EUROPEAN CHITIN SOCIETY

NEWSLETTER

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August, 2008
No. 25

EDITORIAL

This year is a somewhat fallow year for chitin enthusiasts as following the postponement of the Asia-Pacific Symposium that was scheduled to be held in Yangon, Myanmar in May there are no international chitin conferences this year, although there are a number of national meetings. This gives us all a breathing space to scrap together enough funding to attend at least one of next year's major conferences: EUCHIS '09, which is the 9th international meeting of the European Chitin Society and will be held in Venice in May, and the 11th ICCC meeting to be held in Taiwan in September. The postponed APS meeting will now be combined with the ICCC meeting. So start your personal conference saving fund and plan to attend these two conferences next year. Details of both of them are available on our Society's website and there is also further information on EUCHIS '09 in this edition of the *Newsletter*.

Remember that there is financial assistance in the form of travel grants available to research students to help their attendance at EUCHIS conferences. Full details, including how to apply, are available on our website www.euchis.org.

The ECS is in a reasonably healthy state, both as regards finances and membership. However one area where there is considerable scope for improvement is in items from members for printing in the *Newsletter*. This is something that has been raised frequently in editorials over many years but has resulted in a near zero response each time. There must be many members out there who have something to say on various issues relating to the Society and I would ask you to take the time to e-mail your ideas and suggestions. For example, 2011 will be the 200th anniversary of the discovery of chitin by Braconnot; should the Society celebrate this bicentenary and if so, what form should the celebrations take? Please e-mail any suggestions you might have – 2011 is not really that far away.

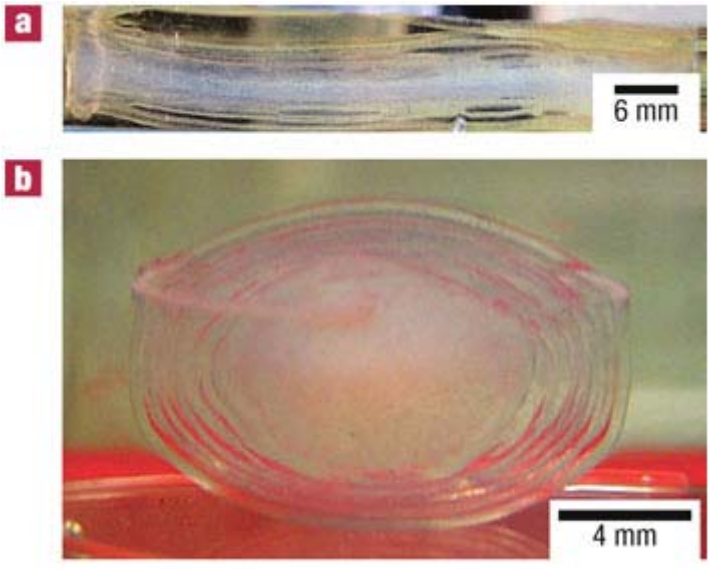
George Roberts

Honorary Secretary

New Chitosan Gels

The last winner of the Braconnot prize, awarded at the 8th EUCHIS meeting in 2007, was Sebastien Ladet (see the summary of his PhD-thesis in EUCHIS Newsletter No. 23 and the Braconnot Prize 2007 article in EUCHIS Newsletter No. 24). Based on results from this thesis, a letter entitled 'Multi-membrane hydrogels' was recently published in Nature (Ladet, S. David, L. & Domard A. (2008) *Nature* **452**, 76-79).

In this publication, Ladet *et al*. report how chitosan gels can be prepared to make structures with specific geometries/shapes and with internal spaces that may be potentially useful in biomedical applications, such as introduction of cell culture for new systems for tissue engineering. A relatively high molecular weight chitosan with a degree of acetylation of 2.5% (F_A=0.025) was used in this study. The principles applied in the preparation of the new multi-layered ('onion-like') chitosan gels are based on the their previous studies of a chitosan alcohol gel (see *Biomaterials* (2005) **26**, 933-943 and *Biomacromolecules* (2005) **6**, 3227-3237).



Multi-membrane hydrogels from chitosan showing
a tubular structure (a) and an onion-like structure (b)

To achieve the layers in the chitosan gels two different kind of gels are prepared. One is a fully neutralized gel that shrinks and therefore forms a gel layer with a high chitosan concentration. In between these fully neutralized gel layers a less dense (lower chitosan concentration) alcohol gel is prepared. This is achieved by first preparing an alcohol chitosan gel that is placed in an alkali solution and where the concentration of NaOH was used to control the the properties of the gel layers. These two different types of chitosan gels and the understanding of the gelation mechanism and kinetics have been essential to successfully prepare the multi-membrane layers.

