

☒ Patents/Apps ☐ Non-Patent Literature

SEARCH
RESEARCH
MPEP 2.0
TOOLS & RESOURCES
ACCLAIM IP
HELP

The Sephadex story.

Abstract:

The environment and the circumstances leading to the Sephadex group of products for gel filtration (SEC, size exclusion chromatography) are described. The importance of Porath's and the author's experience from zone electrophoresis is stressed. To be able to get high resolution the technique for packing columns was of decisive importance for electrophoresis. It paved the way for the observation of the separation properties of dextran gels. The synthesis of dextran-based gels at AB Pharmacia and the development of varieties of them for varying applications are described together with the project leading to their marketing in 1959. The work of the author on gel filtration is summarized. The story is based on publication and memory. Aids to the latter have been laboratory notebooks and other documents still in the possession of the author.

Subject:

Electrophoresis (Research)
Colloids (Product development)
Filtration (Research)

Author:

Flodin, Per

Pub Date:

08/01/1998

Publication:

Name: Polymer Engineering and Science **Publisher:** Society of Plastics Engineers, Inc. **Audience:** Academic **Format:** Magazine/Journal

Subject: Engineering and manufacturing industries; Science and technology **Copyright:** COPYRIGHT 1998 Society of Plastics Engineers, Inc. **ISSN:** [0032-3888](#)

Issue:

Date: August, 1998 **Source Volume:** v38 **Source Issue:** n8

Accession Number:

21187765

Full Text:

INTRODUCTION

In 1950, after having finished a fil.kand. (B.Sc) exam at the University of Uppsala, I approached Professor Arne Tiselius at the Institute of Biochemistry there to become a graduate student under his supervision. I was admitted, but he was initially reluctant because of the limited lab space he had at his disposal. It was caused by the stream of foreign researchers coming to his lab after he received the Nobel Prize in 1948. However, I was allowed to use about one meter of a laboratory bench and shared access to a fume cupboard in a small lab that was the last left for his private use. I shared the lab with an American and a Norwegian guest researcher. In spite of, or maybe because of, the overcrowding, enthusiasm and creativity were on a very high level.

ELECTROPHORESIS (1950-53)

After a small project that failed, Professor Tiselius asked me to study paper electrophoresis. After the success of paper chromatography many researchers had tried to separate substances by electrophoresis in filter paper hanging in a chamber saturated with water vapor in a manner similar to that used with solvents in paper chromatography. However, the heat evolved by the electric current made electrophoretic separations difficult because of evaporation and subsequent uncontrollable buffer streaming. In particular, determination of mobility and isoelectric points. The idea of Tiselius was that by performing the experiment between glass plates these disturbances could be avoided.

I took over after Dr. Henry Kunkel, who had spent a post-doc year in Uppsala trying to improve the method. The information he left was sparse, so I had to start almost from scratch. A paper by Kunkel and Tiselius appeared a year later (1) that was of great help when Tiselius and I wrote a review paper (2).

I worked hard with the glass plate electrophoresis method but the progress was slow. I was not able to eliminate the deformation of zones of the blood serum proteins I used as test objects. Severe tailing and adsorption to the filter paper could not be avoided by any of the numerous treatments of both the paper and the glass plates I tried. However, the experiments led to a discovery that was of use in my later work. More about it later on.

Half a year after me, Jerker Porath came to the Institute. He was fil. lic. (PhD) in organic chemistry and supposed to introduce more pure chemistry into an institution dominated by physical chemists working with heavy equipment like ultracentrifuges, boundary electrophoresis apparatuses, and so on. Tiselius was inspired by his friend Prof. C.H. Lee from the University of California, Berkeley, and it led to Porath's work with peptide hormones.

In spite of the different directions of our work Porath and I found each other and spent many hours discussing various subjects, not least relating to the ongoing work and ideas based on it. A result was a method to detect amino acids and other buffering substances in paper chromatograms. It led to our first joint publication (3) and to the decision to alternate as first names on our future papers. The agreement was kept for more than 20 years.

