

MATERIAL

Field of Invention

- 5 The present invention generally relates to the removal of beta-2 microglobulin in biological fluids, and more particularly relates to particulate porous materials for efficient removal of beta-2 microglobulin in biological fluids.

Background

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The listing or discussion of a prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

- 15 Beta-2 Microglobulin (B2M) is a small protein (99 amino acids, 11.8 kDa) normally located on the surface of nucleated cells as part of the antigen complex MHC-I (major histocompatibility-I complex). As it is present on many cellular surfaces, B2M is naturally shed into the bloodstream continuously. B2M is generated at a rate estimated at 0.159 mg/h per kg of body weight (approximately 200-300 mg per day) (Portales-Castillo, I., *et al.*, *Kidney360*, **2020**, 1, 1447-1455).
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- Under normal circumstances, 99.9% of B2M is removed by the kidneys through glomerular filtration and re-adsorbed by catabolism into amino acids. In cases of kidney failure, patients are reliant on dialysis for B2M removal. Due to the limitations of current dialysis membranes,
- 25 B2M is inefficiently removed and it can accumulate to exceed the normal serum level 60-fold. An average patient of 70 kg is estimated to accumulate 73 g of B2M per year. The cumulative retention of 400-600 g B2M is associated with the onset of the disease Dialysis Related Amyloidosis (DRA). Conventional 4 h, 3.5x per week hemodialysis (HD) regimen removes only approximately 100-150 mg of B2M per day. At best, this only represents 50% removal,
- 30 leading to DRA.

- The pathogenicity of DRA involves the aggregation of B2M to form a polymer macrostructure, amyloid fibrils. The B2M fibrils are frequently found to deposit in critical areas of the body such as the kidneys and joints among other tissues, leading to severe abnormalities including
- 35 kidney stones, bone cysts and carpal tunnel syndrome. Interpreted as foreign matter by the immune system, the fibrils incite macrophage recruitment and inflammation, causing serious damage to tissues and the central nervous system, as well as organ failure.

These complications are associated with serious symptoms including excruciating and debilitating pain, resulting in a reduced level of activity and quality of life. A study of 1704 hemodialysis patients found that predialysis serum B2M was a predictor for mortality, where each 10 mg/L increase in serum B2M corresponded with an 11% increase in mortality.

The main remedy available to the patient is not without its own challenges and risk, with the patient required to undergo invasive surgery to remove the fibrils and treat the damaged tissue.

Unfortunately, DRA is not an uncommon occurrence in dialysis patients. Studies in the US have found that over 95% of long-term dialysis patients (>15 years) suffered from DRA, while a European study of hemodialysis patients found that as many as 20% of short-term (2–4 years) and 100% of long-term (>13 years) patients were afflicted with the disease (Scarpioni, R., *et al.*, *Int. J. Nephrol. Renov. Dis.*, **2016**, 9, 319-328).

Traditional high-flux membranes have been designed with a pore size distribution for effective removal of water and small solutes, while preventing removal of beneficial large protein solutes such as albumin. Unfortunately, this pore size distribution is ineffective in the removal of middle molecules like B2M (Zhang, Z., *et al.*, *Membranes*, **2022**, 12, 253).

Recently developed Medium Cut Off (MCO) dialyzers aim to overcome the limitations of high-flux membranes by shifting and narrowing the pore size distribution to increase the removal of middle molecules, while retaining larger proteins like albumin. Interestingly, a recent study comparing the efficacy of four MCO dialysers with a traditional high-flux membrane did not find a significant difference in B2M removal. The main improvement observed in MCO membranes was in removal of higher molecular weight middle molecules (17-45 kDa), at a cost of higher albumin losses (2-3 times higher) (Maduell, F., *et al.*, *Clin. Kidney J.*, **2022**, 15, 2292-2299). Additionally, only patients undergoing HD are able to benefit from this technology. This type of invention is of limited applicability to peritoneal dialysis patients, who also suffer from DRA.