It is quite unusual to read publications related to chitosan in high impact journals such as Nature (see EUCHIS Newsletter No. 18 regarding published chitin/chitosan articles related to journals and impact factors). The authors should be congratulated for their contribution to an elevated quality of chitosan related research. It will indeed be interesting to follow reports on future biomedical applications of the new chitosan gels.

Kjell M. Vårum

Personal reflections on the 1st International Conference on Chitin/Chitosan

One of my strongest memories from the 1st ICCC is of sheer terror. It was my first-ever conference and I had just finished given my presentation and the session chairman asked if there were any questions. One of the audience started walking towards the microphone and although he took only a few seconds to get there it seemed an eternity and I could feel my pulse racing faster and faster and my mouth drying up despite gulping down a glass of water. When he began his question with "That was a very useful lecture…" I was so relieved I could have jumped down and hugged him. However, having been so tense and nervous I didn't take in his question and had to ask him to repeat it. Since then I have come to realise that it is worse having no questions at the end of a presentation.

The 1st ICCC held in Boston, Massachusetts, on April 11th-13th 1977, was the first major conference devoted entirely to chitin and chitosan. It was hosted by the MIT Sea Grant Program and the Massachusetts Science and Technology Foundation. The Sea Grant Program was funded by the National Oceanic and Atmospheric Administration, a part of the US Government, and one of its objectives was to find applications for chitin and chitosan so as to overcome the pollution being caused by the shellfish processing industry in various locations around the US coastline. Although the conference was hosted by MIT the Chairman was Professor RAA Muzzarelli of the University of Ancona, Italy. It was a seminal event and had a galvanising effect on research in the subject, leading to a rapid increase in the number of publications annually. In fact over the course of the subsequent twenty years annual publications increased by a factor of 10, and the number continues to rise each year. It also gave rise to several series of international conferences: the ICCC series that has been held every three years since the second one that was held in Japan in 1982; the biennial Asia-Pacific Symposia begun in 1994 in Malaysia; the biennial EUCHIS meetings begun in 1995 in Brest, France; and the now biennial Ibero-American meetings begun in Havana, Cuba in 1997. In addition a number of national Chitin Societies hold annual meetings. So nowadays there is no shortage of chitin/chitosan conferences to attend.

In some ways it was very similar to most of the chitin conferences today. The lectures covered a wide range of scientific disciplines but despite a similarly wide spread of interests among the participants there was a 'family' feeling to it all. This was helped by the fact that they all had at least one common link – the molecule itself – and that everyone, apart from a few locals from Boston, were staying in the conference hotel so it was easy to meet people for further discussions. One intriguing point of similarity was the attendance. There were about 230 attendees, which is not far off what you would expect at an ICCC meeting nowadays, despite the 10-fold plus increase in research activity as measured by publications and patents compared with 1977. Obviously conference attendance has not kept pace with research activity, a fact that has enabled our conferences to retain the friendly atmosphere over the years. Perhaps it is due to the large number of meetings that are run nowadays so we are spoiled for choice. This after all was the first international conference to be devoted to chitin since its discovery in 1811. Imagine one conference in your field in 166 years – it would be a bit like waiting to see Haley's comet but with double the time period between sightings – obviously you would

not miss the opportunity of a lifetime. This incidentally is the line of argument I used to persuade my institution, which was not accustomed to pay for staff to attend conferences, to pay for me to go to it. Indeed I think I was the first member of staff to be supported to attend a conference outside the UK.

There were also several differences. The first was that there were only 55 presentations. This meant that there was no need for parallel sessions, which to my mind was a good thing. I find that no matter how carefully you plan your moves between parallel sessions you always miss the start of one or two of them. There was no conference outing although the delegates were left to their own devices to wander around Boston one afternoon after a couple of lectures were cancelled because of the non-attendance of the authors. The majority of papers were either biological in nature, as chemists were only beginning to really study chitin, or practical and on topics such as sources of chitin, industrial production routes and analysis of production costs. The nationality split was also markedly different with only a dozen countries represented. As the host nation it is perhaps not surprising that the USA had by far the largest number of attendees, although this was not the case in 1991 when they hosted the 5th ICCC at Princeton. Japan was strongly represented also, and after that came, in alphabetical order, Belgium, Canada, Chile, India, Italy, Japan, Nigeria, South Africa and the UK, none with a large contingent. Surprisingly there were no delegates from France or Norway whose researchers have made such major contributions to our knowledge of chitin and chitosan since then. In fact the UK had the biggest contingent of any European country – *sic transit gloria*! Neither were there any delegates from Korea or Thailand, now both major centres of chitin research. Obviously from the nationality point of view the chitin family has expanded dramatically even if it does not show up in conference attendance numbers.