Zone Electrophoresis

Starch. Tiselius received the Nobel Prize for the development of boundary electrophoresis and its use to detect components of blood serum. The apparatus was complicated and expensive because of the measures that had to be taken to avoid disturbances arising from density fluctuations and the use of an advanced optical detection system. The components were not separated but displaced relative to each other. Complete separation and thus purification of the components was a dream, and several attempts to solve the problem had been made. Two approaches were tested in the early 1950's. In one of them, columns packed with glass beads were used Haglund and Tiselius (4). They were the first to displace the column content and collect it in a fraction collector. It proved to be difficult to obtain good separations because of the poor stabilization of the glass beads.

In an other approach Svensson and Brattsten used a narrow square glass trough packed with glass beads for continuous electrophoresis (5). Buffer was pumped in at the top of the vessel and a narrow sample stream in the middle. Perpendicular to the buffer stream an electric field was applied. In the bottom, the flow was divided into a number of outlet tubes and the electrophoretically separated components were supposed to come in different tubes. Although the idea was good, and fairly good separations of blood serum proteins were obtained, the glass beads made it very difficult to keep steady conditions over long periods.

In about 1952, the Institute of Biochemistry moved into a new laboratory building, and I could enjoy having a laboratory of my own. In one of our many discussions Porath and I got the idea that starch could be the stabilizer to use because its particle size was much smaller than the glass beads used by the electrophoresis people. In my lab we assembled an apparatus consisting of reversible Ag-AgCl electrodes as used in boundary electrophoresis and a column with a sintered glass filter in the bottom [ILLUSTRATION FOR FIGURE 1 OMITTED]. The column was packed with potato starch, a filter paper placed on top of the packing, a sample was added and allowed to enter the column. The next part of the procedure was to displace the sample down about one third of the column length with buffer to allow for electroosmosis. Finally, the column was filled with buffer, connected to the electrode vessels, and the current applied. After electrophoresis the apparatus was disassembled and the column eluted with buffer into a fraction collector (6).

It is apparent that since the separation takes place in the comparatively small volume in the column, the packing must be very homogenous to allow almost perfect plug flow. The better the packing, the narrower the zones and the better the separations that were obtained. Consequently a lot of work was spent on the packing procedure and the type of starch and its preparation before packing. To test the packing we used hemoglobin and dinitrophenyl amino acids to be able to visualize the zone movement through the column. To our surprise they traveled at different rates and the rate appeared to be independent of the concentration. Since electrophoresis was the main object of our research we did not appreciate the possibilities at that time but we told visitors about the observation. Among them were Lindqvist and Storgards (7), who later used starch for separation of peptides from whey.

What we learned in the work with packing of starch columns proved to be very important for the future work of both of us. Porath applied the starch electrophoresis method for

VF Exhibit 1032

his pituitary gland hormones and I used it for separation of blood serum proteins. However, owing to adsorption effects and soluble contaminants stemming from starch, new and better stabilizing media were constantly searched for.

Cellulose. As mentioned above, one of the approaches to improve the performance of paper electrophoresis was to reduce adsorption of the proteins. The filter paper we used was made of [Alpha]-cellulose and contained a few carboxylic groups. In attempts to esterify them with methanolic HCl, I found that the paper structure was lost and a powder remained. Since starch had limitations as stabilizer in zone electrophoresis in columns, commercial paper powder was tried as packing material. However, adsorption of basic proteins was still observed. It was when Kupke and I used methanolic HCl to treat first paper powder and later cotton linters and ordinary cotton as starting materials that real improvements as regards adsorption were made (8). In addition, in columns packed with the cellulose powder thus obtained, all water in the columns was accessible to proteins. It was of course due to the non-gel structure of the cellulose powder. Both the paper powder and the electrophoresis apparatus were commercially available for many years.

DEXTRAN (1954-62)

In late 1953, I got a licentiate degree with a thesis with the title 'Zone Electrophoresis in Packed Columns.' Soon after I was offered a job at AB Pharmacia, which had moved from Stockholm to Uppsala a few years earlier. One of their main products was the plasma extender Macrodex, which was made from the polysaccharide dextran.

In January 1954 I began working in their research laboratory in close collaboration with the research manager, Professor Bjorn Ingelman, one of the inventors of Macrodex. My task was loosely defined as "to develop new products based on dextran."