Further, hemoperfusion columns have been administered as standalone extracorporeal devices or in conjunction with hemodialysis therapy. Although the addition of hemoperfusion column improved B2M removal by 1.5-2x, the device volume is very large (150-500 mL). A large extracorporeal volume leads to anemia, hypotension, etc. Additionally, some reduction in albumin, leucocytes and platelets were reported.

Protein adsorption and recognition is considered to be a challenging feat in the Molecular Imprinted Polymers (MIPs) discipline, since that would require the imprinting and removal of a macromolecular template, a time consuming and difficult endeavour. Moreover, in the MIP approach to protein recognition, only aqueous systems are considered since the crosslinker, functional monomer and template need to be soluble in the same medium. A recent review was not able to identify any MIPs targeting adsorption of B2M or other middle-molecules (Ma, Y., *et al.*, *Microporous Mesoporous Mater.*, **2021**, 319, 111035).

Therefore, there exists a need for new materials for efficient removal of B2M in biological fluids, which can overcome at least one of the aforementioned problems.

Summary of Invention

Disclosed herein is a material which surprisingly has high selectivity and adsorption capacity for B2M. In addition, the present invention can be produced cheaply, and has high chemical and physical stability.

Aspects and embodiments of the invention are provided in the following numbered clauses.

1. A particulate porous material that comprises a crosslinked polymeric matrix including a first set of constitutional units formed from ethylene glycol dimethacrylate monomers wherein:

the particulate porous material comprises a plurality of pores having a pore diameter of from about 1.6 nm to about 50 nm; and

the particulate porous material has a porosity of at least 3% (v/v) and a single point total pore volume of at least 0.15 cm³/g.

2. The particulate porous material according to Clause 1, wherein:

(a) the particulate porous material has a Brunauer-Emmett-Teller adsorption average pore size of from 3 to 15 nm, such as from 3.5 to 10 nm (e.g. 3.6 to 9.4 nm); and/or

(b) the particulate porous material has a porosity of at least 6% (v/v) and a single point total pore volume of at least 0.3 cm³/g.

3. The particulate porous material according to Clause 1 or 2, wherein the crosslinked polymeric matrix further comprises up to 30 mol % of a second set of constitutional units formed from a hydrophilic monomer, optionally wherein:

(a) the hydrophilic monomer is selected from one or more of the group consisting of itaconic acid, 2-(trifluoromethyl)acrylic acid, and allylurea, and/or

(b) the second set of constitutional units are present in an amount of from 2 to 25 mol % of the crosslinked polymeric matrix.

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4. The particulate porous material according to Clause 1 or 2, wherein the crosslinked polymeric matrix is formed from a homopolymer consisting of constitutional units formed from ethylene glycol dimethacrylate monomers.

10 5. The particulate porous material according to any one of the preceding clauses, having a porosity of from 8 to 30% (v/v), such as from 10 to 28% (v/v) (e.g. from 14 to 24% (v/v)).

6. The particulate porous material according to any one of the preceding clauses, having a single point total pore volume of from 0.36 to 0.85 cm³/g, such as from 0.4 to 0.8 cm³/g (e.g. from 0.45 to 0.75 cm³/g).

7. The particulate porous material according to any one of the preceding clauses, having a porosity of from 8 to 30% (v/v) and a single point total pore volume of from 0.36 to 0.85 cm³/g;

20 optionally a porosity of from 10 to 28% (v/v) and a single point total pore volume of from 0.4 to 0.8 cm³/g.

8. The particulate porous material according to any one of the preceding clauses, wherein the particulate porous material comprises a plurality of pores having a pore diameter of from about 1.6 nm to about 10 nm.

9. The particulate porous material according to any one of the preceding clauses, wherein the particulate porous material has a nitrogen adsorption isotherm that is Type II or Type IV.

30 10. The particulate porous material according to any one of the preceding clauses, wherein the particulate porous material has a D50 of less than 200 µm.

11. The particulate porous material according to any one of the preceding clauses, wherein the particulate porous material has a total protein binding capacity that is less than 0.2g/g.

