



Coordinator:
Prof. Carlo Alessandro Cavallotti

DOCTORAL PROGRAM IN INDUSTRIAL CHEMISTRY AND CHEMICAL ENGINEERING

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 of the doctoral program is the application of the chemical and physical knowledge to all the activities related to the 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 to:

- (1) design and manage industrial processes at any size scales;
- (2) develop new technical applications;
- (3) 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 macroscopic 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, though the program is also open 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 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 30. About ~80% of the CIIC graduated PhD students now work in industry, with the remaining employed in the university or government research centers. These data demonstrate the important interconnection existing between the performed research 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, ENI, Flamma, Isagro, LPE Epitaxial Technology, Mapei, Mare, Pirelli, RSE, Solvay Specialty Polymers, Tecnimont.

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Σ-HOLE INTERACTIONS: A JOURNEY FROM D TO P-BLOCK ELEMENTS

Miriam Calabrese – Supervisor: Prof. Giuseppe Resnati

Noncovalent interaction is one of the main characters of the chemistry of the present century due to its impact in many biological, chemical and physical processes.

σ-Hole interactions are a subclass of noncovalent interactions and represent the focus of this PhD research activity. Concerning the wide field of supramolecular chemistry, i.e., the chemistry of intermolecular bonds, crystal engineering is the subject that mostly investigates and exploits σ-hole interactions for potential practical applications. Indeed, different interesting fields, such as drug design, supramolecular chemistry and functional molecular materials, provide evidences of their relevance. The rationalization of σ-hole interactions is attributable to the anisotropic distribution of the electron density on the outer regions of bonded atoms. This affects the surface electrostatic potential of molecules and this determines the occurrence, on atoms surface, of areas with higher and lower electron densities where the electrostatic potential is respectively negative and positive. Regions where the electrostatic potential is positive are called σ-holes and they can act as electrophilic sites, interacting potentially with

nucleophilic sites.

The birth of the concept of the σ-hole dates back to 1990s, when Brinck et al. discovered the presence of a region of positive electrostatic potential on the surface of monovalent halogen atoms, Group 17 (Fig.1). The IUPAC definition of this specific σ-hole interaction, defined Halogen Bond (HaB), has strongly contributed to the development of this research field.

As a confirmation of the generality of this phenomenon, it has been discovered that also other elements belonging to different groups of the *p*-block of the Periodic Table are able to act as σ-hole donors. A systematic classification of the σ-hole interactions has been based by referring to the group to which

the electrophilic atom belongs to, in order to identify them unambiguously along with their peculiar features. Namely, the terms Chalcogen, Pnictogen and Tetrel Bond are used to refer to interactions where, respectively, elements of groups 16, 15 and 14 are the electrophiles. Recently, a similar mindset has been adopted for the investigation of the ability of the *d*-block elements to establish σ-hole interactions. Initially promising outcomes have emerged, for instance, for elements of Groups 12 and 11, respectively defined as Spodium and Coinage Bonds, nevertheless the *d*-block has been generally less explored than the *p*-block. Moreover, in the last decades the interest in the field of anion

supramolecular chemistry has grown. In this context, the family of σ-hole interactions have been extensively used in anion recognition for anion-binding applications. For the design of the synthetic receptor the use of different σ-hole donor groups offers a wide possibility to obtain anion-binding selectivity. In particular, the attention has been focused on the anion-anion interactions involving σ-holes. Given this background, this present work started with a deeply investigation of σ- and π-hole interactions involving transition metals. The outcomes reached expand their landscape, initially in neutral and then in anionic compounds. Specifically, groups 11, 8, 7 and 5 have been explored in compounds with useful applications, e.g. catalysts, as osmium tetra-oxide and

methyltrioxorhenium (MTO)

Figure 2, or drugs as gold-based derivatives. Subsequently, considering the distinctive results obtained with charged compounds, e.g. in the case of perchlorate anion, a similar mindset has been adopted to expand the knowledge also in the historically more investigated *p*-block, focusing on group 17 and 14. This has allowed new insights to be achieved such as the anion-anion interactions involving halogen atom, as in the case of periodate anion. The experimental results have been obtained by combining several tools. Preliminary Cambridge Structural Database (CSD) surveys have allowed to identify interesting scaffolds in which atoms belonging to the *p/d*-block are potentially able to form σ-hole interactions. Different

characterization techniques have been employed for the analyses of the obtained structures displaying σ-hole interactions, mainly the Single Crystal X-Ray Diffraction that provides the geometrical parameters of the noncovalent forces under investigation. Notably, the experimental evidences obtained are strongly supported by computational studies, e.g. Molecular Electrostatic Potential (MEP) and QTAIM/NCIplot analyses. These allow to analyse the energetic features of the observed assemblies and to prove the σ-hole nature of the primary interactions driving the solid-state packing. Overall, the obtained results underline the importance to employ efforts in this research field with the aim to achieve a deeper knowledge of this phenomenon and exploit its potential, which may result in new catalysts and materials.

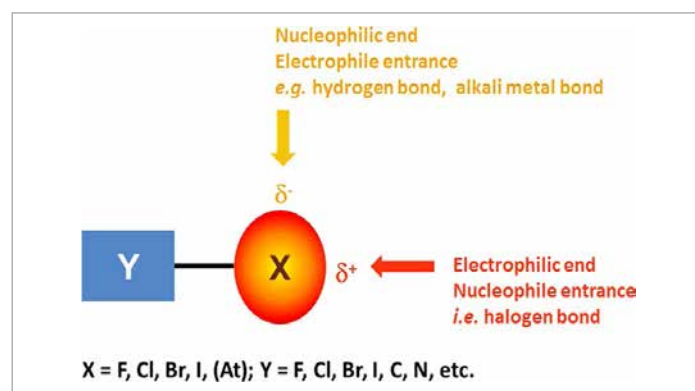


Fig. 1 - Schematic representation of the σ-hole formed opposite to the covalent bond involving halogen atom.

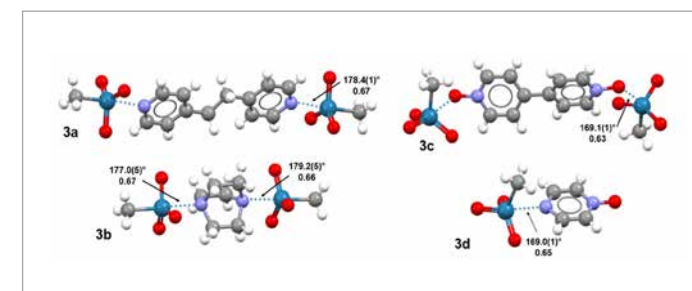


Fig. 2 - Ball and stick representation of MTO crystal structures 3a (MTO/1,2-dif(pyridine-4-yl) ethane)), 3b (MTO/DABCO), 3c (MTO/4,4-bipyridine-1,1'-dioxide), and 3d (MTO/pyrazine 1-oxide); σ-hole interactions are colored dotted lines. Nc and interaction angles are given close to the σ-hole interactions. Color code: whitish, hydrogen; gray, carbon; indigo, nitrogen; red, oxygen; petroleum green, rhenium.

SYNTHESIS AND CONFORMATIONAL ANALYSIS OF HYDANTOIN-BASED UNIVERSAL PEPTIDOMIMETICS

Alessio Maria Caramiello – Supervisor: Prof. Alessandro Volonterio

The first and central focus of this research is the design, development, and characterization of a universal peptidomimetic scaffold based on a central hydantoin ring. Peptidomimetics have emerged as promising alternatives to natural peptides, combining the advantages of enhanced stability, bioactivity, and a wide range of applications. The hydantoin-based scaffold was chosen for its potential to mimic the structural and functional properties of peptides while offering increased chemical stability and synthetic versatility (Fig.1). Moreover, hydantoin-based drugs are already present in the market and are used to treat several, very important, medical conditions. A robust synthetic strategy was devised to efficiently construct the hydantoin-based scaffold. Reaction conditions were optimized to achieve high yields and purity, with the aim of reducing also the final purifications, being the latter a point of time and resources consumption. The successful synthesis of the scaffold was confirmed through rigorous spectroscopic characterization, including nuclear magnetic resonance (NMR) and mass spectrometry (MS).

To evaluate the structural

and conformational properties of the synthesized peptidomimetic compounds, computational calculations were employed. Molecular modeling techniques, such as molecular dynamics simulations and quantum mechanical calculations, were utilized to predict the ground-state conformations and energetics of the peptidomimetics. The computational models provided valuable insights into the preferred conformations, allowing for a deeper understanding of the structure-activity relationships. Furthermore, experimental techniques including NMR spectroscopy (VT-NMR and DMSO titrations), circular dichroism (CD) and Attenuated Total Reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) were employed to characterize the secondary structure, folding patterns, and stability of the

peptidomimetics. Solid-state characterization techniques such as X-ray crystallography, provided valuable insights into the three-dimensional structure and packing arrangement of the scaffold (Fig.2). These techniques provided valued information on the conformational preferences, secondary structural elements, and stability profiles of the synthesized compounds. Additionally, the potential for functionalization was explored to expand the scaffold's versatility and tailor its properties for specific applications. Additional functionalization sites were introduced, allowing for the incorporation of various side chains and moieties. The successful functionalization of the scaffold was confirmed through spectroscopic techniques and analytical methods, demonstrating the attachment of specific

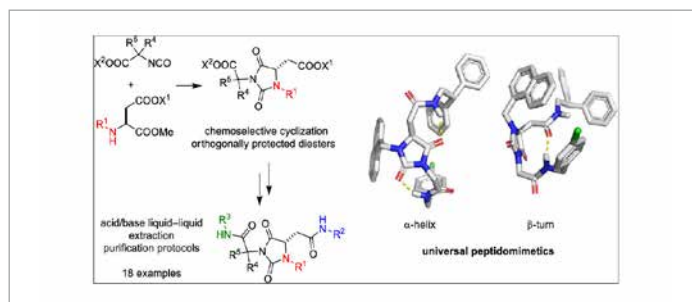
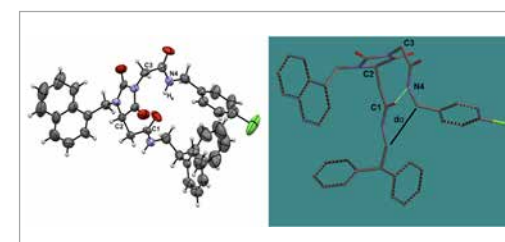


Fig. 1 - Hydantoin-based scaffold as universal peptidomimetic.

functionalities to the hydantoin core. The synthesis of the first examples of potential antimicrobial Hydantoin-based peptidomimetics was achieved, and the preliminary biological tests provided information about the capacity of the scaffold to work in this sense.

During a visiting PhD period, a parallel research endeavor focused on the conjugation of a Ruthenium-based cytotoxic anticancer compound with Guanidinoneomycin. Guanidinoneomycin, known for its ability to enhance cellular internalization, was selected as a targeting moiety. The rationale behind this conjugation strategy was to leverage Guanidinoneomycin's propensity to selectively internalize the cytotoxic compound through the heparan sulfate pathway, which is often overexpressed during carcinogenic events. The successful synthesis of the conjugated compound was achieved through a multi-step synthetic approach, which involved functionalizing Guanidinoneomycin with a suitable linker followed by conjugation with the Ruthenium-based cytotoxic compound (Fig.3).