Dextran is a very high molecular weight bacterial polysaccharide. To be useful as a plasma extender it had to be hydrolyzed and fractionated to a well defined molecular weight range just above the kidney threshold (around 30,000 Dalton). The high molecular weight fractions were recirculated, but the low molecular weight ones had at that time no use and were waste material.

Several years earlier Ingelman had tried to make the low molecular weight dextran useful for clinical use by reaction with epichlorohydrin. Invariably he obtained gels. One of my first tasks was to study the reaction in more detail. With the low molecular weight fractions I was no more fortunate than he had been. I tried lower and lower molecular weights and, finally, with mono- and disaccharides and sorbitol, soluble polymers were obtained, provided their reducing groups were protected (9). Much work was done especially with sucrose and already in 1955 pilot plant quantities were made (10). Many years later it led to commercialization of a product called Ficoll. It is still on the market.

During the next few years my work was extended to encompass not only dextran chemistry but also epichlorohydrin reactions. Thus ion exchange resins were synthesized from amines and epichlorohydrin. One object was to make some pharmaceuticals, like salicylic acid, more endurable by patients taking large quantities (9, 11).

Many derivatives of dextran were synthesized and some evaluated in animal experiments (12). The most successful one was calcium carboxymethyl dextran, which was marketed as a veterinary specialty (13).

SEPHADEX

The observation. Since Porath had been working in the U.S. and I had moved to Pharmacia, our interests had diverged. However, when he returned to the Institute of Biochemistry he resumed work on zone electrophoresis. Our discussions were much less frequent but they did occur occasionally. In early summer of 1956 Porath told about his difficulties with adsorption in zone electrophoresis in cellulose columns and with other supporting materials he had tried. I suggested that he try a new type of gel that I had made. He agreed and I went back to the laboratory to get permission to make and supply Porath with gels. Pharmacia was then and is still a pharmaceutical company, and non-pharmaceuticals were looked upon with great skepticism. Since no pharmaceutical products could be visualized I was allowed to use only up to 10% of my time and I was not permitted to tell Porath how the gels were made. Further conditions were that the work was to be kept strictly secret and that the right to products, if any, should belong to Pharmacia. No written agreement was made but the oral one was adhered to until 1959, the year Sephadex was introduced.

The making of gels suitable for electrophoresis was a slow and boring process. The gels themselves were easily made but they had to be ground, dehydrated, dried, and **sieved** to obtain particles of a suitable size, i.e., small enough to stabilize electrophoresis columns but large enough to permit flow at start and after electrophoresis when the column was eluted. A number of experiments were necessary to get sufficient material to pack a column (9).

Sometime in the late summer of 1956 Porath made the first evaluation of a dextran gel for electrophoresis. Afterwards he reported to me that adsorption of basic peptides (or proteins) was improved compared with other stabilizing media he had tried but not completely eliminated. However, it had the same drawback as starch in that small and large molecules migrated at different rates when just eluting a mixture through the column. This observation started a mental process on what the observed phenomenon could be used for.

Immediately after the observation we did not appreciate the possibilities but were rather much in doubt whether it could be used at all. The reason was the small separation volume of less than a column volume, in fact at most 30-40% of it. It was a rule of thumb among chromatographers that two peaks should be separated by at least 2.5 column volumes to be useful. However, in the work on zone electrophoresis we had learned how to pack and elute columns efficiently so that even small differences in electrophoretic migration could be used to isolate components.

At that time Porath was working on his PhD thesis, which was to be published and defended in public in 1957. His main interest was in electrophoretic separation of peptide hormones, which was the main method used in his thesis. Consequently, when we decided how to continue the exploration of the gels, he took on electrophoresis and, as we then called the method, "molecular **sieving**" (after the term used by Barrer for separations using zeolites) of peptide hormones and small proteins. In my laboratory at Pharmacia I was to work with separation of very large from very small molecules, corresponding to dialysis, polysaccharides, and blood serum proteins. All gels were to be made by me as required by the company.

The concept. In order for me to be allowed to continue, the company wanted me to identify a market for the gels that was large enough to motivate further work. When the idea of using the method for dialysis emerged, a commercial opportunity could be visualized. It gradually developed into a conviction that this was the concept to use as the main line for further development.

