

We have synthesised some amphiphilic carbohydrate substituted porphyrins with different carbohydrate moieties. The compounds were synthesised by reaction of glycosyl imidates with the Nickel complex of 5-(4-hydroxymethylphenyl)-2,8,13,17-tetraethyl-3,7,12,18-tetramethylporphine in good yield and characterized by NMR and MS-spectroscopy. Investigation of the binding constant to different plasma proteins (LDL, HDL, VLDL) revealed, that the carbohydrate subunit is of great importance for the binding properties. Furthermore, time-resolved fluorescence spectroscopic measurements confirm that only a small amount of the porphyrinic sensitizer is associated with the apoprotein unit and most of the sensitizer is incorporated into the lipid compartment. These results are of great importance for the development of new sensitizers with enhanced tumour cell selectivity. Binding of sensitizers to the apoprotein unit may alter the interaction of LDL with cancer cells and has to be avoided.

### Cellulases in the Textile Industry – An Overview

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Cellulases are well established in textile wet processing as agents for fibre and fabric surface modification. The most known applications are the ageing of fabric surfaces, like the stone washed look of Denim garments, and also the cleaning and renewing of fabric surfaces from microfibrils, fuzz and loss fibres. Apparently these opposite effects can be obtained with the same enzymes. However, cellulases are a multi-component enzyme system, with endoglucanases (EGs) that hydrolyze randomly cellulose chains, cellobiohydrolases (CBHs) that hydrolyze cellobiose from cellulose ends and cellobiases that hydrolyze cellobiose to glucose. The different effects can be obtained with different enzyme compositions, EG or EG rich preparations are best for ageing and defibrillation of fibre surfaces while complete cellulase systems are best for cleaning and dippilling effects. The finishing effects delivered by cellulases are always obtained in process (rotating drum washers and jets) where strong mechanical agitation into the fabrics are provided during the treatments. In this paper a overview is done about the actual knowledge of the processes and future directions of this research field.

### Construction of Recombinant BHK Cell Lines Expressing Wild-type and Mutants of Human $\alpha$ 1,3/4-fucosyltransferase

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Stable BHK-21 cell lines were constructed expressing i) the wild-type form of human  $\alpha$ 1,3/4-fucosyltransferase (FT3T2), ii) the secretory form of the enzyme where amino acids 46-361 (S2FT3TS) were coupled at their amino terminus to the signal sequence of interleukin-2, and iii) a membrane bound form (FT3NPT2) where the amino acid residues Cys-16, Gln-23, Cys-29, and Tyr-33 from the transmembrane domain of the enzyme were replaced by Leu residues.

Cell lines expressing similar amounts of total fucosyltransferase activity were used to localize the three constructs

by immunofluorescence microscopy studies. The S2FT3T2 was detected as small vesicles in the cells. The FT3T2 was found to be present within the Golgi and trans-Golgi-network. Most of the FN3NPT2 was detected on the plasma membrane of the recombinant cells. These results suggest that the amino acid residues Cys-16, Gln-23, Cys-29 and Tyr-33 residues of the transmembrane domain of the  $\alpha$ 1,3/4-fucosyltransferase specify location of the enzyme in the Golgi.

The S2FT3T2 was purified on GDP-Fractogel resin and its specificity towards oligosaccharides, N-glycans, glycolipids, glycopeptides and glycoproteins was studied. The soluble forms of  $\alpha$ 1,3/4-fucosyltransferase may be used for *in vitro* synthesis of the Lewis<sup>x</sup> determinant on carbohydrates and glycoproteins, whereas Lewis<sup>x</sup> and sialyl-Lewis<sup>x</sup> structures cannot be synthesized.

### Cyclodextrins, Supramolecular Devices for Drug Transport and Targeting

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Cyclomaltooligosaccharides (cyclodextrins, CDs) are almost ideal supramolecular devices for the bioavailability enhancement of bioactive compounds, in view of their almost starch like innocuity, and their ability to form inclusion complexes with a wide variety of poorly water soluble molecules, and their recent availability at low costs from well handled biotechnological processes. Problems with such carriers are however still encountered, related with their relatively low solubility in water which affects their solubilization properties, relatively high hemolytic character at least for the more common heptose entity which limit their parenteral use, and their absence of recognition sites *in vivo*.

Selective chemical modification, still almost restricted to the narrower primary hydroxyl side of the torus, have been designed in order to overcome these shortcomings. Substitution at C-6, as with branched mono- and per-(6-O and 6-S) linked glycosyl-CDs enhances drastically the solubility and solubilization properties. Interestingly, the thiourea functionality, which was initially introduced as spacer, enhances by itself the solubility as shown with the 6<sup>l</sup>-methylthioureido derivative which shows solubility improvements  $\times 43$  as compared to  $\beta$ -CD, probably due to the hydrogen bond interaction between thiourea NH protons and water molecules. Bioactive compounds, belonging to various therapeutic classes, have been considered as guests in order to define the optimal parameters for their transport in biological fluids. Using NMR spectroscopy as a main tool, it was shown that a balance between inclusion parameters and solubilization properties had to take into account, not only the size of the cavity, but also the possibility of interaction with the primary hydroxyl bearing side of the torus. In situations where the stabilization of the complex involves the formation of hydrogen bonds, the 6<sup>l</sup>-branched derivative exhibits larger binding constants as compared to the persubstituted analog. In addition, when the guest compound interacts from the primary hydroxyls side of the host, as it is the case with the potent anticoagulant 2-phenylindane-1,3-dione, the steric hindrance of the C-6 substituent reduces the affinity. Conversely, the solubilization properties are greatly improved when the host-guest interaction occurs from the secondary hydroxyls side, as with the analgesic carbamazepine or the antidepressant dothiepine. Stabilisation of the trilactonic active form of the

antithrombotic drug ginkgolide B was achieved, with optimal simultaneous solubilization, using 6'-S- $\alpha$ -maltosyl-6'-thiocyclomaltoheptaose. Water solubility enhancements of the almost insoluble anticancer drug Taxotere<sup>®</sup> up to 4.5 g L<sup>-1</sup> were obtained with 6'-methylthioureido- $\beta$ -CD.

