# MONOLITHS – A NEW GENERATION OF CHROMATHOGRAPHIC SUPPORTS[#]

**Igor Mihelič, Tine Koloini**
*Faculty of Chemistry and Chemical Technology, University of Ljubljana, Aškerčeva 5, SI-1000 Ljubljana, Slovenia*

**Aleš Podgornik, Miloš Barut, Aleš Štrancar***
*BIA Separations d.o.o., Teslova 30, SI-1000 Ljubljana, Slovenia*

[#]This paper is dedicated to Professor Dr. Roman Modic on the occasion of his 90th birthday

*Received 30-05-2001*

## Abstract

Monolithic stationary phases are becoming very important in the field of liquid chromatography. Methacrylate based CIM Convective Interaction Media® monolithic columns are produced via radical polymerization, which results in a rigid and chemically very stable porous monolithic structure. Some characteristics of small-scale monolithic columns and an example of extremely fast separation of biomolecules are presented in the paper. However, the preparation of large and homogeneous monolithic columns represents a big problem, because the heat release during the polymerization causes distortion of the monolithic structure. A mathematical model employing the polymerization kinetics for the prediction of the temperature profiles and a comparison with the experimental results is presented with the emphasis on the conversion and the rate of the heat release profiles. Finally, the characteristics of a large-scale monolithic column are presented.

## Introduction

High-performance liquid chromatography is today one of the most important analytical methods, especially with the growing demands for fast chromatographic separations. Fast separations are essential for a fast screening of different combinatorial chemistry or peptide synthesis compounds, as well as for a fast and reliable in-process control of various biotechnological processes. Consequently, many new stationary phases, which enable a fast exchange between mobile and stationary phases have been developed and introduced to the market in the past ten years.

One of the most intensively studied types of stationary phases are monoliths. They consist of a single piece of organic or inorganic highly porous material with well defined distribution of pore sizes. Pores are highly interconnected channels through which the mobile phase flows. The fact that the predominant transport mechanism is a

convection rather than a diffusion leads to a flow-unaffected resolution and dynamic binding capacity[1] of monolithic supports. Among few commercially available monoliths, the methacrylate based CIM Convective Interaction Media® monoliths can be obtained in the largest number of different chemistries and volumes.[2] They are mechanically and chemically very stable[3] and have been successfully used for a fast separation and purification of different molecules, such as proteins,[3-13] DNA,[14] smaller molecules such as organic acids,[15] hydroxybenzoates,[16] oligonucleotides and peptides.[16,17]

The preparation of glycidylmethacrylate-ethylenedimethacrylate (GMA-EDMA) monoliths is performed by the bulk radical polymerization of both monomers in the presence of pore producing solvents. The result of the polymerization is a highly porous (60 % porosity) polymer with a well-defined pore size distribution. The rigid polymeric structure allows the possibility of preparation of extremely short columns, which were theoretically presented to be the best solution for an efficient separation of proteins.[18] In the past, many different authors have shown that the column length does not have a pronounced influence on the separation resolution of proteins in the conditions of a linear gradient elution.[19,20,21] This served as a basis for the development of the so-called high performance membrane chromatography in the early 1990s.[22,23] As it was practically impossible to efficiently pack extremely short conventional columns with the micrometer sized porous particles, a group of researchers in Russia and Czech Republic developed the so-called monolithic polymer, which was very promising for an efficient protein separation already in the early development phase.[24]

To obtain the monoliths with homogeneous structure the control of the temperature increase during the exothermal polymerization is essential. For this reason thermostated water bath is usually applied. Since the polymerization is highly exothermic and carried out in the bulk, where the heat transfer mechanism is mainly conduction, the removal of generated heat often becomes a difficult task. In case of polymerization in small diameter moulds, where small volume monolithic columns are prepared the conductive-based heat transfer is effective enough, however, in case of large diameter moulds the rate of heat release exceeds the rate of heat removal, which

causes the increase of temperature, especially around the center of the mould. The temperature increase leads, accordingly to the Arrhenius law, to a faster kinetics, which additionally intensifies the heat release. This auto-accelerated process causes large differences in characteristics of the monolith in radial direction and eventually leads to the distortion of the structure. Due to this very complex behavior very few attempts for the production of large volume monolithic columns have been described so far,[25,26] and only recently a successful approach based on the steady-state heat balance analysis has been introduced.[27] Here, large volume monolithic columns are made of several annuluses of small thickness and joined together. Using this approach, the temperature increase is no longer a problem because it is theoretically possible to produce a large volume column of numerous thin annuluses. However, joining annuluses together represents another problem, therefore, it is essential to find the optimal annulus thickness. In order to accomplish this, a detailed knowledge about the polymerization kinetics and heat transfer characteristics is required. Employment of this data in the proper mathematical model,[28] enables an accurate prediction of the temperature profiles during the polymerization in moulds of different shapes and at different experimental conditions. Results obtained from the modeling can be of great importance in the search for optimal polymerization conditions and moulds dimensions for the production of monolithic columns with homogeneous structure.

