NEW DEVELOPMENTS IN INDUSTRIAL POLYSACCHARIDES

EDITED BY

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GORDON AND BREACH SCIENCE PUBLISHERS

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Proceedings of the
Conference on New Developments in Industrial
Polysaccharides held at the Stevens Institute of Technology,
Hoboken, New Jersey, May 31–June 1, 1984

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Gordon and Breach Science Publishers
New York London Paris Montreux Tokyo

Gordon and Breach Science Publishers

P.O. Box 786
Cooper Station
New York, NY 10276
United States of America

P.O. Box 197
London WC2E 9PX
England

58, rue Lhomond
75005 Paris
France

P.O. Box 161
1820 Montreux 2
Switzerland

14-9 Okubo 3-chome,
Shinjuku-ku,
Tokyo 160
Japan

Library of Congress Cataloging in Publication Data

Conference on New Developments in Industrial Polysaccharides (1984: Hoboken, N.J.)
New developments in industrial polysaccharides.

Includes index.
1. Polysaccharides—Industrial applications—Congresses. I. Crescenzi, V. (Vittorio), 1942–
II. Dea, I. C. M. (Iain C. M.), 1943–
III. Stivala, Salvatore S., 1923– . IV. Title.
TP979.5.P6C66 1984 661′.81 85-5592
ISBN 2-88124-032-1

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PREFACE

Starch is perhaps the most widely distributed substance in the vegetable kingdom. It belongs to the group of carbohydrates in which sugars, gums, and cellulose are included. The principal applications of various starches, such as those derived from rice, maize, arrowroot, potatoes, and wheat, are in (a) industry, e.g., sizing in the textile industry and thickening mordants, (b) food, (c) laundry, and (d) pharmaceutics and cosmetics.

Cellulose, the main ingredient of the cell walls of higher plants, comprises at least one-third of all vegetable matter. Cotton and wood are the principal sources of cellulose of industrial interest. Cellulose and its derivatives have been known for more than a century; the first stable derivatives, nitrate and acetate, were prepared in 1883 and 1869, respectively. These were French products, as was cuprammonium rayon, the first regenerated cellulose fiber, which was introduced in 1890. In 1892, the more important viscose rayon (cellulose xanthate) was developed in England. Celluloid, a homogeneous colloidal dispersion of cellulose nitrate and camphor, was developed in the United States in 1870. Enormous development took place after 1920 in the industrial use of cellulose for the production of rayon, cellophane, plastics, lacquers, and adhesives.

While the exploitation of starch and cellulose was the first stage in the industrial utilization of polysaccharides, a wide range of different polysaccharides currently find commercial applications. These include (a) plant gums and mucilages, e.g., gum arabic, pectin, and locust bean gum, (b) polysaccharides of marine origin, e.g., alginate, carrageenan, and chitin, (c) microbial polysaccharides, e.g., xanthan, dextran, and levan, and (d) ionic polysaccharides from mammalian tissues, e.g., heparin, hyaluronic acid, and chondroitin sulfate. Today, countless industrial companies throughout the world are devoted to the extraction, purification, and modification of these polysaccharides for use in a wide range of applications. It is, therefore, not surprising that there has been a proliferation of technical publications dealing with not only product development and industrial processes but also the fundamental elucidation of the chemistry of these complex polysaccharides, covering biosyntheses, physicochemical characterization, degradation, chemical modification, reaction kinetics and mechanisms, structure determination, and structure-function relationships in biological applications. The last mentioned has led to an increased level of basic and applied research pointing towards emerging applications of polysaccharides in the development of human vaccines and drugs.

Because of these factors, *The International Workshop on New Developments in Industrial Polysaccharides,* held at the Stevens Institute of Technology, Hoboken, New Jersey on May 31 and June 1, 1984, was most timely. The workshop was organized to provide a forum for the communication of results and the exchange of ideas among all the participants. The program was divided into four consecutive sessions, each session having only three invited speakers followed by one hour of open discussions, each being led by a guest discussion leader. The format of the program may be found towards the end of this book. The workshop, chaired in its entirety by Dr. R. L. Whistler, was attended by scientists active in polysaccharide research. The round-table discussions following each session were lively, with short communications from many of the participants. This volume contains the written versions of the materials delivered by the invited speakers and the short communications that evolved from the participants during the open discussions.

