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PhD Yearbook | 2022

Chair:
Prof. Carlo Alessandro Cavallotti

DOCTORAL PROGRAM IN INDUSTRIAL CHEMISTRY AND CHEMICAL ENGINEERING (CIIC)

The Doctoral Program in Industrial Chemistry and Chemical Engineering (CIIC) is designed for students aiming to get a deep expertise in research activities related to the development and design of chemical processes.

The general topic for the doctoral program in Industrial Chemistry and Chemical Engineering is the application of the chemical and physical knowledge to all the activities related to the synthesis, design, production and transformation of chemical substances and materials (like advanced inorganic chemistry, polymers and biomaterials). These studies involve not only the synthesis processes but also the related plants, here analyzed starting from laboratory tests and pilot plant experiments up to the industrial size, always including the careful evaluation of the related energetic, safety, and environmental issues.
The final goal is to provide to the PhD student the tools and the skills: (1) to design and manage industrial processes at any size scales; (2) to develop new technical applications and (3) to create and to characterize new products and services.

To provide a few examples, the research activity may be focused on one of the following topics:
• study of processes from the microscopic to the mascrocopic scale, by analyzing the thermodynamic and kinetic aspects of the process at the fundamental and applied levels;
• methodologies and criteria for process and plant design and operation, considering the choice of raw materials, costs, safety issues, and sustainability;
• design and control of unit operations and of the whole plant through mathematical modelling and computer simulation techniques;
• synthesis, characterization, process technologies, and use of substances;
• development of innovative chemical processes;
• catalysis and bio-catalysis;
• innovative energy technologies, with particular attention to energy consumption;
• biotechnology and chemistry of natural compounds.

The abstracts reported in this Yearbook provide further examples of the topics covered in the CIIC doctoral program.

Students holding “Laurea Magistrale” degrees in Chemical Engineering, Materials Engineering, Industrial Chemistry, Chemistry, and Safety and Prevention Engineering are the natural CIIC PhD students. The program is though open also to graduates from other scientific faculties.

The CIIC program covers three years during which, in addition to developing the research project, the PhD student follows courses offered by the PhD school, seminars, summer schools, and workshops. The courses offered in the CIIC program cover both soft skills as well as the some of the most advanced scientific subjects in the field of Industrial Chemistry and Chemical Engineering.

Since 2001 (XVII cycle) the CIIC program graduated more than 250 students. The number of enrolled students has risen steadily in the last 10 years, increasing from about 10 to more than 20. About ~80% of the CIIC graduated PhD students now work in industry, with the remaining being employed in the university or government research centers. These data demonstrate the important interconnection existing between the performed researches and the industrial application. In fact, during the years, numerous research topics were directly supported by industrial companies through the Industrial PhD program. Among the sponsors of these programs were Biochemtex, Bracco, ENEL, Flamma, Isagro, LPE Epitaxial Technology, Mapei, Pirelli, RSE, Solvay Specialty Polymers, Tecnimont.

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A PERIODIC DESIGN: Σ-HOLE INTERACTIONS AT WORK

Andrea Daolio – Supervisor: Prof. Giuseppe Resnati

It is no exaggeration that the IUPAC definition of Halogen Bond in 2013 represented a milestone for noncovalent interactions development, paving the way for the understanding and rationalization of a large number of inter- and intra-molecular forces. It was soon discovered, in fact, that halogens were not alone on the periodic table in establishing attractive interactions with nucleophiles, as many other groups rapidly joined the "σ-hole family". As of 2021, the chalcogen bond too received an IUPAC definition, while noble gas bond, pnictogen bond, tetrel bond and triel bond are routinely used terms to designate interactions involving atoms of the remaining groups of the *p*-block of the Table. Lately, even some evidence highlighting the role of σ-holes in transition metal compounds are being reported and published. A great number of processes and mechanisms have been elucidated based on the studies of noncovalent interactions, so that many supramolecular systems based on the polarization of covalent bonds are being used to impart specific properties to materials and drugs. It is even tempting to forecast a future where this rationale will invest most of the interactions taking place in the solid state.

The understanding of these processes as the result of the polarization of atomic species and the subsequent formation of localized areas of positive electrostatic potential on the extension of covalent bonds has come a long way too. This process was known for more than a century for hydrogen, but the explanation of the noncovalent adducts formed by halogens, while discovered almost at the same time, occurred just thirty years ago with the studies of Peter Politzer. Once the concept of halogen bond (and Σ-hole) had been established and sufficiently developed, it was recognised that Group XVIIth was not alone in expressing that behaviour, as several other atoms were known to show the same features. Around 2010, the interest in this field grew immensely: in no time, not only the geometrical and energetical characteristics of the noncovalent adducts formed by all atoms of the *p*-block were examined and clarified but exploited too. Halogen and chalcogen bond have now found relevant applications in medicinal chemistry and catalysis in the same fashion of the hydrogen bond in previous years, while known natural and industrial practices are being rediscovered and elucidated under the new light of σ-hole interactions. The body of work of my doctorate thesis is part of this field of study.

Namely, a work on adducts formed between iodoperfluoroakanes and uncommonly naked fluoride anions provides compelling evidence of how crystal engineering can benefit from this vision and how a clever design of the experimental conditions can result in the formation of peculiar and otherwise unobtainable halogen bonded adducts, a working example of the ability of such interaction to impact crystal formation and the field of crystal engineering.

A large portion of the work is also devoted to exploring a specific supramolecular synthon benzothiazole derivatives engage in and its ability to affect not only the crystalline habits of this class of compounds, but also the biological activity of these pesticides, antimicrobial agents, and drugs. Specifically (building on the previous work of our research group) I focused on benzoisothiazolinones and cyanines engaging concurrently in chalcogen and hydrogen bonds. The possibility to tune the interaction strength by slightly modifying the molecular scaffold, for example substituting the chalcogen atom sulphur or selenium, is also considered and examined.

It is known that smaller and less polarizable atoms are less prone to engage in Σ-hole interactions. Nonetheless, the ubiquitous presence of carbon in biomolecules poses the question if tetrel bond may be a factor impacting some key bioprocesses.

Our work on model systems like hexamethonium and bis-pyridinium methylene provides convincing evidence of the potential of this inherently weak force to play a role in the self-assembly of crystalline adducts. The geometrical features and implications of the distinction between tetrel bond and hydrogen bonds are discussed for both methyl (-CH_3) and methylene (-CH_2-) moieties, also thanks to a rigorous computational approach, at times making good use of the Hirschfeld Atom Refinement (HAR) method, providing both general geometrical distinction and a working method that could be applied in future works for the study of these or other moieties.

The knowledge gained through the study of chalcogen and tetrel bonds is employed in a broad investigation of some biomolecules such as choline derivatives. Given the pivotal role of many of these compounds in the biochemistry of living organism, a rigorous study of their interaction landscape is of great interest and could give more insights into their biological pathway and in the structure-function relationship of their many receptors.

The last portion of the thesis deals with the exploration of the presence of σ-hole interactions in elements owning to the *d*-block of the periodic table. Often overlooked, the expansion of the subset of σ-hole carrying atoms is of great interest because it allows to extend the rationalization of the processes transition metals take part in, possibly explaining coherently the catalytic behaviour and habits of those compounds. The last part of the work assesses the presence of localized areas of positive electrostatic potential on the back end of various polarized metal-nonmetal bonds and the possibility of such areas to engage in the formation of stable noncovalent adducts.

In order to investigate those aspects of the interactions multiple tools are employed. First and foremost, often Cambridge Structural Database, CSD (and more rarely, Protein Data Bank, PDB) surveys and data mining tools are employed to obtain insights about the key features of the interaction, as well as understanding the general behaviour and preferred partners in the synthons of interest in the solid state. In a second phase, model compounds and adducts are selected, designed and, when not available commercially, synthetized. The ability of the selected adducts to act as ×-hole interaction donor is first assessed crystallographically with the obtainment of X-Ray single crystal structures that are studied holistically and analysed in their geometrical features. The analysis of the crystal structure is often associated with a spectroscopic characterisation of the adducts, IR and NMR (^{1}H, ^{13}C, ^{17}N, ^{77}Se), are the most used techniques.

In collaboration with other research groups, often computational analyses were employed, both for selecting suitable model compounds (for example with the analysis of the electrostatic potential in the gas phase of relevant molecular species) or for gaining additional understanding of the nature and energetical features of the interactions taking place in the solid state. Between the large and ever-expanding list of suitable tools for this kind of application, Quantum Theory of Atoms In Molecules (QTAIM) analysis and Noncovalent Interaction Index (NCI) were often the preferred ones. When in need to obtain a better resolution of the position of the hydrogen atoms on X-Ray structures, Hirschfeld atom refinement (HAR) was employed in place of the widely used Independent Atom Model (IAM) in collaboration with other research groups.