In the first part of this work, the small-scale CIM® monolithic columns developed for extremely fast liquid chromatography of biomolecules are presented. The second part presents the emphasis on some aspects of modeling of the temperature and conversion profiles during the polymerization of large volume monolithic columns. Finally, the construction of an 80 mL tube monolithic column based on the results of the mathematical model and its application for a fast preparative separation of proteins is presented.

## Results and discussion

*A. Application of small-scale CIM® monolithic columns for the separation of proteins*

After solving many technical problems concerning the mechanical stability of the polymer formed, its proper structural characteristics (pore size distribution) and batch-to-batch reproducibility, the monolithic rigid GMA-EDMA polymer was finally introduced to the market in the 1998 by a company BIA Separations (Ljubljana, Slovenia) under the trade name of CIM® Convective Interaction Media [3] (Fig.1).

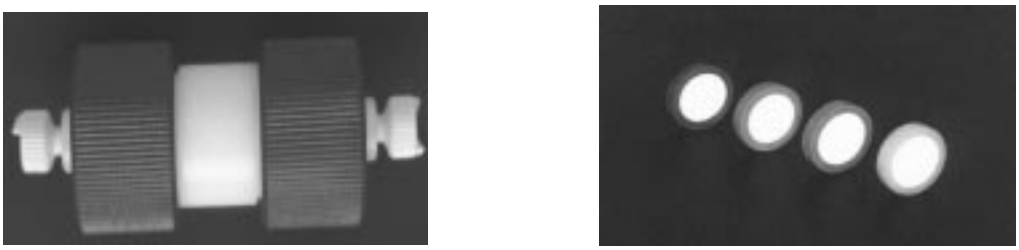

**Figure 1:** CIM® disk housing and CIM® disks. CIM® disk (right) consist of the white monolith in the middle of the disk and of a non-porous self-sealing ring. Different colors represent different chemistries. CIM® disk is placed in a CIM® housing (left) and used as a chromatographic column.

The new chromatographic support did not only give the possibility of performing efficient separations on very short separation layers (this fact being especially important when dealing with labile therapeutic biomolecules), but had also solved one of the most striking problems connected to conventional porous particles, i.e. the pronounced void volume between individual particles. In contrast to conventional particles, the monoliths are made of a single »molecule« ramified by highly interconnected, fractal, flow through pores. Because the mobile phase and sample are forced to run through these flow through pores where the active groups are located, the mass transfer between the mobile and stationary phase is greatly enhanced. This results in the possibility of performing extremely fast protein separations, even in the range of a few seconds. An example of such a separation is presented in Figure 2, where the separation of a mixture of three test proteins has been carried out within only 10 seconds.[29]

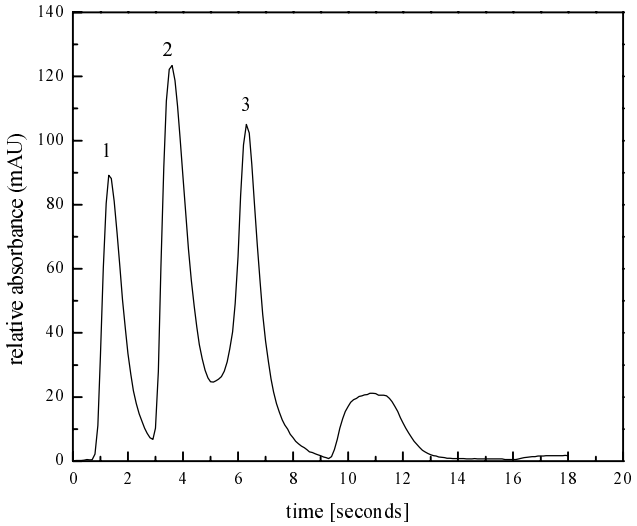


**Figure 2.** Separation of test proteins in seconds on a CIM® QA disk monolithic column. Conditions: Buffer A: 20 mM Tris-HCl buffer, pH 7.4; Buffer B: 1 M NaCl in buffer A, pH 7.4; Flow rate: 10 ml/min; Detection: UV at 280 nm; Gradient: step gradient at 0, 20 and 50 % buffer B for 2 sec each; Sample: 1: myoglobin (0.5 mg/ml), 2: conalbumin (1.5 mg/ml), 3: soybean trypsin inhibitor (2 mg/ml); Injection volume: 20 µl.