During the fall of 1956 and in early 1957 I made gels and studied separation of proteins from salts, in particular hemoglobin from sodium chloride. In spite of the limited time at my disposal, considerable progress was made (9). Owing to the strict secrecy, the number of discussion partners was very limited. The only one outside Pharmacia was Porath; and in the company, I spoke with Ingelman and Drs. Kirsti Granath and Bertil Gelotte. The latter was of great help in formulating the internal report that led to authorization as a project (14). Reading the report now, I am surprised at how many of the future applications were already anticipated at this early date.

The molecular **sieve** project. Pharmacia at that time was a small pharmaceutical company with a turnover of about 10 million dollars. Projects had to be authorized by the top management. Since the molecular **sieve** project was a non-pharmaceutical, objections to authorization were raised by several top management people. However, the president, Mr. Elis Goth, was in favor, probably in part because of the influence of his friend Professor Tiselius, but also and mainly because he, as a chemical engineer, visualized a new large scale unit operation. In due time the project was formalized, which meant that I could devote almost all my time to it and have a lab assistant to help me. However, strict time limits put pressure on us to get results. After one year the project was to be reevaluated.

In the hectic time that followed a number of questions had to be answered. Since the emphasis was placed on "dialysis" type applications, the optimal conditions for these separations had to be found. Thus the optimal degree of crosslinking and particle size had to be found and operating parameters such as column size, particle size, and rate of elution evaluated. In addition, a product specification, analytical methods, and large scale production methods had to be elaborated.

Already in the fall of 1957 the patent applications covering the separation method (15) and the synthesis of dextran gels (16) were filed in Sweden. Both proved to be valuable to the company as they remained unchallenged throughout the time of protection. A policy was later adopted that those who bought the Pharmacia gels automatically got a license to use them for gel filtration.

From 1957 onward, more and more people gradually became involved in the project. In 1958 more than ten people were involved. A steering committee was formed consisting of the export manager Sven Boode, Bertil Gelotte, and me. Our object was to put products on the market in 1959 and get a cash flow into the company in that year. Because of limited resources, we decided to introduce the products in only two markets, the U.S. and Sweden.

Since "dialysis" was the principal application aimed at, the main product chosen was G-25 (G for gel and 25 for a water regain of 2.5 g per g dry gel). It was to be supplied in three different particle sizes: coarse, medium, and fine. As a supplement we chose the somewhat more loosely crosslinked G-50 (water regain 5.0 g/g), also in three different particle sizes.

The manufacturing method was simply a scale-up of the procedure used in the laboratory. Low molecular weight dextran was dissolved in NaOH and epichlorohydrin added. The reaction mixture was poured on shallow baking troughs and heated to about 50 [degrees] C. The exothermic reaction increased the temperature still more and in a few hours the reaction was complete. The yellow to brown gel was neutralized, ground, dehydrated with ethanol, dried and **sieved** to isolate the desired fractions. The procedure was inefficient and lengthy, and much material was wasted.

Other details to be attended to before introduction were the terminology, operating instruction booklets, and sales promotion. Regarding the first, the gels were named Sephadex (Separation Pharmacia Dextran) and the method Gel Filtration as suggested by Professor Tiselius. As a promotion aid, a manuscript by Porath and me was submitted for publication to Nature (17). It was our third joint paper, so his name was first according to our agreement from 1950. Later it became a "Citation Classic."

The introduction. In January 1959 the secrecy was eased and Porath and I gave a seminar at the Institute of Biochemistry in Uppsala. In the same month Tiselius included it in a lecture in India. In June, Porath lectured about the method at a conference in Brugge, Belgium. To our disappointment the response was virtually absent. However, we had planned for the main introduction in the U.S. in July and tried to time the publication in Nature accordingly. Tiselius had arranged an invitation for me to a Gordon Conference on Proteins in New Hampshire, we had a stock of material in New York, and booklets and other promotion material were ready to be distributed.

The Gordon Conference was chaired by Professor Stanford Moore of the Rockefeller University, and a number of the most prominent protein chemists in the U.S. attended. I gave my lecture on the third day. The slides I showed were carefully chosen so as to give no doubt of the generality of the method. After the lecture there was first silence and then a flood of more or less critical questions came from the auditorium. I felt rather uncomfortable trying to answer them. Then Moore came into the lecture hall waving a reprint he just had obtained. It was the note in Nature by Porath and me. With this the mood turned and everybody seemed to accept what I had presented. When I returned to New York two weeks later the stock we had there had been sold out. For the remainder of the year we had to supply the U.S. market by air transport.

