Biomedical Engineering Advances 3 (2022) 100027

Contents lists available at ScienceDirect

Biomedical Engineering Advances

journal homepage: www.elsevier.com/locate/bea

Effect of N-vinylimidazole comonomer on blood plasma protein and endogenous toxin adsorption on mesoporous copolymer beads

Lennart Ljunggren[a,*], Alexander E. Ivanov[b], Farhana Gulalei[a], André Leistner[c], Anke Lehmann[c], Alexandra Klose-Stier[c], Aniela Leistner[c]

[a] Malmö University, Faculty of Health and Society, Department of Biomedical Science, SE-20506 Malmö, Sweden
[b] VitroSorb AB, Medeon Science Park, Per Albin Hanssons väg 41, SE-20512 Malmö, Sweden
[c] Polymerics GmbH, Landsberger Allee 378 A, 12681 Berlin, Germany

ARTICLE INFO

Keywords:
Blood purification
Extracorporeal
Polymer
Pore structure

ABSTRACT

N-vinylimidazole-divinylbenzene (VI-DVB) copolymer beads with a N-vinylimidazole (VI) weight content ranging from 7% to 15% were studied as possible adsorbents for hemoperfusion and compared with the cross-linked polystyrene beads from commercial devices. All the beads had a large volume fraction of mesopores with diameters of 2–50 nm, quantified by nitrogen adsorption, and BET areas from 500 to 1000 m^2/g. Metal-chelate adsorption of copper (II) ions on VI-DVB was high in 0.15 M NaCl (up to 40 μmol/mL), and low in blood plasma (< 1 μmol/mL). Reversible adsorption of blood plasma proteins was high (> 100 mg/mL beads) on all the bead types. Irreversibly adsorbed proteins eluted by 2% SDS included albumin, fibrinogen, fibronectin, and transferrin. Metal chelating contributed to the irreversible adsorption. Unlike the beads from commercial devices, VI-DVB beads adsorbed the LPS endotoxin from plasma. Adsorption of bilirubin and IL-6 was high on all the beads tested, but VI-DVB beads were better adsorbents of bile acids. VI-DVB are promising as components of multifunctional devices for blood purification applicable to treatment of diseases where conventional devices have lower or negligible adsorption capacity.

1. Introduction

Styrene-divinylbenzene (ST-DVB) copolymer beads have been widely studied and used as adsorbents for depletion of low and middle molecular weight toxins from the bloodstream by means of hemoperfusion – an extracorporeal form of treatment, where blood is passed through a device outside the patient's body. Hemoperfusion is a powerful means to treat liver failure or sepsis, it may improve organ dysfunction and shorten the length of stay at intensive care units [1, 2]. Commercially available hemoperfusion devices such as CytoSorb® (CytoSorbents Corporation, USA) [2, 3] and BioSKY^{TM} MD (Biosun Medical Technology Co., China) [4] comprise plastic columns packed with ST-DVB beads, which adsorb several endogenous toxins such as inflammatory cytokines, β2-microglobulin, bilirubin, and bile acids. Depending on pore size distribution and specific surface area resulting from various polymerization techniques, the rates of toxin adsorption as well as the adsorbed amounts differ strongly among the adsorbents [5, 6]. Comparative studies on adsorbents for blood purification [6] are few and far between and hence new investigations are called for. It should be particularly noted that depletion of bacterial endotoxins, i.e., key pathogenesis markers in sepsis, could not be achieved by ST-DVB adsorbents [7]. Alternative biomedical devices such as Toraymyxin^{TM} and oXiris® targeting endotoxin removal comprise polystyrene fibers with the antibiotic polymyxin B covalently attached, or acrylonitrile copolymer hollow fibers with immobilized polyethyleneimine, respectively [7]. Hence it should be advantageous to improve the adsorption specificity of ST-DVB beads by simply adding a new functional monomer, e.g., N-vinylimidazole to the polymerization mixture during synthesis.

A series of new adsorbents based on the cross-linked copolymers of N-vinylimidazole (VI) was developed and patented by Polymerics GmbH (Berlin, Germany) [8]. The adsorbents are spherical porous beads produced by radical copolymerization of N-vinyl imidazole and divinylbenzene containing from 4 to 30 wt% VI in the copolymer. The average pore diameter of the beads is in the range of 100 to 500 Å, and pore volumes fall in the 1 to 2 cm^3/g range. Compared with active carbon, the adsorbents exhibited much higher adsorption rates of albumin-bound bilirubin from 50 mg/mL serum albumin solutions, and higher adsorbed amounts of bilirubin in both static and dynamic adsorption tests. The adsorption of bile acids from 0.9% aqueous sodium chloride on VI-DVB beads was also high. The potential of these adsorbents for hemoperfusion is thus generally good but it needs to be confirmed by several adsorption and desorption tests.

* Corresponding author.
E-mail address: lennart.ljunggren@mau.se (L. Ljunggren).

https://doi.org/10.1016/j.bea.2022.100027
Received 17 November 2021; Received in revised form 16 February 2022; Accepted 21 February 2022
Available online 23 February 2022

Table 1
Chemical composition, specific surface by BET and specific pore volume by BJH (meso- and macropores) and HK (micropores) models.

Bead type	Particle diameter, μm	N-vinyl-imidazole content, weight%	BET surface, m^2/g	Micropore volume, cm^3/g	Mesopore volume, cm^3/g	Macropore volume, cm^3/g	Total pore volume, cm^3/g
AP-100	71–150 (*)	0 (***)	619	0.247	0.708	0.299	1.254
AP-101	56–200 (*)	7	538	0.218	0.662	0.255	1.135
AP-103	71–150 (*)	11	653	0.260	0.999	0.182	1.441
AP-105	71–150 (**)	15	641	0.256	1.086	0.017	1.359
CytoSorb	400–600 (**)	NA	627	0.262	0.887	0.319	1.468
Biosky	550–700 (**)	NA	981	0.381	1.852	0.021	2.254

* The size range where > 95% beads by weight belong.
** The size range where all the observed beads belong as estimated from light microscopy images.
*** Copolymer of divinylbenzene and styrene.

VI-DVB copolymer beads have certain structural features that differ them from conventional ST-DVB adsorbents. The presence of VI monomeric units in the adsorbents may affect the adsorption of plasma proteins and toxins in several ways. Imidazole is a weak base with pK_a of its protonated form of 7.0 [9]. Poly (N-vinyl imidazole) (polyVI) is a water-soluble polymer exhibiting a lower apparent pK_a of ca. 5 [10] owing to repulsion of hydrated protons from the neighboring positively charged VI units, i.e., due to the polyelectrolyte effect. At physiological pH 7.4, only few VI units (5–10 mol%) of the polymer are protonated [10] so its electrostatic interaction with negatively charged plasma proteins is possible, albeit with moderate binding strength. One of the aims of the study was the determination of the ionization degree of VI-DVB beads ionization degree at various pH values, including the physiological pH value.

VI copolymers readily form complexes with transition metal ions. Cu^{2+} and Zn^{2+} are present in blood plasma at concentrations of 10–20 μM [11], and the equilibrium dissociation constants reported for complex formation with cross-linked polyVI gels are in the range of 0.5–1 mM [12], which is much higher than the ion's concentrations in plasma. Some withdrawal of transition metal ions from plasma can thus be anticipated, though the VI-units of the copolymers will probably be far from saturation. The ion binding strength depends on the VI-copolymer structure and can be higher at larger separation of VI-units. Thus, the VI groups were almost saturated by Cu^{2+} in VI-N-vinyl caprolactam (1:9) copolymer at a 1 mM equilibrium concentration of Cu^{2+} [13]. The data suggests a contribution of metal-chelating into the protein adsorption mechanism by VI-DVB beads: VI-containing copolymers loaded with Cu^{2+} ions are known to bind proteins via their amino acids' histidine, arginine, cysteine, and tryptophan [14]. One of the aims of the study was the determination of the copper-binding capacity of VI-DVB copolymer beads in the absence and in the presence of plasma proteins.

The general aim of this study was evaluation of the effect exerted by VI-comonomer on the adsorption characteristics of copolymer beads. For this, comparison of VI-DVB beads with well-established and commercially available beads such as CytoSorb® and BioSKY™ MD was performed by means of measurements of pore size distribution and specific surface area, and estimation of blood plasma protein and toxin adsorption. A reference sample of ST-DVB beads (AP-100) was produced at Polymerics GmbH using the same method as used for production of VI-DVB beads. Here we use the abbreviation AP for all the beads produced by Polymerics.

