Journal of Chromatography, 215 (1981) 1–6
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,818

THE FARADAY SOCIETY'S DISCUSSION AT READING IN 1949 AND THE EXPLOITATION OF MOLECULAR-SIEVE EFFECTS FOR CHEMICAL SEPARATIONS

R. L. M. SYNGE
School of Biological Sciences, University of East Anglia, Norwich (Great Britain)

SUMMARY

Some of the papers presented at Reading, with the resulting discussions, probably hastened the application, beginning during the 1950's, of molecular-sieve effects to chromatography and to electrophoresis. By contrast, applications based on hindered diffusion, dialysis etc. have emerged more slowly and over a very much longer period. Nevertheless, these last may turn out to be just as important.

CHROMATOGRAPHY

In September, 1949, the Faraday Society held one of its discussion meetings in Reading, Great Britain. These meetings were called to discuss various fields of physical chemistry, judged timely for review, and this particular meeting was devoted to chromatographic analysis. Most current aspects of the art came under scrutiny but, in retrospect, three particular papers stand out by having drawn the attention of those present (or who read the resulting publication[1]) to molecular-sieve effects. Barrer[2] gave a contribution on zeolites, Claesson[3] on sorption of various polymers by charcoal etc. and Kunin and Myers[4] on sorption effects in ion-exchange resins as affected by cross-linking. Barrer was most explicit about molecular-sieve effects (in zeolites the channels are so accurately shaped that, *e.g.* in mordenite, methanol may enter with complete exclusion of ethanol). In consequence, for many of the effects which Barrer has been so successful in exploiting, there was never any need for chromatography and simple batch sorption could be used. Claesson and Kunin and Myers both found increase of molecular weight to restrict access of substances to their sorbents, with bad consequences for the chromatographic separations in which they were interested. In introducing the discussion Tiselius[5] (and, in summing up, I[6]) voiced the hope that these effects could be exploited for separations. This seemed particularly important because, at that time, there were no simple procedures (not even preparative ultracentrifugation) for effecting separations of "isochemical"[7] substances purely according to molecular weight.

I remember that Barrer's contribution specially stirred Tiselius' memories of his own work with zeolite crystals, some of which he had himself collected from remote parts of the Faroe Islands[8].

0021-9673/81/0000–0000/$02.50

In a collective review on chromatography published five years later, in 1954[9], I can find no mention by any of the authors of actual or potential use of molecular-sieve effects. Yet the message had gone out, and, in fairly quick succession, we have the use of cross-linked locust-bean gum by Deuel and H. Neukom[10], of swollen starch grains by Lathe and Ruthven[11] and of cross-linked bacterial glucan by Porath and Flodin[12]. The good controllability of this last formed the basis of the "Sephadex" success story in AB Pharmacia. Cross-linked polyacrylamide ("Bio-Gel") soon also became popular with biochemists, and the exploitation of agarose has permitted fractionations in a far higher molecular-weight range than had earlier been possible. Similar fractionations of water-insoluble polymers came almost a decade later[13] than those by the workers cited above, and were called "gel-permeation chromatography", to add confusion to the already confusing nomenclature.*

Moore and Stein[15] had not explicitly recognized molecular-sieve effects in their study of sorption of amino acids by starch grains in water; the small exclusion effect observed on comparing alanine with glycine could then equally well have been explained as increased "negative adsorption". But they were alert to the need for loosely cross-linked ion-exchange resins when they extended their chromatography from amino acids to larger peptides[16].

ELECTROPHORESIS

To exploit molecular-sieve effects electrokinetically, the theoretical considerations were much more complicated than any of us at Reading fully realized. They have since been well set out by Morris and Morris[17], Ogston[18] and Van Oss[19]. As in the chromatographic exploitation, the first useful results were obtained using swollen starch, by Smithies[20] in 1955. A. Tiselius, D. L. Mould and I had an interesting, though frustrating, series of encounters with "secondary-adsorption"[21], sorption, electroendosmotic and ion-exclusion effects in gels, of which I gave a chronological account at a Symposium at Kalamazoo, Michigan, in 1963[22]. I particularly enjoyed that both Tiselius and this year's birthday hero were present there. Since those early efforts, the importance of having a neutral *and* non-adsorbent gel matrix has been properly and widely appreciated. Cross-linked polyacrylamide[23] is now in general favour and often, when proteins are to be separated, their secondary-valence complexes with detergents are used, so that the proteins become electrokinetically "isochemical"[7] and have their migration rates determined solely by molecular size.

Conversely, the development of membranes made from ion-exchange materials has had very great value for improved desalination, electrodialysis etc. procedures.

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* *Cf.* Goethe[14]:

Mephistopheles: Im ganzen, haltet Euch an Worte!
Dann geht Ihr durch die sichre Pforte
Zum Tempel der Gewissheit ein.

Schüler: Doch ein Begriff muss bei dem Worte sein.

Mephistopheles: Schon gut! Nur muss man sich nicht allzu ängstlich quälen;
Denn eben wo Begriffe fehlen,
Da stellt ein Wort zur rechten Zeit sich ein.
Mit Worten lässt sich trefflich streiten,
Mit Worten ein System bereiten...

I hope I have now written enough to justify my belief that that Reading meeting had a decisive influence on the exploitation of molecular-sieve effects in the then rapidly expanding arts of chromatography and electrophoresis.

HINDERED DIFFUSION AND ULTRAFILTRATION

By contrast, the adoption of diffusion and ultrafiltration techniques (which also, at least in part, depend on molecular-sieve effects) has been jerky and unsystematic, despite their potential specificity being perhaps the greater. It has also gone on over a much greater span of time. The main trouble about "counter-current" amplification of diffusion effects is the separate concentration step required at each "plate" —Signer *et al.*[24] seem to have been ready to face this in biochemical work, but in general it has only been seriously undertaken by those who have concentrated $^{235}UF_6$ for evil purposes.

