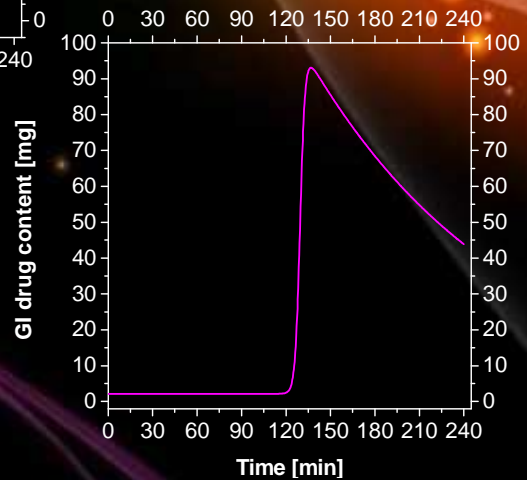
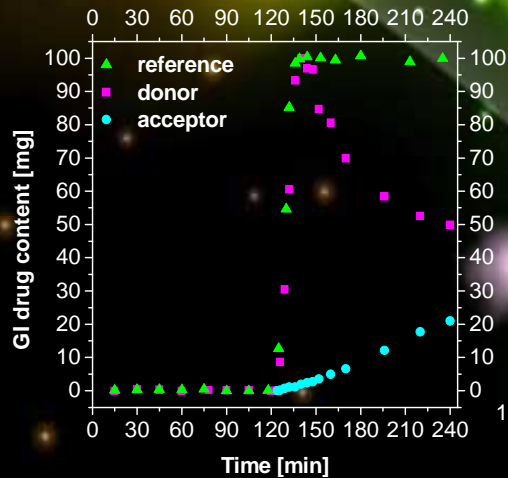


In vitro and *in silico* models in pharmacokinetic studies

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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 pool 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.
 4. **Cascone S.**; Lamberti G.; Paolucci F.; Lamberti G.; Titomanlio G.; "In vitro and in silico approaches to reproduce pharmacokinetic relevant phenomena", Proceedings of *8th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology*, Istanbul, Turkey 19-22 March, 2012
-

References

1. Brink, B., J. Schlegel, and C. Code, *The pressure profile of the gastroduodenal junctional zone in dogs*. British Medical Journal, 1965. **6**(2): p. 163.
2. Kelly, K., *Gastric emptying of liquids and solids: roles of proximal and distal stomach*. The American journal of physiology, 1980. **239**(2): p. G71.
3. MCrear, E., et al., *The normal movements of the stomach*. Experimental Physiology, 1924. **14**(4): p. 379-397.
4. Sheiner, H., *Gastric emptying tests in man*. British Medical Journal, 1975. **16**(3): p. 235.
5. Barker, M., I. Cobden, and A. Axon, *Proximal stomach and antrum in stomach emptying*. British Medical Journal, 1979. **20**(4): p. 309.
6. McConnell, E., H. Fadda, and A. Basit, *Gut instincts: explorations in intestinal physiology and drug delivery*. International journal of pharmaceutics, 2008. **364**(2): p. 213-226.
7. Weitschies, W., et al., *Magnetic marker monitoring: an application of biomagnetic measurement instrumentation and principles for the determination of the gastrointestinal behavior of magnetically marked solid dosage forms*. Advanced drug delivery reviews, 2005. **57**(8): p. 1210-1222.
8. Diakidou, A., et al., *Characterization of the contents of ascending colon to which drugs are exposed after oral administration to healthy adults*. Pharmaceutical research, 2009. **26**(9): p. 2141-2151.

