CSGI

Consorzio Interuniversitario per lo Sviluppo dei Sistemi a Grande Interfase

Report 2004

Introduzione

Il Consorzio Interuniversitario per lo Sviluppo dei Sistemi a Grande Interfase è stato fondato a Firenze il 21 dicembre 1993, ed è ufficialmente riconosciuto dal Ministero dell'Università e della Ricerca.

Presidente è il Prof. Enzo Ferroni, e la carica di Direttore è ricoperta dal Prof. Piero Baglioni. Il CSGI raccoglie sette Università italiane (Cagliari, Campobasso, Catania, Firenze, Pavia, Siena, Udine) che hanno coniugato i loro sforzi per apportare un contributo originale alla Scienza dei Colloidi e delle Interfasi. Più recentemente si sono aggiunti altri laboratori di ricerca: presso l'Università di Bologna, l'Università Statale di Milano, il Politecnico di Milano, l'Università di Napoli, l'Università di Perugia, e L'Università La Sapienza di Roma, in qualità di laboratori affiliati.

Scopo principale del CSGI è la preparazione e studio di nuovi materiali supramolecolari "intelligenti" e sistemi colloidali, e lo sviluppo delle loro applicazioni industriali in campo farmaceutico, bio-medico, delle vernici e degli inchiostri, del petrolio e dell'energia, della metallurgia, della detergenza, nei settori cosmetico, tessile, cartaceo, agro-alimentare, per la salvaguardia ambientale, la conservazione del patrimonio culturale.

Il CSGI ha inoltre lo scopo di fornire al mondo industriale le conoscenze scientifiche e tecnologiche di base per incrementare e ottimizzare specifici processi industriali.

Un tipico esempio è l'uso di nanoparticelle metalliche, ceramiche o composite. Le tecnologie riconducibili alle nanoparticelle sono una valida alternativa ai consueti metodi di produzione di materiali metallici, leghe, catalizzatori, ecc. La possibilità di sfruttare le proprietà delle nanoparticlle in soluzione per processi di tipo ingegneristico è particolarmente promettente, per esempio per la sostituzione di materiali costosi o strategici con materiali a basso costo, caratterizzati dalle stesse funzionalità, per la produzione di nuovi materiali con proprietà altamente innovative, che non possono essere ottenute con i processi tradizionali.

Funzioni sofisticate e interessanti sono mostrate da strutture associate plurimolecolari quali le membrane, le micelle e le vescicole, i cristalli liquidi e gli strati di adsorbimento di monomeri o polimeri alle interfasi solido-liquido o solido-gas. Lo sviluppo di queste strutture aggregate è molto importante per varie applicazioni, come i catalizzatori supramolecolari, i sistemi selettivi di trasporto accoppiato trans-membrana, le proprietà ottiche non lineari, gli endorecettori, i polimeri, le leghe metalliche e non-metalliche con proprietà specifiche di conducibilità, meccaniche e chimiche. Pertanto, le tecnologie innovative e applicative offerte dalla Chimica dei Sistemi a Grande Interfase sono evidenti. Questo tipo di ricerca di base è una premessa fondamentale della ricerca tecnologica o pre-industriale.

Il CSGI è particolarmente impegnato nello sviluppo delle seguenti aree di ricerca:

1. Processi alle Interfasi
2. Catalisi micellare, Catalisi con Membrane, Clusters e Leghe Metalliche, Sistemi Supramolecolari
3. Nanoparticelle (Leghe Intermetalliche per l'Industria Aerospaziale; Nanoparticelle di Carburi, Ossidi, e Nitruri Metallici; Leghe Polimeriche; Superplastiche)
4. Lattici Polimerici
5. Polimerizzazione in Emulsione e Microemulsione
6. Rilascio Controllato di Prodotti Farmacologici e Non-Farmacologici
7. Siti di Reazione Microcompartimentalizzati (Micelle, Vescicole, ecc.)
8. Proprietà Interfasali e Strutturali di Biotensioattivi e Biomolecole
9. Gel, Formazione di Gel e Transizioni Sol-Gel
10. Sistemi Host-Guest e Autoreplicanti

11. Dispersioni Colloidali (Farmaci, Inchiostri, Vernici, Metalli, Carbone, ecc.)
12. Solubilizzazione Micellare
13. Adesione e Lubrificazione, Proprietà Strutturali e Reologiche di Epilamine
14. Schiume Polimeriche (Materiali Compositi, Materiali Antiurto e Antifiamma)
15. Miscele Multicomponenti (Carta, Plastiche, Ceramiche, ecc.)
16. Stabilizzazione di Emulsioni, Microemulsioni e Gel per Applicazioni Alimentari, Farmaceutiche e Cosmetiche
17. Proprietà Interfasali di Materiali Tessili e Cartacei; Compatibilità di Fibre Cellulosiche con altre Fibre Naturali o Artificiali
18. Proprietà Termodinaniche e Reologiche di Sistemi Polifasici
19. Nuovi Materiali costituiti da Strutture Supramolecolari Orientate
20. Detergenza.

Il CSGI promuove progetti di ricerca e formazione ad alto livello nel settore della Scienza dei Colloidi e delle Superfici in collaborazione con altre università, centri di ricerca e industrie, con il contributo scientifico dell'Unione Europea, e del Ministero dell'Università e della Ricerca.

Ogni anno le Unità Operative del CSGI si riuniscono in un Convegno Annuale dove vengono presentati e discussi i risultati dei lavori effettuati, con comunicazioni orali o presentazioni poster. L'edizione 2002 si è svolta a Campobasso, quella del 2003 a Pavia, e la riunione del 2004 ha avuto luogo a Cagliari.

Nel presente resoconto, i progetti di ricerca svolti all'interno del CSGI sono classificati in sei categorie:

1A: Hard Matter (Nanomateriali e Interfasi Solide)
1B: Soft Matter (Nanomateriali e Interfasi Liquide)
1C: Aspetti Teorici e Modelli
2A: Restauro di Opere d'Arte, Salvaguardia Ambientale e Chimica Tessile
2B: Metalli e Prodotti Ceramici
2C: Biotecnologie (Prodotti Alimentari e Farmaceutici, Bioprocessi).

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Introduction

The "Consorzio Interuniversitario per lo Sviluppo dei Sistemi a Grande Interfase" (Center for Colloid and Surface Science) was established in Florence (Italy) on Dec 21, 1993, and is officially recognized by the Italian Ministry of the University.

The President is Prof. Enzo Ferroni, and the Director is Prof. Piero Baglioni. CSGI gathers seven Italian Universities (Cagliari, Campobasso, Catania, Florence, Pavia, Siena, Udine) that joined their efforts in order to bring their original contribution to Colloid & Interface Science. More recently other research laboratories at the State University of Milan, Politecnico di Milano, University of Naples, University of Perugia, and University of Rome joined CSGI as affiliates.

The main goal of CSGI is the preparation and study of new supramolecular “smart” devices and colloidal systems, and the development of their different industrial applications such as pharmaceutics, bio-medical, inks and paintings, oil and energy, metallurgic, detergents, cosmetics, textile, paper, food industry, de-polluting procedures, conservation of cultural heritage, and so forth. CSGI is also intended to provide Industrial companies with the basic scientific and technological knowledge to improve and optimize specific industrial processes.

The theory of complex phenomena of dispersed systems has finally substituted a multitude of empirical methods, and the use of standard pseudo-conventional measurements. Today the optimization of the preparation and control of dispersed systems can be considered sufficiently achieved. This means that they can be extended to several other technological processes involving different activities.

A typical example is the use of metallic, ceramic or composite nanoparticles. Nanoparticle-related technologies will be a valid alternative to the usual methods for the production of metallic materials, alloys, catalysts, etc. The possibility of exploiting nanoparticles properties in the solution of Engineering processes is particularly interesting, for example in the substitution of expensive or strategic materials with cheaper materials, characterized by the same performances, in the production of new materials with highly innovative characteristics, that cannot be obtained with conventional processes.

Sophisticated and interesting functions are shown by plurimolecular associated structures, such as membranes, micelles, vesicles, liquid crystals, monomer or polymer adsorption layers at the solid-liquid or solid-gas interfaces. The development of these aggregated structures is crucial for several different applications, such as supramolecular catalysts, selective coupled trans-membrane transport systems, non-linear optical properties, endoreceptors, polymers, metal or non-metal alloys with particular properties (conducting, super-conducting, mechanical or chemical). This kind of basic research is a fundamental premise of the technological (or "pre-industrial") research.

CSGI is particularly devoted to the development of the following research areas:

1. Processes at the Interfaces
2. Micellar Catalysis, Membrane Catalysis, Clusters and Metallic Alloys, Supramolecular Systems
3. Nanoparticles (Intermetallic Alloys for Aerospace Industry; Metal Carbide, Metal Oxide, and Metal Nitride Nanoparticles for tools; Polymeric Alloys; Super-plastics)
4. Polymeric Lattices
5. Polymerization in Emulsions and Microemulsions
6. Controlled Release of Pharmacological and Non-pharmacological Products
7. Microcompatimentalized Reaction Sites (Micelles, Vesicles, etc.)
8. Interfacial and Structural Properties of Bio-surfactants, Biomolecules

9. Gels, Formation of Gels and Sol-Gel Transition
10. Host-Guest and Self-Replicating Systems
11. Colloidal Dispersions (Drugs, Inks, Varnishes, Metals, Coal, etc.)
12. Micellar Solubilization Processes of Polluting Agents
13. Adhesion and Lubrication, Structural and Rheological Properties of Epylamines
14. Polymeric Foams (Composite Materials, Shockproof and Fireproof Materials)
15. Multicomponents Mixtures (Paper, Plastics, Ceramics, etc.)
16. Stabilization of Emulsions, Microemulsions and Gel for Food, Pharmaceutical and Cosmetic Industries
17. Interfacial Properties of Textiles and Paper; Compatibility of Cellulose Fibers with other Natural or Artificial Fibers
18. Thermodinamic and Rheological Properties of Polyphasic Systems
19. New Materials from Oriented Supramolecular Structures
20. Detergency.

CSGI promotes research projects and high level educational training in the field of Colloid and Surface Science in collaboration with other Universities, Research Centers and Industries, with the scientific contribution of the European Union, of the Italian Ministry of University and Scientific Research.

Every year all CSGI members gather together for the Annual Meeting, where the scientific results are presented and discussed, as oral communications or poster presentations. The 2002, 2003, and 2004 Meetings were held in Campobasso, Pavia and Cagliari.

In this Report, the specific projects carried out at CSGI are classified into six categories:
1A: Hard Matter (Nanomaterials and Solid Interfaces)
1B: Soft Matter (Nanomaterials and Liquid Interfaces)
1C: Theory and Modelling
2A: Art Restoration, Environmental Remediation and Textile Chemistry
2B: Metals and Ceramics
2C: Biotechnology (Food, Pharmaceutics, Bioprocesses).

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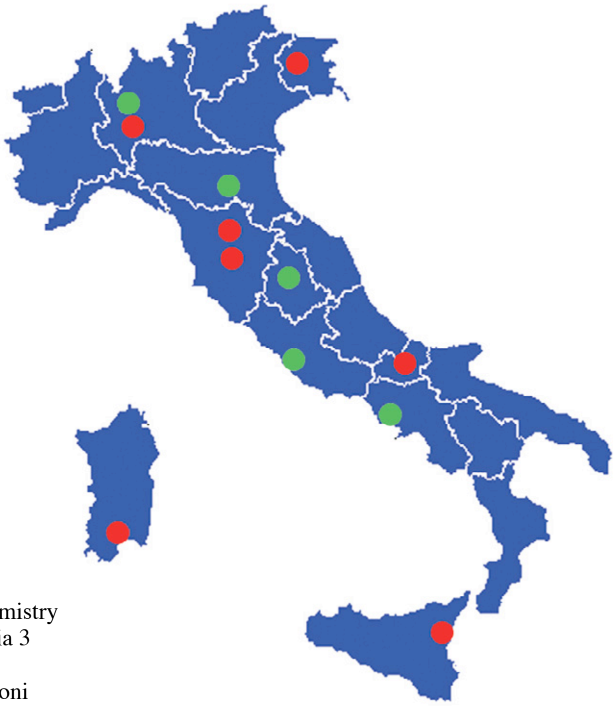
CSGI Units



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1A - Multilayer Thin Films for molecular opto-electronics

G. Caminati, F. Gambinossi, B. Mecheri, P. Baglioni

Aims

Organized molecular assemblies containing surface-active chromophores show specific and useful responses which cannot be achieved in randomly dispersed systems. Molecular design of functional compounds and molecular control of their arrangements will be essential for the construction of highly efficient and functional materials. Langmuir-Blodgett deposition is one of the best methods to prepare highly organized molecular thin films, in which various molecular parameters such as distance, orientation, extent of chromophore interaction or redox potential can be controlled independently in each monolayer. We have been studying photochemical and electrochemical properties of LB films containing electrochromic or photochromic moieties in order to construct molecular electronic and photonic nanodevices.

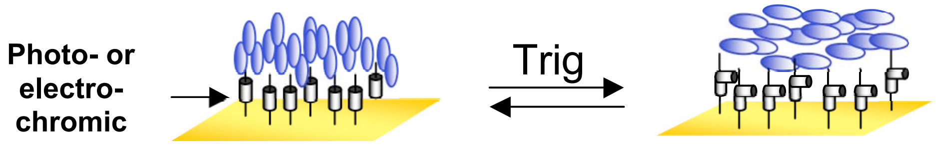

Fig. 1 Schematic representation of an example of the working principle of a “smart” molecular devices.

Results

a) Electrochromic nanodevices

The potential sensitive fluorescent molecules, Di-n-ANEPPS are known to respond to changes in the surrounding medium by modifying their electronic properties.

We studied the interfacial behaviour of Di-4-ANEPPS and Di-8-ANEPPS (figure 2) and we found that, like other styryl dyes, these molecules exhibit a strong tendency to aggregate in monolayer and in multilayer systems. In the attempt to avoid undesired aggregation phenomena in the monomolecular layer, we studied mixtures of the dyes with an amphiphilic matrix.

We used different double and single chain amphiphilic compounds both with charged and uncharged head groups.

The film forming properties of the dye in the mixtures were investigated at the water-air interface by means of surface pressure (p) and surface potential (DV) versus molecular area isotherms.

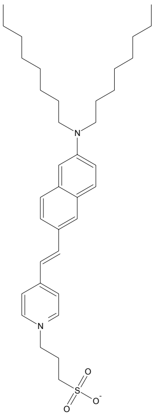

Fig. 2 of monolayers containing Di-8-ANEPPS can be controlled by the appropriate choice of the amphiphile and of the mixing ratio used: stable monolayers were obtained using either DHP (dihexadecylphosphate) and OTMA (Octadecyltrimethylammonium bromide), although p-A isotherms reveal lower stability and higher fluidity in the case of OTMA.

The spectroscopic behaviour of pure and mixed films at the liquid-air interface as well as in LB films was investigated and compared with the electronic spectra of Di-8-ANEPPS in solution.

The absorption spectra of the pure dye and of the mixtures at liquid-air interface show the formation of domains of H-aggregates within the amphiphilic matrices. In particular, in the case of Di-8-ANEPPS/OTMA mixtures with low content of the chromophore no monomer species are detected in Langmuir and in LB films neither by absorbance spectroscopy nor by fluorescence emission. Interestingly, no aggregates were detected in the absorption and emission electronic spectra of the 4:1 Di-8-ANEPPS/OTMA monolayer, either at water-air interface and in LB film; in both cases we determined that the chromophoric part of the dye molecule resides in a polar environment.

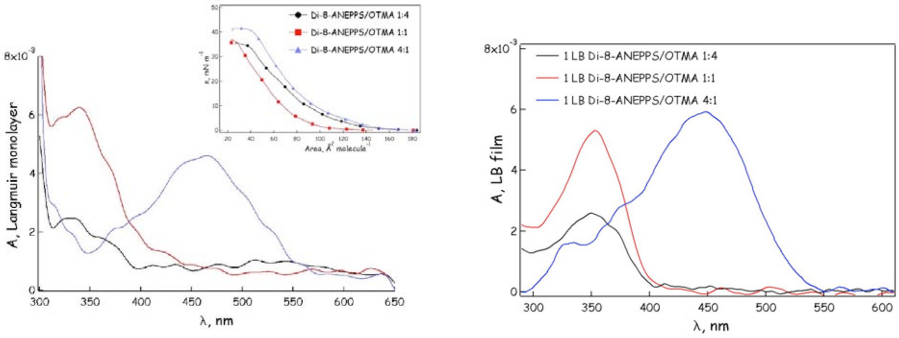

Fig.3 UV-Vis absorption spectra of some Di-8-ANEPPS/OTMA monolayers at water-air interface **(left)** and as LB films **(right)**.

These results on the aggregation behaviour in an organic matrix appear particularly relevant since the understanding of energy migration and transfer in monolayers and LB systems is essential for the rational design and fabrication of microelectronic devices based on vectorial energy and electron transport.

b) Photochromic nanodevices

Photochromic materials in organized molecular assemblies have been the subject of extensive investigations in recent years because of their potential applications in optical switches, memories and displays. When photochromic moieties are introduced in a polypeptide matrix their photoisomerisation reactions can induce conformational changes of the whole macromolecule, amplifying the perturbation due to the single chromophore. Therefore, photochromic polypeptide assemblies can be highly promising materials for photomodulated devices.

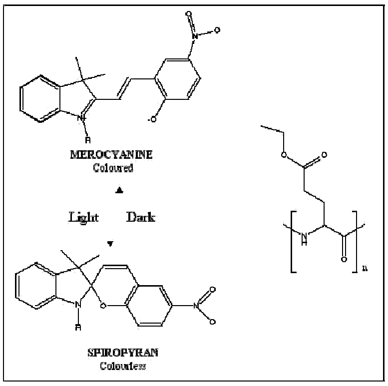

Fig. 4 Chemical structure of PSG. The reversible isomerization between the open merocyanine form (trans) and the close spiropyran one (cis) is driven by the molecule irradiation.

We focused our studies on the investigation of the structural and optical properties of bi-dimensional systems of a merocyanine-substituted poly (L-glutamic acid), PSG (see figure 4). In hexafluor-2-propanol solution, the irradiation with visible light promotes the reversible isomerization of the merocyanine units to spiropyran form. We found that the photochromic biopolymer, PSG, forms stable floating monolayers at the water air-interface that were characterised by means of Surface Pressure-Molecular Area (p-A) and Surface Potential-Molecular Area (DV-A) isotherms, typical results are reported in figure 5.

The shape and the features of the isotherms are different in the case of dark-adapted and irradiated monolayers, indicating that the photoisomerization between merocyanine and spiropyran occurs also at the water-air interface.

Moreover we transferred polypeptide monolayers onto solid supports, by means of the Langmuir-Blodgett technique.

UV-Vis Absorption and Circular Dichroism Spectra, acquired on dark-adapted and irradiated LB films, showed how the biopolymer films respond to the sunlight irradiation.

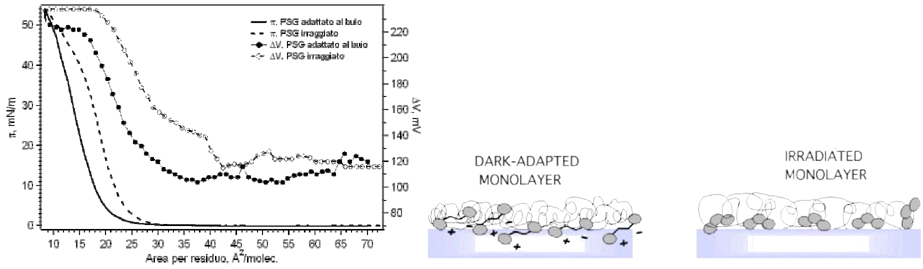

Fig. 5 (left) Spreading isotherms p-A e DV-A of dark-adapted and irradiated PSG monolayers. **(right)** Scheme of the different packing of the PSG molecules in monolayer induced by irradiation.

Fig. 6 UV-Vis Absorption Spectra (inset Circular Dichroism Spectra) of typical LB film of PSG transferred onto quarz. Solid line: dark-adapted film. Dashed line: irradiated film.

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1A - Hydration kinetics and Microstructure of Portland Cement pastes

P. Baglioni, E. Fratini, F. Ridi, F. Mannelli, L. Dei,
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Aims

Hydration Kinetics and Microstructure Characterization
Dynamics of water during the cement's curing
Effect of different additives on Microstructure and Hydration Kinetics

Results

Cement is one of the most used material in human activities. The main components of anhydrous Portland cement are Alite (C_3S, about 50-70%), Belite (C_2S, about 15-30%), Aluminate Phase (C_3A, about 5-10%), and Ferrite phase (C_4AF, about 5-15%); in the chemical nomenclature C=CaO, S= SiO_2, H=H_2O, A=Al_2O_3, and F=Fe_2O_3. It is generally recognized that setting and hardening of the cement are due to calcium silicate hydrate. The setting of cement takes place in several steps. The most important steps can be described according to the following kinetic models:

i) an induction period, that lasts a few hours, is due to the formation of initial products coating the C_3S grains;

ii) a nucleation and growth period related to the formation of C-S-H gel and of C-H products at the surface of C_3S grains; and

iii) a diffusion-limited period, where the C-S-H gel continues growing only inward into the C_3S grains (inner products).

A great deal of information about the C-S-H microstructure has been obtained and refined during the last decade. The basic unit of C-S-H gel has a diameter of about 50 Å. Closed packed globules are formed from the basic units according to the mechanisms above reported and can be characterized by two fractal dimensions. A mass fractal dimension, which increases during the hydration process and involves a development from a very ramified structure (low density, LD) with fractal index of about 1.9 to more compact structures with fractal index of about 2.8, and a fractal surface with a fractal index of about 2.8, that is conserved during the growth process of the globule. Despite the large amount of data, the hardening process and the role of water mobility and structure is still unclear. Moreover, chemical substances (superplasticizers (SP), usually polymers) are added in most of cement applications in order to improve workability and increase the strength decreasing porosity. It is not clear at all how SP interact with C-S-H, how they affect cement hydration, where they distribute during the setting process, how they affect the globule size and interaction, and therefore the final porosity of cement.

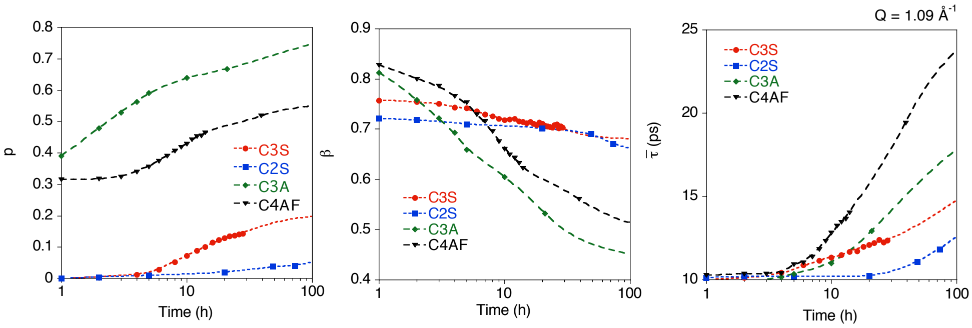
A novel and simple approach has been developed on the basis of differential scanning calorimetry to follow the hydration kinetics of cement pastes in real time from the decrease of the free water index. FWI has been obtained by measuring the fraction of water in the C_3S paste that can solidify and melt. The results account well for the hydration process described by two kinetic stages, the first according to an Avrami-Erofeev nucleation and growth law, and the second one to a three-dimensional diffusion equation. Activation energies and rate constants were also computed. Although quasi-elastic neutron scattering and NMR are more powerful and provide additional information on the water dynamics, we believe that this new simple method will become very popular in all applied investigations, where the knowledge of FWI is essential.

The Differential Scanning Calorimetry (DSC) approach has been used to study the hydration kinetics of tri-calcium silicate (C_3S) in the presence of superplasticizers. We studied some of the most common additives employed in the cement industry, i.e., a sulfonated naphthalene-formaldehyde polycondensate, a polycarboxylate and a polyacrylate. The overall hydration kinetics of the superplasticized pastes have been obtained and compared to that of C_3S cured in pure water. Superplasticizers do not change

the kinetic laws involved in the hydration processes. However, the induction times of the tri-calcium silicate paste are affected by the presence of additives. The polycarboxylate and the polyacrylate additives reduce the temperature dependence of the so-called “dormant period”. The activation energy of the nucleation and growth stage is about doubled in the presence of superplasticizers, and is the largest for the polyacrylate additive, that is the most effective additive. DSC analysis shows that a larger amount of water reacts with C_3S during the acceleration period in the presence of polycarboxylic and the polyacrylic additives, meaning that the water is more available, i.e., the paste is more fluid. SEM images show that the acrylic additive produces a change in the morphology of the formed hydrated calcium silicate gel, from a fiber-like structure to a sheet-like structure. The quantitative determination on how additives affect the curing process of cement is reported for the first time, allowing a determination of a scale of additives efficacy based on activation energies of the nucleation process.

SEM micrographs of C_3S suspensions (w/c) 50), cured at 25 °C for 24 h: (a) C_3S in pure water, bar =1 µm; (b) C_3S in water with NSF, bar=3 µm; (c) C_3S in water with HSP111, bar=2 µm; (d) C_3S in water with HSP114, bar=1 µm.

Moreover, single-particle dynamics of water molecules in hydrated calcium silicates and aluminates, as functions of temperature and aging and in the presence of an additive that retards the curing process. Spectra of incoherent quasi-elastic neutron scattering from hydrogen atoms were measured using a four-chopper spectrometer having an energy resolution of 20-28 μeV, thus probing mainly the translational dynamics of water molecules. The spectra were analyzed with an explicit dynamical model. The model takes into account the existence of two types of water: "immobile water" (type one), presumably water bound inside colloidal particle component of the cement paste, and "glassy water" (type two), water imbedded in gellike component filling spaces between the colloidal particles. The model fits very well all normalized spectra in an absolute scale over a wide range of spectrum covering an energy transfer range of at least 300 eV. We deduced, from these fits, three important parameters as functions of temperature and aging and in the presence of an additive: (1) the Q-independent fraction of the immobile water (p); (2) the Q-independent stretch exponent (β), and (3) the Q-dependent average relaxation time (τ) of the "glassy water". From trends of the age dependence of these three parameters, we obtain a quantitative picture of the kinetics of the hydration process and the structural relaxation of the glassy water. These parameters, obtained for the first time for C_3A and C_4AF, show striking difference from that obtained for calcium silicate pastes (C_2S and C_3S).



Time evolution of the QENS parameters for the investigated cement constituents.

Furthermore, an alternative approach in which we analyze the susceptibility function in the frequency domain instead of dealing with the dynamic structure factor has been proposed and tested. Both methods account very well for the cement hydration process, but the second method is intuitively more appealing and simpler.

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1A - Biosensors based on nano-structured organic films

G. Caminati, B. Mecheri, F. Gambinossi, M. Puggelli, S. Morandi

Aims

General aim of the present project is to gain a deeper knowledge on the mechanism of molecular recognition in interfacial biomimetic systems: spreading monolayers at water-air interface and ordered multilayer nanostructures. This type of investigation represents the basis for the development of biosensors using organized hybrid film technology: Self-Assembled Monolayers (SAM)/Langmuir-Blodgett (LB) devices and alternate LB structures of organic/inorganic materials.

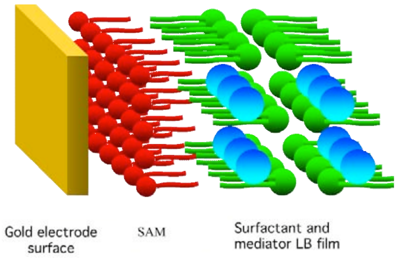


Fig. 1 Scheme of the multilayered architecture signed for the realization of the biosensors.

We focused our studies on the recognition of food-contaminants whose widespread use is posing increasingly high risks to the health of the population.

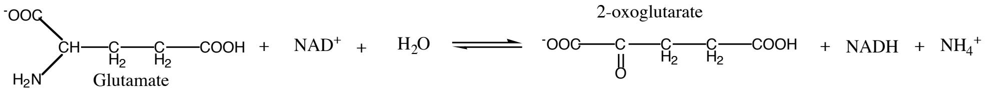
The system described in figure 1 was used for the detection of food contaminants by means of electro-active molecules embedded in the hybrid nanostructure. In particular we investigated two main classes of contaminants: a) monosodium glutamate (MSG), heavily employed in food processing

and responsible of disorders to central nervous system, and b) two of the most used families of veterinary antimicrobial substances, i.e. tetracyclines (TC), and rifamycins (RF). These compounds are well known to induce allergic syndromes and strain resistance to antibiotics. The long term goal of the present work is to develop new methods for the determination of toxic substances in waters and in edible materials.

Results

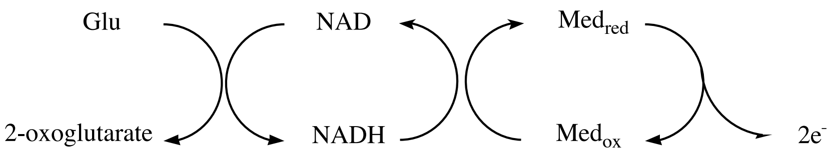
a) Biosensors for MSG.

Much effort has been devoted to the detection of glutamate in fluids not only for its biological relevance but also for the insurgence of allergic syndromes in the population due to its presence in food. Current detection methods are based on the reaction of oxidative deamination of glutamate, catalysed by glutamate dehydrogenase (GlDH) in the presence of nicotinamide adenine dinucleotide (NAD):



$$^{-}OOC-CH(NH_2)-CH_2-CH_2-COOH + NAD^+ + H_2O \rightleftharpoons {}^{-}OOC-C(=O)-CH_2-CH_2-COOH + NADH + NH_4^+$$

The NADH content, directly proportional to the glutamate concentration, can be determined either through direct spectroscopic observation or by electrochemical measurements. The electrochemical determination of MSG requires the use of a suitable redox mediator to facilitate the long-range electron transport from the analyte molecule to the electrode surface according to the following scheme:



Electrode coating with organic ultra-thin films can be successfully used to immobilize such redox mediators. Organized thin films offer several advantages for the realization of novel modified electrodes: first of all the presence of hydrophobic compact layers may act as a selective barrier that can improve control of electrode processes. Secondly, the electrode coating with lipid layers allows immobilization of biomolecules such as enzymes. We focused on the use of different types of organized thin films in preparing sensitive layers containing electroactive molecules: Self-Assembled Monolayers (SAMs), Langmuir-Blodgett (LB) films and Hybrid Films (HFs). HF structures were realized by means of concurrent use of SAM and LB procedures in order to combine high molecular order and architectural control of the LB technology with chemical, mechanical and thermal stability of SAMs (see figure 1). Tipically, dipalmitoylphosphatidic acid (DPPA) was used as lipid matrix for LB fabrication whereas octadecylmercaptane (ODM) and octylmercaptane (OM) were used for the preparation of SAMs on gold surfaces.