A NOVEL APPROACH FOR THE NON-INVASIVE DETECTION OF PROSTATE CANCER BASED ON URINE ODOUR ANALYSIS BY AN ELECTRONIC NOSE

Carmen Bax – Supervisor: Prof. Laura Capelli

Prostate Cancer (PCa) is the fifth most common cancer worldwide and its incidence is expected to considerably increase up to 2040. Current diagnostic protocols are poorly accurate with an overall accuracy of about 58%, which results in patients' over-treatment. There is, thus, an urgent need for innovative and more accurate tools for PCa detection.
Many researches proved the existence of a correlation between urine alteration and the PCa presence, thereby suggesting to investigate urine as source of information for diagnostic purposes. The most promising results published up to now were obtained relying on trained dogs, who achieved an accuracy above 97% in discriminating urine samples from controls and PCa patients. However, trained dogs are not suitable for developing large-scale diagnostic tools, due to dogs' training costs and lack of compliance with hospital protocols.
This PhD, carried out in collaboration with the Humanitas Mater Domini Hospital in Castellanza (VA), aimed to transfer results achieved by Dott. Taverna with trained dogs to an instrumental method. The project focused on the development of an electronic nose (eNose) for the analysis of urine odour (Figure 2). 534 subjects (205 controls and 329 PCa patients) were involved in the study to define the experimental protocol, train the eNose and validate the developed predictive model. eNose training was designed according to a principle of progressive complication of the system: the population included men suffering from non-metastatic prostate cancer (i.e., PCA group) and baby premature girls, young women, and healthy men between 20 and 60 years old (i.e., Control group). The inclusion of female participants and the gradual introduction of young and older men as controls allowed evaluating the eNose responses to a progressively more complicate system that approached gradually the condition of men suffering from PCa.
The experimental protocol for urine odour analysis by eNose was defined by deeply investigating the influence of choices concerning sample preparation on the eNose diagnostic capability tby exploiting different conditions (e.g., distinction of urination portions, dynamic or static sampling, conditioning temperature and storage time), and consisted of 5 phases: Urine collection and storage at -18°C; Thawing; Urine headspace enrichment at 60°C for 1h; Static headspace extraction and modulation of moisture content; eNose analysis.
Concerning data processing, this PhD proposed a predictive model specifically developed for overcoming the problem of drift, which has up to now limited long-term eNose applications. A drift correction model based on OSC was developed and applied to the dataset, comprising urine headspaces analysed over 9 months, prior to feature selection.
Later, a double-phase classifier was built. The model firstly classifies unknown samples as control (S) or prostate cancer (PCa). Then, in case of PCa, it assesses tumour aggressiveness by differentiating among Low- (L), Intermediate- (I) or High- (H) aggressive cancers.
The classification performance achieved showed the opportunity of developing a non-invasive, reliable, and cheap diagnostic tool for PCa detection based on the analysis of urine odour. The predictive model proved the capability of the eNose to detect PCa with an accuracy of about 80%. Compared to current diagnostic protocol, the eNose prototype resulted to be considerably more powerful, especially for the specificity achieved (i.e., 71% compared to 33%) (Figure 3). Thus, the proposed tool might provide in the future an effective solution to patients' overtreatment.
A unique result achieved concerned the capability of the eNose to stage the prostate cancer with an accuracy of about 70%. It is worthy to underline that the information about cancer aggressiveness, currently provided by histopathological evaluation of the prostate tissue, is fundamental in the definition of patients' prognostic pathway. According to current guidelines, men suffering from low aggressive PCa undergo active surveillance, while patients affected by high-aggressive PCa immediately undergo surgery.

Fig. 1
Trained dog sniffing urine samples aimed at non-invasive prostate cancer diagnosis.

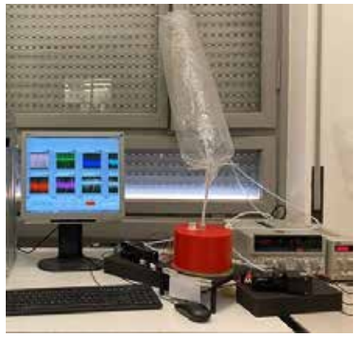

Fig. 2
eNose prototype developed within this PhD project.

To validate the developed predictive model, this PhD involved the execution of double-blind verification tests, which proved the capability of the eNose to early diagnose the PCa, even if 1-year old sensors were involved. An accuracy close to 80% was achieved, thereby confirming results achieved by a new sensor array not subject to drift. Despite the undoubtable importance of result validation by means of blind tests, it is the first time that the execution of such type of tests is reported in the literature in the field of cancer diagnosis by means of eNoses.
Results achieved led to the filing of a European patent request (EP 19160856.1) in March 2019, which has been extended to an International patent request (WO 2020/178284 A1) in March 2020.
Within this PhD, the possibility to combine eNose analysis with the chemical characterization of urine headspaces to identify compounds to be used as eNose calibrants, was also investigated, and Acetone and 4-Heptanone seemed to be suitable as specific calibrants for urine analysis. The possibility to use specific urine calibrants would simplify the selection of sensors with good sensibility towards urine headspaces, and the certification procedure for the eNose as new diagnostic tool, representing the first step for the commercialization of a diagnostic device.
To speculate about eNose use in the clinical practice, a clinical trial needs to be carried out. This multi-centric study will involve thousands of subjects, who will undergo the ordinary diagnostic procedure at the same time they will be examined by eNose, to compare the obtained results, monitoring their clinical status and evaluate if the introduction of the eNose analysis can positively affect their clinical pathway.
Another aspect that needs to be addressed before eNose may become large-scale PCa diagnostic tool is the transfer ability of prediction models.

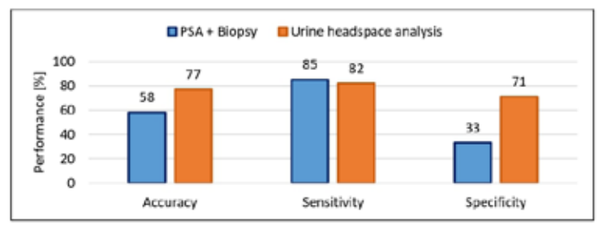

Fig. 3
Comparison of accuracy, sensitivity and specificity achieved by eNose urine headspace analysis with the current PCa diagnostic procedure (PSA + biopsy).

THE ROLE OF FLUID FLOW ON BIOFILM FORMATION BY BACTERIA ISOLATED FROM BILIARY STENTS

Cindy Lorena Cardenas – Supervisor: Prof. Roberto Rusconi

Bacterial biofilms, recognized as the most successful form of life on earth, have implications in a broad range of fields including, but not limited, to clinical microbiology, industry, and marine ecology. Biofilms have important consequences for human health because even in individuals with competent innate and adaptive immune responses, biofilm-based infections are rarely resolved.
The complex of bacteria and the extracellular matrix that they produce constitute what we refer to as the biofilm, and this new structure is up to 1000 times more resistant to antibiotics compared to bacteria in the planktonic state.
In this context, biofilms prevention is challenging because the mechanisms behind biofilm formation are not completely understood. Traditional microbiological techniques study bacteria in static and uniform conditions at the population scale, that do not mimic the actual physiological environments of biofilms. Microfluidics instead offer the current needed mechanism to study biofilm formation in dynamic conditions by enabling observations at high spatial and temporal resolution in carefully controlled microenvironments. In this work, the potential of microfluidics was exploited to investigate how fluid flow influences biofilm formation processes by clinically relevant bacteria.
It was designed and optimized a microfluidic platform to examine bacterial responses to different shear rate and shear stress conditions as well as various nutrient concentrations. Specifically, bacterial attachment kinetics and extracellular polymeric substances secretion at the population-scale were monitored using phase-contrast and epifluorescence microscopy in controlled fluid flow environments. Biofilm formation experiments were performed at different flow for 'strong biofilm-former' strains associated with biliary stents, which naturally face a fluid dynamic environment. The relevant strains were identified in a clinical study performed in collaboration with Humanitas Research Hospital. The main bacteria genera found were Klebsiella, Escherichia, Enterococcus, Enterobacter and Citrobacter, and the strains associated with major postoperative complications were *Enterobacter cloacae, Klebsiella oxytoca*, and *Citrobacter freundii*. It was found that hydrodynamic stresses stimulate bacterial organization, quantified through a lacunarity analysis and the production of extracellular DNA on a surface compared to the static condition. Additionally, conditions characterized by a high flow rate and nutrient depletion accelerate extracellular polymeric substances secretion despite a lower coverage of the confining surface. These results represent a first step towards the understanding of mechanisms for bacterial survival and proliferation in clinical environments.
These data then pushed the project towards studying whether the production of extracellular substances under flow may trigger the development of threadlike biofilms, known as streamers. It is described the capacity of strains associated with biliary stents to form thread-like biofilms, in microfluidic channels with turns or with the presence of a pillar at the center of the channel. All the strains of interest showed the ability to form streamers. Finally, the microfluidic set-up was used to evaluate the antibiofilm effect of different formulations in collaboration with the group of Prof. Maria Rescigno at Humanitas. Two postbiotics and two controls were analyzed by identifying their interaction with three bacterial strains. All the tests were carried out for each bacterial strain using different strategies to recognize whether the postbiotic affects bacterial attachment or biofilm growth. Also, dose-response as well as the duration of the treatment were evaluated and quantified. This microfluidic approach allows understanding the working principle of these new treatments and studying each component separately.

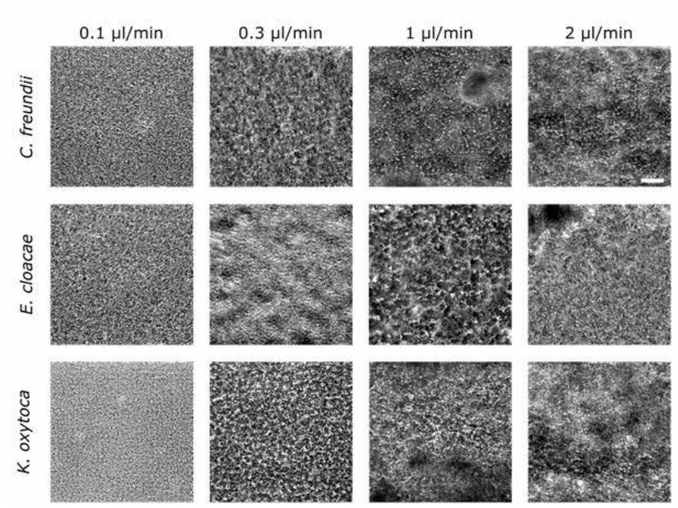

Fig. 1
Phase contrast images of Citrobacter freundii, Enterobacter cloacae and Klebsiella oxytoca after 16 hours of growth in a microchannel (H=115 µm and L=800 µm) with a constant flow of tryptone broth at different flow rates of culture media. Scale bar 100 µm.

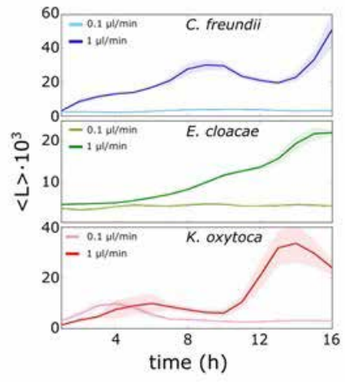

Fig. 2
Weighted lacunarity values (L) reported as ×10^-3.: images of Citrobacter freundii, Klebsiella oxytoca, and Enterobacter cloacae at 1 µl/min and 0.1 µl/min taken during 16 hours of culture.