9. Jantratid, E., et al., *Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update*. Pharmaceutical research, 2008. **25**(7): p. 1663-1676.
 10. Dressman, J.B. and H. Lennernäs, *Oral drug absorption*. 2000: Marcel Dekker.
 11. Grassi, M., *Understanding drug release and absorption mechanisms: A physical and mathematical approach*. 2007: CRC.
 12. Amidon, G., et al., *A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability*. Pharmaceutical research, 1995. **12**(3): p. 413-420.
 13. Banakar, U., *Pharmaceutical dissolution testing*. Drugs and the pharmaceutical sciences, 1991. **49**: p. 1-426.
 14. FDA, *Guidance for industry. Extended release oral dosage forms: development, evaluation, and application of in vitro/in vivo correlations*. Center for Drug Evaluation and Research (CDER). 1997.
 15. XXIII, U. *The United States Pharmacopeia*. 1995.
 16. Pillay, V. and R. Fassihi, *Unconventional dissolution methodologies*. Journal of Pharmaceutical Sciences, 1999. **88**(9): p. 843-851.
 17. Stricker, H., *Die Arzneistoffresorption im Gastrointestinaltrakt - In vitro-Untersuchung Lipophiler Substanzen*. Pharm Ind, 1973. **35**(1): p. 13 - 17.
 18. Savalle, B., G. Miranda, and J. Pelissier, *In vitro simulation of gastric digestion of milk proteins*. Journal of Agricultural and Food Chemistry, 1989. **37**(5): p. 1336-1340.
 19. Minekus, P.M., R. Havenaar and J. H. J. Huis in't Veld, *A Multicompartmental Dynamic Computer - controlled Model Simulating the Stomach and Small Intestine*. Alternatives to Laboratory Animals, 1995. **23**: p. 197 - 209.
 20. Minekus, M. and R. Havenaar, *In vitro model of an in vivo digestive tract*. 1996, US Patent 5,525,305.
-

21. Minekus, M., et al., *A computer-controlled system to simulate conditions of the large intestine with peristaltic mixing, water absorption and absorption of fermentation products*. Applied Microbiology and Biotechnology, 1999. **53**(1): p. 108-114.
 22. Blanquet, S., et al., *The 'biodrug' concept: an innovative approach to therapy*. Trends in biotechnology, 2001. **19**(10): p. 393-400.
 23. Cardot, J., E. Beyssac, and M. Alric, *In Vitro-In Vivo Correlation: Importance of Dissolution in IVIVC*. Dissolution Technologies, 2007. **14**(1): p. 15.
 24. K.Tam, Y. and K.E. Anderson, *Simulated Biological Dissolution and Absorption System*. U. S. Patent 6,022,733, 2000.
 25. Wickham, M. and R. Faulks, *International Publication Number WO 2007/010238*. 2007.
 26. Rozga, J. and A. Demetriou, *Artificial gut*. 2002, US Patent 6,379,619 B1.
 27. Garbacz, G., et al., *Irregular absorption profiles observed from diclofenac extended release tablets can be predicted using a dissolution test apparatus that mimics in vivo physical stresses*. European Journal of Pharmaceutics and Biopharmaceutics, 2008. **70**(2): p. 421-428.
 28. Garbacz, G., et al., *Comparison of dissolution profiles obtained from nifedipine extended release once a day products using different dissolution test apparatuses*. European Journal of Pharmaceutical Sciences, 2009. **38**(2): p. 147-155.
 29. Garbacz, G., et al., *Investigation of the dissolution characteristics of nifedipine extended-release formulations using USP apparatus 2 and a novel dissolution apparatus*. Dissol Tech, 2009. **16**: p. 7-13.
 30. BOGATAJ, M., G. COF, and A. MRHAR, *PERISTALTIC MOVEMENT SIMULATING STIRRING DEVICE FOR DISSOLUTION TESTING*. 2010, WO Patent WO/2010/014,046.
 31. Schulze, K., *Imaging and modelling of digestion in the stomach and the duodenum*. Neurogastroenterology & Motility, 2006. **18**(3): p. 172-183.
-