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12. A sorbent cartridge suitable for use in purification of a bodily fluid (e.g. a kidney dialysis treatment, such as a haemodialysis treatment, a peritoneal dialysis treatment or a

hemoperfusion treatment), wherein the sorbent cartridge comprises the particulate porous material according to any one of the preceding clauses.

13. The sorbent cartridge according to Clause 12, further comprising one or more of:
 5 cation exchange particles (e.g. zirconium phosphate);
 anion exchange particles (e.g. hydrous zirconium oxide);
 activated carbon;
 a buffer (e.g. calcium carbonate); and
 a urease.

14. A method of performing dialysis (e.g. haemodialysis, peritoneal dialysis or hemoperfusion), comprising a step of passing a dialysate through a sorbent cartridge as defined in Clause 12 or 13.

15. Use of a particulate porous material as defined in any one of Clauses 1 to 11, or a sorbent cartridge according to Clause 12 or 13, to adsorb beta-2 microglobulin.

16. A method of removing beta-2 microglobulin from a fluid, comprising:
 providing a fluid comprising beta-2 microglobulin; and
 20 exposing said fluid to a particulate porous material as defined in any one of Clauses 1 to 11.

17. A method of preparing a particulate porous material as defined in any one of Clauses 1 to 11, the method comprising the steps:

25 (i) providing mixture comprising a first monomer that is ethylene glycol dimethacrylate, an initiator, a porogen and optionally a second monomer that is a hydrophilic monomer;

(ii) conducting a polymerisation reaction to form a bulk porous material; and

30 (iii) grinding or pulverising the bulk porous material to provide a particulate porous material.

18. The method according to Clause 17, further comprising washing the particulate porous material to remove the porogen.

35 19. The method according to Clause 17 or 18, wherein the porogen is an organic solvent selected from the group consisting of toluene, chloroform, acetone, dimethylformamide, dimethylsulfoxide, dioxane, ethyl acetate, and acetonitrile.

20. The method according to Clause 17 or 18, wherein the porogen is an organic solvent having a Hansen distance (R_a) of up to 10 MPa^{1/2}, where $D_{\text{polymer}} = 17.0$, $P_{\text{polymer}} = 9.7$ and $H_{\text{polymer}} = 4.9$.

21. The method according to any one of Clauses 17 to 20, wherein step (ii) is performed at a temperature of from 50°C to 70°C.

Drawings

FIG. 1 depicts the synthesis of porous Poly-EGDMA particles.

FIG. 2 depicts the nitrogen porosimetry results for Poly-EGDMA, Poly-DVB, Poly-TRIM, and AC.

FIG. 3 depicts the illustration of Poly-EGDMA particles.

FIG. 4 depicts the labelled scanning electron microscopy (SEM) image of Poly-EGDMA surface. Inset: overall view of a microparticle.

FIG. 5 depicts the labelled Barrett-Joyner-Halenda (BJH) pore size distribution plot.

FIG. 6 depicts the synthesis of porous Poly-EGDMA monolith.

FIG. 7 depicts the SEM images of monolithic polymers: (i) 181 – Toluene; (ii) 210 – Chloroform; (iii) 221 – Acetone; (iv) 223 – Dimethylformamide (DMF); (v) 224 – Dimethylsulfoxide (DMSO); (vi) 225- Dioxane; (vii) 226 – Ethyl Acetate; and (viii) 229 – Acetonitrile (MeCN).

FIG. 8 depicts the SEM images of particulate polymers: (i) 212 – n-Hexane; (ii) 222 – Methanol; (iii) 227 – iso-Propanol; and (iv) 228 – Ethanol.

FIG. 9 depicts the nitrogen porosimetry results for 2B Chloroform, 2C Hexane, 2F DMF, and 2L Acetonitrile.

FIG. 10 depicts the pore size distribution according to BJH model for 2B Chloroform, 2C Hexane, 2F DMF, and 2L MeCN.

FIG. 11 depicts the hydrophobic and hydrophilic monomers.

FIG. 12 depicts the equilibrium adsorption isotherm.

5 **FIG. 13** depicts a schematic of the device test.

FIG. 14 depicts the particle size distribution data.