Several redox mediators were screened on the basis of their redox features. In the case of non-amphiphilic redox mediators a preliminary study on the co-spreading process with the lipid matrix is performed at the water-air interface. Monolayers containing the desired redox mediator are transferred by LB deposition either directly on solid slides (LB), or on top of previously self-assembled monolayer (HF). Two different immobilization procedures were tested for the immobilization of the mediator in the LB film: a) co-transfer, i.e. direct deposition as LB film of the mixed monolayer; b) incubation, i.e. immersion of preformed thin films of the lipid in a solution containing the mediator. Such LB structures are characterized in terms of their electrochemical and spectroscopic behaviour. In the case of tetramethylbenzidine (TMB), the results indicate that the mediator is present in the film with unaltered electrochemical activity as reported in figure 2.

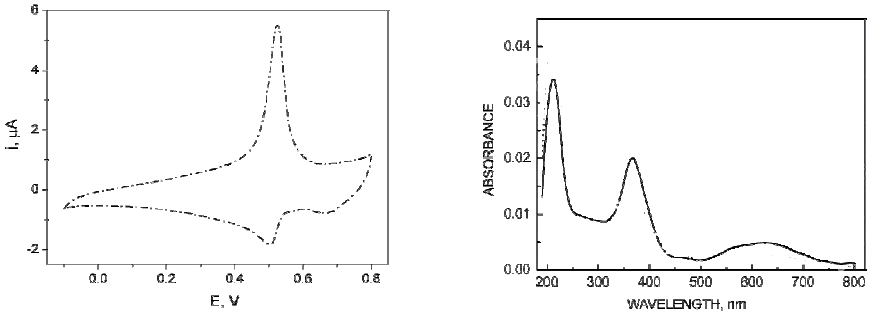


Fig. 2 (left) i-V profile and (right) absorbance of a typical phospholipid LB film containing TMB.

We tested the electrochemical response of such nanostructures for molecular recognition of NADH in solution.

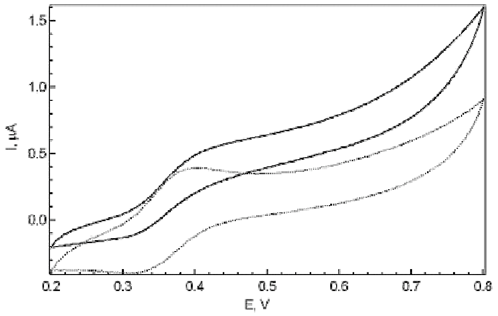


Fig. 3. Cyclic Voltammograms of a typical DPPA:TMB LB film in the absence (dotted line) and in the presence (solid line) of NADH in solution.

The figure shows that the electrochemical signal due to TMB in the film increases when NADH is added in solution; this finding demonstrates that the LB film is able to recognise the NADH molecules. NADH and MSG determination was also performed by means of spectroscopic techniques: the experimental results reveal that the designed architecture of the nanostructure strongly influences the interaction between the biomolecules and the film.

The body of the results indicates that LB nanostructures appear extremely interesting for molecular recognition of biological molecules by properly tuning the architecture and the composition of the film.

b) Biosensors for veterinary antibiotics.

Drug's residues deriving from the veterinary treatment of animals can survive the manufacturing cycle contaminating animal-derived food and the environment. Antibiotics are one of the most ubiquitous families of veterinary drugs which, on over-exposure of the consumer, rapidly induce the development of antibiotic resistance in the population. A rapid and effective control of the presence and level of antibiotic residues involves their fast identification both in animal-derived food and in polluted waters. The paradigm underlying the present research line is the study of the interaction of a suitable bio-reactant incorporated in a Langmuir-Blodgett system with the toxic compounds in the surrounding aqueous medium. The resulting multilayered nanosensor can be directly coupled to the detection devices: we explored both spectroscopic and electrochemical methods. Two typical antibiotic molecules studied are reported in figure 4.

We examined spreading monolayers of several amphiphiles in the presence of the antibiotic in the subphase by means of surface pressure-area and surface potential-area isotherms. We screened several phospholipids differing in the polar head group or in the length of the alkyl chain.

In the case of tetracycline, we found that interactions with the phospholipid molecules is highly dependent on the electric charge of the antibiotic and the ionisation state of the lipid. Significant interactions are established only between the negatively charged form of dipalmitoylphosphatidic acid (DPPA) and the protonated form of tetracycline although penetration through the hydrophobic layer can be excluded. In the case of rifamycins, a systematic study allowed to select dipalmitoylphosphatidyl glycerol sodium salt (DPPG-Na) as the most interactive lipid on the basis of the experimental characterization at water-air interface.

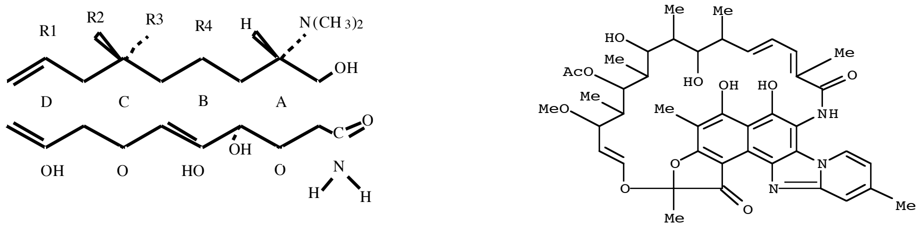


Fig. 4 Chemical structures of tetracycline **(left)** and of rifampicin **(right)**.

LB films of the selected matrix phospholipid were prepared and immersed in a solution containing the antibiotic under examination to allow incorporation of the drug. The resulting films were removed from the solution and characterized by means of contact angle and ellipsometric measurements. UV-Vis and FTIR/ATR spectroscopy as well as cyclic voltammetry were performed on the samples and provided direct evidence of the presence of the antibiotic in the film. For tetracycline, the results showed that TC diffuses from the solution towards the film crossing the alkyl chain layers to reach the interlayer hydrophilic zone. Analysis of the UV-Vis spectra and FTIR/ATR spectra (figure 5) in the Amide I and II region, showed that a specific interaction occurs between the A ring of the tetracycline molecule with the polar head group of DPPA.

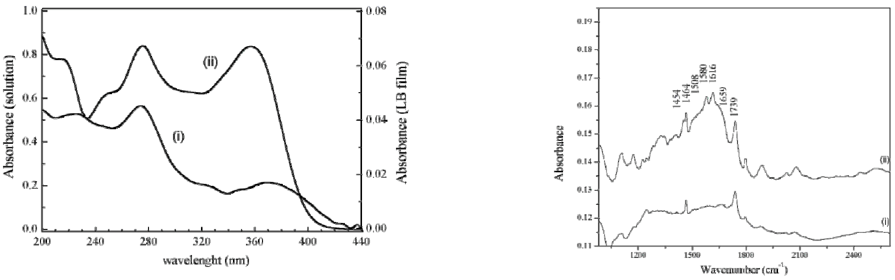


Fig. 5 (left) UV-Vis spectra of 5 DPPA LB layers immersed in TC solution (curve i) together with TC solution spectrum (curve ii); (right) FTIR-ATR spectra on germanium slides: (i) one LB layer of DPPA; (ii) one LB layer of DPPA/TC.

Similarly, studies performed on rifampicin indicated the insertion of the antibiotic into the LB film (figure 6a) and demonstrated a better affinity of RF for the alkyl chains (figure 6b).

Preliminary results as a function of the antibiotic concentration in the examined solution revealed that the LB-based sensor can be used todetermine the antibiotic concentration. In figure 7 we report the absorbance recorded for the LB film after removal from the TC solution. The results obtained with the incubation method open a new perspective for the realization of sensors for antibiotic residues in food by means of the LB technique coupled with a variety of detection systems such as UV-Vis and Fluorescence spectroscopy, Surface Plasmon Resonance and Quartz Crystal Microbalance.

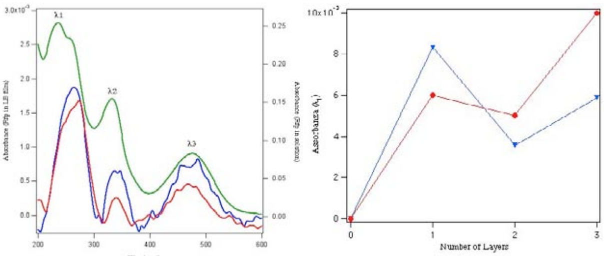


Fig. 6 **(a)** UV-Vis spectra of one DPPG-Na LB film transferred at 25 mN m^{-1}(red line) and 35 mN m^{-1} (blue line) immersed in 1 x 10^{-5} M Rfp solution together with Rfp solution spectra (green line); **(b)** absorbance maxima vs. number of LB layers transferred at 25 mN m^{-1} (red line) and 35 mN m^{-1} (blue line).

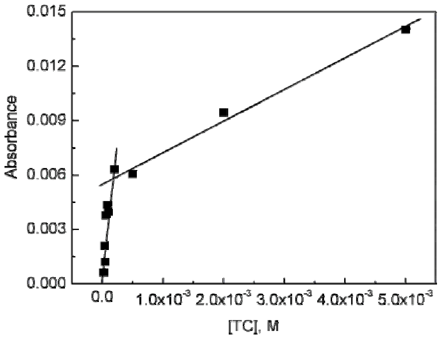


Fig. 7 Absorbance maxima for 3 DPPA/TC LB layers vs. TC concentration in solution.

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1A - Nanostructured coatings for engineering tribological applications

P. Matteazzi, in collaboration with MBN (I), HEF R&D (FR), Liebeherr Aerospace Tolouse S.A. (FR), IfU GmbH Privates Institut für Umweltanalysen (D), Poeton Industries Ltd (UK), Ecole Nationale d'ingenieurs de Saint Etienne (F)

Aims

Novel nanophased powders materials to be applied for fabrication of nanostructured solid lubricants coatings with enhanced wear-resistance at high temperatures and loads;

Energy beam synthesis of nanostructured wear resistant coatings for machine-building industry with the following parameters: thickness100-500 μm; operating temperature up to 900°C, allowable Hertz pressure up to 1100 Mpa, friction coefficient 0.06-0.20;

Thermodiffusion synthesis of solid lubricant compositions based on refractory metals disulphides for aerospace and automotive industries with the following parameters: htickness1-100 μm; operating temperature up to 800°C, allowable Hertz pressure up to 1200 Mpa, friction coefficient 0.01-0.12.

Results

The activity foreseen the development of advanced high-performance Solid Lubricant Coatings (SLC) through materials engineering approach in which required properties will be finely tuned by: designing an appropriate matrix for the solid lubricant as a high performance material (nanostructured, functionally graded), and synthesising by Mechanomaking (the industrial evolution of mechanical alloying and mechanosynthesis) of nanophased powder materials to be applied for coating fabrication. In order to obtain powders best suited for Tribo applications an air-classifier has been developed and assembled. Subsequently it has been equipped with a Jet-mill systems (low energy milling) in order to improve the shape of the particles: it permits to obtain particles more rounded (see *Fig.1*) with better flowability.

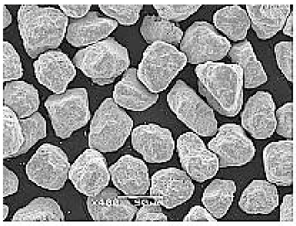

Fig.1 WC/Co powder

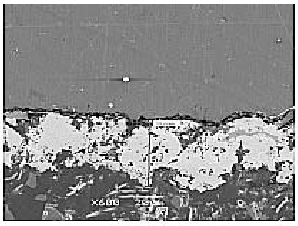

Fig.2 WC/Co coating

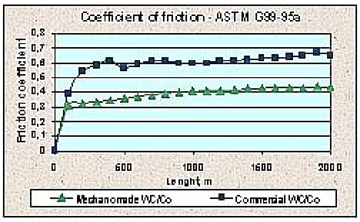

Fig.3 Friction analysis

Different processes of spraying have been used and different results have been obtained. The wear tests are performed for Plasma Spraying, HVOF (High Velocity Oxy fuel) spraying, laser cladding, D-gun spraying and thermo-diffusion synthesis.

The coatings, obtained with nanophased powders, demonstrate advantages in terms of structure, porosity rate (about 1%), pore distribution and result of tribological tests are promising (see *Fig.3*). In order to improve the lubrication performance, a new powder with addition of solid lubricant (WC/Co/CaF_2) has been produced by energy milling and processed by HVOF spraying. The results of erosion and tribological tests on these coatings are expected. Full-scale industrial test (under conditions imitating real exploitation environment), will be performed if the tribolological tests will be positive.

1A - Thermal spraying of nanophased powders

P. Matteazzi, in collaboration with MBN (I), PyroGenesis (EL), KUL (B), Schlumberger (FR), Aprilia (I), NEORION SY (EL)

Aims

Scientific objectives: 1) methodology of designing materials suited for the applications and for thermal spraying processing; 2) synthesis of nanophased powders 3) depositing nanophased coatings having crystal sizes below 100 nm. Technological objectives: 1) plant and processing concepts for thermal spraying of nanophased powders (HVOF, APS); 2) highly corrosion/wear protective surface layers. Technical achievements: 1) parts for motorcycles (cylinder), marine (crankshaft, sleeves) engines; 2) drilling equipments (valve seats, sensor elements).

Results

This project intend to develop the technology of thermal spraying of nanophased materials (grain sizes less than 100 nm), by adapting to each other both the powder materials and the spraying technology. Coatings have been specifically designed for the protection of industrial components operating under severe and extreme wear and/or corrosion conditions (parts of motorcycles and marine engines, oil drilling equipments).

The project is based on two main lines of work: a) designing and producing (by Mechanomaking) nanophased materials powders best suited for the application and thermal spray processing; b) modifying and adapting currently existing HVOF and APS plants to spraying of such powders. Up to now the powders produced and processed (generally with a strong matrix and a binder) are divided in the following systems: nanostructured cermet (WC/Co, WC/Co/Cr), nanocomposites and (Fe/Cu/WC/Co) and alloy (Fe/Cu).

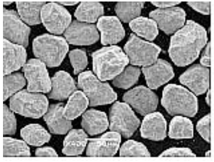

Fig.1 WC/Co powder

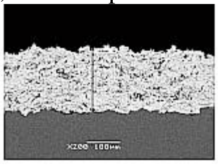

Fig.2 WC/Co Coating

Fig.3 Coating detail

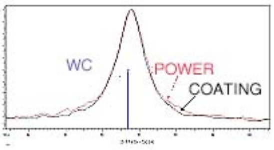

Fig.4 X-ray diffraction

See in *Fig.1* the SEM image of a WC/Co powder is shown and in the *Fig.2-3* the relevant coating processed by HVOF (High-Velocity Oxy-fuel). In *Fig.4* the comparison between powder and coating WC peaks: both with crystal size less than of 20 nm. See in following figures another example of nanocomposites powder (Fe/Cu/WC/Co): *Fig.6* comparison of coating peaks with different methods of HVOF spraying (all nano dimension crystal size). TEM investigation confirms the crystal sizes values obtained by X-ray diffraction.

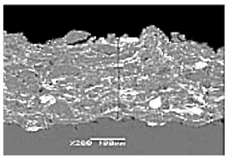

Fig.5 Fe/Cu/WC/Co coating: SEM image

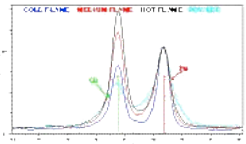

Fig.6 X-ray diffraction: Fe and Cu peaks

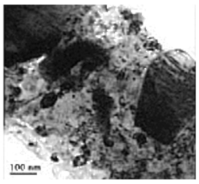

Fig.7 Fe/Cu/WC/Co TEM image

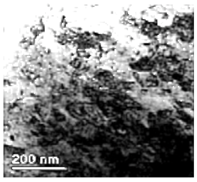

Fig.8 Fe/Cu/WC/Co TEM image

1A - Flame Spraying of Microemulsions

Massimo Bonini and Piero Baglioni, in collaboration with: Niccolo' Baldanzini, Lorenzo Calabri, Lorenzo Ciarpaglini, Paolo Citti (DMTI, Faculty of Engineering, Univ. of Florence)

Aims

Aim of this project is to demonstrate on a lab-scale the feasibility of the flame spraying of microemulsions technique. This new methodology has been recently patented by CSGI [1] and it allows the preparation of nanostructured coatings or powders, constituted by discrete sub-units as small as a few nanometers. An image of a gold film synthesized on top of a silica wafer by means of this innovative process is reported in figure 1.

Results

On a lab-scale, flame spraying has already proved to be an effective synthetic route to obtain really nanostructured coatings, i.e. films constituted by discrete nanodimensioned sub-units [2]. Stable suspensions of nanoparticles are synthesized in reverse micelles and then used to feed the flame spraying system. Thanks to the flexibility of the microemulsion technology, the chemical composition and the dimensions of the nanoparticles can be easily tuned, making so possible to design a surface both form a chemical and a physical point of view.

A prototype intended to produce coatings by means of flame spraying of microemulsions was recently designed and assembled at the CSGI unit of Firenze (see figure 2). This machine can produce uniform nanostructured coatings on objects, as large as 500 mm in diameter and 800 mm in length, with a maximum weigth of 40 kg, i.e. a largege number of mechanical parts used especially in the automotive industry can be treated.

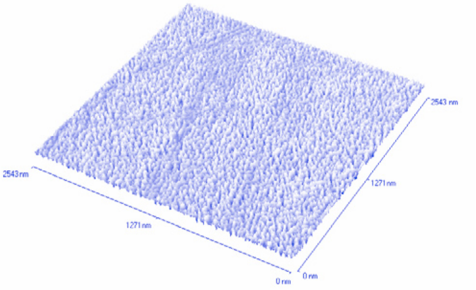

Fig 1. 3D AFM image of a silice wafer coated with gold nanoparticles by flame spraying of microemulsions. Mean diameter of the nanoparticles is about 35 nm.

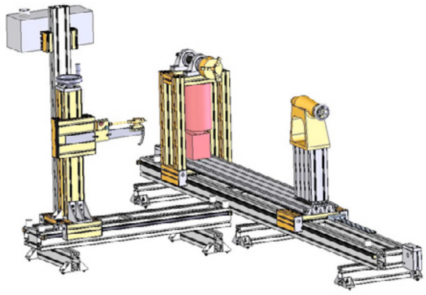

Fig 2. The Flame Spraying of Microemulsion System at the CSGI – Florence.

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1B - Study of the phase behavior of some fluorocarbon/hydrocarbon mixtures and of semifluorinated FmHn copolymers

P. Lo Nostro and S.H. Chen (MIT, Cambridge, USA)

Aims

Phase diagram of fluorocarbon/hydrocarbon mixtures
Self-assembly of semifluorinated alkanes in selective solvents

Results

The mutual conformational incompatibility between fluorocarbons (FC) and hydrocarbons (HC) generates a set of interesting phenomena in all states of matter. These are expression in micro-phase separation (Figure 1), segregation and self-assembly. For example, semifluorinated alkanes (F_mH_n) form ordered smectogenic liquid crystals, adsorb at the air/hydrocarbon interface, and produce gels (Figure 2: gels from F_8H_{16}/C_8F_{18} mixtures) in different organic liquids (fluorocarbons, hydrocarbons, dimethylsulfoxide, dimethylformamide, tetrahydrofurane and so on). F_mH_n can also behave as surface active agents in selected solvents. The nature of common surfactants is based on the 'chemical' antipathy of surfactant head and the tail (reminiscent of the archaic and vague concept of 'affinity'), and on their opposite 'sympathy' for water molecules. Here the mutual repulsion is simply due to the different conformations allowed—the concept and consequences of structural incompatibility are essential. SFA molecules act as "emulsifying" agents in FC/HC mixtures, increasing the miscibiliy of the two liquids, and producing a shift in the upper consolute temperature to lower *T*.

Fig. 1

Fig. 2

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1B - Specific ion effects

P. Lo Nostro, B. W. Ninham, L. Fratoni, P. Baglioni

Aims

Effect of cations and anions at different interfaces (“Hofmeister series”).

Results

We examined the characteristic experimental parameters of some different systems in the presence of concentrated salt solutions (i.e. between 0.1 and 1 M).

In particular, we studied:

the formation and precipitation of polypseudorotaxanes obtained from α- or β-cyclodextrins and PEG or PPG derivatives with different molecular weights in the presence of different electrolyte solutions (measured parameter: threading time; figure 1)

the coagel-to-micelle or the coagel-to-gel phase transitions induced in aqueous mixtures of ascorbyl-alkanoates at different concentrations in the presence of different salts, sugars and urea (measured parameters: transition temperature and enthalpic change; figure 2)

the water absorbency of natural wool fibers in controlled conditions (temperature and relative humidity) in the presence of different salt solutions (measured parameter: weight gain after immersion in water or solution; figure 3)

the growth of Staphylococcus Aureus (a halophilic bacterium) in standard conditions, and in the presence of some water solutions of electrolytes (measured parameter: growth rate of bacteria; figure 4).

Fig. 1

Fig. 2

Fig. 3

Fig. 4

In each case we checked the variation of the examined characteristic parameter as a function of some physico-chemical properties that are directly related to the dispersion interactions (i.e. lyotropic number, free energy of hydration, radius of hydrated ion, partial molar volume, molal surface tension increment, polarizability and molar refractivity).

The results indicate that in all these cases the phenomenon is ruled by dispersion forces, that originate from the different microscopic atomic properties of the single ions (namely polarizability and ionization potential).

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1B - Phase behavior, microstructure and dynamics of surfactant systems: micelles, liquid crystals, microemulsions, and emulsions

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A. Chittofrati (Ausimont CRS, Milano), O. Soderman (Lund, Sweden),
A. Khan (Lund, Sweden), B.W. Ninham (Canberra, Australia)

Aims

Phase diagrams characterization
Relationship between surfactant shape and microstructure
Microstructural transitions in microemulsions:percolative behavior

Results

Some perfluopolyether carboxylates having Na^+, K^+ or NH_4^+ counterions and hydrophobic chains constituted by perfluoropropilene oxide units and mostly Cl-C_3F_6O-terminated, were investigated for phase behavior through surface tension, conduxctivity, optical microscopy and NMR. Different hydration and binding degree of the different counterions cause variations in the extension of the micellar region and in the type of liquid crystalline phases. This, in turn, implies variation of the effective packing parameter v/al.

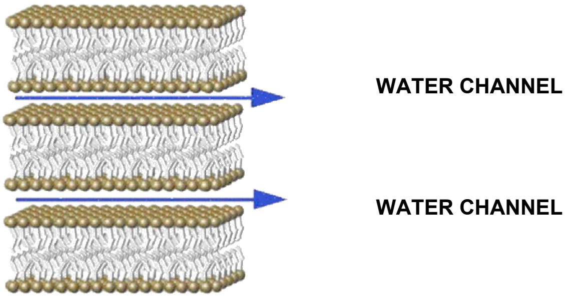

Fig. 1 Sketch of a lamellar phase

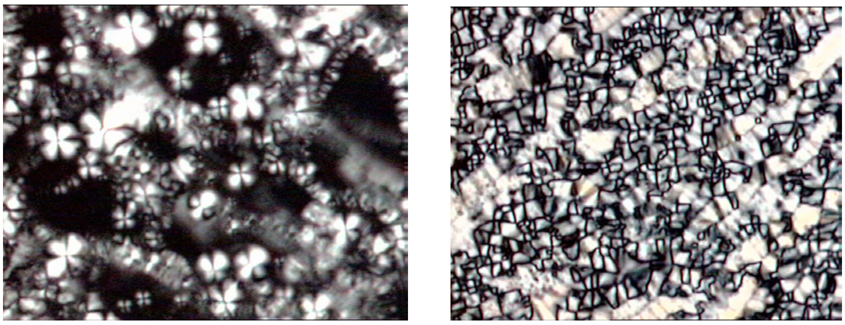

Fig. 2 Optical micrographs in polarized light of different lamellar phases

Investigations on the microstructural transitions occurring in the w/o microemulsions phase formed by Na-AOT and Ca-AOT with decane and isooctane oils were carried out through NMR self-diffusion measurements. Diffusion data along with conductivity measurements allowed to establish that w/o spherical droplets form only in a very limited range of compositions and close to the oil corner.

Several studies were carried out on ternary systems, based on DDAB surfactant.

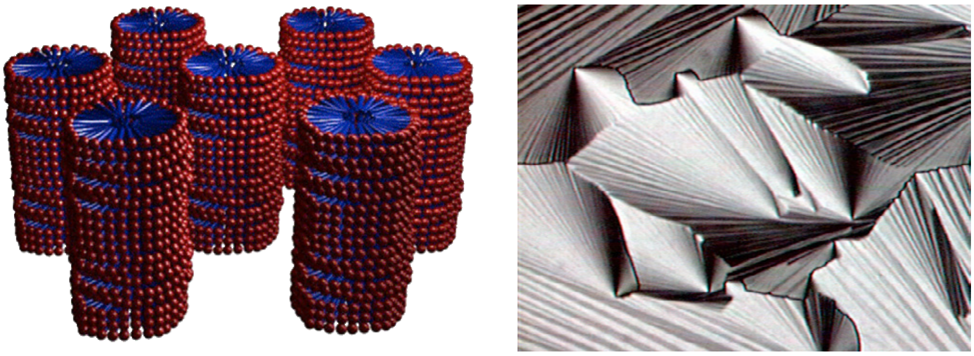

Fig. 3 Hexagonal Liquid Crystals and typical pattern at the Optical microscope in polarized light

Phase diagrams were characterized in the presence of some aromatic oils such as toluene and trifluoro-methylbenzene, and semifluorineted linear alkane oils such as C_4F_9 C_4H_9 and C_8F_{17} $C_{16}H_{33}$, and also a fully perfluoropolyether oil, or perfluorooctane oil. Aromatic oils allow the formation of microemulsion regions characterised, at high oil content, by the presence of DDAB molecules almost molecularly dispersed. This arises from the strong aromatic oil stacking. The presence of semifluorinated or fully fluorinated oils induces the formation of higly stable emulsions which were characterised through NMR self-diffusion, and optical microscopy . The most significant result in the case of a semfluorinated oil (C_4F_9 C_4H_9) is that w/o droplets having bimodal size distribution form [3]. Conversely, in the case of fully fluorinated oils oil-in-lamellar liquid crystals (DDAB/W lamellar phases) emulsions occur.

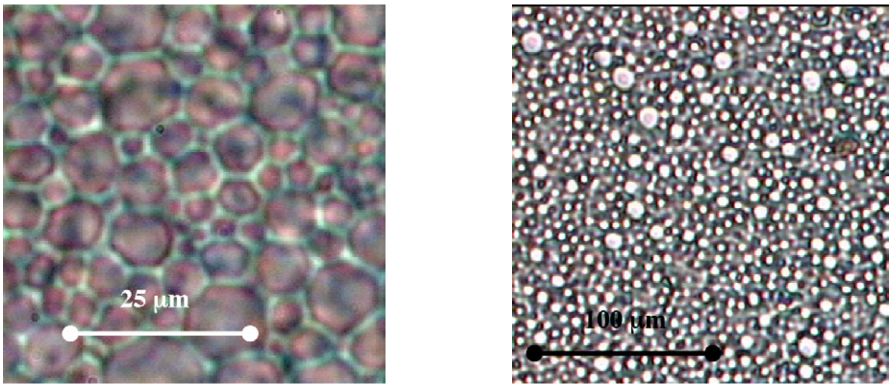

Fig. 4 Optical micrographs of droplets in concentrated emulsion samples

Some DDAB/water/oil (decane, dodecane, toluene) microemulsions were investigated along oil dilution lines through ^{14}N NMR relaxation. The analysis of relaxation data in terms of the two step model along with percolation theory allowed to quantify the range of the correlation times which characterise the interactions (staic and dynamic percolation regimes) among the DDAB aggregates as a function of the oil content.

A review paper on the microstructural transitions occurring in concentrated w/o microemulsions allowed to emphasize furthermore that w/o spherical droplets seldom occur. In many cases, particularly for surfactants having packing parameters close to unity, non spherical closed water domains are often present at low volume fractions of the dispersed phase also.

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1B - ‘Weak’ physical gel in organic media

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G. Colafemmina, U. Olsson

Aims

Microstructure of micellar networks
Phase behavior of lecithine-water-oil ternary systems
Dynamic investigations on shear-induced phase transitions

Results

Microstructure of Lecithin organogels in Isooctane as deduced by surfactant self-diffusion NMR experiments. (**A**) Network of long worm-like micelles with few junction points (branches), whose mean density increases with increasing both micellar volume fraction and water content (**B** and **C**).

The shear-induced Isotropic _ Nematic transition in Lecithin organogels in Cyclohexane presents the unusual property – for a micellar system – to have a very long relaxation time for the process of re-entanglement back to the disordered phase.

This phenomenon, which is reminiscent of a mechanism where nematic state slowly re-melts to isotropic, has been recorded by performing time-resolved SANS experiments as shown below:

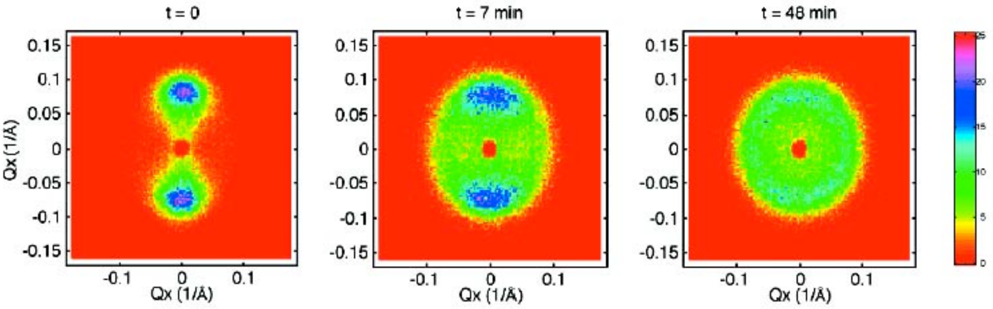

Fig. 1 The detailed phase diagram investigation of the system Lecithin-Water-Cyclohexane at 25°C.

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1B - Structure and dynamics of membrane proteins

G.Palazzo, A. Mallardi, G. Venturoli, R. Piazza

Aims

Study of the coupling of intra-protein electron transfer to protein dynamics in bacterial reaction centers embedded in glassy or polymer matrix

Insight on protein-protein and protein-detrgent interactions in the case of membrane proteins

Results

We have investigated the coupling of protein motions to intra-protein long-range electron transfer and to inter-protein interactions. All the investigations were carried out on a given protein: the *bacterial photosynthetic reaction center* of purple bacterium *Rhodobacter sphaeroide*s. This protein provides an excellent model system for the detailed study of reaction substates and the free-energy barriers between them. The reactions can be probed by means of spectroscopical techniques, and it is possible to initiate electron-transfer in reaction centers (RC) with a pulse of light. This allows synchronized, single turnover measurements over a wide range of time scales. Reactions are reversible, allowing signal averaging.

The reduction of protein dynamics by incorporation into trehalose glasses and in polyvinylalcohol films has been used to investigate the coupling of long-range electron transfer to internal protein dynamics. Therefore the measurements here reported have the advantage of studying electron transfer in the reaction centers, when the protein dynamics approaches the one of a harmonic solid *at room temperature*.