2. Materials and methods

2.1. Materials

AP-adsorbents were produced at Polymerics GmbH (Berlin, Germany), their characteristics are listed in Table 1. BioSKY™ MD columns were supplied by Biosun Medical Technology Co. Ltd. (Foshan City, Guangdong Province, China). CytoSorb® columns were supplied by CytoSorbents Corporation (New Jersey, USA). The commercial hemoperfusion columns were dismantled, the beads were removed and conditioned in the same way as AP-adsorbents, see Methods. Sterile water was from Baxter Healthcare SA (Zurich, Switzerland) and sterile sodium chloride solution (9 g/L, 0.15 M NaCl) was from Fresenius Kabi (Sevres, France). Pellets for the preparation of phosphate buffer saline (PBS, pH 7.4) were supplied by Medicago AB (Uppsala, Sweden). 2-Propanol (puriss. p.a.) was purchased from Honeywell Specialty Chemicals (Seelze, Germany). 2% SDS solution was from Pierce™, BCA Protein Assay Kit and Imperial™ Protein Stain were products of Thermo Scientific, USA. 1,4-Dithiothreitol (DTT) was a product of Bio-Rad Laboratories (Hercules, CA, USA). Bradford reagent and bovine serum albumin were products of Sigma-Aldrich (Saint Louis, MI, USA). Alizarin Red S was from Merck (Darmstadt, Germany).

2.1.1. Blood and blood plasma

Fresh blood and citrate blood plasma from healthy donors was used in the adsorption experiments. Ethical approval for the implementation of the study was obtained from the Ethical Council of the Faculty of Health and Society, Malmö University (Ref: HS2019 No. 38/45, 2019–03–01) and the study was performed according to the Helsinki declaration (2013). All participants gave their written consent.

2.2. Methods

2.2.1. Synthesis of VI-DVB copolymer beads

The synthesis was performed as described previously [8]. Briefly, a solution of divinylbenzene (18.7 g), N-vinylimidazole (in the 3 to 12 g range) and 0.25 g azobisisobutyronitrile in ethyl acetate (18.7 g) was added to 0.15 L of a 0.2% poly (vinyl alcohol) (Mw =49,000 g/mol) solution in 5% sodium chloride at 70 °C. The mixture was subjected to vigorous mechanical stirring. The emulsion was stirred for 60 min at 70 °C until a stable droplet size was established and then heated to 80 °C and polymerized at this temperature for 10 h. The obtained suspension was cooled to room temperature and the solids were separated from the liquid phase on a sintered glass filter. The filter cake was washed with deionized water, ethanol and vacuum dried for 12 h at 100 °C.

2.2.2. Conditioning of the beads

The beads were immersed in isopropanol, agitated in the solvent by mechanical stirring and kept for 8–10 min for sedimentation of the main fraction, suspending of the finest beads and air bubbles. The supernatant was decanted, and the procedure was repeated using 50% and 20% isopropanol in sterile 0.15 M NaCl, each time twice. The beads were then rinsed by 20% isopropanol on a sintered glass filter and kept at room temperature in this solvent. Before the experiment, the beads were rinsed with sterile 0.15 M NaCl on a sintered glass filter, transferred into a vacuum flask, degassed and kept either in sterile, degassed 0.15 M NaCl or in PBS made with sterile water. The wetting of beads was adequate as confirmed by comparison of the weights of wet and dry beads, see Supplementary Materials, Section S1.

2.2.3. Material characterization by nitrogen adsorption and elemental analysis

Specific surface area and pore size distribution were determined by nitrogen adsorption and desorption isotherms recorded at liquid nitrogen temperature (–196 °C) at relative pressures p/p_0 between 0.001 and 1.0 using an ASAP 2010 porosimetry system (Micrometrics Instrument Corp., Norcross, USA). The specific surface area was calculated using the Brunauer, Emett, Teller (BET) equation [15]. The total micropore volume (pore size < 2 nm) was calculated with the Horvath-Kawazoe (HK) method [16], whereas the mesopore volume (2–50 nm) was obtained via the Barrett, Joyner, Halenda method (BJH) [17]. The pore size distribution (PSD) functions were calculated using the equation proposed by Nguyen and Do for carbon adsorbents [18] and modified by Gun'ko to study adsorbents with cylindrical and slit-shaped pores and voids between spherical nonporous particles or certain mixtures of these pores [19]. Adsorbents containing vinyl imidazole were subjected to elemental analysis (C, H, N) and the vinyl imidazole weight fraction was calculated from the nitrogen content. Dispersion of functional groups within a copolymer sample was qualitatively characterized by staining of the beads by Rhodamine B isothiocyanate dye, see Supporting Materials S2.

2.2.4. Potentiometric titration of VI-DVB copolymer beads

Wet filtered-off beads (0.2 g) were added to 10 mL 0.15 M NaCl solution and the pH of suspension was adjusted to 12 ± 0.1 by adding 1 M NaOH, with magnetic stirring. The suspension was titrated by 100 μL-aliquots of 0.1 M HCl, each time the pH was allowed to stabilize at the new value before adding a new aliquot of HCl. The same kind of titration was made with 10 mL 0.15 M NaCl without beads, as a reference. From the amount of acid spent to reach a certain pH of the bead suspension, the amount of acid spent to reach the same pH in the reference sample was subtracted. The difference was the amount of HCl needed to ionize VI-units of the copolymer at the particular pH. The titration was done until the pH value reached 2–2.5, and the amount of HCl spent for the ionization of VI-DVB copolymer was divided by the beads' weight to get its ion-exchange capacity.

2.2.5. Metal-chelate adsorption capacity of VI-DVB copolymer beads

Metal-chelate adsorption capacity of the beads was determined by depletion of copper (II) ions from a 0.18 mM $CuSO_4$ solution in 0.15 M NaCl (2–15 mL), or in blood plasma brought into contact with the beads (30 mg wet) conditioned as described above. Estimation of copper ions in the sodium chloride solution was done using 0.6 mM aqueous solution of Alizarin Red S (ARS), according to method [20]. Briefly, an aliquot of $CuSO_4$ solution was incubated with the beads at room temperature (21 °C) and after orbital shaking for 60 min the suspension was centrifuged at 18,800 rcf for 1 min to separate the beads. The supernatant (0.2 mL) was combined with 10 mM Na-acetate buffer (pH 5.0) (0.3 mL), then 0.25 mL of 0.6 mM ARS was added to the mixture and its absorbance at λ_{max} = 500 nm was measured. The copper ion concentration was calculated as

$$\left[Cu^{2+}\right] = \left(A_{500} + 0.007\right)/\varepsilon_{500} \quad (1)$$

using the extinction coefficient $\varepsilon_{500} = 2000\ M^{-1}\ cm^{-1}$ found from the linear calibration graph (see Supporting Materials, S3). The adsorbed amount of copper (II) ions (Q) was further calculated as the difference between the content in the starting 0.18 mM solution and in the supernatant in equilibrium with the beads:

$$Q_{ads} = \left(\left[Cu^{2+}\right]_{ST} - \left[Cu^{2+}\right]_{SUP}\right) \times V_{ads}/m \quad (2)$$

where V_{ads} is the volume of adsorption mixture and m the weight of wet beads. Q_{ads} was plotted against $[Cu^{2+}]_{SUP}$ to get the adsorption isotherm.

The desorbed amount of copper was determined spectrophotometrically (λ_{max} = 630 nm) after washing the copper-loaded beads (30 mg wet) with 0.15 M NaCl (1 mL) and further treatment with 10% aqueous ammonia (0.45 mL) for 60 min on an orbital shaker. This amount was calculated as

$$Q_{des} = \left(A_{630} + 0.002\right) V_{des}/\varepsilon_{630} \times m \quad (3)$$

using the extinction coefficient $\varepsilon_{630} = 62\ M^{-1}\ cm^{-1}$ found from the linear calibration graph (see Supporting Materials, S4), and the volume of desorption mixture V_{des} = 0.5 mL.