10 **FIG. 15** depicts (A) and (B) the Hansen Solubility Sphere (experimental). ●: bad solvents. ▲: good solvents.

FIG. 16 depicts the Hansen Solubility Sphere (predicted). ●: bad solvents. ▲: good solvents.

15 **FIG. 17** depicts the polymerisation mechanism.

Description

It has been surprisingly found that a porous polymeric sorbent based on crosslinked poly-EGDMA has unexpectedly high adsorption capacity and selectivity for B2M.

20 Thus, in a first aspect of the invention, there is provided a particulate porous material that comprises a crosslinked polymeric matrix including a first set of constitutional units formed from ethylene glycol dimethacrylate monomers

wherein:

25 the particulate porous material comprises a plurality of pores having a pore diameter of from about 1.6 nm to about 50 nm; and

the particulate porous material has a porosity of at least 3% (v/v) and a single point total pore volume of at least 0.15 cm³/g.

30 In embodiments herein, the word “comprising” may be interpreted as requiring the features mentioned, but not limiting the presence of other features. Alternatively, the word “comprising” may also relate to the situation where only the components/features listed are intended to be present (e.g. the word “comprising” may be replaced by the phrases “consists of” or “consists essentially of”). It is explicitly contemplated that both the broader and narrower interpretations
35 can be applied to all aspects and embodiments of the present invention. In other words, the word “comprising” and synonyms thereof may be replaced by the phrase “consisting of” or the phrase “consists essentially of” or synonyms thereof and *vice versa*.

The phrase, “consists essentially of” and its pseudonyms may be interpreted herein to refer to a material where minor impurities may be present. For example, the material may be greater than or equal to 90% pure, such as greater than 95% pure, such as greater than 97% pure, such as greater than 99% pure, such as greater than 99.9% pure, such as greater than 99.99% pure, such as greater than 99.999% pure, such as 100% pure.

As used herein, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a composition” includes mixtures of two or more such compositions, and the like.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

The term “particulate porous material” refers to particles that each include pores.

The term “constitutional units” may refer to the polymerised repeating units (and crosslinks) obtained from the use of one or more monomeric materials and, if necessary separate crosslinking agents, to form the crosslinked polymeric matrix.

As will be appreciated, the crosslinked polymeric matrix may be formed only from a polymeric material obtained from the polymerisation of ethylene glycol dimethacrylate monomers. This is because one of the methacrylate functional groups in a monomer may partake in the formation of a polymeric chain (i.e. this functional group forms part of a carbon skeleton of the polymer), while the other methacrylate functional group that is pendant to the carbon skeleton may (or may not) form a bond with a further pendant methacrylate group on a separate polymeric chain, thereby crosslinking the two polymeric chains together. As will be appreciated, one or more of the pendant methacrylate chains may form crosslinks.

As will be appreciated, the crosslinked polymeric matrix may be a homopolymer. In this case, only constitutional units formed from ethylene glycol dimethacrylate monomers may be present (with or without the use of a separate crosslinking agent).

5 The term “homopolymer” refers to a polymer consisting of identical monomer repeating units.

A “crosslinking agent” when referred to herein is a material that may form links between adjacent polymeric chains. This may be in any suitable form. For example, this may be in the form of forming covalent or ionic bonds between the polymer chains. A crosslinking agent may
 10 be a separate material that is added to form said crosslinks. As will be appreciated, ethylene glycol dimethacrylate can self-crosslink, so the presence of a separate crosslinking agent is optional.

For the avoidance of doubt, it is explicitly contemplated that where a number of numerical
 15 ranges related to the same feature are cited herein, that the end points for each range are intended to be combined in any order to provide further contemplated (and implicitly disclosed) ranges.

As noted above, the particulate porous material may have a pore diameter of from about 1.6
 20 nm to about 50 nm. In more specific embodiments, that may be mentioned herein, the particulate porous material may have a pore diameter of from about 1.6 nm to 10 nm.