Some other personal memories from that conference:

- The sheer enthusiasm of a graduate student from Brown University who had travelled to Boston to attend one session solely to hear Professor Charles Jeuniaux give a 30 minute lecture. She could hardly believe that she was actually going to see him in person.
- A group of us being invited to Professor Maria Bade's house one evening for coffee and a relaxed discussion about chitin.
- The shock effect of a couple of slides of "exploded" mice in a talk on the influence of chitin and chitosan in wound healing.
- The 'conference lunch' for which delegates paid an additional $25. Having lived for a year in Boston I was aware of its reputation for seafood and since this was a chitin conference I looked forward to perhaps lobster or crab, or at the very least shrimps – instead, for the main course we were each served a large meatball!

As what might be called a "conference virgin" at the time it was a great experience for me and obviously left a deep impression, as shown by the fact that of the further 24 conferences I have attended since then, admittedly not a lot over the course of thirty years, 22 have been specialist chitin/chitosan conferences.

George A. F. Roberts

Characterization of the Thermosensitive System Chitosan-Glycerol Phosphate and Applications for Gene Delivery

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Defended August 9th, 2007.
Supervisor: Professor Michael D. Buschmann

Chitosan is a natural polysaccharide derived from chitin, the second most abundant biopolymer after cellulose. Chitosan has been used extensively in the biomedical field due to its abundance, biodegradability, biocompatibility, low toxicity and its mucoadhesive properties. For instance, implants of mixtures of blood and of a thermosensitive solution composed of chitosan and glycerol phosphate have been shown to promote cartilage repair. Chitosan is also used as a gene delivery vector and it as been able to induce DNA-immunization through oral and nasal routes. Chitosan is a linear polymer composed of glucosamine and N-acetyl glucosamine and is defined by its degree of deacetylation (DDA or f_D), i.e. the fraction of its monomer that are glucosamine, as well as its molecular weight. The physico-chemical and biological properties of chitosan depend strongly on the DDA and the MW. As an example, an accurate measure of DDA is required in potentiometric titration experiments where the concentration of glucosamine monomer must be known accurately.

In the first part of this thesis, we report the validation of a method for the determination of the degree of deacetylation of chitosan by ^{1}H NMR spectroscopy. Chitosans with DDAs ranging from 48% to 100% have been used for the validation. The method is found to be simple, rapid and more precise than other known techniques like IR or titration for DDA measurements. The precision, ruggedness, robustness, specificity, stability and accuracy of the technique are discussed in this chapter. Results obtained suggest that ^{1}H NMR should be the standard technique for DDA measurement of any soluble chitosan. The results of this study were published in Journal of Pharmaceutical and Biomedical Analysis (Lavertu, Xia et al. 2003).

The objective of the second study of the thesis is to characterize the acid-base behavior of chitosan and to assess the ability of the nonlinear Poisson-Boltzmann cylindrical cell model to predict the apparent dissociation constant of chitosan at finite concentration, its

pK_{ap}, and the pK_{ap} dependence on chitosan ionization, level of deacetylation, concentration of chitosan and medium ionic strength. In this study, we performed temperature-controlled titration and dilution experiments on chitosan solutions with f_D of 0.72, 0.85, and 0.98 at concentrations ranging from 1.875mM to 30mM concentration of its glucosamine monomer and with 0 to 150 mM added salt. Light transmittance measurements were performed during titration to detect precipitation. We found that the apparent proton dissociation constant of chitosan (pK_{ap}) 1) increases strongly with

increased salt 2) increases strongly with increased chitosan concentration in low salt conditions and 3) decreases weakly with increasing f_D. All of the above influences on chitosan pK_{ap} were accurately predicted using a mean-field Poisson–Boltzmann (PB) cylindrical cell model where the only adjustable parameter was the temperature-dependent chitosan intrinsic monomeric dissociation constant p$K_0(T)$. The resulting chitosan pK_0 values at 25°C were in the range of 6.63 to 6.78 for all chitosans and ionic strengths tested.

The degree of ionization of chitosan when it precipitates upon addition of a strong base was measured to be in the range of 0.25 to 0.55 and was found to 1) increase with increased salt concentration and 3) increase strongly with f_D. The salt effect was accounted for by the PB model, while the influence of f_D appeared to be due to acetyl groups impeding attractive chain-to-chain association to increase solubility and require reduced ionization levels to precipitate. The results of this study were published in Biomacromolecules (Filion, Lavertu et al. 2007).

