Journal of Chromatography, 488 (1989) 25–30
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4567

CHROMATOGRAPHY BEYOND ANALYTICAL CHEMISTRY

IVO M. HAIS

Department of Biochemistry, Faculty of Pharmacy, Charles University, 501 65 Hradec Králové (Czechoslovakia)

SUMMARY

Although chromatography is one of the most important branches of analytical chemistry, it also serves purposes that cannot strictly be considered as part of analytical chemistry: it may be a model of natural processes, a method for the study of surface properties of molecules, for the collection of data in quantitative structure–activity relationship studies and for preparative separations, a teaching aid and sometimes even a kind of visual art. Some speculations and proposals are summarized.

INTRODUCTION

The drawing of frontiers between various disciplines and sub-disciplines depends on conventions and definitions, part of them likely to become quickly outdated. It is therefore subjective and serves the needs of convenience, such as the establishment of professorial chairs and university departments. One of the functions of classification, which is not entirely useless, is to serve as a stimulus for talk and speculation. That is why I have chosen this subject.

Tswett [1,2] did not consider himself to be an analytical chemist when for the solution of biological problems he was using physical and physico-chemical methods: spectroscopy, liquid–liquid extraction, adsorption including dynamic adsorption (chromatography), which he inaugurated, interactions between macromolecular and small-molecular substances, etc. His idea of using adsorption for the separation of chloroplast pigments was inspired by similarities between the leaves and their model, a paper sheet impregnated with a pigment mixture [1]. In the early 1930s, the years of the rebirth of chromatography [3,4], it was mainly a preparative and purification method, especially in the study of natural substances, a discipline now included in bio-organic chemistry. Partition chromatography was invented and ion-exchange chromatography developed to meet the requirements of the chemistry of proteins, peptides and amino acids [5,6]. Several substances, which later became of considerable biological importance,

0378-4347/89/$03.50

were first discovered as spots on paper chromatograms: let us recall, as examples, the finding of 4-aminobutyric acid in the brain [7,8] and of imidazolylacrylic acid in sweat [9,10] and the epidermis [11]. Minor purine and pyrimidine bases were revealed on chromatograms and this led to the recognition of the biological function of methylation of nucleic acids. The detection and identification of drug metabolites could hardly have proceeded at the pace they did if these substances had not first been seen as chromatographic spots or peaks [12,13].

Analysts gradually appropriated the possibilities which chromatography had opened up for them: the progress of physics, especially of spectrometric methods, ensured that almost every substance could be analysed with high accuracy, but the specificity was generally inadequate unless the substances had been physically, materially separated in space. Chromatography, electrophoresis and to a certain extent mass spectrometry were the methods serving this purpose. It is not surprising that in the statistics on the proportions of various methods employed in published papers dealing with analytical chemistry chromatography has occupied first place.

Professional analytical chemists applied their standard concepts to chromatography, namely the concepts of specificity (which, in the case of chromatography, is related to resolution), sensitivity, recovery, reproducibility, accuracy, analysis of error, optimization, etc. Today chemometric methods and computers are used for these purposes which accelerated the development of chromatography as a science. However, in some cases chromatography is used beyond the strictly analytical context. In this paper I shall consider some of these cases in more detail.

CHROMATOGRAPHY AS A MODEL OF NATURAL PROCESSES

If liquid or gaseous mixtures penetrate through geological strata, some of the components may be retarded owing to interactions (adsorption, ion exchange) and appear in the fluid emerging from the bed after the sorbent has been saturated or an eluent or displacer has desorbed them. Changes in the composition of crude oil caused by adsorption during penetration through earth strata were postulated by early geochemists. Engler and Albrecht [14], Day [15] and Kvitka [16] can be mentioned in this context. Devices proposed to demonstrate such phenomena in a laboratory closely resembled chromatographic columns and the above workers were considered to be predecessors of chromatographers.

After the discovery of chromatography these ideas were further developed. There is no need to go into more detail, as there are reviews by Janák [17,18] and Vigdergauz and Kozyurek [19].

