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Research of algal polysaccharides and related natural compounds in Estonia

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Abstract. The results of the investigations of the structure and composition of real natural algal polysaccharides, their fractionation, and chemical modification are summarized against the general scientific background. Elucidation of a suitable agarose matrix from the red alga *Ahnfeltia tobuchiensis* and the synthesis of affinity carriers on this basis are shown. Elaboration of technological schemata of red algae processing, immunochemical investigations, and lectin research are covered as well.

Key words: algal polysaccharides, gelling galactans, alginates, agarose, affinity carriers, furcellaran.

INTRODUCTION

Although some natural algal polysaccharide mixtures have been used already for centuries, their fractionation and structural investigations were started quite recently. Utilization of agar in bacteriology began more than a hundred years ago since the famous works of Robert Koch [1]. Structural research of agar took its beginning from investigations of Araki [2] and lasted about thirty years, until the end of the 1960s [3]. In the course of this troublesome study series, which involved purely chemical methods, a unique component sugar, 3,6-anhydro-galactose, was discovered [4] and an idealized extreme structure type of agarose (Fig. 1) was defined [5].

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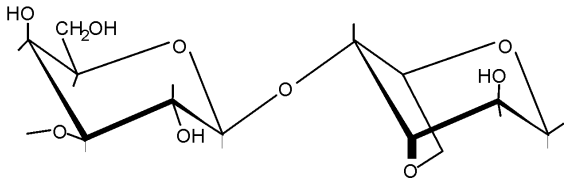

Fig. 1. Idealized disaccharide repeating unit of agarose.

Full complexity of agars as a continuous spectrum of polysaccharides, depending on their natural source, was established by Duckworth & Yaphe in the early 1970s [6, 7]. Also, some generalizations regarding the structure of gelling galactans were made. Among them was the hypothesis of "masked repeating structure" [8] inherent in all the galactans, and extensive elucidation of gelling properties, principles, and mechanisms [9] in hydrated galactan systems.

Briefly, such was the general scientific background in the field of galactan chemistry of the middle of the 1970s when the research project of red algal galactans was started at the Institute of Chemistry, Estonian Academy of Sciences. This research into galactans, especially agars, was the beginning of investigations of polysaccharides in Estonia. Later, some other related subjects such as alginate and lectin chemistry, applications in affinity chromatography, immunochemical investigations, etc. were added to the main galactan topic. Also, the scope of galactan research was broadened to include carrageenans of the Baltic Sea, especially the furcellaran from the red alga *Furcellaria lumbricalis*.

It should be mentioned that the scope of all the activities described below was exceptionally wide. The whole research process might be viewed from the following aspects.

1. *Structural types of the compounds* investigated: galactans (agars, agaroses, carrageenans) and products of their modification, polyuronates (alginates) and lectins.

2. *Types of methods used*: purifying, fractionating, and beading techniques; methods for the determination of the structure and composition of polysaccharides; structure modification; activation and cross-linking methods; technological elaborations on the pilot production level; immunological methods involved; etc.

3. *Major research topics* (connected with usability and/or application parameters of elaborated polysaccharide systems and carriers): kinetics (of algae extraction, gel forming, and activation); rheology (viscosity, gel strength); separation techniques tested (gel filtration, affinity chromatography, fractionating precipitation, etc); immunology (especially separation and specific activity preservation of antibodies); and some others.

4. *Biological background*: investigated algae species and habitats; dependence of the polysaccharide content of algae on the collection time; expeditionary activities of the groups involved; etc.

5. *Organizational activities* and institutions involved; cooperation projects; participation in (or organizing of) symposiums and other meetings; etc.

Of these five aspects, the two last are discussed together in the next section. The remainding three aspects are treated later.

The aim of this article is to analyse the development of the research into polysaccharides and related compounds in Estonia and to summarize the main innovative and scientific trends and results of 25 years of this complex activity.

EXPEDITIONARY AND ORGANIZATIONAL PRELIMINARIES

The whole cycle of investigations concerning algal polysaccharides was introduced by elucidation of promising seaweed species containing a nearly neutral galactan fraction close to agarose (Fig. 1). For collecting samples of various algae several series of expeditions were organized. Because of the political situation of Estonia in the 1970s, the area for possible expeditions was strictly limited to the territory of the then Soviet Union. However, this large region had a very extensive coastline and many bodies of water.