CO₂ VALORIZATION THROUGH INNOVATIVE CATALYTIC ROUTES TO LOWER OLEFINS

Chiara Coffano – Supervisor: Prof. Luca Lietti

Carbon dioxide (CO₂) is a greenhouse gas which significantly contributes to the rise in global temperatures and climate change. The anthropogenic release of CO₂ in the atmosphere mainly arises from the combustion of fossil fuels: the reduction of their utilization is thus crucial to preserve the climate and to meet the goals of Paris Agreement. To reduce CO₂ emissions, a possible route involves its capture and storage/utilization. Carbon Capture and Storage (CCS), consisting in the permanent storage of CO₂ in appropriate geological areas, is currently considered technically feasible at commercial scale, but it faces high energy consumption, high costs, and uncertainties on the stability of the CO₂ reservoirs. On the other hand, Carbon Capture and Utilization (CCU), which focuses on the reutilization of CO₂ to make added value products, is less technologically established, but it is considered more promising in terms of economics and societal acceptance. CO₂ can be considered a renewable and abundant carbon feedstock and can be exploited either as a vector to store renewable energy, thus balancing the intermittency typical of these technologies, or as raw material for chemical production. In fact, the surplus

renewable energy can be used to produce “green” hydrogen from electrolytic water splitting, and this renewable hydrogen, in turn, can be used to hydrogenate CO₂ to valuable products. In particular, lower olefins (viz. C₂=C₄= olefins) are highly appealing products, being important industrial intermediates for the production of a wide variety of compounds, including polymers and solvents, among others. Traditionally, lower olefins are produced by naphtha steam cracking in the petrochemical industry, with massive emissions of carbon dioxide. The production of olefins from CO₂ is a promising option to reduce the carbon footprint of these molecules. The goal of this thesis is to develop a one-pot catalytic process for the synthesis of lower olefins utilizing CO₂ and renewable H₂. Literature suggests different

alternatives for the direct CO₂ hydrogenation to light olefins: both a methanol-mediated route and a modified Fischer-Tropsch approach can be applied for this purpose; in this thesis work the methanol mediated route has been selected as potentially attractive. Accordingly, reference catalytic systems have been selected as model systems for the methanol synthesis from CO₂ and for the subsequent Methanol-To-Olefin (MTO) process, respectively, and tested in the direct olefin synthesis from CO₂. In particular, In-based and SAPO-34 catalysts were considered as reference materials for the methanol synthesis from CO₂ and for the MTO process, respectively. In-based catalysts were prepared by different methods including coprecipitation of indium and zirconium nitrates, and

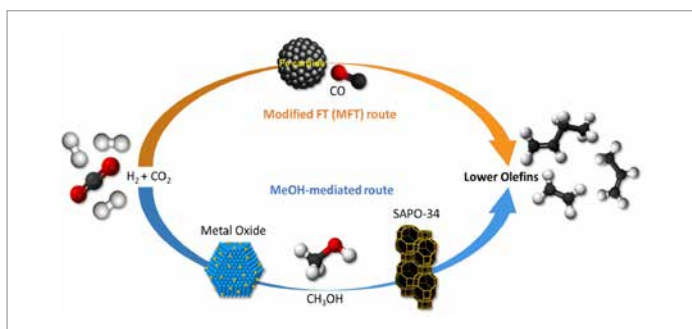


Fig. 1 - Schematic representation of different routes for CO₂ conversion to lower olefins.

impregnation of indium nitrate on ZrO₂ commercial support. A commercial ZnCr₂O₄ was also used as benchmark methanol synthesis catalyst. Concerning the zeolite, different commercial SAPO-34 zeolites were purchased from external suppliers. Preliminary catalyst characterization (CO₂- and MeOH-adsorption and MeOH reactivity at atmospheric pressure) pointed out the superior properties of In-Zr coprecipitated samples and of one of the purchased SAPO-34 samples.

The reactivity of the In-Zr catalyst in the methanol synthesis from CO₂ and of the zeolite in the MTO reaction has been initially investigated, starting from CO₂/H₂ mixtures in the case of the methanol synthesis catalyst and from methanol in CO₂/H₂ in the case of the zeolite. The In-Zr catalyst exhibits very good selectivity to methanol and high activity, reaching thermodynamic equilibrium above 380°C at the investigated space velocities. The zeolite sample showed high activity as well but the presence of H₂ increases the paraffin selectivity at the expense of the olefins, especially at low space velocity. Besides, the zeolite catalyst tends to deactivate at low space velocity and low temperatures. It is therefore clear that a trade-off has to be identified when testing the bi-functional catalytic samples in order to guarantee high methanol formation and good olefins production in the one-pot process.

With this in mind, the reactivity of different catalysts combinations

under various experimental conditions has been analyzed. The reference test was done with a physical mixtures (1:1 weight ratio) of In₂O₃-ZrO₂ and SAPO-34. It has been found that CO₂ conversion increases with temperature, while olefin selectivity showing a maximum around 380°C. In fact, at lower temperatures methanol formation is favored, but the zeolite is not active in the MTO reaction, whereas at high temperatures CH₄ and CO formation prevails. Pressure and space velocity also require a trade-off: higher pressure favors methanol synthesis, but also secondary hydrogenation of olefins into paraffins; high space velocity increases olefins/paraffins ratio, but has a negative impact on the methanol synthesis, leading to an increase of CO and CH₄ among the products.

The change in the relative loadings of the CTM and MTO catalysts has pointed out that the best results are achieved with a CTM/MTO weight ratio near 1. In fact a CTM/MTO weight ratio near 2 has increased methane and paraffins selectivity, while a ratio of 0.5 has slightly increased olefins selectivity among hydrocarbons, but increased overall CO.

The interaction (or “proximity”) between the two catalysts has also been proven as a parameter of paramount importance. Both increasing and decreasing the proximity between the catalyst particles with respect to the physical mixing decrease the catalytic performances. Increasing the

proximity is in principle beneficial in exploiting the shift in the chemical equilibrium of the methanol synthesis, but it leads to a decrease in the catalytic performances possibly due to an interaction between the two catalytic materials. Similarly, the decrease in the catalyst proximity decreases the efficiency, possibly because methanol is not readily involved in the MTO process. Along similar lines, when a double bed in tested instead of a physical mixture, almost no olefins are produced.

Finally, aiming at a precise configuration of the catalytic systems, structured systems have been tested, obtained by coating the two active phases on a cordierite honeycomb monolith. Such novel systems showed good activity for low time on stream (ToS), comparable with the physically mixed sample, but, on the other hand, this arrangement suffers from low stability, with decreasing performances after around 100 h.

The results discussed in this thesis work confirm that a bifunctional catalytic system obtained integrating an In-based catalyst and a SAPO-34 zeolite, is able to directly convert CO₂ into lower olefins in a one-pot process. However, additional efforts are required to rationalize the process and to increase the yields of the lower olefins, both in terms of optimization of the catalytic materials and operating conditions, and configuration of the catalytic systems.

A CFD-BASED INVESTIGATION OF THE TRANSPORT PROPERTIES IN OPEN CELLULAR STRUCTURES FOR CATALYTIC APPLICATIONS

Claudio Ferroni - Supervisor: Prof. Enrico Tronconi

Open cellular structures are regarded as potential substrates for intensification of catalytic reactors affected by fluid-solid heat and mass transfer limitations. They are characterized by high void fractions, leading to low pressure drops compared to packed beds, high surface areas for catalyst deposition and intense fluid-solid interaction, which leads to enhanced fluid-solid transfer rates compared to the state-of-the-art structured substrate, i.e., the honeycomb monolith. The main aim of this work is thus the development of novel structured substrates for intensification of catalytic reactors, specifically for fluid-solid mass transfer limited processes. Despite their great potential, their application in industry is hindered by the limited understanding of the transport phenomena. In particular, few literature correlations are available for the prediction of momentum and mass transfer, emphasizing the necessity of a fundamental study of such mechanisms. My PhD thesis aimed at filling this gap through a numerical approach rooted on Computational Fluid Dynamics (CFD) simulations. In my work, detailed CFD simulations are exploited as in-silico experiments

for the fundamental investigation of the transport properties of cellular structures, and the hierarchical approach is employed to derive CFD-based engineering correlations for their prediction. By virtue of the developed correlations, porous media CFD simulations and macroscopic reactor models are introduced for the simulation of structured catalytic reactors based on cellular substrates. By employing the CFD-based engineering correlations as closure models, these simplified approaches fully retain the accuracy of detailed simulations, allowing for a faithful prediction of the reactor behaviour. The different CFD approaches are coupled with a comprehensive geometrical analysis of the structures and reactors, in view of obtaining adequate computational domains representative of their complex geometry. The work was organized in three parts. In the first part, a fundamental investigation of the fluid-solid mass transfer and pressure drop was carried out on Periodic Open Cellular Structures (POCS) having Diamond and Tetrakaidekahedral (TKKD) unit cell, which were identified as the most promising lattice shapes based on a literature review. A

systematic procedure for the detailed simulation of lattices was developed. In particular, periodic boundary conditions were introduced for compressible reacting flow in the three-dimensional space, to simulate POCS in fully developed flow and concentration profiles. Then, a detailed analysis of the transport mechanisms and pressure drop was carried out. The analysis of the pressure drop was performed in combination with dedicated experimental activity to cross-validate numerical and experimental results. To do so, the virtual models for numerical analysis were accurately reproduced by means of 3D printing. A parametrical investigation of the effect of the

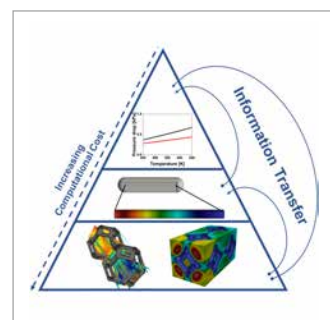


Fig. 1 - Schematic representation of the workflow adopted in this work based on the hierarchical approach for cellular substrates. Bottom: detailed CFD simulations (high hierarchy), centre: porous media CFD simulations (medium hierarchy), top: conventional reactor models (low hierarchy)

geometrical properties (porosity, cell size) on the transport properties and pressure drop was carried out, and the numerical and experimental evidences were employed to formulate engineering correlations. Thanks to them, the performances of POCS and other structured catalytic substrates were compared in terms of fluid-solid transport coefficients and of the trade-off between fluid-solid transport and pressure drop. Accordingly, the Diamond lattice was identified as the best geometry offering the highest transport coefficient compared to open cell foams and the state-of-the-art honeycomb monolith, although, a higher pressure drop resulting in a worse trade-off between the two properties was obtained compared to the honeycomb.