Fig. 1 Kinetics of charge recombination following continuous illumination of RCs embedded in trehalose glasses. Panel A. P^+ decay following a laser pulse (trace a) and continuous illumination of 300 ms (trace b) and 3.5 s (trace c). Continuous curves are best fit to the sum of two power laws. Panel B. Amplitude of the slow component of charge recombination as a function of the duration of photoexcitation at increasing degree of dehydration of the trehalose matrix.

When the above internal motions are slowed down on the time scale of the studied reaction a continuous spectrum of reaction rates is observed (inhomogeneous reaction kinetics). This spectrum reflects the heterogeneity of the ensemble of the protein molecules frozen in different conformational substates, each characterized by a different reaction rate. This means that non-orthodox analyses are required to extract from the observed reaction kinetics pieces of information.

Fig. 2 From left to right: pictures of RC/DDAO samples at pH=6.5 taken 200 s, 12 h, and 4 days after mixing, compared to a sample at pH= 8.0 (last image), remaining fully transparent for indefinite time.

We have also found that, in solution, the presence of a strong electrostatic interaction between the protein and cationic detergents, leads to the phase segregation of reaction centers into mesoscopic ''droplets,'' with a typical size of the order of a few μm and a relatively narrow size distribution. Such a phase-segregation is coupled with a conformational transition of the reaction center. To the best of our knowledge this is the first report on liquid-liquid phase separation tacking place in membrane protein solutions.

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1B - Photoresponsive Surfactants

Massimo Bonini, Debora Berti and Piero Baglioni

Aims

Aim of the project is the investigation of the photoresponsive properties of cationic bolaphorm surfactants, where an azobenzene moiety connects two identical hydrophobic chains terminated by quaternary ammonium groups. Such molecules forms supramolecular assemblies in water in a way similar to ordinary surfactants, but both interfacial and associative properties can be modulated or even fine-tuned by shining the sample with appropriate wavelength light. Since the isomerization is accompanied by a change in conformation, whose extent is determined by the derivatization of the chromophore, they represent excellent candidates for optical storage devices, where the information is contained in the light induced alteration of conformational properties.

Results

Azobenzene derivatives present unique electronic properties, since their cis-trans isomerization, driven by light absorption in the UV-Vis range, presents an elevate reversibility, thus allowing the cycling of photoconversion.

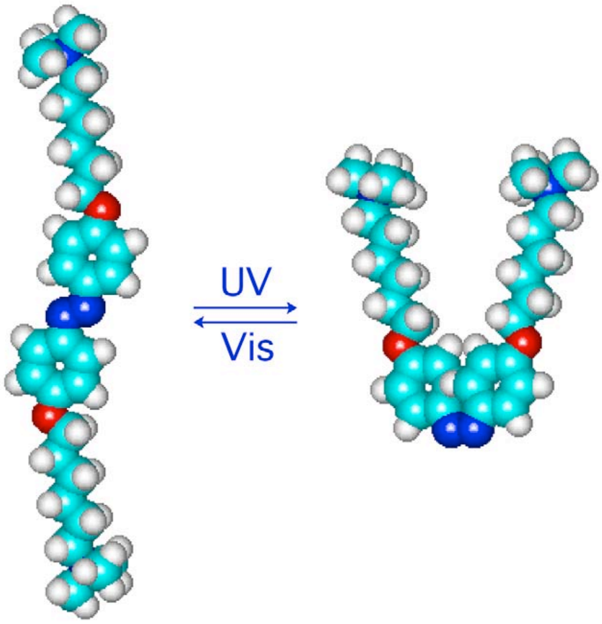

Fig. 1 Isomerization of the BTHA molecule.

Isomerization kinetics have been explored both in binary and in ternary systems (mixed with SDS at different mole ratios) in water. To map the photostationary states in different supramolecular assemblies, a photoconversion cycle has been devised as follows, obtaining a control of the extent of isomerization, as revealed by the reversible UV-Vis spectra.

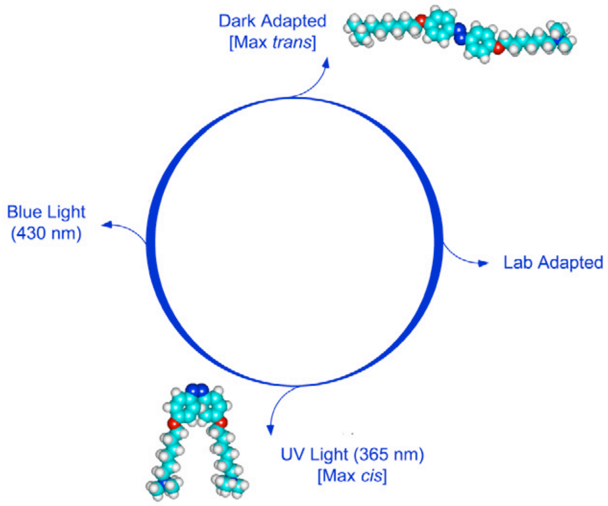

Fig. 2 Sketch of the photo-isomerization cycle.

Isomerization kinetics show a distinct dependence on surfactant concentration for binary systems and on SDS/BTHA mole ratio for ternary systems

Isomerization kinetics show a distinct dependence on surfactant concentration for binary systems and on SDS/BTHA mole ratio for ternary systems.

This is due to the fact that concentration drives self-aggregation and isomerization itself is strictly connected to the packing constraints of the active molecule, dependent on size and shape of the aggregates.

Therefore, in order to gain a deeper insight on this mechanism and also to design supramolecular architectures with the desired structure/performance properties it was necessary to perform a structural investigation, employing the classical techniques of soft matter physical chemistry.

Mesoscopic length scales, that is the dimensional realm of nanosciences, is traditionally investigated by means of scattering techniques, in our case Photon Correlation Spectroscopy and Small Angle Neutron Scattering. For some selected samples spectra have been recorded as a function of the position on the photostationary surface.

Very different results have been obtained for the binary and the ternary system.

This characteristic shed light on the isomerization behavior and also opens the way for posible product formulation.

For bynary systems Dynamic Light Scattering yields results that are consistent with the presence of aggregates with characteristic dimensions typical of the mesoscopic scale. However each intensity autocorrelation functions is not a simple single-exponential function, indicating the presence of different populations. This prevents a straightforward and precise analysis of the data by means of DLS.

Further evidence can be inferred from direct imaging techniques. In Figure 3 two cryo-TEM representative images of 1 mM BTHA in lab-adapted conditions are reported. Closed hollow structures, spherical and rod like micelles are present.

Simple packing considerations regarding the structure and sterical hindrance of the surfactant in its two isomeric forms (see Figure 1) are consistent with the fact that micellar structures can be formed preferentially by the *cis* isomers, while *trans* molecules can favorably pack in locally planar structures that eventually close in spherical vesicles. The presence of two well-defined environments, one for each isomer, deduced by the isosbestic presence, further reinforces this hypothesis.

Fig. 3 Cryo-TEM micrographs of a BTHA/water solution.

For ternary systems, we have found that a particular composition (that is SDS:BTHA 2:1, yields the preferential formation of bilayered structures when *trans* conformation is prevailing, i.e. catanionic photoresponsive vesicles that provide distinctive features to photoisomerization properties. Since *cis* conformation has packing preferences toward micellar aggregates, this is the composition that brings about the highest changes in bulk solutions.

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1B - Bottom-Up strategies for the Self-Assembly of Biologically Inspired Surfactants

Debora Berti, Francesca Baldelli-Bombelli, Martina Fortini and Piero Baglioni

Aims

We have undertaken a structural study of supramolecular structures formed by the self-assembly of recently sinthesized nucleolipids that is double-chain phospholipids undergone to an enzymatic modification that transfers a nucleoside on the polar head. This work is motivated by the interest in the expression of molecular functions in self-organized systems, where the driving force of aggregation has a hydrophobic nature, which is the exclusion of hydrocarbon chains from the contact with aqueous medium. The molecular information (i.e. packing parameter and molecular recognition capacity) is amplified in the supramolecular arrangement and rules phase behavior. Their self-assembly can be fine-tuned by a proper choice of the alkyl chains of the lecithin in the synthetic step. But most important is the molecular information contained in the polar head, standing its biological relevance: like in nucleic acids its functionality is triggered by the macromolecular organization, in our case is the self-aggregation that makes possible base-base interactions

Results

In 1994 we started to implement a remarkably elegant to perform an enzymatic functionalization of phospholipids with nucleic acids in a one-pot combination of natural building blocks.

The reaction proved to be quite flexible and allows the exchange of a choline headgroup in a lecithin with a nucleoside: the resulting derivative has a nucleotide in the polar head reproducing the charge of each DNA monomer and is called phosphatidylnucleoside. The initial interest in such derivatives was boosted by their possible use as liphophilic nucleoside analogues derivatives in mieloyd leukemia treatment. More recently the use AZT (3'-azido, 3'-deoxythymidine, a modified nucleoside) in HIV therapy and the realization that the "bare" nucleoside is inefficient in entering the blood-brain barrier (the most important reservoir of HIV is the central nervous system), sparkled the use of "smart prodrugs". Phosphatidylnucleosides are transformed in nucleoside-triphosphates, that exert antiviral action by inhibiting the reverse transcriptase, in a metabolic pathway carried on by Phospholipases.

A morphoplogical characterization of bilayer-forming phosphatidylnucleoside by TEM techniques, showed a correlation between stacking and H-bonding efficiency of the base on the polar head and the morphology of the aggregate: we would like to stress the attention on the fact that these lipids have a net negative charge per monomeric unit, just like nucleic acids, while the studies until then concerned interactions between ***neutral or oppositely charged groups***. How the stacking H-bonding network propagates along the mesosocopic interface is still unclear and subject of debate.

We have studied phosphatidylnucleosides' aggregation and molecular recognition properties in some details at the water-air interface. A dependence of the limiting areas per molecule on stacking attitudes of the base was found and deviations from the ideal behavior when two complementary-base derivatives were mixed in monolayer reported, suggesting specific interactions between complementary bases.

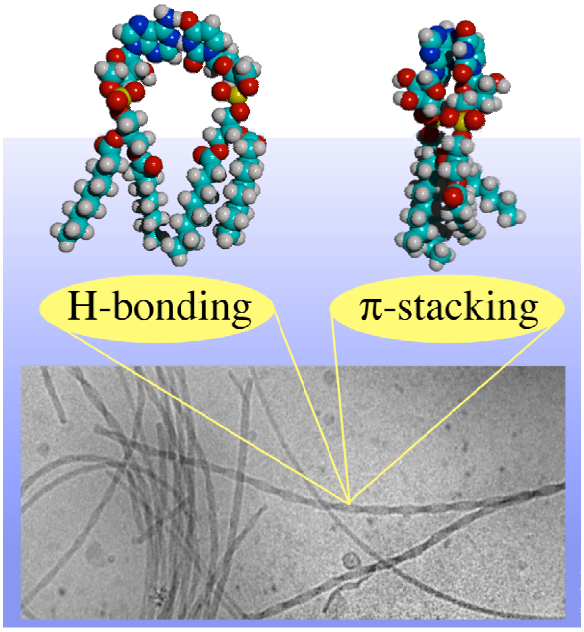
The extension of the study of molecular recognition to mesoscopically extended interfaces was the obvious development of these studies and stable liposomes formed from unsaturated phosphatidylnucleosides have been investigated. Monodisperse vesicles have been prepared from DOP-Adenosine, DOP-Uridine, DOP-Cytidine and DOP-ethenoAdenosine (ε- Adenosine). Uracil and Adenine are RNA complementary bases. Cytidine is a pyrimidinic base like Uridine, and it has a similar sterical hindrance, while ε-Adenosine is a 1,N^6 etheno bridged modification of Adenosine where the amino group of the purine is no longer available for H bonding. The UV spectra of vesicular solutions of DOP-Adenosine, DOP-Cytidine and to a lesser extent DOP-ε-Adenosine, show consistent hypochromicity if compared to those obtained dissolving the vesicles by action of a detergent to obtain mixed micelles. Upon mixing two equimolar vesicle populations formed from complementary bases, changes in Circular Dichroism and UV spectra were detectable on the same time scale, indicating "excess" in the stacking interactions. The whole process occurred without fusion or alteration of the size distribution of the liposomes. The time-scale indicated a mixing process mediated by monomeric exchange. At equilibrium spectroscopic features were identical to those shown by vesicles prepared by hydration and extrusion of a mixed DOP-Adenosine/DOP-Uridine films. The evidence of H-bonding was however indirect, since NMR spectra are not sufficiently resolved in lipid vesicles due to large spectral broadening. The ideal behavior of DOP-Cytidine/DOP-Uridine systems indicated a Watson-Crick selectivity pattern. DPP-nucleoside derivatives exhibited a time-dependent annealing morphology and eventually evolved to elongated non-liposomal structures.

The search for molecular recognition at mesoscopically extended interface evolved toward micellar aggregates where aggregation, shape, and size are ruled by thermal equilibrium and can be altered through the variation of a control parameter. Vesicular aggregates can be considered static metastable structures while micelles are equilibrium assemblies deriving from spontaneous association of monomers as a result of delicate balance of hydrophobic and hydrophilic interactions. This means that either the onset of aggregation and the structural properties of the aggregates can give insights into base-base interactions. Another important aspect concerns the feasibility of ^{1}H-solution NMR investigations, since the fast tumbling of micellar aggregates allows a narrowing of the broad lines observed for bilayered structures and a direct detection of recognition patterns typical of nucleic acids.

The structure of micelles formed by dioctanoylphosphatidylnucleosides (diC$_8$P-Adenosine, diC$_8$P-Uridine and their 1/1 mole ratio mixture) has been studied by Small Angle Neutron Scattering as a function of pH and amphiphile concentration. Several indications of deviation from ideal behavior can be found in the cmc, in the area per polar head and in spectroscopic properties of bases. Selective and preferential interactions have been identified between adenine and uracil moieties, according to a pattern resembling molecular recognition in nucleic acids. In particular NMR, UV-Vis and CD spectroscopies indicate that in mixed micelles, formed from diC$_8$P-Adenosine and diC$_8$P-Uridine phosphatidylnucleosides, both stacking and hydrogen bonding interactions are present between the bases at the micellar surface. NMR indicates that a H-bonded Watson-Crick adduct is formed despite the exposure of the bases to the highly competitive aqueous environment.

A similar characterization of micelles from monoalkyl-phosphoryl nucleosides (C$_{16}$-AMP, C$_{16}$-CMP and C$_{16}$-UMP) has highlighted excess stacking interactions in the ternary system formed by Watson-Crick complementary nucleolipids. The same aggregates have also been investigated by means of NMR techniques. Some selective interactions between bases have been found in the mixed micellar system and an average orientation of the aromatic system parallel to the micellar surface has been deduced.

A recent interesting progress concerns the fromation of flexible polymerlike micellar structures by dilauroylphosphatidylnucleosides. The interfacial curvature is intermediate between globular micelles and bilayers, and like in natural nucleic acids, very different length scales are interplaying. The size and shape of aggregates show strong dependence on the nucleolipid volume fraction and on the nature of the polar head. In fact strong stacking interactions between neighboring bases have been found for DLP-Adenosine, while DLP-Uridine has in the aggregate electronic properties similar to the momomer. Some twisted ribbon-like helical structures have been found for DLP-Adenosine at low volume fractions (see Figure below), while for higher concentrations flexible entangled objects are formed.



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1B - Thermophoresis in macromolecular solutions and colloidal suspensions

Sara Iacopini, Andrea Guarino, Benedetta Triulzi, and Roberto Piazza

Aims

Thermal diffusion, or Ludwig-Soret effect, is the relative matter flow induced in fluid mixtures by a temperature gradient, driving the components in opposite thermal gradient, is a closely akin effect taking place both for airborne particles and in complex liquids like colloidal suspensions, polymer solutions, or biological fluids. Transport of fluids or particles driven by thermal gradients takes place in many situations of basic and practical interest. Thermal diffusion in simple mixtures is generally a weak effect, but still it dramatically lowers thermal convection thresholds, playing therefore a crucial role in many naturally-occurring convective processes like thermohaline convection in oceans, and component segregation in oil wells, volcanic lava, and the Earth mantle. Particle thermophoresis in macromolecular solutions or colloidal dispersion is a much stronger effect: in DNA solutions, for instance, synergy of thermal convection and thermophoresis may lead to amplification of the local macromolecular concentration up to a thousand fold, suggesting the feasibility of Soret-driven bio-reactors. Thermophoresis is known since a long time, yet it still lacks a clear microscopic picture: for instance, in most cases particles diffuse towards the colder region, but examples of reverse behaviour are common, and no model can so far predict the direction of thermodiffusive motion. In dilute suspensions (particle weight fraction $w << 1$), the mass flow J can be written as $J = -D\nabla c - cD_T\nabla T$, where c is particle concentration in mass per unit volume, D is the usual Brownian diffusion coefficient, and D_T is called the coefficient of thermal diffusion. In the absence of convection, and assuming that ∇T is directed along z, Soret-coupling of heat and mass transfer leads therefore to a steady-state concentration gradient given by

$$dc/dz = -cS_T dT/dz$$

where $S_T = D_T / D$ is called the Soret coefficient. S_T is therefore positive when particle are "thermophobic" (they move to the cold) and negative when they are "thermophilic" (drifting to the hot side).

Aims of the investigation is:

- Unraveling the microscopical roots of thermophoresis
- Pointing out the role of particle-solvent interfacial properties in driving thermophoretic motion.

Results

In order to investigate thermophoresis in disperse systems, we have used a beam-deflection method, exploiting the deflection of a laser beam due to the concentration, and therefore refractive index gradient induced by the imposed temperature field. Our experimental apparatus consists first of all of a thermal--diffusion cell, made of two horizontal closely spaced plates separated by an optical--glass frame, with an optical path length of 40 mm and a sample volume of about 300 μl. Tuning of plate temperatures is achieved using two independently-controlled Peltier modules, placed in closed thermal contact with the plates. Typically, a temperature difference $\nabla T \approx 0.5 - 1^\circ C$ is applied in a time scale of few tens of seconds between the initially isothermal plates, and kept fixed within ±5 mK up to several hours. A laser beam is mildly focused through the plate gap, and the position of the transmitted beam is monitored by a position-sensitive detector with a resolution of few μm, placed far from the cell. The beam suffers first a very rapid downward deflection Δz_{th} due to the temperature dependence of the refractive index of the solution, followed by a much slower change $\Delta z_s(t)$ due to the progressive build-up of the Soret-induced concentration gradient, reaching exponentially an asymptotic limit Δz_s with a time constant τ set by the particle Brownian diffusion time over the plate separation distance. Due to the wide separation of the time scales between thermal expansion and Soret effect, S_T can be simply determined from the ratio of these two displacement.

Charged colloids. By measuring the Soret coefficient of SDS micelles, we have shown that thermophoresis in charged micellar solutions has a very distinctive behaviour. In the limit of very low concentration, S_T sensibly drops by adding salt. In other words, the single particle Soret effect strongly increases with the Debye-Hückel screening length λ_{DH}. However, intermicellar interactions play a strongly conflicting role, to such an extent that even at moderately low SDS concentration the situation gets totally reversed, and S_T *increases* with increasing salt concentration. Quantitatively, the single-particle Soret coefficient S_{T0} is found to scale as the square of λ_{DH}. Collective effects show the same ionic-strength dependence as the solution osmotic compressibility: they increase, or conversely reduce the Soret coefficient compared to S_{T0} depending on the fact that intermicellar interactions are, respectively, attractive or repulsive. Both single particle behaviour and collective contributions can be understood in terms of model originally proposed by Eli Ruckenstein, relating thermophoresis to gradients of the particle-solvent interfacial tension γ. According to this view, thermophoresis can be envisaged as a microscopic "thermocapillarity effect": attractive (repulsive) solvation interactions U_s 'pull' particles along (`push' particles down) ∇U_S, because of unbalanced tangential stresses in a thin sublayer close to the particle surface, which can be envisaged as an effectively unbalanced γ.

Fig.1 Soret coefficient for SDS micellar solution, in the dilute limit as a function of λ_{DH}. The insert shows a typical beam-deflection signal

Thermophoresis in protein solutions. Proteins in `salting-out' conditions, that is in the presence of a sufficient amount of added salt, display strongly temperature-dependent solution properties. We have found out a very puzzling behaviour of thermophoresis in lysozyme solutions [3]: particle motion can indeed be *tuned* from thermophobic to thermophilic by decreasing temperature. Moreover, by lowering T the absolute value of S_T increases exponentially, with a growth parameter that weakly depends on the ionic strength. Finally, a strong correlation of S_T with lysozyme equilibrium solubility was observed. Later [4], we have further expanded the analysis to include effects of particle charge and of the addition of different salts, and analysed transient effect to derive the temperature dependence of the thermal diffusion coefficient D_T, which was found to grow linearly with temperature, showing as S_T sign-reversal at a temperature that weakly depends on pH or ionic strength. Presently we are performing measurements suggesting that a very similar behaviour is shared by a wide class of aqueous disperse systems, ranging from synthetic polyelectrolytes to amphiphilic aggregates and rigid latex particles. In particular, the observed functional form of the temperature dependence for the Soret coefficient seems to be an universal feature of aqueous colloidal suspensions. Although we are still on the way of extending Ruckenstein's suggestion to a wider class of colloidal systems, all preliminary theoretical results seem to point out that thermophoresis takes place because of interfacial stress developing in a thin layer close to the particle surface where the solvent properties differ from the bulk. The net hydrodynamic effect of such stresses can be envisaged as a violation of the equilibrium stick boundary conditions, resulting in an effective particle slip (this is quite similar to what happens for other "phoretic" effects like electro- and diffusiophoresis) [5]. Were this interpretation correct, thermophoresis could be used as an useful probe of particle-solvent interfacial properties

Fig.2 Soret coefficient versus temperature for c= 7 g/l lysozyme solutions at pH= 4.65, in the presence of 7.5 (open squares), 20 (full squares), 100 (full dots), and 400 mM open dots) NaCl. Full lines are fits to the exponential law proposed in ref. [3]. Inset: Soret contribution to the beam-deflection signal for lysozyme solutions at various temperatures, indicated to the right in °C: Δz_s is conventionally drawn as positive if it goes along with Δz_{th}, and each transient is plotted versus t/τ for graphical convenience.

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1B - “Thermal-lensing” method for measurements of thermophoresis in aqueous colloidal dispersions

Roberto Rusconi, Lucio Isa, and Roberto Piazza

Topic

The phenomenon of “thermal-lensing”(TL) occurs when a laser beam goes through a fluid medium which shows a partial absorption at the given light wavelength. The local heating induced by light absorption leads to the build up of a temperature (T) profile and consequently of a refractive index(n) profile. A quantitative analysis gives as a result that, for small distance r from the beam axis, the refractive index decreases with the square of r as in an ordinary lens. This leads therefore to a “self-defocusing” of the propagating beam which can be accurately measured by detecting changes in the central beam intensity. Such a description can be extended from simple liquids to colloidal suspensions or micellar solutions. In this case we have that the dispersed particles experience a thermal gradient created by the laser beam absorption: this induces thermophoretic motion (or Soret effect) within the gradient itself (see contribution by Iacopini *et. al.*). Particles thermal diffusion brings therefore an additional refractive index variation. This means that, because of thermophoresis, the laser beam undergoes a second “self-(de)focusing” due now to the presence of a concentration(c) profile. At variance with the “thermal-lens”, this “Soret-lens” can be either negative or positve depending on the thermal-diffusive properties of the colloids. Because the used particles have usually a greater refractive index than water, if they tend to move towards the hot (*thermophilic behaviour*), which in other words means towards the optical axis, their motion has a focusing effect; on the contrary, if they show a *thermophobic behaviour*, their motion gives an additional defocusing effect. Heat and mass diffusion for colloidal particles suspended in water take place on well separated time scales and so these two lensing effects can be effectively distinguished. The main advantage of this method stands in the possibility of faster measurements of “big” particles (100nm or more) within respect to other techniques for the mass-diffusion spatial scale is set by the beam-spot size. Such method was first used by Giglio and Vendramini in 1974 for a strong absorbing mixture; in fact this method seems to be useless for the case of aqueous suspensions because of water’s trasparency to visible light.

Aims

- Developing an experimental set-up allowing to perform measurements of thermal diffusions in suspensions of large colloidal particles.
- Analysing convection effects in thermal-lensing measurements on fluid mixtures or macromolecular solutions.

Results

TL Apparatus

We set up an apparatus aimed to overcome the aforementioned setback by using an infrared laser at 980nm which corresponds to a water absorption peak, so that the thermal profiles can be “written” without the need of absorbing dyes as in other measuring techniques. The apparatus can be described as follows (Fig.1): 980nm laser light is provided by a semiconductor photodiode(3) which is controlled by a current driver (1) and cooled by a Peltier device driven by a temperature controller(2). The photodiode exit is coupled with a monomodal fiber(4) which guarantees that the laser beam is in its fundamental mode TEM_{00} (a gaussian shape of the beam is a compulsory requirement for TL experiments). The fiber ending is connected with a collimator (5) which gives a divergence limited beam with a radius of about 1.1mm. Then a small portion of the beam is withdrawn by a beam-sampler (6) for monitoring use. The main part passes through a shutter(7) and then is opportunely collimated by a lens(8) in the measure cell(9). The cell is made of optical glass and has a cylindrical shape with optical windows of 4.5mm of diameter and thickness ranging from 0.2 to 10mm; it's also surrounded by a thermalization jacket which allows to perform temperature controlled measurements. Finally it's mounted on a precision translation stage which allows positioning of the cell in order to maximize the effect. Once the beam has gone through the cell its peak intensity is measured by a photodiode put beyond a pinhole. The collected signal is amplified(11) and then sent to a PC to be processed.

Numerical simulations.

Unfortunately, "thermal-lensing" is associated with the presence of horizontal thermal gradients which unavoidably leads to convective instabilities. Such motions of the fluid can easily modify or even destroy Soret-induced concentration profiles and so controlling them is a basic step to obtain stable measures. The basic strategy to limit convection effects is reducing the focused spot of the laser beam, so that on its spatial scale diffusion is much faster than convection. A safe criterium is keeping $wU / D \ll 1$, where w is the beam-spot size and U is the typical

convection velocity that can be evaluated by balancing viscous and buoyancy forces. In order to get more quantitative results, we performed extensive numerical simulations and mathematical modelling, which confirm that convection can be safely reduced via focusing the beam and decreasing the cell thickness. For instance, in Fig.2 we show the velocity fields for horizontal or vertical positioning of the cell; in the former, the spatial scale on which buoyancy forces act is set by the diameter (4.5mm), while in the latter is the thickness (0.5mm) which plays the same role. This difference leads to a two-three orders of magnitude difference in convective velocities. Therefore, an appropriate choice for the apparatus is a set up where the optical axis is positioned vertically. In addition, we found that, in the presence of moderate absorption, radiation pressure effects are far from being negligible. It is therefore particularly useful to send the laser beam in the direction of gravity (pointing down), to partially counteract buoyancy. By taking into account these previous prescriptions, we have been able to minimize convective effects and therefore to perform measurements on suspensions of colloidal particles up to a particle radius of 100 nm.

Thermal Lens signals.

To give an example of a typical TL experiment, the following figure shows the signal obtained from micellar SDS solutions. One can notice a first quick decrease in the peak intensity due to the thermal effect and then a slower one given by thermophoretic motion. The inset shows the full thermal signal pointing out the different time scale. By comparing the extents of thermal an Soret de-focusing one can extract the value of the Soret coefficient of the analysed particles. A detailed analysis of thermophoresis from SDS solution gave results that are fully consistent with those obtained by Guarino and Piazza using a beam-deflection apparatus (see contribution by Iacopini *et. al.*).

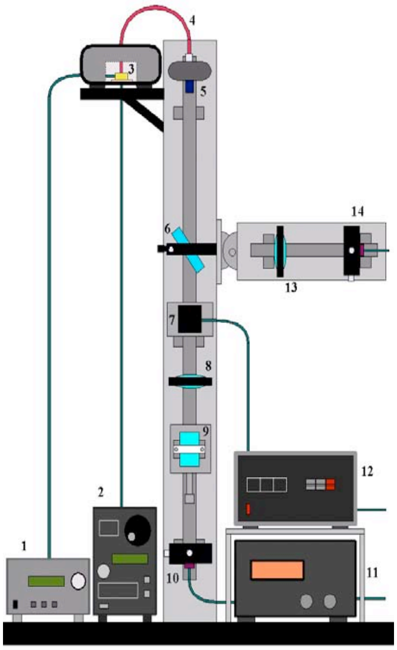

Fig.1: Thermal–lensing apparatus

Fig.2: Numerical simulation of convective patterns.

Fig.3: TL signal from SDS solutions.

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1B - Pickering Emulsions

E. Vignati, R. Piazza
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Aims

It is well known that stable emulsions can be readily obtained by adding surfactants to immiscible liquids. Yet, starting from seminal studies by Pickering, evidence has been gathered that emulsion stability does not necessarily require surface-active agents, but can also be efficiently promoted by dispersed particles in the colloidal size-range. ”Pickering” emulsions play a crucial role in many industrial applications. For instance, stabilization of oil/brine emulsions encountered during oil recovery processes, which must be broken before crude oil refining, is brought about by clays, asphaltenes, and other dispersed particles. Presumably similar natural colloids cooperate in emulsifying oil spills, leading to serious environmental problems. Until a few years ago, most experimental studies aimed at providing a macroscopic, semi-quantitative description of the stability diagram of emulsions stabilized by naturally-occurring, but not so well-characterized colloidal particles. In spite of their limitations, these studies have allowed some general conclusions to be drawn , and led to the formulation of a basic stabilization mechanism, invoking steric-hindrance to droplet-droplet coalescence provided by interface-adsorbed colloids. According to this mechanism, coalescence requires the solid particles to be removed from the contact region, presumably by lateral displacement.

Aims of the investigation is:

- exploring the basic features of Pickering emulsions, using as the stabilizing agent “model” colloidal particles
- measuring the interfacial tension between a colloid-stabilized droplet and the continuous phase.
- detecting interfacial adhesion processes, providing a description of the colloidal surface-layer morphology.
- analyzing the effects of particle surface `roughness' on emulsifying efficiency.

Results

We have used model silica particles, obtained using a modified Stöber synthesis method yielding a fluorescent core, which allow interfacial adhesion processes to be detected and quantified by video-microscopy. We have prepared two colloidal systems, primarily differing in the particle-surface structure. The first one is composed of monodisperse, surface-smooth spherical colloids. Particles of the second batch, although still monodisperse, display noticeable surface “roughness”, mimicking an important, and so far neglected, feature of natural colloids. Particle wetting properties have been tuned by exposing them for different time to a silanizing agent.

First of all, we have tried to measure directly the interfacial tension between a colloid-decorated oil droplet and the aqueous continuous phase. To this aim, we have designed and built a micro-tensiometer based on a micropipette manipulation system, exploiting the direct relation between the interfacial tension γ of an emulsion drop and the negative pressure needed to suck the drop in a micropipette. Using this apparatus, we have shown (see Fig. 1) that surface adsorption of the colloids does not affect γ, so that stabilization stems primarily from steric mechanisms.