This time frame of protein separations with CIM® monolithic columns that is currently limited not by the column itself, but mainly due to limitations in the available HPLC equipment, gives the possibility of performing real on-line in-process controls of various biotechnological processes.[6,15,30,31]

Another striking features of the CIM® columns that are due to the characteristic monolithic structure are the flow unaffected resolution and dynamic binding capacity. The resolution is the measure for the degree of separation between two consecutive peaks and it mainly depends upon parameters like temperature, mobile phase composition and the efficiency of the column. With conventional HPLC columns, the resolution is highly dependent on the applied flow rate of the mobile phase and it generally decreases with increased flow rate due to the decrease of the column efficiency. On the other hand, as shown in Figure 3, the resolution on monolithic columns is practically unaffected by increased flow rate in both, isocratic[15, 17] and linear gradient elution.[3]

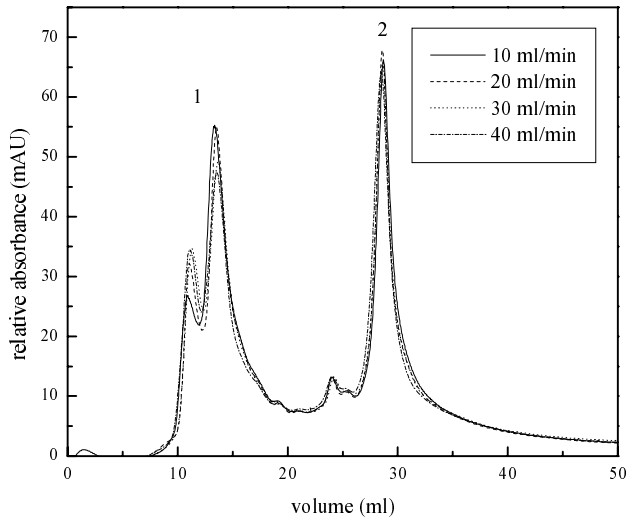


**Figure 3.** Influence of the flow rate on the resolution of conalbumin and soybean trypsin inhibitor on a CIM® DEAE-8 tube monolithic column. Conditions: Buffer A: 20 mM Tris-HCl buffer, pH 7.4; Buffer B: 1 M NaCl in buffer A, pH 7.4; Flow rate: 10, 20, 30 and 40 ml/min; Detection: UV at 280 nm; Gradient: linear gradient from 0 to 100 % buffer B in 60 ml; Sample: 1: conalbumin (2 mg/ml), 3: soybean trypsin inhibitor (4 mg/ml); Injection volume: 100 µl.

This, again, gives the possibility of performing very fast separations without sacrifying the separation resolution, thus highly increasing the throughput, which is becoming increasingly more important with increased use of combinatorial chemistry and peptide synthesis approaches. On the other hand, the dynamic binding capacity is a kinetic property of HPLC columns that depends mainly on the mass transfer characteristics of the column concerned. Dynamic binding capacity is a measure for the amount of protein that can bind to the column at defined chromatographic conditions. From the practical point of view, this value should be large enough to allow efficient and fast purification of target protein. Because of the diffusional limitations (diffusion driven process of the analyte from the mobile phase to the inner surface of closed pores containing active groups) not all of the sorbent active groups can be reached by the analyte at higher flow rates, thus, the dynamic binding capacity is drastically reduced. On the other hand, as already expressed in the case of the resolution, the mass transfer in monolithic material is driven by much faster convection. Therefore, even at higher flow rates, the breakthrough curve (as a typical measure of the dynamic binding capacity) of the

protein binding to the monolithic column, when normalized to the elution volume, remains steep and located in the same position, see Figure 4.[1]