Further development. The limits of exclusion of the first gels were about 10,000 and 50,000 for G-25 and G-50, respectively. For protein and polysaccharide separations, higher exclusion limits and thus a separation interval was needed. More loosely crosslinked gels could easily be made by the technique, but they became soft and difficult to handle. Columns packed with loose gels were compressed already at moderate pressures, causing flow to stop completely. However, G-75 and G-100 could be managed, and they were put on the market in 1960.

A leap forward was achieved when a method to make spherical beads was developed. Already in early 1959 experimentation was started based on the well-known suspension polymerization of styrene and other monomers. We used a reverse phase reaction in which the dextran solution in NaOH was suspended in a non-miscible organic phase in which a stabilizing polymer was dissolved. The crosslinker epichlorohydrin was then added and the reaction completed at a moderately high temperature (18). The product was then recovered by a simple filtration.

The bead method had several advantages. The particle size could be regulated almost at will by varying the concentration of stabilizer and/or the rate of stirring. In the production equipment nearly monodisperse particles were obtained and the yield was almost quantitative; only a small amount of very free particles had to be removed by flotation. To be able to keep the specification, it was necessary to blend several batches having bead diameters around a specified average. Since a small amount of

NaB[H.sub.4] was added to the dextran solution the reducing end groups were eliminated and color of the product was white.

There were also a number of advantages on the application side. Spherical, almost monodisperse particles packed well and gave much less resistance to flow than did the irregularly shaped ones made earlier. It meant that smaller particles could be used, and thus better resolution obtained. Another effect was that more loosely crosslinked gels could be used, permitting separation in higher molecular weight regions. Thus efficient fractionation of blood proteins could be made with Sephadex G-200 (19).

Ion exchangers. In 1958 Sober and Peterson (20) had introduced ion exchangers based on cellulose from chromatography of proteins. Like them we had hydroxyl functional groups and derivatization by etherification was a fairly straightforward operation. Since our materials were covalently crosslinked we could reach considerably higher ion exchange capacities than was possible with cellulose. In June 1959 we filed two patent applications, one covering cation exchangers, carboxy methyl, and sulfoethyl ethers of crosslinked polyhydroxyl compounds (21), and the other covering tertiary amine ethers of similar gels (22). They were marketed in 1960 under the names CM-, SE-, and DEAE-Sephadex, respectively.

Further developments. There was a steady flow of ideas for new products once the first Sephadex had been established. In addition to those already mentioned, attempts were made to make gels for use in organic solvents. By addition of propylene oxide to the dextran gels the LH-Sephadex types were initiated. Once the Sephadex had been introduced on the market, customers responded with suggestions for improving the products and ideas for new ones. Thus Sephadex G-10, G-15, and G-150 saw the light.

GEL FILTRATION

Group separation. It was mentioned above that the applications we expected to yield a market large enough to motivate the development of Sephadex were those corresponding to dialysis, i.e., separations of very large from very small molecules or ions. We called it group separation since no attempt was made separate species of closely related sizes. The first Sephadex types were developed with this application in mind. Since I directly supervised both the synthesis of gels and the application studies {in the first phase I made both types of experiments myself}, the feedback from separation to synthesis was a minor problem.

Part of the results were presented in a paper in J. of Chromatography (23) (it became one the twenty most cited papers in the 500 first volumes of the journal), in my PhD thesis (24), and in the Pharmacia booklets on Gel Filtration {1959}. The apparatus used was very simple, as shown in Fig. 2, consisting of a column, an eluant reservoir, and a fraction collector. The packing of the column and the application of samples were critical points, but the experience gained in zone electrophoresis was of great help.

The influence of particle size and rate of elution on the efficiency of columns as measured by the EHTP were as expected for a diffusion controlled process. An unexpected property for a chromatographic process was that the concentration of solutes was of little importance provided the viscosity was low. Thus very high concentrations of, e.g., globular proteins could be treated at high flow rates {see below under Automated gel filtration}.