Fig. 1: Droplet interfacial tension as a function of particle volume fraction. The inset shows a droplet captured by a micropipette, just before being sucked in.

We also found that surface roughness appreciably lowers particle emulsifying power, and that no straightforward relation exists between the degree of droplet surface-coverage and macroscopic emulsion stability. Indeed, long-term stabilization can be obtained even with a degree of droplet surface coverage of the order of 5-10%. Conversely, fully covered droplets may not yield a macroscopically stable emulsions. This means that, at variance with the current interpretation, steric stabilization is not brought in by a rigid static layer, but rather that hydrodynamic effects, related to particle-redistribution on the droplet, must play an important role.

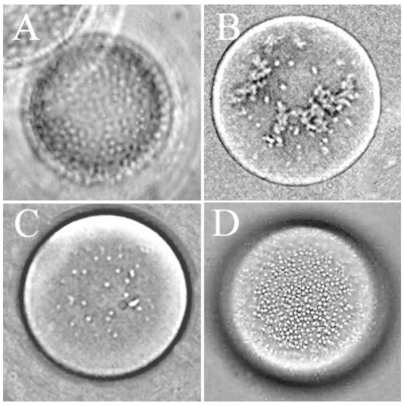

Fig.2: Examples of surface morphology for octanol emulsions stabilized by "rough" particles silanized for different times. A) Dense surface layer on a droplet of an *unstable* emulsion (t_{sil} = 10'); B) "Colloidal lumps" (t_{sil} = 15'); C) Stable emulsion showing very weak interfacial trapping (t_{sil} = 50'). D) Comparison with octanol droplets stabilized by "smooth" colloids of similar size, showing no particle clustering.

We have finally studied surface diffusion of the trapped particle, and directly shown that particle redistribution (and, likely, exchange) effects take place in the emulsified state.

These complex hydrodynamic-capillary effects, which may play a primary role in Pickering emulsion stabilization, are still to be investigated.

Fig.3 Time-sequence (from A to D, in order) of detachment and drifting apart of two particle-stabilized octanol droplets. Brighter regions on the smaller drop indicate trapped-particle location.

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1B - Cryo-TEM of Liposomes loaded with boron-containing compounds intended for Boron Neutron Capture Therapy (BNCT)

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Aims

Cryo-TEM investigation of structure and stability of liposomes intended for drug delivery in BNCT.

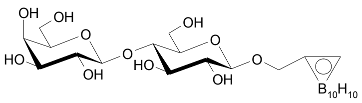
ESR analysis of phospholipids bilayer of liposomes loaded with amphiphilic drugs.

Results

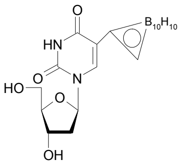
Electron microscopy constitutes an powerful method for the determination of aggregate morphology in dilute lipid solutions. Lipid aggregates, such as liposomes and micelles, are, however, very labile and extremely sensitive to small changes in concentration and composition. Cryo-transmission electron microscopy (Cryo-TEM) is a relatively new technique that offers unique possibilities for direct visualization of labile microstructures in dilute aqueous solutions [1,2]. This technique makes it possible to obtain images that are two-dimensional projections of all of the aggregates in water suspension without the need of treating the sample with fixation reagents or other chemicals. The cryo-TEM involves ultra-fast cooling of thin liquid films (by rapidly plunging the samples into liquid ethane held at ~ 100K) to vitrify the samples, that are examined in the electron microscope at cryogenic temperatures.

We used Cryo-TEM to obtain structural information on the effect of boronated drugs on aggregate structure in samples containing small unilamellar liposomes (diameter =100 nm) with varying bilayer composition. In addition, the Electron Spin Resonance (ESR) spectroscopy of lipophilic spin labels (5-, 7- and 16- doxyl stearic acid, n-DSA, cholestane-doxyl derivatives) give us interesting information on the dynamic perturbation of the liposome bilayer after boronated drug insertion within membrane. ESR spin labelling has, in fact, proved to be a powerful technique for studying liposome bilayers and drug-lipid interactions [3-5].

The compounds used are based on a boron rich carborane cage: the lactosyl carborane [1,2-dicarba-closo-dodecarboran(12)-1-ylmethyl] (D-galactopyranosyl)-(1→4)-glucopyranoside (LCOB) (kindly provided by Prof. L. Panza, Università del Piemonte Orientale) and β–5–*o*-carboranyl-2'-deoxyuridine (CDU) (kindly provided by Prof. Schinazi, Emory University Atlanta, USA).



LCOB



CDU

In LCOB the carborane moiety has been coupled to an hydrophilic lactosyl moiety [6] which increases the solubility in water of the compound and escorts and binds the boronated compound to the tumor cell surface.

The second compound, CDU, is a non-toxic pyrimidine nucleoside analogue designed for BNCT of brain tumors, which has been extensively studied for its cytotoxicity, anticancer, antiviral activity and cellular uptake [7]. Since both compounds have high hydrophobicity because of their ortho-carborane domain they are expected to locate inside the hydrophobic region of the membrane.

We investigated and characterised unilamellar liposomes with different membrane composition [8]: *i)* egg phosphatidylcholine (EPC), *ii)* distearoyl phosphatidylcholine (DSPC); *iii)* EPC/Cholesterol and DSPC/Cholesterol at molecular ratio 55/45. In many pharmaceutical formulations, cholesterol is included in the lipid mixture to further increase the *in vivo* stability. In addition, saturated phospholipids are preferred when considering the long-term stability of the liposomes.

ESR results proved that CDU insertion into the liposome membrane gave rise to a strong variation of the fluidity and order degree of the bilayer. The EPC bilayers became more packed in the presence of the carboranyl-nucleoside and this effect took place both in the hydrophilic glycerol moiety and in the deepest interior of the hydrocarbon regions. This effect was stronger in EPC liposomes than in EPC/Chol liposomes, where it occurred at lower CDU concentration.

In LCOB-loaded liposomes LCOB insertion into the phospholipid bilayer increased the packing order in the vicinity of the water/hydrocarbon interface whereas the inner hydrophobic region of the bilayer was unaffected.

Cryo-TEM micrographs revealed a significant change in the size distribution when the LCOB molar fraction increased in the liposomes dispersion. The lactosyl-carborane induced the formation of extremely small liposomes with diameters ranging from 10 to 30 nm in coexistence with liposomes in the size range of 80-100 nm. A second interesting effect caused by the incorporation of LCOB was that both populations of the liposomes developed a tendency to aggregate and form clusters, a phenomenon which was proved by turbidity measurement. When the LCOB concentration was further increased, the number of small liposomes increased. At x_{LCOB} =0.82 the sample contained only a limited number of intact liposomes and most of them showed bilayer openings from which originated short threadlike micelles, a few tens of nanometers in length (figure 1).

Figure 1.

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1B - Lipoplexes as non viral vectors for gene delivery

Laura Ciani, Giacomo Martini, Sandra Ristori and Anna Salvati, in collaboration with Annarosa Arcangeli (Department of Pathology, Univ. of Florence)

Aims

Physico-chemical characterization of several lipoplexes to establish valuable systems for non toxic and highly efficient gene transfection.

Results

Human genome characterization and recombinant DNA technology have created tremendous opportunities for gene therapy. However, delivery of the large anionic bioactive DNA into the cell is not simple, because it must arrive intact to the nucleus, after crossing the cell and nuclear membranes. In recent years, cationic lipid/liposomes have emerged as useful carriers,[1,2] because they exhibit ease of manipulation, reproducibility, and versatile preparation. We made use of positively charged unilamellar vesicles, formed by a cationic surfactant, DOTAP or DC-Chol, and a zwitterionic phospholipid, DOPE, as helper lipid.

Model lipoplexes were first prepared by using small oligonucleotides, either in the form of a single strand (PolyA and PolyT) or of the corresponding double strand. Their characterization was performed by Zeta Potential, Microcalorimetry and Electron Spin Resonance.[3] Both zeta potential and microcalorimetric titration showed that a stable complex could be formed between oligonucleotides and cationc liposomes. In particular, zeta potential measurements showed that: for DOTAP/DOPE liposomes, the two single-strand oligos had the inversion point of the zeta potential (corresponding to the formation of a neutral complex) at 0.50 charge ratio. This meant that neutralization took place when the negative phosphate groups of the added oligo reached half the concentration of the cationic lipids. We thus deduced that, in this case, only the lipids located on the liposome external surface interacted with DNA fragments. A similar behaviour was observed in the titration of DC-Chol/DOPE, but in this case the inversion point was different for different oligos: This was due to specific interactions between the DNA bases and the external surface of the DC-Chol/DOPE liposomes.

The thermodynamic data of the complex formation, obtained by microcalorimetry, where in agreement with zeta potential data.

The status of the lipid bilayer as a function of oligonucleotide addition was monitored at molecular level by introducing appropriately tailored ESR spin probes. In particular, 5-doxyl stearic acid showed that interactions between oligos and liposomes did not change either the dynamics or the structural properties of the bilayer. (fig. 1). ESR probes also showed enhanced rigidity in the DC-Chol/DOPE bilayer with respect to the DOTAP/DOPE bilayer, which is in line with the well known condensing effect that cholesterol and its derivatives exert on fluid lipid assemblies.

From the above analysis the following scheme of lipoplex could be deduced for single strand oligos interacting with both type of cationic liposomes, and for the double strand oligo interacting with DC-Chol/DOPE liposomes: DNA fragments simply “wrapped up” the external surface of liposomes, which remained substantially intact. On the contrary, DOTAP/DOPE liposomes showed a more complicate behavior when interacting with the double strand oligo. This difference could be traced back to the higher fluidity of their bilayer, that allowed more marked deformations.

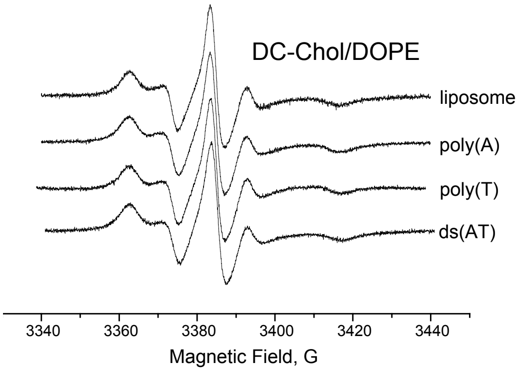

Fig 1. 310 K ESR spectra of 5-DSA inserted in plain DC-Chol/DOPE liposomes and in lipoplexes containing single and double strand oligonucleotides.

Transfection experiments toward CHO cells were then performed with lipoplexes made up by liposomes and the pEGFP plasmid, that carries the information to produce a fluorescent protein. Preliminary results showed that: i) marked transfection was achieved by the lipoplexes; ii) DOTAP/DOPE liposomes were less toxic, but also less efficient, than DC-Chol/DOPE liposomes and (iii) Lipoplex charge and lipoplex/cell ratio were critical parameters for transfection efficiency. Figure 2 shows a comparison between experiments performed at two different lipoplex charge values, with constant cell/lipoplex ratio.

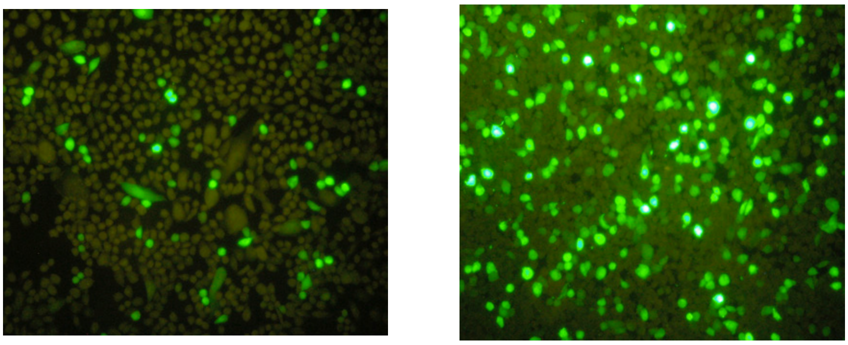

Fig 2. Fluorescence microscopy images of cell transfected with the pEGFP plasmid/DC-Chol/DOPE lipoplex. (a): DNA$^-$/lipide$^+$ = 0.07 and (b): DNA$^-$/lipide$^+$ = 0.7 48 h from the injection.

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1B – Nanostructured Magnetic Fluids

Massimo Bonini and Piero Baglioni, in collaboration with Albrecht Wiedenmann (HMI, Berlin)

Aims

Aim of this project was the synthesis of magnetic fluids constituted by magnetic nanoparticles dispersed in both water and organic solvents. We focused in preparing magnetic nanoparticles stabilized either by a surfactant shell, by the charges present on their surfaces or by an outer shell of an inert material.

Results

Ferrofluids are stable dispersions of magnetic nanoparticles in a liquid carrier. During this project, three different synthetic strategies were explored: synthesis in reverse micelles, preparation from super-saturated solutions and thermal decomposition. These techniques lead to different results in terms of dimensions, polydispersity and maximum amount of dispersed solid material [1]. In order to characterize the ferrofluids, microscopy (TEM and AFM) and scattering (DLS, SAXS and SANS) techniques were used.

A particular attention was devoted to the preparation of core-shell nanoparticles from supersaturated solutions, the core being constituted of cobalt-ferrite, $CoFe_2O_4$, while the outer shell was silica. These particles are of particular interest because of their bio-compatibility, due to the inert silica shell. A detailed characterization was obtained by means of Small Angle Scattering of Polarized Neutrons (SANSPOL), a recently developed technique available at the Berlin Neutron Scattering Center, Hahn-Meitner Institute, Berlin (Germany). Results showed that core-shell cobalt-ferrite nanoparticles present a uniform silica shell that can be obtained by a very simple synthetic procedure. The thickness of this coating does not depend on the size of the cobalt-ferrite particles and it doesn't change the shape of the coated particle. Moreover the thickness of the outer shell can be easily tuned during the synthesis.

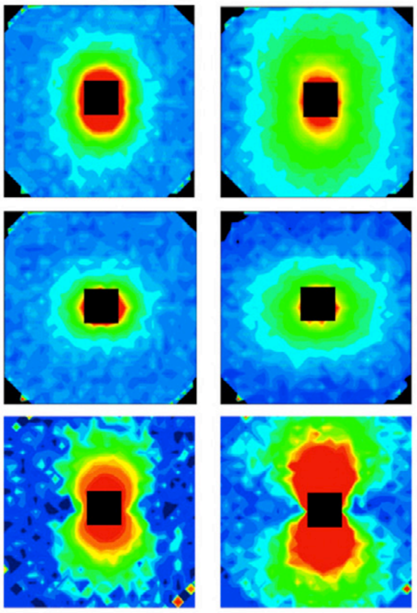

Figure 1. SANSPOL iso-intensity plots of silica-coated cobalt ferrite nanoparticles.

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1C - Conformational Analysis of Biomolecules in Solution Using Theoretical and Experimental NMR and Molecular Simulation Methods

Claudia Bonechi, Silvia Martini, Maso Ricci, Alessandro Donati, Claudio Rossi

Aims

Determination of geometrical constraints for the structures in solution using experimental 2D-NMR techniques.

Identification of one stable conformation or families of conformations for molecules with important biological properties such as protein, nucleic acid, peptides, drugs, self-assembling polymers, etc.

The study of the relationship between structure determination and biological functions.

Results

The conformational and dynamical studies in solution, carried out with nuclear magnetic resonance techniques, are of great importance to obtain information on molecular mechanisms for biologically active molecules.

The structural determination of biological macromolecules is a fundamental step for further studies at the molecular level for their interactions with other structures. This differentiation in terms of molecular structure can be connected to the difference of biological functionality of these compounds.

Combined use of NMR experimental results, theoretical simulations of two-dimensional NMR spectra by Complete Relaxation Matrix Analysis (CORMA) and molecular dynamics calculations, represents a powerful approach in the determination of the structure of organic compounds in solution.

NOESY (Nuclear Overhauser Effect Spectroscopy) experiments are usually used to identify spatial connectivities between nuclei which are dipolarly coupled, since the size of the NOE depends inversely on the distance between two interacting spins. Geometrical constraints were obtained from 2D-NOESY and/or 2D-ROESY cross-peak intensities using the MARDIGRAS program. The derived proton-proton and proton-carbon distances were used in the molecular dynamics simulations and the accuracy of the final structures was obtained generating the theoretical 2D-ROESY spectra by CORMA program. From the protons cartesian coordinates of a molecular structure with CORMA program it is possible to determine quantitatively the entity of the dipolar interactions, including the phenomenon of spin-diffusion. In the refinement procedure the accuracy of the models obtained after molecular dynamics calculations was evaluated on the basis of the crystallographic-type R-factor, comparing experimental and theoretical NOESY spectra.

In order to obtain one single conformation or a family of conformations it is important to apply the molecular and dynamic calculations using the experimental geometrical distances as constraints. In order to take in account the conformational complexity of macromolecules with biological interest during the structural determination in solution we optimized various approaches.

For the structure calculations different methodologies have been applied. We used the restrained molecular dynamics (rMD) with the aim to explore the entire conformational space a Montecarlo method. In particular the approach based on molecular dynamics, known with the name of MDtar (Time Averaging Molecular Dynamic), has provoked remarkable interest. In this procedure the geometric constraints are not satisfied at every step of the simulation but only for a specific definable time.

This approach was applied to the study of different macromolecules and bio-molecules in solution.

We investigated the conformational properties of rifaximin and its derivative, rifaximin OR, which belongs to a more recent class of ansamycin derivatives. The conformational analysis allowed the identification of one stable conformation for rifaximin and highlighted that the brake of the aliphatic bridge of rifaximin didn't give raise to strong conformational modifications, nevertheless some important structural rearrangements involving the aliphatic chain of rifaximin OR have been found.

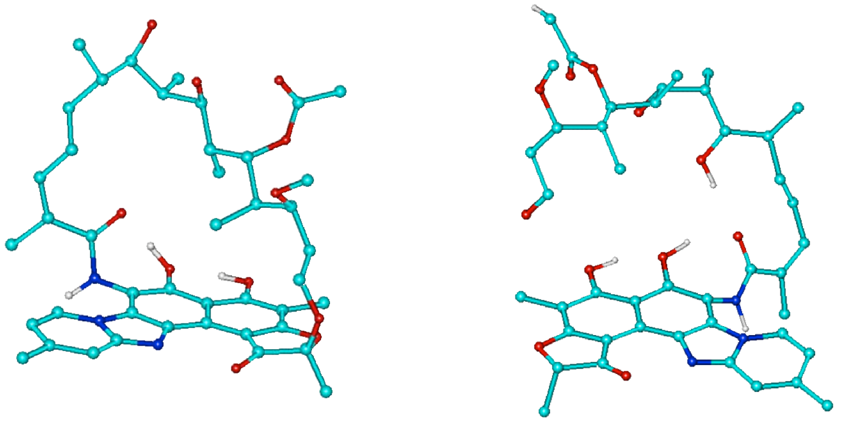

Fig. 1 Structure of rifaximin and rifaximin Or obtained by experimental NMR and theoretical caluclations methods.

We analized the conformation of natural products as anandamide. This compound mimics the pharmacological effects of D^9-tetrahydrocannabinol. In this case the results show that a single conformation is not present in solution. By cluster analysis procedure we found twenty-two conformational families able to describe the behavior of anandamide in solution.

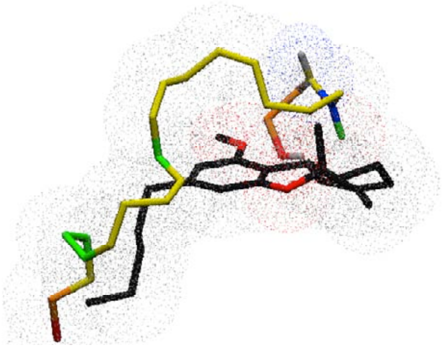

Fig. 2 Comparisons of one structure of anandamide (obtained by cluster analysis) and D^9-THC structures (RMS=1.64).

This approach has been used to analyze the conformational properties of different bioactive compounds, such as peptides (bradikinin), oligosaccharides (hyaluronic acid), drugs (carborane, antibiotics, calyx[n]areni), natural products, etc.

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1C - Solution Behavior of a Sugar-based Carborane: a Nuclear Magnetic Resonance Investigation

Alessandro Donati, Claudia Bonechi, Silvia Martini, Maso Ricci, Claudio Rossi

Aims

Analysis of physico-chemical properties of carborane molecules in solution.

Determination of intra-molecular CH…X(N, O) bonds in crystals, and their persistence in solution were observed in molecules containing, carba-borane cage and amidic groups, by X-ray and NMR techniques.

Validation of experimental data by theoretical ab initio calculation.

Results

Polyhedral boranes and heteroboranes have been the subject of widespread interest for at least fifty years and this was mainly due to the ability of boron atoms to catenate and form molecular networks with rather peculiar geometries. Nuclear Magnetic Resonance has proven to be a suitable technique for the physico-chemical characterization at a molecular or micellar level, and it has been used since long to obtain valuable information on surfactant-based systems

The existence, the persistence in solution and the chemical properties of unusual hydrogen bonds are recently emerged as an argument of extensive debate, reflecting the extreme interest of this topic, both for theory of chemical bonding and its applications in several fields. Multiple withdrawing groups attached to a sp^3-hybridized carbon, should be the necessary condition to make the carbon a proton donor. New techniques and refinement of theoretical calculation allowed a more accurate description of this subtle interaction.

We analize the behaviour of C-H donor included in the cage of a carbaborane compounds, designed to develop an alternative mechanisms of action in Boron Neutron Capture Therapy (BNCT). In these molecules the CH enclosed in the cage, which presents H-donor behaviour, can form intra-molecular C-H···O bond and C-H···N bond, which surprisingly appears to be both persistent in solution. Nuclear Magnetic Resonance data showed that the conformation in which C-H···O interaction occur is favored, even if other reasons, than intrinsic C-H···X strength only (i.e. steric hindrance), are co-responsible for this behaviour.

X-ray crystallography, 2D-NMR and *ab initio* calculations confirmed this finding. Structure of both species has been proposed together with detailed geometries of the respective CH···X bonds.

Regarding the persistence of CH···X(O,N) bonds in solution, at the best of our knowledge this is the first case in which an intra-molecular CH···N interaction has been recognised with a very high degree of confidence and the geometrical parameters determined. On the other hand the persistence of the CH···O bond interaction is a clear confirmation of other recent findings in the field.

The occurrence and the persistence in solution of CH···X(O,N) bonds has been demonstrated for carba-boranyl derivatives of pharmaceutical interest. The activated CH group of the carborane cage results as an important probe for the evaluation of chemical behaviour of this non-classic interaction, with group of biological interest. In this case crystallographic studies demonstrated that the amidic oxygen was involved as hydrogen acceptor in the bond, allowing the determination of accurate geometric parameters.

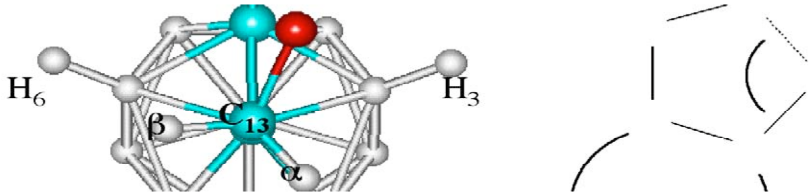

Fig. 1 (a) View of the carborane cage along the C_{13}-C_1 bond of the GCOB model optimized by *ab initio* calculation. (b) Sketch of the five member ring in which the proposed bond is involved.

All the experimental data presented in this research were in agreement for describing the carborane molecules as remarkably rigid objects, whose two main portions (the sugar rings and the carborane cage) were held fixed with respect to each other by means of a H-C···O bond. Temperature variations and aggregate formation were not able to disrupt this intramolecular bond, and thus did not introduce any substantial change at a molecular level. In this respect carborane molecule,has a different behaviour from classical surfactant systems, where structural changes have been shown to take place as a consequence of micelle formation. Quantum mechanical calculation strongly supported this model.

On the other hand, the analysis of nuclear relaxation parameters indicated that in aggregating condition an equilibrium existed between monomers and micelles, which is in line with the picture of typical surfactant behaviour.

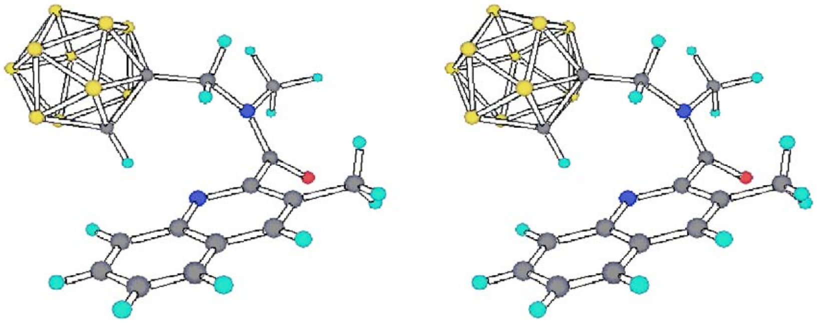

Fig. 2 Stereo view of DDQC *cis* model optimized by ab initio calculation at RHF/6-31G** level. Boron bonded hydrogens were omitted for clarity.

The occurrence and persistence in solution of CH···X(N,O) bonds was demonstrated for the two rotamers of a carba-boranyl derivative of potential pharmaceutical interest. The activated CH group of the carborane cage can thus be considered an important probe for the evaluation of the chemical behaviour in this non-classic interaction, especially when groups of biological interest such as the amide are involved. Crystallographic studies demonstrated that the amide oxygen was involved in the bond as hydrogen acceptor. This allowed the accurate determination of geometric parameters, from which it was possible to extract information on the forces involved in the hydrogen bond. Moreover, this intra-molecular CH···O interaction was demonstrated to persist in solution, showing its importance in driving conformational properties of bio-macromolecules and supra-molecular complexes.

The occurrence of a CH···N bond in solution was also demonstrated by NMR studies and was confirmed by *ab initio* calculations. The strength of both these interactions and their geometrical parameters were found to be in the same range of classical hydrogen bonds. In particular, in the case of the CH···N for the cis rotamer, the H atom directly pointed toward the nitrogen sp^2 lone-pair, whereas in the case of the CH···O bond for the trans rotamer a higher deviation from planarity was observed ($\theta=10.2^\circ$ and $\theta=41.2^\circ$ respectively). However, for the CH···O bond we should consider that a seven member ring was formed, in which geometrical constraints are more effective.

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1C - Ligand-Macromolecules Interaction as observed by Nuclear Relaxation Analysis

Silvia Martini, Claudia Bonechi, Alessandro Donati, Maso Ricci, Claudio Rossi

Aims

Definition of experimental parameters from selective nuclear relaxation analysis in order to evaluate the strength of the overall complexation behaviour of small ligands (drugs, xenobiotics, etc.) toward biomacromolecules (Nucleic acids, Proteins, etc.).

Identification of the functional groups of the ligands involved in the binding sites and the effects of the bound ligand on macromolecule conformation by the combined use of different spectroscopic techniques (NMR, IR).

Extension of the methodology to solvent-macromolecule interaction studies, in particular water-protein interactions.

Results

Biological reactions are mostly concerned with selective interactions between small ligands and macromolecular receptors. In particular interactions with bioactive molecules are of primary interest for defining the biological role of large biopolymers and for the activation of specific chemical processes.

In order to be able to compare the extent of recognition processes occurring between ligands and macromolecules, an "Affinity Index", representing the global affinity between the ligand and the receptor, was calculated from selective relaxation rate measurements. We applied this methodology to different systems in order to compare the strength of the interaction processes between the same ligand and different receptors, as well as between a macromolecules and different ligands. The investigation is based on the comparison of selective (R_1^{SE}) and non-selective (R_1^{NS}) proton spin-lattice relaxation rate analysis of the ligand in the presence and absence of the macromolecular receptor. The formation of intermolecular adducts affects R_1^{NS} and R_1^{SE} to different extents, depending on the dynamical parameters (i.e. the correlation time tc), assuming fast chemical exchange between the bound and the free environments with respect to both chemical shift difference and proton relaxation rate (Figure 1). In particular, the slower ligand dynamics in the ligand-macromolecule complex mostly affects R_1^{SE}. In the presence of well resolved proton resonances R_1^{SE} can be easily determined in different systems.

We investigated the interaction between lamotrigine , an antiepileptic drug, and some plasma proteins such as albumin, α-globulin, β -globulin and γ -globulin. The selective (R_1^{SE}) and non-selective (R_1^{NS}) spin-lattice relaxation rates analysis, allowed the identification of interaction processes between lamotrigine and these plasma proteins. In particular the calculation of the *Affinity Index*, suggested that the interaction was selective and the drug had specificity for certain proteins (Figure 2).

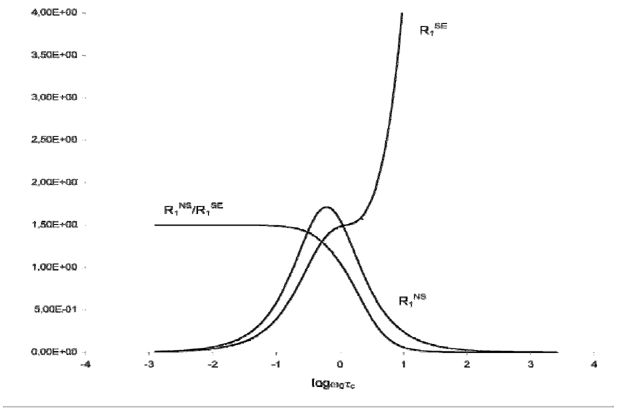

Fig. 1 R_1^{NS} and R_1^{SE} of a proton pair in relation to $\omega_H\tau_c$ assuming a constant r $_{i\text{-}j}$ distance for the i-j dipolar interaction.

We also investigated the effects of a second ligand (lamotrigine in this case) on the interaction process between carbamazepine (another antiepileptic drug) and albumin. The results suggested that lamotrigine is able to play a different role at low concentrations, reinforcing the carbamazepine-albumin interaction, in respect to higher concentrations producing competitive effects. The cooperative role of lamotrigine at low concentration was also revealed by the higher value of the "*affinity index*" in the ternary system with respect to carbamazepine-albumin. The synergic effect was probably more evident at low lamotrigine concentrations, since at higher concentrations the carbamazepine and lamotrigine ligands could compete for similar receptor sites.

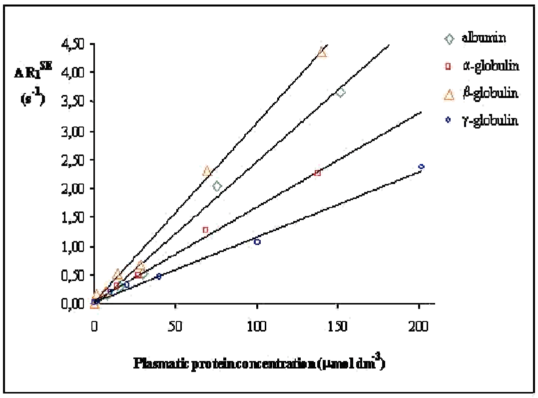

Fig. 2 Linear relationship between H4' selective relaxation enhancements and ovine plasma protein concentrations.