The pore diameter of the particulate porous material may be derived, for example, from the
 25 33-datapoint adsorption branch of the N₂ isotherm. Miromeritics MicroActive software version 5.02 may be used to analyse the data according to the Barrett-Joyner-Halenda model with Halsey-Faas correction, in order to derive the pore size distribution and average pore diameter data (see Barrett, E. P., Joyner, L. G. & Halenda, P. P., *J. Am. Chem. Soc.* **1951**, 73, 373–380; and Rouquerol, J., *et al.*, *Adsorption by powders and porous solids: principles, methodology and applications*. **2013**: Academic press). Details on the calculations performed
 30 by Miromeritics MicroActive software version 5.02 may be found at https://www.micromeritics.com/Repository/Files/Calculations_-_2020_ASAP_Jan_2019_.pdf.

The term “porosity” as used herein refers to the ratio of the pore volume to the tapped density of a material. As will be appreciated, porosity may be calculated via the below equation:

35 Porosity (%) = Single point total pore volume (cm³/g) * tapped density (g/cm³) x 100

Tapped density may be measured by mechanically tapping the dry polymer samples in a 5 mL measuring cylinder, and allowing the sample to settle to a final volume under its own weight. The tapped density may be calculated as follows:

$$\text{Tapped density} = \text{Sample mass (g)} / \text{Final Volume (cm}^3\text{)}$$

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Specific details are provided in Example 4 below.

When used herein, the single point total pore volume is taken to mean the total volume of liquid N₂ adsorbed by the sample, at a datapoint near saturation pressure of the N₂ isotherm (P/P₀ > 0.95).

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In some embodiments of the invention that may be mentioned herein, the particulate porous material may have a porosity of at least 3% (v/v), such as at least 6% (v/v). For example, the particulate porous material may have a porosity of from 3 to 30% (v/v), such as from 3 to 28% (v/v), such as from 3 to 24% (v/v), such as from 3 to 14% (v/v), such as from 3 to 10% (v/v), such as from 3 to 8% (v/v), such as from 3 to 6% (v/v), such as from 6 to 30% (v/v), such as from 6 to 28% (v/v), such as from 6 to 24% (v/v), such as from 6 to 14% (v/v), such as from 6 to 10% (v/v), such as from 6 to 8% (v/v), such as from 8 to 30% (v/v), such as from 8 to 28% (v/v), such as from 8 to 24% (v/v), such as from 8 to 14% (v/v), such as from 8 to 10% (v/v), such as from 10 to 30% (v/v), such as from 10 to 28% (v/v), such as from 10 to 24% (v/v), such as from 10 to 14% (v/v), such as from 14 to 30% (v/v), such as from 14 to 28% (v/v), such as from 14 to 24% (v/v), such as from 24 to 30% (v/v), such as from 24 to 28% (v/v), such as from 28 to 30% (v/v).

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The term “single point total pore volume” as used herein refers to the total volume of the pores in a material, as determined by the single point calculation method. Specific details are provided below and in the examples.

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The term “Brunauer-Emmett-Teller adsorption average pore size” as used herein refers to average pore size as determined by the Brunauer, Emmett and Teller method. Specific details are provided below and in the examples.

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The single point total pore volume, and Brunauer-Emmett-Teller adsorption average pore size may be determined, for example, by following the dinitrogen adsorption-desorption experimental methods disclosed in ISO 9277-2022, which is incorporated in its entirety by reference herein. Briefly, measurements can be performed at 77.3 K on a Micrometrics ASAP

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model 2020 porosimeter, and can be calculated using methods provided by Micromeritics MicroActive software version 5.02.

More specifically, the single point total pore volume may be calculated at a single point approaching saturation pressure $p/p^0 > 0.95$, which is an application of the Gurvich rule (Rouquerol, J., *et al.*, *Adsorption by powders and porous solids: principles, methodology and applications*. **2013**: Academic press). The Brunauer-Emmett-Teller (BET) adsorption average pore size may be calculated via the Gurvich rule and BET method:

$$D = 4V/S,$$

Where D = average pore diameter, V = single point total pore volume, and S = specific surface area determined by BET model.