2.2.6. Adsorption of specific blood plasma proteins

Wet polymer beads (50 μL) pre-washed with PBS were placed in 1.5 mL-Eppendorf tubes and 1 mL of fresh citrate plasma was added prior to incubation at room temperature (21 °C) using an orbital rotator. Samples of the supernatants (10 μL) were taken after 5, 15, 30, 45 and 60 min of contact followed by quick centrifugation (1 min) aknd transferred into new Eppendorf tubes containing 90 μL of PBS buffer. The diluted samples were frozen until analysis of fibrinogen and albumin. After 1 h incubation, the beads were washed with 1 mL of PBS buffer, centrifuged and washed again with fresh PBS, the procedure was repeated five times. The beads were dried by suction with a filter paper, thereafter 200 μL of 2% SDS solution was added to the beads to extract irreversibly adsorbed proteins from the polymer beads. The extraction was performed by rotating for 5 min followed by quick centrifugation, and the supernatants were transferred into new tubes. The same technique was used for extraction of the adsorbed proteins with 50 mM EDTA.

The total protein content in the plasma before and after its contact with the adsorbents was determined using the Pierce™ BCA Protein Assay Kit from Thermo Scientific (Rockford, IL, USA). Albumin concentration was estimated with the Albumin Bromocresol Green Kit AB 362 Randox Laboratories (Crumlin, UK). Plasma fibrinogen concentration was estimated by Human Fibrinogen ELISA Kit (ab108842) from Abcam (Cambridge, UK).

The adsorbed amounts of specific proteins (Q_P) and endotoxins were calculated using the equation:

$$Q_P = \left(C_{st} \times V_{st} - C_t \times V_t\right)/m \quad (4)$$

where C_{st} is the starting concentration of the analyzed substance in plasma or serum, C_t is its concentration at time t, V_{st} is the starting volume of plasma or serum, V_t is the volume of liquid phase in the adsorption mixture at time t, and m is the weight of the wet adsorbent. V_t was calculated as described in Supplementary Materials, Section S1.

2.2.7. Adsorption of bilirubin, bile acids and lipopolysaccharide from bovine multisera

The adsorption experiment was carried out as described in Section 2.2.6 using 100 μL wet polymer beads and 1 mL Bovine Multisera Level 3 from Randox Laboratories (Crumlin, UK) with total protein content of 80.4 g/L. Total bilirubin was analyzed using Total Bilirubin Kit T-BIL from Randox Laboratories (Crumlin, UK). The starting bilirubin concentration in the serum was 66 μM. Bile acids were analyzed using Total Bile Assay from Diazyme Laboratories (Poway, CA, USA). The starting concentration of bile acids in the serum was 78 μM. The lipopolysaccharide (LPS) content was analyzed using the Pierce LAL chromogenic endotoxin quantification Kit from Thermo Scientific (Rockford, IL, USA). The initial concentration of LPS was 2000 EU/mL. For the estimation of LPS the sera samples were diluted 1:20 in endotoxin free water and heated for 15 min at 70 °C prior to analysis.

2.2.8. Adsorption of IL-6 and lipopolysaccharide (LPS) from cell culture medium

The test medium was produced by activating THP-1 cells (a monocyte like cell line) using LPS, letting the cells grow for three days, and thereafter the cell culture medium was collected. The medium was aliquoted in 10 mL portions and kept at −80 °C until evaluation of the adsorption capacity of LPS and IL-6. The THP-1 cells were cultured in RPMI 1640 supplemented with 10% calf serum. The cells were activated

by adding 1 μg LPS/mL medium. LPS, E. Coli O111:B4 was from Sigma-Aldrich (Saint Louis, MI, USA).

The adsorbents (100 μL) were transferred into 1.5 mL Ellerman tubes and washed at least three times with 1 mL PBS buffer, thereafter dried with filter paper strips to remove excess buffer. Test medium (1 mL) was added to each tube with adsorbent and incubated at room temperature (21 ˚C) in a rotator for 1 hour. After incubation, the adsorbents were spun down and the supernatants were transferred into new tubes. The supernatants were analyzed for IL-6 using an Invitrogen Elisa kit (Thermo Scientific, Rockford, IL, USA). For analysis of LPS 50 μL medium was diluted by 450 μL LAL free water and kept at 75 ˚C for 10 min and thereafter centrifuged at 18,800 rcf for 10 min. The supernatants were assayed after additional dilutions 1:5000 and 1:10,000 for LPS using the Pierce LAL chromogenic endotoxin quantitation kit (Thermo Scientific, Rockford, IL, USA). The initial concentration of LPS in the cell culture medium was 7640 EU/mL (1.53 μg/mL).

2.2.9. Polyacrylamide gel electrophoresis (PAGE) of the sds extracts

The SDS extracts were assayed by SDS-PAGE both in reduced and non-reduced forms. The electrophoresis was run on 12% Mini-Protean® TGX™ Precast Gels in TRIS-glycine-SDS buffer (Bio-Rad Laboratories, Hercules, USA). The samples were prepared with Laemmli Sample Buffer (Bio-Rad Laboratories, Hercules, USA) either in non-reduced or reduced form, in the latter case using 1,4-dithiotreitol. The molecular weight standard was Precision Plus Protein™ Kaleidoscope™ Standard (Bio-Rad Laboratories, Hercules, USA). The electrophoresis was run at 300 V for approximately 15 min. The gel was washed with deionized water and stained with Bio-Safe™ Coomassie G-250 Stain (Bio-Rad Laboratories, Hercules, USA).

2.2.10. Scanning electron microscopy

SEM micrographs were obtained from the beads glued to the sample carrier and sputtered with 4 to 7 nm-layer of gold. The SEM images were recorded using a Zeiss DSM 982 GEMINI scanning electron microscope equipped with Kamer SE Detector, at 0.3 Pa pressure, using an accelerating voltage of 4 – 7 kV and working distance of 7 – 25 mm.

3. Results and discussion

3.1. Physical and chemical characteristics of the beads

The chemical compositions, particle sizes, specific surface areas and specific pore volumes of the adsorbent beads are listed in Table 1. The AP-series adsorbents have smaller particle sizes than those of CytoSorb and BioSKY. Particles with diameters larger than 300 μm are needed for unhindered filtration of whole blood [21], although 100 μm diameter beads can still be used for blood plasma filtration [22]. The surface and pore characteristics were calculated from the nitrogen adsorption isotherms obtained at 77 K, see Section 2.2.3. As follows from the table, all the beads studied had a large volume fraction of mesopores with diameters 2–50 nm, and minor fractions of micropores (< 2 nm) and macropores (> 50 nm), the latter were almost absent in AP-105 and BioSKY beads. Specific surface area as a function of pore size is illustrated in Fig. 1. The surface area of mesopores belongs mostly to the pores with diameter of ca. 30 nm, which can be attributed to the spaces between the nanoparticles visible in SEM images, see Graphical Abstract, Fig. 2 and Fig. S8. This mesopore fraction is typical of polystyrene hemoperfusion adsorbents [5, 6]. Moreover, the mesopore size distribution underlies the adsorption efficiency in removal of middle molecular weight toxins as well as albumin-bound toxins [6]. The mesopore volume and the total pore volume as well as the BET surface area were the largest in BioSKY beads.

3.2. Potentiometric titration of VI-DVB copolymer beads

Titration curves of VI-DVB beads AP-101 and AP-105 reflected significant difference between the adsorbents (see Fig. S5). AP-105 contained more ionizable VI groups than AP-101, in accordance with their chemical compositions, see Table 1. AP-101 beads were ionized completely at pH below 3, and the ion-exchange capacity was ca. 170 μmol/g wet beads. About 10% of the VI units seemed to be ionized at physiological pH 7.4, which agreed well with the ionization pattern of soluble polyVI [10], see Introduction. In contrast, AP-105 beads could still bind hydrochloric acid even at pH about 2, whereas the fraction of VI units protonated at pH 7.4 was certainly below 10%. Therefore, both the bead types can be classified as weak anion exchangers. Electrostatic interactions between the protonated VI units and plasma proteins are not likely to cause high protein adsorption in the presence of physiological 0.15 M NaCl since weak anion exchangers are known to release the adsorbed proteins at similar ionic strengths, i.e., 0.1 – 0.2 M [23].

3.3. Metal-chelate adsorption capacity of copolymer beads

Copolymers of VI have considerable affinity for transition metal ions [13, 14], which may be depleted from blood and further affect the adsorption of plasma proteins and toxins. On the other hand, the proteins themselves can bind the metal ions [24] too, enabling distribution between the solid phase of adsorbent and the liquid phase of blood, during hemoperfusion. We have studied the adsorption of copper (II) ions from copper sulfate solution in 0.15 M NaCl and from blood plasma spiked with copper sulfate as described in Section 2.2.4 (see Eq. (1) and 2). The adsorption isotherms were linear in the range of copper (II) concentrations, typical of those in blood plasma and higher up to 100 μM, see Fig. S6. The distribution coefficients were as high as 380 and 650 for AP-101 and AP-105, respectively, i.e., the copper ions were effectively removed from the aqueous solution by the adsorbents. The copper (II) amounts desorbed by 10% ammonia agreed well with the adsorbed amounts but less so at higher copper (II) loads.