Another study concerned the interaction between ovine serum albumin and two amphenicolic antibiotics [cloramphenicol (CAP) and tiamphenicol (TAP)], using a combined approach based on NMR and IR methodologies, furnishing complementary information about the recognition process occurring within the two systems. The two ligands, despite their similar structures, showed a different affinity toward albumin. The results obtained by both NMR and IR spectroscopy suggest that the driving force for the albumin-ligand interactions is in general a recognition process with low specificity. In particular we found that both the CAP and TAP ligands interact with the albumin mainly via aspecific interactions, the interaction with TAP has a more denaturing effect on the protein than that occurring with CAP and the ligand portions responsible for the binding with the protein are mainly the aromatic and amidic regions.

Water relaxation behaviour can be used to monitor the ligand-macromolecule interaction as well as the protein hydration. The results obtained from a study involving water-albumin system showed the existence of a significant contribution to the bulk water molecules in the presence of the protein. This contribution contains two terms: the first arises from the long-lived buried water molecules surrounded by the macromolecular constituents, the second is due to water molecules present in the hydration shell around the macromolecular surface which are affected by some extent in their dynamical properties by the presence of the protein.

The same approach was used to evaluate the strength of the interaction between anandamide (AEA) and cannabinoid receptors. In particular, different interactions are analysed using three brain sections, the whole brain, the cerebral cortex and the cerebellum. Knowing the distribution of the CB1 receptors in the brain is useful in the analysis of the molecular recognition phenomena between a specific ligand and different sample obtained from three zone of brain.

Selective and non-selective proton spin-lattice relaxation rate analysis of anandamide in the presence of different cerebral receptors allowed the identification of interaction processes occurring at different strengths. In particular AEA was found to interact preferentially with cannabinoid receptors present in the cerebellum in respect of these found in the whole brain and cerebral cortex. This study confirms the biological role played by AEA in simulating trans-delta-9-tetrahydrocannabinol effects on cerebral receptors.

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1C - The cosurfactant role in four component microemulsions: microstructure determination and model prediction

A. Ceglie, G. Palazzo, G. Colafemmina, F. Lopez, M. Giustini

Aims

Structural determination in multicomponent microemulsions
Full comprehension of the cosurfactant role in surfactant systems

Results

The microstructure of the quaternary water-in-oil microemulsion CTAB/water/n-pentanol/n-hexane has been investigated over a wide range of composition. The dependence of the interface composition on the overall composition is described satisfactorily by a simple partition equilibrium. This permits a quantitative analysis of the experimental data. To achieve this result, a new strategy has been developed for two composition paths: (i) water dilution lines, where the interface composition remains constant; (ii) paths where the interface composition is changed at fixed W_0 (due to the partition equilibrium, such paths correspond either to cosurfactant dilution either to oil dilution). All the data recorded along different paths can be analysed by the same “master plot”. Moreover, the behaviour of nucleic acids solubilized in such microemulsion was studied in detail.

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1C - NMR data analysis in complex and simple liquid mixtures

A. Ceglie, L. Ambrosone, G. Palazzo, G. Colafemmina,

Aims

Determination of size distribution in emulsions by NMR
Deconvolution of echo decays coming from overlapping peaks
Analysis of anomalous diffusion

Results

We presented a new method to analyse pulsed gradient spin-echo (PGSE) NMR data from a mixture of compounds sharing the same NMR resonance (e.g. polymer solutions or mixtures of aliphatic compounds). If all the spin-bearing species undergo Brownian motion, their contribution to the experimental echo decay is exponential.

For the case of more than one diffusing species at a given chemical shift, the echo attenuation is the Laplace transform of the distribution function of the self-diffusion coefficients. Applying the algorithm previously developed by us, we solve the integral equation, obtaining the distribution function of the diffusion coeffcients. The method is tailored for small data sets (10 – 30 points) typical of PGSE-NMR measurements.

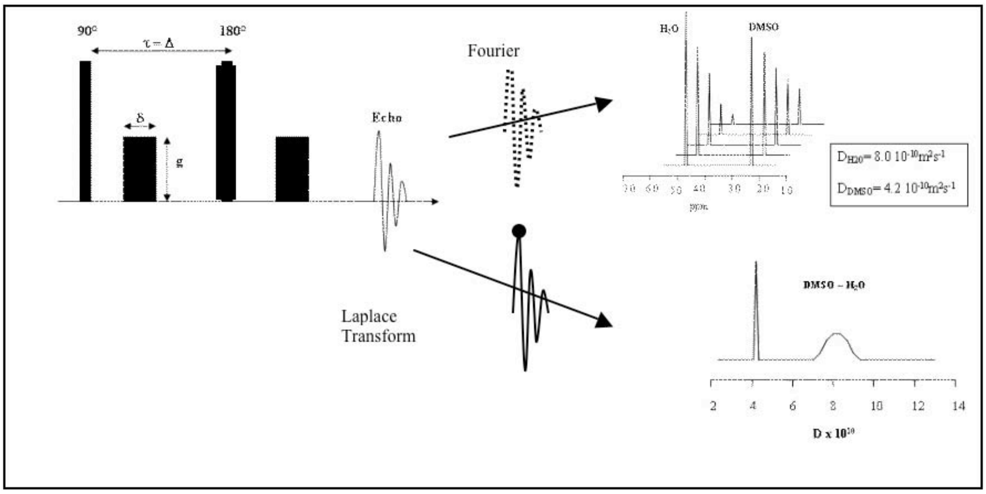

Fig. 1 Decays at the frequency of the ^{1}H peaks characteristic of the three compounds and of the aliphatic signal (on the left), and the diffusion spectrum coming from the analysis of only the aliphatic echo decay (on the right).

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2A - Physical, Chemical and Limnological characterization of aquatic ecosystems. Study of impacts of solar radiation (UV and Visible): in situ and laboratory measurement and model descriptions

Luca Bracchini, Steven Loiselle, Arduino Massimo Dattilo, Stefania Mazzuoli, Andres Cozar, Antonio Tognazzi, Silvia Focardi, Claudio Rossi

Aims

Determination of the temporal and spatial distribution of the optical characteristics of natural aquatic ecosystems

Determination of the impact of UV and Visible radiation on aquatic ecosystems

Models elaboration to examine the impact of the radiation on living systems

Results

The flux of solar UV (290 nm-400 nm) and visible (400 nm – 700 nm) radiation is one of the most important flux that determines ecosystem behaviour. UV radiation is the most energetic, for each incident photon, and damaging for the ecosystem. This radiation can have a significant impact on the metabolic activity of the primary producers, on the chemical characteristics of the biotic and abiotic compartments and on the biological functions of the fauna. Visible radiation is the fundamental energy source for all photosynthetic activities and therefore is the building block for the trophic web.

The measurement of the flux of radiation is particularly important in aquatic ecosystems. The manner in which the available radiation is attenuated depends upon the optical path that the electromagnetic wave follows within the study medium. In an aquatic ecosystem, besides the optical path of the water, there are a number of other important elements that need to be considered as having an important impact on the optical properties of the water medium. Dissolved organic matter, phytoplankton and suspended solids can cause a significant modification of the optical characteristics of the ecosystem. It is therefore important to measure the concentrations of the main components of the aquatic medium. These physical chemical characteristics will vary seasonally and spatially, as well as be influenced by the activities of man in and around the ecosystem. Man's activities can directly impact the material and energy flows that determine the ecological and chemical-physical status of natural systems. In fact, increases of allocthonous matter and changes in the distribution of solar radiation are the main threats to aquatic ecosystems both nationally and internationally. Both of these flows have a direct effect on the quality and productivity of these systems through modifications in the radiation field available within the water body.

More recently, to study the effect of different level in qualities (photon wavelength) and quantities (number of photons) on abiotic and biotic community of the aquatic ecosystems, The group is initiating a study with a large enclosure (10 m^3) constructed in the lake. With help of selective filters that cut-off a part of solar spectrum and a part of lamps spectrum (with well know spectral emission) it will be possible to study the effects of the modified radiation on the biotic and the abiotic systems, and the interaction between them.

Different aquatic ecosystems are interested by the research of the group: Iberà Lake in North East of Argentina, Victoria Lake in Uganda/Kenya, Salto Lake in Rieti province, Pontine lagoons in Latina Province, Montepulciano Lake in Siena Province and many other sites (eg in Manfredonia Gulf, Puglia). The laboratory studies consist in the analysis of optical characteristic of the filtered (absorption and scattering of dissolved matter) and unfiltered (scattering and absorption of the total suspended solid) water sample and the analysis of the mortality and development of organism in controlled and repetitive condition. In aquatic ecosystems, in particular in wetland lake, the spatial, with a non Gaussian distribution, and temporal variation of the physical and chemical condition suggest a non total deterministic georeferenced maps representation (Kriging with help of variogramm model) of measurement and algorithm developed. The attenuation coefficients of the environmental solar radiation give many information on the difference of habitat in the same lake (local scale) and give much information on functionality of the boundary (eg wetland, agricultural and urban activities).

Some example of the results of the researches group are represented in the followed figure:

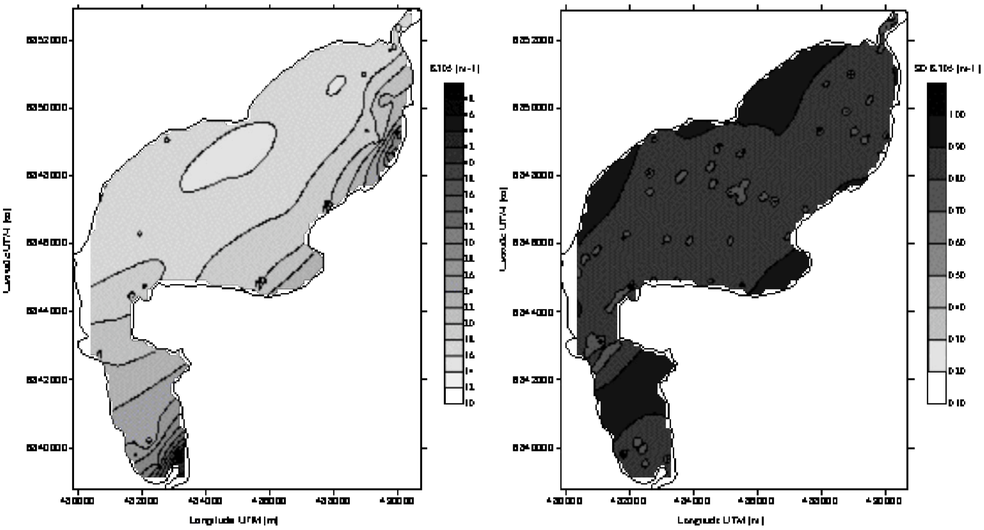


Fig. 1 Map representation of 305 nm attenuation (K305, m^{-1}) and standard deviation (SD, m^{-1}) of interpolating grid of 77 stations in the Iberà Lake

The extremely variation of the 305 nm attenuation coefficient have suggest us to divide the lake in three distinct basins: south, centre and north. The resulting map is well correlated with the Dissolved Organic Matter (DOC, mg l^{-1}, figure 2) measured from a sample at 0.25 m from surface of the lake:

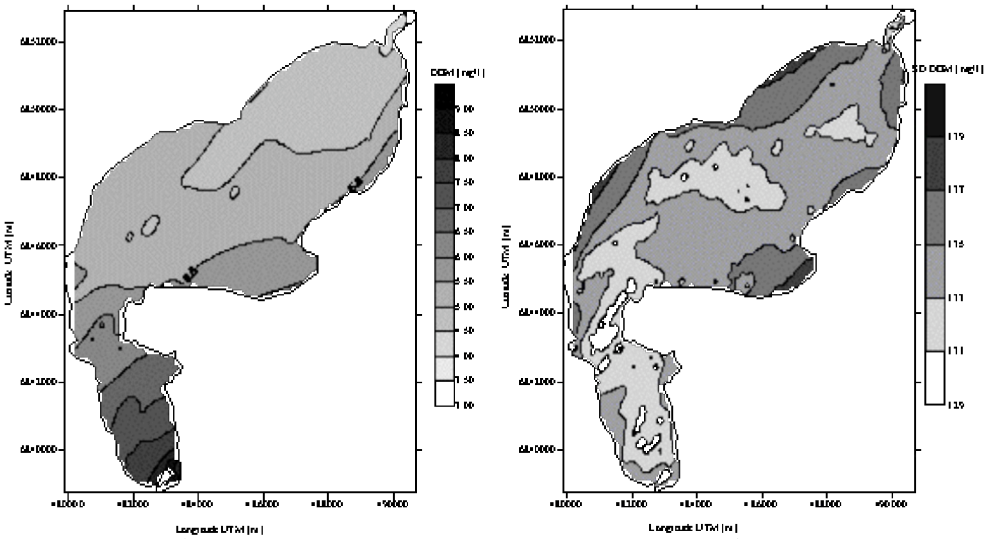


Fig. 2 DOM [mg l^{-1}] distribution in Iberà Lake and Standard Deviation [mg l^{-1}] of interpolating grid

The attenuation distribution of the Visible radiation (with a PAR sensor) in the Katonga bay and Bumjako bay in North West of Victoria Lake (Uganda) is presented in figure 3:

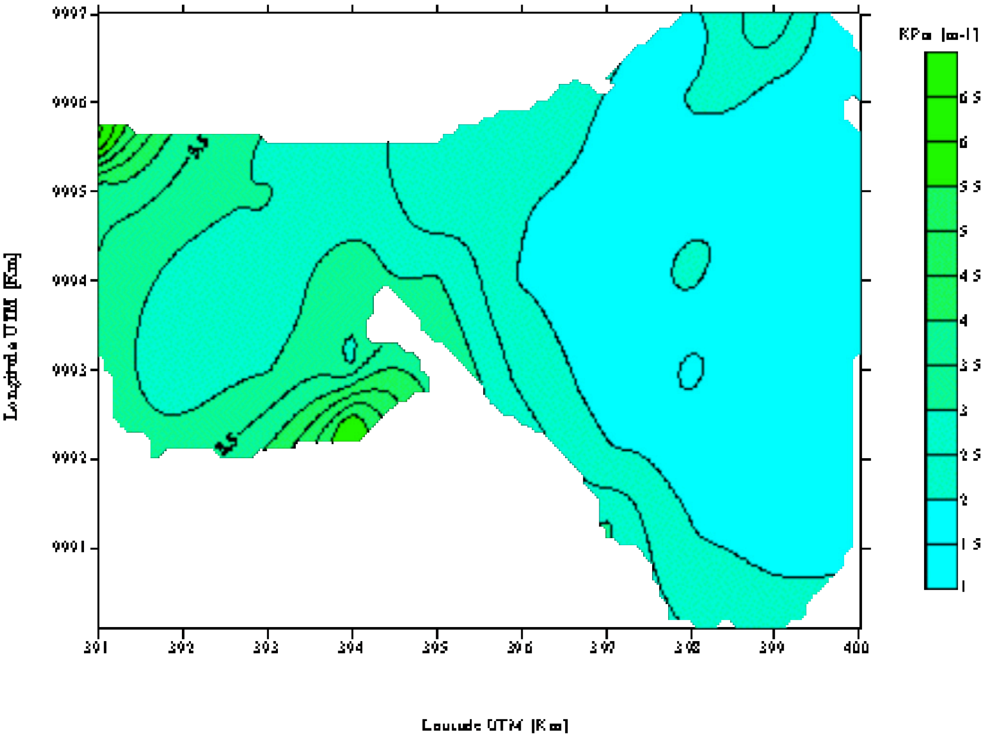


Fig. 3 Attenuation of Visible radiation in Victoria Lake

With the same statistical technique it is possible represent the Turbidity and Fluorescence measurements (figure 4) obtained from sample of water collected at 0.25 m of depth

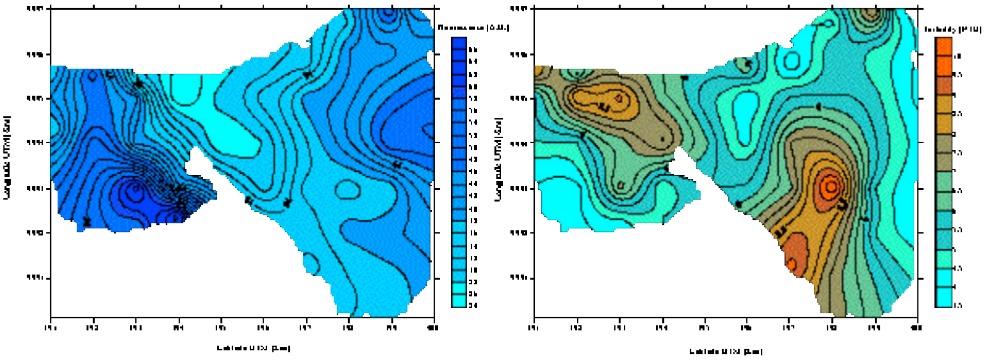


Fig. 4 Fluorescence [Arbitrary Units] and Turbidity [Nephelometric Turbidity Units] in two bays of Victoria Lake

The correlation between the in situ measurements of Chlorophyll α and the florescence and turbidity permit the determination of the distribution of fitoplankton in all studied section of the lake (figure 5):

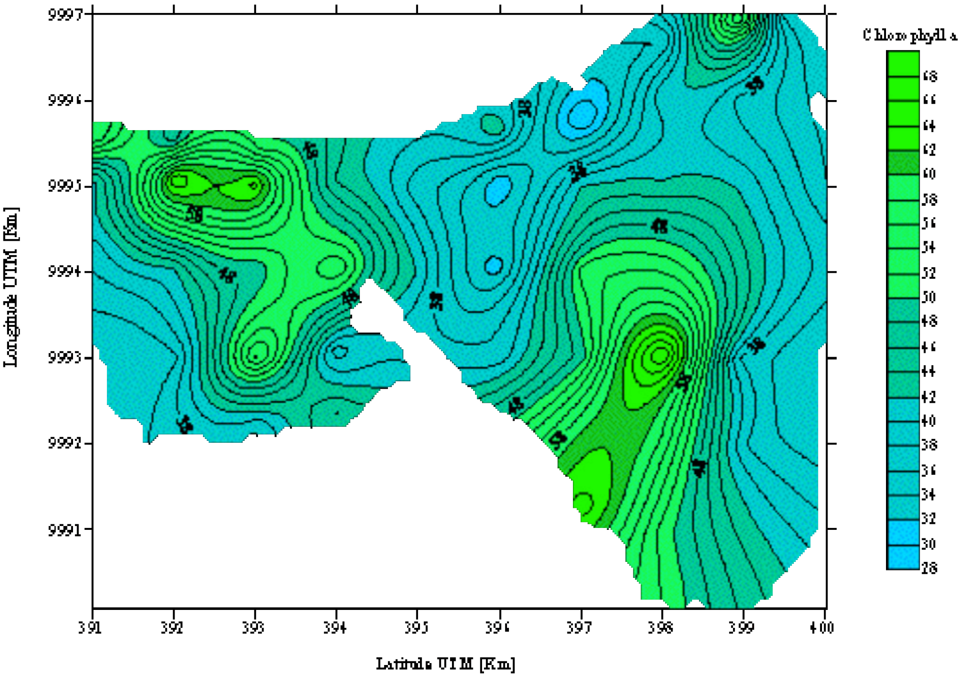
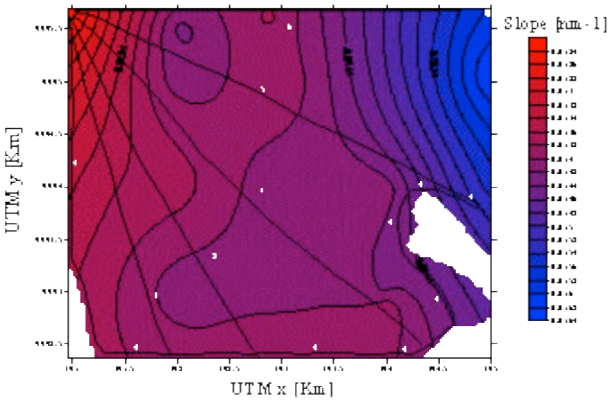


Fig. 5 Chlorophyll a distribution obtained from multiple linear regression of the Turbidity, Fluorescence and in situ measurement of fitoplankton

In the same way it is possible represent the effect of rivers and wetlands on aquatic environment. In particular it is possible to study from the molecular weight and the photobleaching of dissolved organic matter, the chemical quality of the water (figure 6 and 7)

Fig. 6 DOM chemical quality transported by two river in the Iberà Lake. The maximum values of photobleaching was found in central part of Lake



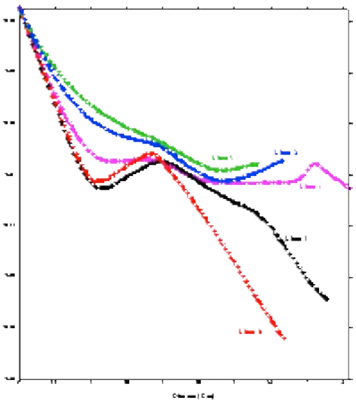


Fig. 7 Effect of the wetland on DOM release (Victoria Lake, Uganda)

The group is interested to modelling the light environment. In particular was develop a model to estimate the upwelling radiation (equation 1) with help of Secchi disk depth measurements (figure 8)

$$A = -\frac{1}{2} Ln\left(\frac{I_{uw}}{I_o}\frac{1}{r(\lambda)}\right) = 1.78 \pm 0.03 \qquad (1)$$

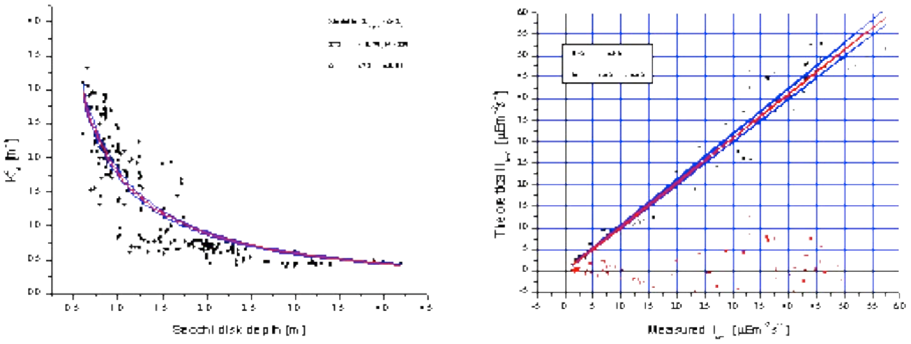


Fig. 8 Secchi disk model results

With the synergism between in situ and laboratory optical measurements it is possible to distinguish the role of the dissolved and suspended matters in the attenuation of solar radiation as function of wavelength (figure 9a and 9b):

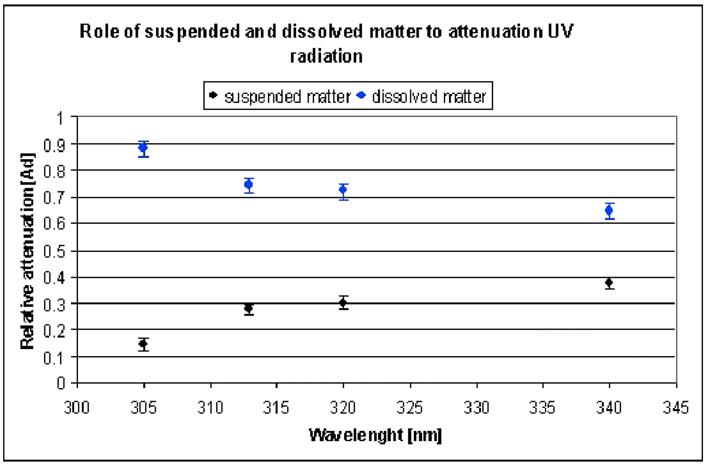


Fig. 9a Importance of the dissolved and suspended matter to the attenuation of UV radiation in eutrophic Lake (Montepulciano Lake)

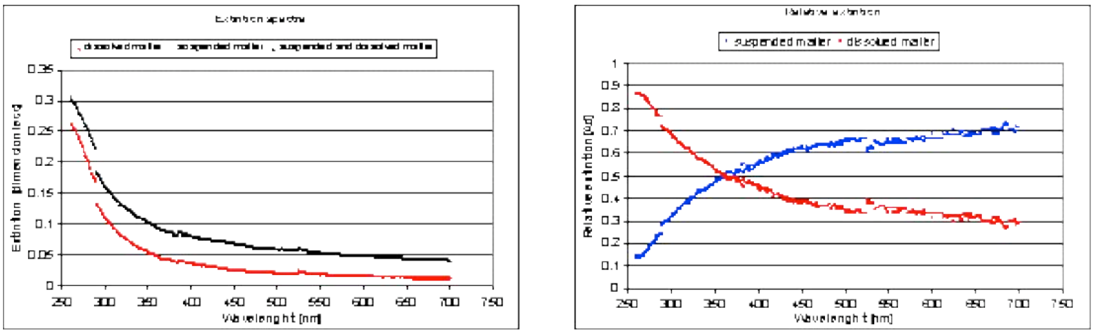


Fig. 9b Extinction spectra and relative extinction of UV and Visibile radiation in Montepulciano Lake

With the extinction properties it is possible obtain some information about in solution and suspended matter (figure 10)

Fig. 10.

The impact of UV radiation (wave centered on 310 nm) on living systems was performed in laboratory; the resulting mortality model (eg. on *Artemia Franciscana*) is showed in figure 11a (Mortality in the adult stage) and 11b (Mortality in the Naupliar stage):

Fig. 11a Mortality of Artemia franciscana in adult stage exposed to a different doses of artificial UV radiation.

Fig. 11b Mortality of Artemia franciscana in naupliar stage exposed to a different doses of artificial UV radiation.

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2A - The use of satellite based optical sensors to examine the chemical, physical and biological properties of aquatic ecosystems

Steven Loiselle, Andres Cozar, Arduino Massimo Dattilo, Luca Bracchini, Stefania Mazzuoli, Claudio Rossi

Aims

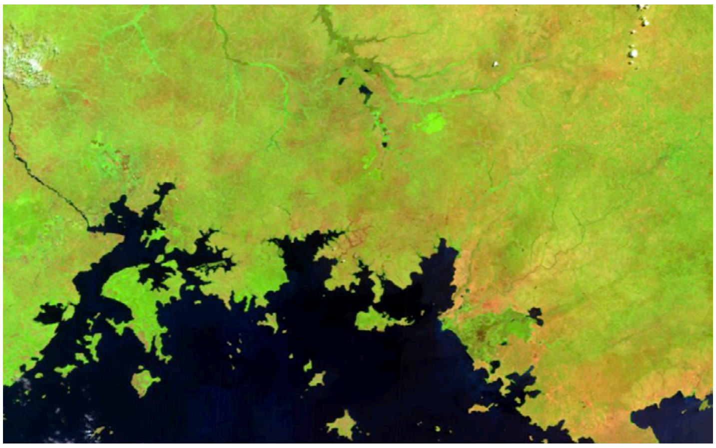
The combination of biological, chemical and optical measurements with the satellite based emission and reflectance measurements is an important tool in the study of the spatial and temporal characteristics of aquatic ecosystems. The CSGI Siena environment group has used these instruments in collaboration with researchers in a number of important research projects studying Lake Victoria in East Africa, the Ibera wetland in Argentina, the Nembuucu wetland in Paraguay and the Lake Montepulciano in Italy. The objective is usually to examine season and spatial changes in water and habitat quality and model these changes to understand the impact of changes in local and global climate, resource uses and hydrological conditions. Doing so, satellite derived information about lake shape, distribution, optical characteristics (Secchi depth, turbidity, concentration of dissolved organic matter) can be used to examine the role of resource utilisation on ecosystem quality. The satellite systems most often used are the SAC-C satellite MMRS sensors, the EO1 ASTER sensors and the Landsat ETM sensors. The wavebands that are usually used are visible and infrared. The availability of a time series of images from satellites with similar characteristics allows for a macro-scale comparisons related to the temporal co-variation of the ecosystem characteristics.

Results

Lake Victoria, Africa

SAC-C images were used to perform the classification of the wetland areas of the Ugandan coast and the Kenyan coast acquired on 23/06/2002 and 04/09/2002 . Images were georeferenced using the geocorrection file for each acquisition date. At sensor radiances were obtained using the published values. Path irradiance was removed by using 1% dark object subtraction. The surface reflectance was calculated by determining the sun elevation at the centre of each scene at the time and the MMRS spectral responses supplied by CONAE. Clouds present in the resultant images were then removed and those areas impacted were not considered in the analysis.

Plots of known vegetation composition in the Kirinya and Nabugabo wetlands were then used as training areas. A classification was performed using four main classes; wetland dominated by *Cyperus papyrus* with *Tyhpa latifolia* and *Phragmites domenginensis,* wetlands dominated by *Miscanthidium violecium* with areas of *Typha latifoli* and *Phragmites domenginensis*, non wetland areas (forests and agricultural areas obtained using land use maps from 1992) and open water areas. Plots (17) in the Yala and Katonga wetlands were used to control the accuracy of the abelled pixels of the two classified images created. The overall accuracy of the two wetland classes were 64 % (papyrus dominated) and 66 % (miscanthidium dominated).

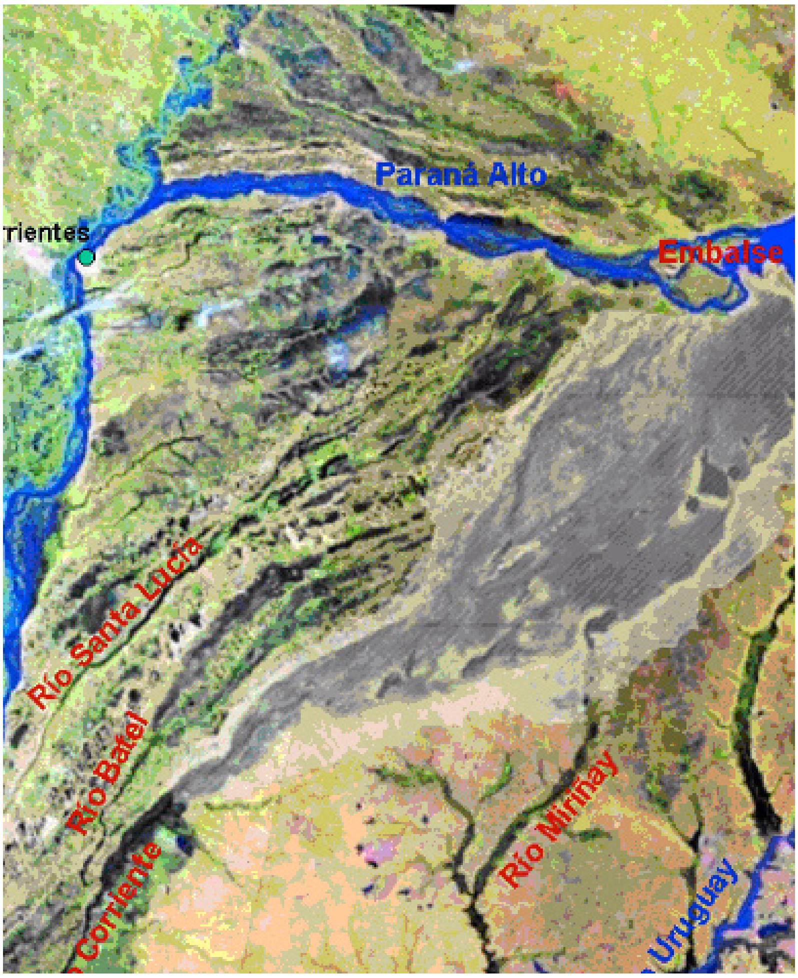


The results of the analysis indicate that the wetlands along the western shore have a higher density than the northeastern shore. This is most likely the result of water and air circulation in this equatorial setting as well as the increased population density along the northern shore. The resulting impact on water quality in the Lake is presently being investigated, but the combination of lower wetland presence and increase population density will undoubtedly lead to poorer water quality in the northern bay areas where much of the lake population lives.

