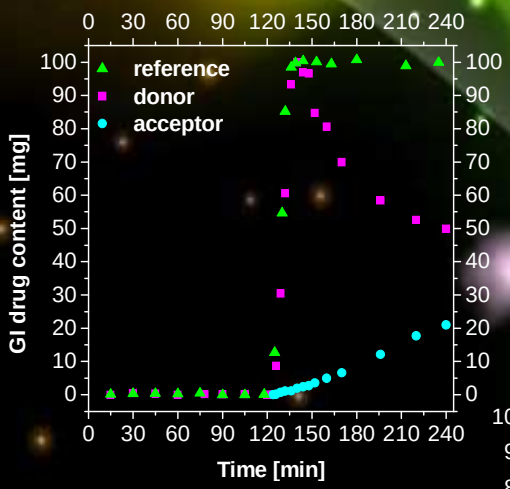
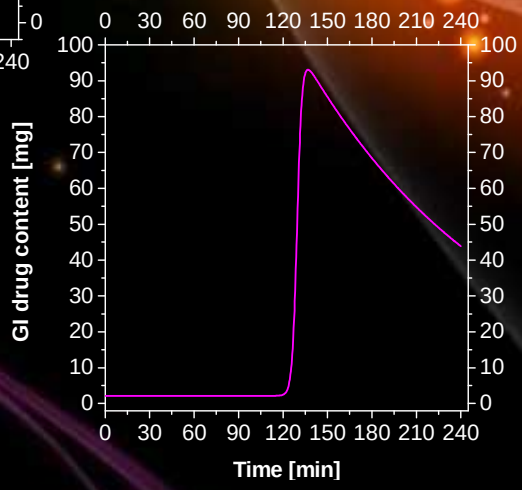
In vitro and *in silico* models in pharmacokinetic studies





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In silico and *in vitro* models in pharmacokinetic studies

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La cornacchia e la brocca

Una cornacchia, mezza morta di sete, trovò una brocca che una volta era stata piena d'acqua. Ma quando infilò il becco nella brocca si accorse che vi era rimasto soltanto un pò d'acqua sul fondo. Provò e riprovò, ma inutilmente, e alla fine fu presa da disperazione. Le venne un'idea e, preso un sasso, lo gettò nella brocca.

Poi prese un altro sasso e lo gettò nella brocca.

Poi prese un altro sasso e lo gettò nella brocca.

Ne prese un altro e gettò anche questo nella brocca.

Piano piano vide l'acqua salire verso di sé, e dopo aver gettati altri sassi riuscì a bere e a salvare la sua vita.

"A poco a poco si arriva a tutto."

Esopo

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Abstract

One of the aims of the thesis was to design and realize an *in vitro* device able to reproduce the gastrointestinal behavior.

To reproduce the temperature and pH history an USP apparatus II coupled with a control system was used. The temperature was kept constant using the USP apparatus, a pH probe was inserted in the dissolution medium to measure the pH. The measured pH was compared (by a software) with a set point. Proportionally at the mean error, a quantity of an acidic or basic solution was inserted, by pumps, in the dissolution medium adjusting the pH at the desired value. Using the real pH history of the gastrointestinal tract, which provide a decrease in the pH value from 4.8 to about 2.0 during the first two hours of dissolution, and then an increase to 6.8, the release pattern from tablets was evaluated. The release patterns of these tablets obtained with the new device were compared with those obtained using the conventional method (which provides a pH 1 during the first two hours of dissolution, and then the neutralization at pH 6.8) and it was found that the drug released during the first two hours was higher in the case in which the real pH history was reproduced. This is due to the fact that the higher pH in the first stage damages the coating of the tablet.

Once the chemical and thermal conditions were reproduced, the reproduction of the transport across the intestinal membrane was faced. An high throughput device which is able to reproduce continuously the exchange between the compartments has been necessary. The USP apparatus was equipped with a device composed by an hollow filter (which simulate the intestinal wall) and two pumps for the fluids simulating the intestinal content and the circulatory system surrounding the gastrointestinal tract content. The fluids enter in contact in the filter and the fluid rich in drug content (that simulates

the intestinal content) gives the drug to the fluid poor in drug (simulating the blood content). The release patterns obtained by the use of this device were studied and compared with those obtained following the conventional dissolution method. Moreover these release patterns obtained using the real pH evolution were coupled with the effect of mass exchange and compared with those obtained using the conventional methods. The results showed that the effect of the real history of pH is higher in the first stage of dissolution, than the effect of the mass exchange is dominant.

The reproduction of the mechanical history of the stomach is than faced. The peristaltic waves were reproduced using a lattice bag (elastic and compressible) connected to a camshaft which, with its rotation ensured the contraction of the bag. The bag was shrunk by connectors and the right position was ensured by guides. Changing the rotation speed of the shaft, the frequency of the contractions could be adjusted. The release pattern of a commercial tablet in the new device was evaluated and compared with the conventional one. The results showed that the non-perfect mixing of the stomach was satisfactory reproduced and this lead to a release pattern completely different. Moreover, the effect of the frequency of the contractions on the release pattern was evaluated.

