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Abstracts

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Antisense Drugs: Low-cost Multi-ton Manufacture for Market

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Over the last decade, the biotechnology industry has witnessed a tumultuous growth in antisense based therapeutics. This is evidenced by a significant rise in the number of oligonucleotides entering into human clinical trials. Therefore, antisense technology represents a major paradigm shift for the biotech-based drug discovery in the nineties, with impressive capabilities to target a host of diseases well into the coming millennium.

Interestingly, all of the first generation antisense drugs belong to a common class of modification, popularly known as Phosphorothioates. The first generation drugs are manufactured from four simple phosphoramidite building-blocks. These blocks are currently prepared from natural 2′-deoxynucleosides, which are generally isolated from fish milt. Recently, however, chemical synthesis of thymidine has been accomplished on multi-ton scale and synthesis of other 2′-deoxynucleosides is under investigation.

The availability of raw materials and the recent advances made in automated solid-phase synthesis of oligonucleotides, now enables the large-scale manufacturing of antisense drugs at relative ease. This presentation will focus on the various technological advances made at Isis. Particularly, development of new synthetic approaches for effective assembly of oligonucleotides on solid-support, use of novel reagents for improved efficiency and lowering the cost of oligonucleotides, state-of-the-art purification techniques that allow enhanced full-length purity of oligonucleotides and excellent recovery yields. In summary, we will describe our vision of low-cost multi-ton manufacturing of antisense phosphorothioate oligonucleotide for the market.

Approaching Sucrose Crystallization at a Molecular Level: Role of Water Structure and Interactions

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IT is well known that molecular associations take place in sucrose solutions. We have made the demonstration of the existence of these interactions and of their different nature (water-water, water-sucrose, sucrose-sucrose) as a function of concentration some years ago. The clustering of hydrated sucrose molecules in swarms called "protonuclei" was also found to occur in saturated sucrose solutions.

The rate of sucrose crystallization in supersaturated solutions is known to include at least two steps: the diffusion of sucrose from the bulk solution to the thin layer at the interface crystal/solution and the incorporation of sucrose molecules in the crystal after the release of their hydratation water.

Among the energy barriers encountered in the "hurdle race" of the crystallisation process, viscosity seems to be a minor one and the desassociation of hydration water a major one. These hypothesis were given some 20 years ago by Professor Andrew Van Hook.

We will attempt to show that the dehydration of sucrose molecules prior to their incorporation to the crystal plays an important part in the crystallization process and propose to conceive the mechanism of crystal growth as mainly based on the release of water molecules and their diffusion in the bulk solution rather than a migration of sucrose from the solution to the crystal.

Biopolymers Used in Microspheres and Microcapsules

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Microcapsules are defined as particles with sizes in the range of 50 nm to 2 mm consisting of a polymer matrix and an 'encapsulated' or bound active component. Polymer microspheres are described as empty microcapsules. Interest in the fundamental and applicative research of these materials is immense. The choice of the manufacturing process depends on the nature of the starting materials. Frequently used methods are suspension-emulsion polymerization from monomeric materials or suspension-emulsion crosslinking from polymeric starting materials.

Traditionally, microspheres were produced from synthetic polymers such as polyacrylates. Also, agarose and cellulose-based supports in chromatographic packing materials were applied. Although there were attempts in grafting of polysaccharides with synthetic polymers such as in starch-polyacrylate microparticles, recently microspheres were prepared solely from biopolymers such as chitosan and starch.

Starch is used in various non-food products such as bioplastics. Starch is available in large quantities, renewable and cheap. At ATO-DLO a range of starch-based microspheres were prepared by several new routes based on emulsion crosslinking or polymerization. The size of the particles range from 100 nm to several mm. The particle size could be controlled by the amount of energy during emulsification and emulsion composition. A polydispersity can be obtained of less than 30%. Furthermore, they have unique colloidal and emulsifying properties.

Starch microparticles were prepared with a broad range of structural and functional differences. This opened the door to

new applications for starch although many of these opportunities remain to be explored. Areas of interest are packaging, textiles, controlled release, cosmetics, pharmaceuticals and flocculation.

Carbohydrate Chemistry Focused on Agrochemical Application

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In the last few years we have been devoted to the synthesis of biomolecules potentially interesting as agrochemicals. We now describe the preparation of the sugar moiety of Amipurimycin, of Miharamycinn and of its analogues. These two molecules are antibiotics known to inhibit *Pyricularia oryzae*, responsible for the rice blast disease.