My roll of honour starts, of course, with Graham in 1861, with his discovery of dialysis[25]. He used "parchment paper" ("vegetable parchment") and sometimes mucus from the stomach of a pig, and not pig's bladder, as textbooks tend to repeat. One of his conclusions was: "The crystallizable principles, thein, salicin, and amygdalin, appear greatly more diffusible than gallo-tannic acid, or than gum... Such inequality of rate is likely to facilitate the separation of vegetable principles by the agency of dialysis."

My roll continues with Martin, who in 1896 invented ultrafiltration[26]. I got to know Sir Charles Martin more than 40 years later, when he supervised my work, on behalf of the International Wool Secretariat. Even as an old man, he would turn his hand to almost anything that fell within his unusually wide field of interests[27]. Apropos of ultrafiltration, he noted perceptively, after contrasting the behaviour, relative to one another, of urea and glucose in dialysis and in ultrafiltration, that substances which can pass into an ultrafiltrate tend to do so at equal rates, regardless of their molecular weight[26].

I go on to Elford, who handled collodion gels with great skill, to produce ultrafilter membranes of quite remarkably uniform porosity[28]. From his work are derived many of the membranes now in commercial circulation; concentration by ultrafiltration has been called by some people "reverse osmosis" (*cf.* again ref. 14).

Then came, during the 1940's, the remarkable improvements of serological precipitin techniques, associated particularly with the names of Oudin[29], Elek[30,31] and Ouchterlony[32], who caused the reactions to occur during diffusion of antigens and antibodies within gels. Earlier workers had observed similar phenomena but tended to explain what they had observed as Liesegang rings[31].

Next I cite Craig *et al.*[33], who did not just use cellophane sausage skins for dialysis, as had somehow become the general practice of biochemists, but used various techniques to modify cellophane and studied the effects of their modifications, as well as the peculiar behaviour of the various substances whose diffusion across the membranes they studied.

Finally, and inextricably tangled with these applications to separative work, are those workers who studied the permeability of artificial membranes in order better to understand the behaviour of the membranes encasing living cells. The Donnan equilibrium, the Teorell–Meyer–Sievers concept of the role of fixed charges

in membrane permeability and the related experimental work of K. Sollner were all based on sound concepts in physical chemistry[34,35].

SOME PERSONAL REMARKS

I find mirrored, from the patchy and erratic progress of this last branch of scientific understanding, the patchy and erratic nature of my own scientific education. That despite the fact that it was centred around the scientifically distinguished University of Cambridge. There, you had to make your studies in a good broad sweep of subjects. But you learnt different things in different Departments from teachers who took a rather proprietorial attitude to what they taught. So the things you learnt tended to get stored away by you in different mental compartments. Thus, dialysis was done in "Biochemistry", invariably in sausage casing, of which we occasionally got bad batches. Precipitin reactions were done in "precipitin tubes" and were "Serology" or "Pathology". Though my microbiological colleagues grew a lot of organisms on gels, they didn't often think about how the various antigens, toxins and so forth moved about in the gel. (My colleague Muriel Robertson[36], at the Lister Institute, was an exception in that matter, but neither was she at all proficient in chemistry.) Ultrafiltration membranes were "Virology" (occasionally "Bacteriology"). And permeability of membranes, viewed in relation to those of living cells, was partly "Physiology" and partly "Colloid Science". Edsall has later well characterized (in the *Tiselius Festschrift*[37]) the equivocal influences of "Colloid Science" on the study of proteins. But in those earlier days it was inspiration, as well as fun, to attend E. K. Rideal's[38] lectures in that last-named department. We biochemists used for it the pejorative nickname "Superficial Chemistry" and Marjory Stephenson[39], in particular, used to fulminate with the words "Don't talk to me about permeability!"[40]. In return, she was accused of regarding bacteria as no more than little bags of enzymes which often, unfortunately, could only be brought into solution by ball-milling. As for electroendosmosis, it was never mentioned in any of the courses or laboratories which I frequented, and I first met it when one compartment of a diaphragm cell[41], which I was using for an electrophoretic separation, had overflowed overnight. So I looked through Freundlich's *Kapillarchemie*[42], a copy of which had been given to me by my fellow-student J. H. Humphrey, who had inherited it from his father, H. A. Humphrey of Brunner, Mond & Co. (later Imperial Chemical Industries). I learnt that electroendosmosis had been discovered by F. F. Reuss in Moscow in 1807. I read *Kapillarchemie* quite thoroughly after that, and found that "colloid science", viewed from one angle at least, had always been well-rooted in reputable physical chemistry.

What a contrast it was to go to Uppsala in 1946! Svedberg and Tiselius were certainly extraordinary people, and leaders in the application of physico-chemical principles to practical problems. But the Scandinavian countries have altogether produced a quite disproportionate number of good physical chemists in that line — Arrhenius, Bjerrum, Brønsted, Linderstrøm-Lang, Onsager and Sørensen, to mention only a few more from among the dead.

As I make it out, the Scandinavian success has come because, besides maintaining high academic standards in each university department, their professors and other senior academic staff have usually maintained close interdepartmental contacts, and

fairly good relations with one another. Moreover, they have been closely consulted about industrial, medical and agricultural problems, as and when they arose. That is how derivatives from the bacterial glucan which used to gum up sugar refineries found their way into blood transfusions and, later on, to the shelves of every biochemical laboratory in the world. It is good to couple heartiest birthday greetings to Jerker with the hope that the University of Uppsala will carry on its great work through untold future years.

ACKNOWLEDGEMENT

I thank Prof. P. G. H. Gell for his help in tracing the history of precipitin reactions in gels.

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