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Review

Short monolithic beds: history and introduction to the field

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Abstract

The history of the development of short monolithic beds is described.

Keywords: HPLC; Quasi-steady state; HPMC; Monolith

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1. History and introduction to the field

In the middle of the 1980s, numerous scientific groups studied very thoroughly gradient approaches to the chromatography of proteins using chemically different stationary phases, different parameters of elution as well as different columns geometries. This intent interest was conditioned, in general, by practical needs stimulated by rapid growth of recombinant technology. Fundamental theory of gradient HPLC [1,2] generated by pioneering works of Snyder and co-workers [3–6] can be counted as a very strong promoting factor of this research. In particular, this theory has pointed out that with gradient elution the length of the column often had only a very negligible influence on the separation of large molecules like proteins. The same conclusion has been drawn from several experimental investigations [7–10]. For example, Moore and Walters demonstrated excellent separation of a model protein mixture using columns of 0.16–4.5 cm length. Surprising for that time was the result of quite a small change in protein separation efficiency at almost 30-times decrease of column length [8]. A similar effect has been observed by a Russian group for the separation of proteins by reversed-phase HPLC on capillary columns of widely varied length [11].

The next step in the comprehension of the discussed phenomenon has been taken by Yamomoto et al. who presented a very detailed study on protein separation with the use of gradient ion-exchange HPLC. These authors have suggested the model of the so-called quasi-steady state of migrating along the column chromatographic zones [12]. According to this theoretical notion, at some "point" of peak elution (defined by gradient conditions, e.g. flow rate and gradient time) the effect of zone squeezing becomes equilibrate the effect of its broadening. Such balance leads to the quasi-steady state resulting in the absence of further broadening of migrating chromatographic zone. At quasi-steady state, the velocity of the movement of protein zone is equal to that of the migration of a displacer. After this point, the separated zones move along the column with the same velocity and the distance between them does not change, or R_S value stays constant. In other words, at the fixed gradient slope and elution velocity, the resolution increases along the column length only until some moment. The distance needed to reach this position de-

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pends on a gradient slope and, at very sharp gradients, can be much shorter than a geometric length of a column. Thus, the separation can be realized on relatively short starting part of column, whereas the rest of its length does not improve the resolution. In contrast, it makes it even worse because the gradual spreading of a gradient will result the decrease of squeezing of both peak's front (at adsorption chromatography $K_D > 1$) and peak's tail (at the transition from adsorption to exclusion mechanism $K_D \leq 1$).

The correlation between Z (that is the stoichiometric factor corresponded to the number of displacer's molecules needed for solvation of all adsorption sites both on a sorbent and protein molecule) and time (or volume) parameter is the most important condition. Time correlation governs the efficiency of "single theoretical plate" [13], whereas the volume one indicates the existing of critical (or operative) thickness of separation (adsorptive) layer [14,15] (OTAL) X_0 [16]. The latter confirms the non-stationary character of starting step of separation process carried out by gradient mode and is defined as a distance at which the quasi-steady state can be reached. This fact was finally counted as a concept of thin separation beds (extremely short-packed columns or membranes) needed for protein chromatography.

Despite the undoubted practical attractiveness, the liquid chromatography of the 1980s had very serious limitations. In fact, it was as a rule a rather slow process causing significant product (protein) degradation and requiring expensive separation media and large volumes of solvents. It is easy to imagine that the diffusive mechanism of molecular penetration into the inner space of porous particle, especially when the molecule is large and has a small coefficient of free diffusion, significantly limits the speed of separation. The undertaken efforts to improve the situation by introducing of improved porous design of dispersed separation media (micropellicular [17–20], superporous [21], superficially porous [22], non-porous [23,24] and gigaporous, namely, perfusion [25–27] and "gel in a shell" [28], supports) allowed faster exchange of a solute between the mobile and stationary phases. However, besides the absolute predomination of diffusive transport of separated substances, any dispersed sorbent fills not more than 28% of total column volume (theoretical calculation for ideal, e. g. monodisperse, microbeads). Interphase transport, in its turn, significantly influences the broadening of chromatographic peak and, respectively, leads to decreasing of separation efficiency. Thus, the suggested materials enabled partially solving of the problem of fast and efficient separations.