To evaluate the adsorption of copper (II) ions from blood plasma, the plasma was spiked with copper sulfate solution to obtain 0.18 mM Cu^{2+} concentration, *i.e.*, about ten times higher than the natural concentration of copper (II) ions [11]. Nevertheless, the desorbed amounts were quite low, *i.e.*, below 1 μmol/mL beads at all the studied beads-to-plasma ratios, compared with the desorbed amounts of 7 – 25 μmol/mL beads in Fig. S4. Adsorption of copper (II) ions on AP-101 from blood plasma was, therefore, insignificant even at artificially high concentration of the metal, probably related to the competing metal ion complex formation with plasma proteins. This suggests that copper will not be withdrawn from blood during hemoperfusion using these adsorbents. Neither CytoSorb nor BioSKY nor AP-100 beads adsorbed any copper (II) ions even from 0.18 mM $CuSO_4$ in 0.15 M NaCl, *i.e.*, in the absence of plasma, confirming the absence of affinity for copper (II) displayed by the ST-DVB hemoperfusion media.

3.4. Albumin and total plasma protein adsorption

Polymer beads intended to bind endogenous toxins may non-specifically adsorb many other plasma proteins [6] and blood cells resulting in lower platelet counts, lower total protein levels and lower albumin levels observed, e.g., in patients treated with hemoperfusion using CytoSorb® [25]. Fibrinogen, a mediator of platelet adhesion to biomaterials, is known to adsorb strongly to polystyrene [26]. Other proteins important for blood coagulation such as antithrombin III could also be depleted due to adsorption on ST-DVB beads, and the adsorbed amounts of which will be dependent on the composition and pore characteristics of the beads [5]. To evaluate these accompanying processes of blood purification, we studied the kinetics and adsorbed amounts of several plasma proteins on AP-adsorbents, CytoSorb, and BioSKY.

Adsorption of serum albumin, the most abundant protein in blood plasma, was studied using the dye bromocresol, which specifically forms a colored complex with albumin [27]. Kinetic profiles of protein adsorption are shown in Fig. 3a. As follows from the graphs, the albumin adsorption reached equilibrium with AP-beads in about 30 min or less.

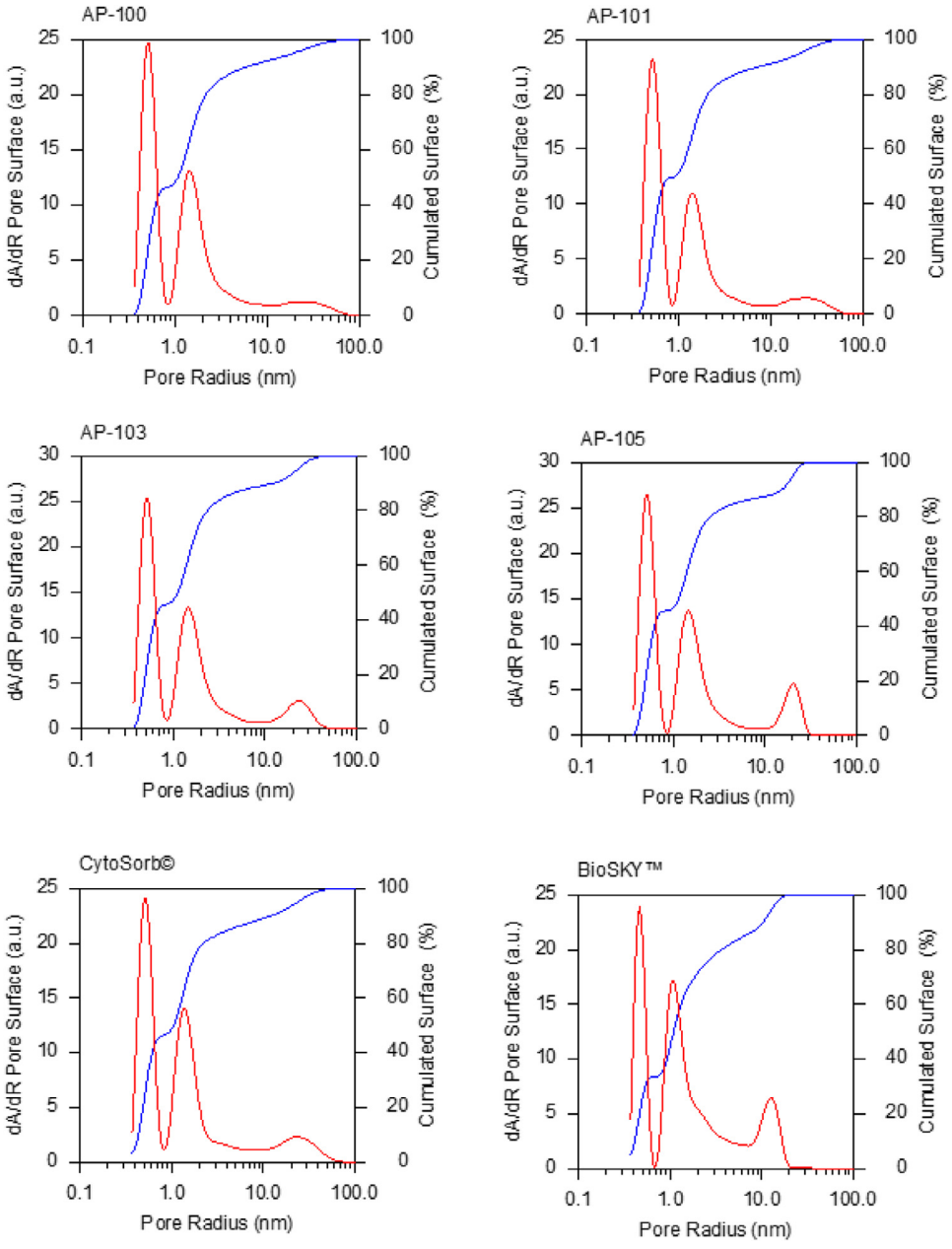

Fig. 1. Specific pore surface distribution of the beads as a function of pore radius (red lines). Blue lines are integral curves of the cumulated surface.

The albumin adsorption on BioSKY and CytoSorb did not reach equilibrium after being in contact with plasma for 60 min, probably due to the beads' large size.

Similar patterns of total protein adsorption from plasma were observed as a decrease of the plasma absorbance (λ = 280 nm) due to protein adsorption when plasma was in contact with various types of beads (Fig. S7). The highest rate of adsorption and liquid exchange was exhibited by the beads of the AP-series because of their smaller size (see Table 1) and faster diffusion of both protein and the buffered saline to and out of the beads, respectively. The lowest protein adsorption rate was exhibited by BioSKY beads. The reason could be related to the large bead size as well as a small fraction of macropores (see Table 1). The lower total protein adsorption on BioSKY during the first hours of its contact with plasma may be an advantageous feature for hemoperfusion. After a 22 h contact time the adsorption equilibrium seemed to be established.

The adsorbed amounts of albumin on various beads are summarized in Table 2. The high adsorbed amounts of albumin are typical of other hydrophobic mesoporous materials such as activated carbons [28]. Some loss of plasma proteins was, therefore, unavoidable under the conditions of hemoperfusion either with the commercial adsorbents CytoSorb® and BioSKY^{TM} MD or with AP-beads.

3.5. Fibrinogen adsorption

Adsorption of fibrinogen (Fb) on surfaces of biomaterials is a phenomenon crucial for adhesion and activation of platelets and, further, clotting events. The chemical composition of materials exerts strong effects on fibrinogen adsorption from blood plasma: the adsorbed amounts ranged from 5 to 800 ng Fb/cm^2 among 16 various polymer samples [26]. Fibrinogen adsorption, platelet adhesion and activation were found to decrease with increasing hydrophilicity and there was a good correlation between fibrinogen adsorption and platelet adhesion [29].