Expeditions were organized yearly from 1976 to 1990, mainly in summer or autumn, in some years simultaneously by different groups (later less often). As a final result, all the large bodies of water were observed on the whole territory of the Soviet Union. At the end of the investigations attention was focused on the Far East. The major destinations of expeditions by years were as follows:

– The region near Vladivostok (Primorye and/or islets in Amur Bay, the Sea of Japan: Popov Island, Reineke Island): 1976–78, 1981–84, 1988.

– South Kamchatka: 1977, 1978, 1982, 1987, 1988.

– Sakhalin and/or the South Kuril Islands (Kunashir and Iturup): 1978, 1983–86.

The extreme North-East Asian region was the target of investigations in 1977 (Bering Island, Provideniya), in 1979 (Anadyr, Uelen, Ratmanov Island), and 1981 (Cape Schmidt, Nutepelmen). The region of Lake Baykal in Siberia was investigated in 1982.

In Europe, extensive shorelines of the Black Sea and Caspian Sea were researched mainly in 1980 and White Sea regions (Solovets Islands, Arkhangel′sk) in 1985. The Baltic Sea areas (Kassari Bay, Saaremaa Island, etc.) were studied mainly in 1993–2000. Also some other trips were made, for instance to the Aral Sea, Lake Il′men (close to Novgorod), the Barents Sea (near Murmansk), and Lake Sevan (Armenia).

As a result of the expeditionary activities, an exceptionally wide area of Eurasia was fully sampled, especially from the standpoint of resources for potential industrial use of red macroalgae containing gelling galactans of various structure and rheological properties. In case of need, algal strata were observed and procured by using aqualungs (including in such extreme northern regions as the Chukotsk Peninsula). Local practices of storing, preparation, and processing

of seaweeds were studied on the spot (all the major agar/agaroid factories of this tremendous territory were visited). Seaweeds were gathered by researchers themselves, which guaranteed the necessary quality and sure geographic origin of the samples. Later, the samples served as a suitable raw material for pilot production of agarose and affinity chromatography carriers. A variety of large algae samples for voluminous extracting investigations were collected. Some preliminary experiments on artificial cultivation of red macroalgae (*Ahnfeltia tobuchiensis* and *Gracilaria verrucosa*) were also undertaken (in Tallinn). However, artificial cultivation appeared to be too expensive for extensive use.

Persons and groups participated very dynamically in the course of the development of the polysaccharide programme. The whole project was initiated by Andres Kollist in 1975; some key persons in Tallinn (Aavo Aaviksaar, Jaan Paris, Merike Vaher) and Tartu (Tõnu Püssa) were involved from the very beginning. From time to time, numerous persons and groups from several biochemical and medical research institutions in (or outside) Estonia joined in. For example, all in all 55 persons took part in the complex programme "Agarose and spheron carriers for chromatography" supervised by Kollist [10], and accomplished in 1986–90. They were mainly from the Institute of Chemistry of the Estonian Academy of Sciences and the University of Tartu (in addition, some people from the Institute of Chemical and Biological Physics, Tallinn, and from N. F. Gamaleya Institute of Epidemiology and Microbiology, Moscow, Russia, were involved).

Nevertheless, the main group of algal polysaccharide researchers at the Institute of Chemistry was not numerous. The group was formed and supervised by Andres Kollist in 1975–90 (since 1991 by Kalle Truus) and consisted of 11 persons as a maximum. Two smaller groups were very important as well, especially in the 1980s: one at the University of Tartu (Tõnu Püssa, Peeter Toomik, Riina Mahlapuu and others) and the other at the Institute of Chemical and Biological Physics (Mati Haga and others).

The members of the groups from the Institute of Chemistry and from the University of Tartu took part in organizing expeditions and conferences. The 11th International Lectin Meeting (Tallinn–Tartu 1989) with participants from 35 countries [11] deserves special mentioning.

ALGAL EXTRACTING AND TESTING OF POLYSACCHARIDES

One of the initial aims of the red algal galactan investigations was to elucidate some important matrix properties of polysaccharides in the seaweeds growing on the territory studied. The characteristics of main interest were charge density of the matrices and average molecular mass of the polysaccharides. These characteristics determine many important properties in practical work, such as rheological behaviour (viscosity, gel strength, etc.), interactions with proteins, and so on. On the other hand, the molecular as well as rheological properties of the galactans from algae are closely connected to the extracting dynamics of

algae, because seaweeds do not contain any certain compound but a wide spectrum of similar polysaccharides. The group had also an aim to elaborate isolation and purification methods for the fractionation of possibly homogeneous galactans.