The third part of the thesis is devoted to the study of the thermosensitive solutions composed of chitosan and glycerol-phosphate (GP) that undergo a sol-gel transition when heated. As indicated above, these solutions are used as implants for cartilage repair. The first proposed gelling mechanism for these solutions was based on increasing hydrophobic interactions with temperature. Subsequently, an investigation of ionization and precipitation behavior of chitosan revealed important differences in the temperature dependence of the pK_a of chitosan versus GP and led us to propose an alternative hypothesis for the mechanism of gelation in chitosan-GP systems whereby heat induces transfer of protons from chitosan to glycerol phosphate thereby neutralizing chitosan and allowing attractive inter-chain forces to form a physical gel.

In order to investigate this specific molecular thermogelling mechanism, temperature ramp experiments on dilute chitosan-GP solutions were performed. Chitosans with f_D of 0.72 and 0.98 were used to prepare solutions with a range of molar ratios of GP to chitosan glucosamine monomer of 1.25 to 10 and with 0 or 150 mM added monovalent salt. Light transmittance measurements were performed simultaneously to indicate precipitation in these dilute systems as a surrogate for gelation in concentrated systems. Measured temperatures of precipitation ranged from 15 to 85ºC, where solutions with less GP (used in a disodium salt form) had higher precipitation temperatures. The theoretical model developed in the second part of this thesis was used to calculate the degree of ionization of chitosan (α, the fraction of protonated glucosamine monomer) as a function of temperature and showed a significant decrease in α with increased temperature due to

proton transfer from chitosan to GP. This heat-induced proton transfer from chitosan to GP was experimentally confirmed by ^{31}P NMR measurements during temperature ramp experiments since the chemical shift of ^{31}P of GP is an indicator of its level of protonation. By assuming average temperature independent values of α at precipitation (α_p) that were calculated from measured temperature at precipitation (T_p), the model was able to accurately predict measured T_p of all chitosan-GP mixtures. The resulting α_p were temperature independent but increased with increased chitosan f_D and with increased salt. Measurements and theory revealed that T_p can be adjusted in a predictable manner by changing the chitosan/GP molar ratio and thereby systematically tailored to obtain a large range of precipitation temperatures.
Similar temperature ramp experiments using inorganic phosphate and MES instead of GP demonstrated that the temperature-induced precipitation of chitosan also occurs with these buffers, confirming that the key feature of the buffer used with chitosan is its ability to absorb heat stimulated release of chitosan protons and facilitate chitosan neutralization. A theoretical expression for the variation of chitosan ionization degree with temperature in a system composed of two titratable species (chitosan and buffer) was derived and allowed us to establish the required characteristics of the buffer for efficient heat stimulated proton transfer between a chitosan and the buffer. These results provide a useful explanation for the mechanism of heat induced gelation of chitosan-based systems that could be exploited for numerous practical applications. The results of this study were published in Biomacromolecules (Lavertu, Filion et al. 2008).

The last part of the thesis is an in vitro gene delivery study using chitosan. The objective of this study was to obtain insight into the influence of the molecular weight (MW) and DDA of chitosan on transfection efficiency. To examine the influence of these parameters on gene transfer, we produced chitosans with different DDAs (98, 92, 80 and 72%) and depolymerized them with nitrous acid to obtain different MWs (150, 80, 40, 10 kDa). We produced 64 formulations of chitosan/pDNA complexes (16 chitosans, 2 amine-to-phosphate (N:P) ratios of 5:1 and 10:1 and 2 transfection media pH of 6.5 and 7.1), characterized them for size and surface charge, and tested them for gene transfection in HEK 293 cells *in vitro*. Several formulations produced high levels of transgene expression while two conditions, 92-10-5 and 80-10-10 [DDA-MW-N:P ratio] at pH 6.5, showed equivalence to our best positive control. The results also revealed an important coupling between DDA and MW of chitosan in determining transgene expression. Maximum expression was obtained with a certain combination of DDA and MW that depended on N:P ratio and the pH, but similar expression levels could be achieved by simultaneously lowering MW and increasing DDA or lowering DDA and increasing MW, suggesting a predominant role of particle stability, through co-operative electrostatic binding, in determining transfection efficiency. The results of this study were published in Biomaterials (Lavertu, Méthot et al. 2006).