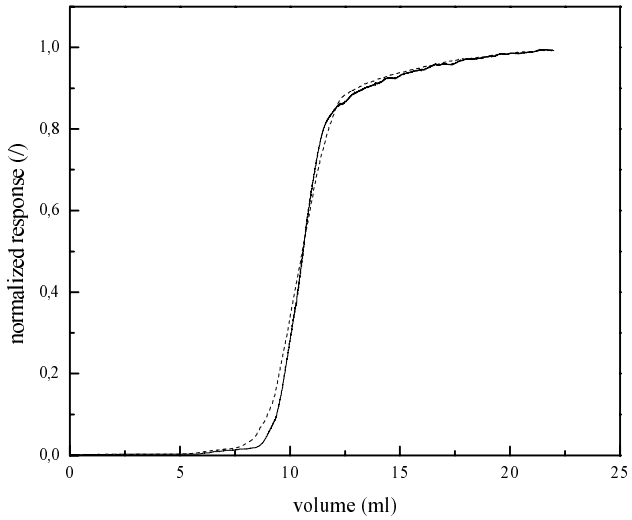


**Figure 4.** Normalized breakthrough curves for binding human serum albumin to a CIM QA disk monolithic column. Conditions: Binding buffer: 20 mM tris-HCl buffer, pH 7.4; Elution buffer: 1 M NaCl in buffer A, pH 7.4; Flow rate: 4 and 8 ml/min; Detection: UV at 280 nm; Sample: human serum albumin dissolved in buffer A (1 mg/ml).

*B. Mathematical model for the prediction of temperature profiles during the polymerization in large volume monoliths*

A mathematical model employing the polymerization kinetics[28] for the prediction of temperature profiles during the polymerization in cylindrical geometry can be described as non-steady heat conduction with the generation of heat:[32]

$$\frac{\partial T}{\partial t} = \frac{\alpha}{r}\frac{\partial}{\partial r}\left(r\frac{\partial T}{\partial r}\right) + \frac{\dot{S}}{\rho c_p} \qquad (1)$$

With the initial and boundary conditions:

$T = T_0$ $\quad 0 \le r \le r_0$ $\quad t = 0$

$\frac{dT}{dr} = 0$ $\quad r = 0$ $\quad t \ge 0$

$T = T_\infty$ $\quad r = r_0$ $\quad t \ge 0$

The generation of heat is caused by exothermal polymerization. Since the kinetics and the heat of polymerization are known[33] the generation of heat can be expressed as:

$$\dot{S} = \frac{\partial}{\partial t}\left[\rho \Delta H_r \left(1 - e^{-A\int_0^t e^{-E_{a,app}/RT}dt}\right)\right] \qquad (2)$$