The volumes of samples to be treated were also evaluated. It proved that sample volumes of up to 30-40% of the total column volume could be used for a degree of purification of better than 950/0.

Fractionation. For Pharmacia, knowledge of the molecular weight and its distribution for dextran was of great importance for the manufacture and control of the plasma extender Macrodex. Thus a considerable amount of effort was devoted to molecular weight determination by end group determination and light scattering and fractionation by precipitation and subsequent viscosimetric characterization of the fractions. This was made in the physical chemical laboratory under supervision of Dr. Kirsti Granath. The fractionations were tedious (often a fractionation could take several weeks to complete) and required extreme care to give meaningful results. Consequently we could very early visualize great savings in labor and time if the fractionation could be made by gel filtration.

At the IUPAC Symposium in 1959 (25) and in (26) we reported the results of gel filtration of dextran fractions on three gels with different water regain values. The experiments were made before specifications for G-25, G-50, and G-75 were finalized but the gels used had nearly the corresponding separation properties. With the loosest gel (corresponding to G-84) dextrans with [M.sub.n] up to 40,000 could be separated. Eluates were collected in test tubes in a fraction collector and the contents of each tube analyzed for dextran concentration. At intervals the [M.sub.n] was determined by end group analysis and the [M.sub.w] by light scattering.

In Fig. 3 the results are shown in a semilogarithmic manner. A single straight line represented the data for some gels. In the most loosely crosslinked gel the data could be resolved into three straight lines.

With the advent of spherical beads and still less crosslinked gels the dextran molecular weight distributions were routinely determined by gel filtration, Molecular weights up to about 200,000 were separated. Gels made in the original way and separating still higher molecular weights were very difficult to use and it was only when the Sepharose gels (made from agarose) appeared that the separation range could be materially displaced upwards.

Oligosaccharides. Two goals to further develop gel filtration were obvious, to extend the range of molecular size to be separated upwards, and to increase the efficiency so that species with close molecular weights could be separated. The latter was tested with oligosaccharides at the Institute of Biochemistry where a column measuring 4.5 by 150 cm was packed with Sephadex G-25 Fine (200-400 mesh dry sieved). The individual species of a celloextrin sample from glucose up to cellobiose were isolated as separate peaks. Even the hepta- and oktaoses could be seen as unresolved peaks [ILLUSTRATION FOR FIGURE 4 OMITTED] (27).

When similar long high resolution columns were used with acidic oligosaccharides derived from chondroitin sulfate and hyaluronic acid by enzymatic degradation with hyaluronidase, the results were poor when distilled water was used as eluant. Using 0.1 M NaCl improved resolution but results comparable to those obtained for neutral oligosaccharides in distilled water was obtained only when the salt content was increased to 1M NaCl (28).

Proteins. From my early days at the Institute of Biochemistry I had been interested in proteins and their isolation in pure form. It was a disappointment when the first Sephadex types had an upper exclusion limit of about 70,000 and could not be used to separate larger ones, Therefore it was a great relief when the spherical beads were developed because with G-100 and G-200 in bead form blood serum proteins could be fractionated. In Fig. 5, a comparison of G-75, G-100, and G-200 is shown in which experimental

conditions were made as comparable as possible (29).

In a paper later to become a Citation Classic, Killander and I made an extensive study of the separation of blood serum proteins (30). Fractions obtained by gel filtration were further investigated by paper electrophoresis, analytical ultracentrifugation, immunoelectrophoresis in agar gels, double diffusion in agar gels, and immunotitration. A number of components were thus identified and their position in the chromatogram established.

In another paper by Gelotte et. al. (31), human plasma proteins were separated by gel filtration in combination with zone electrophoresis in columns packed with Sephadex G-25 and ion exchange chromatography in DEAE-Sephadex. The latter method was found to be very efficient in combination gel filtration and at least two components (transferrin and 7S [Gamma]-globulin) could be isolated in a nearly pure state.

Automated gel filtration. In early work it was noted that the experiments were easy to reproduce and that the columns, once packed, could be used over and over again. For group separations fairly large sample volumes could be used and the solvent had the same composition throughout the experiment. It led to the idea to automate the process. The apparatus for automatic gel filtration is shown in Fig. 6. A column packed with Sephadex, one pump for the sample, one for the eluant, and a timer to regulate the pumps and valves. The critical point was how to insert the sample just above the bed top surface without causing turbulent mixing with the solvent standing above the surface. The trick was to use the higher density of the sample and to distribute it as evenly as possible on the surface, A device was designed to achieve this (32).