The workshop was supported, in part, by the Department of Chemistry and Chemical Engineering and by the Polymer Processing Institute at the Stevens Institute of Technology. We wish to express our appreciation to Dr. Francis T. Jones, Head of the Department of Chemistry and Chemical Engineering, and to Dr. Luigi Z. Pollara, President of the Polymer Processing Institute, for their continued support and encouragement. We extend our thanks to Dr. R. L. Whistler, the invited speakers, the round-table discussion leaders, and the participants, all of whom helped to make this workshop an unqualified success.

The workshop was organized while V. C. was visiting professor at the Stevens Institute of Technology and I.C.M.D. was visiting professor at the Polytechnic Institute of New York.

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EARLY HISTORY OF STARCH AND CELLULOSE RESEARCH

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In 1811 Gay-Lussac and J. Thénard pointed out that starch and "ligneous matter" had an elemental composition equal to the sum of carbon and water and suggested the term "carbohydrate" for such substances.[1] At that time nothing was known about molecular structure, but Gay-Lussac noted in 1814 that "the composition of acetic acid does not differ significantly from that of ligneous matter. Here are two substances composed of carbon, oxygen and hydrogen in the same proportions which have quite different properties. This is a new proof that the atomic arrangement in a compound has the greatest influence on its character...Sugar and starch lead to the same conclusion."[2]

The observation that starch is converted in acid solution to a sugar was made accidentally in 1811 by Kirchhoff[3] who, for reasons unknown, hoped to convert starch into rubber. The discovery of the blue starch-iodine complex, made only three years after the discovery of iodine[4] was to prove a valuable analytical tool. In 1833 Biot and Persoz[5] found that the degradation of starch by acid or an enzyme which they called diastase, leads to a strongly dextrarotary product, which they named dextrin. This was at first thought to be an intermediate in the conversion of starch to sugar, but Musculus[6] showed that dextrin and sugar were produced concurrently. The sugar resulting from the enzymatic degradation of starch was identified in 1876 by O'Sullivan[7] as a disaccharide which he named maltose.

By 1879 Brown and Heron[8] wrote that they had a list of 400 publications dealing with starch. Research in this area was favored by the availability of three independent analytical methods employing the starch-iodine complex, the optical activity and the reducing power. Brown and Heron concluded that starch molecules contained at least ten maltose residues; these split off one maltose unit at a time with equilibrium being attained at 80% maltose and 20% dextrin.

In 1899 Brown and Morris[9] obtained a cryoscopic molecular weight of 6400 for dextrin, which they considered to be one-fifth of the starch molecule. However, ten years later the influence of the "colloid chemistry school" had made Brown and Millar[10] write that "the method of freezing is not applicable to colloidal products." Instead they lightly oxidized dextrin to a "dextrinic acid" and found that the carboxyl content suggested a chain of forty glucose residues in the dextrin and 200 in the starch. The distinction between amylose and amylopectin was clarified only at the beginning of this century by Maquenne.[11]

Strangely enough, the chain character of starch came again into disrepute as colloid concepts gained the upper hand. The influential organic chemist Karrer claimed[12] that starch is in colloidal solution analogous to colloidal silver solutions-true solutions are obtained for starch acetate just as with silver nitrate. He thought that the disappearance of the starch-iodine color, "a typical colloid phenomenon" when starch is acetylated was proof of this concept. As late at 1921, Karrer and Nägeli[13] argued that it made no sense to assume that plants would synthesize a polysaccharide which would have to be degraded again to the sugar used in the metabolism of the plant.

The history of cellulose research starts with Payen's discovery[14] that Gay-Lussac and Thénard were wrong in claiming that wood and starch had ths same elemental composition, but that after extraction with dilute nitric acid a substance with an elemental composition identical to that of starch can be isolated. This substance, characteristic of cell walls, was named cellulose. Payen believed that starch and cellulose differed merely in their states of aggregation whild Frémy[15] advanced the view that they were isomers.

In the early years "cellulose" was used to include a variety of polysaccharides since techniques to differentiate between different sugars were limited. Flechsig[16] found that hydrolysis of cotton yielded only glucose and Schulze[17] suggested that the designation of cellulose be restricted to "constituents of cell walls resistant to dilute acid and alkali, soluble in ammoniacal copper solution and yielding grape sugar on hydrolysis." Skraup and König[18] isolated in 1901 cellubiose octa-acetate from the acetolysis of cellulose but Hess[19] claimed as late as 1928 that the concept of cellulose built up from cellobiose residues was erroneous.