Advanced designs have then been investigated in the following part of the work, in view of the intensification of catalytic applications affected by the trade-off between fluid-solid mass transfer and pressure drop, such as environmental catalysis applications. Two design strategies have been

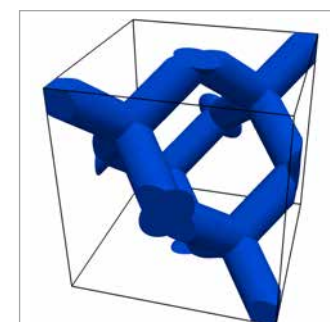


Fig. 2 - An example of POCS unit cell (Diamond unit cell).

examined at the POCS unit cell level. Firstly, an advanced Diamond design was obtained by changing the struts orientation to the flow direction. In doing so, the unit cell was stretched along the flow direction modifying the fluid-solid interaction. This solution enables to boost the trade-off between fluid-solid mass transfer and pressure drop by a factor of 2 compared to the honeycomb, potentially allowing for the intensification of mass-transfer limited catalytic reactors. As a second solution, streamlined elliptical struts lined up to the flow direction have been envisioned. Also in this case, this solution benefits the pressure drop versus mass transfer trade-off enabling to outperform the state-of-the-art honeycomb monolith, while offering at the same time higher specific surface areas for catalyst deposition. In the last part of the work, the engineering models previously developed through fundamental investigations are employed for reactor design, presenting a new technology for a realistic exhaust aftertreatment system based on cellular materials for DeNO_x-SCR. To limit the pressure drop, an alternative configuration of the reactor based on radial flow was considered in this case, hence, the conventional device equipped with the state-of-the-art honeycomb substrate was substituted with the radial flow reactor equipped with cellular substrates. Such a configuration is only possible for cellular substrates, since they are characterized

by isotropic permeability in contrast to the segregated flow in the honeycomb channels. The new reactor was found to be a compelling solution, offering higher NO_x conversions than the standard device by virtue of the cellular substrates higher transport coefficient, but with comparable or even lower pressure drop than the honeycomb due to the radial flow. Overall, the advanced POCS unit cell designs and the alternative radial flow reactor highlight that cellular substrates flexibility in design may pave the way to improved reactor solutions for Process Intensification.

THERMO-RESPONSIVE POLYMERS IN NON-POLAR MEDIA

Gianmaria Gardoni – Supervisor: Prof. Davide Moscatelli

Thermo-responsive polymer nano-objects able to dynamically change their properties in response to modifications in the local temperature are finding growing attention in various fields, especially in the biomedical and oil&gas sectors. *Chapter 1* describes the tremendous potential and enhanced performances that thermo-responsive polymers can impart to a target substrate. The review addresses some of the most important applications of this class of materials, such as cell culture, chromatography, colloidal stabilization, and enhanced oil recovery. Many examples from the literature are used to show the state of the art, criticisms, and primary benefits of this technology above traditional tactics, highlighting the unique functionalities that these active surfaces can achieve.

As clearly shown by the examples reported in *Chapter 1*, most of the syntheses of highly controlled thermo-responsive nano-objects are performed via Controlled Radical Polymerization (CRP) techniques in water. However, the possibility of synthesizing thermo-responsive copolymers with a well-defined phase separation and bulk response in organic

solvents is becoming more and more critical for applications in the oil&gas field and as lubricants for heat engines. Here, the missing step is translating the wide research that has been conducted on the so-called waterborne smart materials also to these non-polar media.

Chapter 2 provides a convenient strategy for synthesizing modular thermo-responsive block copolymers dynamically self-assembling into nano-objects with different morphologies in a poorly polar solvent, *i.e.* a 50:50% v/v decane:toluene hydrocarbon blend (dectol). In particular, the thermo-responsive behavior can be finely tuned through Reversible Addition-Fragmentation Chain Transfer (RAFT) polymerization by changing some simple parameters such as the solid content and number of units of both the thermo-responsive and solvophilic block.

Once demonstrated the possibility to efficiently synthesize modular thermo-responsive copolymers in a non-polar solvent, *Chapter 3* further expands the research by taking into account the influence of the solvophilic block on the final properties, an aspect

which is often neglected. In particular, the living feature of RAFT is once again exploited to carefully control the copolymer structure, more precisely the number of units of both thermo-responsive and solvophilic block and the length of the alkyl side chains. In this way, it is disclosed the relationship between the structural parameters of the copolymers and their thermo-responsive and colloidal behavior, which can be determined a priori and tailored according to the needs.

As highlighted by the first chapters, RAFT proved to be a valid strategy to synthesize well-defined thermo-responsive copolymers in non-polar media due to its outstanding control over the copolymer structure and, as a consequence, properties. However, RAFT (and CRPs in general) is still far from being widely diffused on an industrial scale due to its high costs and low polymerization rate.

Therefore, in *Chapter 4*, it is proposed a method to synthesize well-defined thermo-responsive polymers via Free Radical Polymerization (FRP), the preferred technique on industrial level.

The notorious problem of compositional drift of the

copolymer chains in FRP is solved by implementing a Power Feed strategy. Through this process, it is possible to produce thermo-responsive polymers with a carefully controlled composition, reflected in a controllable cloud point and sharp phase separation while maintaining high productivity

THERMODYNAMIC ANALYSIS OF NOVEL SYSTEMS FOR SUSTAINABLE PROCESSES

Fabio Giudici – Supervisor: Prof. Laura Annamaria Pellegrini

The rising focus on a more sustainable future, and hence on technologies to reduce greenhouse gas emissions and their relative global impact, has increased interest in CO₂ capture processes and processes for biofuels production. Thus, the present doctoral thesis, which fits into the actions undertaken to achieve the Sustainable Development Goals of the 2030 Agenda, aims to study the thermodynamic behaviour of mixtures relevant in mentioned sectors and to have a practical relevance for process modelling and simulation for obtaining safer and economically advantageous plants. The main objective is to fill the lack of reliable models on these issues.

As regards carbon dioxide separation processes, they can be carried out by several techniques, including low-temperature methods. In order to improve the performance of these technologies, it is essential to accurately predict the thermodynamic behaviour of the mixtures of interest, especially in the presence of solid CO₂. For this reason, a numerical code based on the theoretical approach developed by Span and Wagner and by Jager and Span was developed to depict the thermo-chemical properties of

pure carbon dioxide, with a focus on the solid phase. In addition, correlations for the calculation of CO₂ transport properties (surface tension, viscosity, thermal conductivity) were also implemented. The accuracy of the algorithm was then verified by analysing different data sets available in the literature. The outcomes point out that the numerical code is able to reproduce the thermodynamic properties of CO₂ with an AAD% less than 0.8%, except for the melting pressure, which shows an AAD% equal to 3.6%. The AAD% related to the transport properties, instead, is not greater than 4.6% (the higher AAD% values are mainly due to the fewer experimental measurements considered). All the results achieved highlight the reliability of the numerical code developed in the present work. The study of the thermodynamic behaviour of pure CO₂ was followed by the thermodynamic analysis of two binary mixtures containing carbon dioxide: the CO₂ + H₂ and the CO₂ + N₂ systems. As regards this topic, different set of experimental data related to the two binary systems have been selected after a literature search and used to calibrate an existing algorithm based on a thermodynamic

approach for the simultaneous computation of stability and multiphase equilibrium of CO₂ mixtures with hydrocarbons and/or other components. The outcomes highlight that the original algorithm predicts very accurately the values of the bubble and dew curves for the CO₂ + H₂ system (with an AAD% not higher than 2.7%), while for the CO₂ + N₂ mixture the agreement between computed results and experimental data is less satisfying at higher temperatures and pressures. In order to overcome this problem, various modifications have been assessed. Of these changes, only the alpha correction by Mathias

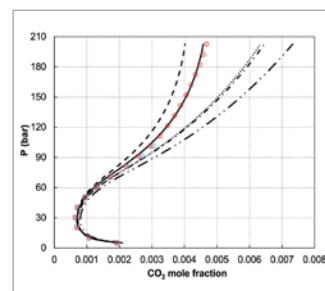


Fig. 1 - Comparison between results obtained with the original algorithm (dashed line), the code with the Poynting correction (dash-dotted line), the algorithm with the alpha correction by Mathias and Copeman (solid line), the code with the solid molar volume correction (dotted line), the algorithm with the solid fugacity correction (dash-dot-dotted line), and the experimental SVE measurements (light blue squares refer to Sonntag and Van Wylen, while red dots refer to Smith et al.) at 150 K.

and Copeman has improved the efficiency of the algorithm, especially for the solid-vapor equilibrium (SVE), as displayed in Figure 1.

As previously mentioned, this research has also dealt with the study of bio-processes. In particular, a thermodynamic analysis of bioalcohols, which are of particular interest because of their gasoline-like characteristics, was carried out. Considering the renewed attention paid to the production of bioalcohols, new equilibrium measurements, which are not available in the existing databases or in the literature, were collected for different binary and ternary systems, such as: ethanol +

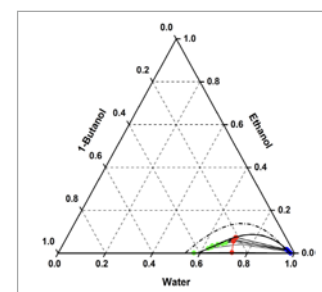


Fig. 2 - Comparison of experimental VLE data for the water + 1-butanol + ethanol system at 0.5 bar (blue dots refer to the aqueous phase, green dots refer to organic phase and red dots refer to vapor phase) with the values predicted by the UNIFAC (dash-dotted line), NRTL (dashed line) and UNIQUAC (solid line) models for the non-isothermal binodal curve (black lines) and for the vapor line (red lines).

water (VLE data at 1.03, 1.5 and 2.0 bar), 1-butanol + water (LLE data at atmospheric pressure in the temperature range 313.55–353.15 K), 1-butanol + 3-pentanol (VLE data at 1.01 bar), water + 1-butanol + 3-pentanol (VLE and VLLE data at 1.01 bar, and LLE data at atmospheric pressure in the temperature range 303.15–313.15 K) and water + 1-butanol + ethanol (VLE and VLLE data at 0.5 bar). The whole activity took place at the *Process Thermodynamics laboratory* at the Department of Chemistry, Materials and Chemical Engineering “G. Natta” of Politecnico di Milano and within the *Equilibrio entre fases* research group at the Chemical Engineering Department of University of Alicante. The first two systems were also tested at pressures at which experimental data are available in the literature, in order to validate the apparatus and experimental procedure. The experimental data were then utilised to calibrate the NRTL, NRTL-RK and UNIQUAC models, implemented in the most modern process simulators, which can then be used to design or optimise new separation strategies. Satisfactory results were obtained from the modelling point of view for almost all the systems investigated (Figure 2 shows the outcomes obtained

for the VLLE data of the water + 1-butanol + 3-pentanol system). On the other hand, the 1-butanol + 3-pentanol and water + 1-butanol + 3-pentanol mixtures proved to be the most difficult to adapt to the models.