Ibera wetland, Argentina

The "Esteros del Iberá" is one of the largest pristine inland wetland ecosystems in South America. The analysis of the wetland was made to determine the seasonal changes in water quality in the large number of wetland lakes. Landsat TM and SAC-C MMRS satellite images were processed using radiometeric correction models created for each satellite system utilised. Digital numbers were transformed to radiances on the sensor according to the calibration data for each scene. The path irradiance of the measured exo-atmospheric radiance was removed by using the dark object subtraction. The reflectance was calculated by determining the sun elevation at the centre of each scene at the time and date of each image. Lake morphology and lakes distribution (geomorphologic analysis) was performed thought the spectral analysis of the near-infrared band of the MMRS sensor of the SAC-C satellite. A single image of spring 2001 was used to determine the size and location of all major open water bodies (> 0.5 km^2) in the wetland area. The reflectance data of each image was then sampled for each lake and each lake subsection. Samples of permanent water bodies just beyond the wetland borders were also sampled for comparison purposes. A database of 63 lake sections of a total of 25 water bodies was obtained. Using the corrected at-surface reflectance of each lake section, the average reflectance for all sections in the visible and infrared wavelengths was then recorded for 39 image dates from March 1997 until November 2001. When clouds covered a particular lake section, a weighted average of the preceding and following images was used to complete the matrix.

The determination of optical properties of the lake waters by satellite measured reflectances was made by comparing on site measurements with coincident satellite images. The on site measurements were made over a two year period in two of the permanent wetland lakes, Laguna Ibera (54 km2) and Laguna Galarza (14 km2). Georeferenced satellite images obtained in the same period (± 3 days) were used to create a series of algorithms that related the reflectances in the visible wavebands (bands 1,2,3 on all sensors) and infrared wavebands (band 4 and 5) to measured water optical properties. These included the Secchi depth, the nephelometric turbidity and the concentration of dissolved organic matter, which consisted mostly of dissolved humic and fulvic acids.

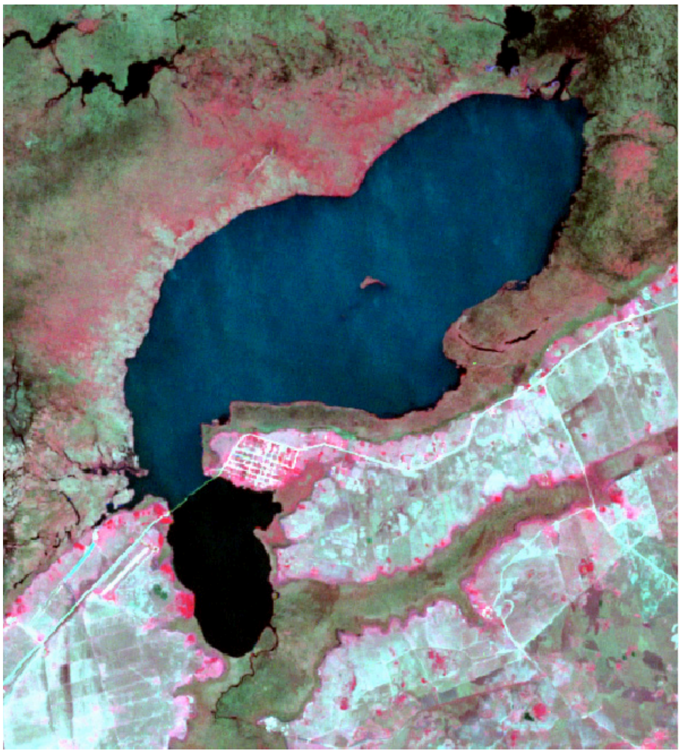


On the basis of the changes in lake and wetland morphology, the wetland was divided into three regions and the qptical characteristics of each region were determined. The spatial distribution of the optical properties show strong similarities with the geomorphogical regions. The water quality of the lakes of Southern Region generally showed higher transparency, that is, higher S_d values (media e SD). The most transparent waters were located in the central zone of this Region, in particular in Laguna Medina and the northern sections of Laguna Trin. The lakes of Northeast and Northwest basin showed lower transparency. Laguna Luna and Laguna Iberá in the Northeast basin shows a different behaviour with respect to the “north-south” pattern of transparency in the macrosystem. The lakes selected outside the wetland in the active floodplain of the Mid Parana river showed the lowest transparency of all the sampled areas.

Temporal analysis

The seasonal dynamics of the lacustrine systems within the wetland were analysed using the EOF methodology of the series of Secchi depth estimations. The first EOF mode (first principal component) extracted from the covariance matrix of S_d explained the 56.4% of the variance of the collection of temporal series. The remainder of the modes always explained less 44% of the variability. The first mode corresponded with the seasonal nature of the wetland, showing transparency maxima during June and minima during January (fig. 4.9). This pattern approximately coincides with the maxima and minima of solar irradiance in the region as well as measured lake water temperature. The monthly maxima and minima of water temprature respectively coincided with the maxima and minima of S_d. Phytoplankton dynamics of two of the wetland lakes also displayed similar dynamics (L. Galarza and L. Ibera south basin).

To compare the temporal variations in the lake optical characteristics in different lakes, the temporal trend in each lake section was compared to the first EOF mode. The deviation in the temporal trend of each site when compared to the EOF mode was used to examine the differences in the temporal trends of each lake section. The lakes present in the border agricultural and population centres (L. Mercedita y Cambí Retá) show an even larger deviation ($R < 0.80$). These areas are subject to nutrient flows that may lead to changes in the seasonal cycle of phytoplankton. The analysis of the seasonal trend within the artificial reservoir of the hydroelectric dam (Lago Yacyretá) on the Paraná River demonstrated a completely different behaviour in relation to the EOF mode ($R < 0.50$) and wetland lakes. The rain periods coincided with a increase in water transparency, apparently due to the "washing" of the basin when the Paraná river passes through the floodplain. Similar observations have been made in Venezuela in the Orinoco floodplain (Castillo 2000).

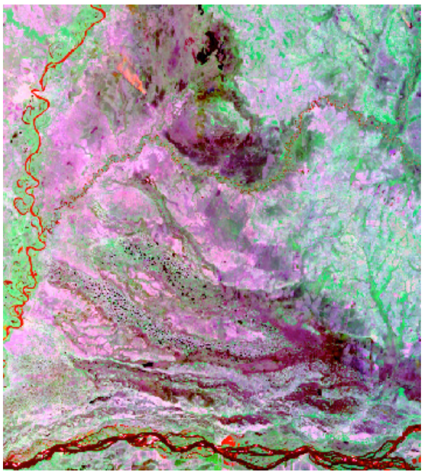


The analysis of the spatial and temporal trends of the lacustrine ecosystems of the Iberá wetland system showed a clear north-south difference. In the north, differences in water quality along the east-west gradient were also determined. In the spatial analysis, this was clearly determined in the cluster analysis of the water optical properties using the monthly averages of the four years of satellite data. Those wetland lakes that strayed from this behaviour were those that were located in border areas in which agricultural activities were present. These three regions (NE, NW and S) within the wetland were differentiated by both water quality measurements as well as geomorphology.

In summary, the Ibera wetland can be defined as a system of lakes and marshes, whose characteristics have regional and temporal gradients. These gradients are effected by the flowrate and retention time of each lake, the presence of agricultural activities near the lake and the geomorphological characteristics of the bio-geographic region.

Other areas under study are the Lake Montepulciano (Italy) and the Nembuucu wetland in Paraguay.

Lake Montepulciano, Italy (IKONOS satellite)



Nembuucu wetland and Paraguay river (SAC-C MMRS)

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Rocchini, D., Chiarucci, A., Loiselle, S. (accepted for publication 2003) Testing the Spectral Variation Hypothesis by using satellite multispectral images. Acta Oecologica

2A - Surface modification of textile materials

P. Lo Nostro, L. Fratoni, P. Baglioni

Aims

Modification of the surface of textile materials

Results

Modification of the surface of textile materials, mainly wool and cellulosic fabrics (cotton and Tencel®), was obtained through:

- grafting of a cyclodextrin derivative in order to endow the surface with empty hosting cavities that can be used for uptake/release of fragrances, insect repellents, antimicrobials, etc. (Figure 1)
- deposition of metal oxide nanoparticles (mainly ZnO and TiO_2) for anti-UV shield and comfort upgrade (Figure 2)
- flame retardancy through surface reaction of cellulosic fibers with ammonium sulfammate and urea water solutions

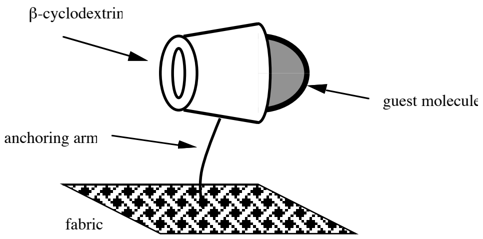

Fig. 1

Fig. 2

The formation of stable inclusion compounds at the fabrics' surface was tested with several techniques: UV-VIS spectrophotometry (with integrating sphere), back-extraction with organic solvents, calorimetry, aroma testing and insect repellency tests (in collaboration with the Italian National Institute of Health, Rome). Figures 3 and 4 show a specimen of Tencel® treated with monochlorotriazinyl-b-cyclodextrin and then with DEET, a common insect repellent.

Fig. 3

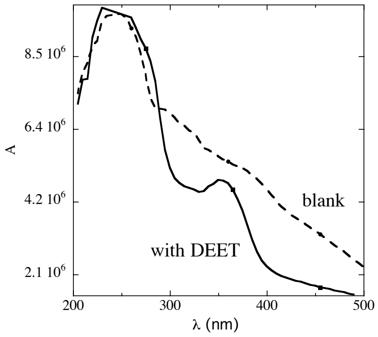

Fig. 4

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2A - Enzymes, Synzymes and their application to waste processing and bioremediation.

E. Sanjust, A. Rescigno, S. Murgia, A. Salis (CSGI), in cooperation with F. Sollai, G. Vivona, J.V. Bannister (University of Malta, Msida, Malta)

Aims

Synthetic techniques: selection of macromolecular supports and redox mediators for synzyme synthesis.

Characterization procedures for enzymes and synzymes.

Applied techniques: treatment of polluted water from agrofood industries, biotransformations and biomass production and analysis.

Results

Many wastes from agrofood industrial processes contain high concentrations of organic products, requiring at least one oxidation step to be safely disposed. Chemical oxidations are often depending on aggressive chemicals such as hypochlorites, ozone and so on; high costs and/or formation of toxic byproducts could be a serios limitation. On the other hand, biological treatments take place under relatively mild conditions but their application is usually prevented by long times and/or poor availability and high costs of the used enzymes. Therefore an integrated project is now in progress, devoted to optimise the production of selected highly active oxidases from edible white-rot fungi such as *Pleurotus*. The fungus could be directly employed in bioconversion processes, leading to formation of edible biomass and substantial reduction of the recalcitrant substrates. Alternatively, the involved oxidases could be extracted form suitable culture media, purified, and possibly immobilized on suitable supports. Among fungal oxidases, laccases and tyrosinases have drawn our attention and are under study as regards their induction, substrate specificity, action mechanism(s) and operational stability.

Synzymes are artificial enzymes, made by assembling naturally occurring moieties such as proteins and coenzymes, or also assembling suitable non-proteinaceous polymers and redox mediators, both natural or synthetic. Our attention is now focused on quinonoid synzymes, acting as amine oxidases to deaminate primary aliphatic amines to aldehydes, and especially to artificial peroxidases, capable of oxidatively degrading a wide range of aromatic molecules by means of hydrogen peroxide or also molecular oxygen. A number of such artificial peroxidase have been synthesised starting from water soluble or water insoluble polymers, coupled with both natural and synthetic metalloporphins and metalloporphyrins. Preliminary work has shown the promising features of these proparations as it regards ease of preparation, low cost, stability and catalytic activity. Also good substrate specificity towards non-phenolic substrates has been reached with ceratin preparations. The synzyme technology is obviously not limited to bioremediation and could find deep application in other technological processes such as drug biosynthesis: to this purpose, a flavosynzyme acting as a $NAD(P)^+$ regenerating tool has been recently synthesized and characterized.

A review on bioremediation through microbial agents was published.

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2A - Colloidal Science and Nanotechnology for Cultural Heritage Conservation

P. Baglioni, G. Bitossi, E. Carretti, L. Dei, V. Errico, R. Giorgi, A. Macherelli, M. Mauro, B. Salvadori

Aims

Nanomaterials for wall paintings and stones conservation.
Nanocontainer solutions for wall painting and stone surfaces.
Innovative gels for easel paintings conservation.
Nanotechnology for paper deacidification.
Anti-scaling molecules for inhibition of salt crystallization in porous media.
Stone investigation by Nuclear Magnetic Resonance techniques.

Results

CSGI is involved in several projects aimed to improve materials and techniques for the conservation of cultural heritage. The city of Florence is one of the most appropriate "environments" for these studies. After the 1966 Florence flood, the research group directed by the CSGI co-founder Prof. Enzo Ferroni was one of the first Academic Institutions that applied a rigorous scientific approach to the investigation of Cultural Heritage conservation. The peculiarity of the research in the Science for Cultural Heritage resides in its multidiscipinarity, where basic studies are usually associated to technological researches.

Several conservation workshops have been carried out with CSGI scientific consultancy, and using innovative methodologies developed in the CSGI Laboratories (Masaccio's wall paintings in Cappella Brancacci, and Beato Angelico's wall paintings in San Marco Abbey, in Florence, Piero della Francesca's wall paintings in Arezzo, etc.).

CSGI contribution mainly consists of a co-operation with conservators and private and public institutions for experimentation of the innovative methodologies; this approach provides a continuous improvement of the conservation procedures. Moreover, CSGI provides also physicochemical diagnostics of materials: in particular, CSGI expertise is related to the characterization of pigments, dyes, and binders used for wall paintings or easel paintings, and also of the degradation products, as salts, varnishes and aged adhesives, in stones and wall paintings.

Fig. 1 San Marco Abbey (Florence), wall paintings by Beato Angelico. Conservation carried out with the Ferroni-Dini method (ammonium carbonate plus barium hydroxide)

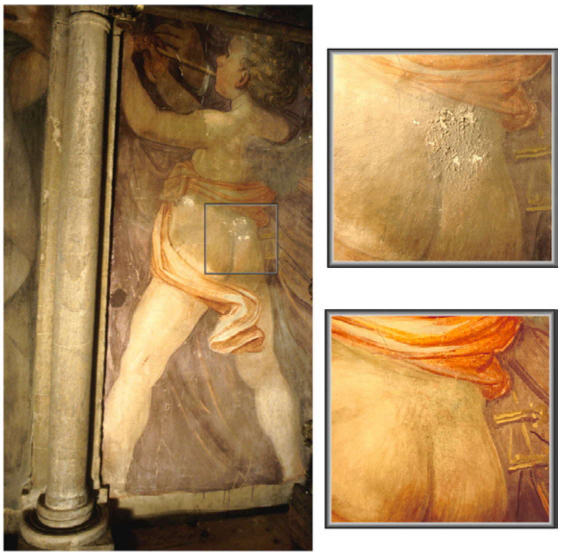
Fig. 2 San Marco Abbey (Florence), wall paintings by G. D. Sogliani: Dino Dini at work

Nanomaterials for wall paintings and stones conservation

Calcium hydroxide is probably the best solution for reinforcement of wall paintings, since it is physicochemical compatible and it is the 'original' binder used by the artists. Unfortunately the poor solubility of calcium hydroxide in water has prevented a large use of lime water. More concentrated systems, based on lime as binder, could be prepared as a dispersion, but lime dispersions in water are not stable, actually sedimentation rate is very fast. The formation of white glazing over painted surfaces is the main consequence of this behaviour.

Ca(OH)2 nanoparticles and stable dispersions, in nonaqueous media, have been obtained by several synthetic approaches. Scanning and Transmission Electron Microscopy coupled with X-Rays Diffractometry (SEM/TEM, XRD) showed crystalline, hexagonally shaped, nanoparticles with hexagon sides in the range 50-300nm. Atomic Force Microscopy (AFM) evidenced that the thickness were in the range 2-40 nm.

Calcium hydroxide stable dispersions were successfully applied, instead of organic glues, as fixatives to re-adhere lifted paint layers during several restoration workshops: wall paintings by Santi di Tito in the Cathedral of Florence, wall paintings by Filippo Lippi in the Cathedral of Prato, wall paintings by Conrad Albrizio in the State Exhibit Building Museum of Louisiana, stone materials of the Santa Prisca in Aventino Apse Church in Rome and of the Bell Tower at Impruneta near Florence.



Cathedral of Florence, Santi di Tito's wall paintings: conservation carried out by means of lime nanodispersions

Fig. 3 Santa Maria Novella Basilica in Florence, wall paintings by Andrea da Firenze: conservation carried out by means of lime/alcohol dispersions

Nanocontainer solutions for wall painting and stone surfaces

Oil-in-water microemulsions have been formulated for the application to wall paintings, easel paintings and stones, in order to remove hydrophobic materials (acrylic resins, wax, fats, etc.) from the works of art surfaces. Past treatments, based on the use of hydrophobic synthetic materials, failed since of their degradation. Actually, they lose its efficacy as protective and/or binder, and change in color producing dramatic side effects. The only way for removing them is, at the moment, limited to the usage of toxic mixtures of solvents.

Three (water, oil, surfactant), four (water, oil, surfactant, and co-surfactant), and five components (water, oil A, oil B, surfactant, and co-surfactant) systems were set up and checked in laboratory as 'cleaning agents'. The best formulation was tested in some case studies in the framework of the restoration workshops of the wall paintings by Filippo Lippi in Prato Cathedral and Spinello Aretino in the Guasconi chapel in San Francesco Church, Arezzo. The removal of hydrophobic polymeric resins from the fresco surfaces was complete and any side effects come out. Recently 250 m2 of the Conegliano (near Venice) Cathedral Façade mural paintings have been conserved using a micellar solution developed at the CSGI Center (and baptized 'Conegliano's potion') to clean the wall painting from vynil and acrylic polymer used in past interventions in 1950 and 1989.

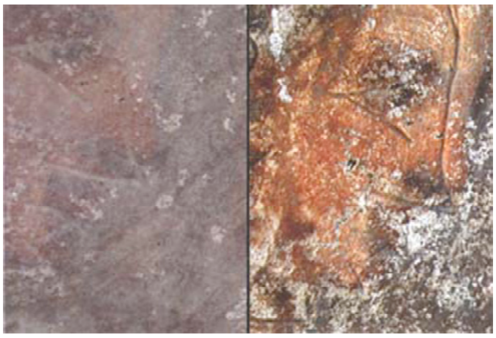


Fig. 4 Particular of the fresco by Pozzoserrato (XVI century) in the Conegliano's Cathedral after the cleaning with the micellar solution developed ad hoc for this workshop.

Innovative gels for easel paintings conservation

In the last two years the Florence unit of CSGI has developed new gel systems with potential application for eesel paintings conservation. The basic idea is to overcome the problems associated to gelators with high molecular weight (very high viscosity creates diffuculties during the removal from the painted surfaces) by developing system with a gelator precursor (polyallylamine) that becomes a true gelator after CO_2 bubbling with consequent formation of the polyallylammoniunm carbamate which behaves as a very good gelator for many solvents commonly used in easel paintings cleaning. Then the gelator can be easily removed disrupting the gel network by simple weak acid hydrolysis that gives back the polyallylamine precursor. This approach seems to have very stimulating outputs for potential application in cultural heritage conservation: important tests are in progress in cooperation with Museums and Galleries in Florence and Siena.

Nanotechnology for paper deacidification

Acidity is the main source of degradation in paper. Many different techniques and products have been studied or developed in order to eliminate acidity from paper. Unfortunately, up to now the problem is far from to be satisfactorily solved, and the specialists are looking for new products against the acidity present in paper.

Despite its scarce usage, calcium hydroxide is an excellent deacidifying agent. Calcium hydroxide is physico-chemically friendly to paper and, once converted to calcium carbonate, works efficiently as alkaline reservoir. The best way to apply is as a suspension shape.

Unfortunately, commercially available calcium hydroxide powders have a size distribution was quite broad and larger than several micrometers. Smaller particles were around 0.5 microns, 80% were greater than 1 micron. This originated two problems: 1)kinetic stability is good but not appropriate for some application procedures, for example spraying; 2)because of particle sizes, a white glaze (or spots) on the treated surfaces could form. Large calcium hydroxide particles showed a poor adhesions and penetrability into the cellulose fibers. Deacidifying agents must ensure good adhesion with fibers, so that the alkaline reservoir acts efficiently, and excellent penetrability through cellulose fibers. These requirements are ensured by the use of particles smaller than micron size.

Calcium hydroxide nanoparticles dispersed in alcohol offer an innovative solution for paper deacidification. The used solvents are environmentally friendly. Moreover, nanoparticles can be applied by different techniques, as brushing or spraying, providing excellent results.

Nanosized magnesium hydroxides and oxides have been recently made. Their application in paper deacidification is currently under investigation.

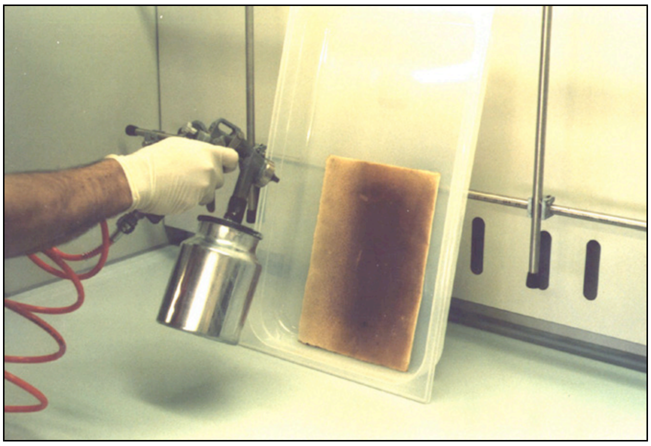


Fig. 5 Deacidification of paper by sprayed lime nanodispersions

Innovative methodologies for inhibition of salt crystallization in porous media

The growth of crystal phases within a porous matrix is one of the main sources of the degradation affecting the historical building materials. The formation of salts is connected to the environmental conditions and, therefore, in many cases they are not completely removable. Less disrupting effect, inside the porous matrix, would be obtained if we were able to minimise the growth of crystals (and the volume). Inhibitor molecules or habitus modifiers, adsorbing on crystalline growing faces or over the crystallization nuclei, provide the right way to do it.

The research project is devoted to the study of inhibition or modification effects by some classes of molecules. The research project is planned as follows:

1 - preparation of laboratory samples impregnated by saturated salt solution;

2 - physicochemical characterization of the crystalline phases, and the porous materials;

3 - monitoring of the crystallization process and analysis of the inhibition activity of additives after crystallization-dissolution cycles carried out in climatic chamber.

This approach aims to control the growth of crystalline phases, by reducing the mechanical stresses of the pore walls. The project contribution to the conservation field is remarkable because it offers new tools for prevention of the degradation processes, instead of mechanical cleaning of surfaces affected by salt crystallization.

Stone investigation by Nuclear Magnetic Resonance techniques

Deterioration of the porous building materials can be considered as a sort of "stress-corrosion process" in which chemical and physical attacks are combined, giving a mechanical alteration of the stone. Water amount inside porous stones plays a crucial role in most of the decay processes. Water is involved in many chemical processes, such as pollutants chemical reactions (i.e. sulphur dioxide), frost attacks, salt crystallization cycles (in particular sulphates and nitrates), and physical attacks, like pore gelivity, or biological degradation due to the action of fungi, bacteria, and so on.

Experiments have been performed, by using innovative experimental approach based on nuclear magnetic resonance (NMR) techniques. NMR exploits the magnetic properties of nuclei (hydrogen in water in our case) under an external high magnetic field. By studying the time length needed for the spin system magnetization to reach the equilibrium value, after a selected perturbation obtained by a radio-frequency pulses sequence, a plenty information on the energy exchanges between the system and its surroundings is obtained. By proper pulse sequences, high-resolution 2D-images of water distribution inside low porous materials (1-3% open porosity) were obtained. Analysis of water proton relaxation rates provided direct information on the porous structure.

This point is meaningful since we allowed the investigation of the stone features after a restorative treatment, in not invasive way. The importance of the physicochemical compatibility among chemicals used by restorers and the original materials was completely neglected in the past. By this reason, wrong conservative restorations have been performed. NMR techniques probe in real time the water filling of stone and provide a direct correlation with the pore structure in restored materials.

Fig. 6 Salt crystallization effect on Italian limestone

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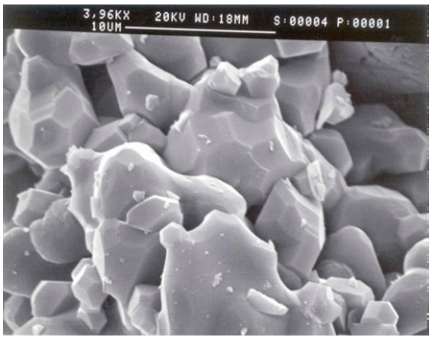
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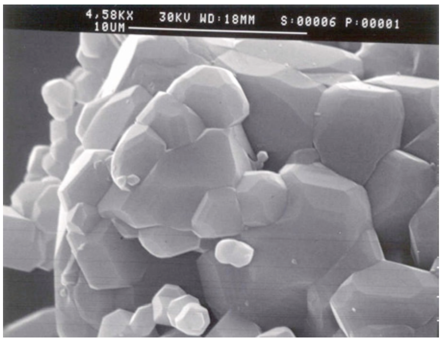
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magnetic properties were investigated measuring hysteresis loops. For the activated mixtures, the mass loss is over at ~600 °C i.e. well below the temperature where $BaCO_3$ spontaneous decomposition is complete (T>850 °C). Such a noticeable temperature lowering is a consequence of the high energy milling enhancing the formation of $BaFe_2O_4$. After heating the milled mixture to 850 °C, $BaFe_{12}O_{19}$ was rapidly formed from the $BaFe_2O_4$ and residual Fe_2O_3. Starting from an unmilled mixture, only minor amounts of $BaFe_{12}O_{19}$ were formed by heating to 850 °C. The favourable formation of barium hexaferrite, when starting from milled powders, has been confirmed by micro-Raman spectroscopy. The powder from the activated sample was shown to have far better magnetic properties than the unactivated sample.

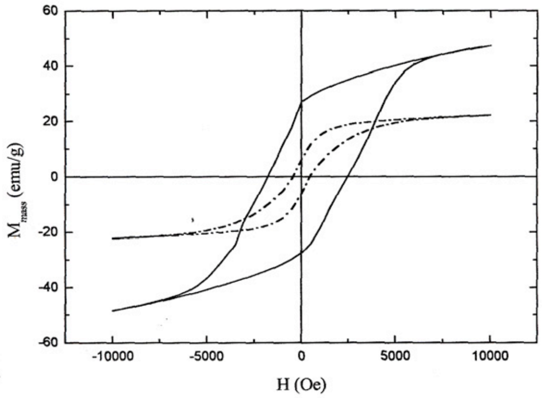

Fig. 2 Hysteresis loop at r.t. obtained from unmilled (dashed line) and milled (solid line) BaC_2O_4+$6Fe_2O_3$ mixtures annealed at 850 °C

Barium metatitanate ($BaTiO_3$) is widely used because of its high dielectric constant, ferroelectric properties and positive temperature coefficient of electrical resistivity. The compound has been synthesized by milling and annealing mixtures of $BaCO_3$ and TiO_2 (rutile) [19]. High energy milling of $BaCO_3$-TiO_2 equimolecular mixture resulted in a noticeable drop of the temperature where the reaction can occur. The formation of single phase $BaTiO_3$ was confirmed by thermogravimetric analysis (TGA) (both alone and coupled with Fourier transform infrared spectroscopy (FT-IR) evolved gas analysis), by differential scanning calorimetry (DSC) and x-ray powder diffractometry (XRPD). The formation of phases like Ba_2TiO_4 (orthotitanate), which are known to be detrimental for $BaTiO_3$ end performances, has only been revealed as an intermediate minor phase that disappears by annealing at temperatures as low as 750 °C. At such temperatures the crystal size of the product is ~400 Å. $BaTiO_3$ formation occurs either by a slow heating (2 K/min) of the milled mixture, or by a rapid heating followed by an isothermal annealing.

By thermoanalysis (TGA and DSC) and X-ray powder diffraction it has been shown that the compounds $SrTiO_3$ and Sr_2TiO_4 can be prepared by mechanical activation of, respectively, 1:1 and 2:1 $SrCO_3$ –TiO_2 (rutile) mixtures followed by annealing for 12 h at 3 2 800–850 °C [20]. These compounds could not be obtained by heating the physical mixtures to temperatures as high as 1000 °C. Moreover, the enthalpies of the reactions leading from Sr carbonate and rutile to the formation of these compounds have been determined. By combining these data with the enthalpy of $SrCO_3$ decomposition, also obtained in this work, the enthalpies of formation of $SrTiO_3$ and Sr_2TiO_4 have been calculated. On the contrary, no $Sr_3Ti_2O_7$ was shown to form, by the same annealing procedure, when starting from a mechanically activated mixture. DSC and XRD results agree in indicating that a mixture of Sr_2TiO_4 and $SrTiO_3$ forms instead.

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2B - Y_2BaNiO_5 and related compounds

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Aims

Structural determination by ab initio procedures from X-ray powder data

Cationic substitution and lattice stability by X-ray diffraction and NMR

Vibrational characteristics and phase stability range of doped samples by micro-Raman technique

Chemical bonding study in Ca substituted compounds by analysis of charge density distribution

Results

The procedure of *ab-initio* structure solution is successfully applied to Y_2BaO_4 [5] and a series of $Y_{2-x}Ca_xBaNiO_5$ compounds ($0<x<0.33$) [6]. The cation substitution does not influence the success of structure determination by using the EXPO code for direct method procedure. The structural parameters obtained for samples with different x values are compared with those reported in the literature. It is pointed out that the knowledge of the dependence of lattice parameters and cell volume on the cation site occupancy factors allows the direct determination of the degree of substitution of calcium on the yttrium site of the sample [5, 6].