ADDUCTS OF OXY HYDROXIDES AND PYRROLE COMPOUNDS AS FILLERS FOR ELASTOMERIC NANOCOMPOSITES

Daniele Locatelli - Supervisor: Prof. Maurizio Stefano Galimberti

Elastomer composites play a key role in the human life. They cover a wide span of applications and cannot be replaced by any other type of materials.
Elasticity is the key property of elastomeric materials. Charles Goodyear said: "There is probably no other inert substance the properties of which excite in the human mind an equal amount of curiosity, surprise and admiration". However, elastomer composites would be almost useless without mechanical reinforcement (possibly achieved with reinforcing fillers). Nowadays, the most common reinforcing fillers are carbon black and precipitated silica.
Precipitated silica, that is considered as an oxy-hydroxide inorganic filler, is able to confer lower hysteresis to the rubber composites for tires. The reduction of the hysteresis leads to lower dissipation of energy and thus to lower rolling resistance for a tire and, consequently, a lower fuel consumption and a lower impact on the environment, in terms of CO_2 emission and carbon footprint.
To reduce the hysteresis of rubber composites, the filler has to be homogenously dispersed into the rubber matrix and has to be connected with the polymer chains through chemical bonds. In order to obtain homogeneous dispersion of silica (hydrophilic filler in general) into rubber matrix, coupling agent has to be used. In particular, coupling agents are molecules able to react through covalent bond with both filler and rubber matrix. The covalent reaction between filler and rubber confer to composites lower hysteresis and dissipation of energy.
Nowadays, in the tire industry, silanes are the molecules used as coupling agents for precipitated silica. The most used chemical substance is bis(triethoxysilylpropyl)tetrasulfide (TESPT). The Si-OR groups of TESPT react with the silanols of silica through the so called "silanization reaction" (Fig.1). The sulphur atoms take part in the vulcanization reaction and promote the polymer-silica chemical bond, by means of sulphur bridges. However, silanization reaction show many problems: low reaction yield (from 52 % to 72 %), possible occurring of side reactions and the release of ethanol, which is the co-product of the silanization reaction. Industrially, in tire factories, the ethanol produced has to be burned, thus releasing CO_2 into the atmosphere. As reported in the web, the global market for tires is forecast to reach 2.7 billion units by 2025 (average mass of tire equal to 10 kg), that correspond to 87,3 tons of CO_2 released in the atmosphere (by considering 8% of TESPT with respect to silica). In order to reduce the large environmental impact of CO_2 released, innovative coupling agents between the inorganic filler and the elastomer chains were used in this PhD research: pyrrole compounds, whose chemical structure is in Fig.2.
The functionalization of the inorganic oxy-hydroxides occurred with high yield: 92 % and 83 % for silica and sepiolite, respectively. The pre-industrialization process for the preparation of the adduct between silica and pyrrole compounds was done. In particular, for the adduct silica/SP, a spray dry technique was found suitable for kilograms scale production. In the case of APTESP, a water-based procedure was used, operating at low temperature.
The mechanism for the formation of the silica/SP and silica/APTESP adducts was studied. In the case of APTESP, the chemical reaction occurred between the Si-OEt of APTESP and the silanols of the inorganic filler.
In the case of SP, the chemical reaction occurred between the C-OH of SP and the silanols of the inorganic filler. Hence, in the case of SP, the adduct was obtained with high atom economy, high functionalization yield and without ethanol emission. In the case of SP, a lower amount of ethanol, with respect to the traditional silane TESPT, was released.

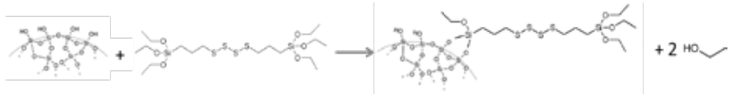

Fig. 1
Silanization reaction.

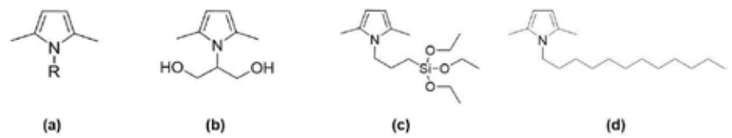

Fig. 2
Chemical structure of: (a) pyrrole compound; (b) 2-(2,5-dimethyl-1H-pyrrol-1-yl)propane-1,3-diol (SP); (c) 2,5-dimethyl-1-(3-(triethoxysilyl)propyl)-1H-pyrrole (APTESP).

For both the adducts, the reactivity of the pyrrole ring with sulfur was preserved after the functionalization reaction.
Functionalized oxy-hydroxides inorganic fillers with pyrrole compounds were used as reinforcing fillers in rubber composites based on diene elastomers and crosslinked with a sulphur-based system.
Silica/SP and silica/APTESP adducts were compared with the traditional silica/TESPT system. Sepiolite/PyC adduct was used in partial replacement of silica/PyC.
The composites filled with silica/PyC adducts have shown similar or better dynamic-mechanical properties with respect to the corresponding composites with silica and TESPT.
The same results were also obtained by using functionalized sepiolite with pyrrole compounds as reinforcing filler in rubber composites. By using functionalized sepiolite/PyC in silica-based rubber composites as partial replacement of silica, high reinforcement and lower dissipation of energy was obtained with respect to the silica-based rubber composites with the system sepiolite/TESPT.
These results can be explained with the ability of the pyrrole compound, a *Janus* molecule, to react with the silanol groups of the inorganic oxy-hydroxide and with sulfur during the vulcanization.
It can be thus concluded that a pyrrole compound such as SP and APTESP acts as a coupling agent between an inorganic oxy-hydroxide and an unsaturated elastomer. The pyrrole compound as coupling agent allows at least to reproduce the properties of traditional silica-based compounds used at the industrial scale, reducing their environmental impact, thanks to the elimination of ethanol release, at least in the case of SP (Fig.3).

		Silica/TESPD	Silica/SP
For 100 kg of rubber composite	Alcohol emission	0.39 kg	0
	CO_2 emission	0.74 kg	0
For 1 ton of rubber composite	Alcohol emission	3.87 kg	0
	CO_2 emission	7.40 kg	0

Fig. 3
Concept of "green tire" by using pyrrole compounds as coupling agent

NOVEL TECHNOLOGIES FOR H_2S VALORIZATION

Elvira Spatolisano - Supervisor: Prof. Laura Annamaria Pellegrini

The growing energy demand, together with the depletion of sweet gas reservoirs, impose the monetization of ultra-sour natural gas fields with a high H_2S content. Due to the increasing sulphur concentration in processed oil and gas together with the stricter environmental regulations, hydrogen sulfide is becoming a critical issue to handle. To date, H_2S is removed from natural gas through amine washing and it is converted to sulphur in the Claus process. The Claus process is the leading H_2S conversion technology for large-scale applications. Regarding small-scale facilities, scavengers are the most efficient and widely spread choice. On the other hand, present middle scale options show quite high operating costs. Therefore, research efforts are devoted to developing new intermediate scale alternatives with lower costs and easier operability. These novel alternatives, intending as "novel" those technologies not yet marketed but still at the development stage, are often aimed at the simultaneous H_2S abatement and its conversion to valuable chemicals. Among them, the HydroClaus technology, patented by Eni S.p.A., deserves attention. The process aims at converting H_2S and SO_2 into a hydrophilic mixture of sulphur and sulphur-rich compounds, the polythionates (Fig.1), to be used as a fertilizer and soil improver. Polythionates are extremely valuable chemicals, since they can find applications in several fields (i.e., solutions for chemical milling of magnesium and its alloys; lubricants – coolants for metal machining; eluting agents in gold leaching processes; fertilizers for alkaline soils). Polythionates' kinetics in the Wackenroder reaction, on which the HydroClaus process is based on, has been analyzed.
The influence of operating conditions, i.e., temperature and residence time, on the system has been investigated through experimental data collected in a bench scale reactor. The experimental apparatus behavior has been assessed by means of a step tracer experiment. As the reacting system is a gas-liquid one, the controlling regime has been identified thanks to a qualitative analysis of the available tests. Due to the very complex nature of the reacting system and the large number of chemical species involved, a single and two-lumped models have been considered to describe the phenomenon. Studied the system's kinetics and in view of the process scale up to the industrial level, some critical issues detected at the bench scale have been deepened, as the reactor fouling, the CO_2 management and the process water management. Regarding the reactor fouling, an ad-hoc experimental campaign has been performed through an off-line glass-made apparatus, to understand the fouling causes and suggest possible solutions. Concerning the CO_2 management, to determine its effect on the process performance and establish whether any purification of the feed stream is necessary, different configurations have been proposed through the Aspen Plus V9® simulator software. The outcomes show that the CO2 presence in the feed stream does not hinder the normal process operation. The HydroClaus reaction is CO_2 tolerant, being the carbon dioxide inert in the reaction zone. The carbon dioxide effect is related to the downstream separations only and its optimal inlet content can be opportunely tuned after a suitable sensitivity analysis, for which the present study is intended to pave the way.
In addition, an improved configuration able to solve the issue related to the management of process water has been proposed. After an explorative experimental campaign at the bench scale, a process scheme has been set up and its performances have been discussed in terms of heat and material balances and CO_2 emissions. Results reveal that the modified HydroClaus can be a valid solution for an effective H_2S valorization. The technology shows negative CO_2 emissions, thus being carbon-negative in the wider plant context. Moreover, since only electric power is required, a further reduction of the equivalent CO_2 emissions is expected, if renewable sources can be exploited for the purpose.
Together with the HydroClaus, as an alternative to the H_2S valorization (Fig.2), processes for the simultaneous H2S valorization and hydrogen production have been analyzed, focusing on the Hydrogen Sulfide Methane Reformation (HSMR) and the non-thermal plasma, which show the highest readiness level. A pre-feasibility study has been developed for the non-thermal plasma, given the technology maturity and the lack of application of plasma reactor at the industrial scale. On the other hand, hydrogen sulfide methane reformation is based on traditional chemistry and equipment, which facilitate its scalability. Hydrogen Sulfide Methane Reformation major concerns are the possible coke formation in the reaction zone and the lack of active and selective catalysts. For the reaction phenomena understanding, a deep thermodynamic analysis has been performed to explore the system behavior as a function of temperature, pressure, and inlet feed composition. In this way, the optimal process operating conditions to avoid carbon lay down have been identified. Assessed the system's thermodynamics, a preliminary process scheme has been developed and simulated in Aspen Plus® V11, considering hydrogen production and its distribution in pipeline with methane. Its performances have been evaluated as a function of the CH_4/H_2S inlet molar ratio. Material and energy balances are presented for each considered case, together with a detailed process economic assessment. Results in terms of hydrogen cost show the strong system's dependence on the CH_4/H_2S inlet molar ratio. If opportunely optimized, the process can be competitive with respect to the traditional methane steam reforming, also considering that no direct CO_2 emissions are produced. The advantages of the H_2S methane reformation are discussed, to pave the way for future process optimization.