METAL-EXCHANGED CHABAZITES AS EFFECTIVE DENOX CATALYSTS FOR LEAN DIESEL ENGINES APPLICATIONS: A FUNDAMENTAL AND KINETIC MODELING INVESTIGATION

Umberto Iacobone – Supervisor: Prof. Enrico Tronconi

Nowadays, there is a growing awareness to shift towards a more sustainable society, hence emission regulations have been implemented worldwide to contain the environmental pollution and enhance the air quality. Nitrogen oxides (NO_x), primarily present as nitric oxide, nitrogen dioxide and nitrous oxide (NO, NO₂, N₂O), are widely recognized as harmful gaseous substances responsible for a broad range of detrimental effects (e.g. respiratory system irritation, cardiovascular damage, ozone layer depletion, acid rain formation, global warming). Among the various sectors of human activity, the transport one stands out as the main contributor to anthropogenic NO_x release, specifically through fossil fuel combustion. Therefore, governments have introduced dedicated emission standards and modern vehicles must comply with them to be commercialized. This has spurred the development of technologies aimed, among others, at reducing and removing NO_x from vehicle exhausts, forcing positive declining emission trends (e.g. the global European Union NO_x emissions have roughly halved over the past decade). However, in view of future more stringent regulations, further enhancements in the

aftertreatment technologies are required. To this end, this PhD work intends to investigate and broaden the fundamental mechanistic understanding of metal-promoted zeolite (Cu- and Pd-chabazites) catalysts for deNO_x technologies, namely NH₃-Selective Catalytic Reduction (NH₃-SCR) and Passive NO_x Adsorbers (PNAs), under low temperature (low-T) conditions. NH₃-SCR, utilizing Cu-promoted chabazites (Cu-CHA) catalysts, is currently the most effective technology for controlling NO_x emissions in diesel-fueled vehicles under warm engine conditions. However, the cold start phase (T < 200°C) presents a challenge as the catalytic deNO_x activity is limited, leading to the release of significant pollutant emissions. Numerous studies have emphasized the redox features of the Standard-SCR reaction ($4 \text{NO} + 4 \text{NH}_3 + \text{O}_2 \rightarrow 4 \text{N}_2 + 6 \text{H}_2\text{O}$) over the Cu ions (Cu²⁺ ↔ Cu⁺) which act as the active site precursors. However, the exact nature of these sites and of the two half-cycles (reduction and oxidation) in the redox mechanism are still subject to debate in the SCR field. Therefore, a significant portion of this thesis is dedicated to investigating the low-T NH₃-SCR Reduction Half Cycle (RHC):

this work was conducted in collaboration with Johnson Matthey and the University of Virginia, USA. This research mainly utilizes a dedicated Transient Response Method (TRM), based on CO to CO₂ oxidation, to probe the formation of multinuclear Cu²⁺ species, under various conditions and with different Cu-CHA catalyst formulations. The initial application of such a technique showcased a strict relationship between the CO₂ production during CO exposure and the NH₃ pre-saturation. The ammonia solvation of Cu²⁺ ions (namely ZCu²⁺(OH)⁺ and Z₂Cu²⁺) plays a crucial role in the SCR chemistry as it provides varying degrees of intra- and inter-cage mobility, allowing for the formation of dimer-like structures. This aspect justifies the net carbon dioxide enhancement observed when NH₃ is present. The CO oxidation is an inherently dual-site process that necessitates the co-reduction of two neighboring Cu²⁺ ions within a dimer-like complex. NH₃ promotes the formation of such structures, thereby enhancing the process. However, due to its slow rate, CO conversion remains incomplete even after a 90-minute exposure. Consequently, a kinetic analysis of carbon dioxide production has

been conducted to investigate the nature of the active sites involved. This approach, performed on different catalysts, demonstrates that ZCu²⁺(OH)⁺ ions serve as the sole precursors of active sites in the absence of water, while Z₂Cu²⁺ cations seem inactive. In fact, the analysis of integral CO₂ production with an NH₃-saturated catalyst revealed that the maximum CO conversion corresponds to half the population of ZCu²⁺(OH)⁺ ions, aligning with the dual-site mechanism. However, when water is introduced into the feed stream, a net positive effect is observed, resulting in an asymptotic titration corresponding to half the overall Cu²⁺ content. This finding, combined with additional Transient Response techniques, theoretical modelling (DFT), and spectroscopic techniques, converged to indicate the presence of a viable hydrolysis mechanism. This pathway involves the reversible interconversion of Z₂Cu²⁺ species into ZCu²⁺(OH)⁺, facilitated by the simultaneous presence of H₂O and NH₃. Consequently, under these conditions, the overall copper content acts as a unified cation population, specifically in the form of NH₃-solvated Cu²⁺(OH)⁺, thereby promoting the formation of Cu²⁺-dimers that now include also the previously inactive Z₂Cu²⁺ ions. Although the water effect assessment was initially conducted on a single Cu-CHA catalyst formulation, additional testing has been performed, validating the occurrence of the hydrolysis phenomenon across a broader range of

catalysts with varying Cu loading. Consequently, regardless of the Cu fraction, once NH₃ and H₂O are co-present, Cu²⁺-dimers originate encompassing the whole ions population, regardless of the Cu loading. Finally, a 24-hour CO oxidation test experimentally verified the exclusive involvement of ZCu²⁺(OH)⁺ cations during the CO oxidation chemistry in the absence of water. This finding validates the proposed dual-site chemistry and supports a Cu²⁺ pair mediated low-T SCR-RHC pathway, in contrast to single sites mechanisms traditionally proposed. In addition to the comprehensive investigation of the Reduction Half Cycle (RHC), the SCR catalyst aging has been investigated in collaboration with Cummins Inc.. Hydrothermal aging (HTA) conditions commonly occur in various NH₃-SCR systems, leading to changes in catalyst performance and a concomitant irreversible transformation of the acid sites in Cu-CHA catalysts. Using again Transient Response Methods (TRMs), we addressed this issue by studying two Cu-SSZ-13 samples exposed to increasing levels of HTA. The collected evidence points to a straightforward stoichiometry for mild HTA processes, specifically involving the conversion of ZCu²⁺(OH)⁺ to Z₂Cu²⁺, concurrent to a consumption of Brønsted sites (ZH⁺). These findings further consolidate and align well with previous observations regarding the Cu²⁺-pair mediated SCR-RHC chemistry. Additionally, the results obtained in this study demonstrate that TRMs can

serve as viable alternatives to more complex experimental techniques to characterize the catalyst evolution (e.g. upon HTA, sulfur poisoning). Achieving further insights in the herein inquired aspects is crucial for the development of high-performance catalysts and for enhancing the emission control technologies. Synergic to the NH₃-SCR investigation, the examination of Pd-exchanged chabazite (Pd-CHA) catalysts for low-temperature Passive NO_x Adsorber (PNA) applications has been undertaken in collaboration with Johnson Matthey. These catalyst systems are designed to capture NO_x at low temperatures and subsequently release them thermally when downstream reduction units (such as NH₃-SCR) become active. Despite extensive research, the mechanistic pathway of NO_x adsorption and desorption on such systems remains a subject of controversy. To address this issue, transient kinetic analysis was employed based on a simple Pd-redox mechanism (Pd²⁺ ↔ Pd⁺) for NO adsorption, as reported in a previous experimental study. While some limitations are still present in the analysis, the results hereby obtained are encouraging. A successful description of the PNA system under different reacting conditions and Pd-loadings supports the simple Pd-redox scheme herein adopted and validates the modeling approach for the description of PNA materials and their functionality.

MULTISCALE INVESTIGATION OF REACTIVE FLUIDIZED TECHNOLOGIES FOR THE ENERGY TRANSITION

Daniele Micale – Supervisors: Prof. Matteo Maestri, Prof. Mauro Bracconi

Heterogeneous catalysis has emerged as a crucial technology to address the challenges associated with the reduction of the human footprint and the optimization of natural resources usage and energy consumption in order to meet the Sustainable Development Goals set by the United Nations. To meet the objectives posed by the energy transition, innovative reactors have to be designed and the existing technology must be optimized. In this context, the fluidization technology, with its unique characteristics such as homogeneous reaction environment, high heat transfer rates, and effective management of solid particles, is acknowledged as a promising route for achieving high productivity, selectivity, and energy efficiency in various catalytic processes promising for the energy transition. Indeed, fluidized systems, particularly fluidized bed reactors, have shown promise in the context of CO₂ capture and utilization technologies, valorization of natural gas, and integration of plastics in the circular economy. A comprehensive understanding of the interactions between fluid dynamics and catalytic reactions is however required to properly design fluidized systems. Computational modeling, coupled

with selected experiments, has emerged as a promising approach to gain insights into these complex systems and facilitate the design, optimization, and scale-up of fluidized processes. In particular, the multiscale modeling, which combines the first-principles descriptions of all the phenomena occurring in a reactive environment, represents a key tool for the computational analysis of heterogeneous catalytic reactors, providing a comprehensive understanding of their performance. In this context, my Ph.D. work aims at increasing the understanding of complex fluidized systems by developing dedicated multiscale numerical methods with the focus of fundamentally analyzing innovative fluidized technologies

promising for the energy transition. Challenging issues, such as the accurate description of fluid dynamics, particle shape representation, management of chemical kinetics, and computational cost reduction, have been addressed for this purpose. Consequently, two numerical multiscale frameworks based on Eulerian-Lagrangian and Eulerian-Eulerian descriptions of multiphase flow have been developed. These frameworks enable the simulation of fluidized units from lab- to industrial scale, offering insights into complex multiphase fluid dynamics, detailed descriptions of chemical kinetics, and their interplay. On the one hand, the multiscale Euler-Lagrange framework enables the fundamental investigation of novel processes

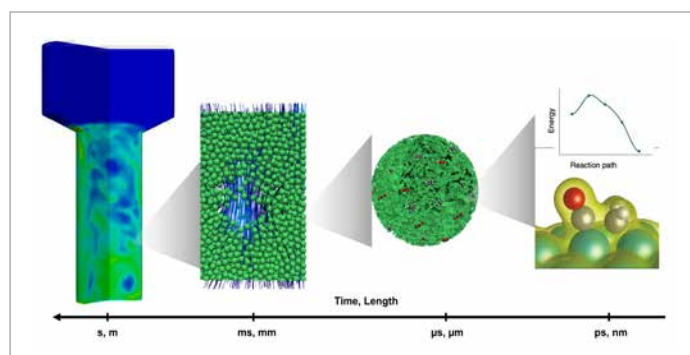


Fig.1 - Schematic representation of all the phenomena accounted for in multiscale simulations of catalytic fluidized units: gas and solid transport in the reactor (left), particle-particle and gas-particle mass, momentum and heat transport (middle), detailed description of the reactivity (right).

and fluidization concepts in lab-scale units and the development of engineering correlations, based on the first-principles description of the phenomena, which can improve the accuracy of less detailed numerical strategy (i.e., Eulerian-Eulerian one). On the other hand, the multiscale Euler-Euler framework provides a deep knowledge of the interplay between the physical and chemical phenomena occurring in pilot or industrial units, representing a promising tool for studying the design and scale-up of novel fluidized processes and for optimizing the existing industrial units. The potentials of the multiscale frameworks have been finally demonstrated through fundamental investigations of novel reactor technologies, including dynamically structured fluidization, and processes, such as plastic waste gasification and synthesis of turquoise hydrogen and advanced carbon materials via methane pyrolysis, paving the way for the rational design of innovative fluidized technologies. The work was organized in two parts. In the first part, the fundamental investigation of novel processes and fluidization concepts at the lab-scale has been performed through the multiscale Eulerian-Lagrangian framework. After its development, a combined experimental and numerical campaign has been carried out to first assess the reliability of the strategy in the prediction of lab-scale unit performances and, at the same time, to show the insights regarding the gas-solid reactive

environment that it is able to provide. Then, the Eulerian-Lagrangian framework has been adopted for investigating under a reactive standpoint the dynamically structured fluidization, a novel fluidization concept aiming at easing the scale-up of fluidized units, demonstrating a strong relation between the flow characteristics and the conversion of such systems and the advantages that it can provide in managing highly exothermic processes. Finally, the multiscale Eulerian-Lagrangian framework has been extended to manage particles of arbitrary shape (e.g., cylinder, Raschig rings), and used to fundamentally analyze the effects of the particle shape on both the fluid dynamic and reactive behaviors of bubbling fluidized bed reactors. In the following part, the fundamental investigation, screening of the operating conditions, and design of pilot and industrial scale processes promising for the energy transition have been carried out using the multiscale Euler-Euler framework. This latest tool has been first developed together with proper numerical strategies (i.e., Multiphase Operator Splitting, Dynamic Load Balancing) to allow for the computationally affordable simulation of the whole transient behavior of such systems. Then, an industrial-scale bubbling bed reactor for the Oxidative Coupling of Methane has been analyzed with the developed framework showing the strong relation between the distribution of the gas and solid phases and

the concentration of the main reactants and reaction products. The screening of the operating conditions of a fluidized bed reactor for the gasification of two plastic wastes (i.e., polyamide, polyvinyl chloride) has been subsequently carried out to understand the effects of the conditions on both the plastic degradation kinetics and the evaporation phenomena. Finally, the floating catalyst chemical vapor deposition reactor for the synthesis of turquoise hydrogen and advanced carbon material via methane pyrolysis has been optimized by combining the multiscale framework with experimental campaigns conducted at Rice University. Specifically, the gas injection system has been redesigned through the combined effort of computational modeling and experimental campaign providing the enhancement of the productivity of the system, and the fluid dynamic of the units has been fundamentally analyzed to reduce the inert content of the feed, with the consequent improvement of the process energy efficiency, without losing in productivity. All in all, this work showed that multiscale modeling is able to grant fundamental insights into the interplay between the catalytic chemistry and the transport phenomena unraveling crucial pieces of information needed for the advanced design of fluidized processes promising for the energy transition.