The renal tubule also shows some analogies to a chromatographic column, although its function is much more complicated. Active secretion and absorption are not known in chromatography. Kidney basement membranes, however, have similar sieving properties to those in polyacrylamide gel electrophoresis or gel permeation chromatography.

CHROMATOGRAPHY AS A METHOD FOR THE STUDY OF SURFACE PROPERTIES OF MOLECULES AND OF INTERMOLECULAR INTERACTIONS

There are many methods, most of them spectroscopic, which give information on the structure of molecules; examples are infrared, magnetic resonance, Raman and possibly ultraviolet and circular dichroism spectroscopy, and also some electrochemical techniques. Chromatography is unique or at least especially important as it depends on the surface properties of molecules and ions which are involved in interactions with their environment (constituents of the stationary and mobile phases). Retention data provide information on these surface properties. Unlike NMR, where individual signals can be assigned to individual atoms, but similarly to UV–visible spectra, chromatographic retention is a value that results from several structural features. In order to split up this value into the individual contributions, it is necessary to evaluate a number of independent chromatographic systems and to compare a whole series of substances. In a first approximation, the contributions of various functional groups to the R_M or log k' value [$R_M = \log (1/R_F - 1)$; $k' =$ capacity factor] are considered to be additive ("linear free energy relationship"). As the analysis proceeds further, one encounters further structural features and interactions between the groups. The number of unknowns and hence the number of equations to be solved increases. Their effect need not be additive, but may have to be expressed as a mathematical function, which would have to be specified.

COLLECTION OF DATA FOR STUDIES ON QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS

As attractions and repulsions localized on the surface of the molecule decide on the interactions of the molecule with various "acceptors" and receptors in biological systems, their existence and strength influence the response of biological systems (by acceptors we mean here loosely substances that interact more or less non-specifically, whereas receptors interact specifically and are converted to a form that triggers a chain of events leading to an observable effect). No wonder quantitative relationships between chromatographic retention and chemical structure are reflected by quantitative relationships between chemical structure and biological activity [20–23].

In some of the relevant papers, biological activity was not simultaneously studied, but the relationships between the R_M value or the logarithm of chromatographic retention on the one hand and the logarithm of partition coefficient (for the octanol–water system) on the other was tested. In these papers, it was taken for granted that the relationship between partition coefficient and biological effect had already been defined by previous studies.

In many instances biological activity was correlated with the overall R_M value, retention index or the logarithms of partition coefficient or k' and the possibility of correlating biological activity with the contributions of individual functional groups (or of other structural features) (ΔR_M, $\Delta \log k'$ or π values) was neglected. This should be corrected, as the hydrophobicity or polarity at the individual sites

may be correlated with the strength with which they are bound by or their effect on biological receptors, rather than the overall hydrophobicity or polarity which, of course, may also be important in the partition between membranes and liquid phase.

PREPARATIVE APPLICATIONS

One of the shortcomings of chromatography as practised by Tswett was his failure to accumulate sufficient material of the pigments that he was studying to enable him to investigate them by the classical methods of macrochemistry known in his time. Further workers have amply proved the preparative power of chromatography. This extends from the submicro level (e.g., in isolating minimum amounts of radioactively labelled substances) to large-scale industrial applications [24,25]. Frontal analysis and displacement are often used to reduce solvent volumes. There is no doubt that chromatography can achieve separations which are unattainable by other methods. What are the disadvantages responsible for the slow acceptance of large-scale chromatography? One may be the cost of the solvents and, if these are regenerated by distillation, then the energy spent on distillation. When water can be used and easily purified, chromatography becomes cheaper. Another disadvantage against other procedures may be the batchwise operation of the column. Continuous analytical monitoring of the effluent and automatic operation of the dosing, mobile phase introduction, fraction collection and regeneration of the column should not be difficult in the computer age.