The first results of the work were presented at meetings of young scientists, at conferences of the Baltic States and of the USSR (13 abstracts in 1977–80). A series of six articles [12–17] was published in 1980.

Seven most promising algae species from this extensive Eurasian region were selected and treated in publications. Gelling substances were isolated from the following seaweeds [12]: (1) *Furcellaria lumbricalis* I (Hiiumaa Island in the Baltic Sea), (2) *Ahnfeltia plicata* and (3) *F. lumbricalis* II (Solovets Islands in the White Sea), (4) *Phyllophora nervosa* (Odessa Region in the Black Sea), (5) *Ahnfeltia tobuchiensis* I and (6) *Gracilaria verrucosa* (Gulf of Peter the Great in the Sea of Japan), and (7) *A. tobuchiensis* II (Kunashir Island, the Kurile Archipelago in the Pacific). According to the extraction kinetics of gelling galactans, the studied seaweeds were divided into two groups (algae species 1–4 and 5–7). Further, the changes of galactan parameters in the course of algae processing and in the fractionation of the gelling substances were investigated. Special attention was paid to the dependence of gel strength on the homogeneity and sulphur content of the galactans [13].

It was shown that the characteristics of laboratory agars and their quality strongly depend on the extraction time. A large number of algae species were tested and some fractions of very high gelling properties were selected. Agars with the highest gel strength and satisfactory electroendoosmosis (EEO) properties were obtained from certain fractions of the red algae *A. tobuchiensis* (I and II) and *G. verrucosa*. The qualities of these agars surpass the corresponding qualities of all commercial agars [14].

At that time the relationships between various gelling properties of algal galactans were not exactly known yet, especially in the case of the seaweed species mentioned. For this reason, some correlations of rheological parameters were elucidated in the following papers of this series [15–17]. Also, some agarose fractions were already isolated from agars of *A. tobuchiensis* [15] and characterized by their EEO properties and zone electrophoresis of human serum proteins [16]. Very promising results in this field led to some practical solutions as well: synthesis of affinity carriers on an original agarose matrix basis never used for this purpose before. These agarose sorbents found wide use in biochemistry and medicine from the early 1980s onwards (will be observed later). Further, some more extensive generalizations were presented [18] about correlations between the gel strength, average viscometric molecular mass of galactans, and their EEO characteristics ($-m_r$).

It was shown that the gel mechanical strength ($\lg G$) for chemically similar agaroses increases linearly with increasing average viscometric molecular mass of the polysaccharide. When correlating the deviations ($\Delta \lg G$) of the points for agars and agaroses with the EEO characteristics it was observed that the points

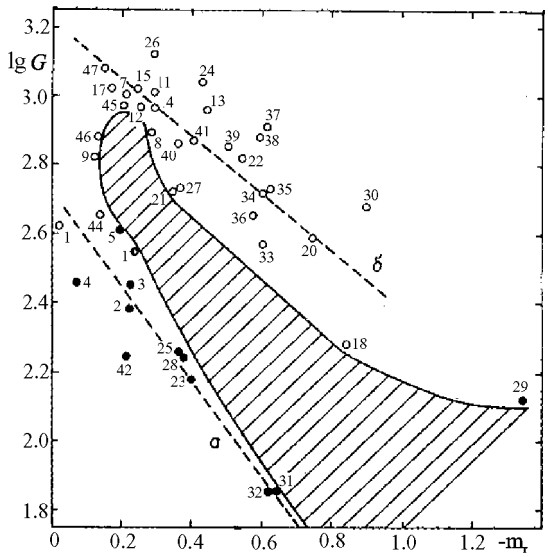

Fig. 2. Correlations between mechanical strength and electroendoosmotic properties of agarose gels: formation of two domains.

fall into two groups. In the interval where $-m_r > 0.2$ an increase in $-m_r$ is accompanied by a decrease in gel strength and at the values of $-m_r < 0.2$ the opposite occurs (Fig. 2). The voluminous experimental results revealed also that the algae investigated initially do not contain gelling galactans of average molecular mass in the interval of 60–140 thousand [18]. Later, the conclusions of this work were analysed by Norwegian scientists [19]. Mostly on the basis of the six articles cited, Kollist defended his Cand.Sci. degree in 1981 [20].

