ELSEVIER

Journal of Chromatography B, 689 (1997) 117–122

JOURNAL OF CHROMATOGRAPHY B

Novel polymeric solid-phase extraction material for complex biological matrices
Portable and disposable artificial kidney

V.A. Davankov[a,b,*], L.A. Pavlova[a,b], M.P. Tsyurupa[a,b], D.R. Tur[a,b]

[a]*Institute of Organo-Element Compounds, Russian Academy of Sciences, Moscow 117813, Russia*
[b]*Ultrascan Corp. Moscow, Russia*

Abstract

The replacement of conventional hemodialysis treatment for a patient with malfunctioning kidneys by providing him/her with a portable and disposable "artificial kidney" which is connected to implanted artery and veneous cannulas on a patient's wrist is discussed. During a continuous flow of blood, plasma diffuses through a plasmapheretic membrane, passes through a bed of plasmacompatible sorbent Styrosorb, and arrives at an ultrafiltration membrane. Vacuum-operated ultrafiltration removes excess water together with dissolved urea and other small molecules, and Stryosorb removes medium-sized toxic substances. The hemocompatibility of the sorbent is improved by chemical modification of the surface.

Keywords: Polystyrene; Artificial kidney; Styrosorb

1. Introduction

Beginning with the discovery of chromatography by Mikhail Tswett during his impressive studies on the dye components of green plants, the most exciting and probably the most productive fields of application for chromatography have been the life sciences. Modern day health care and drug research, understanding of assimilation and metabolic processes and the enormous progress of biotechnology and genetic engineering would all be absolutely unthinkable if chromatography had not contributed in such a revolutionary manner. Vice versa, pressing requirements to isolate and analyze particular groups of compounds have stimulated rapid and successful development of novel chromatographic techniques. Thus, interchange of ideas and methods can be recognised in the development of amino acid analysis and high-performance ion-exchange, isolation of enzymes and affinity chromatography, separation of enantiomers and chiral ligand exchange chromatography, protein and polynucleotide analysis and gel electrophoresis.

The development of multidimensional and hyphenated chromatographic techniques, which significantly increased the number of resolvable components, has led to a tremendous improvement in the handling of biological matrices, as has the development of selective sample preparation techniques, which reduce the complexity of mixtures prior to the separation. Thus, numerous selective solid-phase extraction procedures have been introduced, where immune adsorbents exploiting antigen–antibody com-

*Corresponding author.

PII S0378-4347(96)00307-6

plementary interactions represent the highest level of specificity.

Although the role of chromatography in modern health care is enormously important, very few chromatography-related procedures are applied in the direct medical treatment of a patient.

Besides the occasionally applied hemosorption and plasma perfusion procedures, only hemodialysis is used on a regular basis. However, our present knowledge of normal and pathological metabolism has increased greatly, and the variety of developed adsorbents which are suitable for correcting the composition of complex biological matrices, should allow researchers to develop body-compatible chromatographic procedures for providing immediate medical attention to patients.

In this paper, we describe some preliminary approaches to the development of portable adsorption/ultrafiltration systems which could help patients with malfunctioning kidneys and replace, sooner or later, the unpleasant procedure of hemodialysis. This approach is based on the latest achievements in the development of biocompatible materials and novel adsorbing polymers. No experiments with the proposed "artificial kidney" have been performed in vivo, as yet.

2. Ultrafiltration instead of hemodialysis?

The kidneys are important excretory organs whose main functions are to remove the superfluous water and toxic materials accumulating in the blood. Approximately 180 l per day of blood passes through the kidneys producing about 1.5 l of urine. Urine contains hundreds of organic compounds, most important of which are the protein digestion and metabolism products, urea, creatinine, uric acid etc.. When kidneys cannot operate properly, toxic materials accumulate in blood and other physiological fluids, leading to death within 10–12 days.