Unlike the total protein and albumin adsorption, Fb adsorption reached high values within only five minutes of plasma contact with all types of the beads, see Fig. 3b. The adsorbed amounts were quite

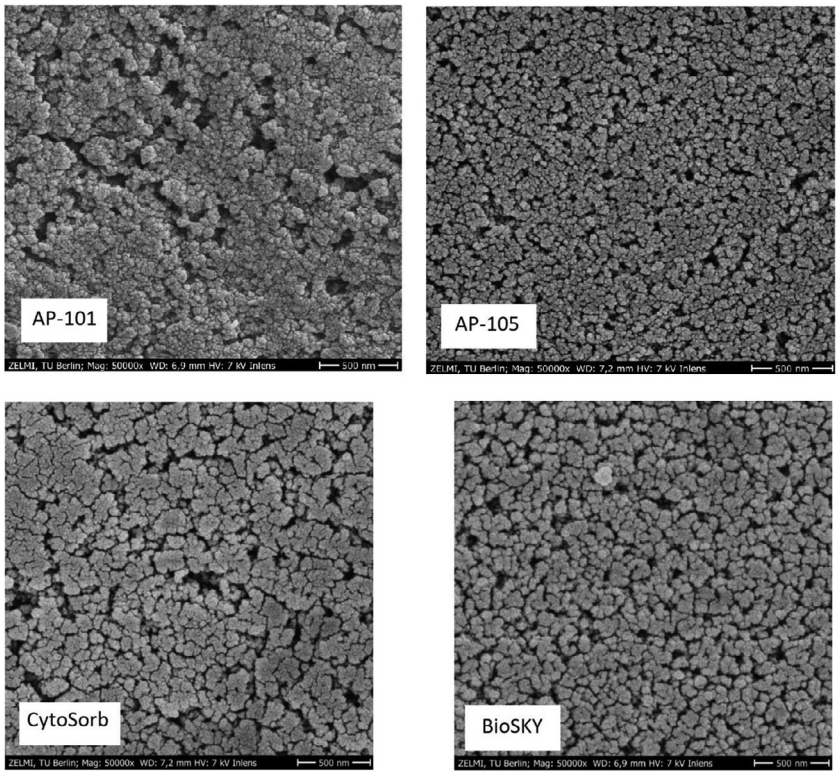

Fig. 2. SEM images of the adsorbent's surfaces. The scale bars are 500 nm.

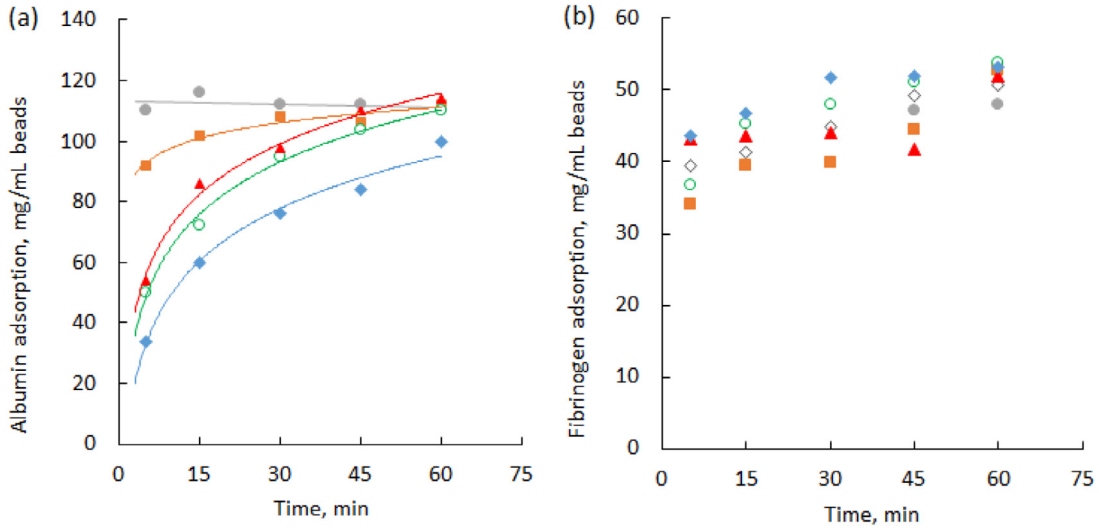

Fig. 3. Adsorbed amounts of albumin (a) and fibrinogen (b) as functions of time during contact of blood plasma (1 mL) with various beads (50 μL). (♦) – BioSKY, (▲) – CytoSorb, (◊) - AP-100, (●) – AP-101, (■) – AP-103, (○) – AP-105.

Table 2
Adsorbed amounts of albumin, total protein, bilirubin, bile acids and LPS on various beads (*).

Bead type:	AP-100	AP-101	AP-103	AP-105	CytoSorb	Biosky
Albumin, mg/mL beads	110	110	112	110	114	100
Total protein, irreversible, mg/mL beads	2.6	2.9	1.8	1.9	1.1	0.5
Bilirubin, μmol/mL beads	0.64	0.53	0.55	0.59	0.44	0.54
Bile acids, μmol/mL beads (**)	0.66	0.72	0.65	0.61	0.42	0.11
LPS from bovine multisera, μg/mL beads (***)	0.80 ± 0.05	1.92 ± 0.05	ND	0.48 ± 0.05	0.16 ± 0.05	ND
LPS from cell culture medium, μg/mL beads (****)	14.9 ± 0.05	15.1 ± 0.05	13.1 ± 0.05	15.0 ± 0.05	13.0 ± 0.05	13.7 ± 0.05

See Supplementary Materials S9 for evaluation of experimental errors.

* Contact time 60 min.

** Variation coefficient of Diazyme Total Bile Acids Assay is within 4% according to the manufacturer.

*** Starting LPS concentration 2000 EU/mL (0.4 μg/mL).

**** Starting LPS concentration in the medium 7640 EU/mL (1.53 μg/mL). ND – not detected as the concentration in the contacting sera did not change.

high (ca. 50 mg/mL beads) and did not correlate neither with the outer particle surface area nor with the volume of macropores appropriate to accommodate the large Fb molecules (340 kDa). Assuming that adsorption took place on the outer surface of 100 μm-beads, the adsorbed amount of 50 mg/mL beads would correspond to 160 μg/cm^2, i.e., a value many times higher than the above range of values 5–800 ng/cm^2. It seems that the outer surface of the beads was quickly populated by Fb molecules, which did not form a monolayer but likely a kind of multilayer due to hydrolysis of Fb with thrombin, formation of fibrin and its further polymerization. Polystyrene beads with adsorbed Fb were earlier shown to aggregate under the action of thrombin due to inter-bead fibrin dimerization [30]. In another study, ST-DVB beads with 75–120 nm pore diameter and microscopically visible large surface pores reduced the Fb concentration in plasma from 2.2 mg/mL to 1.2 mg/mL at a 1 : 9 bead-to-plasma volume ratio [31]. Beads with smaller pore diameters, 24–38 nm, and a smooth surface, adsorbed much less Fb [31]. At the gel-to-plasma volume ratio of ca. 1 : 10, protamine-Sepharose depleted up to 30% fibrinogen from plasma [32]. Our results are, therefore, comparable to those obtained with other macroporous beads of various chemical composition. No significant differences were found between CytoSorb, BioSKY and AP-adsorbents regarding Fb adsorption, which was quite high in all the cases.

3.6. SDS-PAGE assays of irreversibly adsorbed plasma proteins

Adsorption of plasma proteins described above was partially irreversible, i.e. some fractions of the adsorbed proteins could not be washed out from the beads by means of PBS, even after several repeated washing cycles, see Section 2.2.6, but could be extracted by 2% SDS. The composition of the irreversibly adsorbed protein layer may be of high importance during the processes of hemoperfusion. Pre-treatment of ST-DVB adsorbents with human serum albumin resulted in lower adsorption of fibrinogen and the coagulation inhibitor protein C, thus increasing the biocompatibility of the adsorbents [31]. On the other hand, adsorption of IgG was shown to be positively correlated with the activation of coagulation factor XII, a trigger of contact activation at negatively charged surfaces, whereas no correlation with complement activation was found [33] Fig. 4.a shows the non-reduced, stained SDS-PAGE gels. Clear bands at ca. 60 kDa and 75 kDa are typical of serum albumin and transferrin, whereas two major bands above 250 kDa can be attributed to fibrinogen (340 kDa) and fibronectin (440 kDa) found in the extracts. The weaker bands at ca. 80 kDa and 140 kDa might be attributed to vitronectin and high molecular weight kininogen, respectively. Under reducing conditions (Fig. 4b), the bands of serum albumin were accompanied by the neighboring bands of fibrinogen β- and γ-subunits with Mw of 55 and 50 kDa, respectively. It is relevant to note that a typically strong band at 25 kDa, characteristic of the IgG light chain, was very weak in the reduced gel. This indicated a negligibly low adsorption of IgG on any of the studied beads, unlike, for example, its adsorption from plasma on poly(vinyl alcohol) membranes [34].