CHEMICAL CHARACTERIZATION AND TOXICOLOGICAL EVALUATION OF ODOROUS VOLATILE ORGANIC POLLUTANTS EMITTED FROM INDUSTRIAL PLANTS: FOCUS ON WORKERS INVOLVED IN DYNAMIC OLFACTOMETRY

Elisa Polvara – Supervisor: Prof. Selena Sironi

Odour pollution is a growing problem, as suggested by the increasing scientific interest in this topic and the rising attention by regulatory authorities. Nowadays, dynamic olfactometry is the reference method in Europe for quantifying odour emissions, standardized by EN 13725. This technique is a sensorial analysis and leverages the capability of the human nose to be stimulated by an odorant. As a result, dynamic olfactometry involves direct human examiners, called *panellists*, to determine odour concentration. During the analysis, panellists are directly exposed, at increasing concentrations, to the diluted emission sample, which may contain potentially hazardous pollutants. By this, they are exposed to an occupational risk. The exposure assessment is therefore fundamental to define appropriate measures to protect the health of the panellists and thus to safely conduct olfactometric analyses. However, limited guidelines are prescribing in the standard and the only valuable protection measure that can be adopted to guarantee panellists safety is the definition of a *minimum dilution value* (MDV) not to be overcome during olfactometric analysis. However, despite the relevance

of this topic, at the current state, a standardized method for estimating the risk and MDV value is not yet described in the standard. In addition, the methodologies for conducting the risk assessment for panellists described in scientific literature, once applied to real case studies ad real exposure scenario, present some critical aspects. Therefore, my PhD project was focused on the investigation of risk assessment for workers involved in dynamic olfactometry analysis. To deal with this problem, two different research topics, connected with industrial hygiene and chemical problems, were investigated. Regarding the industrial hygiene side, it was necessary to investigate the risk, proposing a method of risk determination. Indeed, after a preliminary

application of literature methodologies to real case, new risk assessment model, robust, conservative yet user-friendly, was constructed, with the aim to obtain the most comprehensive determination of occupational risk, seeking to overcome the criticalities observed in the application of previous models. However, after the application of the developed method to real odorous samples, it appeared clear that another critical point of risk assessment for panellists is the definition of specific exposure time and dose of examiners during their working activity. Indeed, the exposure parameters (exposure time (ET), frequency (EF) and duration (ED)) are fundamental to evaluating the real risk, and, at this stage of my research, these parameters were evaluated, in the literature, by an average exposure. In

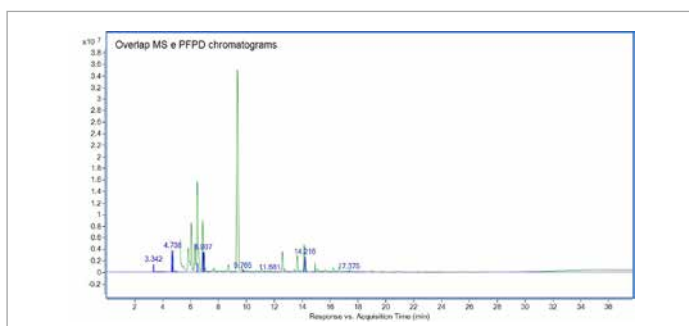


Fig.1 - Overlap of chromatograms obtained with new GC instrumental configuration.

addition, an evaluation of the variability of samples (in terms of nature and composition of the gas) is not considered by adopting a deterministic risk assessment method. Therefore, the constructed deterministic risk assessment method still presents a degree of uncertainty due to the particular activity performed by these workers. To resolve these problems, the adoption of a probabilistic approach for performing panellists' health risk assessments focusing on their real-case occupational exposure scenarios appears a useful solution to overcome these criticalities. Therefore, a new probabilistic approach, based on randomisation of exposure parameters by using a Monte Carlo simulation, was proposed to overcome the deterministic models and describe the exposure risk for olfactometric workers in terms of probability. This PhD research, therefore, is the first attempt to investigate the occupational risk for these workers in detail, proposing and evaluating solutions to address this problem and conduct olfactometric analyses

while protecting the health of examiners. About the chemical issue, obtaining the complete chemical characterisation of complex odorous is necessary to evaluate the exposure risk for panellists. To identify and quantify the species present in odorous emissions, the most diffuse analytical technique is gas chromatography coupled with mass spectrometry (GC-MS). Despite the potential of this technique and the extensive application of this technique in the field of odour emissions, in different situations it shows some critical aspects, in particular, correlated with the quantification and detection of specific compounds (e.g. sulphur compounds). Therefore, my research investigated the applicability of a new instrumental configuration, constituted by a gas chromatograph coupled with three different detectors, to chemically characterized odorous mixtures. In parallel, another important step to obtaining the complete chemical characterization of odorous emissions is the choice of sampling materials. Indeed, the selection of the most suitable

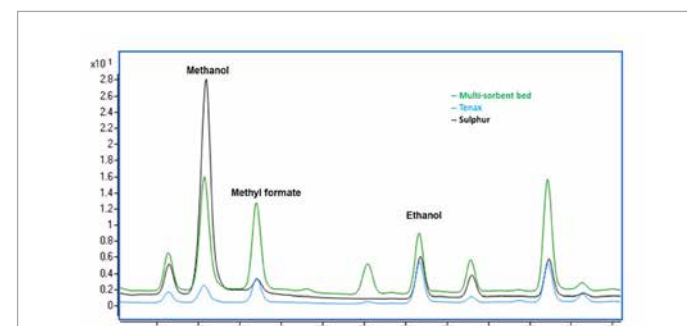


Fig.2 - Performance comparison of different sampling material - real odorous sample

sampling method has a great influence on the obtained results. Focusing on sampling and analysis of odorous emissions, three methodologies are mainly adopted: sorbent tubes, stainless steel canisters and polymeric bags. These three methods differ in portability, applicability, sampling volumes and storage of samples. Each method, however, has certain criticalities, but in the absence of specific regulation all can be used equally for the chemical analysis of odorous emissions. Therefore, a comparison of different sampling methods was conducted to investigate the performance of different sampling materials applied to collection of real odorous samples. From the obtained results, the combination of different detectors, in parallel and in support of the mass spectrometer, can be extremely useful to better characterize and quantify the odorous molecules responsible for malodours and obtain a more complete characterization of odorous emissions. Discussing sampling methods, the selection of the sampling materials could be a critical point for the chemical analysis of odours, due to their different performance/usability: about sorbent tubes, the selection of the most suitable sorbent material can drastically influence the retention and storage of specific compounds. From the comparison between sorbent tubes and polymeric bags, the performances appear similar.

SYNTHESIS OF FLUORINATED POLYMERS FOR DRUG/GENE DELIVERY AND ¹⁹F-MRI IMAGING

Carola Romani – Supervisor: Prof. Alessandro Volonterio

In the last years, the development of new polymeric non-viral vectors for gene delivery able to overcome both the cytotoxicity issues and the transfection efficiency limitation which are often linked to the non-viral vectors exploited so far has gained great attention. The principal goal of this thesis is to develop multifunctional fluorinated systems for gene/drug delivery that could possibly overcome this drawback being, at the same time, highly efficient, low cytotoxic, and with a possible implementation in ¹⁹F-MRI imaging (Fig.1). Two main projects characterized the thesis, each one composed of sub-projects, dealing with 1) functionalization of PAMAM dendrimers with ad hoc designed fluorinated Michael acceptors, and 2) synthesis of fluorinated block-copolymers. Polyamidoamine (PAMAM) dendrimers are among the most studied cationic polymers as non-viral gene delivery vectors. An "ideal" PAMAM-based gene delivery vector is still missing because of the paradox efficiency/cytotoxicity associated to their generation (the higher the generation, the higher the efficiency and cytotoxicity of the polymer) along with high manufacturing costs. In this contest, the first project

is focused on the synthesis of multifunctional PAMAM conjugates 1) via functionalization of the outer primary amines of low generation and medium generation PAMAM (G2 and G4) with building blocks bearing fluorinated moieties along with a guanidino functional group and 2) by crosslinking the same dendrimers with a fluorinated stimuli responsive linker containing a disulfide bond. For the functionalization, we have designed different fluorinated Michael acceptors able to be straightforwardly "clicked" to PAMAM dendrimers without the need of coupling reagents and/or catalysts. The employed "click" functionalization resulted to be a smooth and tunable tool, guarantying higher control of the reaction outcome by varying the equivalent of fluorinated Michael acceptors with respect to the outer amines of dendrimer. Efficient plasmid DNA complexation and negligible cytotoxicity is observed for the obtained conjugates, showing improved gene transfection efficiency as compared to undecorated PAMAM dendrimers, to the corresponding unfluorinated PAMAM conjugates, and, remarkably, to gold standard bPEI 25 kDa. In a second project, the functionalization

of terminal primary amines of PAMAM G2 and G4 with a new fluorinated ibuprofen (IBU)-Arg Michael acceptor is described. The obtained fluorinated IBU PAMAM-Arg conjugates were tested as fluorinated pro-drug systems designed to overcome conventional drug formulation. When comparing the IBU release profiles of these systems with the release of the encapsulated drug using fluorinated Arg-PAMAM conjugates, which were previously synthesized by our team, we observe a superior efficiency in controlled and pH-dependent drug release behavior with the fluorinated prodrugs. This superiority is evident in both weak alkaline and physiological buffer solutions compared to the drug encapsulation strategy. These results emphasize the

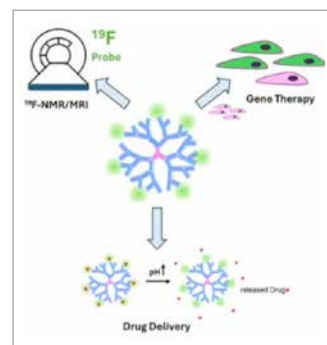


Fig.1 - Multifunctional fluorinated systems for gene/drug delivery with a possible implementation in ¹⁹F-MRI imaging.