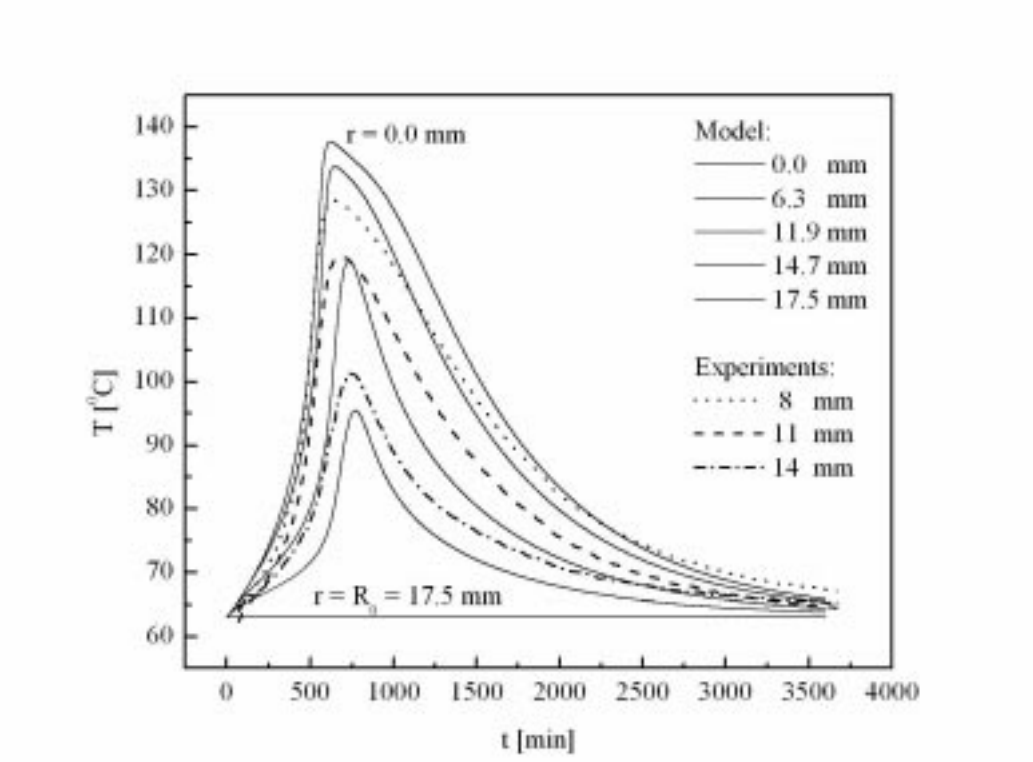


**Figure 5.** Comparison between experimentally measured temperature profiles and the mathematical model predictions. Experimental conditions: cylindrical mould of 35 mm diameter, water bath temperature 63 $^0$C. Model parameters: $c_{p,0}$ = 2.1 J/gK, $c_{p,f}$ = 1.74 J/gK, $\alpha_0$ = 1.3 $10^{-7}$ m$^2$/s, $\alpha_f$ = 0.7 $10^{-7}$ m$^2$/s, $\Delta H_r$ = 190 J/g, $T_0$ = 63 $^0$C, $T_\infty$ = 63 $^0$C, $\Delta t$ = 0.5 s, $\Delta r$ = 0.7 mm. Radial positions of thermocouples are given in the legend.

By applying the initial and boundary conditions together with the data of physical properties of the polymerization mixture[33] the numerical solution of a model can be obtained using finite difference method. A comparison between mathematical model and measured temperature profiles is given in Figure 5. It can be seen that the model describes a complex behavior quite well. A detailed description of the mathematical model together with a comparison of the experimental results is published elsewhere [28].

For the production of large volume monolithic columns, besides the temperature increase, also the estimation of the time for polymerization completion is important. Due to the temperature gradients in radial direction, the reaction rate varies in radial direction and in time. As a consequence the extent of reaction close to the mould wall is always lower then the extent near the center of the mould. Consequently, experimental determination of the time needed for the polymerization completion is a challenging task. On the other hand, the presented mathematical model enables the predictions of the rate of generated heat profiles and the extent of reaction profiles, which closely correlates with conversion. In Figure 6 calculated rate of generated heat and conversion profiles are presented. Since the presented profiles in Fig.6 were calculated using the same parameters as in Fig. 5, both figures are directly comparable.

It can be seen (Fig.6) that the polymerization time required for the complete conversion is quite long and depends on the radial distance. Such observation shows an evident advantage of the model, because considering only the measured temperature profiles (Fig.5) one could speculate that the polymerization is completed much earlier as shown in Figure 6.

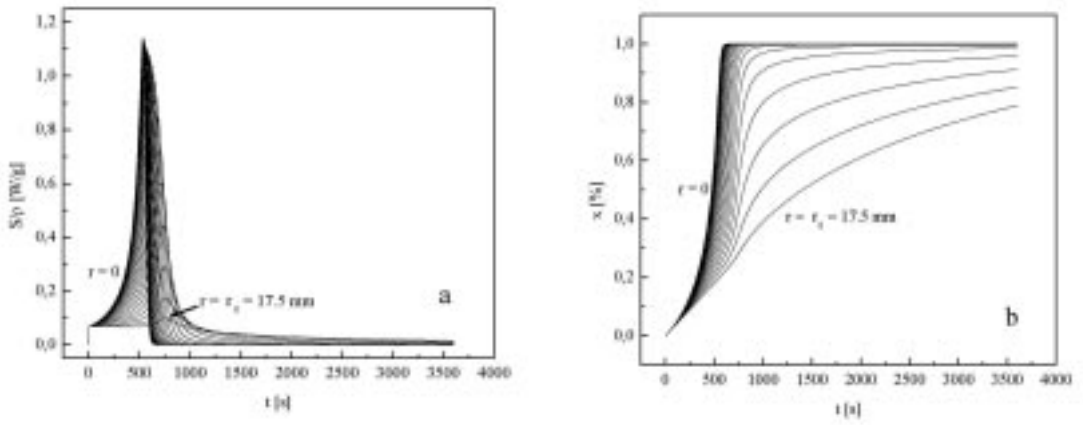


**Figure 6. a.** Calculated rate of the heat generation at different radial positions. The interval between two lines is equal to Δr, e.g. 0.7 mm. Model parameters are the same as in Fig.5. **b.** Calculated conversion profiles at different radial positions. The interval between two lines is equal to Δr, e.g. 0.7 mm. Model parameters are the same as in Fig.5.

Another important feature of the mathematical model is the ability for the prediction of the maximal temperature increase as a function of the mould diameter.

This is extremely important information for the production of homogenous large volume monolithic columns, where the maximal temperature increase is the critical parameter [27]. Since the thermal diffusivity at the beginning of the polymerization is a function of the mould diameter, because of the enhanced conductive heat transfer by natural convection inside the mould[28], such calculations become more complex. However, the results are presented in Figure 7, which represents the dependence of the maximal temperature increase at the center of the cylindrical mould on the cylinder diameter at different temperatures of the water bath. The dashed line indicates the maximal theoretical temperature increase, which is determined by the heat of polymerization ($\Delta H_r$ = 190 J/g) and the average specific thermal coefficient ($c_p$ = 1.9 J/gK) of the polymerization mixture throughout the polymerization.