32. Goldsmith, H.S. and H. Akiyama, *A comparative study of Japanese and American gastric dimensions*. Annals of surgery, 1979. **190**(6): p. 690.
 33. Pal, A., et al., *Gastric flow and mixing studied using computer simulation*. Proceedings of the Royal Society of London. Series B: Biological Sciences, 2004. **271**(1557): p. 2587-2594.
 34. Pal, A., J.G. Bresseur, and B. Abrahamsson, *A stomach road or "Magenstrasse" for gastric emptying*. Journal of biomechanics, 2007. **40**(6): p. 1202-1210.
 35. Ferrua, M. and R. Singh, *Modeling the fluid dynamics in a human stomach to gain insight of food digestion*. Journal of food science, 2010. **75**(7): p. R151-R162.
 36. Ferrua, M.J., F. Kong, and R.P. Singh, *Computational modeling of gastric digestion and the role of food material properties*. Trends in Food Science & Technology, 2011.
 37. Jain, R., et al., *Kinetics of uptake, distribution, and excretion of zinc in rats*. Annals of Biomedical Engineering, 1981. **9**(4): p. 347-361.
 38. Gueorguieva, I., I. Nestorov, and M. Rowland, *Reducing whole body physiologically based pharmacokinetic models using global sensitivity analysis: diazepam case study*. Journal of pharmacokinetics and pharmacodynamics, 2006. **33**(1): p. 1-27.
 39. Nestorov, I., L. Aarons, and M. Rowland, *Physiologically based pharmacokinetic modeling of a homologous series of barbiturates in the rat: a sensitivity analysis*. Journal of pharmacokinetics and pharmacodynamics, 1997. **25**(4): p. 413-447.
 40. Yu, L. and G. Amidon, *A compartmental absorption and transit model for estimating oral drug absorption*. International journal of pharmaceutics, 1999. **186**(2): p. 119-125.
 41. Yu, L., et al., *Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption*. Advanced drug delivery reviews, 1996. **19**(3): p. 359-376.
-

42. Agoram, B., W. Woltosz, and M. Bolger, *Predicting the impact of physiological and biochemical processes on oral drug bioavailability*. Advanced drug delivery reviews, 2001. **50**: p. S41-S67.
 43. Jamei, M., et al., *Population-based mechanistic prediction of oral drug absorption*. The AAPS journal, 2009. **11**(2): p. 225-237.
 44. Jamei, M., et al., *The Simcyp® population-based ADME simulator*. 2009.
 45. Di Muria, M., G. Lamberti, and G. Titomanlio, *Physiologically Based Pharmacokinetics: A Simple, All Purpose Model*. Industrial & Engineering Chemistry Research, 2010. **49**(6): p. 2969-2978.
 46. Grass, G., *Simulation models to predict oral drug absorption from in vitro data*. Advanced drug delivery reviews, 1997. **23**(1-3): p. 199-219.
 47. Willmann, S., et al., *A physiologic model for simulating gastrointestinal flow and drug absorption in rats*. Pharmaceutical research, 2003. **20**(11): p. 1766-1771.
 48. Willmann, S., A. Edginton, and J. Dressman, *Development and validation of a physiology-based model for the prediction of oral absorption in monkeys*. Pharmaceutical research, 2007. **24**(7): p. 1275-1282.
 49. Willmann, S., et al., *A physiological model for the estimation of the fraction dose absorbed in humans*. J. Med. Chem, 2004. **47**(16): p. 4022-4031.
 50. Plusquellec, Y., et al., *A pharmacokinetic model for multiple sites discontinuous gastrointestinal absorption*. Medical engineering & physics, 1999. **21**(8): p. 525-532.
 51. Kamlet, M.J., et al., *Linear solvation energy relationships: 36. Molecular properties governing solubilities of organic nonelectrolytes in water*. Journal of Pharmaceutical Sciences, 1986. **75**(4): p. 338-349.
 52. Stewart, B.H., et al., *Discrimination between drug candidates using models for evaluation of intestinal absorption*. Advanced drug delivery reviews, 1997. **23**(1): p. 27-45.
-