Fig. 1
Fresh HydroClaus reaction product, with a focus on the micelle structure in the hydrophilic micellar colloids.

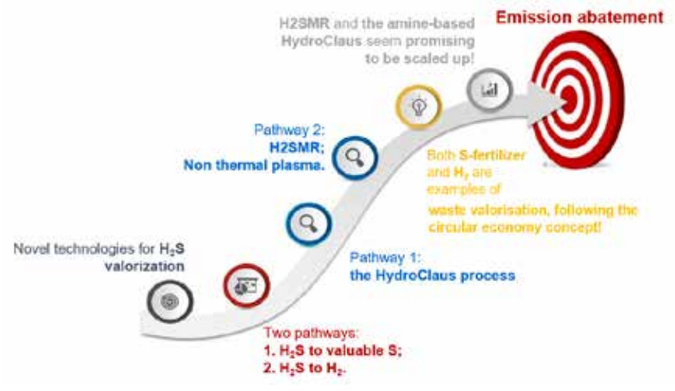

Fig. 2
Pathway followed during the PhD Project.

DISCONTINUOUS PROCESS SAFETY: PASSIVE PROTECTIONS AND TRANSITION TO CONTINUOUS PROCESSES

Federico Florit - Supervisor: Prof. Renato Rota

The chemical process industry has evolved during the years from the sole production of commodity chemicals to more specialised products. Discontinuous (batch) reactors were mainly used to produce fine chemicals with a high added value, such as pharmaceutical intermediates and polymers. Nowadays, the demand of these chemicals has increased and industries want to produce them more efficiently or in larger quantities. Therefore, attention is drawn to safety issues which may arise because of the larger volumes involved in the reaction processes using discontinuous reactors, which suffer from poor thermal efficiencies. Proper safety devices/measures must be included in the reactor design to face accidental scenarios originating from possibly undesired, exothermic reactions, which could lead to runaway (loss of thermal control). These methods can be grouped in means called "passive protections", those countermeasures which take place once the accidental scenario is happening. Another solution is to design new processes which are intrinsically safer, by exploiting reactors that are more thermally efficient and possibly with reduced volumes. This can be done by shifting the discontinuous process to a continuous one, as done in the so-called "process intensification". Continuous reactors involve smaller volumes with respect to discontinuous ones and also gain the advantage of producing chemicals with a constant quality (no batch-to-batch differences). The transition from discontinuous to continuous processes belongs to the "inherent safety measures", those protections which are applied at the design phase, to make the system intrinsically safer. Even though inherently safer measures are more efficient than passive protections, small industries may not be able to afford a process revamping/redesign, thus both protection methods are of interest to industries.

The design of protections can be done in several ways. The use of phenomenological models can greatly help the understanding of the physical-chemical processes involved in the studied system, providing reliable predictions of the system behaviour under different conditions (even during accidental scenarios), but requiring a large amount of information. For this reason, these methods can be called "data-intensive". The information required by such models can be obtained experimentally, but often with infeasible times (and costs) for industries, which prefer to rely on rules of thumb. Consequently, the knowledge of the phenomena involved in many industrial processes is limited.

The aim of this research project is to develop new protection methods requiring few information, with easy applicability by the industrial practitioner. These methods will be based on phenomenological models (thus obeying the physical constraints of the system) but will not require as much information as the original models. For this reason, these methods will be called "data-meagre". Regarding passive protections, a data-intensive model for a fire accidental scenario (jet fire impingement on a batch reactor, triggering runaway as a domino effect) is developed to determine the maximum intervention time within which some countermeasure should be taken. A data-meagre method for the design of emergency relief systems is also proposed, in order to quickly design the filling level of the reactor which guarantees a safe discharge of the contents using the pre-installed emergency system in the case of a runaway accidental scenario.

The amount of information required by the first method is way larger than the second method: the former requires the complete knowledge of physical, chemical, and geometrical properties of the system, while the second requires few, easily-measurable quantities.

For what concerns inherently safer designs, a data-intensive model is developed for the polymerisation of acrylic acid in a semi-batch reactor, which is then converted into a (safer) continuous process. A method for the kinetics-free transition of an existing semi-batch reactor into a continuous one (using a tubular reactor with distributed and local injections) is developed. This method is effectively able to transform any discontinuous process into a continuous one without requiring the user to know the chemical kinetic details of the synthesis involved. The method is accompanied by a study on the proper design of a coiled, tubular reactor (in terms of residence time distribution) in order to provide a practical tool for the batch-to-continuous transition.

A data-meagre method for the exploration of a chemical design space is developed to provide a simple mean to develop a new process in a continuous fashion, by exploiting transient experiments in a tubular reactor. This last method can thus be used to obtain a good indication of the optimal reaction conditions of a chemical process.

The full modelling of the process required much information (regarding the reaction mechanism) and many experiments for the determination of the kinetic parameters. On the contrary, the developed methodology for the batch-to-continuous transition requires very few, easily measurable quantities. The developed method for the design space exploration does not require any information regarding the chemical system and thus becomes a very powerful tool for optimisation in practice.

The comparison outlined in this research shows how the use of data-meagre methods can be an effective tool for industrial practice. The demand of fewer information can help industries saving time (and money) and thus protecting their processes according to needs.

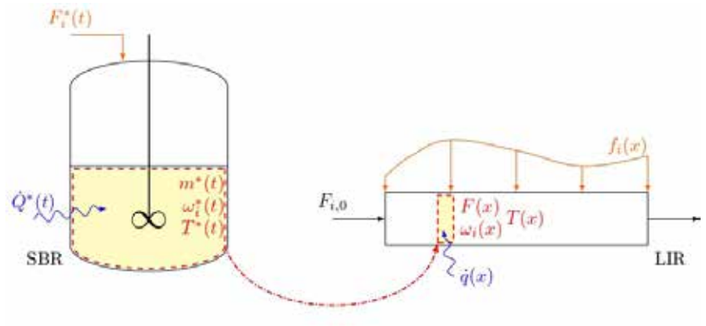

Fig. 1
General diagram for the transformation of a semi-batch reactor into a lateral-injection reactor.

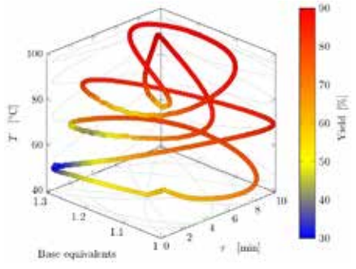

Fig. 2
Example of chemical design space exploration for the evaluation of the effect of reactant equivalents, reaction time, and temperature on the yield of the desired product.

BIOMOLECULE-FRIENDLY GRAFTING-FROM ATOM TRANSFER POLYMERISATIONS TO CONTROL THE MOLECULAR ARCHITECTURE OF PROTEIN-POLYMER CONJUGATES

Filippo Moncalvo - Supervisor: Prof. Francesco Cellesi

The impact of therapeutic proteins in healthcare is steadily increasing, due to advancements in the field of biotechnology and a considerable understanding of several pathologies. However, the safety and efficacy of their administration is often limited by poor pharmacokinetics, short circulation half- life, denaturation and immunogenicity. The conjugation of biocompatible polymers to proteins represents a successful strategy for overcoming these limitations.
In this thesis work, a site-specific protein modification by Activator ReGenerated by Electron Transfer (ARGET) Atom Transfer Radical Polymerization (ATRP) was investigated to obtain proteins conjugated with hydrophilic biocompatible polymers of predetermined molecular weight and topology. The main goal was to develop a synthetic platform to generate a new class of protein-polymer conjugates, for half-life extension of biotherapeutics. The novelty lies in the investigation of poly(glycerol monomethacrylate) (PGMA) as PEG alternative for half-life extension, as well as in the synthesis of a library of new multifunctional ATRP initiators to obtain PGMA and PEG brushes with linear or two-arm architectures, aiming to investigate the effect of polymer composition, molecular weight and topology on protein activity and stability.

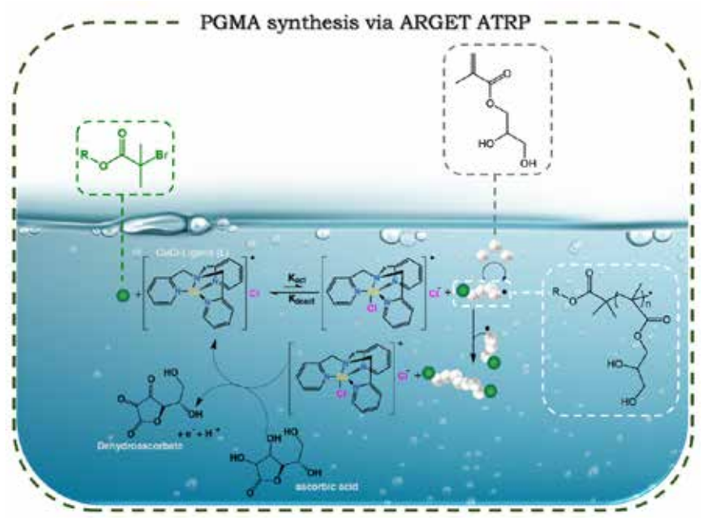

Fig. 1
ARGET ATRP of GMA in phosphate buffer.

A biologically friendly approach was firstly developed for the synthesis of PGMA through aqueous ARGET ATRP, under organic solvent-free conditions which are well tolerated by active proteins (Fig.1). Secondly, PGMA of different length and architecture (linear and two-arm) was compared with poly (poly (ethylene glycol) methyl ether methacrylate) (PPEGMA) for the synthesis of lysozyme-polymer conjugates. Mono- and two-arm functional ATRP initiators were designed and selectively attached to lysozyme at N-terminus, via reductive amination (Fig.2). The enzymatic activity and stability in serum and trypsin showed a clear dependence on the type of repeating unit and on the macromolecular architecture, confirming the potential of PGMA as a PEG alternative for half-life extension of biotherapeutics.
Finally, the aqueous ARGET ATRP of GMA was extended to the synthesis of polymeric sacrificial initiators, drug-polymer conjugates and engineered surfaces of carbon nanotubes. Preliminary results showed the versatility of this approach for biomedical application.