Fig 1. left: A comparison between observed (dots) and calculated (solid line) pattern after Rietveld refinement for $x_{Ca}=0.5$ shows very low residuals as difference curve (lower line). **Right:** The trend of the experimental Ca content (x, closed circles) as a function of the nominal x_{Ca}, content is well compared with literature data (open circles) in a wide range of values.

Cation substitution by Mg and Zn on the Ni site of Y_2BaNiO_5 compound has been investigated by NMR and micro-Raman techniques. The presence of Mg makes possible to resolve the signals associated to different Ni ions in the Haldane chains of this compound, the finite length being created by substituting Ni^{2+} (S=1) by Mg^{2+} (S=0) [13].

Zn substitution on Ni site induces a structural change for x>0.13, while for x<0.13 two structural types coexist. The lack of the inversion symmetry on the Zn-substituted structure causes the appearance of peculiar Raman modes in the frequency region between 260 and 340 cm^{-1}, shown in figure below.

Energy and intensity of these modes are discussed taking into account the role of the doping level and of the phase abundance of the samples. A possible assignment of the modes is proposed [34].

We investigate how the presence of Ca^{2+} substituted in Y^{3+} site can influence the electronic charge distribution and the bond character in the Y_2BaNiO_5 compound. The electronic density map (r(r)) is derived from powder X-ray diffraction patterns with a novel procedure based upon a correlative use of two methods: Maximum Entropy Method (MEM) and Whole Powder Profile Fitting (WPPF). The substantial contraction of NiO_6 octahedra with increasing Ca content is related to an Ni-O bond which becomes more ionic as x increases up to x=0.24. The covalency becomes stronger above x=0.24 particularly along some Ba-O bonds [31].

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2B - Magnetic investigations in Mn substituted titanates

V. Massarotti, D. Capsoni, M. Bini

Aims

EPR determination of Mn oxidation state
XRD characterization of perovskitic solid solutions
Microstructure and grain boundary study of substituted $SrTiO_3$

Results

Electron paramagnetic resonance (EPR) and static magnetization data on Mn substituted (up to 3 mol%) strontium titanate samples are used to determine the Mn oxidation states: Mn^{4+} and Mn^{2+} states are observed. The behavior of their intensity vs temperature is also determined and suggests the presence of antiferromagnetic interactions in heavily Mn doped grain boundaries. X-ray diffraction analysis shows two coexisting perovskite-type phases, whose abundance depends on the Mn content [14].

XRD patterns of samples (a) 1 mol%Mn, (b) 2.5 mol%Mn and (c) 3 mol% Mn

When La and Mn are simultaneously substituted (up to 10 mol%) in $SrTiO_3$ the EPR signals indicate the nearly complete stabilization of the Mn^{3+} oxidation state. X-ray diffraction analysis shows in each sample a single cubic perovskite phase with lattice parameter depending on the degree of substitution.[15]

Electron paramagnetic resonance (EPR) on polycrystalline $CaCu_3Ti_4O_{12}$ have been performed and are discussed within a crystal-field approach. A symmetric signal centred at $g=2.15$ is observed for $T>25$ K, with no evidence of hyperfine structure. At this temperature an antiferromagnetic transition is observed by static magnetization measurements.

V, Cr, Mn and La substituted samples are also investigated to understand the nature of the observed paramagnetic centre. A strong copper-hole delocalization may justify the EPR response [42].

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2B - Thermal stability and properties of pure $LiMn_2O_4$

V. Massarotti, D. Capsoni, M. Bini, V. Berbenni, A. Marini

Aims

Properties-composition relationships in $LiMn_2O_4$ spinel obtained from sol-gel and solid state syntheses

Understanding of the mechanisms of thermal decomposition of the spinel phase as a function of oxygen partial pressure in the T range 25-1050°C

Results

Lithium manganese spinel $Li_{1+y}Mn_{2-y}O_4$ ($0.00<y<0.07$) synthesized by the sol-gel process is studied with a focus on the effects on the changes of formation conditions which can sensibly determine the mean crystallite size from a few nanometers to several hundreds nanometers. Changes of stoichiometry and abundance of the spinel phase can be observed in the $573<T_A/K<1073$ range of annealing temperature (T_A). Comparing spinel phase obtained by sol-gel and solid state synthesis evidences the correlation among composition, structure and electric and magnetic properties [8].

The structural evolution of $LiMn_2O_4$ spinel was followed from 320 K down to 10 K. The structural transformation takes place near room temperature with a significant hysteresis: the high-T cubic phase transforms into a superstructure orthorhombic cell.

The study indicates that the nuclear structure is stable down to 10 K, while neutron diffraction patterns below 80 K show the rise of a magnetic ordering. From Mn-O bond length analysis of the MnO_6 octahedra, a temperature-independent charge ordering can be deduced [9].

Reversible and irreversible transformations in $LiMn_2O_4$ are studied under different atmospheres (air, O_2 and N_2) in the temperature range 25-1050°C. In air and O_2, a reversible cation exchange occurs. For T>800°C Mn^{2+} ions substitute Li^+ at the tetrahedral (8a) position and Li^+ shifts to interstitial octahedral (16c) sites. Under N_2 flow, a first decomposition occurs between 600 and 800°C and yields Mn_3O_4, orthorhombic $LiMnO_2$ and O_2. Minor O_2 release occurs when heating above 900°C, which is accompanied by the transformation of o-$LiMnO_2$ into a cubic $Li_xMn_{1-x}O$ solid solution ($x<0.5$), consistent with a decrease in the average oxidation state of Mn ions. This cubic phase decomposes upon cooling (T<800°C) giving Mn_3O_4 and o-$LiMnO_2$ [33].

The electrical conductivity of $Li_{1+y}Mn_{2-y}O_4$ ($-0.36<y<0.16$) spinels is analyzed. Li-rich spinels show conductivity values higher than that observed in stoichiometric $LiMn_2O_4$. A conductivity drop, associated with a structural phase transition at about 280 K in $LiMn_2O_4$, progressively disappears by decreasing Li content, while it is absent in Li-rich samples. Stoichiometry effects on the concentration of charge carriers and on the available sites for the hopping transport process are evaluated. The role of the Jahn-Teller effect on the conductivity behavior is also considered [7].

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2B - Stability and properties of spinel related phases

V. Massarotti, D. Capsoni, M. Bini

Aims

Identification of the Mn_5O_8 Raman spectrum

Investigation on structural transformation thermally induced in Mn_5O_8 lattice by laser beam

Results

The Raman spectrum of the metastable Mn_5O_8 phase, obtained from slow oxidation of Mn_3O_4 at low temperature, is analyzed for the first time and the thermal stability is monitored by the changes in Raman spectra due to laser induced thermal treatments. A structural transformation toward the spinel phase Mn_3O_4 is observed at T>1000 K. Other Mn oxides, characterized by intermediate Mn oxidation states, are not detected below o during the transformation. A compositional model of the sample grains is also proposed by comparing Raman data with X-ray diffraction and scanning electron microscopy measurements [10].

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2B - Doped $LiMn_2O_4$ spinel and Jahn-Teller distortion

V. Massarotti, D. Capsoni, M. Bini

Aims

Study of the inhibition of the Jahn-Teller cooperative distortion in $LiMn_2O_4$ by doping with transition metal and Ga ions.

Characterization of the homogeneous dilution of doping ions and the valence state of Mn ion in the spinel lattice.

Determination of the charge distribution model related to the cationic substitution.

Results

The study of the occurrence near room temperature of Jahn-Teller (J-T) transition in $Li_{1.02}M_xMn_{1.98-x}O_4$ with $0.0<x<0.06$ and M=Ni, Co, Cr and Ti [22] and with Ga ions [32] is performed. The EPR spectra and magnetic susceptibility data are related to the valence state of M and Mn, and to the homogeneous distribution of the dopant. The J-T distortion, which is associated with a drop in conductivity with decreasing temperature is suppressed by substituting Mn with 3% Co^{3+} or Cr^{3+}, with 2% Ga^{3+} or by substituting an even smaller amount of Ni^{2+} (1%). It is found that the J-T distortion inhibition is possible when $r=|Mn^{4+}|/|Mn^{3+}| \geq 1.18$. Doping with the tetravalent cation Ti^{4+}, which always decreases the r value, does not suppress the J-T transition [22].

NMR measurements indicate that Ga^{3+} ion substitute chiefly in the octahedral sites, Mn^{4+} ions occupy regular and distorted octahedral sites. At the same time, the value of the lattice parameters remain unchanged. Mn^{2+} ions are present in the tetrahedral site and Li^+ increases in the octahedral sites as a consequence of Li-Mn/Ga inversion. Such mechanism increases the efficiency of J-T inhibition by Ga doping in comparison with transition cation having the same valence [32].

Al-doped lithium manganese spinel showed homogeneous distribution of Al^{3+} ions in both cationic sites by XRD, NMR and EPR analysis [41]. The J-T distortion is shifted towards lower temperature by very low Al-substitution.

The charge distribution in Mg-doped lithium manganese spinel is discussed and compared to those of the other M-doped samples: the sensitivity of the cationic sublattice in displaying electronic and magnetic changes is remarked [40].

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2B -Formation of elemental metal powders by room-temperature mechanosynthesis

Riccardo Ricceri, Paolo Matteazzi

Aims

Mechanosynthesis has been shown to be very effective for the preparation of many metals with high melting points and ceramic materials (1,2). The aim of this work was to produce elemental metals like tungsten and boron using magnesium and metal oxides as starting materials by a mechanochemical process. Both these metals are presently produced by processes based on long and expensives routes: the reported process takes place ion both cases at room temperature, and is relatively unexpensive, requiring also a simple equipment in comparison with the industrial processes in use. The studied reactions are:

$B_2O_3 + 3\ Mg \rightarrow 2\ B + 3\ MgO$ and

$WO_3 + 3\ Mg \rightarrow W + 3\ MgO$

Mechanosynthesis reactions were performed in a vibratory ball mill (Spex 8000 Mixer Mill) using carbon steel balls as grinding media under argon atmosphere.

Results

Elemental boron powder of submicron size has been obtained with 82% yield after very short milling times (as short as 9 minutes): the powder was crystalline and of very high purity (>99%), after leaching with diluted HCl of the milled powders in order to remove the unwanted MgO product. In fig.1 the XRD spectrum of the purified elemental crystalline boron powder is reported. Elemental tungsten was obtained thanks to a similar procedure: submicron crystalline metallic tungsten powder was obtained after 8 minutes milling with 84% yield: a high purity metal powder (>99% also in this case) was obtained after leaching of the milled powders with diluted HCl in a similar way to that reported for boron powders. In fig.2 the XRD spectrum of the purified elemental crystalline tungsten powder is reported.

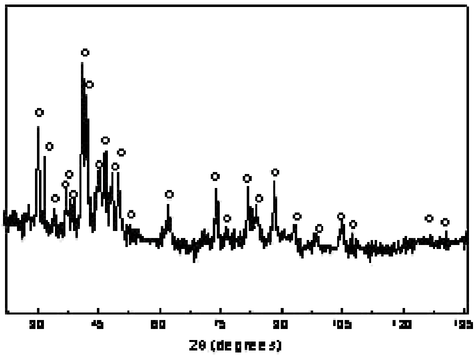

Fig.1 XRD trace of purified boron. (o) metallic B.

Fig.2 XRD trace of purified tungsten. (*) metallic W.

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2B - Microscale fabrication of graded materials components

P. Matteazzi, R. Rolli, in collaboration with MBN (I), INASCO (EL), Ltm (F), LZH (D), SIEMENS (D), TIL (UK), IMNR (RO)

Aims

Scientific research objectives: 1) realize nanophased powders materials (3-10μm); 2) conceive a laser/powder head being capable of spatial resolution of better than 50 μm; 3) microscale fabrication of materials with gradients resolution in the 10-50 μm range. Technological research objectives: 1) micromaking machine for graded materials, with a productivity in the order of 0.1 g/s; 2) fully dense micromade components with graded materials (tolerances within 10 μm). Technical achievements: 1) micromade recess tools for the aeronautics fasteners industry, with improved wear resistance and service life; 2) micromade tools inserts for plastics injection molding, with graded wear resistance, thermal conductivity and cooling channels; 3) process conditions of Micromaking for laser sintering of nanophased powders.

Results

The activity is divided into different parts to develop the small scale sintering technology employing a fusion produced by the laser beam with the coaxial powder injection into the action zone. Another large part of the activity is the design of materials for some demonstrative applications and the development of the suitable powder.

The Micromaking machine (see *Fig.1*) has been assembled and some nanophased powders have been tested. One of the main objectives has been reached: starting from laser tracks (2 Dimensional) now it is possible to make objects 3D with a spatial resolution of 50 μm (see *Fig. 2-3-4*) using the Micromaking machine.

In order to obtain a powder best suited for Micromaking application the air-classifier (already designed and assembled) has been equipped with Jet-mill system (low energy milling): it permits the control of sizes distribution of particles (range 3-10 μm) and their shape (more rounded to obtain improvement of flowability).

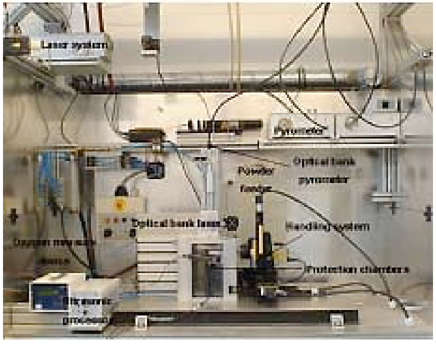


Fig.1 Micromaking machine prototype

Fig.2 Gear: SEM image

Fig.3 Gear: SEM image

Fig.4 Frame: SEM image

2C - “In vivo” NMR studies of cell metabolism

Maso Ricci, Alessandro Donati, Claudia Bonechi, Silvia Martini,
Fiora Bartolini, Claudio Rossi

Aims

Characterisation of ^{13}C enriched substrates metabolism in prokaryotic and eukariotic cells.

Development of a mathematical model for the cellular metabolism.

Analysis of metabolism response to exogenous stimuli.

Results

The kinetic behaviour of complex organism, like cells culture, are often difficult to analyse. Carbon-13 NMR spectroscopy together with selective 13-C sugar substrate enrichments were used to study bacteria and yeast metabolization processes

In vivo NMR techniques and substrates selectively enriched with ^{13}C were used to follow the step-by-step metabolism of sugar to elucidate the metabolic pathway and to compare the metabolic behaviour of different organism.

For the interpretation of experimental results we developed a compartmental model alternative to classical modelling approach.

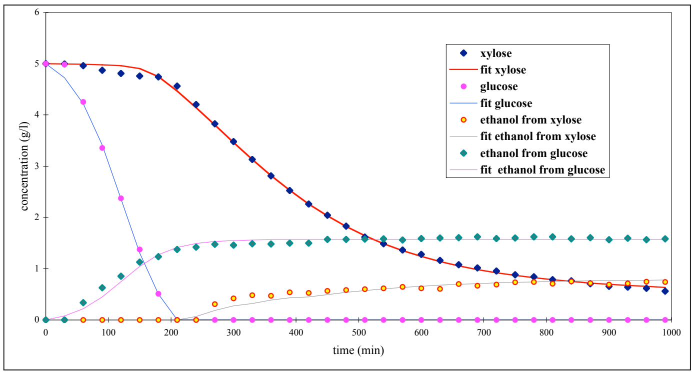
The compartimental model considers the cellular metabolic reactions resulting from interactions between glucose and active cells and between active cells and the end-products (feed back action)

The kinetics constant within the model have a precise biochemical meaning of great utility to compare the efficiency of a metabolic process in different species or to identify biological functions.

The models tested on different cell cultures were flexible enough to fit specific metabolic activities.

Several studies were carried out with different cells and different enriched substrates.

Our studies pointed out the potential of this methodology for biochemical and biophysical interpretation of metabolic process in cells.



We analysed the bacteria (*Klebsiella planticola*) metabolism in presence of a mixture of two substrates (glucose and xylose). Cell metabolism was described as an overlap of two kinetic process: glucose fermentation, which depends on the number of active cells and xylose fermentation, an autocatalitic related to the number of active cells and to xylose concentration. In this model xylose and glucose fermentation are assumed to be independent and the model highlights two different metabolic pathways for the fermentation of glucose and xylose. Both the ethanol productions from xylose and glucose can be followed simultaneously due to the different enrichment position of ^{13}C in the substrates. The model took in account the inhibition due to the total ethanol concentration present in the batch.

The metabolism of yeast *Saccharomyces cerevisiae* was also investigated using the compartmental model. Two samples at different sugar concentration were analysed using in vivo ^{13}C-NMR spectroscopy.

In this study glucose consumption is described as the overlap of two kinetics processes. An autocatalytic one and the other dependent only on the active cell concentration. Inhibition of the ability of yeast to convert glucose, due to the presence of ethanol is proportional to the ethanol concentration which acts as controller (feed back action). It is evident that the autocatalytic route is predominant at the beginning of fermentation. The part that is proportional to the number of the cells becomes dominant towards the end, meaning that part of the glucose is consumed as a “required minimum”.

The comparison of our modelling approach with other classical kinetics dynamics highlighted the potential of our methodology. In fact, other modelling approach like Michaelis Menten dynamics gives information on the speed of the reaction and the rate of degradation of substrate, which is insufficient when we need information about interactions between cells, substrates and products. We have extended this approach to the investigation of eukaryotic cells control mechanisms.

The behaviour of complex systems, as eukaryotic cells in response to external stimuli, can only be completely understood by considering the complex network of interactions that take place within the system.

Saccharomyces cerevisiae is an important model to understand the eukaryotic responses to environmental stresses.

In this project, the effects of increasing concentrations of ethanol on Saccharomyces cerevisiae glucose degradation have been investigated with the approach based on the combined use of in vivo 13C-NMR spectroscopy and mathematical modelling.

This approach provides chemical and biological information indicating the strategies used by Saccharomyces cerevisiae in response to exogenous stress.

The simplifications offered by the modelling approach allows to consider the system as composed by elements that interact with each other exchanging energy and matter. Non-linear equations were developed in order to describe the dynamics of glucose consumption, ethanol production, and cellular activity behaviour.

The cellular activity (figure above) , behaves as the result of two contributions: at the beginning of fermentation the high glucose concentration induces the increase of cellular activity, on the other hand an opposite effect is induced by the produced ethanol which inhibits the fermentation process causing a decrease in the cellular activity.

Despite of the few metabolites included, the model describes correctly the dynamical trends of glucose degradation and ethanol production and allowed to evaluate the “cellular activity” trend, whose value is not correlated only to the biomass concentration but it depends to the initial exogenous ethanol concentration and to the ethanol stress during the fermentative process. The robustness and flexibility of the model enables it to work correctly at different initial exogenous ethanol concentrations.

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2C - Phase transitions and structure-properties in organic compounds

V. Massarotti, D. Capsoni, M. Bini et al.

Aims

Investigation of solid state properties of a tetrahydro hydrochloride naftalene derivative (CHF1035), a new drug for the treatment of hearth failure

Study of the racemic compound CHF1035 with binary phase diagram with one enantiomer

Characterization of polymorphism by spectral methods: Raman and ^{13}C NMR in solution and solid state

To put into evidence the different high temperature polymorphs by powder X-ray diffractometry and thermal methods

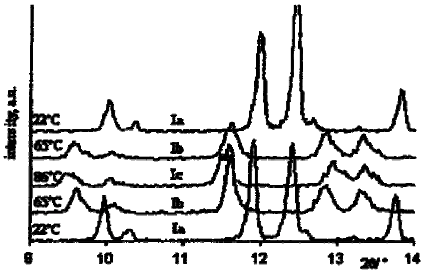
Results

The polymorphism of CHF1035 is investigated. Three different crystal forms (I, II and III) were obtained by recrystallization procedures from common organic solvents. The polymorphs have been characterized by Raman and ^{13}C NMR spectroscopy, in solution and solid state (cross polarization-MAS), powder X-ray diffractometry and thermal methods (DSC, hot stage microscopy and TGA). The diffraction patterns of Form I collected at controlled temperatures give evidence of the presence of two reversible structural rearrangements at $60<T<75$°C.

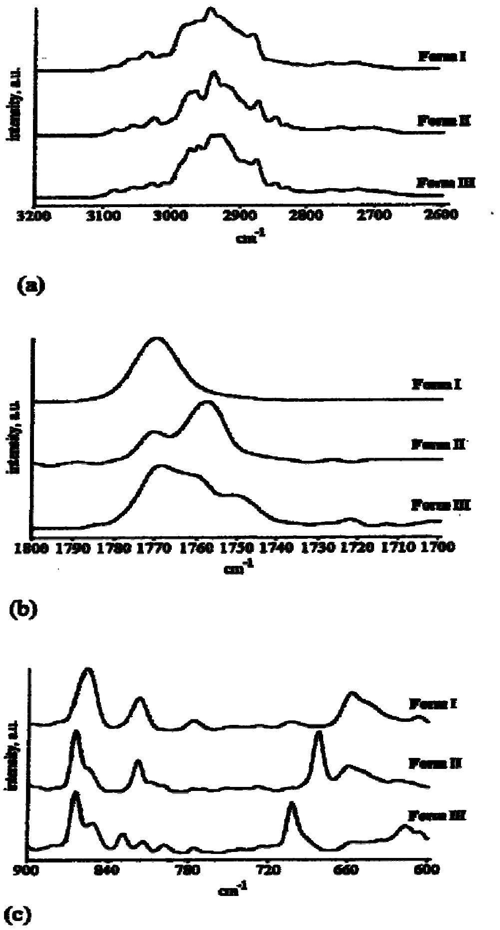
The structural variations are confirmed by DSC and hot-stage spectroscopy technique.

The analysis of the Raman spectra allows the identification of peculiar absorption bands for each polymorph. Form III is the stable crystal form at room temperature.

The polymorphism of paracetamol was investigated. Two metastable polymorphs (II and III)were obtained by appropriate thermal procedures from binary mixtures containing 10% (w/w) of hydroxypropylmethylcellulose and controlling the re-heating process it was possible to address the recrystallization into form II or III. Form III transforms either into form II or III depending on the preparatio method. The polymorphs have been characterized by micro-Fourier transform infrared spectroscopy (MFTIR) and powder X-ray diffractometry, both temperature controlled.



Powder X-ray diffraction patterns of CHF 1035 Form I: details of the 9–14°2θ range collected at RT, 65°C, and 86°C during the heating/cooling cycle (RT → 86°C → RT).



Details of Raman spectra of CHF 1035 polymorphs: (a) 3000–2600 cm^{-1} region; (b) 1800–1700 cm^{-1} region; and (c) 900–600 cm^{-1} region.

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2C - Physico-Chemical Properties of Pharmaceutical systems

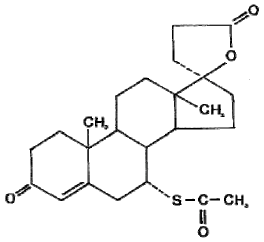
A. Marini, V. Berbenni, G. Bruni, P. Cofrancesco, A. Maggioni,
C. Margheritis, C. Sinistri

Aims

Polymorphism, pseudopolymorphism and stability of solid state drugs
Host-guest systems
Drug-excipient compatibility
Phase diagrams characterization
Thermal decomposition

Results

Spironolactone is a steroidal diuretic which can be obtained in four different solid forms [1]. An investigation of "melting" in spironolactone under different experimental conditions [16], demonstrates that the melting is always accompanied by loss of mass, and by an endothermic and temperature-activated "decomposition" process.



Spironolactone

However, experiments performed under different atmospheres reveal a more complex phenomenology than expected for a simple melting/decomposition process. It is shown that the byproducts of decomposition may undergo an exothermic process of "oxidation". The "oxidant" may be the oxygen of the atmosphere or may be provided by decomposition. By combining a number of experiments, the melting enthalpy, the specific enthalpy of decomposition and that of oxidation have been estimated. It is shown that an "adiabatic" balance between decomposition and oxidation is eventually attained. The concept of "adiabatic balance" provides simple explanations for several experimental observations.

Dipyridamole

A physico-chemical characterization of dipyridamole ($C_{24}H_{40}N_8O_4$), a widely used anti-aggregating agent, has been performed by using a combination of thermoanalytical (DSC) and spectroscopic (XRPD and FT-IR/PAS) techniques [27]. A solid state transition, already reported in literature, has been ascribed to the breaking of an intramolecular H-bonds network.

The rupture of a network of intermolecular H-bonds is thought to accompany the fusion. The solid state transition has been shown to be reversible provided the sample has not undergone melting. Mechanical milling and thermal annealing have been shown to decrease melting temperature and enthalpy. The effect brought about by mechanical and thermal treatment on the solid state transition is different. In the milled samples the transition peak shifts towards lower temperatures and its enthalpy suggests that all intramolecular H-bonds have been transformed into intermolecular H-bonds.

GV118819X (Fig 1), a novel tricyclic b-lactam antibiotic of GlaxoWellcome, is a racemic mixture of two diastereoisomers, **A** and **B** [12] **.** Of the two diastereoisomers, only **A** is available as a pure compound. By analyzing mixtures of GV118819X and **A** (Fig 2)**,** a partial phase diagram is constructed, which indicates the presence of a eutectic when the **A** fraction is ~39%. Moreover, the melting enthalpies of the eutectic mixture and of diastereoisomer **B** can be estimated. With the exception of the pure **A** form, all mixtures undergo important modifications in morphology and microstructure as a consequence of thermal treatments, which induce melting- amorphization of the eutectic, and crystallization of the **A** form. Analyses of the sieved fractions of GV118819X demonstrate that it consists of acicular crystals of different composition, with the larger crystals having a larger **A** fraction than the smaller ones. Grinding causes melting/amorphization of the eutectic and, following hours-long treatments, the formation of a substantial fraction of submicron particles with unusually low melting temperatures.

Fig 1 SEM pictures of GV118819X as received (a) and after cooling from melt (b).

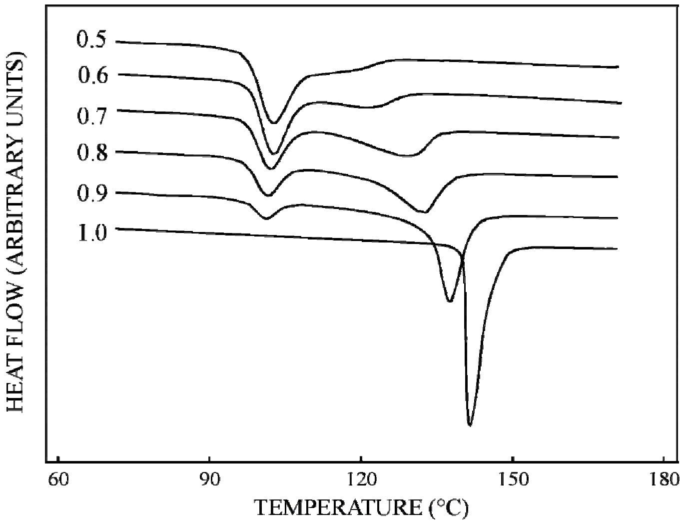


Fig. 2 DSC traces of mixtures of GV118819X and diastereoisomer A. The numbers on each curve represent the total molar fraction of A in the mixture.

Thermoanalytical (differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), thermogravimetric analysis coupled with Fourier transform infrared spectroscopy (TG/FTIR)) and spectroscopic (X-ray diffraction (XRD), ultraviolet–visible (UV–Vis), mass spectrometry (MS) and Fourier transform infrared diffuse reflectance (DRIFT)) measurements have been used to characterise solid-state retinoic acid (RA) from a chemico-physical point of view [18].

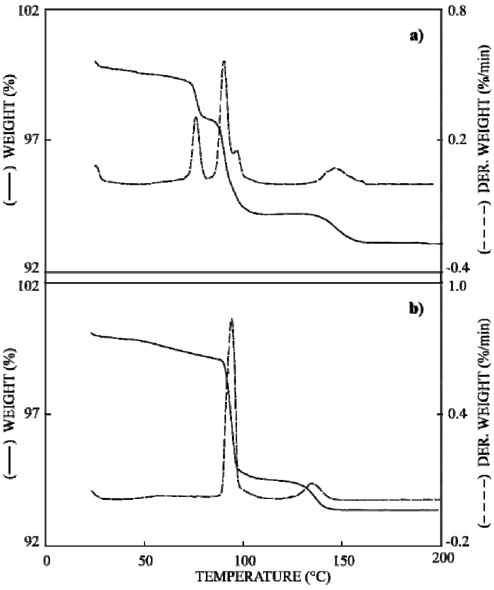
Between 130 and 160°C, a phase transition takes place that does not correspond to the transition between the known monoclinic and triclinic phases (DSC and XRD evidences).

By annealing in air (in the 130–160°C temperature range and for different times), an exothermic oxidative degradation occurs that, depending on the thermal treatment, competes with the mentioned phase transition (TGA evidence). Spectroscopic techniques (UV–Vis, MS and DRIFT) allow one to conclude that the new solid phase is still constituted by retinoic acid with a different orientation of the side chain (Fig. 3). Finally, RA does not undergo stable melting: the fragmentation patterns, both in air and in nitrogen, have been examined by TG/FTIR.

Fig 3 Diffuse Reflectance IR spectra of : (a) a commercial sample of all-trans retinoic acid; (b) same sample after thermal treatment (66 h at 130 °C in nitrogen).

GV150526A is a new drug recently developed by GlaxoWellcome. It belongs to a novel group of 2-carboxyindole derivatives and is therapeutically effective in the treatment or prevention of CNS disorders resulting from neurotoxic damage. A deep physicochemical characterization of the three solid forms (hydrates) of GV150526A has been carried out mainly by thermal methods (Fig. 4) [17].

It is shown that: (1) the different forms can be qualitatively and quantitatively distinguished by their thermal properties; (2) dehydration is followed or accompanied by a structural relaxation of the substrate that takes place at different rates and with different enthalpies in the three forms; (3) two of the solid forms can be converted into a phase that is thermodynamically stable at room temperature by partial dehydration/rehydration in a


102
97
92
0.8
0.2
-0.4
a)
(——) WEIGHT (%)
(– – – –) DER. WEIGHT (%/min)
102
97
92
1.0
0.4
-0.2
b)
(——) WEIGHT (%)
(– – – –) DER. WEIGHT (%/min)
0
50
100
150
200
TEMPERATURE (°C)

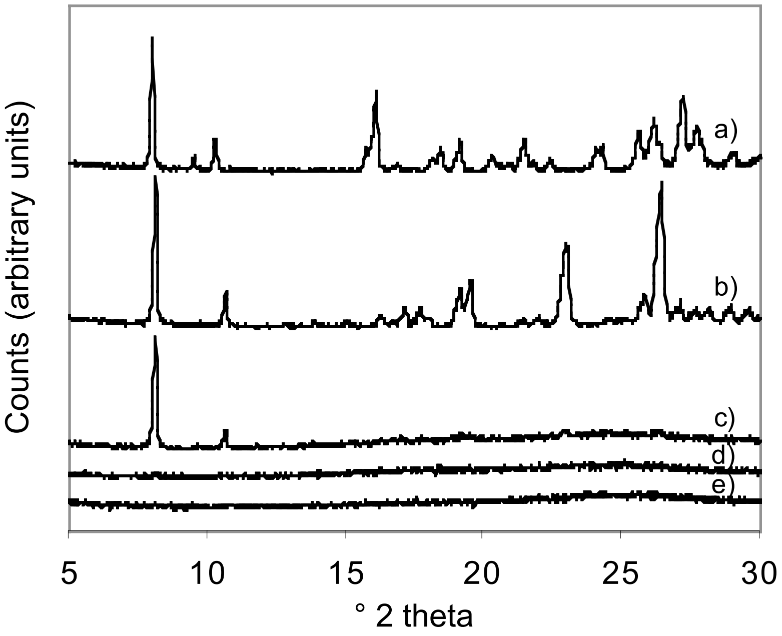


Fig. 6 – Evolution of X Ray patterns of BBR2778 during heating-cooling cicle: a) original sample at 27 °C; b) 120 °C; c) 190 °C; d) 190 °C after two hours annealing; e) room temperature after cooling.