Second, but not secondary, aim of the thesis was to develop an *in silico* model (physiologically based) which is able to simulate the plasma concentration of drugs.

The model is composed by seven compartments, which simulate the human organ, tissue, or a group of them. The compartments are interconnected between them and seven differential equations (with their initial conditions) describe their behavior. Once the parameter are obtained (by fitting or in literature), using an *in vitro* release pattern, the model is able to simulate the concentrations in all the compartments, including the plasma compartment.

The plasma concentration are simulated both in the case in which the new release pattern (with the real pH history) is used as input, and the case in which the conventional one is used. The results show that in the real case the plasma concentration is very different both in value and in shape than the expected.

The model then was used to simulate the fate of several molecules simultaneously in the human body (i.e. if a racemic mixture is

administered or if the drug is metabolized to another molecule). The system of differential equations is expanded to describe the fate of each molecule. Then, the physiological parameters, such as gender and age, were integrated in the model; in this way, the dependence of the model parameter on the physiological parameter was evaluated.

Finally, the gastrointestinal concentration simulated with the *in silico* model was successfully compared with the drug concentration measured with the *in vitro* model. It could be concluded that the combined approach which uses the *in vitro* and the *in silico* models is a powerful tool in the pharmacokinetic studies.

Publication List

Publication concerning this activities:

1. G. Lamberti, **S. Cascone**, M. Iannaccone, G. Titomanlio, "In-vitro simulation of drugs intestinal absorption", *International Journal of Pharmaceutics*, 439(1-2) 165-168 (2012).
2. G. Lamberti, **S. Cascone**, G. Titomanlio, "An engineering approach to biomedical sciences: advanced testing methods and pharmacokinetic modeling", *Translational Medicine @ UniSa*, 4 (4) 34-38 (2012).
3. **S. Cascone**, F. De Santis, G. Lamberti, G. Titomanlio, "The influence of dissolution conditions on the drug ADME phenomena", *European Journal of Pharmaceutics and Biopharmaceutics*, 79 382-391 (2011).
4. M. Grassi, G. Lamberti, **S. Cascone**, G. Grassi, "Mathematical modeling of simultaneous drug release and in vivo absorption", *International Journal of Pharmaceutics*, 418 (1) 130-141 (2011).
5. **S. Cascone**, G. Lamberti, G. Titomanlio, "A rule of thumb in designing in-vitro systems to simulate the intestinal absorption", submitted to *Heat and Mass Transfer*.

Conference proceedings concerning this activities:

1. **Cascone S.,** Lamberti G., Titomanlio G.,"Modelli in silico ed in vitro per analisi farmacocinetiche" Proceedings of *GRICU 2012* Montesilvano (PE), Italy, 16-19 settembre 2012

2. **Cascone S.**, Dalmoro A., Lamberti G., Barba A.A. "Metodi innovativi di preparazione e testing per sistemi farmaceutici", Proceedings of *GRICU 2012* Montesilvano (PE) Italy, 16-19 settembre 2012

3. **Cascone, S.**; De Santis, F.; Lamberti, G.; Titomanlio, G.; Barba, A.A.; "Alternatives to Laboratory Animals: In Vitro and In Silico Approaches", Proceedings of *8th CESPT*, Graz, Austria, September 16th-18th 2010.

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Short curriculum

Sara Cascone was born on March 23rd, 1985. She gained the high school degree in 2004 and started to study Chemical Engineering at University of Salerno. She achieved the bachelor degree in Chemical Engineering, cum laude, in 2007. Then, she obtained the master degree in Chemical Engineering, summa cum laude, in November 2009 with a project dealing with microencapsulation of biopolymer by ultrasonic assisted atomization. Since November 2009 she attends the Doctorate course in "Science and technologies for chemical, pharmaceutical and food industry" (XI cycle 2009-2012), with a project dealing with *in silico* and *in vitro* models in pharmacokinetic studies. She produced 10 papers on international journals and 10 communications to international conferences.

Papers

1. **S. Cascone**, G. Lamberti, M. Cafaro, G. Titomanlio, "Measurements of water content in cellulose derivative (HPMC) based hydrogels via texture analysis", *Carbohydrate Polymers*, 92(1) 765-768 (2013).
2. G. Lamberti, **S. Cascone**, M. Iannaccone, G. Titomanlio, "In-vitro simulation of drugs intestinal absorption", *International Journal of Pharmaceutics*, 439(1-2) 165-168 (2012).
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5. **S. Cascone**; Lamberti, G.; Titomanlio, G.; Barba, A.A.; d'Amore, M. Microencapsulation effectiveness of small active molecules in biopolymer by ultrasonic atomization technique, *Drug Development and Industrial Pharmacy*, 38(12) 1486-1493 (2012).

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