The amounts of irreversibly adsorbed plasma proteins differed strongly among different beads, see Table 2, whereas these adsorbed amounts were much lower compared to those found for reversible protein adsorption. In general, the proteins were irreversibly adsorbed at higher quantities on AP-beads, probably due to their smaller size and, therefore, larger fraction of available pores located near the surface. As follows from Fig. S5, the adsorption equilibrium may take up to 24 h to be attained. In this situation, the higher outer surface area of AP-beads could support the higher adsorption capacities obtained during 1 h incubation. The lowest irreversible adsorption of proteins found on BioSKY beads agrees well with the low reversible adsorption and can be ascribed to a small fraction of macropores in these beads, see Table 1.

Fig. 4c and d show images of the stained SDS-PAGE gels obtained with the plasma proteins desorbed from the beads by 50 mM EDTA, a strong chelator of transition metal ions. Under non-reducing conditions, the bands of ca. 200–250 kDa obtained in the extracts from AP-101 and AP-105 may correspond to fibrinogen and/or fibronectin, proteins known to bind transition metal ions [35,36]. These bands were weaker and less clear in the extract from AP-100 containing no metal-chelating VI-groups. The gels obtained under reducing conditions show bands at 70 kDa and 55 kDa, corresponding to α- and β-subunits of fibrinogen, respectively, well expressed in earlier electrophoretic studies of plasma proteins [34]. The strongest bands were obtained with the extract from AP-101 beads. The bands at 150 kDa could be due to α2-macroglobulin [34], which was earlier reported to have high affinity to copper and zinc chelating Sepharoses [35]. The results indicate a possible influence of metal chelation on the mechanism of irreversible protein adsorption by both VI-DVB and ST-DVB beads. In the first case, the metal ions could bridge the imidazole groups of the beads and the proteins. In the second case, the metal ions could bridge the adsorbed protein molecules.

3.7. Adsorption of bilirubin and bile acids

Bilirubin and bile acids are toxins accumulating in the blood because of diminished detoxification capability of the liver. These metabolites are albumin-bound, and thus they cannot be removed by diffusive or convective extracorporeal blood purification systems but rather require adsorptive techniques for depletion [37]. The adsorption of bilirubin and bile acids was studied using Bovine multisera Table 2. summarizes the amounts of toxins adsorbed on various beads. All the adsorbents tested exhibited well-expressed binding of bilirubin from serum. The highest adsorption of bilirubin was observed with AP-100 beads, perhaps due to their higher hydrophobicity and small size. The other AP-beads containing VI monomer units might be significantly hydrated and thus less hydrophobic.

Adsorption of bile acids was also reasonably high, though AP-adsorbents were clearly advantageous over both CytoSorb and BioSKY. The highest adsorption was observed on AP-101 with a moderate content of VI-units. Interestingly, the bile acid adsorption was positively correlated with the amount of irreversibly adsorbed total protein with the lowest amounts adsorbed on BioSKY and the highest on AP-101. Since bile acids are albumin-bound toxins, their adsorption might involve interaction with irreversibly bound albumin. ST-DVB mesoporous beads are typically good adsorbents for bilirubin and bile acids ensuring 70–95% depletion of bilirubin under conditions similar to ours [5, 6, 31, 38], the smaller beads (6 μm) exhibiting higher adsorption rates than the larger ones (28 μm) [5]. We have found, however, that much larger beads, e.g., AP-adsorbents (50–200 μm), CytoSorb (400–600 μm) and BioSKY (550–700 μm) were still capable of effective bilirubin depletion from plasma during a 60 min contact time.

3.8. Adsorption of lipopolysaccharide (LPS) and IL-6

Adsorption of LPS was studied in two different sets of experiments, the first one carried out with cell culture medium containing 10% calf serum, and the second one with Bovine multisera. As follows from Table 2, LPS from the cell culture medium adsorbed quite well on all the tested beads, better depletion of LPS was found with AP-100, AP-101 and AP-105, compared with CytoSorb and BioSKY. Adsorption of LPS from Bovine multisera was much lower, probably due to the very high concentration of competing proteins (80.4 g/L). Nevertheless, depletion of LPS from the sera was still successful, especially when performed with AP-100 and AP-101 adsorbents. Their adsorption capacities of 0.8–2 μg LPS/mL beads were high compared with those exhibited by regular hemoperfusion devices, *i.e.*, oXiris® and Toraymyxin®, active volume about 300 mL, removing 6.9 μg and 9.7 μg LPS from 0.5 L blood plasma, respectively, during 120 min circulation and a total LPS quantity in the plasma (0.5 L) 15.8 μg [7].

Ion-exchange interactions seem to be the main mechanism involved in LPS binding to polyethyleneimine (PEI), the functional ligand of oXiris®, from aqueous media. Elution of biopolymers adsorbed from plasma on an oXiris® device by 1 M NaCl was followed by SDS-PAGE,

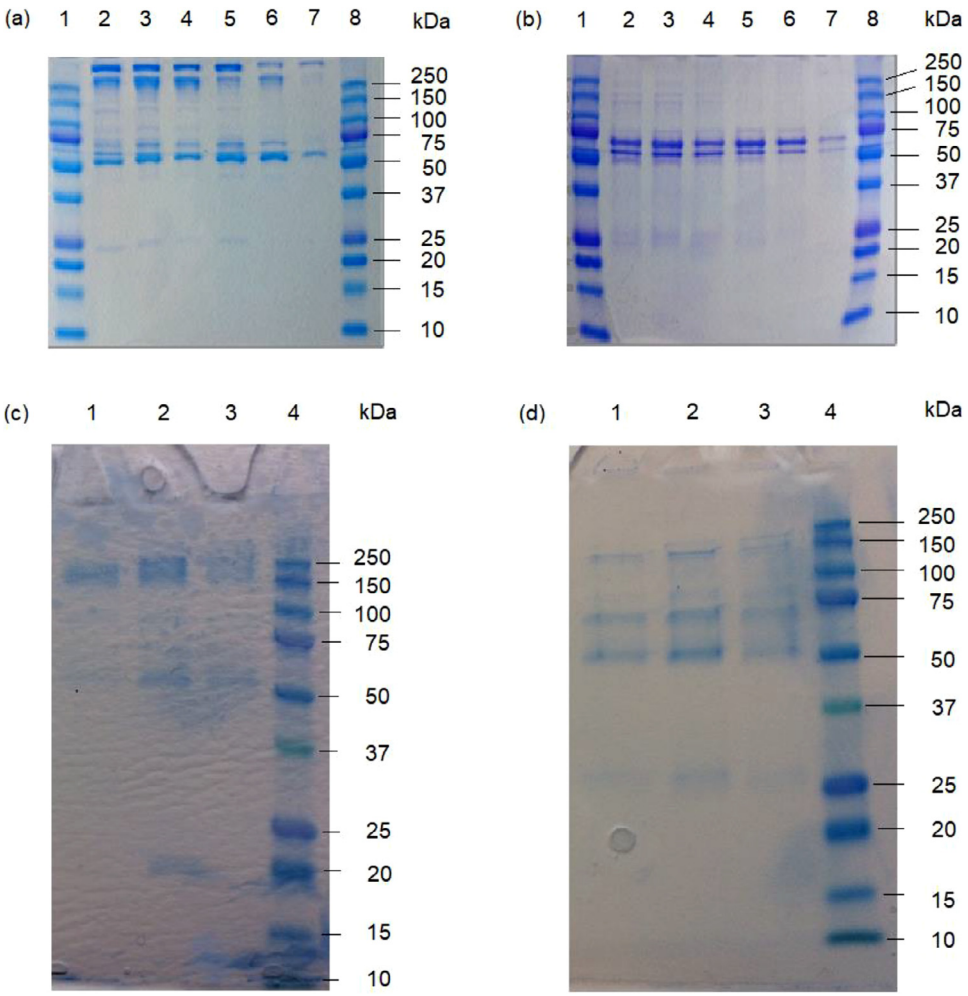

Fig. 4. (a,b): SDS-PAGE analysis of the SDS-extracts under non-reducing (a) and reducing (b) conditions. Wells 1 and 8 contain Mw standards, wells 2–7 contain extracts from samples AP-100, AP-101, AP-103, AP-105, CytoSorb and BioSKY, respectively; (c,d): SDS-PAGE analysis of the EDTA-extracts under non-reducing (c) and reducing (d) conditions. Wells 1–3 contain the extracts from samples AP-105, AP-101 and AP-100, respectively. Wells 4 contain MW standards. The proteins were stained with Bio-Safe™ Coomassie G-250 Stain and Imperial™ Protein Stain under the non-reducing and reducing conditions, respectively.

revealing albumin, fibrinogen, and several other proteins [7]. More specific elution of the LPS adsorbed on PEI-polyacrylamide porous monolithic gels could be done with 2 M NaCl [39]. As discussed in the Introduction, poly(N-vinyl imidazole) is a weaker base than PEI. Significant adsorption of LPS on both AP-100, which is a neutral ST-DVB copolymer, and AP-101 suggests that LPS is adsorbed on the latter due to hydrophobic interactions, possibly strengthened by electrostatic attraction to the positively charged VI units of the adsorbent.