significance of trifluoromethyl groups and suggest the potential for synergistic biomedical applications, such as drug/gene delivery. Consequently, we embarked on an investigation into the catalytic activity of fluorinated macromolecular species. Specifically, we focused on G2 and G4 PAMAM dendritic structures, linear polyethylenimine (IPEI), and branched polyethylenimine (bPEI), aiming to develop a fully multifunctional medical tool. This research line aligns with antisense therapy, which relies on the selectively binding of antisense oligonucleotide to mRNA sequence to prevent or silence the the transcription of disease-related proteins. The investigation presented in the thesis shows that PAMAM G2 and overall PAMAM G4 dendrimers partially functionalized with the designed linkers incorporating trifluoromethyl and guanidino groups are able to efficiently catalyze the cleavage of a phosphodiester model compounds demonstrating their possible application in antisense oligonucleotide. The second main project of PhD project relies on the synthesis of biodegradable cationic polymers for biomedical applications, namely gene

delivery and ¹⁹F-MRI, and is splitted in two sub-projects. In the first study, ionizable amino-polyesters were synthesized via ring opening polymerization and free radical polymerization of lipophilic component, producing positively charged polyesters. Sixteen stable nanoparticles were obtained exploiting fluorinated zwitterionic-based polymers by varying the number of the fluorinated units. Depth study over the size of the different NPs obtained were done, followed by a preliminary transfection test *in vitro* using the polyplexes that have shown the most suitable characteristics. A degradation study via DLS investigation demonstrated the degradability of NPs. Furthermore, a deeply investigation on the influence of the number of the fluorinated units inside zwitterionic backbone outlined a remarkable independent control linked to linear correlation between fluorine intensity, NPs size, and fluorinated units, enforcing RAFT and ROP coupled strategies in NPs development by simply modulating the stoichiometry of polymerization reaction. The second sub-project reported the synthesis of fluorinated PEGylated polymers as novel nano-contrast agents detectable via ¹⁹F-MRI. Two reactive PEGylated fluorinated

macro-RAFT agents were prepared through acylation and living polymerization reactions and used for subsequent polymerization induced self-assembly (PISA) reactions to synthesize fluorinated and rhodamine labeled NPs. A preliminary *in vitro* intracellular investigation of the nanotracers revealed an increased cellular internalization with the number of fluorinated units inside the polymeric systems, guarantying an extremely low cytotoxicity.

DEEP EUTECTIC SOLVENTS: NEW TOOLS FOR BIOMASS VALORIZATION

Letizia Anna Maria Rossato – Supervisor: Prof. Paola D'Arrigo

In this PhD thesis, deep eutectic solvents (DESs) were investigated as new tools for biomasses valorization thanks to their versatility and unique properties. DESs are easily prepared by mixing together a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA) at high temperatures (50–100 °C) for few hours. For many years, DESs were considered similar to ionic liquids (ILs) as “new green solvents”, but recently DESs have gained more relevance because they offer some advantages over ILs, such as no-toxicity, biocompatibility, biodegradability and cost-effectiveness. Consequently, DESs are largely exploited in many fields, such as biocatalysis, biomass fractionation, CO₂ capture and metallurgy. However, despite their widespread exploitation, the scientific community is highly debating about what can be considered a DES and what a simple mixture of compounds, which are the main parameters to consider in defining a DES and how the presence of water influences the DES formation. In fact, the term “DES” is often abused because of the limited number of publications devoted to understand DESs nature, and the absence of a standardized, cost-effective method for their

characterization. For this reason, we set up a new analytical tool based on the NMR technique for the study of trimethylglycine betaine-based DESs. In particular we focused on understanding how the relaxation time (T_1) of this set of DESs changes with the HBD molar fraction, and how progressive additions of water might modify this correlation. The first DESs application here reported involves their utilization in the biocatalytic synthesis of polar head-modified phospholipids. In particular DESs in biocatalysis are known to work as co-solvents with water, as pure solvents or as solvent and substrate of the reaction. In the latter case, these DESs are referred to as reactive DESs (RDESs), and we

exploited them for the enzymatic transphosphatidylation of phosphatidylcholine (PC). PC is the most abundant natural phospholipid and is esterified at *sn*-1 and *sn*-2 positions with saturated and unsaturated long chain fatty acids, which constitute the lipophilic moieties, and in *sn*-3 with a phosphate diester as the polar head group. In particular, the enzyme phospholipase D (PLD) catalyzes the transphosphatidylation of the substrate in presence of an alcohol (X-OH) leading to PX formation. So, in this work, we investigated the potentialities of alcohol-based RDESs for the enzymatic transformation of PC into two polar head-modified phospholipids of pharmaceutical interest.

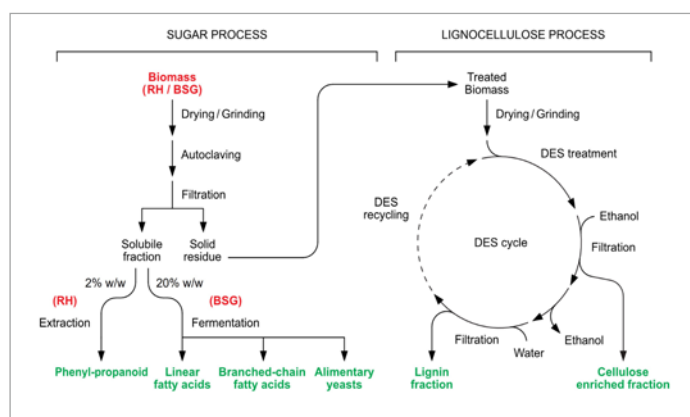


Fig.1 - Schematic representation of the multistep DES-mediated lignocellulose fractionation.

The second application addressed has been the set-up of a multistep DES-mediated process to fractionate two abundant regional agrifood waste biomasses: brewers' spent grain (BSG) and rice husks (RHs). The primary objective was the recovery and exploitation of their main fractions, with particular attention on recovered lignins. In detail, the developed process was based on an initial biomass pretreatment in hot water in autoclave that led to the formation of a soluble fraction and of an insoluble one. For BSG, the soluble fraction (accounting for 25% of the initial biomass) consisted of sugars subsequently exploited for the preparation of growth media for microbial fermentation, instead, in RHs cases, we recovered by extraction phenyl-propanoid acids (2–3%). For all biomasses, after filtration, the insoluble fraction was successively submitted to a DES-mediated fractionation process, which allowed the recovery of the cellulose-enriched fraction and the lignin. The recovered lignin fractions (almost 15–20% of the initial biomass) have been deeply characterized, and a preliminary evaluation of their potentiality as precursors of cement water reducers gave encouraging results.

The third part of this PhD project was focused on the exploitation of lignin recovered from waste biomasses for nanoparticles (NPs) preparation. Initially, the set-up of the NPs synthesis protocol using the technical lignin Protobind has been studied thanks to its quite low cost and large availability in research laboratory. The final aim of this work was to set-up a sustainable scaffold for enzyme immobilization that could be employed in phospholipids biotransformations. In particular, stable lignin nanoparticles were synthesized starting from hydroxymethylated lignin, and then PLD was immobilized on them by direct adsorption without use of any crosslinkers. The immobilized PLD was employed in the biotransformation of PC in three natural high-value polar head-modified phospholipids: phosphatidylglycerol, phosphatidylserine and phosphatidylethanolamine.

OPTICAL INVESTIGATION OF NON-EQUILIBRIUM PROPERTIES OF SOFT SOLIDS

Vincenzo Ruzzi – Supervisor: Prof. Roberto Piazza

The aim of this PhD project is the experimental study of the dynamical and structural properties of two fundamental types of *Soft Solids*: **2D Colloidal Crystals** and **Polymer Physical Gels**. Besides the interest in studying their physical properties, often not fully understood, these materials are useful in several fields. Colloidal crystals for instance are fundamental in photonics, since they are used as photonics band-gap materials or optical filters. The possible usages of hydrogels are even wider: biological hydrogels are the actual best candidates as surrogates of the extracellular matrix because of their low toxicity and high biocompatibility. Among them, thermoreversible gels are the most used due to the simplicity of the sol-gel transition that occurs by raising or lowering the temperature and the possibility of tuning the mechanical properties by simply changing the temperature. Noteworthy is also the growing use and engineering of gels as smart sensors, thermal switches and drug delivers. The research was conducted using various optical techniques: microscopy, “standard” light scattering techniques and more recent experimental methods that combine microscopy and

scattering, such as Photon Correlation Imaging, which allows for discrimination of the temporal correlation of the scattered intensity from different regions of an image of the scattering volume. Among the techniques used in my doctoral work, the **optothermal stimulation** method is certainly noteworthy, in which a partially absorbing laser beam – in the infrared range in the case of aqueous solvents – impinges on the sample, locally heating it. In this way, it is possible to study the thermophysical properties of dispersed and arrested systems exploiting a self-effect on laser propagation known as *Thermal Lensing*, as well as to apply thermal stresses of easily variable intensity to Soft Solids, whose microscopic properties can then be assessed with scattering techniques. This strategy has been initially employed to concentrate colloidal suspensions of particles confined along the vertical direction, which move in a temperature gradient due to *thermophoresis*. By using thermophilic silica particles (which move toward regions of higher temperature), we studied the **2D crystallization** of a system of hard spheres. The nucleation process of the aggregate, its ordering, and the growth kinetics of the formed

crystal were investigated using optical microscopy methods, varying the applied temperature gradient, accurately measured using a temperature-sensitive fluorescent probe. We found that ordering takes place when the cluster reaches an average surface density that is still below the upper equilibrium limit for the fluid phase of hard disks, meaning that we did not detect any sign of a proper “two-stage” nucleation from a glass or a polymorphic crystal structure. The crystal obtained at late growth stage displays a remarkable uniformity with a negligible number of defects. By switching off the thermal field, we observe the development upon crystal melting of defects that are a distinctive signature of the formation of a hexatic phase. As for polymer physical gels, a first part of my doctoral work focused on **biological hydrogels**, whose constituent blocks are peptide chains. In particular, the **gelation kinetics in solutions of amyloid fibrils** of β -lactoglobulin was studied, induced by the perfusion of a saline solution through an osmotic membrane. We observed that the propagation velocity of the gelation front and the microscopic dynamics of the arrested phase depend on the final ionic strength of the

system, which determines how the stresses accumulated during gel formation relax over time. The second investigated protein gel system consists of solutions of **self-assembling peptides**, which aggregate in a basic environment through hydrophobic interactions. In this study, we determined the aggregation kinetics of peptides in solution and the consequent gel formation by utilizing the urea hydrolysis reaction controlled by urease, which allows for gelation times slow enough to follow the aggregation process and at the same time produce structurally and dynamically uniform gels. We found that, in diluted and concentrated solutions, gelation follows different pathways. At high concentrations, a strong gel is formed, made of relatively thick and rigid branches that firmly entrap nanoparticles. By contrast, the gel formed in dilute conditions is weaker, characterized by entanglements and crosslinks of very thin and flexible filaments. The gel is still able to entrap nanoparticles, but their motion is not completely arrested. These different gel morphologies can potentially be exploited for controlled multiple drug release. Within the framework of the study of physical gels, **thermoreponsive systems** have played an important role in my project. In this context, we studied the viscoelastic properties of polymer solutions and Mebiol® gels, a widely used thermoreversible system for cell cultures, using **passive microrheology**. While Mebiol® exhibits Newtonian behaviour in the dilute regime, at higher

concentrations, the viscoelastic moduli show a non-monotonic trend with temperature, with a pronounced peak around the value above which, in the dilute limit, the individual Mebiol® chains are fully compacted. Such a distinctive trend persists within the gel, which therefore becomes “softer” above that temperature. Furthermore, the heating rate significantly influences the phase diagram of the polymer solution: it is possible to form a hard gel by rapidly heating a “low concentration” sample that would remain fluid when heated slowly to the same temperature. This behaviour actually mirrors the effect of quenching rate observed in simple glass-forming liquids and it is consistent with a simple model of competition between chain compaction and increase of the crosslinks strength. Indeed, if the heating rate is much faster than this intrinsic restructuring time, the chains, already fully entangled at low temperature, remain frozen in a network having the same topology but with very strong inter-chain bonds, forming a strong gel. Mebiol® was also studied using the previously mentioned Thermal Lensing method to observe the temperature dependence of the **thermal expansivity** of the system in both the fluid and gel phases. These measurements were also carried out on other thermoresponsive systems, studying the *Volume Phase Transition* in PniPAM microgels and the *micellization* in poloxamer solutions. For both these different kinds of structural transitions we observed a significant peak

in the solute contribution to the thermal expansivity indicating a decrease in the overall solution density, a rather counterintuitive evidence that can nevertheless be attributed to the dehydration of the polymer chains. The technique of *optothermal excitation* using an infrared laser was finally combined with the Photon Correlation Imaging apparatus to possibly unravel the microscopic dynamics and the strain field of physical gels subjected to local thermal stresses. Preliminary measurements on Mebiol® gel suggest that, even for moderate heating, a dramatic change of the microscopic gel dynamics, accompanied by the evidence of consistent strain effects, takes place. On switching off the laser, the gel slowly reverted to its original state, but only if the IR irradiation is kept for a limited time. For longer exposure, structural deformation became permanent, hence the gel displayed a plastic behavior.