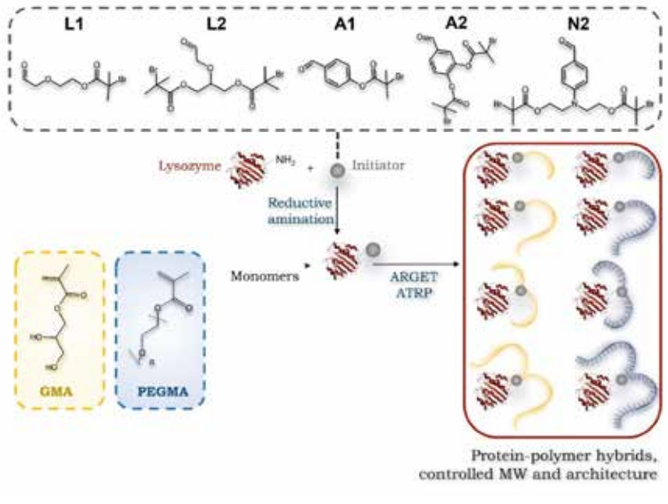

Fig. 2
Synthesis and characterisation of Lysozyme-PGMA and Lysozyme-PEGMA Conjugates.

A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL TO IMPROVE AND EXTEND THE CLINICAL ADMINISTRATION OF DRUGS TO PATIENTS WITH IMPAIRED RENAL FUNCTION

Giuseppe Pesenti - Supervisor: Prof. Davide Manca

Pharmacotherapy is one of the most common therapies in medicine and consists in using pharmaceutical drugs to prevent, treat, and cure diseases. When prescribing or administering a drug to a patient, doctors must determine the dosage that will achieve the desired therapeutic effect. Since drug overdose can lead to toxic, potentially life-threatening side effects, dose selection is critical in clinical settings, and finding the optimal dose is an essential aspect of pharmacotherapy. Due to the significant degree of inter- and intra-individual variability of their pharmacokinetic response, however, standard dosing protocols are not suitable for every patient. This variability is connected to the physiological processes that determine the individual pharmacokinetic trends, i.e., absorption, distribution, metabolism, and excretion (ADME processes).

For drugs that are mostly renally excreted (*i.e.*, about 31% of approved drugs), elimination from the human body mainly depends on renal excretion. Renal function declines with age due to progressive glomerulosclerosis and nephrosclerosis, and elderly people usually suffer from a varying degree of renal impairment. Furthermore, the decline of renal function (*e.g.*, due to kidney diseases, diabetes, poisoning, vascular damage) eventually leads to chronic kidney disease and renal failure. As a consequence, renal impairment is a very common condition and about 10% of the general world population suffers from the loss of more than half of the normal kidney function. In case of kidney impairment, renally excreted drugs will have a slower elimination rate, leading to sustained high blood levels and potentially toxic effects. In this case, finding an appropriate dosage becomes critical, and standard dosing protocols employed in clinical settings are often suboptimal. Drug dosing errors in this population are therefore common, leading to adverse effects, additional healthcare costs, and reduced life quality and life expectancy.

Pharmacokinetic models describe the drug material balances and ADME processes and can be used as clinical support tools to suggest individualized, optimal dosages according to each patient's characteristics. While these models are widely applied in the pharmaceutical industry for drug development studies, their adoption in clinical settings has been so far slow and limited.

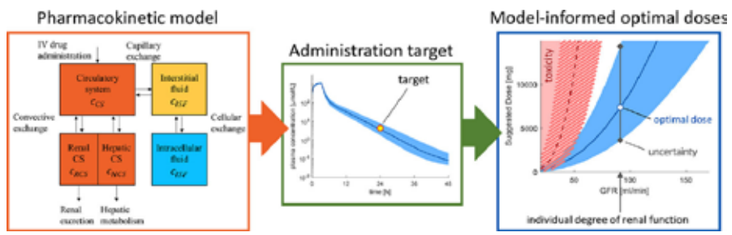

Fig. 1
The pharmacokinetic model (left) describes drug administration, its distribution and exchanges within the model compartments, and its excretion and metabolism. The model can predict individualized concentration trends (center) according to the specific features of an individual. If an administration target is defined, the model can be applied to suggest model-informed optimal does (right) and the associated uncertainty.

The goal of this study is the development of a predictive pharmacokinetic model for the intravenous administration of renally excreted drugs in adults, and the application of this model as a clinical support tool able to suggest individualized optimal dosages in patients with varying degrees of renal function. The study was carried out in collaboration with the Unit of Lymphoid Malignancies, Department of Onco-Hematology at IRCCS Ospedale San Raffaele, Milan, Italy.

The investigation considers the case study of high-dose methotrexate administration to develop an innovative minimal physiologically-based pharmacokinetic model with a detailed description of renal excretion that accounts for the physiological processes of glomerular filtration, secretion, and reabsorption. The model features dedicated compartments for plasma, interstitial fluid, and intracellular fluid, and extensively characterizes the capillary and cellular exchanges among them. The specific characteristics and clinical data of each patient (*i.e.*, body weight, height, age, sex, serum creatinine, and hematocrit) allow estimating *a priori* the individualized values of volumes, flows, and other pharmacokinetic parameters for each patient. Organs and tissues of the human body are lumped to reduce the complexity of the model and keep the number of adaptive parameters as low as possible. Only four parameters, which characterize capillary and cellular exchanges, cannot be assigned *a priori* and are determined through nonlinear regression.

The model is successfully identified and validated using a large experimental dataset from the literature, using bootstrap analysis and a population approach. A comparison with a pharmacokinetic model from the literature demonstrates improved predictions, which better capture the wide intra- and inter-individual variability of high-dose methotrexate and do not present significant bias in a wide range of degrees of renal function. The model appears suitable to describe the pharmacokinetic dose-response in patients ranging from healthy to functionally impaired.

Next, the model is applied to develop a clinical decision-support system to improve drug administration in clinical settings. The model allows obtaining model-informed optimal doses according to different administration targets while performing a concurrent assessment of methotrexate toxicity risk. Monte Carlo analysis allows estimating the uncertainty associated with both suggested doses and toxicity thresholds. Model-informed optimal doses obtained for patients with varying body weights and degrees of renal function highlight the major role played by the individual degree of renal function.

Results confirm findings of other literature studies and indicate that standard high-dose methotrexate protocols lead to suboptimal treatment in patients with varying renal function, such as the functionally impaired. This demonstrates how pharmacokinetic models can support clinical drug administration by identifying room for improvement in current drug dosing and guiding the design of improved personalized clinical treatments.

This work outlines and demonstrates a general approach for the development and application of pharmacokinetic models to improve and extend the administration of renal-excreted drugs to clinical patients with varying degrees of renal function.

NANOCELLULOSE: PRODUCTION AND APPLICATIONS OF AN INNOVATIVE ECO-SUSTAINABLE RESOURCE

Laura Riva - Supervisor: Prof. Carlo Punta

Nowadays, nanocellulose is considered a great innovative material for the development of a wide range of advanced applications. Nanocellulose-based materials are also attracting an increasing interest for the positive role they could play in sustainable development, being originated from renewable resources. Moreover, cellulose is characterized by a high potential of recycling from both post-consumer waste and industrial waste. All these factors result extremely favorable also in the perspective of circular economy.
These premises were the key points for the development of my Ph.D. project, whose main objectives were definitely two: the production of cellulose nanofibers and their application in several fields.
For the production of cellulose nanofibers, I explored the combinations of enzymatic and chemical pre-treatments with different mechanical treatments. The choice of these processes was the result of the combination of a careful analysis of the literature, the skills concerning oxidative systems and polysaccharides of the research group where I did my Ph.D., the O^{SCM}Lab, and the chemical-mechanical competences in the field of paper industry of Innovhub SSI - Area Carta e Cartone, the other institution supporting my Ph.D.
After their production, I also performed a careful characterization of cellulose nanofibers from the point of view of environmental impact through a Life Cycle Assessment analysis in collaboration with Mat4En2 group of Politecnico di Milano.
Regarding the applications, the goal of this Ph.D. project was to use the produced nanofibers in the most virtuous way possible in different fields.
The areas in which I tried to develop the use of cellulose nanofibers synthesized starting from virgin and waste sources were: the field of green building and the synthesis of nanostructured materials in which nanocellulose played a key role as a building block.
Concerning the application in the bio-building sector, I used cellulose nanofibers as an additive in non-renewable matrices (cements and blast furnace slags) and in renewable building materials (raw earths), analyzing their behavior in different building materials and trying to understand how the source and the treatment performed to produce the nanofibers could influence the performance of these renewable additives.
Regarding nanostructured materials, the aim of this second part of the Ph.D. project focused on the synthesis and the applications of cellulose-based aerogels produced starting from TEMPO-oxidized cellulose nanofibers and branched polyethyleneimine. These nanosponges, previously synthesized within the O^{SCM}Lab, have shown good mechanical, morphological and metal adsorption properties. So, the central goal was to exploit their potentialities exploring different fields.
Considering the good performances of cellulose-based nanosponges in adsorbing heavy metals, I thought to consider a similar application for these cellulose-based materials by removing dyes from wastewaters. We tested cellulose-based nanosponges as potential sorbents for organic dyes, comparing these alternative sorbents with the most common activated carbons. The sorption performance was tested on four commercial dyes (Orange Sodium Salt II, Brilliant Blue R, Naphtol Blue Black, and Cibacron Brilliant Yellow), differing for both molecular dimension and the number of sulfonate groups. The sorbent was effective also at a slightly basic pH, even if under these conditions the nanostructured sponge was negatively charged. This result suggested that the sorbent-solute interaction should not be simply ascribed to electrostatic attraction between opposite charges, but other intermolecular interactions could occur. The role of bPEI in the network was crucial, as cellulose alone was not able to reproduce significant sorption. Isotherm and kinetic investigation, modelled with Langmuir and pseudo-second order equations respectively, revealed a molecular-size dependence of sorption performance, as the smallest dye was much more trapped on the material. Nevertheless, these studies also showed that the strength of sorbent-solute binding was higher when two or more sulfonate groups were present on the dye. This evidence was also confirmed by conducting regeneration and reusability tests, as once again the smallest dye was much more easily removed from the nano-sponge under alkaline conditions, so that the sorbent system could be reused several times, by maintaining its sorption efficiency.
Another field explored during my Ph.D. project was the use of cellulose-based nanostructured aerogels as heterogeneous sensors for the detection of fluoride anions. These materials were tested by dipping them into a DMSO solution containing the target analyte. Interestingly, the sponge-like materials were able to recognize with high selectivity the presence of fluoride by the *naked-eye* over common interfering species. Effective sensing of fluoride was obtained up to about 0.05 M concentration and another important property of these materials was that they can be easily reused for several times without apparently losing any sensing ability by washing them with deionized water and methanol.
The last application explored for these nanocellulose-based systems was related to heterogeneous catalysis. In recent years, heterogeneous cellulose-based catalytic systems have been developed for different chemical reactions. Following this trend, I exploited the cellulose-based nanosponges as catalytic systems. Considering their high versatile properties, especially their micro and nano-porosity, and the considerable amount of amino groups on the aerogel's surface, I predicted that these nanostructured cellulose-based materials could also be used as heterogeneous solid catalysts for amino-catalyzed reactions, choosing Knoevenagel and Henry reactions as a model of study. The results were encouraging: Knoevenagel reaction in presence of our cellulose-based catalyst generally gave excellent conversions (80% - 100%) using water and methanol as reaction solvents. Henry reaction also gave very good conversions. Moreover, in this last case, by varying the solvent and the temperature of the reaction, a different selectivity was obtained. Both reactions gave excellent yields also using aliphatic aldehydes as reagents; in the case of Henry reaction also aliphatic and aromatic ketones shew good reactivity. For the Henry reaction, re-use tests were performed, demonstrating that the cellulose-based nanosponges could be re-used as catalyst several times after washing in organic solvents, still obtaining good conversions.