The concentration of IL-6 in the cell culture medium was at realistic level of 73 pg/mL, which was higher than the normal 5–7 pg/mL in healthy individuals, but lower than about 1000 pg/mL found in sepsis [40]. We have observed 84% removal of IL-6 by BioSKY beads, 98% removal by CytoSorb beads and 100% removal by AP-adsorbents. In adsorption studies performed at 1000 pg/mL IL-6 concentration in a spiked plasma, about 60% of IL-6 was removed by CytoSorb at an adsorbent/plasma volume ratio similar to ours [6].

4. Conclusions

We have studied a series of porous N-vinylimidazole-divinylbenzene copolymer beads (AP-beads) with varied content of N-vinylimidazole (VI) as potential adsorbents for hemoperfusion and compared them with the beads from the commercial devices CytoSorb® and BioSKY™ GM, regarding pore size and specific surface characteristics, and adsorption of endogenous toxins and blood plasma proteins. A reference sample of styrene and divinylbenzene copolymer (AP-100) was made under the same polymerization conditions as VI-beads. All the beads had a large volume fraction of mesopores with diameters of 2–50 nm, and minor fractions of micropores (< 2 nm) and macropores (> 50 nm). Metal-chelate adsorption of copper (II) ions on VI-DVB beads from 0.15 M NaCl solution was high with distribution coefficients from 380 to 646, whereas no copper (II) adsorption from blood plasma was registered, probably due to metal complex formation with plasma proteins.

Irreversible adsorption of plasma proteins was higher on the AP-series beads, whereas BioSKY beads exhibited the minimal one. The irreversibly bound proteins included albumin, fibrinogen, fibronectin, and transferrin, but almost no immunoglobulin, as found by SDS-PAGE. Metal chelating contributed to the irreversible protein adsorption. Reversible adsorption of fibrinogen was high (ca. 50 mg/mL beads) on all the beads studied. Adsorption of bilirubin was highest on AP-100 and lowest on CytoSorb. Adsorption of bile acids positively correlated with the amount of irreversibly adsorbed protein and was higher on AP-beads compared with the commercial adsorbents. All the adsorbents exhibited good removal of the cytokine IL-6. Adsorption of lipopolysaccharide (LPS) was highest (ca. 2 μg/mL beads) on AP-101 containing 7% VI units. Hence, AP-101 appears to be suitable for removal of LPS from blood plasma, with characteristics comparable or even superior to those of commercial devices oXiris® and Toraymyxin®.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution Statement

Lennart Ljunggren: Conceptualization, Methodology, Supervision, Investigation, Resources. Alexander E. Ivanov: Methodology, Investiga-

tion, Visualization, Writing. Farhana Gulalei: Investigation, Validation. André Leistner: Conceptualization, Pore Size Analysis, Resources. Anke Lehmann: Synthesis of Adsorbents, Purification, Toxin Adsorption, Nitrogen Adsorption. Alexandra Klose-Stier: Synthesis and Modification of Adsorbents, Toxin Adsorption, Polymer Characterization. Aniela Leistner: Conceptualization, Methodology, Supervision, Investigation, Funding Acquisition

Funding

This work was supported by Sweden's innovation agency VINNOVA [Ref. 2018–02329] and by the Federal Ministry for Economic Affairs and Energy of Germany (grant no. ZF4573201TS8).

Data availability: Research data

Raw and processed data confirming the pore characteristics of the adsorbents have been deposited at Mendeley under the accession number

https://data.mendeley.com/datasets/nw8g9thhg4/1

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.bea.2022.100027.

References

[1] Z. Huang, S.-.R. Wang, W. Su, J.-.Y. Liu, Removal of humoral mediators and the effect on the survival of septic patients by hemoperfusion with neutral microporous resin column, Ther. Apher. Dial. 14 (2010) 596–602, doi:10.1111/j.1744-9987.2010.00825.x.

[2] B. Hinz, O. Jauch, T. Noky, S. Friesecke, P. Abel, R. Kaiser, CytoSorb, a novel therapeutic approach for patients with septic shock: a case report, Int. J. Artif. Organs. 38 (2015) (2015) 461–464, doi:10.5301/ijao.5000429.

[3] M.C. Gruda, K.-.G. Ruggeberg, P. O'Sullivan, T. Guliashvili, A.R. Schreirer, T.D. Golobish, V.G. Capponi, P.P. Chan, Broad adsorption of sepsis-related PUMP and DAMP molecules, mycotoxins and cytokines from whole blood using CytoSorb® sorbent porous polymer beads, PLoS ONE 13 (1) (2018) e0191676, doi:10.1371/journal.pone.0191676.

[4] Biosky MG Series Disposable Hemoperfusion Cartridge. Biosun Medical Technology Co., Ltd. https://www.oriontamajaya.com/biosky-mg/

[5] V. Weber, I. Linsberger, M. Hauner, A. Leistner, A. Leistner, D. Falkenhagen, Neutral styrene divinylbenzene copolymers for adsorption of toxins in liver failure, Biomacromolecules 9 (2008) 1322–1328, doi:10.1021/bm701396n.

[6] J. Chen, W. Han, J. Chen, W. Zong, W. Wang, Y. Wang, G. Cheng, C. Li, L. Ou, Y. Yu, High performance of a unique mesoporous polystyrene-based adsorbent for blood purification, Regen. Biomater. 4 (2017) 31–37, doi:10.1093/rb/rbw038.

[7] B. Malard, C. Lambert, J.A. Kellum, In vitro comparison of the adsorption of inflammatory mediators by blood purification devices, Intensive Care Med. Exp. 6 (2018) 12, doi:10.1186/s40635-018-0177-2.

[8] A. Leistner, A. Leistner. Adsorbing material for blood and plasma cleaning method and for albumin purification. EP1578526B1 (2011). https://patents.google.com/patent/EP1578526B1/en

[9] , Dissociation constants of organic bases and acids, in: D.R. Lide (Ed.), CRC Handbook of Chemistry and Physics, 88th edition, CRC Press/Taylor & Francis Group, Boca Raton, FL, 2007, pp. 8.42–8.51. http://www2.chm.ulaval.ca/gecha/chm1903/4_acide-base/organic_acids.pdf.

[10] S. Asayama, T. Sekine, H. Kawakami, S. Nagaoka, Design of aminated poly(1-vinylimidazole) for a new pH-sensitive polycation to enhance cell-specific gene delivery, Bioconjugate Chem 18 (2007) 1662–1667, doi:10.1021/bc700205t.

[11] W. Niedermeier, J.H. Griggs, R.S. Johnson, Emission spectrometric determination of trace elements in biological fluids, Appl. Spectrosc. 25 (1971) 53–56, doi:10.1366/000370271774370949.

[12] B.L. Rivas, H.A. Maturana, M.J. Molina, M.R. Gomez-Anton, I.F. Pierola, Metal ion binding properties of poly(N-vinylimidazole) hydrogels, J. Appl. Polym. Sci. 67 (1998) 1109–1118 6%3C1109::AID-APP19%3E3.0.CO;2-V, doi:10.1002/(SICI)1097-4628(19980207)67.

