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Review

# Developments in the use and fabrication of organic monolithic phases for use with high-performance liquid chromatography and capillary electrochromatography

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**Abstract**

Capillary electrochromatography suffered in its development because of difficulty in producing stable columns with good permeability. Variability in frit characteristics gave rise to non-reproducible capillaries whose fabrication was extremely difficult and time consuming. Monolithic stationary phases gained popularity in the early 1990s due to the fact that they were easy to fabricate and required no retaining frits. They were also able to be manufactured in a wide variety of chemistries which made them very interesting to the analytical chemist who is constantly looking for materials with different selectivity to the popular silica-based stationary phases.


*Keywords:* Capillary electrochromatography; Micro HPLC; Organic-based monoliths; Methacrylate-based monoliths; HILIC phases; Acrylamide-based monoliths; Styrene-based monoliths

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## 1. Introduction

There has been a tendency over the last 10–15 years to reduce the column dimension in HPLC in order to capitalise on reduced solvent consumption and disposal, increased sensitivity and the ease of coupling to MS to name just a few advantages. This miniaturisation process gathered pace due to the advent of proteomics when reduced sample size was a significant problem. As demand for ever higher throughputs with higher efficiencies grew, the obvious solution was to lower the particle diameter, reduce the column length and increase the flow rate. However, the downside to this was increased backpressure. In order to overcome the problem of backpressure, researchers began to develop monolithic materials. These materials differ from conventional particle-based supports in that their structure consists of a network of interconnecting channels through which the mobile phase flows. Because the monolith totally fills the column, this eliminates any interparticle void volume resulting in increased efficiency due to enhanced mass transport as a result of all of the mobile phase flowing through the specially designed pores i.e. reduced C-term. Capillary liquid chromatography is typically performed in columns that are 50–500 μm I.D. and packed with conventional HPLC phases of between 3 and 10 μm diameter. However, the fabrication of such materials in small I.D. columns is not trivial. All packed columns need retaining frits and the ability to produce these with reproducible porosities, lengths and inertness is difficult. In contrast, because a monolith is a continuous porous material, it requires no retaining frits and because these can now be made by a one-step process in situ, their fabrication is considerably simpler. Monolithic materials have some distinct advantages over their particulate counterparts.

Although Hjertén [1] first reported the preparation of a compressed soft polyacrylamide gel in 1989, it was a complex process requiring multiple steps including a compression process. Hjertén et al. [2] subsequently reported the fabrication of highly crosslinked acrylamide polymers which were anchored to the capillary surface following modification with γ-maps (3-(trimethoxysilyl) propyl methacrylate.) However, this approach was complex and later work by Hjertén and co-workers [3] reported much-simplified procedures involving the use of a surfactant in order to solubilise the hydrophobic monomers. This early work was followed by that of Svec and Fréchet [4,5] who reported for the first time a much simpler procedure for the fabrication of rigid monolithic columns, whereby the capillary was filled with the monomer mixture including AIBN as initiator and heated in order to form a rigid porous polymer.

Capillary electrochromatography is a technique that uses electroosmotic flow to drive a mobile phase through a packed bed. Because there is no pressure drop across the capillary, this allowed the use of small particles packed into long capillaries. Although this is a very high efficiency technique, its development has been held up because of difficulties with fabrication.

The primary cause of problems is once again associated with frit formation within the capillary [6–8].

On the other hand, monolithic materials are easy to produce, can be formed with a wide variety of chemistries and require no retaining frit. Monolithic materials therefore solved all the problems associated with the manufacture of capillaries for μ-HPLC and CEC in particular those associated with frit formation and problems with excessive backpressures.

Organic monolithic materials include those that are styrene based, acrylate or methacrylate based or acrylamide based. The actual polymerisation process is initiated either thermally or by photo induction of a mixture consisting of monomers and porogenic solvents. The compositions of the monomers and porogens have a profound effect on the overall morphology of the resulting monolith. Because the porous structures of these monoliths result from a phase separation of the solid polymer from the porogen mixture during polymerisation, the pore characteristics depend heavily on the solvency of the porogens, and their content in the polymerisation mixture. Solvents that have good solvency for the forming polymer will result in the formation of small pores whereas macroporogenic solvents, which show poor solvency for the forming polymer, will result in the formation of macro-sized pores [9,10].

## 2. Monoliths

Monolithic stationary phases consist of continuous beds of interconnected channels. Because the mass transfer process is significantly higher than for conventional particle-based stationary phases, the van Deemter plots of plate height versus mobile phase linear velocity are shown to be very shallow which means that fast separations can be performed without compromising efficiency. The mass transfer contribution in monolithic materials is low even at high velocities and this is a result of the lack of interparticular voids in the column which results in all of the mobile phase flowing through the separation medium rather than around it as is the case with particle packed capillaries. Thus, the column efficiency is improved by convection, which has a positive effect on the mass transfer. In addition, these same monoliths show high permeability which means that use at high flow rates are readily tolerated. The important knock-on effect of this is that these materials can be used with conventional HPLC equipment because they do not have to be operated at the optimal flow conditions in order to provide maximum efficiency. It also allows the use of long packed capillaries, which can still be used at high linear velocities because of their high permeabilities.

### 2.1. Hydrophobic monoliths

Both methacrylate/acrylate and styrene based monoliths are most often prepared as hydrophobic monoliths. Most recent studies on these phases were focused on their development and application in reversed-phase HPLC and CEC.

Coufal et al. [11] described the co-polymerisation of butylmethacrylate (BMA) and ethylenedimethacrylate (EDMA) within a 320 μm I.D. fused silica capillary which they subsequently tested using capillary liquid chromatography.