Pseudo-C-nucleosides are another group of compounds which have been investigated by us, in order to obtain new structures possessing bioactivity. In this work we also describe the synthesis and bioactivity of some thiazoles, tetrazoles, triazoles and pyrazoles, obtained by chain elongation of some sugar precursors.

The α,β-unsaturated-γ-lactone unit is known from the literature to confer a great diversity of biological effects. We present the synthesis of fungitoxic sugar molecules, containing this unit in their structure, via Reformatsky-type reaction of the appropriate carbonyl compounds with ethyl α-bromomethyl acrylate and zinc. The relationship between structure, conformation, configuration of the molecules and the bioactivity detected will be evaluated.

Carbohydrate Liquid Crystals

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Emil Fischer reported the *double melting points* of some long chain n-alkyl pyranosides, but failed to connect the observation with liquid crystal formation. It was not until nearly forty years later that these compounds were shown to form liquid crystals. This observation seemed to be overlooked, since some thirty years later none of the text books or reviews of the subject mentioned carbohydrates. The revival of interest came in 1984 when 17 examples were reported. Now a recent Liquid Crystal Data Base lists over 2000 Carbohydrates.

Liquid crystals fall into two classes; thermotropic and lyotropic. The thermotropic may be calamatic, discotic or chiral. The lyotropic phases, formed on contact with water, may be laminar, cubic or hexagonal.

Carbohydrates which illustrate these phases will be described. The potential commercial uses will be discussed.

Carbohydrate Polymers as Wound Management Aids

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Polymeric materials composed of carbohydrate units, i.e. polysaccharides, are a diverse group of biological macromolecules that are showing increasing application in areas of wound management. A variety of neutral polysaccharides, e.g. dextran and starch, basic polysaccharides, e.g. chitin and chitosan, acidic polysaccharides, e.g. alginic acid and hyaluronic acid, and glycosaminoglycans, e.g. dermatan sulphate and hepafin, and their respective derivatives, have been the focus of much interest with respect to biomedical and particularly woundcare applications over recent years, however there has been no directive that any one chemical structure is more efficacious than any other. To be suitable as a wound management aid a dressing material should exhibit a number of properties including abilities to: maintain high humidity at the wound-dressing interface provide protection against secondary infection, be able to remove excess wound exudate and toxic components, and maintain its strength when sterile and wet.

More recent investigations have examined some of the ore unusual polysaccharides isolated from plant, bacterial and animal sources which possess potentially useful biological properties that may make them suitable for woundcare applications, e.g. arabinoxylan and β-D-(1 $\rightarrow$ 3)-glucan derivatives. The precise structures of such unusual polysaccharide based materials are often unknown and detailed compositional and structural characterisation is required in order to satisfy regulatory authorities. The carbohydrate structure of a novel polysaccharide based material, namely Sterigel® (Seton Healthcare), used as a wound management aid has been determined. Enzymic hydrolysis and methylation analysis have shown the carbohydrate structure to be a highly substituted β-D-(1 $\rightarrow$ 4)-xylan. This polysaccharide backbone is substituted with α-L-arabinofuranoside residues and α-D-glucopyranosyluronic acid residues. The total amino acid content of the Sterigel, as determined after acid hydrolysis, is 4.4% w/w with the amino acid hydroxyproline accounting for 0.22%. The cinnamic acid derivative ferulic acid has been identified in both alkaline (0.40%) and enzymic (0.25%) hydrolysates of the polysaccharide.

Carbohydrate Substituted Porphyrins. Synthesis, Characterization and Lipoprotein Binding Properties

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The development of new photosensitisers for photodynamic therapy of tumours (PDT) is one of the most important fields of porphyrin chemistry today. PDT is a treatment that is based on the selectivity of porphyrinic compounds to tumour tissue and on the production of singlet oxygen by irradiation of the sensitiser with visible light. Thus formation of singlet oxygen in tumour cells causes cell death and tumour necrosis. Besides long wavelength absorption solubility and water and high selectivity to tumour cells are requirements a new photosensitiser has to fulfill. Therefore a number of different carbohydrate substituted porphyrins were synthesised. Although the mechanism of sensitiser uptake is not yet clarified, there is evidence that amphiphilic porphyrins associate to LDL and are introduced into the tumour cell via receptor medicated endocytosis.