[13] P. Arvidsson, A.E. Ivanov, I.Yu. Galaev, B. Mattiasson, Polymer versus monomer as displacer in immobilized metal affinity chromatography, J. Chromatogr. B 753 (2001) 279–285, doi:10.1016/S0378-4347(00)00558-2.

[14] B. Mattiasson, A. Kumar, A.E. Ivanov, I.Yu. Galaev, Metal-chelate affinity precipitation of proteins using responsive polymers, Nat. Protoc. 2 (2007) 213–220, doi:10.1038/nprot.2006.440.

[15] S. Brunauer, P.H. Emmett, E. Teller, Adsorption of gases in multimolecular layers, J. Am. Chem. Soc. 60 (1938) 309–19, doi:10.1021/ja01269a023.

[16] G. Horvath, K. Kawazoe, Method for the calculation of effective pore size distribution in molecular sieve carbon, J. Chem. Eng. Jpn. 16 (1983) 470–475, doi:10.1252/jcej.16.470.

[17] E.P. Barrett, L.G. Joyner, P.P. Halenda, The determination of pore volume and area distributions in porous substances. I. Computations from nitrogen isotherms, J. Am. Chem. Soc. 73 (1951) 373–378, doi:10.1021/ja01145a126.

[18] C. Nguyen, D.D. Do, Effects of probing vapors and temperature on the characterization of micro-mesopore size distribution of carbonaceous materials, Langmuir 16 (2000) 7218–7222, doi:10.1021/la991596a.

[19] V.M. Gun'ko, R. Leboda, J. Skubiszewska-Zięba, B. Gawdzik, B. Charmas, Structural characteristics of porous polymers treated by freezing with water or acetone, Appl. Surf. Sci. 252 (2005) 612–618, doi:10.1016/j.apsusc.2005.02.075.

[20] M. Nejati-Yazdinejad, Spectrophotometric determination of trace amount of copper (II) ion based on complexation with an anthraquinone derivative, Anal. Sci. 22 (2006) 617–619, doi:10.2116/analsci.22.617.

[21] V. Davankov, L. Pavlova, M. Tsyurupa, J. Brady, M. Balsamo, E. Yousha, Polymeric adsorbent for removing toxic proteins from blood of patients with kidney failure, J. Chromatogr. B 739 (2000) 73–80, doi:10.1016/S0378-4347(99)00554-X.

[22] L. Blomberg, T. Bratt, M.A. Chester, B. Hansson, Improved removal of anti-A and anti-B antibodies from plasma using blood-group-active haptens, Vox Sang 65 (1993) 126–135, doi:10.1111/j.1423-0410.1993.tb02129.x.

[23] A.E. Ivanov, V.P. Zubov, Adsorption and separation of proteins on composite anion-exchangers with poly(*N,N*-dimethylethylacrylamide) bonded phases, J. Chromatogr. A 673 (1994) 159–165, doi:10.1016/0021-9673(94)85034-8.

[24] W. Bal, M. Sokolowska, E. Kurowska, P. Faller, Binding of transition metal ions to albumin: sites, affinities and rates, Biochim. Biophys. Acta 1830 (2013) 5444–5455, doi:10.1016/j.bbagen.2013.06.018.

[25] D. Schädler, C. Pausch, D. Heise, A. Meier-Hellmann, J. Brederlau, N. Weiler, G. Marx, C. Putensen, C. Spies, A. Jörres, M. Quintel, C. Engel, J.A. Kellum, M.K. Kuhlmann, The effect of a novel extracorporeal cytokine hemoadsorption device on IL-6 elimination in septic patients: a randomized controlled trial, PLoS ONE 12 (10) (2017) e0187015, doi:10.1371/journal.pone.0187015.

[26] T.A. Horbett, Fibrinogen adsorption to biomaterials, J. Biomed. Mater. Res. A 106 (2018) 2777–2788, doi:10.1002/jbm.a.36460.

[27] D. Webster, A.H.C. Bignell, E.C. Attwood, An assessment of the suitability of bromocresol green for the determination of serum albumin, Clin. Chim. Acta 53 (1974) 101–108, doi:10.1016/0009-8981(74)90357-X.

[28] A.E. Ivanov, O.P. Kozynchenko, L.I. Mikhalovska, S.R. Tennison, H. Jungvid, V.M. Gun'ko, S.V. Mikhalovsky, Activated carbons and carbon-containing poly(vinyl alcohol) cryogels: characterization, protein adsorption and possibility of myoglobin clearance, Phys. Chem. Chem. Phys. 14 (2012) 16267–16278, doi:10.1039/C2CP42869E.

[29] S.N. Rodrigues, I.C. Goncalves, M.C. Martins, M.A. Barbosa, B.D. Ratner, Fibrinogen adsorption, platelet adhesion and activation on mixed hydroxyl-/methyl-terminated self-assembled monolayers, Biomaterials 27 (2006) 5357–5367, doi:10.1016/j.biomaterials.2006.06.010.

[30] G.S. Retzinger, Adsorption and coagulability of fibrinogen on atheromatous lipid surfaces, Arterioscler. Thromb. Vasc. Biol. 15 (1995) 786–792, doi:10.1161/01.ATV.15.6.786.

[31] S. Harm, D. Falkenhagen, J. Hartmann, Pore size – a key property for selective toxin removal in blood purification, Int. J. Artif. Organs 37 (2014) 668–678, doi:10.5301/ijao.5000354.

[32] J.T. Parker, D.S. Beutler, S. Sukavaneshvar, N. Jacobs, K.A. Solen, S.F. Mohammad, Mitigation of coagulation by removing clotting factors. Part 1: in vitro feasibility study, ASAIO J 53 (2007) 415–420, doi:10.1097/MAT.0b013e3180cab642.

[33] S. Huang, A.E. Engberg, N. Jonsson, K. Sandholm, I.A. Nicholls, T.E. Mollnes, K. Fromell, B. Nilsson, K.N. Ekdahl, Reciprocal relationship between contact and complement system activation on artificial polymers exposed to whole human blood, Biomaterials 77 (2016) 111–119 http://dx.doi.org/, doi:10.1016/j.biomaterials.2015.10.067.

[34] K.E. Ryu, H. Rhim, C.W. Park, H.J. Chun, S.H. Hong, J.J. Kim, Y.M. Lee, Plasma protein adsorption to anion substituted poly(vinyl alcohol) membranes, Macromol. Res. 11 (2003) 451–457, doi:10.1007/BF03218975.

[35] M.J. Sinosich, M.W. Davey, J.G. Grudzinskas, Interactions of pregnancy associated plasma protein-A and α2-macroglobulin on metal chelate affinity chromatography, Protides Biol. Fluids. 31 (1984) 19–22, doi:10.1016/B978-0-08-030764-0.50008-2.

[36] M. Zachariou, M.T.W. Hearn, Adsorption and selectivity characteristics of several human serum proteins with immobilised hard Lewis metal ion–chelate adsorbents, J. Chromatogr. A 890 (2000) 95–116, doi:10.1016/S0021-9673(00)00462-3.

[37] W. Laleman, A. Wilmer, P. Evenepoel, C. Verslype, J. Fevery, F. Nevens, Review article: non-biological liver support in liver failure, Aliment. Pharmacol. Ther. 23 (2006) 351–363 https://doi:, doi:10.1111/j.1365-2036.2006.02765.x.

[38] T. Eichorn, A.E. Ivanov, M.B. Dainiak, A. Leistner A, I. Linsberger I, H. Jungvid, S.V. Mikhalovsky, V. Weber, Macroporous composite cryogels with embedded polystyrene divinylbenzene microparticles: synthesis, characterization and adsorption of toxic metabolites from blood, J. Chem. 348412 (2013) http://dx.doi.org/, doi:10.1155/2013/348412.

[39] A. Hanora, F.M. Plieva, M. Hedström, I. Yu. Galaev, Bo Mattiasson, Capture of bacterial endotoxins using a supermacroporous monolithic matrix with immobilized polyethyleneimine, lysozyme or polymyxin B, J. Biotechnol. 118 (2005) 421–433 http://dx.doi.org/, doi:10.1016/j.jbiotec.2005.05.006.

[40] A. Spittler, M. Razenberger, H. Kupper, M. Kaul, W. Hackl, G. Boltz-Nitulescu, R. Függer, E. Roth, Relationship between interleukin-6 plasma concentration in patients with sepsis, monocyte phenotype, monocyte phagocytic properties, and cytokine production, Clin. Infect. Dis. 31 (2000) 1338–1342 http://dx.doi.org/, doi:10.1086/317499.