There are two areas of preparative chromatography to which special attention should be paid here: the use of biospecific sorption (affinity chromatography) and the separation of enantiomers. Affinity chromatography was generally considered to be a preparative method. Only within the last few years have the sophistication of affinity chromatography and the introduction of high-performance affinity chromatography led to its analytical application. In bulk preparative processes affinity chromatography would be of special advantage in cases in which it could retain the desired substance from an initial impure mixture: the mixture would then be greatly simplified and the subsequent purification steps could proceed on a smaller scale. Affinity chromatography is predicted to expand beyond the preparative and analytical procedures towards modelling and a better understanding of supramolecular interactions occurring in living systems.

The chromatographic separation of enantiomers is certainly an important asset in analytical chemistry. The whole area is rapidly expanding and assuming prime importance in pharmaceutical chemistry and biochemical pharmacology. Whether in large-scale preparative applications it will compete favourably with the traditional methods of crystallization of diastereoisomeric salts or of diastereoisomeric covalent derivatives remains to be established.

CHROMATOGRAPHY AS A TEACHING AID

Phillips [26] pointed out that it may be easier to understand chromatography than chemistry. Harsch and Bussemas [27] proposed a game with dice which

models the probability character of the distribution of molecules in each elementary cell.

The world in which we live consists of innumerable compartments containing mixtures of compounds. Generally, we do not see these compounds. Chromatography not only reveals them, but also characterizes them by their retention and possibly by their optical and other properties; it may therefore help children and other uninitiated people to conceive the world in chemical terms. I admit the chemical changes (reactions), which are the essence of chemistry as a dynamic science, play a subordinate role in chromatography. This shortcoming can be remedied by employing chromatography to demonstrate the reaction which has taken place.

CHROMATOGRAPHY AND VISUAL ART

The inclusion of this section in this article may seem out of place, but I shall take the risk. There is no doubt that the arrays of bands, rings or spots in planar chromatography or electrophoresis and the elegant curves drawn by a recorder are often beautiful. However, what is beauty? I view attempts at aesthetic generalizations with suspicion, so I shall abstain from defining it. In addition, the relationship between art and beauty is by no means simple.

In science, the term beauty is usually given a meaning different from that in art and is connected with the information content of the pattern. In chromatography and electrophoresis, beauty usually means well resolved spots or peaks. This may be illustrated by the following quotation [28] concerning an autoradiogram of a two-dimensional ionophoretic separation: "... (he) came into my laboratory brandishing a beautiful sheet of film with clear, round, well separated spots on it. This was certainly exciting after the streaky unresolved pictures we had been getting before".

From among the forerunners of chromatography, Runge [27,29,30] generated circular patterns reproducibly by applying solutions of inorganic salts on paper impregnated with another salt. His main object was aesthetic and he recommended these patterns for various decorative purposes. He attributed them, in agreement with his natural-philosophical principles, to the "creative urge of Nature". They may be considered as examples of abstract art before that term was coined.

In present-day paper and thin-layer chromatography, with both linear and radial development, the patterns, although often also beautiful, are seldom viewed as works of art. I only once read about an American artist who was reported to employ chromatograms as part of his language of art, but I unfortunately never saw these pictures.

To a certain extent it is surprising that chromatography has not found wider acceptance in art. Some art movements have developed that use signs and symbols, originally intended for communication, as part of their idiom: let us remember "lettrism" and the use of numbers in modern art or islamic, Chinese and Japanese caligraphy. The inclusion of media photographs in some pop-art pictures is based on a similar idea. The pattern, in addition to its aesthetic qualities,

may also serve as a means of communication and calls forth associations, this producing a calculated thrill in these art categories. A chromatogram, which also carries qualitative and quantitative information, would be another example of such a hybrid between visual art and information transfer (communication). Remember that poetry in general is based on the combination of verbal communication and sound and, in some instances, the visual image it evokes.