In my PhD thesis (24) some results are reported. They were obtained from fractionation experiments with Ficoll, the sucrose epichlorohydrin copolymer described above. The low molecular weight tail and salts from the synthesis were to be removed. In each cycle a sample the size of which was 20-30 vol% of the total bed volume (31.4 liters) was added. The cycle time was 2 hours and 398 cycles were run before the experiment had to be terminated. After a back-wash to eliminate fines, the experiment was resumed.

The technique was later used on much larger scale for purification or sometimes removal of proteins, e.g., allergenic ones from penicillin.

Concentration of proteins. Since dry Sephadex swells in contact with water it can be used to concentrate solutes, which do not penetrate into the inner volume of the gel particles. We used this method to concentrate proteins, in particular proteins that are sensitive to evaporation or freeze drying. The Sephadex was suspended in the protein solution, centrifuged in a basket centrifuge, and washed by spraying with water or buffer (33). High recoveries ([greater than]90%) were obtained in experiments with tenfold to twentyfold concentration. An advantage with the method is that the concentration of the gel penetrating substances do not change. Thus the pH and ionic strength remain essentially constant.

It can be mentioned that dry dextran gels later were successfully used for wound care under the name Debrisan.

Theoretical In my internal report of May 1957 (14), I used the term molecular **sieving** for the separation process. It was Barrer's work on zeolites (34) that gave Porath and me the idea of the mechanism by which the gels acted, i.e., that the size of the molecules determined the depth to which they could penetrate the gel particles. An experimental evidence was in favor of this hypothesis, from static experiments to studies of column efficiencies (23).

In my PhD thesis I elaborated the concept further (24). Since the swollen gels imbibe more liquid (water) than was present at crosslinking, the glucose units have been separated until physical constraints set a limit. In this state the density of glucose units is highest near the crosslinking sites (covalent or by entanglement). It increases gradually to a maximum value, which corresponds to the exclusion limit of the particle. In two dimensions it can be visualized as a cone [ILLUSTRATION FOR FIGURE 7 OMITTED] into which a solute can diffuse until its size approaches that of the pore. If it is supposed that the diffusion coefficient is nearly the same in the open part of the pore as in the surrounding medium but decreases rapidly at the size limit, it can easily be understood how the gel works.

Gel filtration can be seen as a liquid-liquid partition chromatographic method in which the mobile and stationary phases have the same composition. A partition coefficient, $[K_{sub.d}]$, can be defined which can take any positive value. Values above one indicate that adsorptive forces are operating. For true gel filtration in which $[K_{sub.d}]$ is independent of concentration, its values are between one and zero, meaning that the concentration in the stationary phase, $[c_{sub.i}]$, is equal to or smaller than the concentration in the mobile phase, $[c_{sub.o}]$. In terms of the exclusion hypothesis mentioned above, it means that the concentration is the same as $[c_{sub.o}]$ in the part of the $[v_{sub.i}]$ that is available to the solute in question. For this reason the more suitable name for the coefficient $[K_{sub.av}]$ (av for available) was coined later.

FAREWELL TO PHARMACIA

In the middle of 1961 the steering group proudly told the top management of Pharmacia that the total cash flow in the project now was positive and that in the future it could generate money for the company. Toward the end of the year I was approached by another company offering a challenging job and generous conditions. Pharmacia did not counter so I accepted the offer on the condition that I could wait for six months.

Pharmacia was very generous in that I was allowed to use much of the material I had collected during the years in a PhD thesis. I already had a few published papers and what I had to do was to write a summary of my work and publish some more papers and finish the running projects. It was agreed that the summary was to be written in such a manner that it could be used as promotion material for Sephadex and Gel Filtration. Thus it was printed in more than 3000 copies, which were given to customers on request. In an amazingly short time the stock was out (a few are still in my possession).

My thesis was defended in public on May 16, 1962, with professor K.O. Pedersen as faculty opponent. Two weeks after the defense of my thesis I left Pharmacia and Uppsala to begin a new, completely different job with new challenges.