INVESTIGATIONS OF THE COMPOSITION AND STRUCTURE OF ALGAL POLYSACCHARIDES

Some technical analyses of the composition (ash and sulphur content mainly) of galactans have been performed almost from the beginning of the project. The group started investigations on the integral structure of agarose in the second half of the 1980s. The findings were first presented at some all-USSR symposiums and at Eurocarb VI and published in the abstracts of these meetings [21–23]. The results of the study of the structure of galactans, mainly agaroses, with some additional aspects were published also elsewhere [24, 25].

These structure investigations in Estonia were based already on ^{13}C-NMR spectroscopic methods, which have been applied to gelling polysaccharides from the end of the 1970s. This approach, developed by two independent groups [26, 27], was found to be exceptionally fruitful and is now an obligatory

component for the elucidation of galactan structures instead of very troublesome chemical procedures. In our first studies, ^{13}C-NMR spectroscopy was used to investigate structural differences between commercial preparations of agarose of various firms. By detecting the basic signal assignments of the disaccharide unit with high accuracy it was shown that the quite low methylation degree actually present in agaroses does not cause, contrary to earlier results, differences in chemical shift values in the cases of real matrices [24].

In spectroscopic investigations various methods of methoxyl and sulphur content determination were comparatively applied and a large number of agars and agaroses were tested [K. Truus & M. Limberg, unpubl.] since the content and location of methyl and sulphate groups in disaccharide units are the major causes of structural and rheological differences between various galactans. Later, chemical analysis combined with NMR spectroscopic methods was applied also to other polysaccharides, particularly to furcellaran [28–30]. At present, alginates from the Baltic brown alga *Fucus vesiculosus* (Fig. 3) are being studied by these methods.

Reliability and advantages of various methods of functional group analysis in algal galactans have been investigated during a long period. The data were summarized and analysed with structure determination methods including NMR spectroscopy and X-ray diffraction analysis [31]. Receiving high-quality agarose films and their orientation (stretching out) prior to X-ray analysis caused some problems. This orientation is important for obtaining regular and informative microzones in isotropic agarose films. As a matter of fact, there was only one research paper concerning agarose orientation [32] at that time. Nevertheless, stretching methods were elaborated and satisfactory röntgenograms were achieved [33]. Also, a computer-controlled stretching device was constructed,

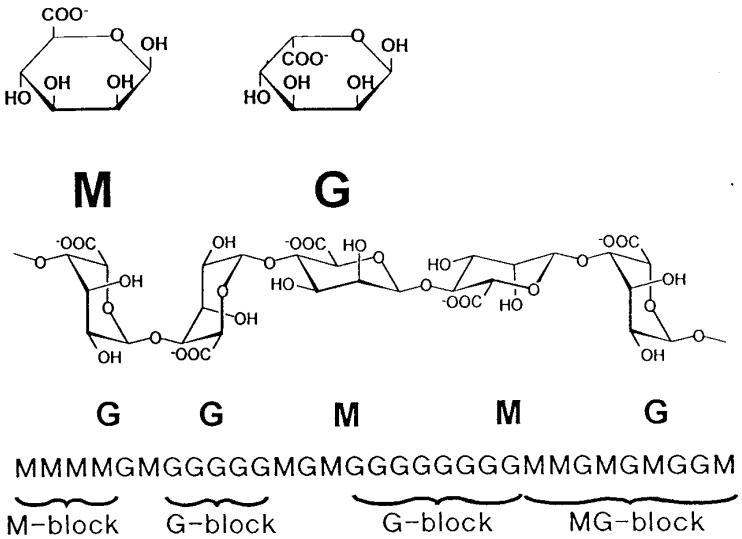

Fig. 3. Structure of alginates: an example of alginate chain fragment. M, β-D-mannuronate; G, α-L-guluronate.

and a package of programs was compiled for calculating cylindrical Patterson functions with their graphical presentation and finding the atom coordinates of agarose by the method of linked atoms [34].

Structural investigations of carrageenans (Fig. 4) from Baltic red macroalgae were carried out in the second half of the 1990s. The results were presented at some European symposiums [28–30, 35] and summarized in papers [36–38]. Also some aspects of the structure–functionality relationship were treated in these papers.