I believe that characterisation and modelling of the chitosan acid-base behavior achieved in my doctoral thesis will be useful to determine the ionisation degree of chitosan under various conditions and will enhance control of chitosan solutions properties. I developed a theoretical model that correctly predicts the precipitation temperature of chitosan-GP mixtures and therefore constitutes a very useful tool to guide

the preparation of chitosan solutions with predictable gelation properties covering a wide range of gelation temperatures, salt conditions and chitosan f_D. The mechanism of gelation by proton transfer could be used to design gels using other hydrophobic polyelectrolytes. The influence of DDA and MW on chitosan transfection could also guide the synthesis of chitosan-DNA nanoparticles with specific properties adjusted for various applications and routes of administration. This influence of DDA and MW on the stability of chitosan-DNA complexes could be combined with other strategies to improve transfection efficiency of non-viral vectors including the use of targeting ligands, nuclear localisation sequence (NLS) peptides, endosomolytic peptides and steric stabilisation methods.

Depolymerization Mechanism and Polyelectrolyte Complexes of Alginate and Chitosan

Hilde K. Holme

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Defended: June 27th, 2008.
Supervisor: Professor em. Olav Smidsrød. Co-supervisor: Dr. Are Kristiansen

Polysaccharides such as chitosan and alginate are susceptible to a variety of degradation mechanisms causing cleavage of the glycosidic bond and a consequent reduction in molecular weight. This work examines the kinetics and mechanisms of depolymerization of alginate and chitosan in the solid state and in aqueous solution at elevated temperatures. The work also includes a study of the enzymatic hydrolysis specificity of chitosan using NMR techniques. Finally, the formation of polyelectrolyte complex (PEC) involving alginate and chitosan is studied, together with an investigation of chitosan stability to lysozyme catalyzed hydrolysis when chitosan is interacting with alginate.

Depolymerization kinetics were followed by measuring the time course of the apparent viscosity and the intrinsic viscosity. The initial rate constants for depolymerization were determined from the intrinsic viscosity data after these data had been converted to a quantity proportional to the fraction of bonds broken. The initial rate constants were found to increase markedly with increasing degree of acetylation for chitosan chloride in the solid state. The activation energies of three chitosan chlorides in the solid state with degrees of acetylation (F_A) of 0.02, 0.16, and 0.35 were determined from the initial rate constants to be 114 ± 11 kJ/mol, 112 ± 5 kJ/mol, and 109 ± kJ/mol, respectively. The activation energies of G-rich alginate and M-rich alginate in the solid state were found to be 114 ± 6 kJ/mol and 126 ± 12 kJ/mol, respectively. The activation energies of the aqueous chitosan chloride and chitosan glutamate solutions at pH ~5 and the same degree of acetylation (F_A = 0.14) were determined from the initial rate constants to be 76 ± 13 kJ/mol and 80 ± 11 kJ/mol, respectively.

The present work shows that acid hydrolysis and β-elimination, caused by alkaline conditions, are the primary mechanisms involved in the thermal depolymerization of sodium

alginate in the solid state, and that the thermal depolymerization of chitosan chloride in the solid state is driven mainly by acid hydrolysis. Thermal depolymerization of aqueous chitosan salt and sodium alginate solution at pH 5-8 occurs primarily by oxidative-reductive depolymerization (ORD) in the presence of transition metal ions, but by both acid and alkaline depolymerization when the samples are highly purified. The reducing end in each polymer chain appears to play an important role in the ORD reaction in aqueous solution in the presence of catalytic ion. This observation is of scientific importance and deserves further attention in order for definitive conclusions to be drawn.

High-field ^{13}C NMR spectroscopy was used to determine the identity of the new reducing and non-reducing ends and the variation in their nearest neighbors. Egg white lysozyme was found to depolymerize partially *N*-acetylated chitosans by preferentially hydrolyzing sequences of acetylated units bound to site C_L, D_L, and E_L of the active cleft (A-F), while there was no specificity for acetylated or deacetylated units at site F_L. *Bacillus* chitosanase was found to hydrolyze partially *N*-acetylated chitosan by preferentially attacking sequences of three consecutive deacetylated units (hypothetical subsites C_C, D_C and E_C), where cleavage occurs between sugar units bound to subsites D_C and E_C. A hypothetical subsite F_C on the chitosanase showed no specificity with respect to **A**- or **D**-units.

Polyelectrolyte complexes (PECs) of alginate and chitosan were formed by dropwise addition of 0.1% sodium alginate solution (pH ~ 6.5) to 0.1% chitosan chloride solution (pH ~ 4.0), or vice versa, under high shear by rapid mixing. The properties of the polymer and the preparation procedure were varied, and the effects on PEC size, particle surface charge (zeta potential, Zp), and pH of the dispersion were determined using dynamic light scattering (DLS) and measurements of turbidity, electrophoresis, and pH. Some of the complexes were also visualized by atomic force microscopy (AFM) in tapping mode. This work shows that the selection of rotational speed and diameter of the dispersion element of the homogenizer, as well as the molecular properties of the polyelectrolytes, should be carefully chosen in order to optimise the particle size and the Zp of alginate-chitosan complexes. The charge ratio was found to affect the particle size, Zp, and pH of the dispersion, and the molecular weight was found to affect the particle size. At charge ratios close to one (neutrality), the particle sizes proved to be less reproducible. The smallest particles were obtained by using polymers of low molecular weight and adding one of the two polymer solutions into an excess amount of the other solution. Adjusting the pH of the PEC mixture above 7 significantly increased the particle size. PECs showed good stability over the temperature range of 4 – 37^{o}C. Increasing the ionic strength in the PEC mixture to 0.15 M NaCl produced PECs of less reproducible size and reduced Zp. The