When x-ray crystallography was applied to cellulose fibers, the crystallographic unit cell was found to be surprisingly small. Although Polanyi[20] had pointed out that this cell could accommodate <u>either</u> two cyclic disaccharides <u>or</u> two disaccharide segments of polysaccharide chains, the second alternative was forgotten for a number of years. Later, Polanyi recalled that the fact "that the elementary cell contained only four hexoses appeared scandalous"[21], since it was inconsistent with the physical properties of cellulose. Yet, as in the case of starch, there was a strong tendency to assume that cellulose was an association colloid, and the small crystallographic unit cell was rationalized by assuming that "cohesive forces attain the order of magnitude of valence forces so that any distinction between them ceases to be meaningful".[22] Only in 1926 did Sponsler and Dore[23], who were convinced that a "satisfactory formula for cellulose must account for its physical properties", show that the x-ray diffraction pattern of cellulose is consistent with polysaccharide chains. Although their structure was incompatible with a chain built up from cellobiose units (they assumed alternating 1,1 and 4,4 linkages between anhydroglucose residues) they felt that the chemical evidence for this structure was inconclusive. However, Meyer and Mark[24] showed in 1928 that the x-ray structure is, in fact, compatible with chains built up from cellobiose residues. Their data could not distinguish between parallel and antiparallel chains, but the parallel arrangement was adopted since it seemed unlikely that a living organism could produce the antiparallel structure. A remarkable deduction of physical properties from this crystal structure was realized in the next few years when Mark[25] calculated the tensile strength of an "ideal cellulose" (which turned out to be about eight times the value for the best cellulosic fibers) while Meyer and Lotmar[26] estimated the elastic modulus from crystallographic and spectroscopic data.

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CAPSULAR POLYSACCHARIDES OF GRAM NEGATIVE BACTERIA

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Capsular polysaccharides of Gram-negative bacteria are discussed with particular reference to those of the genus *Klebsiella*. The polysaccharides from this genus are noteworthy for the diversity of their structural patterns, several of which are illustrated. It is now generally accepted that bacterial polysaccharides have highly regular structures and it is appropriate to think in terms of repeating units which, in the case of *Klebsiella* polysaccharides, may contain three to seven monosaccharide residues. Bacterial viruses, or bacteriophages, contain highly specific endoglycanases capable of depolymerizing capsular polysaccharides into oligosaccharides, corresponding to the repeating units, which will still contain labile groups such as pyruvate acetals or ester groups, if they are present in the original polymer. These capsular polysaccharides, and their related oligosaccharides, are easily prepared and represent useful models for studying the relationship between chemical structure and viscosity and as starting materials for the preparation of vaccines.

1. INTRODUCTION

Research groups studying the structures of non-mammalian polysaccharides are finding a wealth of interesting data in the bacterial kingdom. In order to appreciate the significance of current results and to put these investigations into perspective it is useful to review briefly the history of polysaccharide structural determinations.

The determination of the primary chemical structure of polysaccharides dates from the second decade of this century following the introduction of the Haworth method of methylation. Substances studied initially were those that were readily available in large quantities, suitable for the methodology of the day - fractional distillation of methylated sugars and the preparation of crystalline derivatives - and included cellulose, starch and glycogen. Each of these is a neutral polymer of D-glucose and is, thus, a homoglycan. There then followed a period when many plant gums, such as acacia, tragacanth and mesquite gums, were examined and these were found to be acidic heteroglycans, where the acidity is due to uronic acids. Another type of acidic polysaccharide is represented by that from algae where the anionic character is caused by sulfate groups.

During the 1950 and 1960's many research groups shifted their focus to other plant polysaccharides, the hemicelluloses, both those from annual plants and from trees. It is only within the last 15 years or so that systematic studies on bacterial polysaccharides have been carried out by many different groups and the results have given a new dimension to the meaning of "polysaccharide structure". Why is this so?

Despite the excellent early work on plant polysaccharides the results were inconclusive in an exact sense since these types of polymers do not have regular structures and,