Taking into account all previous considerations, the idea of development of specially designed ultra-short columns, or adsorptive layers, was born. Since the creation of thin layers by packing of dispersed particles would definitely meet such difficulties as irregularities in a package, excessive channeling, etc. the novel approaches for their preparation were needed.

Additionally to previously discussed theories, plus the stoichiometric model of protein chromatographic retention suggested by Regnier and co-workers [29–31], it could be easily imagined that the wide distribution of velocities of protein desorption caused by significant differences of adsorption energy of the most strong protein ligand (adsorptive domain), will result practically in a single-step gradient separation of these solutes [32]. In this case, protein separation at gradient conditions realized in the absence of long column and repeating acts of adsorption–desorption can be logically predicted.

This theoretical possibility has been materialized in novel methodology called membrane chromatography and carried out with use of membrane-like adsorptive and absolutely permeable for the through liquid flow layers of stationary phase. Such devices were called as membrane adsorbers. This term came from membrane filtration and related very well to the geometry of stationary phase, e.g. filter-like membrane [32–38]. The chromatographic interactions in the membrane are similar to those in the porous particulate material. The main difference between them is the intraporous hydrodynamics. Membrane-based chromatographic process can generally be distinguished from porous particle-based chromatography by the fact that the interaction between a solute (for example, a protein) and a matrix (immobilized adsorptive ligand) does not take place in the dead-end pores of a particle, but mainly in the through-pores of the membrane. While the mass transport in dead-ended pores necessarily takes place by diffusion, the liquid moves through the pores of the membrane by convective flow [39–41], drastically reducing the long diffusion time required by particle-based chromatography. As a consequence, membrane separation processes are generally very fast, in fact, one order of magnitude or more faster when compared with columns packed with corresponding porous particles [42]. Moreover, since the membranes are very thin beds compared to chromatographic columns packed with porous particles, reduced pressure drops are found along the chromatographic unit, allowing dramatically increased flow rates and consequently higher productivity.

In fact, the chromatography on membrane adsorbers can be accepted as the first step in the direction to more carefully designed media of a higher degree of continuity (called later monoliths) [43]. Obviously, that the use of such type of adsorptive matrixes filling totally the column volume enables significantly decrease the factor of interparticle void volume influencing the separation efficiency. The most important feature of this kind of sorbents is that all the mobile phase is forced to flow through the large pores of rigid medium representing, in fact, one gigantic particle of different geometry (rod, disk or tube) but similar carefully designed porous space. As a consequence, mass transport is enhanced by convection and has a positive effect on the separation. The detailed theoretical description of this phenomenon was recently published [44,45].

It is now over a decade since the introduction of novel chromatographic supports based on discussed here monolithic structure. Historically, there were three main groups active in this field and introduced the supports of different chem-

istry and different characteristics. These were the groups of Nakanishi [46] and Hjertén [47] which tried to replace standard chromatographic column with the new type of continuous support allowing convective mass transport, whereas the third group [48,49] realized the idea of combining of the advantages of convective mass transport on continuous bed supports and the theory of short column layer [14,15,50].

Monolithic supports intended for protein separations offered an ideal solution to avoid mentioned recently packing and channeling problems at minimizing of column length, which led the authors (Tennikova, Belenkii, Svec) to develop 1 mm thick membrane-like layers made of rigid macroporous methacrylate polymer. The realized model separations seemed to be very efficient. The name high-performance membrane chromatography (HPMC) for the technique using short chromatographic layers with high-resolution power has been introduced at that time. To avoid confusion and mixing such type of the supports with real membrane separation units (see above), the change of this name to the name high-performance monolith chromatography has been suggested later by Tennikova and Freitag [16].

