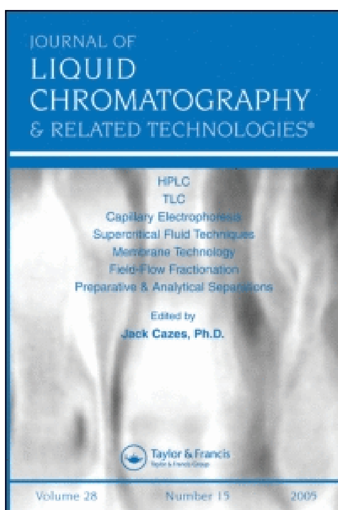
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Preface

Stellan Hjertén[a]
[a] Department of Biochemistry, University of Uppsala, Biomedical Center, Uppsala, Sweden

To cite this Article Hjertén, Stellan(1995) 'Preface', Journal of Liquid Chromatography & Related Technologies, 18: 18, xv — xviii
To link to this Article: DOI: 10.1080/10826079508014609
URL: http://dx.doi.org/10.1080/10826079508014609

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(18&19), xv-xix (1995)

PREFACE

A study of the history of electrophoresis shows that the introduction of a new, important electrophoresis method usually requires the introduction of a new approach to minimize or eliminate convection, i.e., hydrodynamic flows caused by differences in density between a zone and the surrounding medium. In Tiselius' classical moving boundary method, the density below any boundary is higher than that above it, which affords stabilization against convection. However, if the run is prolonged in an attempt to achieve zone separation, the zones will become blurred by convection in the form of sedimentation.

Downloaded At: 13:25 24 January 2011

This disturbing convection can be prevented by rotating a horizontal electrophoresis tube around its long axis. An alternative is to conduct the experiments in an electrophoresis chamber of very narrow cross-section. This approach is the basis of free flow electrophoresis and capillary electrophoresis. The same principle for stabilization against convection can be used for electrophoresis in columns of larger diameters by inclusion of substances which confer a capillary structure on the electrophoresis medium. Grains of starch or plastic; cellulose (and some derivatives) in the form of powder; filter paper, and films; and gels of agar, agarose, starch and polyacrylamide have been employed for this purpose. It should be emphasized that many gels also have molecular sieving properties, i.e., the separations are based not only on differences in zeta

potentials among the substances to be analyzed, but also on differences in molecular size.

An obvious disadvantage of these anti-convection media is that the solutes of interest can adsorb to them. To avoid this, the runs can be performed, instead, in a density gradient, for instance of sucrose, provided that the density of the sample is low enough to avoid sedimentation.

To master convection is important not only in electrophoresis, but also in other (separation) methods, for instance sedimentation (by ultracentrifugation) and chromatography. However, there are many more analogies between these three methods which one can understand intuitively, since the separations they accomplish can be ascribed to differences in the migration velocities of the solutes. In fact, using just migration velocity (v) as a variable in the equation for mass balance, one arrives at the following expression, which is valid not only for electrophoresis, chromatography and sedimentation, but also for other methods based on differences in migration velocities.

Downloaded At: 13:25 24 January 2011

$$M_j^{\alpha} \cdot v_j^{\alpha} - M_j^{\beta} \cdot v_j^{\beta} = v^{\alpha\beta}\left(M_j^{\alpha} - M_j^{\beta}\right)$$

In this preface, it is not necessary to define the different variables in the equation. It is only of importance to know of the existence of an expression which is so general that it is applicable for all of the methods mentioned. This means that any phenomenon appearing in, for instance, chromatography, will appear also in electrophoresis and sedimentation in an analogous way. Therefore, if one has found something methodologically interesting in chromatography, it is worth while to investigate whether an analogous electrophoresis experiment also gives an interesting result. The analogy between electrophoresis and chromatography tempts one to give analogous separation methods

analogous names. Thus, as an example, I suggest the use of the terms displacement electrophoresis (often called isotachophoresis) and displacement chromatography. In doing so, we also honor the pioneers in these fields who employed these notations.

There are no (or very few) similarities between electrophoresis and chroma-tography. I want to stress this since discussions in which these two methods are compared can likewise degenerate and become meaningless. No doubt, we need both of these methods. For some purposes, chromatography is preferable to electrophoresis; for other purposes the opposite is true. The resolution obtained in electrophoresis in free solution (i.e., buffer alone) or in a gel such as agarose or cross-linked polyacrylamide is, however, often higher than that obtained in chromatography. One reason is that a chromatographic medium (bed) is not as homogeneous as these electrophoresis media and, therefore, causes a larger zone broadening.

Free electrophoresis is a milder method than chroma-tography, since the separations are not based on interactions with a bed as in chromatography. The risk of denaturing labile macromolecules, such as proteins, is therefore lower.

I do hope that capillary electrophoresis will not meet the same fate as polyacrylamide gel electrophoresis, the advantage of which is that the experimental conditions (for instance, gel composition and buffer systems) can be varied easily to give optimum resolution for any particular separation problem. This flexibility, however, is seldom utilized. Unfortunately, certain standard conditions are often employed instead. Learning more theory will help the users of capillary electrophoresis to avoid the same trap! They will also characterize the solutes by their mobilities (not migration times) so that data from different laboratories can be compared in a meaningful way. Furthermore, the more one knows about the theory of electrophoresis, the more

Downloaded At: 13:25 24 January 2011

information one gets about the physicochemical properties of the solutes.

The general trend in the analysis of biological material goes toward smaller sample amounts (for instance, the contents of a single cell) and shorter analysis times combined with higher resolution. This is a big challenge, particularly since the latter requirement often is incompatible with classical electrophoresis theory. I believe that the success of capillary electrophoresis will accelerate and promote the development of capillary chromatography, not the least because the preparation of columns with diameters as small as 10 μm presents no problem since the introduction of the so-called continuous beds (the column bed is prepared in the same simple way as a gel is prepared for polyacrylamide gel electrophoresis). For instance, the different minute fractions obtained from a micropreparative capillary electrophoresis can be analyzed sequentially on these micro columns by chromatography based on ion-exchange, hydrophobic interaction, bioaffinity, etc. In doing so, we take advantage of the natural complementarity between electrophoresis and chromatography. The trend toward utilization of mass spectrometry for the analysis of electrophoretic fractions will continue.

Although many different separation techniques are available, there is a great need for novel high-resolving methods for the analysis and fractionation of biological materials. As an illustrative example, it may be mentioned that as much as 90% of the total cost to produce a protein by DNA hybridization refers to the analysis and purification (many biotechnical companies went bankrupt because they were not aware of the difficulties to analyze and fractionate protein mixtures). No doubt, capillary electrophoresis will reduce this cost -- and more generally, increase our knowledge in the field of separation science.

Downloaded At: 13:25 24 January 2011