53. Lennernäs, H., et al., *Comparison between active and passive drug transport in human intestinal epithelial (Caco-2) cells in vitro and human jejunum in vivo*. International journal of pharmaceuticals, 1996. **127**(1): p. 103-107.
 54. Irvine, J.D., et al., *MDCK (Madin–Darby canine kidney) cells: a tool for membrane permeability screening*. Journal of Pharmaceutical Sciences, 1999. **88**(1): p. 28-33.
 55. Di, L., et al., *Development of a new permeability assay using low-efflux MDCKII cells*. Journal of Pharmaceutical Sciences, 2011. **100**(11): p. 4974-4985.
 56. Tavelin, S., et al., *Prediction of the oral absorption of low-permeability drugs using small intestine-like 2/4/A1 cell monolayers*. Pharmaceutical research, 2003. **20**(3): p. 397-405.
 57. Linnankoski, J., et al., *Paracellular porosity and pore size of the human intestinal epithelium in tissue and cell culture models*. Journal of Pharmaceutical Sciences, 2009. **99**(4): p. 2166-2175.
 58. Kansy, M., F. Senner, and K. Gubernator, *Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes*. Journal of medicinal chemistry, 1998. **41**(7): p. 1007-1010.
 59. Avdeef, A., *Absorption and drug development: solubility, permeability, and charge state*. 2003, Hoboken: John Wiley & Sons, Inc.
 60. Wohnsland, F. and B. Faller, *High-throughput permeability pH profile and high-throughput alkane/water log P with artificial membranes*. Journal of medicinal chemistry, 2001. **44**(6): p. 923-930.
 61. Obata, K., et al., *Biopharmaceutics classification by high throughput solubility assay and PAMPA*. Drug development and industrial pharmacy, 2004. **30**(2): p. 181-185.
 62. Sugano, K., et al., *High throughput prediction of oral absorption: improvement of the composition of the lipid solution used in parallel artificial membrane permeation assay*. Journal of biomolecular screening, 2001. **6**(3): p. 189-196.
-

63. Levet-Trafit, B., et al., *Estimation of oral drug absorption in man based on intestine permeability in rats*. Life sciences, 1996. **58**(24): p. PL359-PL363.
 64. LENNERNÄS, H., et al., *A Residence-Time Distribution Analysis of the Hydrodynamics within the Intestine in Man during a Regional Single-pass Perfusion with Loc-I-Gut: In-vivo Permeability Estimation*. Journal of pharmacy and pharmacology, 1997. **49**(7): p. 682-686.
 65. Takamatsu, N., et al., *Human intestinal permeability of piroxicam, propranolol, phenylalanine, and PEG 400 determined by jejunal perfusion*. Pharmaceutical research, 1997. **14**(9): p. 1127-1132.
 66. Barba, A.A., et al., *Synthesis and characterization of P (MMA-AA) copolymers for targeted oral drug delivery*. Polymer bulletin, 2009. **62**(5): p. 679-688.
 67. Marteau, P., et al., *Effect of the microbial lactase (EC 3.2. 1.23) activity in yoghurt on the intestinal absorption of lactose: an in vivo study in lactase-deficient humans*. British Journal of Nutrition, 1990. **64**(01): p. 71-79.
 68. Cascone, S., G. Lamberti, and G. Titomanlio, *A rule of thumb in designing in-vitro systems to simulate the intestinal absorption*. Heat and Mass Transfer. **Submitted**.
 69. Cascone, S., et al., *Microencapsulation effectiveness of small active molecules in biopolymer by ultrasonic atomization technique*. Drug development and industrial pharmacy, 2012(00): p. 1-8.
 70. Barba, A.A., et al., *Simultaneous measurement of theophylline and cellulose acetate phthalate in phosphate buffer by UV analysis*. Canadian Journal of Analytical Sciences and Spectroscopy, 2008. **53**(6): p. 249-253.
 71. Cascone, S., et al., *The influence of dissolution conditions on the drug ADME phenomena*. European Journal of Pharmaceutics and Biopharmaceutics, 2011.
 72. Ikeda, S. and K. Nishinari, *Intermolecular forces in bovine serum albumin solutions exhibiting solidlike mechanical behaviors*. Biomacromolecules, 2000. **1**(4): p. 757-763.
-