NOVEL METHODOLOGIES FOR THE FLOW SYNTHESIS OF ORGANIC SCAFFOLDS

Alessandra Sivo – Supervisor: Prof. Gianvito Vilé

Continuous-flow manufacturing represents an innovative method that revolves around conducting reactions within a constant stream, utilizing microreactors or microfabricated systems in place of traditional batch reactors. In this way, reactants are continuously pumped into the microreactor, where they blend and react, and the resulting product is continuously collected at the reactor outlet. This technology is fundamentally changing how pharmaceutical and industrial organic chemistry are approached. One advantage involves the enhanced performance of these types of reactors, which unlocks new opportunities from a chemical perspective, enabling new reactivities that were previously considered unfeasible, along with the potential for milder reaction conditions. Moreover, there exists an inseparable connection between this new technology and current themes linked to process sustainability and circularity. Flow processing notably reduce waste generation and overall consumption costs, by enhancing mass and heat transfer, as well as by improving mixing conditions and leading to heightened reaction yield and productivity. My PhD thesis has aimed to significantly contribute

to this field, by comprehensively elucidating the fundamental principles and providing practical directives for the creation of highly effective and efficient synthetic procedures. The research detailed in this thesis has underscored the significance of flow chemistry across various processes of key relevance for the industrial sector. This technology significantly enhances reaction efficiency by enabling precise temperature control, particularly in hazardous conditions, and streamlines the scale-up of reactions. This was evident in the first exothermic synthesis of azetidinium salts, where flow technology provided a safer and quicker reaction environment owing to the high surface-to-volume ratio of microreactors. Additionally, the advantages of flow chemistry were apparent in the highly hazardous synthesis of glycidol

and glycidyl derivatives. By employing biphasic mixtures and optimizing work-up steps, the integrated approach demonstrated safer conditions. The telescoped protocol facilitated sequential execution of multiple steps without requiring intermediate isolation and purification, thereby reducing overall time and effort expended in the synthetic process. Since the majority of continuous-flow methods have focused on reactions in the liquid phase, my research aimed to integrate multiple reaction steps in innovative ways, recognizing the necessity to develop more environmentally friendly continuous methods that replace traditional, hazardous, and costly homogeneous catalysts. Therefore, the thesis also emphasized the development of innovative light-driven methods for pharmaceutical synthesis

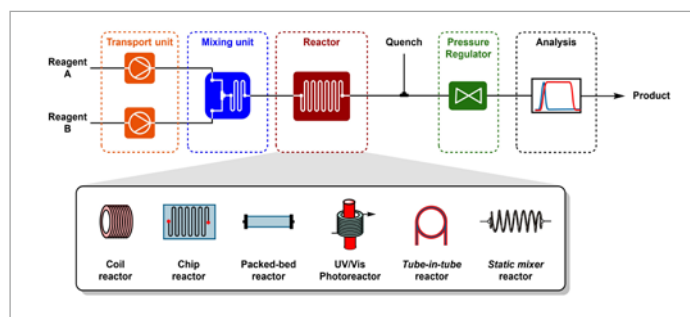


Fig.1 - General scheme of a flow chemistry setup.

using heterogeneous systems. Specifically, in the fabrication of carbon nitride-based materials used in visible light-driven processes, customizing catalyst features was crucial for achieving enhanced catalytic performance. The ability to finely adjust the catalyst's surface area played a pivotal role in optimizing the trifluoromethylation process, a significant transformation in pharmaceutical synthesis. Additionally, exploring the modulation of bandgaps by introducing different metals into these materials provided valuable insights into the theorized reaction mechanism, elucidating factors influencing catalytic performance. The utilization of single-atom catalysts, a novel material class blending features of homogeneous and heterogeneous catalysts, improved catalytic performance in the benzyl alcohol oxidation process. This approach also revealed crucial insights into the role of the photoactive support in the oxidation reaction. These strategies established structure-performance relationships by conducting comprehensive characterization of the prepared nanocatalysts, offering deep insights into their properties and their impact on catalytic performance. The integration of photocatalytic heterogeneous catalysis and flow chemistry poses a significant processing challenge for continuous processing due to suboptimal light penetration within the reactor volume, which limits catalyst-light interactions. To address this, my thesis also

explored the development of nanostructured reactors for flow applications. Particularly, it investigated a newly developed polymer-based material, leveraging enhanced light penetration capabilities due to the presence of carbon nitride as a photoactive component supported by a polymeric matrix. Extensive characterization highlighted the crucial role of the material's structure and porosity in various photocatalytic processes, demonstrating its high photo-stability and recyclability. Overall, the research proves the pivotal role of photocatalysis in harnessing solar irradiation to foster sustainable and eco-friendly processes. This innovative approach holds immense potential to reshape the landscape of sustainable technologies, promising a brighter future for chemistry. The ultimate goal of this PhD project was to establish adaptable procedures for the end-to-end preparation of pharmaceutical intermediates in continuous mode, integrating upstream and downstream steps. Typically, this is the bottleneck of end-to-end manufacturing and the few methods available are often based on the connection of reactive methods with membrane separation technologies. My goal was to reimagine the process by integrating continuous countercurrent chromatography in small molecule synthesis, simulating a "moving bed" which involved the combination of sequential microreactors and a twin-column system. The column loading method ensured that the

product breakthrough from a fully loaded first column was directed to the second one, minimizing waste and technological complexity associated with the use of four-to-six columns typical of simulated moving bed chromatography. In comparison to discontinuous and traditional approaches, this method resulted in higher isolated yields (approximately +60%), increased productivity (approximately +30%), and reduced solvent consumption (approximately -80%). A techno-economic analysis and a life-cycle assessment were also conducted to demonstrate the environmental benefits of the integrated flow process. Overall, the protocol facilitated easier scalability and enhanced suitability for industrial applications, providing an additional tool for designing future sustainable manufacturing processes.

PEPTIDE BASED TECHNOLOGIES FOR EXTRACELLULAR VESICLES ANALYSIS

Alessandro Strada – Supervisor: Prof. Francesca Baldelli Bombelli

Extracellular Vesicles (EVs) are cell-derived membranous structures ubiquitously found in all biological fluids that have been only recently unveiled as fundamental players in intercellular communication. Lying at the cross-roads of biology and nanoscience, EVs are progressively being acknowledged as the new frontier nanoparticles. Yet, many aspects underlining their biological roles remain mostly unexplored due to severe limitations that plague current EV isolation and characterization protocols. Due to the complex nature of biological sample matrices of origin and the physicochemical properties of EVs, accurate and reproducible isolation of EVs still poses significant and mostly unmet challenges, particularly towards the prospective of routinely EVs-based analytical workflows. In this direction, microarrays represent a useful platform for the analysis of extracellular vesicles. This technology allows different simultaneous analysis, low volume consumption of the samples, and high sensitivity. In this technology, peptides have been largely investigated as valuable alternative probes to antibodies, particularly in the immunodiagnostic field, thank to their low cost, stability, synthetic versatility, and tunability of functional groups.

In this thesis work, we set to develop different approaches for the use of peptides in the microarray technology propaedeutic to EVs analysis. In the first case, we present the use of membrane-sensing peptides (MSP) as new effective ligands for directly integrating extracellular vesicles (EVs) isolation and analysis on different microarray platforms. We could show that MSP operate as generic yet selective EVs baits and display higher binding capacity than commonly used anti-tetraspanin antibodies. We reported for the first time the use of peptide microarrays for EVs, where membrane-sensing peptides represent a novel class of molecular ligands for integrated sEV isolation and analysis. Based on the membrane sensing properties of a precursor sequence derived from Bradykinin protein, we performed molecular design that led to the generation of a small library of peptides. In particular, the sequences were developed based on their electrostatic and hydrophobic interactions with the phospholipid membrane of EVs. Our platform showed highly efficient sEV capturing even from unpurified and complex samples such as serum, provided the surface chemistry of peptide-based probes presentation

is accurately tailored. These new molecular probes can be effectively used with protein markers to enhance the isolation and characterization of sEV. Compared to current approaches in EV on-chip analysis based on proteins and antibodies, peptides are characterized by low cost of preparation, remarkable stability, and ease of chemical manipulation, offering virtually unlimited possibilities for experimental design. These molecular probes have been evaluated and compared with each other through tests to capture extracellular vesicles on microarray platform. Furthermore, we here showed how the hydrophobic components of peptide sequence play an important role in membrane sensing. The theoretical model greatly enhanced our understanding of peptide/membrane systems laying the bases for the large-scale computational screening of a virtually infinite amount of newly designed sequences. The results showed that, in addition to a first purely electrostatic approach with the membrane, the presence of hydrophobic anchors within the peptide sequence plays an important role in capturing of these biological nanoparticles. Collectively, these characteristics may encourage

the incorporation of membrane-sensitive peptides into EV affinity-based isolation techniques, enhancing the analytical and pre-analytical EV toolset. Our approach represents the starting point for the application of peptides on other solid support for EVs analysis such as magnetic beads or solid membrane.