It was shown that the galactan mixture in *F. lumbricalis* (furcellaran) from Kassari Bay contains 6-O-methylgalactose and 2-O-methyl-3,6-anhydrogalactose in addition to the unsubstituted residues in their composition (Fig. 5). The main structural features of this complex polysaccharide are presence of kappa carrageenan [37] and absence of iota fragments in furcellaran [38].

The low gel strength of the native (water-extracted) furcellaran may be considerably (about five times) increased by alkaline extraction of the seaweeds. From this standpoint, the most suitable medium for extraction with simultaneous modification of these algal galactans is potassium hydroxide solution at a concentration of 0.16 M. Another red alga occurring in Kassari Bay, *Coccotylus truncatus*, may also be used for the production of a gelling galactan mixture by alkaline extraction without any structural modification of the carrageenans from this species [37]. It was shown that *C. truncatus* contains viscous galactans of iota carrageenan type, their structure is close to those from *Phyllophora brodiaei* (the Black Sea) [39].

This comparative structural approach was summarized as a Master's thesis in 1999 [40]. The thesis elucidated the chemical composition (including micro-elements, sulphur, and monosaccharide residues), the localization of sulphate and methyl groups in the repeating disaccharide units, and the structure of the latter. In this context, the criteria for the gelling ability of the carrageenans were specified.

During a long period the content of micro- and trace elements in algal polysaccharides and in seaweeds has been investigated mainly by methods of neutron activation analysis.

As to agarose, the main aim was to give a possibly complete characterization of the real natural matrix and develop its purification process. The level of biologically active microelements in agarose carriers is also of interest for finding them applications in biochemistry and biotechnology [41]. Depending on

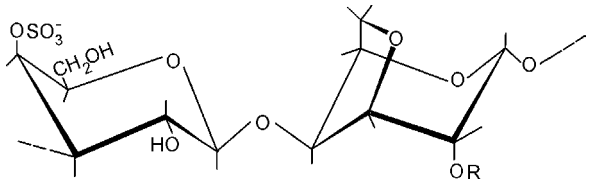

Fig. 4. Disaccharide repeating unit of carrageenan chain consisting of D-galactose and 3,6-anhydro-D-galactose.

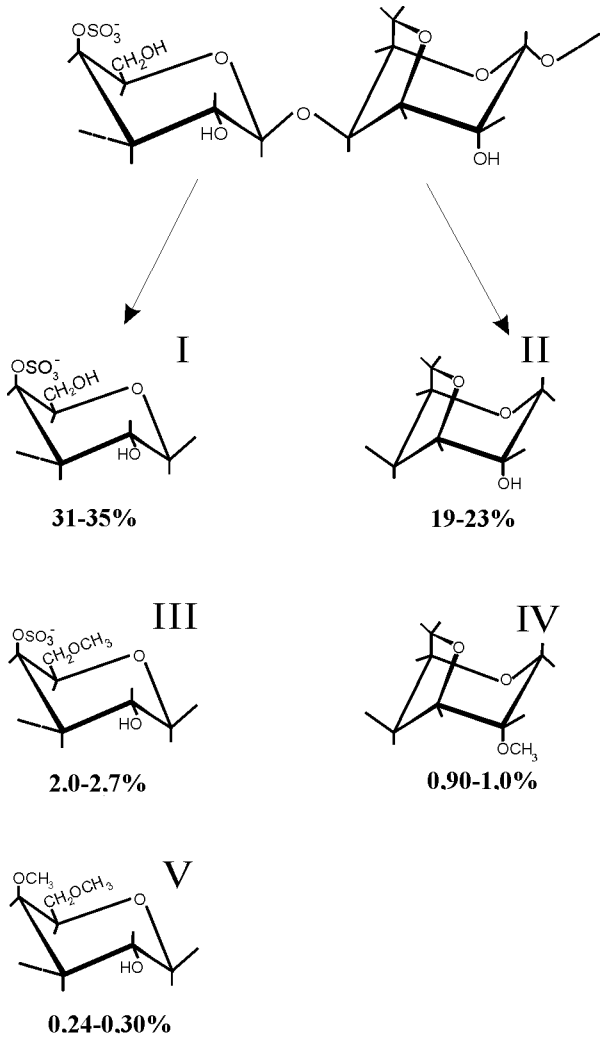

Fig. 5. Monomer composition of furcellaran (from *Furcellaria lumbricalis* of the Baltic Sea; % of air-dry matter).

objects, interests, and possibilities, the content of more than 30 elements has been analysed. To follow the changes in the content of micro- and trace elements in agarose processing, the concentration of 22 elements and the dynamics (purification multiplicity) of elemental composition were studied [42].