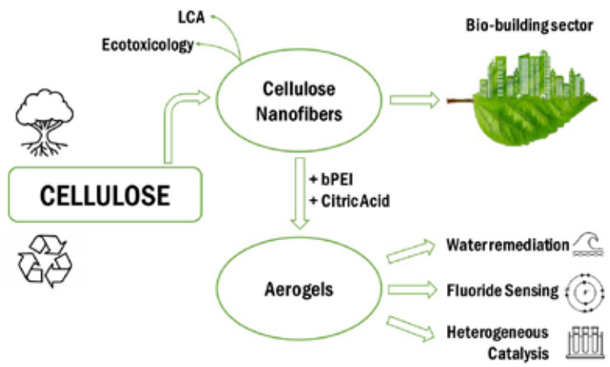

Fig. 1
General scheme of the Ph.D. project, focused on the production of cellulose nanofibers from virgin and recycled sources and their application in several innovative fields.

CHARACTERIZATION OF BIOLOGICAL FLUIDS PROTEINS INVOLVED IN THE INTERACTION WITH FUNCTIONALIZED CARBON NANOTUBE USING PROTEOMIC APPROACH

Maria Nicoletti - Supervisor: Prof. Elisa Fasoli

The field of 'Nanomedicine' is the medical application of nanotechnology for diagnosis, treatment and prevention of disease and traumatic injury, improving human health through molecular tools and knowledge of the human body.
In the last decades, the interest on Carbon Nanotubes (CNTs) has raised in biomedical applications, due to their impressive structural, optical, mechanical, and electronical proprieties.
Carbon nanotubes (CNTs) are allotropes of carbon, formed by one or more cylindrical tubes of graphene in Single-wall Carbon Nanotube (SWCNTs) or Multi-Wall Carbon Nanotubes (MWCNTs).
Their high aspect ratio and extended electronic π-structure enable the surface functionalization with chemical groups, therapeutic and diagnostic agent (drugs, genes, vaccines, antibodies, biosensors, etc.), which are able to increase CNTs biocompatibility, making them excellent vehicles for drug delivery directly into cells.
When exogenous materials such as CNTs enter the body, they interact with different biomolecules, e.g. proteins, present in biological fluids. The binding of cellular proteins to CNTs controls both biocompatibility and the possible toxic effect of drug delivery system.
It is very important to understand the possible interactions between CNTs and physiological proteins, in order to elucidate the relationship between the specific functionalization on CNTs surface and the protein affinity.
The biomolecules layers composed of proteins is called protein-corona (Fig.1), and it is divided in soft and hard corona. The first layer consists of proteins which are not bounded directly to the nanoparticle (NP) itself, but to the hard corona, while the second layer interacts directly with the NP.

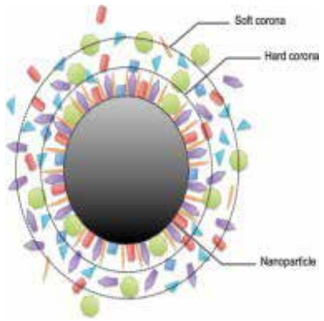

Fig. 1
Protein corona absorption onto CNTs surface divided in soft and hard corona.

An important role is played by specific chemical groups that could be linked to the surface of CNTs, which influences their behavior.
The goal of functionalization is the increase of solubility or dispersibility in biocompatible media, reducing toxic and cytotoxic effects.
The aim of the PhD project is to evaluate of the protein corona developed on differently functionalized MWCNT (*f*-MWCNTs), after their incubation in human plasma (HP) (Fig.2).

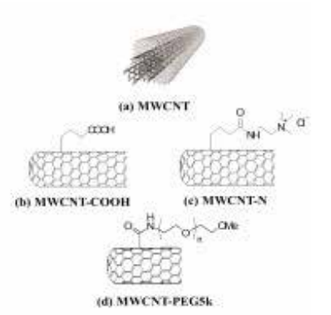

Fig. 2
Chemical functionalization applied on Multi-Wall Carbon Nanotubes: (a) pristine MWCNTs; (b) MWCNT with carboxyl group; (c) MWCNT with ammonium group; (d) MWCNT with PEG group.

The investigation of the interactions between *f*-MWCNTs and the proteins present in biological fluids (human plasma) as well as the proteomic fingerprinting of bio-corona was performed by electrophoretic separation (SDS-PAGE and 2D-PAGE) and mass spectrometry analysis (nLC-MS/MS).
MWCNTs surfaces were firstly modified attaching acid and basic chemical functions, such as carboxyl (MWCNTs-COOH) and ammonium (MWCNTs-N) groups respectively, which were chosen because they are used in various researches as a drug delivery system for cancer treatment.
These functionalizations were used to perform preliminary studies aimed at finding an appropriate incubation protocol (conducted by incubation of MWCNTs with a standard protein mixture), obtaining initial information on the protein corona composition, using a complex protein mixture (E. coli extract and HP); finding relationship between the functionalized group and acid/basic residues of the amino acids that make up a particular protein.
MWCNTs were also functionalized with polyethylene glycol (PEG), chosen considering its well-known biocompatibility in human body, and then incubated in HP to create the bio-corona.
Plasma proteins were eluted by CNTs surface in different conditions by using both native and denaturant buffers, which are useful for characterizing the two protein corona layers, thanks to their ability to break different type bonds.
According to recent studies, protein adsorption onto NPs is controlled by their exposure time to the fluidic media. Moreover, protein corona is a dynamic system that can influence the use of CNTs as drug delivery system.
For these reasons, it was interesting to observe how the protein corona changes at different incubation times.
It was performed a comparison between the HP incubation made with the control (MWCNTs-COOH) and MWCNTs-PEG5k at different times (15 min, 30 min, 45 min, 1 hour, 2 hours, 3 hours, 4 hours and 5 hours). In particular, the observed data was the Average Density of the band contained in each lane (corresponding to a specific time of incubation).
The preliminary experiments (conducted with MWCNTs-COOH and MWCNTs-N) did not show significant correlations between different types of f-MWCNTs and the captured proteins.
Otherwise, it was demonstrated the ability of MWCNTs to interact and to bind plasma proteins, their dependence of protein adsorption on protein shape. Mass spectrometry analysis has identified that 86% of the total proteins were present in bio-corona of all *f*-MWCNTs and 29% were specifically recognized in only one type of *f*-MWCNTs, suggesting that CNTs may contribute to increase the concentration of low-abundance, normally present at undetectable levels in HP.
The interaction between MWCNTs-PEG5k and HP proteins has identified proteins in soft corona with specific functions that involve them in the regulation of immune response and in the CNTs transport, as well as biomolecules in hard corona with a role in the maintenance of host homeostasis.
A deep exploration (using 2D-PAGE separation) has demonstrated that native eluents (such as Tris-HCl and NaCl able to break protein–protein interactions typical of the soft corona without misfolding the proteins) were able to capture proteins of soft corona, characterized by complex secondary structures, and formed by both β-sheets and α-helices domains.
Denaturant buffers have eluted many proteins with a high percentage of the α-helix structure that could be involved in specific interactions responsible for the formation of hard corona.
These data could suggest a possible connection between the secondary structure of proteins and the affinity for CNTs, demonstrating also the potential of PEGylated MWCNTs as future candidates for drug delivery.
The incubations of MWCNTs-PEG5k with HP at different incubation time were made to observe possible changes in the protein corona at a short (from 15 min to 1 hour) and long time (from 2 to 5 hours) exposure.
The proteins probably belonging to the soft corona could interact more strongly with the chemical functionalization than MWCNTs take as control and this may provoke changes in the protein-corona increasing the incubation times. PEG functionalization on the CNTs surface could increase the dynamism of the protein corona. The protein corona changes occurred mainly in proteins concentration and after some time it reached equilibrium system.