73. SADLER, P.J. and A. TUCKER, *pH induced structural transitions of bovine serum albumin*. European Journal of Biochemistry, 1993. **212**(3): p. 811-817.
 74. Matsuyama, H., M. Teramoto, and H. Urano, *Analysis of solute diffusion in poly (vinyl alcohol) hydrogel membrane*. Journal of membrane science, 1997. **126**(1): p. 151-160.
 75. Smith, E.L., et al., *B12 vitamins (cobalamins). 1. Vitamins B12c and B12d*. Biochemical Journal, 1952. **52**(3): p. 389.
 76. Brennan, R.A. and R.A. Sanford, *Continuous steady-state method using Tenax for delivering tetrachloroethene to chloro-respiring bacteria*. Applied and environmental microbiology, 2002. **68**(3): p. 1464.
 77. Mudry, B., et al., *Quantitative structure-permeation relationship for iontophoretic transport across the skin*. Journal of Controlled Release, 2007. **122**(2): p. 165-172.
 78. Sangster, J., *Octanol-water partition coefficients: fundamentals and physical chemistry*. 1997: Wiley.
 79. Pade, V. and S. Stavchansky, *Link between drug absorption solubility and permeability measurements in Caco 2 cells*. Journal of Pharmaceutical Sciences, 1998. **87**(12): p. 1604-1607.
 80. Vaughan, A.D., J.B. Zhang, and M.E. Byrne, *Enhancing therapeutic loading and delaying transport via molecular imprinting and living/controlled polymerization*. AIChE Journal, 2010. **56**(1): p. 268-279.
 81. Lee, H.S., et al., *Simultaneous determination of aceclofenac and diclofenac in human plasma by narrowbore HPLC using column-switching*. Journal of pharmaceutical and biomedical analysis, 2000. **23**(5): p. 775-781.
 82. Scheytt, T., et al., *1-Octanol/water partition coefficients of 5 pharmaceuticals from human medical care: carbamazepine, clofibric acid, diclofenac, ibuprofen, and propyphenazone*. Water, Air, & Soil Pollution, 2005. **165**(1): p. 3-11.
 83. Crommlin, D., J. Modderkolk, and C. De Blaey, *The pH dependence of rectal absorption of theophylline from solutions of aminophylline in situ in rats*. International journal of pharmaceutics, 1979. **3**(6): p. 299-309.
-

84. Pinsuwan, S., A. Li, and S.H. Yalkowsky, *Correlation of octanol/water solubility ratios and partition coefficients*. Journal of Chemical and Engineering Data, 1995. **40**(3): p. 623-626.
 85. Rinaki, E., G. Valsami, and P. Macheras, *Quantitative biopharmaceutics classification system: The central role of dose/solubility ratio*. Pharmaceutical research, 2003. **20**(12): p. 1917-1925.
 86. Mauri, R., *La reologia e il flusso del sangue*.
 87. Weinreb, J., et al., *Portal vein measurements by real-time sonography*. American Journal of Roentgenology, 1982. **139**(3): p. 497.
 88. Bradley, S., et al., *The estimation of hepatic blood flow in man*. Journal of Clinical Investigation, 1945. **24**(6): p. 890.
 89. Takahashi, T. and T. Sakata, *Viscous properties of pig cecal contents and the contribution of solid particles to viscosity*. Nutrition, 2004. **20**(4): p. 377-382.
 90. Lamberti, G., et al., *In-vitro simulation of drugs intestinal absorption*. International journal of pharmaceutics, 2012.
 91. Willis, J., et al., *The pharmacokinetics of diclofenac sodium following intravenous and oral administration*. European Journal of Clinical Pharmacology, 1979. **16**(6): p. 405-410.
 92. Nishihata, T., et al., *Clinical investigation of sodium diclofenac sustained-release suppositories*. International journal of pharmaceutics, 1988. **42**(1): p. 251-256.
 93. Winek, C.L., W.W. Wahba, and T.W. Balzer, *Drug and chemical blood-level data 2001*. Forensic science international, 2001. **122**(2): p. 107-123.
 94. Gupta, S., et al., *Age and gender related changes in stereoselective pharmacokinetics and pharmacodynamics of verapamil and norverapamil*. British journal of clinical pharmacology, 1995. **40**(4): p. 325.
 95. Kim, C., *Asymmetrically coated table*. 2006, Google Patents.
-

96. Abernethy, D.R., et al., *Stereoselective verapamil disposition and dynamics in aging during racemic verapamil administration*. Journal of Pharmacology and Experimental Therapeutics, 1993. **266**(2): p. 904.
 97. Von Richter, O., et al., *Cytochrome P450 3A4 and P-glycoprotein Expression in Human Small Intestinal Enterocytes and Hepatocytes: A Comparative Analysis in Paired Tissue Specimens*. Clinical Pharmacology & Therapeutics, 2004. **75**(3): p. 172-183.
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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).
3. 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).
4. G. Lamberti, **S. Cascone**, G. Titomanlio, A.A. Barba, "Controlled release of drugs from hydrogel based matrices systems: experiments and modeling", *Chemical And Biochemical Engineering Quarterly*, 26(4) 321-330 (2012).