In some embodiments of the invention that may be mentioned herein, the particulate porous material may have a single point total pore volume of at least 0.15 cm³/g, such as at least 0.3 cm³/g. For example, the particulate porous material may have a single point total pore volume of from 0.15 to 0.85 cm³/g, such as from 0.15 to 0.8 cm³/g, such as from 0.15 to 0.75 cm³/g, such as from 0.15 to 0.45 cm³/g, such as from 0.15 to 0.4 cm³/g, such as from 0.15 to 0.36 cm³/g, such as from 0.15 to 0.3 cm³/g, such as from 0.3 to 0.85 cm³/g, such as from 0.3 to 0.8 cm³/g, such as from 0.3 to 0.75 cm³/g, such as from 0.3 to 0.45 cm³/g, such as from 0.3 to 0.4 cm³/g, such as from 0.3 to 0.36 cm³/g, such as from 0.36 to 0.85 cm³/g, such as from 0.36 to 0.8 cm³/g, 0.36 to 0.75 cm³/g, such as from 0.36 to 0.45 cm³/g, such as from 0.36 to 0.4 cm³/g, such as from 0.4 to 0.85 cm³/g, such as from 0.4 to 0.8 cm³/g, such as from 0.4 to 0.75 cm³/g, such as from 0.4 to 0.45 cm³/g, such as from 0.45 to 0.85 cm³/g, such as from 0.45 to 0.8 cm³/g, such as from 0.45 to 0.75 cm³/g, such as from 0.75 to 0.85 cm³/g, such as from 0.75 to 0.8 cm³/g, such as from 0.8 to 0.85 cm³/g.

In particular embodiments of the invention that may be mentioned herein, the particulate porous material has a porosity of at least 3% (v/v) and a single point total pore volume of at least 0.15 cm³/g.

In some embodiments of the invention that may be mentioned herein, the particulate porous material may have a Brunauer-Emmett-Teller adsorption average pore size of from 3 to 15 nm, such as from 3 to 10 nm, such as from 3 to 9.4 nm, such as from 3 to 3.6 nm, such as from 3 to 3.5 nm, such as from 3.5 to 15 nm, such as from 3.5 to 10 nm, such as from 3.5 to 9.4 nm, such as from 3.5 to 3.6 nm, such as from 3.6 to 15 nm, such as from 3.6 to 10 nm, such as from 3.6 to 9.4 nm, such as from 9.4 to 15 nm, such as from 9.4 to 10 nm, such as from 10 to 15 nm.

In some embodiments, the particulate porous material may have a Brunauer-Emmett-Teller adsorption average pore size of from 3 to 15 nm, such as from 3.5 to 10 nm (e.g. 3.6 to 9.4 nm). In some embodiments, the particulate porous material may have a porosity of at least 6% (v/v) and a single point total pore volume of at least 0.3 cm³/g. In some alternative embodiments, the particulate porous material may have a Brunauer-Emmett-Teller adsorption average pore size of from 3 to 15 nm, such as from 3.5 to 10 nm (e.g. 3.6 to 9.4 nm), and the particulate porous material may have a porosity of at least 6% (v/v) and a single point total pore volume of at least 0.3 cm³/g.

The particulate porous material described above may further comprise up to 30 mol % of a second set of constitutional units formed from a hydrophilic monomer. Examples of hydrophilic monomers include, but are not limited to, itaconic acid, 2-(trifluoromethyl)acrylic acid, and allylurea. In some embodiments, the hydrophilic monomer may be selected from one or more of the group consisting of itaconic acid, 2-(trifluoromethyl)acrylic acid, and allylurea. In some embodiments, the second set of constitutional units may be present in an amount of from 2 to 25 mol % of the crosslinked polymeric matrix. In some alternative embodiments, the hydrophilic monomer may be selected from one or more of the group consisting of itaconic acid, 2-(trifluoromethyl)acrylic acid, and allylurea, and the second set of constitutional units may be present in an amount of from 2 to 25 mol % of the crosslinked polymeric matrix.