It was shown that various elements get detached very differently in the course of agarose isolation and purification process. As a rule, no strong dependence upon an element's position in the periodic table was observed. Some of the elements (Na, Mg, Zn, etc.) have a considerable affinity to the agarose matrices and they cannot be removed below a certain level during the purification process.

However, halogens (especially iodine) are easy to separate from agarose [42] as well as from carrageenans [38] and they should not have a noticeable affinity to these galactan matrices. Consequently, the unusually high concentrations of iodine and bromine in algae (typically, 10^{-1}% as an order of content for both halogens) should be related to other chemical structures of the cells.

As to brown algae of the Baltic Sea, it was elucidated that the mineral composition of the biomass from *F. vesiculosus* is rich in light elements (Mg, K, Ca, Na) and halogens (Br, I). Its arsenic content is also high (over 300 ppm); however, the content of other toxic elements (Hg, Sb, Se, etc.) is extremely low. The mineralization of the algal biomass practically does not depend on seasonal varieties. The content of α-L-guluronic acid residues, and therefore the viscosity of alginates from the Baltic *F. vesiculosus*, is relatively low [43].

AGAROSE CARRIERS FOR GEL FILTRATION AND AFFINITY CHROMATOGRAPHY

When our project was started agarose carriers were introduced to gel filtration and affinity chromatography [44]. The main stages of the preparation of novel agarose carriers are as follows: (1) finding a natural agarose matrix suitable for biochemical applications and (2) elaboration of beading (granulation) procedures to give good flowing characteristics for the eluent to the gel filtration media. These two kinds of procedures may be followed by (3) cross-linking of the matrix for raising the mechanical strength of agarose beads and (4) activation of the granulated agarose gel to obtain carriers for affinity chromatography.

For conclusive elucidation of a suitable matrix, some technical problems faced in beading and cross-linking of the novel agarose were resolved and a starting material with good flowing characteristics (for eluent) was obtained. Single agarose gel beads of various concentration (mainly 2, 4, and 6% of the agarose content) were tested for use in gel filtration techniques. Because of the high gel strength of the original agarose from the alga *A. tobuchiensis* (the Gulf of Peter the Great, the Sea of Japan), the granulated medium guarantees a high flowing velocity of the eluent under a liquid column pressure. This galactan matrix has also other advantageous characteristics (particularly, a low charge density) for use in liquid chromatography, being comparable to other best agarose carriers [17].

The novel agarose was activated by methods known before: using cyanogen bromide [45, 46], divinyl sulphone [47], 1,1'-carbonyldiimidazole [48], active dyes [49], and some others (Table 1). Also, some original activating agents were introduced and new affinity carriers were developed.

The activated matrices were used directly or additional spacers via specific groups were coupled. The spacers may permit the use of milder binding conditions or a more suitable attachment of a ligand (due to the specific steric conditions).

Table 1. The main activated agarose matrices used

Activation	Linkage to agarose	Available reactive group	Specificity of group	Bond type; stability of attachment
Cyanogen bromide	Isourea (finally)	Cyanate ester	Amine	Isourea; moderately stable
Carbonyl-diimidazole	Carbamate	Imidazolyl carbamate	Amine	Carbamate; good stability below pH 10
Epichloro-hydrin Epoxy(bis)	Ether	Epoxy	SH > NH_2 > OH	Thioether, sec amine ether; all very stable
Vinyl sulphone	Sulphonyl thioether	Vinyl sulphone	SH > NH_2 > OH	Thioether, sec amine ether; good stability below pH 9

A new structural type of affinity carriers was worked out in collaboration with N. F. Gamaleya Scientific Research Institute of Epidemiology and Microbiology. The carriers called Odigose and Odifil (ethylsulphoactivated agarose, Fig. 6) are preparations with variable degree of active groups, suitable for synthesis of antigenic immunosorbents [50]. It was established that some antibodies and enzymes such as muscle aldolase retain their specific activity after immobilization on these sorbents [51]. Technological regulations for the production of ethylsulphoactivated agarose and antigenic immunosorbents on its basis were worked out at the Institute of Chemistry in 1986–87 [K. Truus, unpubl.]. Also a method for the isolation of medicinal γ-globulin preparations against *Staphylococcus* from human plasma by these immunosorbents was elaborated with N. F. Gamaleya Institute and the Institute of Sera and Vaccines of Ufa (Bashkiriya) [52].