To summarize, the years at Pharmacia were successful from many points of view. I had learned a lot, particularly in polymer science, I had invented and participated in the development of a whole range of products and been acting as one of the leaders of the team that took the products to the market. In fact, what we started was the embryo of what has now become Pharmacia Biotech, a company with 2500 employees around the world and a turnover of almost \$450 million.

REFERENCES

1. H.G. Kunkel and A. Tiselius, A., J. Gen. Physiol., 35, 89 (1951).
2. A. Tiselius and P. Flodin, Adv. Protein Chem., 8, 461 (1953).
3. J. Porath and P. Flodin, Nature, 168, 202 (1951).
4. H. Haglund and A. Tiselius, Acta Chem.Scand, 4, 957 (1950).
5. H. Svensson and I. Brattsten, Arkiv Kemi, 1, 401 (1949).
6. P. Flodin and J. Porath, Biochim. Biophys. Acta, 13, 175 (1954).
7. B. Lindqvist and T. Storgards, Nature, 175, 511 (1955).
8. P. Flodin and D.W. Kupke, Biochim. Biophys. Acta, 21, 368 (1956).
9. P. Flodin, Laboratory Notebooks 1954-56.
10. P. Flodin and B. Ingelman, United States Patent 3,300,474 (1967).
11. P. Flodin, B. Ingelman and B. Spross, Swedish Patent 314,163 (1969).
12. P. Flodin, et al., United States Patent 3,453,364 (1969).
13. P. Flodin, O. Johansson, and L. Johansson, Swedish Patent?
14. P. Flodin, Pharmacia, Internal Report May 18, 1957: "PM on Molecular **Sieving**,"
15. P. Flodin and J. Porath, United States Patent 3,002,823 (1961).
16. P. Flodin and B. Ingelman, United States Patent 3,042,667 (1962).
17. J. Porath and P. Flodin, Nature, 183, 1657 (1959).
18. P. Flodin, United States Patent 3,208,994 (1965).
19. P. Flodin and J. Killaner, Biochim. Biophys. Acta, 63, 403 (1962).
20. H.A. Sober and E.A. Peterson, Federation. Proc., 17, 1116 (1958).
21. P. Flodin, O. Johansson, and S. Carlsson, United States Patent 3,275,576 (1966).
22. P. Flodin, O. Johansson, and S. Carlsson, United States Patent 3,277,025 (1966).
23. P. Flodin, J. Chrom., 5, 103 (1961).
24. P. Flodin, PhD thesis, Uppsala Sweden (1962).
25. P. Flodin and K. Granath, in Proc. IUPAC Symposium on Macromolecules, Wiesbaden, Germany (1959).
26. K. Granath and P. Flodin, Makromol. Chem. 48, 160 (1961).
27. P. Flodin and K. Aspberg, in Biological Structure and Function. Vol. 1, p. 345, Academic Press, London (1961).
28. P. Flodin, J.D.Gregory, and L. Rodin, Anal. Biochem. 8, 424 (1964).
29. J. Porath, and P. Flodin, in Protides of the Biological Fluids, Vol. 10, p.297, H. Peeters, ed., Elsevier (1963).
30. P. Flodin and J. Killander, Biochim. Biophys. Acta, 63, 403 (1962).
31. B. Gelotte, P. Flodin, and J. Killander, Arch. Biochem. Biophys. Suppl. 1, 319 (1962).

32. A. Emneus and P. Flodin, United States Patent 3,265,215 (1966).

33. P. Flodin, B. Gelotte, and J. Porath, Nature 188, 493 (1960).

34. R.M. Barrer, Ann. Repts. Chem. Soc., 41, 31 (1944).

Gale Copyright:

Copyright 1998 Gale, Cengage Learning. All rights reserved.

Previous Article: [The case law on aversive interventions for students with disabilities.](#)

Next Article: [Photochemical modification of polymers - photocrosslinking, surface photografting, and lamination.](#)

Home
Search
Services
Communities
Help
Contact us
Advertise on this Site

© 2004-2017 FreePatentsOnline.com. All rights reserved. Privacy Policy & Terms of Use. A SumoBrain Solutions Company