In particular embodiments of the invention that may be mentioned herein, the particulate porous material may have a porosity of from 8 to 30% (v/v) and a single point total pore volume of from 0.36 to 0.85 cm³/g. For example, the particulate porous material may have a porosity of from 10 to 28% (v/v) and a single point total pore volume of from 0.4 to 0.8 cm³/g.

In some embodiments of the invention that may be mentioned herein, the particulate porous material may have a nitrogen adsorption isotherm that is Type II or Type IV. Without wishing to be bound by theory, a type of Type II isotherms herein may refer to macroporous materials while a type of Type IV isotherms herein may refer to mesoporous materials (e.g. mesoporous molecular sieves). For these Type IV materials, the monolayer-multilayer formation is followed by pore condensation, which means that the gas condenses to a liquid-like state in a pore at a pressure less than the saturation pressure p_0 of the bulk liquid. Type IV isotherms finally reach a saturation plateau. Type IV(a) isotherms with hysteresis can be distinguished from Type IV(b) isotherms without hysteresis. Hysteresis occurs when the pore width exceeds a certain critical width which is depending on the adsorptive and temperature. For nitrogen and argon at 77 and 87 K, respectively, hysteresis occurs for pores larger than ≈ 4 nm. In contrast,

adsorbents having smaller mesopores show a completely reversible adsorption behavior leading to a Type IV(b) isotherm.

5 In some embodiments of the invention that may be mentioned herein, the particulate porous material may have a D50 of less than 200 μm . The term "D50" as used herein refers to a mean or average particle size of a material.

10 In some embodiments of the invention that may be mentioned herein, the particulate porous material may have a total protein binding capacity that is less than 0.2g/g. The term "total protein" as used herein refers to a mixture of dialysate or serum proteins containing ~80% albumin.

15 Without wishing to be bound by theory, it is believed that the properties of some embodiments of the invention that may be mentioned herein may be particularly useful in providing the particulate porous material size selectivity and B2M sieving effect due to the availability of surface adsorption sites.

20 In a second aspect of the invention, there is provided a sorbent cartridge suitable for use in purification of a bodily fluid (e.g. a kidney dialysis treatment, such as a haemodialysis treatment, a peritoneal dialysis treatment or a hemoperfusion treatment), wherein the sorbent cartridge comprises the particulate porous material as disclosed above.

As will be appreciated, the sorbent cartridge may further comprise one or more of:

25 cation exchange particles (e.g. zirconium phosphate);
anion exchange particles (e.g. hydrous zirconium oxide);
activated carbon;
a buffer (e.g. calcium carbonate); and
a urease.

30 Any suitable amount of the components listed above may be used in the sorbent cartridge mentioned herein.

35 The term "cation exchange particles" as used herein refers to particles capable of capturing or immobilizing cationic or positively charged species when contacted with such species, typically by passing a solution of the positively charged species over the surface of the particles. For example, the cation exchange particles may be zirconium phosphate. For example, zirconium phosphate in the sodium or hydrogen form serves as a cation exchanger

and absorbs cations such as ammonium (NH_4^+), calcium (Ca^{2+}), potassium (K^+), and magnesium (Mg^{2+}). In exchange for absorbing these cations, zirconium phosphate releases two other cations, sodium (Na^+) and hydrogen (H^+).

- 5 Zirconium phosphate may be prepared by mixing sodium zirconium carbonate with a phosphate buffer having a desired pH value and in an appropriate ratio, which can readily be determined by a skilled person.

The zirconium phosphate particles may have an average particle size in the range of from
 10 about 10 microns to about 1000 microns, about 100 microns to about 900 microns, about 200
 microns to about 900 microns, about 300 microns to about 800 microns, about 400 microns to
 about 700, 500 microns to about 600 microns, about 25 microns to about 200 microns or from
 about 25 microns to about 150 microns or from about 25 microns to about 80 microns or from
 15 about 25 microns to about 50 microns or from about 50 microns to about 100 microns or from
 about 125 microns to about 200 microns, or from about 150 microns to about 200 microns, or
 from about 100 microns to about 175 microns, or from about 100 microns to about 150 microns
 or from about 150 microns to about 500 microns, or from about 250 microns to about 1000
 microns.