The choice of methacrylate base for developed materials was also not occasional. Approximately, at the same describing time, Institute of Macromolecular Chemistry, Prague, studied thoroughly the synthetic possibilities to prepare macroporous polymer beads of different structure and chemistries. One of intensively investigated material was the copolymer of glycidyl methacrylate (GMA) and ethylene dimethacrylate (EDMA) that, being directly functional solid phase containing the active epoxy groups, represented very strong interest for different purposes including different types of liquid chromatography. In 1986, one of the authors of this paper (T.B.T.) has been invited to this Institute to start the new international project concerned the preparation, optimization and use of polymer separation media. The obtained and published results of this successful cooperation [51–54] appeared to be quite promising from the point of view of development of new criteria of porous structure and surface design of chromatographic sorbents. In fact, the formed group firstly suggested the original approach of bulk polymerization transferred with some modifications from conventional suspension procedure to prepare the monolithic sorbents of different shape. A little bit later, the same approach has been used for preparation of monolithic rod columns [55].

Interesting that the first communication concerning the new separation method has been submitted to *Journal of Chromatography* in 1988. However, the reviewers did not recognize the potential of the new approach. It took many months until the paper was rejected, and its publication considerably delayed. Fortunately, the manuscript was accepted without any problems by *Journal of Liquid Chromatography* and published in 1990 [48]. Surprisingly, this publication stirred up a considerable interest documented by more than 300 (!) cards obtained by authors requested sending the preprint of this paper. It is known that Hjertén had very similar experience with publication of his pioneering work introducing another kind of monoliths—the compressed beds [47]. The most detailed and very often quoted paper on the novel principle of protein separation was published in 1993 [56].

In contrast to some parts of scientific community, the industry has quickly recognized the potential of the separation media in such atypical shape. Thus, Saulentechnik Knauer GmbH, West Berlin, Germany, acquired the rights to produce and market the HPMC technology and the group led by Dr. Reusch and Dr. Josic continued in the product development [57–62]. The first series of Quick-Disks was introduced in the market already in 1991. Unfortunately, this commercial product did not reach wide acceptance on the market due to problems with batch-to-batch reproducibility and bypassing. Also the scale-up strategy based on producing disk with larger diameters has not been resolved.

A major breakthrough in the development of short monolithic layers was represented by Strancar and co-workers [63–67] who also introduced tube monolithic units resolving the problem of scale-up while retaining the idea of short chromatographic pathway. Further work published by Podgornik et al. [68] introduced the “tube in a tube” column design which resolved the problem of the preparation of larger homogeneous monolithic units At present, the young and active company BIA Separation, Ljubljana, Slovenia, successfully developed and offered to the market the wide set of monolithic products under the commercial name Convective Interaction Media (CIM). Their current success relies on a balanced combination of research, development, and marketing.

It is very well known now that the new type of solid phases has such powerful advantage as very fast interphase mass transfer at dynamic conditions that, in its turn, provides high speed and high efficiency of the processes based on this principle [43]. The discussed here short monolithic beds are widely used in different practical fields such as chromatography, high throughput enzyme reactors, flow injection analysis, etc. The compatibility of method and very good results were demonstrated further not only for proteins but also for other kind of large [67,69] and even small [70] molecules. Especially impressing results were obtained in the technologies based on affinity interactions [71–77]. Multidimensional, so-called conjoint liquid chromatography, represents one of the most innovative and advantageous features of discussed supports. It concludes in the possibility of preparing a conjoint LC disk or tube monolithic column by placing disks or tubes (“tube in a tube”) with different active groups into one housing. Such method enables a separation and purification with a very low dead volume within a single step by using, for example, ion-exchange and affinity disks [78]. By placing four different disks, each carrying different ligand, the fractionation of the mixture of four different antibodies in a single step directly from biological mixture becomes possible [76]. Another field of using short monolithic units is solid-phase synthesis and combinatorial chemistry [79–84] which allow direct introduction of biospecific ligands (for example, pep-

tides) into the medium used directly for bioanalysis based on affinity interactions.

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