properties and structure of the resulting PECs were rationalised in terms of a core-shell model. Furthermore, lysozyme did not affect the surface charge of the PECs, and it was found to depolymerize chitosan in PEC at a similar rate as chitosan in solution. However, further investigations are needed in order to draw conclusions about the activity of lysozyme on PEC.

9th INTERNATIONAL CONFERENCE OF THE EUROPEAN CHITIN SOCIETY

EUCHIS 2009

Venice, Italy, 23-26 May 2009

Announcement and Call for Papers

(http://www.isf.univpm.it/euchis2009)

Aim of the Conference

The aim of the conference is to present the results produced very recently in the research, development and applications related to chitin and chitosan. The conference will cover a broad range of topics according to the tradition which distinguishes the European Chitin Society Conference series from others which focus on more specific areas. According to our intention the conference will constitute a forum whereas producers and researchers (chemistry, biology, biochemistry, medicine, pharmacy, dentistry, veterinary, agriculture, textile, food etc.) acting in different but related fields, can meet, exchange information on the new findings and perspectives in the different research and application fields.

History of the International Conference of the European Chitin Society

1st International Conference	Brest, France	11-13 September 1995
2nd International Conference	Lyon, France	3-5 September 1997
3rd International Conference	Potsdam, Germany	Aug 31-Sept 3 1999
4th International Conference	Senigallia, Italy	6-10 May 2001

5th International Conference	Trondheim, Norway	26-28 June 2002
6th International Conference	Poznan, Poland	Aug 31-Sept 3 2004
7th International Conference	Montpellier, France	6-9 September 2006
8th International Conference	Antalya, Turkey	8-11 September

Conference Chairs

Franco Rustichelli, Polytechnic University of Marche, Italy	Chair
Carla Caramella, University of Pavia, Italy	Co-Chair
Sevda Senel, Hacettepe University, Turkey	Co-Chair
Kjell M. Vårum, Norwegian Biopolymer Lab, Norway	Co-Chair

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Francesco Federiconi, Ancona, Italy	
Maria Chiara Girombelli, Ancona, Italy	
Caterina Mokrousova, Ancona, Italy	
Vittorio Rozzi, Ancona, Italy	

Location

The Conference will be held at the S.Servolo Island, Venice, Italy, located in the middle of the Venetian lagoon (http://www.sanservolo.provincia.venezia.it).

Tentative Conference Schedule

Four types of presentations are foreseen:

- Plenary lectures (45 minutes each)
- Key-note lectures (30 minutes each)
- Oral presentations (20 minutes each)
- Poster presentations.

Plenary and key-note lecturers will be invited by the Organizing Committee
The list of speakers and the conference program will be published under the conference web site.
The maximum poster size is 130 cm (height) 90 cm (width).

	May 23	May 24	May 25	May 26
9:00		Welcome		
9:15		Plenary lecture	Plenary lecture	Plenary lecture
10:00		2 parallel sessions	2 parallel sessions	2 parallel sessions
10:45		Coffee break	Coffee break	Coffee break
11:15		2 parallel sessions	2 parallel sessions	2 parallel sessions
13:00		Lunch break	Lunch break	Lunch break
14:30		Poster session	Poster session	Poster session
15:30		2 parallel sessions	End of the session	2 parallel sessions
16:45		Coffee break		End of the Conference
17:15		2 parallel sessions		
18:30	Welcome party	End of the session		
20:30			Social dinner	

Conference Topics

1. Physico-chemical
2. Enzymatic
3. Chitooligosaccharides
4. Biomedical applications
5. Food, Textile and diverse applications

Important Dates and Deadlines

Submission of abstracts	January 16, 2009
Notification of acceptance	February 13, 2009
Advance registration	February 27, 2009
Grant request	February 27, 2009
Refund request	March 27, 2009
Manuscript due	March 27, 2009
On site registration	May 23, 2009

Registration

The registration is available under the conference web site. There one can register, book a on-site room, pay the social dinner and pay the relative amount together with the registration fee. The registration fee includes coffee breaks, lunches, conference materials, conference proceedings, social dinner and the ticket for the boat transportation for the duration of the conference. The registration fee of the accompanying persons includes coffee breaks, lunches, conference materials, social dinner, the ticket for the boat transportation for the duration of the conference and two tours as described below in the social program. A penalty of 20% is applied for payments after February 27, 2009.