From a systematic investigation on the role of the chemical modification with regard to the hemolytic character of cyclodextrins, conclusions have been brought about the charge and the geometry of the modification: i) Substitution at primary hydroxyl groups usually decreases the hemolytic character; ii) introduction of an amino group, resulting in a positive charge at physiological pH, decreases the hemolytic character; iii) negative charges are comparatively less effective; iv) zwitterionic groups seems to enhance the hemolytic character of the cyclodextrin molecule. Most of these data probably relate to interactions with erythrocyte membranes which may result in extraction of components. Taking into account the above results, it is anticipated that convenient functionalization with biological markers, oligosaccharides or proteins, may result in site-specific drug delivery systems based on these supramolecular carriers.

### Determination of Sugars, and Some Other Compounds in Infant Formulae, Follow-up Milks and Human Milk by HPLC-UV/RI

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Progressive attempts have been made by the industry to bring composition of the infant formulae closer to that of the human milk. Follow-up milks are given to infants after 4–6 months of age to make the transition from human or infant formulae to cow's milk. The composition of these artificial milk formulae, relative to some constituents, namely, the sugars, does not correspond to that of genuine cow's milk from which they are originally prepared. A Regulation (91/5/EC Directive, J.O.E.C., #L 175/35, 4.7.91) establishes the type and limits of carbohydrates which can be added. Other milk endogenous compounds such as uric and orotic acids appear naturally in these formulae and because their levels can be good indicators of the quality of cows' milk used their quantification is also important.

In the present study the composition of sugars, uric and orotic acids in infant formulae and follow-up milks commercially available on the local market is reported. The levels found are compared with Portuguese and European Standards and with human and cows' milk composition. 50 samples including all of these products were analysed, using a rapid and accurate HPLC procedure developed for that purpose, which allowed simultaneous determination of lactose, glucose, galactose, saccharose, maltose, uric and orotic acids by HPLC using refractive index and UV detectors in series.

For the analysed sugars all, except two, infant formulae contained exclusively lactose, as happens with human and cow's milk. The two exceptions were lactose free infant formulae used for lactase-deficient infants and contained maltose and glucose. In follow-up milks the prevailing carbohydrate was lactose but they also contained other sugars such as, maltose, saccharose and traces of glucose and galactose, at the levels allowed by regulation. With respect to uric and orotic acid composition no significant differences between their levels were obtained when determined by ANOVA methodology, followed by Fisher's PLSD test ( $p > 0.01$ ).

### Fiber Preparation of N-acylchitosan and its Composite With Cellulose by Spinning Their Sodium Xanthate Solutions

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Chitin, chitosan and cellulose have a structural backbone of (1  $\rightarrow$  4)-linked  $\beta$ -D-glucan. Cellulose xanthate is used widely in the global rayon industry, but chitin xanthate is little used in the textile field. The present paper aims to develop a method of generating novel fibres of N-acylchitosan and its composite with cellulose from their aqueous alkaline sodium xanthate solutions.

A sodium N-acylchitosan solution in aq. 14% NaOH was treated with CS<sub>2</sub> to afford the corresponding aq. sodium N-acylchitosan xanthate solution. The xanthate solution and its clear mixed solution with sodium cellulose xanthate were spun at 45–50°C through a viscose-type spinneret into a coagulation bath containing aq. 10% H<sub>2</sub>SO<sub>4</sub>, 32% Na<sub>2</sub>SO<sub>4</sub> and 1.3% ZnSO<sub>4</sub>.

Eight kinds of novel fibers were prepared from N-acetylchitosan, N-propionylchitosan, N-acetylchitosan-cellulose composites (6:4, 4:6 and 3:7, w/w), and N-Propionylchitosan-cellulose composites (4:6, 5:5 and 3:7, w/w). All the fibers obtained were white and showed absorptions at 1650–1657 and 1550–1554 cm<sup>-1</sup> (C=O and NH of N-acyl) in FTIR spectra (KBr). N-Propionylchitosan-cellulose composite filament had better mechanical properties than did N-acetylchitosan-cellulose composite filament, because of the relatively firm interaction of N-Propionyl groups. These fibers are usable not only as biomedical materials (e.g., controlled digestible surgical suture, tissue wound-dressing etc.) but also as general functional textile materials.

### Modelling Cucose Crystallization – A Simulator for Operator Training

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Here, we report on the study of the operation of an industrial semi-batch evaporative-crystallizer for cane sugar refining, making use of an in-house developed process simulator with the required capacities for development and training in process control. Today, computer-based supervisory and control systems are routinely employed in plants, running applications on-line and in real-time. A major job for the future, which has already started, will be to incorporate new methodologies into those industrial equipment and thus to bring such methodologies into process operation. A practical difficulty hindering development in this direction is that experiments with real industrial processes, both for the assessment of new strategies and for the required operator training, are difficult to carry out for reasons of economy and safety. Such limitations can be overcome with the available technology and theory, building new laboratory environments and tools, focusing on computer control and on the concepts related to the use of information in real-time.

The theory on process modelling and simulation plays a key role in the development of such environments and tools. For a significant number of processes, deterministic models can be written which, with appropriate parameters determined by