Another aim of the thesis was the synthesis of a reference material for the standardization of our platform. For this reason, we investigate a new approach for the production of synthetic extracellular vesicles (synEVs). Bottom-up manufacturing processes use small molecules as the foundational building blocks to create huge, complex structures through a methodical assembling process that combines their physical and chemical features. Specific lipids, ranging from conventional formulations to replicating the makeup of extracellular vesicles, are employed to produce a lipid bilayer before being modified with chemical groups or designed with surface proteins to replicate the structure of natural EVs. As a result, comprehensive knowledge of the roles played by important natural EVs components is necessary for bottom-up approaches. Artificial extracellular vesicles can be pure in composition and have tunable properties by assembling desired components learned from natural EVs. In the second approach, we focused on the use of peptides in the field of hydrogel 3D microarrays. Hydrogels represent ideal semi-wet systems, as

biomolecules can be gently confined within a native-like environment, preserving their structural and functional properties. Hydrogel based immobilization platforms can indeed provide highly versatile systems for the fabrication of functional bio-interfaces on diverse analytical formats: from multi-well plates to microarrays slides. However, some hydrogel properties still limit their widespread use for the creation of bioassays. Herein, we developed a novel nano-materials for the straightforward integration of EVs in multiplexed analytical platforms obtained by mixing a self-assembling peptide with low-temperature gelling agarose. In this approach, agarose represent the core structural component while the presence of peptide is crucial to balance out the rheological properties and the introduction of biofunctionalization. We showed the viability of our 3D approach in real applications by profiling the serum IgG immunoreactivity of Covid-19 patients, which displayed a markedly better signal-to-noise ratio over conventional assays in the 2D format and exceptional specificity. The new composite peptide-agarose hydrogel is uniquely attractive, and the easy control on constituting self-assembling monomers offers virtually unlimited design possibilities to define hydrogels tunable micro-structural and physical properties. The proposed technique overcomes the drawbacks that prevent the widespread use of hydrogels for 3D immunoassays,

such as difficult fabrication, poor biomolecule diffusion, and nonspecific interactions, and has been shown to be user-friendly, resilient, and cost-effective. The 3D assay outperforms standard planar 2D assays in terms of performance due to a more favourable contact between the epitope probes and antibody analytes, as well as greater loading capacity and stability over extended incubation durations. The system allows for immunoreactivity analysis, directly on complex biological matrices, without affecting the molecular recognition properties of entrapped probes nor showing relevant nonspecific interactions. Overall, our findings reinforce the rationales driving the desire for 3D analytical platforms, constitute a step forward toward next-generation bioassays, and, more broadly, demonstrate the potential of new composite (nano)-materials. Finally, we demonstrated the proof-of-concept use of our hydrogel for the physical confinement of EVs under controlled permeability conditions and its use as a platform for the prognostic value of extracellular vesicles as potential biomarkers of bladder cancer. We were able to entrap extracellular vesicles within a biofluid-like environment by a simple mixing step contemporary permitting to other molecules (peptides) the detection of vesicles. The foreseen protocol for analysis is gentle, rapid and likely to preserve those physical and functional properties of EVs.

NEW FRONTIERS IN CHARACTERISATION AND DISPERSION MODELLING OF DIFFUSE ODOUR EMISSIONS

Francesca Tagliaferri – Supervisor: Prof. Selena Sironi

Environmental odours are now subject to regulation and control by the authorities in many countries; for example, in Italy, national guidelines have recently been issued for the monitoring of industrial odour emissions. In this regard, atmospheric dispersion models represent a useful tool for reproducing spatio-temporal distribution of odour emitted by a specific source, thereby quantifying the areas of population exposure. However, their trustworthiness is influenced by various sources of uncertainty. Firstly, the Odour Emission Rate (OER), which has a linear correlation with the fallout concentrations and can be extremely complex to estimate, particularly in the case of diffuse odour emissions, and is therefore considered the major contributor to overall uncertainty. Another significant source of uncertainty is represented by the input meteorological data used to run the simulations in atmospheric dispersion models. In view of this, the PhD project aims to deepen the knowledge about diffuse odour emissions, particularly focusing on different technical aspects related to the characterization of the emission sources and the estimation of the OER, as well as the implementation of input

meteorological data for odour impact assessment studies. The main concerns associated to the characterisation of diffuse odour emissions are:

- the sampling methodology commonly adopted to collect odour samples. For instance, the absence of a well-defined flow directly emitted by the passive sources or the large extent of some emission sources, possibly with a non-homogeneous surface, pose challenges to the sampling approach;
- the possible influence of external variables, strictly related to the specific source considered, on the OER. Indeed, the release of Volatile Organic Compounds (VOCs) from diffuse emissions, and the subsequent dispersion into the atmosphere, is ruled by different complex phenomena, potentially affected by a variety of chemical and physical parameters.

Regarding the odour sampling of diffuse emissions, it is discussed an experimental study to evaluate the performance and optimize, in terms of fluid dynamics and mass-transfer, a recently developed sampling device (i.e. a wind tunnel) by the Olfactometric Laboratory of Politecnico di Milano. Moreover, it is illustrated a comparison between two sampling devices extensively used in case of passive area sources, i.e. the recently developed wind tunnel and a flux chamber with a design inspired by the EPA. As an alternative to the direct sampling system, the potential application of *backward dispersion modelling*, which employs numerical models to inversely estimate emissions by linking observed and simulated concentrations of pollutants, is investigated. Regarding the impact of external variables on the emission from

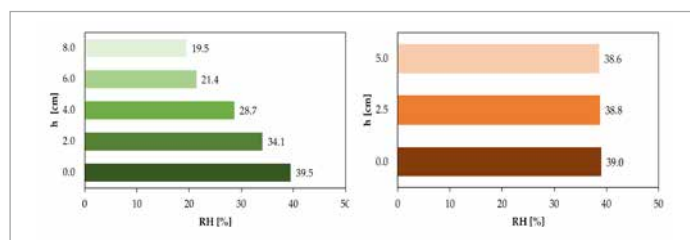


Fig.1 - Vertical concentration gradient at the outlet section of Italian standard wind tunnel (left) and new-proposed wind tunnel design (right).

diffuse sources, the complexity of some specific odour sources (i.e. wastewater tanks and lignocellulosic biomass heaps) is investigated by analysing the possible influence of various parameters on their emission. Very tightly, it is possible to summarise the findings presented in this research. The experimental trials conducted to test the new proposed wind-tunnel design, reveal that the novel device outperforms the Italian standard one in various aspects: a more uniform velocity distribution within the central body and, thanks to an effective outlet mixing system, the absence of a vertical concentration gradient (Figure 1). Concerning the comparison between wind tunnel and flux chamber, the experimental trials conducted on a liquid surface reveal that atmospheric motion cannot be uniquely described as unidirectional or omnidirectional: depending on the instantaneous meteorological conditions, the fluid dynamic is comparable to a wind tunnel or a flux chamber behaviour: it is not possible to exclude a priori one of the two sampling devices. Nevertheless, differently from what the literature suggests, the emission rates estimated by the wind tunnel and flux chamber, in the case of dilute solutions and water-soluble VOCs, appear largely comparable. The numerical model developed to theoretically estimate the emission rate of liquid area sources shows a good overall trend in predicting the experimental measurement, with deviations from the experimental

data generally lower than 50%. Besides the complexity associated with odour sampling, another critical point associated to diffuse emissions is the estimation of the OER, which can be potentially affected by external variables. This study reveals that VOCs emission from liquid area sources seems to be slightly influenced by the wind velocity, whereas the effect of the temperature is much more significant. There is therefore compelling evidence that the commonly proposed approach to evaluate the effect of wind velocity on OER should be revised on behalf of a more consistent approach to experimental evidence. Moreover, it points out that the influence of the liquid temperature should be considered, for instance, by conducting olfactometric campaigns in different climatic conditions. Lignocellulosic biomass is another odour source potentially affected by a variety of factors: surface heterogeneity appears to be the most influential one. Indeed, smoking areas have a much higher odour potential than dry areas, reaching values in the order of 10^5 ou_e/m³. This discrepancy suggests collecting a sufficient number of samples to characterise the emission source. In addition, CO concentration appears to be the parameter most strongly correlated to odour concentration: CO appears to be an auxiliary expeditious parameter for monitoring odorous emissions. Given the challenges of direct sampling and the potential impact

of environmental variables for diffuse odour emissions, WindTrax backward model appears to be a promising tool for indirect estimation of the emission rate. The validation study reveals a general tendency of the model to predict the observed values with a good level of accuracy, especially under stable atmospheric conditions. However, it is worth highlighting that it is not straightforward and to obtain meaningful results it requires a preliminary analysis (e.g. about the sensor position). Lastly, the impact of meteorological data on atmospheric dispersion models are investigated. The most interesting outcome is that in the case of area source, particular attention must be given to the choice of met data for odour dispersion models, as different input meteorological settings lead to discrepancies in odour impact. In particular, it appears that the implementation of measured meteorological data overestimates the odour impact compared to prognostic data or hybrid simulations (blended prognostic and measured data).

DIFFUSION MOTION IN CONFINED SYSTEMS: NMR EXPERIMENTAL APPROACHES

Valeria Vanoli – Supervisor: Prof. Franca Castiglione

The investigation of the diffusion of small molecules represents a cornerstone in scientific inquiry, with implications spanning disciplines from chemistry to biology. As technology and methodologies have advanced, so too has our ability to delve into the intricacies of molecular dynamics. The current state of the art in this field reflects a nuanced understanding achieved through diverse experimental and computational approaches. One of the key advancements lies in the refinement of imaging techniques. Here, NMR spectroscopy has emerged as a cornerstone in the study of molecular diffusion. Pioneering techniques, such as Pulsed Field Gradient NMR, have provided researchers with a non-invasive mean to measure diffusion coefficients. This allows for the precise quantification of how molecules move in different environments, ranging from solutions to biological tissues. NMR's ability to explore diffusion at the molecular level and in complex systems has been instrumental in unravelling the dynamics of small molecules. In recent years, the field of drug delivery has evolved, with researchers focusing on creating novel strategies to enhance the targeted delivery of medications.

As the traditional approach of systemic drug administration often leads to side effects and limited effectiveness, drug delivery now aims for precision and efficiency, introducing a new era in personalized medicine. This work is set in this landscape, where the basic idea came from thinking about the molecular motion of small drugs within a gel carrier, how it can be studied efficiently and its correlation with the macroscopic in vitro release kinetics. As this topic can have a huge number of ramifications, both the choice of analysis technique and of materials was fundamental in order to have a cohesive work. Nuclear Magnetic Resonance (NMR) was used as main technique as it is a fundamental tool in the study of diffusion coefficients in a non-invasive way and data obtained in this way can be easily analysed through various mathematical methods, giving an insight on the type of motion of the molecules under study. A great advantage of the technique is its versatility, both in types of experiments that can be performed and the ability to probe different materials (solid, semi-solid and liquid systems). Using this technique, during my Ph.D. a methodology was developed to study controlled release of drug from gel systems

and how it is related/influenced by drug-polymer interactions. Diffusion at a microscopic level, and consequent molecular interactions, are studied through HR-MAS NMR, while macroscopic release is studied using a technique based on quantitative NMR. To determine the validity and range of application of this methodology, it was important to find different gels following a few basic guidelines: biocompatibility of the components, ease of preparation, ability to dissolve/incapsulate both hydrophilic and hydrophobic drugs and stability at 37°C. Three different systems were then chosen: hydrogels, bijels and eutectogels. The results obtained confirmed that the methodology can be applied to both hydrophilic and hydrophobic systems, chemical and physical gels and used to study the behaviour of different drugs. First, biphasic water/oil porous structures stabilized by hydroxyapatite or nanogel nanoparticles show reduced diffusion coefficients of drugs compared to bulk solutions, showcasing the influence of microstructure and nanoparticle choice on drug delivery behaviour. Second, the development of type V DES/DBS supramolecular eutectogels reveals complex non-covalent interactions between gel

components and drug molecules, affecting solubilization, diffusion, and release profiles, particularly emphasizing thermo/pH-responsive control. Finally, investigation into diffusion regimes within agarose-carbomer and hyaluronic acid-based hydrogels elucidates superdiffusive motion of sodium salicylate due to electrostatic interactions, contrasting with the unrestricted diffusion of ethosuximide. Moreover, simultaneous drug loading unveils drug-drug interactions influencing both microscopic and macroscopic dynamics, emphasizing the necessity of understanding drug dynamics at a microscopic level for effective drug delivery carrier design. The culmination of these studies not only highlights the versatility and significance of advanced drug delivery systems but also the necessity of a multidimensional understanding of drug-polymer interactions for the rational design of next-generation drug delivery platforms tailored to diverse therapeutic needs.