DENDRITIC AMPHIPHILES FOR ^{19}F-MRI AND GENE DELIVERY

Marta Rosati - Supervisor: Prof. Pierangelo Metrangolo

Dendrimers are a class of highly structured three-dimensional macromolecules characterized by a well-defined periodically branched structure, high monodispersity and tunable size and shape. Among them amphiphilic dendrimers, in which two moieties of different hydrophilicity are covalently linked, can self-assemble in aqueous media generating many different types of aggregates whose morphology strictly depends on the balance between hydrophilic and hydrophobic portions. This allows to control their molecular conformation, the aggregation behavior both in bulk and in solution, and thus the final shape of the aggregate formed. The control of these parameters is important for the emergence of specific function. A powerful tool for tuning the self-assembly behavior of dendritic amphiphiles and increasing their colloidal stability, also in biological media, is the introduction of linear or branched fluoroalkyl groups in their molecular structure. This enhances their tendency to segregate and influences their molecular supramolecular organization.
The search for more sustainable fluorinated materials focused attention on shorter chains perfluorocarbons (PFCs). However, simply cutting down chain length does not lead to satisfactory results in balancing environmental impact and performance. Recently, multibranched super-fluorinated derivatives obtained high attention since they combine good biocompatibility with performances assimilable to classical PFCs. Moreover, the presence of a high number of equivalent fluorine atoms in their structure renders them excellent ^{19}F-Magnetic Resonance Imaging (^{19}F-MRI) probes. We decided to exploit the advantages of branched fluorinated chains to generate new polyfluorinated amphiphilic dendrimers suitable for gene delivery and traceable by ^{19}F-MRI, thus obtaining new theranostic systems. Firstly, this thesis gives an overview on the literature with particular attention to amphiphiles, especially dendritic amphiphiles. Moreover, an introduction on ^{19}F-MRI probes and gene delivery vectors is also reported relative to the proposed application of the reported derivatives.
Subsequently, synthesis and characterization of new polyfluorinated dendritic amphiphiles of different generations are discussed reporting on their properties both in bulk and in solution. In order to better understand how the balance between hydrophilic and hydrophobic portions in fluorinated dendrimers can affect their self-assembly behavior, we synthesized a new family of dendrimers by attaching the same branched fluorinated moiety, showing 27 magnetically equivalent fluorine atoms, to 1st, 2nd and 3rd generation 2,2-bismethylolpropionic acid (Bis-MPA) polyester dendrons.
From experimental results we observed that the crystallinity of dendritic amphiphiles decreases with the increase in generation. In fact, single crystal structure could be determined only for the 1st generation dendrimer showing the segregation of fluorinated part from polyester one. Moreover, the generation of the polyester moiety affects the temperature stability and thermal behavior of the three molecules. In fact, the 2nd generation dendrimer showed a higher degradation temperature than 1st one. On the contrary, a decrease in temperature stability was observed for the 3rd generation dendrimer. DSC, POM and SWAX analyses revealed that 2nd generation dendrimer has mesomorphic properties and forms a cubic mesophase, while the 3rd generation one is isolated as a mixture of different polymorphs with different thermal behaviors. The dendrimers are dispersible by the ethanol in water procedure; this allowed us to observe that the generation dependency is confirmed even in solution. In fact, while 1st and 2nd generation dendrimers formed micelles and spherical nanoparticles with a single intense peak in ^{19}F-NMR spectra, important for ^{19}F-MRI applications, the 3rd generation dendrimer tends to form dendrimersomes, multilayer vesicles, which undergo morphology transition to fibers over 48 hours of ageing, with the subsequent switching off of ^{19}F-NMR signal, probably related to reduced mobility of fluorine atoms. By substituting ethanol with trifluoroethanol, transition to fibers is prevented and the switching off of the ^{19}F-NMR signal is avoided. Coarse-grain simulations revealed that the 3rd generation dendrimer can exist in two conformations where the polyester moiety and the fluorinated branched part are respectively directed in the same (*cis*) and opposite (*trans*) directions. With trifluoroethanol, the dendrimer assumes preferably the *cis* conformation while in ethanol the *trans* conformation is preferred. Presumably, this is at the bases of the peculiar self-assembly observed experimentally in solution, suggesting that when the dendrimer self-organizes in the conformation of high curvature, vesicles are preferred and ^{19}F-NMR is on, while the tendency of the amphiphile to self-adjust in the trans conformation in presence of ethanol can be at the bases of the transition to fibers. Gene delivery has become a powerful tool to treat diseases. The need to protect and deliver nucleic acids into cells requires the use of biocompatible and efficient vectors. Among those reported in literature, fluorinated dendrimers attracted high interest since they were shown to improve vector stability and enhance gene endosomal escape. It has indeed been reported that fluorination can stabilize the formation of complexes with genes for similar compounds and facilitate their intracellular release. Therefore, the chemical structure of the 2nd generation polyfluorinated dendritic amphiphile was modified for promoting the interaction with nucleic acids. In this regard, the synthetic approach adopted, and the chemical characterization of the obtained derivative are discussed. Furthermore, self-assembly behavior in aqueous media of the obtained derivative is described with particular attention to the characterization of shape and stability of its aggregates. Finally, gene complexation ability and transfection efficiencies of the new dendritic vector are presented. The new fluorinated gene vector presented is able to bind nucleic acids with good N/P ratios and showed a reduced cytotoxicity and higher efficacy when compared to commercially available polymeric vector. In the last part of the thesis the effects of fluorination on the self-assembly of small molecules are discussed. Here the tendency of fluorinated moieties to microsegregate is combined with the high directional effect driven by halogen bond (XB) to tune liquid crystal phase and thermal stability of new ionic liquid crystalline materials. All the complexes displayed thermotropic liquid-crystalline behavior over a wide range of temperatures, with enantiotropic smectic phases, which are reminiscent of the lamellar phase observed in the crystal state. Our results highlight the ability of XB to work as an efficient supramolecular tool to introduce long perfluoroalkyl chains on imidazolium salts to design novel supramolecular ionic conductors.

DESIGN AND SYNTHESIS OF NOVEL 1D BISPIDINE-BASED COORDINATION POLYMERS FOR ADSORPTION APPLICATIONS

Martina Lippi - Supervisor: Prof. Massimo Cametti

The involvement of porous and non-porous Coordination Polymers in many research fields have been deeply studied over last decades, with much emphasis on the attempt to extrapolate the correlation which exists between their structural features and their properties, relevant to their several fields of application. A clarification in this regard is believed to be of great importance, starting from the influence of CP dimensionality, which is one of the structural features which can be more easily designed by modulating the basic CP components. Due to their specific architecture, the use of 1D CPs could be considered limited to fewer applications as the assembly of linear, zig-zag, helical or ribbon-chains does not lead always to channeled or porous systems. Indeed, for adsorption purposes, the advantage of Porous Coordination Polymers (PCPs, also known as Metal Organic Frameworks, MOFs), which generally result from 2D and 3D structures, is directly associated to their accessible internal space, whereby non-porous materials are not usually considered as promising adsorbents. In contrast with this common view, a detailed review work revealed that a certain number of non-porous 1D CPs are instead able to adsorb large molecules (larger than common N_2, O_2, CO_2 gases). This behavior was explained by an intrinsic high flexibility of these 1D CP structures which allows them to adapt in relation to the external chemical stimulus. Despite the absence of any accessible channel or pore space, a series of one-dimensional CPs demonstrated to have the ability to release/adsorb guest molecules and selectively separate mixture of compounds in solid/liquid or solid/vapor phases thanks to the dynamic aptitude that they can express.
In this research work bispidine molecule (based on the 3,7-diazabicyclo[3.3.1]nonane-1,5-dicarboxylate unit) has been chosen as a rigid and versatile scaffold to build a series of highly tunable organic ligands for the production of a novel family of Coordination Polymers (CPs). The implemented design featured the introduction of divergent pyridines and phenyl-pyridinic moieties to allow the coordinative polymerization. In this thesis work it will be shown that the selected design indeed allows to produce highly crystalline CP materials combining bispidine with metals like Mn(II), Cu(II), Zn(II) and Hg(II). They consist most frequently in 1D CPs, but also to a lesser extent to 2D and 0D systems. In total, 30 novel SCs structures have been obtained, whose X-ray characterization constitutes an important part of this work. Depending on the solvent crystallization mixture used it was possible to obtain mono- and bi-solvated CPs. Single Crystal structural characterization has been reported for all the CPs collected, evidencing mostly the weak interactions that control the overall packing mode of each system. Among all the CPs reported, a series of 1D Mn(II)-ribbon like systems build upon pyridine-based bispidine ligands possessing a different substitution in N7 position (Fig.1), were studied in detail in terms of their adaptable and dynamic features aiming at the collection of novel potential adsorbent materials.

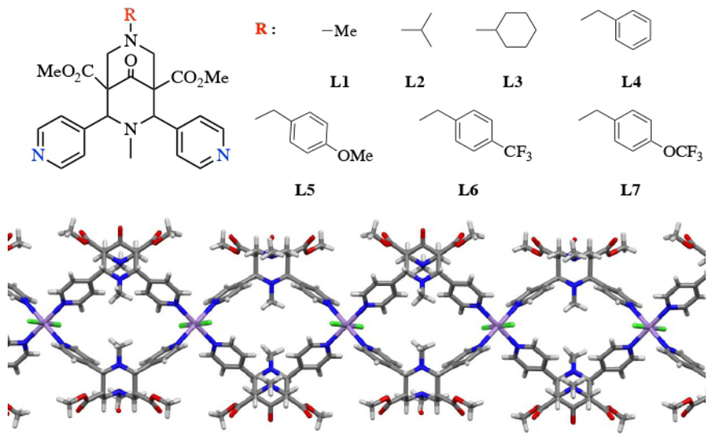

Fig. 1
Molecular formulae of bispidine ligands L1-L7 differently substituted in N7 position. (above) Single Crystal X-ray showing the 1D ribbon-like chain of a Mn(II)-CP. (below)

The extensive structural analysis showed that this class of N7-bispidine-based CPs are composed by non-interpenetrated robust 1D ribbon-chains assembled via weak inter-ribbon interactions, resulting in different packing orientation, mainly depending on the ligand and solvent identities.
Reproduced in form of microcrystalline powder samples, characterized by PXRD, Thermogravimetric Analysis (TGA), NMR spectroscopy and *ab initio* PXRD, these materials showed a remarkable highly dynamic behavior once subjected to heterogeneous solid/vapor and solid/liquid solvent adsorption and exchange reactions. Several solid-state reactions, comprising crystalline-to-crystalline, crystalline-to-amorphous-to-crystalline, single crystal-to-single crystal (SCSC), selective adsorption transformations have been reported demonstrating the significant structural reorganization that 1D ribbon chains can undergo. We have also demonstrated that the dynamic tendency of CPs could be correlated to the aggregation modes and efficiency evidenced by the bispidine ligand alone in the solid-state. Combined structural and theoretical calculations based on SC- X-ray structure of each N7-bispidine ligand showed indeed the possibility to have qualitative indications on the dynamic properties of their corresponding CPs. Preliminary results allowed also to envisage a way to control on whether the ligand/metal assembly leads towards a CP or rather to a molecular complex, simply by choosing among different N7 substituents having varying steric and electronic attributes. Finally starting from the tetra and pentadentate bispidines, largely employed for the synthesis of molecular complexes, a novel ligand design led to the production of other CP structures displaying an additional metal center not involved in the coordinative polymerization but available for potential applicative purposes. Moreover, after several crystallization efforts, the first 1D hetero bi-metallic bispidine-based CP has been obtained, thus opening the way towards a further widening of the fields of application of these bispidine-based CP materials into heterogeneous catalysis.