Replacing the malfunctioning kidney with a healthy one by transplant stimulates the rejection mechanisms of the living body against foreign organ, unless the donor is a near relative. Therefore antirejection drugs must be given to the recipient patient, and they always have harmful side-effects, so a transplanted kidney cannot generally be expected to function effectively for more than five years.

The only alternative way of removing waste from the organism is external hemodialysis (or peritoneal dialysis [1] which is, however, much less efficient). By allowing blood to equilibrate with a special dialysate aqueous solution through a semipermeable polymeric membrane, hemodialysis allows the excess water and small molecules to migrate down the concentration gradient from the blood into the dialysate fluid. Hemodialysis is a slow process, which keeps the patient connected to the dialysis machine for several hours. This procedure has to be repeated three or four times a week. Besides the high consumption of the physiological dialysate fluid (about 120 l), the technique is expensive as well as unpleasant and inconvenient for the patient. The patient will feel unwell both before and after dialysis. Before dialysis the waste products build up in the body, and following dialysis there is a dramatic distortion of the balance of chemical equilibrium and processes in the body due to the rapid removal of the whole pool of molecules of molecular mass <500 Da. Among these molecules are all amino acids, nucleotides, mineral ions and many other useful components. This unavoidable harmful effect would be diminished, if the removal of water and small molecules was slow and constant instead of being carried out three or four times a week with total clearance.

In principle, one can think of an extracorporeal device with an adsorbent removing undesirable components from an appropriate biological fluid, however there are no materials which could efficiently adsorb urea and other small and polar molecules from an aqueous medium. Similarly, no immobilized enzymes could solve the problem of removing the excess water from the organism. Therefore, the only alternative to the discontinuous hemodialysis procedure, which removes water and small toxic molecules, is a continuously functioning adsorption and ultrafiltration device. This idea, recently formulated by Davankov [2], could be realized in an extracorporeal portable artificial kidney system combining (i) a selective adsorption of toxic medium-size and less polar small-size molecules on a polymeric adsorbent with (ii) a non-selective removal of excess water and dissolved small polar molecules from blood by a

vacuum-operated ultrafiltration process. Major components of the system (Fig. 1) are two disposable containers. The first one contains polymeric adsorption material which is situated between two semipermeable membranes. The adsorbent and the membranes of the "artificial kidney" are in permanent contact with the blood or plasma flow of the patient. The second container is an appropriate vacuum container. It provides an easily adjustable driving force for the ultrafiltration process on one of the semipermeable membranes and, simultaneously, serves as an artificial bladder which may be conveniently emptied by the patient. Contrary to some previous totally unrealistic "implantable artificial kidneys" [3,4], this suggested artificial kidney system relies on well established physico-chemical chromatography-related phenomena and depends on the efficiency of the materials used as well as their hemocompatibility.

As shown in Fig. 2, the "chromatographic" part of the system is small and it can be easily connected to an arterial cannula implanted, for instance, in the radial artery of a patient's wrist, and a venous cannula implanted in the cerphalic vein adjacent the radial artery. When not short cut as a loop, the cannulas direct the blood continuously through the artificial kidney due a the pressure difference in the artery and vein of about 5300 to 6600 Pa. The vacuum container, connected through a thin tube to the above main part of the construction, provides slow, but continuous flow through an ultramembrane and removes the filtrate (water, urea and other small molecules and ions). The polymeric adsorbent and the ultrafiltration membranes which are placed in the artificial kidney device are either in direct contact with, or, are separated from the blood flow by an additional hemocompatible membrane. The membrane has larger pores accessible to all plasma components, but impermeable to platelets and other blood cells which tend to coagulate on foreign surfaces.

