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Journal of Chromatography A, 1079 (2005) 50–58
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Review

Homogeneous gels for capillary electrochromatography

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Available online 18 April 2005

This article is dedicated to the memory of Professor Csaba Horváth, whose excellent lectures and brilliant scientific explanations about electrochromatography have always been very stimulating and exploring.

Abstract

Homogeneous gels represent a new type of (electro)chromatographic media possessing unique separation properties unmatched with any other chromatographic beds. It is important to emphasize that they principally differ from continuous beds, polymer rods (better known as monoliths), which are particulate separation media with pores permitting hydrodynamic flow through the columns. Monoliths, thus, are more similar to beds conventionally packed with beads, although the particles building up monolithic columns are usually smaller in size (few submicometers) and covalently linked together. Consequently, homogeneous gels deserve better the term "monoliths" having a non-particulate structure formed by crosslinked free polymer chains (according to a dictionary a monolith is a non-modularized column). The goals of this minireview are to clarify the position of homogeneous gels among the separation media (including polymer solutions), to explain and to exemplify their outstanding (electro)chromatographic properties. This review gives hopefully a complete list of references to homogeneous gels developed for capillary electrochromatography.

Keywords: Homogeneous gels; Monoliths; Capillary electrochromatography; Microdevice

Contents

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doi:10.1016/j.chroma.2005.03.122

1. History

Electrophoresis, the migration of electrically charged species in an electrical field [1–3] is used for the separation of various molecules ranging from bioparticles and biopolymers to low-molecular-weight compounds and ions. It can be conducted in different modes and in carrier-free or anticonvective media, such as polymer solutions or gels, which – if their pores are small enough – also have a size-sieving function.

The early history of electrophoresis is a search for sufficiently good anticonvective media. The analytical device of Tiselius [4], in which the sample ions are subjected to electrophoresis in free buffer, i.e., in the absence of an anticonvection medium, was supplemented in the late forties by the introduction of paper electrophoresis [5], a method which, in contrast to the moving boundary method, affords complete separation of proteins into discrete zones. The gel-based molecular-sieving anticonvection media soon followed the paper strips. Smithies was first to introduce such a medium, a starch gel [6], which, unfortunately, had a low but not negligible content of charged groups resulting in some adsorption of proteins and an electroendosmotic flow. The starch gel was soon replaced by polyacrylamide gels, introduced independently by Raymond and Weintraub [7], Davis and Ornstein [8] and Hjertén [9]. Hjertén [10] has demonstrated the unique properties of polyacrylamide gels and emphasized the importance of varying the pore size to attain optimum resolution. With polyacrylamide as a nearly ideal anticonvection medium the resolving power of electrophoresis was increased considerably, because diffusion was reduced and the sample components migrated as sharp zones. In 1961, Hjertén showed that gels of the neutral agarose are superior to the charged agar gels (which contain sulfate groups) for electrophoresis and immuno-electrophoresis [11,12]. Polyacrylamide and agarose gels are usually used for electrophoresis in a conventional slab or rod format but also in capillary electrophoresis.

The first papers on capillary gel electrophoresis were published in the 1980s by Hjertén [13] and Karger and co-workers [14,15]. They addressed the application area of protein separations in polyacrylamide gels in the presence of sodium dodecyl sulfate (SDS). Since then, it has become evident that almost every method developed for slab gel electrophoresis can easily be transferred to a capillary format with the advantages of fast analysis with high resolution and full automation. However, attempts to perform separations in polyacrylamide gel-filled capillaries have enjoyed only limited success because these gels are not stable during electrophoresis. Gel instability, i.e., bubble formation and clogging of the pores in the gels by precipitated proteins, limits the number of runs [16,17]. Although some research groups have described methods for the preparation of stable, bubble-free polyacrylamide gels for capillary gel electrophoresis [18–22], these have for unknown reasons not become widely used. Other types of cross-linked gels (mostly modified acrylamide matrices, such as poly(*N*-substituted-acrylamides) [23]) have also been introduced [24–26]. Bode showed that polymers entrapped in agarose gels had molecular-sieving properties [27], which certainly prompted the use of molecular-sieving polymer solutions in CE [21,28]. It should be emphasized that gels yield a higher resolution than do polymer solutions [29,30], which, however, have the advantage of being replaceable, i.e., they permit repeated automated analyses, as do methylated agarose gels.

In many respects, capillary electrochromatography (CEC) is a hybrid separation technique with advantages from both high-performance capillary chromatography and capillary electrophoresis [31,32,14]. The earliest use of electroendosmosis in a liquid chromatography experiment was reported by Strain [33] who separated dyes in an alumina column. Electrochromatography in its present form, i.e., for transport of the mobile phase was introduced by Synge and co-workers [34,35]. However, two decades was to pass until the first successful application of electrochromatography in columns conventionally packed with beads was published [36,37]. The current tremendous interest in CEC is probably due to a series of papers by Knox et al. [38–41]. Its popularity has only increased by the introduction and common use of monolithic columns able to generate high electroosmotic flow (EOF) [42]. However, the great advantages of monoliths, compared to conventional packed beds, are that they can easily be prepared in narrow-bore tubes and can be covalently attached to the tube wall, i.e., no disturbing frit is required to support the bed.

Few review articles about capillary electrochromatography and its technology deal with homogeneous gel beds [43–45]. Although the review [43] dedicates a separate section to “Soft gels” it does not differentiate between homogeneous gel beds, particulate monoliths and open tubular CEC. Two other reviews treat some of the papers dealing with homogeneous gels in the sections “Fritless columns” [44] and “Polyacrylamide-based technologies” [45] but do not distinguish clearly between monoliths and gels probably because the conventional in situ polymerization method is employed for both types of beds. Therefore, I will also clarify the differences between homogeneous gels and monoliths.

2. Theoretical considerations

2.1. Capillary electrochromatography

In capillary electrochromatography an electroendosmotically-driven flow is used instead of a pressure-driven flow to propel the mobile phase through the column. The separation mechanism in CEC is primarily based on differential interaction (e.g., partition between two phases). If the solutes are charged their migration velocities also are influenced by the electrical field. CEC offers the same stationary phases with different chromatographic properties and broad range of retention mechanisms and selectivities typical of chromatography without requiring an expensive HPLC pump. As