MULTISCALE MODELING OF REACTING FLOWS IN CATALYTIC FLUIDIZED SYSTEMS: METHODOLOGIES AND APPLICATIONS

Riccardo Uglietti - Supervisor: Prof. Matteo Maestri

Catalytic fluidized systems have played a key role in the operation of challenging processes, where the management of severely exothermic reactions and the easy mobility and replacement of the catalyst are pivotal. In the last years, this technology is gaining an increasing attention. Indeed, fluidization has the potential of acting as a powerful tool in the current energy transition in view of its leading role in the optimal design of processes for methane valorization and CO_2 capture and utilization. Despite the advantages of the fluidization in terms of heat and catalyst management, the required gas-solid flow leads to a complex fluid dynamics, which can severely affect the catalytic performances of fluidized units, thus hampering their design and scale-up. In this view, the research on catalytic fluidized bed processes is focusing on two main aspects. On the one hand, novel fluidization concepts are currently under development, e.g., pulsed and confined fluidization, to engineer and control the chaotic fluid dynamics. On the other hand, the investigation of catalytic mechanisms is ongoing to improve and optimize the chemical performances of the particles. Therefore, an increased fundamental knowledge of these units is required to achieve the best fluidization technology for the catalytic process under investigation.
In this view, CFD based models can be a valid supporting tool for the rational design and optimization of these units. Considering the wide range and time scales involved in fluidized reactors (from the elementary step at the catalytic active site to the macro-scale transport phenomena in the gas-solid flow), the adoption of multiscale modeling is pivotal to gain a deep understanding of the interplay between fluid dynamics and catalysis. However, the coupling between microkinetic modeling and CFD literature models is absent, thus leading to the need for novel multiscale methodologies for the analyses of catalytic fluidized systems in arbitrary complex operating regimes and catalytic mechanisms, possibly arising in novel fluidization designs.
This thesis presents the development and application of multiscale methodologies, which couple non-reactive open-source Euler-Lagrange (EL) and Euler-Euler (EE) literature CFD models of the gas-solid flow with the microkinetic description of the catalytic reactivity for the fundamental investigation of lab and industrial scale fluidized units, respectively.
With respect to the multiscale EL methodology, based on the tracking of the particles in the system, the work starts focusing on the implementation of the microkinetic modeling of catalytic reactivity and the management of the induced hampering computational cost.
With respect to the latter aspect, two speed-up strategies have been developed based on tabulation and agglomeration techniques. Moreover, a selection methodology has been proposed for the identification of the best strategy for the process under evaluation, enabling for the first time the reactive particle tracking simulation of million particles reactor.
Then, the multiscale EL framework has been validated on the basis of experimental methanation data available in the literature. Additionally, an experimental investigation of a catalytic lab scale fluidized bed has been performed at TU Delft to proof the applicability of the developed EL multiscale model in a wide range of fluidization regimes, starting from typically available mechanical and chemical characterization of the catalytic particles, and obtaining an excellent agreement in the whole operative range. Finally, the developed EL multiscale framework has been used for the analysis of the novel pulsed fluidization technology in the context of heterogeneous catalysis, investigating for the first time this fluidization concept in high temperature reactive processes, unraveling the operative limits and the benefits provided by this technology in terms of axial mixing and reaction heat removal efficiency.
With respect to the EE multiscale model, based on the description of the solid as a fluid-like phase, the work focuses on the efficient implementation of the numerical coupling between the extremely fast catalytic steps and the long industrial reactor dynamics, proposing a Multiphase Operator-Splitting algorithm (MOS). Once validated the model with experimentally available data for the Oxidative Coupling of Methane (OCM) reaction at the lab scale, its successful usage for a simple benchmark industrial scale unit has proved its applicability for the modeling at relevant scales of complex catalytic processes.
In conclusion, the proven applicability of the two multiscale models allows for the analysis of systems with complex fluid dynamics, hardly predictable with 1D empirical models of conventional units (e.g., pulsed beds), and enables the description of complex catalytic mechanisms (e.g., OCM), poorly predictable with simplified rate equations.
Additionally, the availability of these multiscale models, will pave the way for the application of machine learning in fluidized systems, enabling the adoption of kMC based kinetics and the usage of EL data for the refinement of EE models.
Consequently, the developed multiscale frameworks represent an important tool for the numerical assisted design and optimization of catalytic fluidized units from lab to industrial scale.

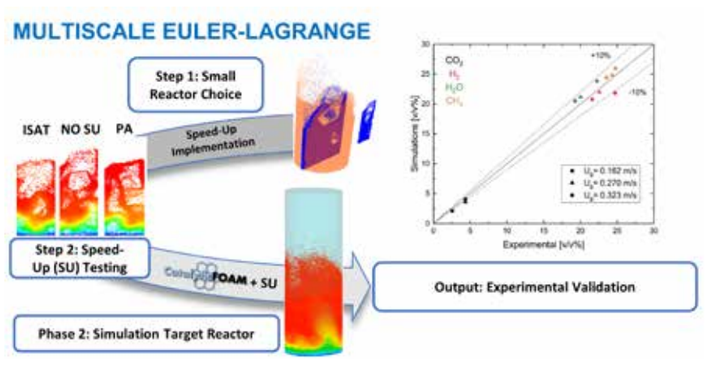

Fig. 1
Scheme of the speed-up (SU) based methodology proposed to efficiently apply the EL multiscale model (on the left), experimentally validated with literature methanation data in a 1.5 cm diameter fluidized bubbling bed reactor as shown in the parity plot on the right.

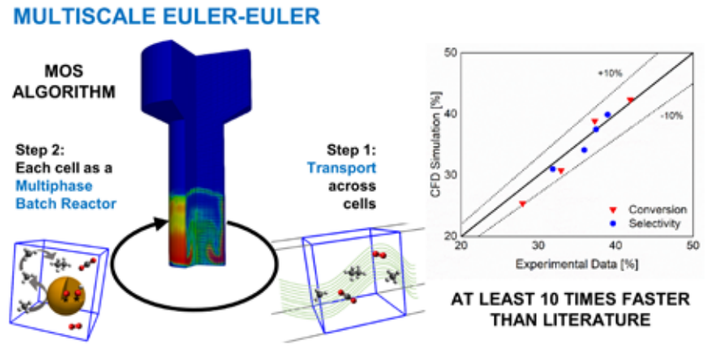

Fig. 2
Scheme of the Multiphase Operator Splitting (MOS, on the left) whose time step application is composed by the sequential solution of species/energy transport across the computational cells and the solution of homogeneous chemistry, gas-solid transport and heterogeneous chemistry in each cell. MOS has been experimentally validated by means of literature OCM data (as shown in the parity plot on the right).

DEVELOPMENT OF MAGNETIC HETEROGENEOUS SYSTEMS FOR THE ELIMINATION OF POLLUTANTS

Ruggiero Maria Pesce - Supervisor: Prof. Davide Moscatelli

The research done in my executive PhD was sponsored by Captive Systems and was developed in close collaboration with Captive's team. Captive System is start-up recognized as a spin-off of Politecnico di Milano, that is developing an innovative technology based on the use of magnetic aggregates for the treatment of wastewaters and gas stream. The main objective is to introduce into the waste management this innovative technology with the aim of promoting an easy way treatment to nullify the environmental impact of waste processes.
In line with this, all the research was done having as a *fil rouge* the objective of an industrial application both regarding the production of the material and their application in waste treatment. Three possible applications were investigated: 1. Metals remediation; 2. Carbon Dioxide capture. 3. Oil adsorption. For each specific application a unique type of magnetic particles was developed.
The first part of the work is focused on the production of different types of magnetic functionalized aggregates. The materials used were chosen according to their ease to be purchased and their low risks MSDS. Among the different production methods, the co-precipitation was chosen as the easiest to scale-up in an industrial production, and literature always reports the use of this technique in batch mode for producing different types of magnetic nanoparticles and their aggregates. The first result achieved by this work was to produce three classes of magnetic nanoparticles and their aggregate (anionic, cationic and lipophilic) by using co-precipitation in a continuous process. A pilot production plant (Fig.1) was developed with a production capacity of 50 Kg per day. Different batches were produced with the pilot plant and their chemical-physical characteristics were analyzed and found consistent with the one produced at the lab scale.
The work continued developing magnetic aggregates to be used for the removal of metals from wastewater. A coating made of bi-carboxylic acid was developed to promote the adsorption of metal ions by electrostatic interaction. The materials were successfully used to treat two real wastewaters coming form an Italian electroplating industry polluted with copper and zinc. Different treatment parameters were analyzed in order to understand the main parameters that control the application of these materials in metals remediation.
Another application that was investigated was the carbon dioxide capture. Different type of materials functionalized with amine groups were produced. TGA/DTA was used to determine their thermal stability, and moreover to evaluate their capacity of absorbing CO_2 and their ability to be regenerated (recyclability). The magnetic particles showed a good recyclability and stability, with a slight loss in performance after several regeneration cycles.
An application dealing with circular economy was also developed. In the specific, the possibility of using mussel shells waste combined with magnetic particles to obtain composite materials for oil adsorption was investigated. Mussel shell waste has a huge economic impact on bivalve aquaculture and moreover their wrong disposal causes environmental problems. In this work, mussel shells, that are mainly made of calcium carbonate (CaCO3), were recycled to synthesize composite magnetic materials made of biogenic CaCO3 and magnetite. Their capacity of adsorbing crude oil was checked indirectly by Chemical Oxygen Demand analysis. The promising results obtained showed that this way could be a valid solution for recycling of mussel shell waste.
Along with research activities, a preliminary executive Business Plan was executed with the aim of positioning the technology developed in this work among the already commercially available technologies for waste treatment. The main figures of the eBP are: 1. Technology description with the related opportunity and risks; 2. Market analysis; 3. Competitive landscape; 4. Go-to-market strategy and 5. Estimation of pricing and the revenues model.

Fig. 1
Pilot production plant.

Fig. 2
Composite materials for oil adsorption.