Any kind of biocompatible specific adsorbents, including affinity adsorbents, immune adsorbents, or immobilized enzymes, can be installed in the main container of the artificial kidney, provided they prove useful in some special treatment of a patient. In a more general case, however, a biocompatible non-specific adsorbent could be used, selected from activated charcoal or polymeric adsorbents, in particular, hypercrosslinked polystyrene-type adsorbent

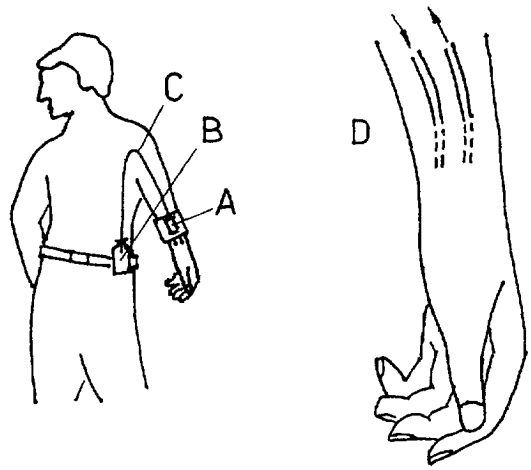


Fig. 2. Possible disposition of the "artificial kidney" (A), "artificial ureter" (C) and "artificial bladder" (B); implanted artereous and veneous cannulas (D).

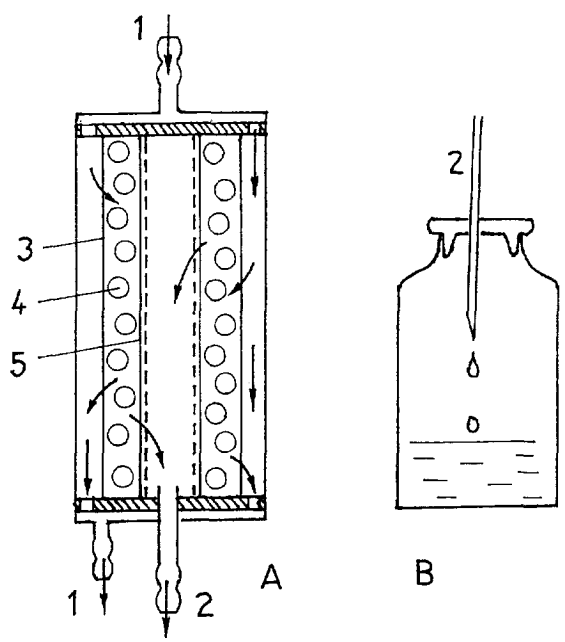


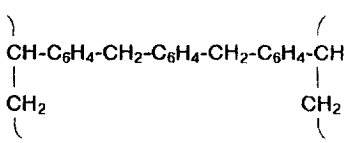
Fig. 1. Principal construction of "artificial kidney" (A) and "artificial bladder" (B). (1) Flow of blood; (2) flow of ultrafiltrate; (3) plasma filtrating membrane; (4) adsorbing material; (5) ultrafiltration membrane.

Styrosorb. The unprecedented high stability and adsorption capacity of the Styrosorb allows for the possibility of miniaturizing the artificial kidney.

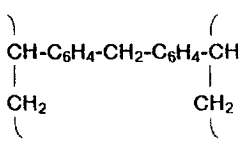
Since the cannulas and the flow-through chamber of the device are expected to be in contact with blood for long periods of time, thromboresistance of the material is of utmost importance. Many new polymers have been developed for this purpose, special types of poly-[bis-(trifluoroethoxy)phosphazene] being particularly promising construction materials and/or coatings [5,6].

3. Styrosorb – a new polymeric SPE material for plasma perfusion

Many years ago, we introduced a neutral adsorbing material based on hypercrosslinked polystyrene [7,8] which showed unprecedented adsorption capacity with respect to organic compounds. Contrary to all other polystyrene-type column packing materials which are manufactured by polymerization or co-polymerization of monomeric styrene, the hypercrosslinked polymer is prepared by post-crosslinking high molecular mass polystyrene. The polystyrene utilized in the form of solution in a good solvent (in particular, ethylene dichloride) or in the form of highly swollen beads of a styrene copolymer with small amounts of divinylbenzene. The post-crosslinking is carried out with large amounts of a bifunctional reagent capable of alkylating phenyl rings and forming conformationally rigid bridges. The most convenient crosslinking agents are xylilene dichloride and monochlorodimethyl ether. According to the Friedel–Crafts reaction, by alkylating two phenyl rings of polystyrene chains, long and rigid bridges of the following structures are formed:



and



respectively.