REFERENCES

1 M.S. Tswett, Khromofilly v Rastitel' nom i Zhivotnom Mire, Tipogr. Varshavsk. Uchebn. Okruga, Warsaw, 1910; reprinted in ref. 2.
2 M.S. Tswett, in A.A. Rikhter and T.A. Krasnosel'skaya (Editors), Khromatograficheskii Adsorbtsionnyi Analiz, Izbrannye Raboty, Academy of Sciences of the U.S.S.R. Press, 1946, pp. 12–13.
3 R. Kuhn and E. Lederer, Ber. Dtsch. Chem. Ges., 64 (1931) 1340.
4 R. Kuhn, A. Winterstein and E. Lederer, Hoppe-Seyler's Z. Physiol. Chem., 197 (1931) 141.
5 A.J.P. Martin and R.L.M. Synge, Biochem. J., 35 (1941) 91 and 1358.
6 S. Moore and W.H. Stein, Annu. Rev. Biochem., 21 (1952) 251.
7 J. Awapara, A.J. Landua, R. Fuerst and B. Seale, J. Biol. Chem., 187 (1950) 35.
8 E. Roberts and S. Frankel, J. Biol. Chem., 187 (1950) 55.
9 I.M. Hais, J. Král and A. Ženíšek, Čas. Lék. Česk., 92 (1953) 974.
10 A. Ženíšek and J. Král, Biochim. Biophys. Acta, 12 (1953) 479.
11 H.W. Spier and G. Pascher, Arch. Klin. Exp. Dermatol., 209 (1959) 181.
12 H.G. Bray, W.V. Thorpe and K. White, 1st International Congress on Biochemistry, Cambridge, Abstracts, 1949, p. 8.
13 I.M. Hais and L. Morávek, Biol. Listy, 31 (1950) 144.
14 C. Engler and E. Albrecht, Z. Angew. Chem., 14 (1901) 889.
15 D.T. Day, Ind. Tech. Pet. Rev., 3, Suppl. to the issue of August 25th (1900) 9.
16 S. Kvitka, Communication in Baku, June 17th, 1900, cited by Kh. S. Koshtoyants and K.F. Kalmykov, Biokhimiya, 16 (1951) 479.
17 J. Janák, in L.S. Ettre and A. Zlatkis (Editors), 75 Years of Chromatography – A Historical Dialogue, Elsevier, Amsterdam, 1979, p. 181.
18 J. Janák, in K.I. Sakodynskii (Editor), Prikladnaya Khromatografiya, Nauka, Moscow, 1984, pp. 268–276.
19 M.S. Vigdergauz and V.I. Kozyurek, in K.I. Sakodynskii (Editor), Prikladnaya Khromatografiya, Nauka, Moscow 1984, pp. 277–286.
20 E. Tomlinson, J. Chromatogr., 113 (1975) 1.
21 R. Kaliszan , CRC Crit. Rev. Anal. Chem., 16 (1986) 328.
22 K. Valkó, Trends Anal. Chem., 6 (1987) 214.
23 Z. Peřina, in M. Šaršúnová and O. Hanč (Editors), Vysokoúčinná Kvapalinová Chromatografia vo Farmácii a Biochémii, Osveta, Martin, 1985, pp. 271–275.
24 2nd International Symposium on Preparative and Up-Scale Liquid Chromatography, Baden-Baden, February 1–3, 1988; J. Chromatogr., 450 (1988) 1–140.
25 B.A. Bidlingmeyer (Editor), Preparative Liquid Chromatography, Elsevier, Amsterdam, 1987.
26 C.S.G. Phillips, in F. Bruner (Editor), The Science of Chromatography, Elsevier, Amsterdam, 1985, p. 343.
27 G. Harsch and H.H. Bussemas, Bilder, die sich selber Malen. Der Chemiker Runge and seine Musterbilder für Freunde des Schönen, Du Mont, Cologne, 1985, 134 pp.
28 F. Sanger, Annu. Rev. Biochem., 57 (1988) 16.
29 F.F. Runge, Zur Farben-Chemie. Musterbilder für Freunde des Schönen und zum Gebrauch für Zeichner, Maler, Verzierer und Zeugdrucker. 1. Lieferung, Dargestellt durch chemische Wechselwirkung, Berlin, 1850.
30 F.F. Runge, Der Bildungstrieb der Stoffe. Veranschaulicht in selbständig gewachsenen Bildern. Fortsetzung der Musterbilder, Selbstverlag, Oranienburg, 1855.