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).
6. **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 (2011) 382-391.
7. M. Grassi, G. Lamberti, **S. Cascone**, G. Grassi, "Mathematical modeling of simultaneous drug release and in vivo absorption", *International Journal of Pharmaceutics*, 418 (1) (2011) 130-141.
8. A.A Barba; M. d'Amore; **S. Cascone**; G. Lamberti; G. Titomanlio Intensification of biopolymeric microparticles production by ultrasonic assisted atomization. *Chemical Engineering and Processing: Process Intensification* 48(10), 1475-1481, 2009.
9. A.A Barba; M. d'Amore; **S. Cascone**; S. Chirico; G. Lamberti; G. Titomanlio. On the behavior of HPMC/Theophylline matrices for controlled drug delivery. *Journal of Pharmaceutical Sciences* 98(11), 4100-4110, 2009.
10. **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 proceeding

1. **Cascone S.**; Lamberti G.; Paolucci F.; Titomanlio G.; "In vitro and in silico approaches to reproduce pharmacokinetic relevant phenomena", Proceedings of *8th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology*, Istanbul, Turkey 19-22 March, 2012.
 2. **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.
 3. **Cascone S.**; Barba A.A.; d'Amore M.; Lamberti, G.; Rabbia L.; Titomanlio G.; "Microencapsulation of active molecules in biopolymers by ultrasound assisted atomization", Proceedings of *CHISA2010/ECCE 7*, Praha, Czech Republic, 28 August - 1 September, 2010.
-

4. Barba A.A.; d'Amore M.; **Cascone S.**; Lamberti G.; Rabbia L.; Titomanlio G.; Grassi M.; Grassi G.; "Pluronic/alginate gels in drug eluting stents preparation", Proceedings of *CHISA2010/ECCE 7*, Praha, Czech Republic, 28 August - 1 September, 2010.
 5. Barba A.A.; d'Amore M.; Rabbia L.; **Cascone S.**; Lamberti G.; Titomanlio G.; Grassi M.; Grassi G.; "Gelification of polymer blends for coating of eluting stents", Proceedings of *7th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology*, La Valletta (Malta), 8-11 March, 2010.
 6. Barba A.A.; d'Amore M.; **Cascone S.**; Rabbia L.; Lamberti G.; Titomanlio G.; "Novel microencapsulation technique of active molecules in pharmaceutical and nutraceutical preparations", Proceedings of *7th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology*, La Valletta (Malta), 8-11 March, 2010.
 7. Barba A.A, d'Amore M, **Cascone S**, Lamberti G, Titomanlio G. Microencapsulation of nutraceuticals and pharmaceuticals by ultrasonic atomization. Proceedings of *Effost 2009*. Budapest, Hungary, 11-13 November, p. 1-4, 2009.
 8. Barba A.A, **Cascone S**, Di Muria M, Lamberti G. Micro-particles by ultrasonic atomization: new strategy towards novel drug carrier. Proceedings of *CRS36*. Copenhagen, Denmark, 18-22 July, 2009.
 9. Barba A.A, d'Amore M, **Cascone S**, Lamberti G, Titomanlio G. Intensification of pharmaceuticals atomization by ultrasonic: experiments and correlations testing. Proceedings of *GPE-EPIC*. Venice, Italy, 14-17 June 2009.
 10. **Cascone S**, Chirico S, Lamberti G., Titomanlio G. Water and theophylline transport phenomena within HPMC based tablets. Proceedings of *Innovation in Drug Delivery*. Naples, 30 September - 3 October 2007.
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