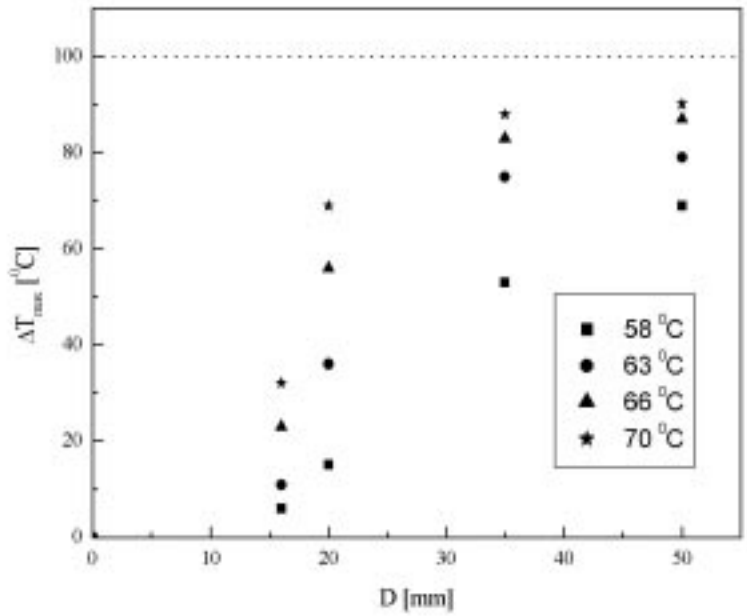


**Figure 7.** Dependence of the maximal temperature increase at the center of the cylindrical mould on the cylinder diameter at different water bath temperatures.

### *C. Construction and application of large volume monolithic columns*

A detail analysis of a heat transfer during the polymerization indicates inevitable heat release and consequently the temperature increase inside the polymerization mixture. Since the monolith structure is seriously damaged[26] a logical scaling-up of the disk

shaped GMA-EDMA monoliths can be achieved by further increasing the disk diameter. Unfortunately, the mechanical stability of such units as well as the difficulties with the uniform sample distribution are two main problems that limit such an approach. Another possibility is construction of long monolithic columns. A column of volume 15 ml and the length of 300 mm was reported[34]. However, the pressure drop of such a unit becomes one of the main problems, as it exceeds 10 MPa even at the flow rate of only 3.5 ml/min [34].

An alternative approach to avoid the problems of mechanical stability in the case of large disk shaped monolith or high backpressure in the case of rods, but still to keep the advantages of short monolithic columns is the tube geometry.

The first monolithic tube with the volume of 22 ml having the dimensions of 53 mm (height) x 23 mm (diameter) with a hole of 1 mm in the middle was described in 1997[25]. In contrast to axial working mode of the monoliths described so far, the monolithic tube works on the principle similar to the radial chromatography. In this type of geometry the height as well as the thickness of a tube can be varied. If the volume is increased only with the variation of the height, the uniform sample distribution might again become a problem. Therefore, it is necessary to increase the thickness, too. A detailed description of the construction of large tube monolithic columns has been published recently[27]. On this basis, an 80 ml CIM® tube, which is commercially available, was designed (Fig. 8).


**Figure 8.** CIM 80 ml monolithic column. Tube shaped monolitih in placed in a stainless still housing. Flow rates up to 250 ml/min can be used.

Despite the capacity of above 1 g of protein per unit, it enables extremely fast separation. It is important to emphasize that in the case of the tube shaped monoliths the resolution is also independent of the tube thickness. This is not the case for radial columns filled with particulate supports, which are typically very thin, and of a large diameter. The reason for such a design is to avoid changes in the linear velocity of the mobile phase passing through the column. Since the diameter of the outer part of the tube is larger than the inner one, the flux increases and consequently the linear velocity increases from outside toward inside. According to Van Deemter equation[35] the column efficiency decreases with the increase of the linear velocity and consequently the resolution becomes poorer with the increased thickness. Because of the flow unaffected characteristics of the monoliths however, the inner hole can be very small. In the case of the commercial 80 ml tube the inner radius is of only 1.5 mm resulting in an increase of linear velocity for almost 12 times. Despite this fact, an excellent separation of proteins can be obtained within a minute (Fig. 9).