A systematic study has been undertaken and applied in several papers, to find and optimize a general method of studying the drug-excipient interactions, with the aim of predicting rapidly and inexpensively the long term stability of their mixtures. The general purpose is to develop a protocol of physicochemical characterisation which reliably predicts the outcome of standard stability/compatibility tests. It envisions analysis of thermal, structural, morphological and spectroscopic data collected under a range of experimental conditions and treatments in the drug, the excipient, and their binary solid mixtures. For each treatment, the data for a mixture are compared with the "superposition" of the responses of pure drug and pure excipient, which is expected under the "no interaction hypothesis". The outcome may be qualitative (existence of a significant difference or not) or may be quantified with numerical values, such as the change (in %) of the enthalpy of melting relative to the expectation. Up to now, our method of studying the drug-excipient interactions has been applied to several drugs and some commonly used excipients [28, 44, 45]. Both qualitative and quantitative interaction indicators have been identified. Moreover, our results show that simultaneous thermal analysis (TG/DSC) is the best suited technique in the search of interaction indicators (Figs. 7-11).

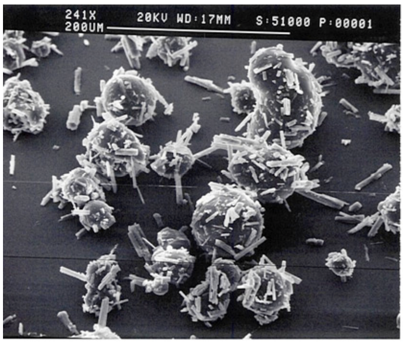


Fig. 7 Left: SEM micrograph of a GW118819X:MgST=20:80 mixture stored 30 min at R.H.>90%. Right: SEM micrographs of a GW118819X:PVP=20:80 mixture stored 30 min at R.H.>90

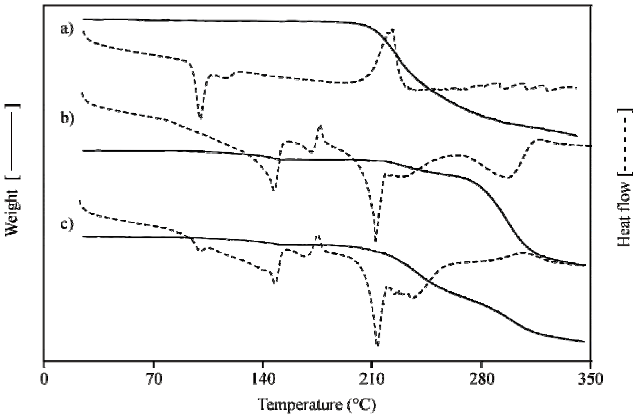


Fig. 8 TG-DSC traces recorded in dry nitrogen at 5 K/min on a) GW118819X, b) MgST and c) physical mixture 20:80 (w:w) of the two components.

Fig. 9 SEM pictures of Indomethacin:PVP (80:20) mixture untreated (left) and moisture treated (right).

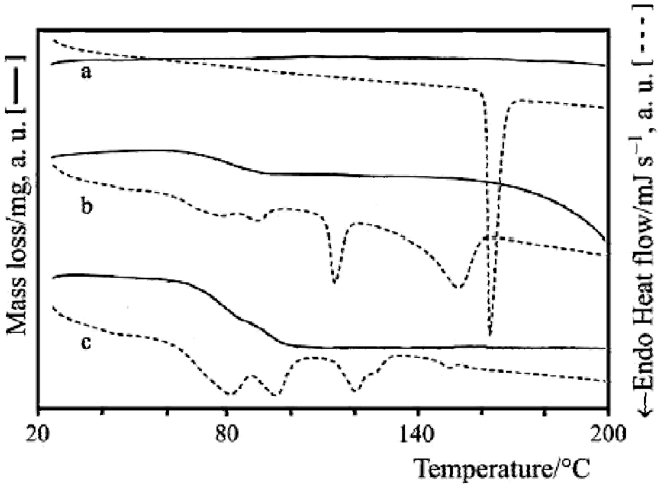


Fig. 10 Comparison of TG-DSC traces of b – Indomethacin:MgST (80:20) mixture with those of a – pure indomethacin and c – pure MgST.

Fig. 11 Indomethacin

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2C - Production of Recombinant Human Monoclonal Antibodies for Diagnostic and Therapeutic Use

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Aims

Production and testing of recombinant monoclonal antibodies against viral or microbial diseases

New antibodies for diagnostic use

New antibodies for infectious disease therapy

Results

Monoclonal antibodies have revealed a great potentiality in both diagnostic and therapeutic purposes. The conventional monoclonal antibodies are produced in murine hybridomas and thus are not always suitable for human therapeutic use. Recently a new technology for the production of human recombinant monoclonal antibodies has been realized (Williamson RA et al. 1997. Use of recombinant human antibody fragmens by Phage Display Technology. Ann. Rev. immunol. 17: 433-55). This technology allows the production of Fab antibody fragments in *E. coli* or other microorganims, with some evident advantages in terms of productivity and specificity of antibodies.

Antibodies have been already produced against Influenza A virus. They recognize a viral antigen common to most influenza viruses, probably the M matrix protein and are suitable for preparing specific diagnostic kits. Other antibodies which are in preparation are those against HHV8, a herpesvirus involved in the Kaposi's sarcoma, and against Parvovirus B19, which is responsible of some general human infectious diseases.

Other antibodies are worthy to be produced against other viral or bacterial diseases.

HHV8, the same virus mentioned above, produces a protein, named *kaposin*, which is necessary for the induction of the cell transformation. It is one of the goals of this project the production of monoclonal antibodies against this protein, with the aim of blocking the transforming activity of HHV8. Other viruses which could be candidates for the production of recombinant monoclonal antibodies are the Blue tongue virus, which infects sheeps and cows, and the African swine pest virus, which is responsible of a severe disease of pigs.

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2C - Formulations based on fully biocompatible surfactant systems for food, pharmaceutical and cosmetic applications

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Aims

Biocompatible and stable formulations
Structural characterizations
Dynamic aspects

Results

Drug delivery systems based on liquid crystalline matrices have been developed in the recent years. The liquid crystals, generally of cubic or hexagonal space group, are formed by naturally occurring lipids such as monoglycerides or polyglycerides and phospholipids. Particularly Glycerol monooleate-based aqueous systems were considered. The aim was the preparation of high-performance biocompatible formulations which might solubilize a large variety of additives. The characterisation of the phase behavior and of the microstructural transitions occurring in multicomponent systems was performed through optical microscopy, NMR and SAXS techniques. Thermal stability of the monophasic regions and microstructural transitions vs. time were investigated, particularly when the volume fraction of the dispersed phase increases. Acyl migration and hydrolysis of the monoglyceride are the most significant phenomena, whose rate increases with increasing temperature and water content. Acyl migration in few months reaches the thermodynamic equilibrium that is G1MO:G2MO = 9:1 approximately. Hydrolysis tends to progress with storage time and often the various GMO liquid crystalline phases (lamellar and cubic) undergo a transition to a reverse hexagonal phase within 6-8 months of storage due to the formation of at least 10-12 wt% of oleic acid.

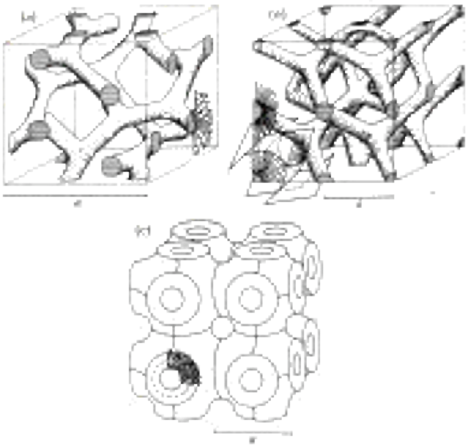

Fig. 1 Bicontinuous cubic phases

Some additives (for instance sodium decanoate) act sinergically with oleic acid thus the reverse hexagonal phase forms within 1-2 month from preparation of the sample. Other additives such as diglycerol monooleate - a lamellar phase forming surfactant - counteract the effect due to hydrolysis products and cubic or lamellar microstructures can be retained for years. Interestingly, GMO based systems can protect for years easily oxidazing substances such as retinol.

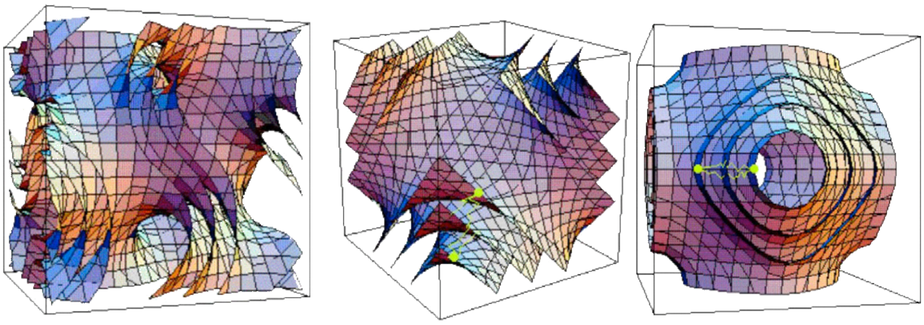

Fig. 2 Mathematical surfaces (IPMS) of bicontinuous cubic phases

Cubic and hexagonal liquid crystalline phases of GMO can be dispersed in nanoparticles which display mostly cubic (cubosomes) and hexagonal (hexosomes) shape respectively as demonstrated by SAXS and also AFM techniques. The nanoparicles can be stabilised in aqueous solution for several months through addition of a triblock non ionic copolymer (polaxamer 407) which, besides the stabilizing action, prevents hydrolysis phenomena of the monoglycerides as shown by NMR spectra.

^{13}C NMR relaxation experiments performed on reverse micellar solutions and various liquid crystalline phases of GMO/water, GMO/water/additive and also on cubosomes and hexosomes showed that the monoolein hydrophobic skeleton is scarsely affected by the nature of the system. The local arrangement and the dynamics are retained in all systems. The significant variations of the relaxation times observed for the polar head were easily related to the interfacial curvature which is zero for lamellar and bicontinuous cubic phases and becomes negative for reverse micellar and hexagonal phases.

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2C - Ovine milk: composition, dairy processing and quality controls, fractionation as a source of raw materials, no food products, and dairy wastes

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Aims

Methodologies for analytical and nutritional certification
Methodologies for selective fractionations
Extraction of valuable by-products from dairy wastes
Selection of autochthonous lactic acid bacteria to be used as starters for ewe's cheeses

Results

A project concerning the characterisation of the ovine milk as a function of the laction stage, the influence of feeding, the dairy processing and the nature of the various wastes, is still in progress. Among the results, all the analytical procedures have been tested and new processing treatments, based on the use of CO_2 in supercritical conditions, have been employed to modify the lipid fraction of the milk.

In parallel, catalytic transesterification reactions, based on the use of immobilised lipases, to produce wax esters and biosurfactants from a milk cream fraction have been tested (see project 2C.CA1). Furthermore production of lysozyme-enriched biomass from cheese industry by-products and re-use of cheese whey to grow engineered yeasts were asceruained.

Methodologies to extract phospholipids from the cheese processing waste and an emulsifying powder from the ricotta processing waste are in progress.

In addition, methodologies to evaluate microbial activity in foods have been also investigated.

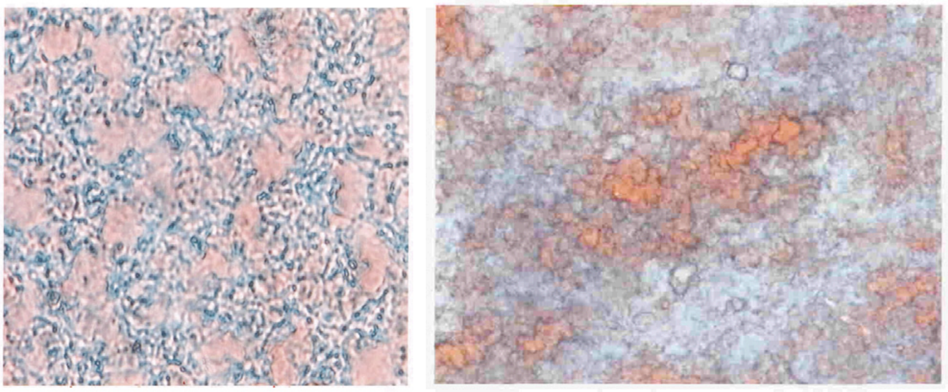

Fig. 1 Optical microscopy of cheese samples

As a part of a project concerning the valorisation of typical Sardinian ewe's cheeses, several strains of lactic acid bacteria and yeasts have been isolated, identified and screened for their technological properties. These strains are now being tested to evaluate their potential as autochthonous starters.

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2C - Biocatalysis through immobilised lipases

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Aims

Immobilisition procedures
Characterization of enzymes activity
Biocompatible processes to obtain no food products
Biosurfactants and Biodiesel

Results

Sucroesters represent a relatively new class of surfactants showing high performances and biocompatibility. The problem is related to traditional synthesis involving organic solvents. New types of synthesis, based on lipase enzymatic catalysis were tested. On the basis of promising results previously obtained to produce wax esters the commercial immobilized lipases (Candida Anthartica, Candida rugosa and Mucor miehei) will be investigated . The effect of loading and enzymatic activity will be investigated in the range of temperature 40-60°C. Optimum conditions of temperature and water activity to produce sucroesters should be ascertained.

Such system has been successfully used to produce selected glycerides.

In collaboration with a Swedish research group of the Chemical Centre of Lund, a small SCF plant will be arranged to test immobilized lipases (Candida Anthartica, Candida rugosa and Mucor miehei) for the sysnthesis of sucroesters based on glucose and galactopyranose together with linear hydrocarbon C12-C18 chains.

The new molecules were characterized for the physico-chemical properties and for the molecular structures by NMR technique.

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2C - Bioconversions in emulsion systems

A. Ceglie, L. Ambrosone, G. Palazzo, G. Colafemmina, G. Cinelli, A. Hockhoeppler, A. Stefan

Aims

Studies on the correlation between the supramolecolar structure and the reactivity induced by biological guest

Results

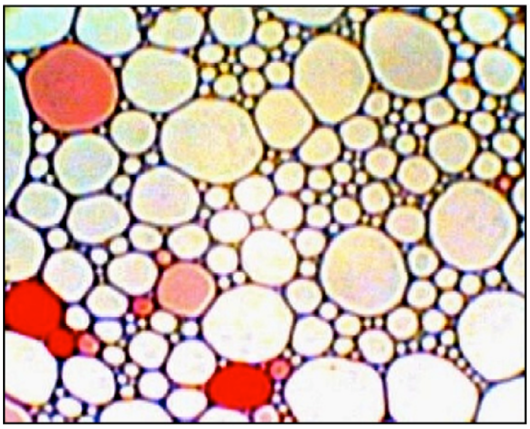


Fig. 1 A SDS/Toluene/Water oil-in-water concentrate emulsion absorbing Sudan IV dye from a toluene mixture in contact over the same slide (obtained by VEM technique).

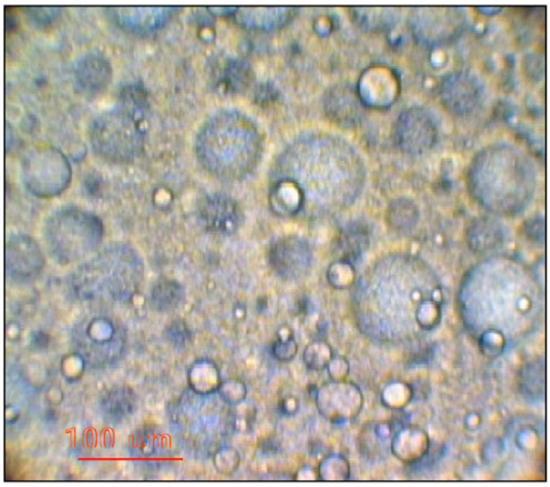
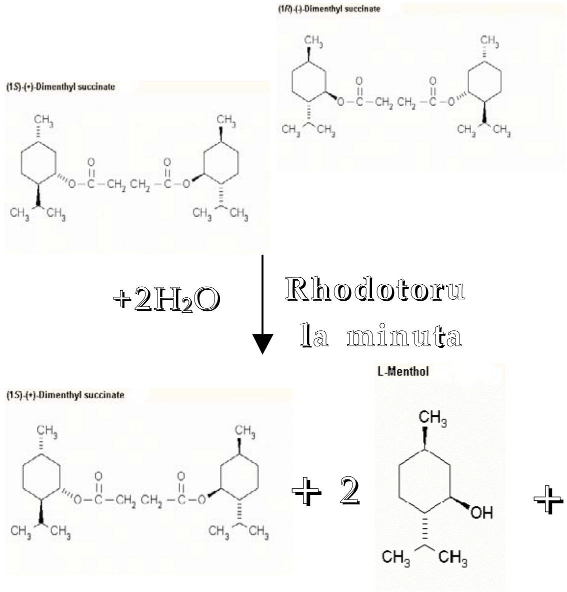


Fig. 2 Water-in-oil emulsion systems sustain viability of a eukaryotic cell. The droplets are stabilized by a Lα phase. When water-insoluble compounds are involved they are solubilized in organic bulk but the diffusion between the interphase allows microorganism growth in the dispersed phase. Microphotograph was obtained by VEM technique.

Fig. 3 The yeast Rhodotorula minuta var texensis grows in YPD medium and the population is composed of single cells and diads (on the right). Upon transfer of this yeast in w/o macroemulsions, cells aggregation occurs (on the left). Microphotographs were obtained by SEM technique



The yeast catalyzes the enantiomeric hydrolysis of (1R)-(-)-Dimenthyl succinate forming a water-insoluble

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2C - Organogels in technological processes

A. Ceglie, F. Lopez, L. Ambrosone, M. Orsini, F. Venditti

Aims

Preparation of organogel based on microemulsion containing a positively charged surfactant

Phase diagram characterization

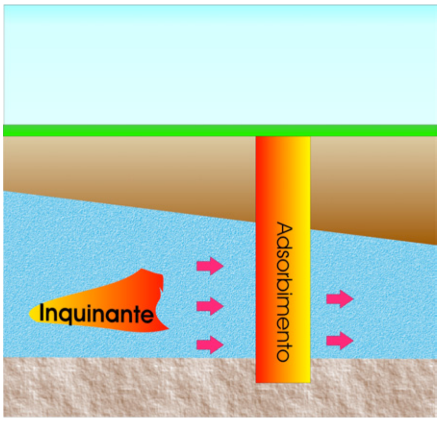
Improvement of the ability of these systems for technological applications

Results

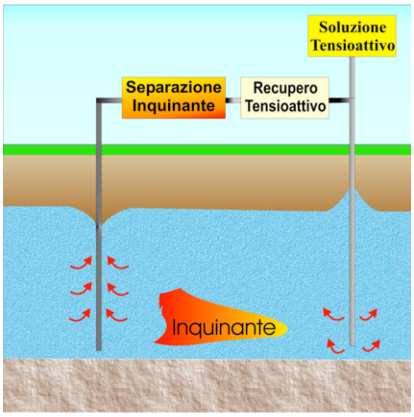
Organogels are highly viscous gels, they originate from microemulsions containing gelatine. Inside this gels, different kinds of biomolecules or dyes may be entrapped. In our lab these gels containing different enzymes are used for the removal of aromatic compounds from water and for synthesis of esters.

Organogel: applications

- Reactions in organic solvents
  (synthesis of esters)



- Reactions in aqueous solution
  (degradation of phenolic compounds)



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2C - Enzyme activity in microemulsions

A. Ceglie, F. Lopez, L. Ambrosone, G. Palazzo, G. Colafemmina, G. Cinelli, A. Hochkoeppler

Aims

Study of the enzymatic activity of lipase at different values of W_0 and P_0 should give insight on the parameters governing the lipase activity on membrane mimetic systems.

Relationship between the enzymatic activity and the composition of the interface.

Results

The enzymatic activity of Lipase VII from Candida rugosa was studied in the quaternary water-in-oil microemulsion CTAB/water/pentanol/hexane. The enzyme-catalyzed hydrolysis of p-nitrophenylbutirate was found essentially independent of the water/surfactant ratio. On the other hand, the enzyme kinetics was strongly affected by the cosurfactant/surfactant ratio.

The turnover number is unaffected by the reverse micelles size and from the interfacial bromide concentration while is strongly dependent on the interfacial composition.

Fig. 1 Initial rate, v_0, for the (lipase catalysed) p-NPB hydrolysis as a function of the overall p-NPB concentration in microemulsions at different P_0

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2C - Structure and stability in food emulsions

A. Ceglie, L. Ambrosone, G. Palazzo, G. Colafemmina, G. Cinelli

Aims

Structure determination in food emulsions via NMR and VEM techniques
Emulsification processes and oil an fat oxidation
Demulsification kinetics and stability of food emulsions

Results

Our approach in analysing PSGE-NMR data (see section NMR data analysis) was firstly applied to emulsion as alternative to previously proposed treatment to obtain the size distribution of water dispersed phase.

Fig. 1 In the figure size distribution of dispersed phase of normal margarine: (a) our method to calculate size distribution by NMR data, (b) traditional method.

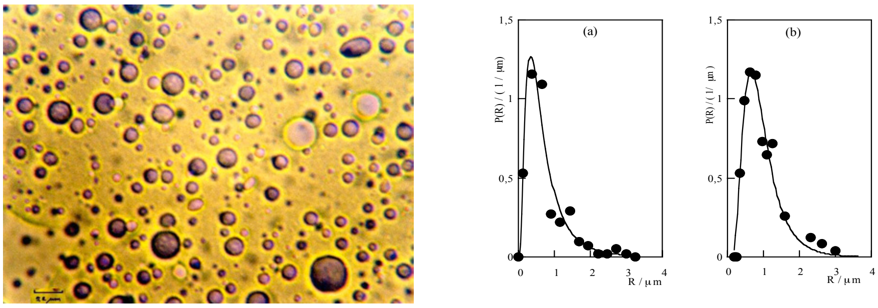

Fig. 2 Results obtained on low cholesterol margarine. Size distribution from VEM (left) and from NMR data (right).

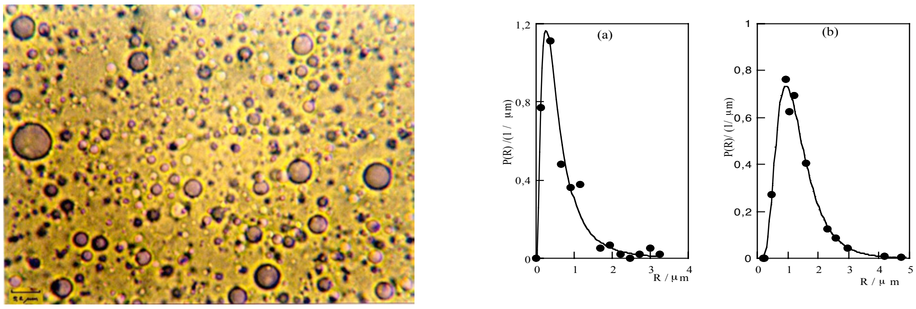

Fig. 3 Results obtained on salted butter. Size distribution from VEM (left) and from NMR data (right).

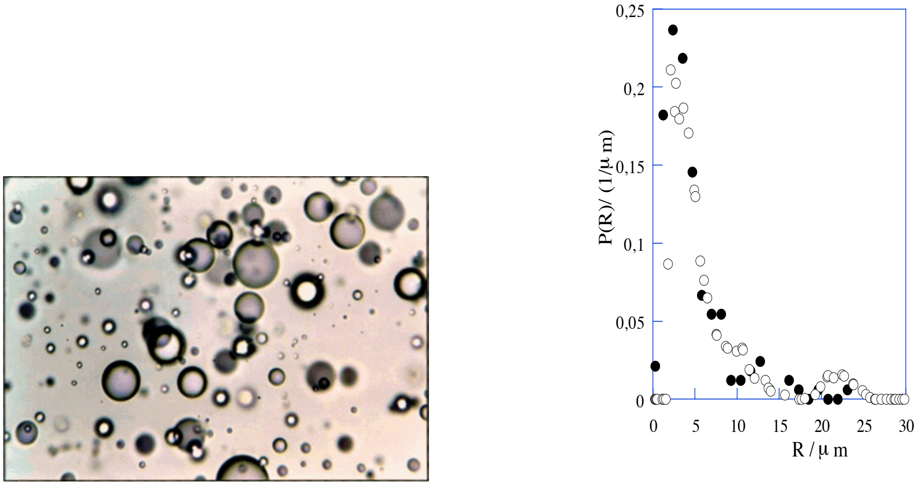

Fig. 4 Results obtained on Water in extra-virgin olive oil analysed by NMR technique (empty circles) and via VEM (filled circles).

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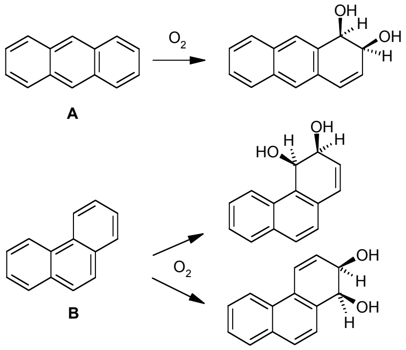
2C - Nanostructured Media for Bacterial Production of Fine-Chemicals

Debora Berti, Silvia Milani and Piero Baglioni, in collaboration with F. Briganti, A. Scozzafava, Dept. of Chemistry, University of Florence

Aims

The purpose of the present study is the optimization of the whole-cell production of cis-dihydroxy-dihydro compounds from other less water soluble polycyclic aromatic hydrocarbons: anthracene (A in scheme 1) and phenanthrene (B in scheme 1). Such substances are converted by the naphthalene dioxygenase employed in the present study, but the biotransformation resulted in very low final yields.



Scheme 1

The production of large quantities of such derivatives will allow the design and synthesis of optically pure new compounds of potential industrial and pharmaceutical interest.

The particular aim of this project is the desing of a nanostructured medium where whole-cell bioconversion of polycyclic aromatic hydrocarbons for the production of fine chemicals can be performed and optimized with respect to traditional aqueous media.

Results

Industrial chemical synthesis can greatly benefit of the capabilities of enzymatic systems in terms of more gentle reaction conditions, reduced environmental pollution, and reduced costs. For oxidation reactions, the use of suitable enzymatic systems allows replacing strong oxidants with the plain oxygen molecule under mild conditions. Our interest is focused on dioxygenases which catalyze the regio- and stereo-selective dihydroxylation of a large variety of hydrocarbons, using whole cell engineered microorganisms have been developed.

Living systems accomplish high levels of selectivity and efficiency using compartmentalization. This is often realized through self assembly of amphiphatic units similar to those of synthetic surfactants which form micellar systems above the critical micellar concentration (C.M.C.). Direct micelles are able to solubilize amounts of apolar compounds in their interior. This nanophases can be seen as chemical microreactors, where hydrophobic substances stored in the apolar core can diffuse to the hydrophilic layer to take part to chemical reactions in the aqueous medium.

Kinetic bioconversion of PAH's have been performed in different o/w microemulsion media, varying surfactant type (Tween, Triton), oil nature (IPP, methyloleate, ethyloleate, glycerol trioleate) and substrate. Solubilization of PAH inside the apolar core of these nanocompartments can lead to a bulk "pseudoconcentration" three orders of magnitude higher than that in aqueous media. For a given substrate the experimental evidences that we have collected so far showed no dependence on micellar microstructure (oil content, surfactant type, etc.) whatsoever. We have interpreted this result as due to the fact that substrate uptake by bacteria was not the rate-determining step meaning that the bioavailability of the substrate was always optimal for that bacteria number density.

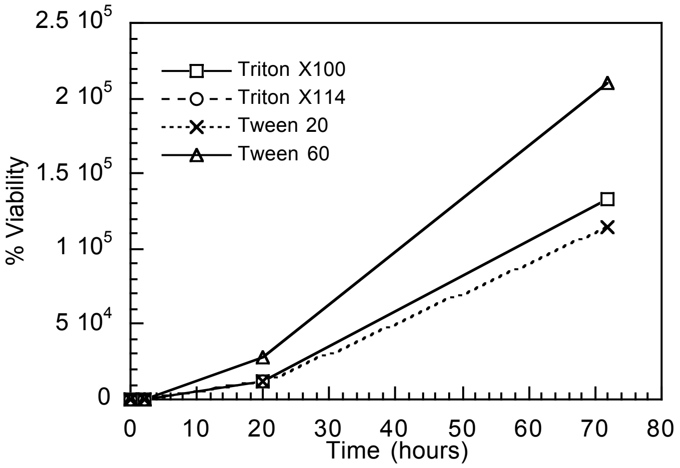


Figure1. Viability of bacterial cells suspended in some selected direct micellar solutions

Bacterial cells maintain their viability and catalytic efficiency when suspended in the different surfactant solutions employed in this study and moreover they retain viability and catalytic activity once removed from the reaction environment and resuspended again in a fresh micellar medium; this feature is rather unexpected and opens the way to scaling up procedures that take into account the reusal of cells, while in plain aqueous media cells are poisoned presumably by reaction products.

Our main achievements in this field demonstrate that the utilization of micellar phases in microbial bioconversions is milder than vigorous mixing of multiphasic media, and the effective improvement of conversion kinetics has been investigated into details for naphthalene. In such a case complete substrate conversion yielding grams of pure product per liter of culture was observed while maintaining high cell viabilities.

Figure2. Nanoenginereed bioconversion of Naphthalene in Tween60 micellar medium compared to what obtainable in aqueous solution with excess solid substrate under vigorous stirring conditions

The latest developments of this project concern the realization and investigation of experimental conditions where some control on reaction kinetics can be exerted by proper tailoring of the micellar microstructure controlled substrate release conditions can have potential use in future application of bacterial conversion, on the other size some useful insight on the hypothesized reaction scheme can be gained.

The mechanism hypothesized consists of different steps: essentially bacteria and micelles are unperturbed by each other's presence, and their only connection is the exchange of aromatic substrate. The upload of substrate occurs from the aqueous medium and the overall conversion rates depend on the comparison of PAH's clearance operated by bacteria with respect to the supply rate to water solution operated by micellar nanocompartments.

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2A Art restoration, Enviromental Remediation, Textile Chemistry

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