Another type of sorbents for medical use was elaborated with the researchers of the Oncological Scientific Centre (Moscow, Russia) for the isolation of a cancer-embryonic antigen from tumour tissue [53].

In many cases traditional carriers were synthesized and used, the reaction dynamics, optimum conditions, and/or possible mechanisms were studied. The activation kinetics of the reaction with some agents such as cyanogen bromide and 4-(β-oxy-ethylsulphonyl)-2-aminoanisole was thoroughly investigated [J. Paris & K. Truus, unpubl.] but insufficiently published [31, 54, 55]. From the extensive investigations only some brief overviews, mainly about the synthesis of agarose-based ion exchangers [56–58], were published. The activation dynamics

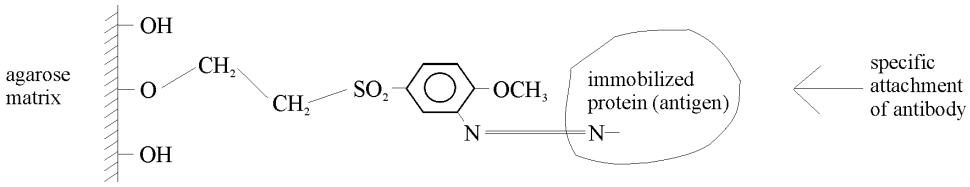

Fig. 6. Structure and use of ethylsulphoactivated agarose with immobilized antigen.

of the agarose matrix with 1,1'-carbonyldiimidazole (CDI) and the yield of the carrier (CDI bound) in various conditions were investigated [59]. An extensive overview on affinity carriers [60] was published by researchers from the University of Tartu at the same time.

APPLICATIONS AND ELABORATION OF TECHNOLOGICAL SCHEMATA

Technological investigations and applications have been of great importance in the group's activities from the very beginning of the project. The first programme of national level in which the 'agarose group' was already involved was carried out in 1976–80. By that time the methods of isolation, fractionation, and pilot production of a novel agar and agarose had been elaborated [61]. The next step was aimed at obtaining a CNBr-activated matrix for the production of a variety of affinity sorbents for practical use in biochemistry. For this purpose, a safe method of cyanogen bromide preparation was introduced [54]. Other activation methods, described in Table 1, were also used to produce numerous affinity carriers.

The main types of agarose preparations produced in Estonia were the following:

1. Agarose-based gel filtration media: beaded 4% or 6% agarose gel, and the same, cross-linked; fractionation range of molecular mass 6×10^4–2×10^7 (for 4% gel) or 1×10^4– 4×10^6 (for 6% gel).

2. Ion exchangers on agarose: cross-linked carriers diethylaminoethyl agarose (anion exchanger) and carboxymethyl agarose (cation exchanger).

3. Affinity chromatography carriers: a variety of agarose matrices of direct activation (Table 1) and those with additional groups (for example, aminohexyl agarose, containing hexamethylene diamine bound to a CNBr-activated matrix); and also dye ligand carriers (containing triazine active dyes or arylvinylsulphone groups, see Fig. 6).

As a rule, detailed methods of preparing, analysis, etc. of these carriers are described in reports [10, 62] and in numerous classified regulations. All the agarose preparations (over 30 different products) have full documentation for manufacturing on a pilot plant scale. The carriers have been produced by one to three Estonian institutions (Pilot-Production Plant of the Institute of Chemistry in Tallinn, firms Kemotex in Tallinn and Aire in Tartu County), particularly in 1983–91. Some of them are still produced though only by Kemotex at present.

The groups at the Institute of Chemistry and the University of Tartu have elaborated some technological schemata for algae processing. The first of them was a scheme of agar and agarose production from the red alga *A. tobuchiensis* of the Far East discussed above.