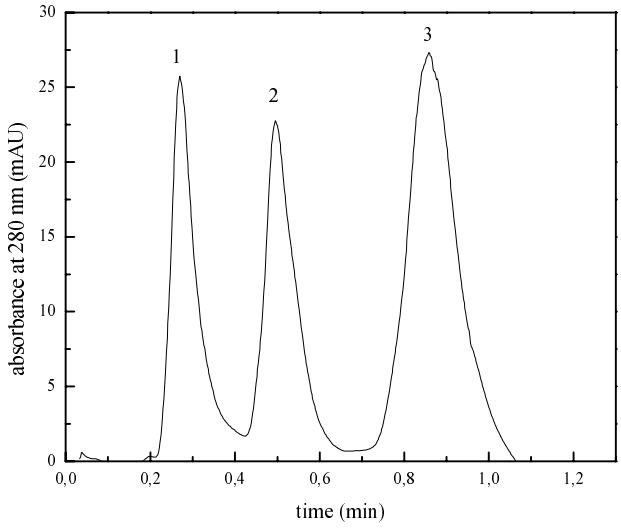


**Figure 9.** Gradient separation of a protein mixture on CIM® SO3 80 ml monolithic column. Conditions: Mobile phase: buffer A: 20 mM Tris-HCl buffer, pH 8.0; buffer B: 20 mM Tris-HCl buffer + 1M NaCl, pH 8.0; Flow rate: 240 ml/min; Gradient: 0-100 % buffer B in 52 s; Sample: 3 mg/ml of myoglobin (peak 1), 3 mg/ml of cytochrom C (peak 2) and 3 mg/ml of lysozyme (peak 3) dissolved in buffer A; Injection volume: 1000 µl; Detection: UV at 280 nm.

## Acknowledgements

We acknowledge to the Ministry of education, science and sport of Republic of Slovenia for support of this work through the projects No. L2-1522-0158-99 and PS-103-510.

## Nomenclature

| | |
|---|---|
| A | pre-exponential factor [$s^{-1}$] |
| $c_p$ | specific heat capacity [J/gK] |
| D | cylinder diameter [m] |
| $E_{a,app}$ | apparent activation energy [J/mol] |
| $\Delta H_r$ | heat of reaction [J/g] |
| $\dot{S}$ | released heat flow per unit volume [W/mL] |
| r | cylindrical coordinate [m] |
| $r_0$ | internal cylinder radius [m] |
| R | gas constant [J/molK] |
| T | temperature [K] |
| $T_\infty$ | water bath temperature [K] |
| t | time [s] |
| x | extent of reaction [/] |
| $\alpha$ | thermal diffusivity [$m^2/s$] |
| $\lambda$ | thermal conductivity [W/mK] |
| $\rho$ | density of the mixture [g/mL] |

## References

1. G. Iberer, R. Hahn, A. Jungbauer, *LC-GC* **1999**, *17*, 998-1005.
2. Dj. Josić, A. Buchacher, A. Jungbauer, *J. Chromatogr. B* **2001**, *752*,191-205.
3. A. Štrancar, M. Barut, A. Podgornik, P. Koselj, Dj. Josić, A. Buchacher, *LC-GC* **1998**, *11*, 660-669.
4. C. Kasper, L. Meringova, R. Freitag, T.B. Tennikova, *J. Chromatogr.* **1998**, 798, 65-73.
5. Dj. Josić, H. Schwinn, A. Štrancar, A. Podgornik, M. Barut, Y.P. Lim, M. Vodopivec, *J. Chromatogr.* **1998**, 803, 61-71.
6. H. Podgornik, A. Podgornik, A. Perdih, *Anal. Biochem.* **1999**, 272, 43-47.
7. G.A. Platonova, G.A. Pankova, I.Ye. Il'lina, G.P. Vlasov, T.B. Tennikova, *J. Chromatogr.* **1999**, 852, 129-140.
8. K. Amatschek, R. Necina, R. Hahn, E. Schallaun, H. Schwinn, Dj. Josić, A. Jungbauer, *J. High Resol. Chromatogr.* **2000**, 23, 47-58.
9. T.B. Tennikova, R. Freitag, *J. High Resol. Chromatogr.* **2000**, 23, 27-38.
10. K. Branović, A. Buchacher, M. Barut, A. Štrancar, Dj. Josić, *J. Chromatogr.* **2000**, 903, 21-32
11. A. Bavec, A. Podgornik, M. Zorko, *Acta Chim. Slov.* **2000**, 47, 371-379.
12. Dj. Josić, A. Štrancar, *Ind. Eng. Chem. Res.* **1999**, 38, 333-342.
13. M. Merhar, A. Podgornik, M. Barut, S. Jakša, M. Žigon, A. Štrancar, *J. Liq. Chromatogr.* **2001**, 24, in press.
14. R. Giovannini, R. Freitag, T.B. Tennikova, *Anal. Chem.* **1998**, *70*, 3348-3355.
15. M. Vodopivec, A. Podgornik, M. Berovič, A. Štrancar, *J. Chromatogr. Sci.* **2000**, *38*, 489-495.
16. A. Podgornik, M. Barut, J. Jančar, A. Štrancar, T.B. Tennikova, *Anal. Chem.* **1999**, *71*, 2986-2991.
17. A. Podgornik, M. Barut, J. Jančar, A. Štrancar, *J. Chromatogr.* **1999**, *848*, 51-60.
18. X. Geng, F.E. Regnier, *J. Chromatogr.* **1984**, *296*, 15-30.
19. R.R.M. Moore, R.R. Walters, *J. Chromatogr.* **1984**, *317*, 119.