Provided that the above post-crosslinking involves the major part of the initial phenyl groups, the bridges are rigid, and the reaction is carried out in excess of a good solvent, networks which display unique properties are formed. They represent a spacious molecular construction, a type of rigid three-dimensional web characterized by low density and high permeability to small and medium-sized molecules with an effective molecular diameter of up to ca. 25 Å.

These hypercrosslinked polystyrene materials serve as excellent adsorbents for manifold organic compounds dissolved in aqueous media [9] or dispersed in air. These adsorbents swell with the adsorbate retained in the course of adsorption, which accounts for the extremely high adsorption capacity amounting, in some favorable cases, up to several grams per gram of the column packing. Another advantage is that, in spite of the fact that the hypercrosslinked polystyrene contains no polar groups, it readily adsorbs both unpolar and polar compounds. Thus, phenol can be concentrated on Styrosorb up to a factor of 1000 allowing an easy quantitation of phenol in tap water on a concentration level of 0.5 ppb [10]. Similarly, this SPE material, efficiently extracts unpolar propranolol together with its polar metabolism products, propranolol sulfate and propranolol glucuranide from blood plasma [9], which is not possible for other known types of hydrophobic SPE materials. Moreover, as a microporous polymer, Styrosorb does not adsorb large proteins, thus acting as a restricted access column packing material [9,11]. Therefore, no preliminary deproteinization of plasma is needed by concentrating drug metabolites on this SPE phase. Some variants of hypercrosslinked polystyrene-type adsorbents have become available, such as the MN-series of "Hypersol Macronet" for large scale adsorption technology and "Purosep" for analytical scale solid-phase extraction (from "Purolite International", Pontyclun, Wales, UK) or pre-packed 1-ml cartridges with "Diapak-Phenol/P" (from "Bio-ChemMack", Moscow, Russia).

A series of preliminary experiments with blood and blood plasma showed Styrosorbs to be promising materials for hemoperfusion and plasma perfusion procedures. No reduction of protein levels, such as fibrinogen content, has been registered. Most probably, the hypercrosslinked polystyrene network repre-

sents an "open work construction" and does not expose a dense hydrophobic surface to the proteins, thus substantially reducing their adsorption. An efficient removal of drugs and medium-sized molecules from both blood and plasma was observed using conventional spectrophotometric analysis at 280 and 254 nm. The pool of medium-sized molecules includes a series of intoxicants which build up in body fluids of patients with malfunctioning kidneys. They are generally not eliminated by the hemodialysis procedure but should be removable by the proposed artificial kidney system.

The adsorbing materials of an artificial kidney are expected to function in contact with blood or plasma for much longer periods of time than is the case with a conventional hemoperfusion or hemodialysis procedure. Therefore, to further improve the hemocompatibility of Styrosorbs, chemical modification of the surface of polymeric beads has been carried out [12]. Provided that the post-crosslinking of polystyrene was made using monochlorodimethyl ether or bis-chloromethyl derivatives of benzene or biphenyl as crosslinking agents, a sufficient amount of pending chloromethyl groups always remain on the surface of the material. During the crosslinking reaction, the surface exposed chloromethyl groups are not in a position to find an appropriate reaction partner, since the high rigidity of the network formed prevents them from "looking" inside the bead where such partners, i.e., aromatic rings, are present in large amounts. These surface-exposed reactive groups provide a unique possibility to selectively modify the surface of beads with a variety of functional groups. The following approaches have been used [12] with the aim of further enhancing the biocompatibility of the polymer.