	Fee
Non EUCHIS Members	600
EUCHIS Members	550
Student – non EUCHIS members	300
Student – EUCHIS members	250
Accompanying person	300

Payment can be made by cash, cheque, wire transfer or credit cards. Detailed instructions are available under the conference web site.

Refund Policy

A 20% service charge will be made for processing refunds. Requests for refunds will be honoured until March 27, 2009. After March 27, 2009, no refunds will be given, although substitutes will be allowed.

EUCHIS awards

The European Chitin Society will provide the following awards:

1) **Poster awards** for posters presented at EUCHIS 2009.

- 1st prize: Euro 500
- 2nd prize: Euro 300
- 3rd prize: Euro 200

The awards will be decided by an award committee. The posters will be evaluated on the basis of their scientific quality and their communication skills (http://www.euchis.org/posterAwards.php).

2) The **Braconnot Prize** is awarded for outstanding contributions and achievements in chitin science and for special merits in the promotion of EUCHIS. The Braconnot Prize consists of a certificate with the name of the recipient and a cheque. The recipient of the Braconnot award must be no more than 36 years old during the year of the award (http://www.euchis.org/braconnotPrize.php).

3) The European Chitin Society will provide a limited number of **travel awards** for attending the EUCHIS 2009 conference. Depending on the number of applicants selected and the funds available, awards may cover travel (economy return air, rail fare or car fuel costs), conference registration, and in some cases accommodation (http://www.euchis.org/travelAwards.php).

General rules:

Applicants must:

- be a PhD student or junior scientist (i.e. medical resident or postdoctoral).
- be an EUCHIS member in good standing (subscription paid).
- make a contribution (oral or poster) to the conference.
- have no other source of funds for this purpose, including supervisor's or University funding. If funds from elsewhere are subsequently obtained, EUCHIS should be informed immediately and the application for EUCHIS funding withdrawn, or the EUCHIS award declined/returned if already made, so that another applicant can be funded.

Application procedure:
The deadline for applications is eight weeks before the deadline for early conference registration. Please send an informal letter of application to:

Prof. Kjell M. Vårum NOBIPOL, Department of Biotechnology Norwegian University of Science and Technology 7491 Trondheim, Norway, email: kvaarum@biotech.ntnu.no

with enclosing

- Proof of status (usually a short statement from the supervisor or Head of Department), including the date or expected date of completion of PhD or medical qualification.
- Evidence of non-availability of other funds (usually part of the statement from the supervisor or Head of Department). Please state if other applications for funding are being made.
- Submitted abstract of the oral or poster contribution.
- Estimates of the costs of travel, accommodation and conference registration.
- Applicant's full address, phone and fax numbers, and e-mail address where available.

Social program

Venice has been defined as an "Unbelievable city to be approached only by boat and coming from far away" (Thomas Mann). Some excursions will be organised for the accompanying persons to give them the feeling of this unique city.

Trip to the Venetian Lagoon Islands. Murano, Burano and Torcello on May 24 (about 8h, including lunch).
Tour around the San Marco area on May 25 with a visit to San Marco Square, San Marco Church and the Doge's Palace (about 4 h).

Both the above mentioned tours are included in the registration fee of the accompanying person.

Abstract Submission Guidelines

Authors are invited to submit an abstract of their contribution to the conference. The authors are requested to submit their abstract through the conference web site (http://www.isf.univpm.it/euchis2009) according to the instructions described there. An abstract book and the conference proceedings will be distributed at the conference.

Commercial exposition

During the whole conference period a dedicated area will be available for exhibition of scientific instrumentation and for science publishers.

Banquet

A banquet will take place on May 24, 2009. The banquet is not included in the registration fee. The price of the menu of the gala dinner with beverages is 70 €. Registration for the gala dinner should be done until May 1, 2009.

Lodging

As you probably know, Venice is visited by about 14 millions of tourists every year, so there are many hotels available with a very large spread of prices. We have booked a limited number of rooms for the participant convenience at the conference venue.