20. G. Vanecek, F. Regnier, *Anal. Biochem.* **1980**, *109*, 345.
21. T.B. Tennikova and F. Švec, *J. Chromatogr.* **1993**, *646*, 279-288.
22. T.B. Tennikova, B.G. Belenkii, F. Švec, *J .Liq. Chromatogr*. **1990**, *13*, 63-70.
23. T.B. Tennikova, M. Bleha, F. Švec, T.V. Almazova, B.G. Belenkii, *J. Chromatogr*. **1991**, *555*, 97-107.
24. Dj. Josić, J. Reusch, K. Löster, O. Baum, W. Reutter, *J. Chromatogr*. **1992**, *590*, 59-76.
25. A. Štrancar, M. Barut, A. Podgornik, P. Koselj, H. Schwinn, P. Raspor, Dj. Josić, *J. Chromatogr*. **1997**, *760*, 117-123.
26. E.C. Peters, F. Švec, J.M.J. Frechet, *Chem. Mater*. **1997**, *9*, 1898-1902.
27. A. Podgornik, M. Barut, A. Štrancar, Dj. Josić, T. Koloini, *Anal. Chem*. **2000**, *72*, 5693-5699.
28. I. Mihelič, A. Podgornik, M. Barut, M. Krajnc, T. Koloini, 6th World Congress of Chemical Engineering, Melbourne **2001**, Australia, accepted for publication.
29. M. Barut, A. Podgornik, H. Podgornik, A. Štrancar, Dj. Josić, J.D. MacFarlane, *Am. Biotechnol. Lab*. **1999**, *17*, 48-51.
30. A. Štrancar, P. Koselj, H. Schwinn, Dj. Josić, *Anal. Chem*. **1996**, *68*, 3483-3488.
31. H. Podgornik, A. Podgornik, P. Milavec, A. Perdih, *J. Biotechnol*. **2001,** *88*, 173-176.
32. F.M. White, *Heat transfer*, Addison-Wesley, London, **1984**, 157-211.
33. I. Mihelič, M. Krajnc, A. Podgornik, T. Koloini, *Ind. Eng. Chem. Res.* **2001***,* in press.
34. F. Švec, J.M.J. Frechet, *J. Chromatogr*. **1995**, *702*, 89-95.
35. J. Van Deemter, F. Zuiderweg, A. Klinkenberg, *Chem. Eng. Sci.* **1956**, *5*, 271-289.

## Povzetek

V zadnjem času se pomen monolitnih stacionarnih faz na področju tekočinske kromatografije hitro povečuje. Izdelava monolitnih kolon na metakrilatni osnovi „CIM Convective Interaction Media® monolithic columns“ poteka z radikalsko polimerizacijo v masi. Pri tem dobimo rigidno in kemijsko odporno monolitno strukturo. V prispevku so predstavljene nekatere lastnosti monolitnih kolon manjših dimenzij in primer izredno hitre separacije biomolekul. Izdelava homogenih monolitnih kolon večjih dimenzij predstavlja velik problem zaradi sproščanja toplote pri polimerizaciji, ki povzroča poškodbe monolitne strukture. Predstavljena je primerjava rezultatov matematičnega modela, ki vključuje kinetiko polimerizacije za napoved temperaturnih profilov, in eksperimentalnih rezultatov. Poseben poudarek je tudi na modeliranju konverzijskih profilov in profilov toplotnih tokov med polimerizacijo. Na koncu so predstavljene lastnosti velike monolitne kolone.