The simplest way involves reaction of the chloromethyl groups with low-molecular-mass compounds containing reactive amino functions, such as Tris-hydroxymethyl-methylamine, glucoseamine, chitooligosaccharides. By a chain of trivial reactions, a lipid-like layer was synthesized on the surface, involving groups of phosphatidyl choline and phosphatidyl serine. According to Ishihara et al. [13], such surfaces adsorb free phospholipids from blood to form an organized structure similar to that of a bilayer biomembrane.

Binding of long hydrophilic oligomeric or polymeric chains onto the polymer surface is another possibility which, when realized in the tentacle-type chromatographic bonded phases, proved to be an efficient way of reducing the adsorption of proteins. Poly(ethylene glycol), polyacrylamide, chitosan and, in particular, heparin chains were bonded in several ways. Heparin, which is known to effectively inhibit activation of the blood complement system and prevent formation of clots, can be alternatively deposited on the surface by a simple electrostatic interaction with some positively charged functional groups.

Coating Styrosorb beads with an appropriate high-molecular-mass polyphosphazene is another very promising approach. This can be easily done by immersing the polymer into a solution of the polyphosphazene in an organic solvent, followed by filtration and washing the beads with methanol and water. The hypercrosslinked polystyrene swells and absorbs the largest part of the solvent, whereas the polyphosphazene macromolecules remain on the surface of the beads.

By using the above mentioned approaches, several dozens of surface-modified Styrosorb batches have been prepared. Of course, biochemical evaluation of the hemocompatibility and detoxification efficiency of these new packing materials, optimization of the permeability of the plasma filtration and ultrafiltration membranes, as well as estimation of the efficiency of the artificial kidney as a whole, in real animal tests, will take additional time and effort. This next stage of research, however, requires a more intensive contribution from the community of biochemists, clinicists and surgeons.

References

[1] Patent FRG 4123001, 1991.
[2] V.A. Davankov, Artificial kidney, Russian Patent 2068707, 1994; USA Patent 5545131, 1996.
[3] USA patent 5 092 886, 1992.
[4] USA patent 4 769 037, 1988.
[5] D.R. Tur, V.V. Korshak, S.V. Vinogradova, N.B. Dobrova, S.P. Novikova, M.B. Ilyina and E.S. Sidorenko, Acta Polymerica, 36 (1985) 627–631.
[6] D.R. Tur, V.G. Kulichikhin, V.T. Pal'chun and A.S. Lapchenko, Surgical suture filament, Russian Patent 2046606 (1995).

[7] V.A. Davankov, S.V. Rogozhin and M.P. Tsyurupa, Patent USSR 299165 (1969); Patent USA 3 729 457; Chem. Abstr., 75 (1971) 6841b.
[8] V.A. Davankov and M.P. Tsyurupa, Reactive Polymers, 13 (1990) 27–42.
[9] M.P. Tsyurupa, L.A. Maslova, A.I. Andreeva, T.A. Mrachkovskaya and V.A. Davankov, Reactive Polymers, 25 (1995) 69–78.
[10] M.P. Tsyurupa, M.M. Ilyin, A.I. Andreeva and V.A. Davankov, Fresenius J. Anal. Chem., 352 (1995) 672–675.
[11] M. Beth, K.K. Unger, M.P. Tsyurupa and V.A. Davankov, Chromatographia, 36 (1993) 351–355.
[12] V.A. Davankov, M.P. Tsyurupa, L.A. Pavlova and D.R. Tur, Modified hypercrosslinked polystyrene sorbents for removing toxicants from blood or plasma, Russian Patent Appl., 96104844, 1996; USA patent application submitted.
[13] K. Ishihara, N. Nakabayashi, K. Nishida, M. Sakakida and M. Shichiri, Chemtech, 23 (1993) N10 19–25.