These on-site rooms at the San Servolo Campus are available for the conference participants. The on-site rooms are comfortable and nice, with private bath, TV and air-conditioning, but no daily room service and breakfast are included. The on-site rooms can be booked through the conference web site and will be allocated on a first-come, first-served basis. Single, double, three, four and five bed rooms are there available and they can be shared upon request. The rooms are offered for a minimum stay of three nights and requests to extend the lodging period are also possible.
Overall room prices for a 3 nights stay. (check-in May 23 – check-out May 26, 2009)

30 single rooms:	225 € (75 € x 3)	
26 double rooms:	330 € (110 € x 3)	Single occupancy 300 € (100 € x 3)
12 three-bed rooms:	360 € (120 € x 3)	
3 four-bed rooms:	420 € (140 € x 3)	

Early booking is strongly recommended

Alternatively, the conference participants are free to find their accommodation in Venice.

A list of websites for hotel booking is also here below reported for any needs.

http://www.venere.it/venezia/index.html.en
http://www.doge.it/affari/hotel.htm
http://www.elmoro.com/UK/frmwhere.html
http://www.venicehotel.com/index.htm

Venice Weather

The weather in Venice is usually warm and humid with average daily temperature between 12 and 21°C.

Travel instructions

By plane to Venice

The Venice International Airport (http://www.veniceairport.it) connects Venice with many european and international direct flights. From the airport there are several services that run to Venice:

- Public boats Alilaguna (http://www.alilaguna.com) offer a service from the airport to Murano, Fondamente Nuove, Lido, Arsenale, San Marco (connection to San Servolo) and Zattere.

Ticket price: 12 €
Travelling time: about 70 minutes

- ATVO buses (http://www.atvo.it/images_doc/linee/air_terminal.pdf). Blue non-stop buses scheduled about twice every hour (from 9.00 to 24.00) from the airport to "Piazzale Roma"

Tickets (3 €) can be purchased at the arrival hall.
Travelling time: about 20 minutes

- ACTV bus n°5. Orange buses scheduled every 30 minutes from the airport to "Piazzale Roma" (last stop) (http://www.actv.it)

Tickets (about 2 €) can be purchased at the arrival hall.

- Water taxis: from the airport to the final destination in Venice. A water taxi costs about 100 €

- Land taxis: from airport to "Piazzale Roma" a taxi costs about 30 €

By car To Venice

People arriving by car will have to park in a private or public garage at Piazzale Roma (daily ticket from 19 to 26 €) or at "Tronchetto" (daily ticket 18 €). It is also possible to park the car before the bridge that connects Venice to the mainland (daily ticket about 4 €) and then reach "Piazzale Roma" by public orange buses, or close to the train station of Venezia-Mestre and then take a train or bus.

By train to Venice

The railway station (Venezia S. Lucia) is placed on the Grand Canal in the north-west corner of the city. Please, pay attention to get out the train at the station Venezia S.Lucia, since trains stop also at Venezia-Mestre station on the mainland.

From "Piazzale Roma" or Railway Station S.Lucia to San Zaccaria

Piazzale Roma is the entry point of the city if one travels by car or with the bus from the airport, while the railway station Santa Lucia is the entry point for those arriving by train.

Take boat n° 1, 41, 51, 61 or 82 at Piazzale Roma or n° 1, 51 or 82 at S.Lucia (railway station) to San Marco boat stop and then proceed walking to the "San Zaccaria" stop (in front of the Londra Palace Hotel, Riva degli Schiavoni) (timetables at http://www.actv.it).

From San Zaccaria To San Servolo island

The island of San Servolo can be reached by public transportation. The public boats n° 20 to San Servolo leave from San Zaccaria (at walking distance from the San Marco square) in front of the Londra Palace Hotel, Riva degli Schiavoni. Alternatively, private taxi may costs from 50 to 75 € one way.

From San Zaccaria to San Servolo
00.25 – 01.30 – 02.10* – 06.45 – 07.15 – 07.45 – 08.15 – 08.45 – 09.15 – 09.45 – 10.45 – 11.45 – 12.45 – 13.45 – 14.45 – 15.45 – 16.15 – 16.45 – 17.15 – 17.45 – 18.15 – 18.45 – 19.15 – 20.15 – 21:15 – 22.15 – 23.15

From San Servolo to San Zaccaria
01.40* – 02.20 – 07.20 - 07.50 - 08.20 - 08.50 - 09.20 - 09.50 - 10.20 - 11.20 - 12.20 – 13.20 - 14.20 - 15.20 - 16.20 - 16.50 - 17.20 – 17.50 – 18.20 - 18.50 - 19.20 - 19.50 - 20.50 - 21.50 - 22.50 - 23.50

* upon request by calling the toll free number 800 845065 at least 20 minutes before the scheduled time

The timetables reported here are valid at the time of the publication of this information, they may undergo some changes according to the companies and the Conference Organizers are not responsible for their changes.

Conference Secretary

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