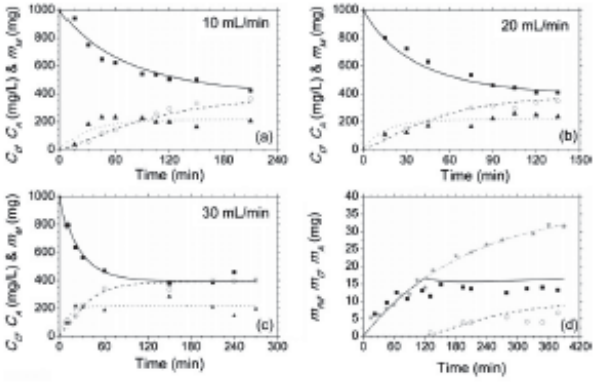

Fig. 2. (a–c) Evolutions of donor and acceptor TP concentrations (donor: full squares, continuous curve; acceptor: open circles, dashed curve) and of TP mass entrapped into the membrane (full upwards triangles, dotted curve). (d) Evolutions of TP masses in reference vessel (stars, dash-dot curve), in the donor vessel (full squares, continuous curve), and in the acceptor vessel (open circles, dashed curve). In all the graphs, symbols are experimental data and curves are model calculations.

As expected, the drug concentration in the donor compartment (which mimics the intestinal lumen much better than how the conventional USP apparatus does) is lower than the drug concentration in the reference vessel. This means that the real concentration values in the intestine will be lower than those measured in dissolution apparatuses. If in these tests the drug reaches levels which are too high (out of the therapeutic window), probably in the real body these levels will be not realized, then the pharmaceutical dosage can be administered without any danger. Furthermore, the concentration evolution measured in the donor compartment could be effectively used as input function in pharmacokinetic models, allowing a better in silico simulation of the real body.

In this work, a conventional USP-approved dissolution apparatus 2 was equipped with an hollow fibers filter, in order to allow the simulation of both the dissolution of pharmaceuticals (which happens in the gastro-intestinal tract after oral administration) and of the absorption (which happens through the intestinal wall). The gastro-intestinal lumen and the gastro intestinal circulatory system were reproduced by two vessels of the USP apparatus (respectively, donor and acceptor compartments), the intestinal wall was reproduced by the filter membrane. The apparatus was tested using solutions of known drug initial concentration, to estimate the transport coefficients, also using a simple mathematical model; then it was used to compare the release kinetics in traditional apparatus (reference test) with the novel test. The concentration of drug in the donor compartment, as expected, was found lower than that obtained in the reference test. This finding could be of aid in the pharmacokinetic modeling, as well as in the design and realization of novel pharmaceutical systems.

Acknowledgements

This work was supported by the Italian Ministry of Education (PRIN 2008 – 2008HCAJ9T) and by Fondazione Cassa di Risparmio Salernitana (Carisal).

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“Chi è incapace di vivere in società,
o chi non ne ha bisogno perché basta a sé stesso,
deve essere una bestia o un dio”
-Aristotele-

La ricerca di un rapporto, è la ricerca di noi stessi e di ciò che non siamo, ma vorremmo, nell’altra persona. L’esigenza di ritrovarsi nella persona vicina si delinea dal desiderio di condivisione con l’altro. La condivisione diventa elemento indispensabile in quanto l’uomo è un animale intrinsecamente sociale e la cui visione della vita è tendenzialmente pessimistica. Ci si ammala di solitudine e la cura è il rifugio nella persona vicina a noi psicologicamente, ma al contempo lontana da noi. Nell’altra persona ambiamo a qualcosa che non è presente in noi stessi; è come l’invidia di questa o quella qualità che non ci appartiene. È però una invidia costruttiva, che induce al continuo auto-perfezionamento e crescita personale, mira dell’uomo dotato di raziocinio.

Mi sono sempre circondata di persone che mi facessero bene nella misura in cui ho appena raccontato. Ringrazio, quindi, qui di seguito, senza un ordine particolare e senza specificare il motivo, perché non necessario, quelle persone che con più o meno difficoltà, hanno fatto parte più o meno corposamente del mio percorso e hanno riempito soprattutto l’esperienza universitaria che sto per lasciare.

Mario

Angela

Salvatore

Margherita

Francesca

Aniello

Enrica

Sara

Elena

Felice

Antonio

Clara

Gaetano

Martina

Antonio

Mirko

Laura

Alessandra

Valentina

Pietro

Pierfrancesco

Piera

Carmen

Giovanni

Carmine

"Respira, respira nell'aria
Non aver paura di preoccuparti
Parti, ma non lasciarmi
Guarda intorno, scegli il tuo terreno
Per quanto vivi e in alto voli
E i sorrisi che donerai e le lacrime che verserai
E tutto ciò che tocchi e tutto ciò che vedi
È tutto ciò che la tua vita mai sarà

Corri, coniglio corri
Scava quella buca, dimentica il sole
E quando alla fine il lavoro è concluso
Non sederti, è il momento di scavarne un'altra
Per quanto tu viva e in alto voli
Ma solo se cavalchi la marea
E in equilibrio sull'onda più grande
Corri verso un precoce sepolcro"

Pink Floyd, "Breathe"-*The Dark Side of the Moon (1973)*