In the first half of the 1990s, particularly in 1993–94, some technological investigations concerning the Baltic red alga *F. lumbricalis* and a commercial carrageenan product from this seaweed were carried out at the Institute of Chemistry. As a result, the basic principles for industrial production of high-

quality carrageenan from this alga species were formulated [36, 37]. In 1995–2000 the group completed an Estonian State Programme (in cooperation with researchers of the Estonian Marine Institute) of industrial utilization of algae resources of the Baltic Sea. The part of the programme concerning red algae of Kassari Bay was fulfilled in 1995–97 [63]. This investigation included also a full technological scheme for processing the algae *F. lumbricalis* and *C. truncatus.*

Some strictly technological investigations, particularly those concerning the effects of drying on the gelling properties of furcellaran, were made later by another group of Tallinn Technical University [64].

SOME OTHER RESEARCH TOPICS AND PERSPECTIVES

As it was briefly mentioned, some topics of interest concerned immunochemistry and blood fractionation [50–53]. The Tartu group was also interested in human plasma treatment [65].

Other objects of research were lectins and their complexes with carbohydrates, which play an important role in sugar research. Lectin from the edible snail *Helix pomatia* and the carbohydrate complexes were investigated in greater detail [66–68]. Also potato lectin [69] and some other lectins were investigated. Expeditions to obtain natural raw material were organized to the other Baltic states (Lithuania, Latvia) at the end of the 1980s and in 1990.

Gelling kinetics and effects of electrolytes, solvents, etc. were investigated mainly during the second half of the 1980s. The findings were presented at conferences in Estonia and elsewhere [25, 70]. Also rate constants of gel formation and energy of hydrogen bonds in agarose gels were calculated. This kind of research will be continued in the future and some visualization techniques for polysaccharide aggregates will be developed.

At the present time, the main investigation themes are connected with the polysaccharic structures in brown algae of the Baltic Sea. Although the Baltic brown algae, particularly *F. vesiculosus*, have been of interest for over 70 years in Estonia, their more extensive research started only in 1998 [71]. Some conclusions from these investigations [43] were mentioned before in this paper. Probably, the minor components of *F. vesiculosus* (fucans, fucoidan, etc) are of greater interest than the alginates of this species. Fucoidans have a very interesting structure and promising biological activity. The fraction of low molecular mass fucan of *F. vesiculosus* has *in vitro* anticoagulant activity [72]. Some fractions of this seaweed show also a high potential as antioxidants [73].

FINAL REMARKS AND ACKNOWLEDGEMENTS

Summarizing the activities and results of the groups, it should be mentioned that most of the time the conditions for creative work were relatively good. The wide area of scientific interests and investigations had found recognition and

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Vetika polüsahhariidide ja nendega seonduvate looduslike ühendite uurimine Eestis

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Käsitletud on puna- ja pruunvetikates sisalduvate spetsiifiliste polüsahhariidide (galaktaanid, alginaadid) ja nendega seonduvate ühendite (lektiinid, teatud valgud, modifitseerivad agendid jt) uurimist Eestis aastatel 1975–2002. Ülevaatlikult on esitatud vastavate uurimuste kõik põhilised, erinevate uurimisrühmadega seotud aspektid.

Ekspeditsioonidel kogutud Põhja-Euraasia punavetikaliikidest selekteeriti sobivad galaktaankomponendid ja määrati nende struktuurispetsiifilised omadused. Näidati, et biotehnoloogiliseks maatriksiks sobib *Ahnfeltia tobuchiensis*'e (Jaapani meri) polüsahhariidskelett. Selle vetikaliigi baasil töötati välja meetodid elektroneutraalse komponendi (agaroosi) eraldamiseks, modifitseerimiseks ning biokeemilisteks rakendusteks. Sünteesiti ja juurutati tootmisse rida uudseid ning spetsiifilisi agarooskandjaid kasutamiseks geelfiltratsioonis, afiinsuskromatograafias, immunotehnoloogias jm.

Laialdaselt on uuritud vetika polüsahhariidide koostist ja struktuuri ning füüsikalis-keemilisi omadusi (reoloogiat, kineetikat). On välja töötatud tehnoloogilised skeemid agaroidide, agarite ja agarooside tootmiseks erinevatest vetikatest. Läänemere liikidest on põhjalikumalt uuritud punavetikaid *Furcellaria lumbricalis* ja *Coccotylus truncatus* ning pruunvetikat *Fucus vesiculosus*.

Kirjeldatud töödega loodi baas polüsahhariidstruktuuride uurimiseks ning arendati erinevate vetikaliikide teaduspõhiseid kasutusvõimalusi Eestis.