- 20 The term "anion exchange particles" as used herein refers to particles capable of capturing or
 immobilizing anionic or negatively charged species when contacted with such species,
 typically by passing a solution of the negatively charged species over the surface of the
 particles. The anion exchange particles may comprise of an amorphous and partly hydrated,
 water-insoluble metal oxide in its hydroxide-, carbonate-, acetate-, and/or lactate- counter-ion
 25 form, wherein the metal may be selected from the group consisting of titanium, zirconium,
 hafnium and combinations thereof. For example, the anion exchange particles may be
 zirconium oxide particles.

The zirconium oxide may be provided in any suitable form, such as zirconium oxide particles.
 30 When in the form of particles, the zirconium oxide particles may have an average particle size
 in the range of from about 10 microns to about 1000 microns, about 100 microns to about 900
 microns, about 200 microns to about 900 microns, about 300 microns to about 800 microns,
 about 400 microns to about 700, 500 microns to about 600 microns, about 10 microns to about
 200 microns or from about 10 microns to about 100 microns or from about 10 microns to about
 35 30 microns or from about 10 microns to about 20 microns or from about 20 microns to about
 50 microns or from about 25 microns to about 50 microns or from about 30 microns to about
 50 microns or from about 40 microns to about 150 microns or from about 80 microns to about

120 microns or from about 160 microns to about 180 or from about 25 microns to about 250 or from about 250 microns to about 500 or from about 250 microns to about 1000.

5 The zirconium oxide may be a hydrous zirconium oxide. Hydrous zirconium oxide may be synthesised by conventional methods, for example by reaction of an aqueous mixture of sodium zirconium carbonate and sodium hydroxide as described in US Pat No 4,256,718. After synthesis of hydrous zirconium oxide, the product may be titrated to a pH of from 12 to 13. This can be done by making an aqueous slurry of the hydrous zirconium oxide and titrating it with 5M sodium hydroxide until the slurry is at a pH of 12 to 13. In some instances, the
10 hydrous zirconium oxide may then be washed until the concentration of leachables in the filtrate was within acceptable levels, and air dried. Alternatively, the hydrous zirconium oxide may recovered directly from the slurry and not washed before being air dried.

When used herein, the term “buffer” refers to a buffer suitable for use in a sorbent cartridge in
15 purification of a bodily fluid (e.g. a kidney dialysis treatment, such as a haemodialysis treatment, a peritoneal dialysis treatment or a hemoperfusion treatment). Any suitable buffer may be used herein. For example, the buffer may be calcium carbonate.

When used herein, the term “urease” is a synonym for the term “uremic toxin-treating enzyme”
20 and both refer to an enzyme able to react with a uremic toxin as a substrate. For example, the uremic toxic-treating enzyme may be an enzyme able to react with urea as a substrate, with uric acid as a substrate, or with creatinine as a substrate. Uremic enzymes can be determined to have this function *in vitro*, for example, by allowing the enzyme to react with a uremic toxin in solution and measuring a decrease in the concentration of the uremic toxin. Examples of
25 uremic toxin-treating enzymes include, but are not limited to, ureases (which react with urea), uricases (which react with uric acid), or creatininases (which react with creatinine).

In a third aspect of the invention, there is provided a method of performing dialysis (e.g. haemodialysis, peritoneal dialysis or hemoperfusion), comprising a step of passing a dialysate
30 through a sorbent cartridge as defined above.

In a fourth aspect of the invention, there is provided a use of a particulate porous material as defined above, or a sorbent cartridge as defined above, to adsorb beta-2 microglobulin.

35 In a fifth aspect of the invention, there is provided a method of removing beta-2 microglobulin from a fluid, comprising:

providing a fluid comprising beta-2 microglobulin; and

exposing said fluid to a particulate porous material as defined above.

In a sixth aspect of the invention, there is provided a method of preparing a particulate porous material as defined above, the method comprising the steps:

- (i) providing mixture comprising a first monomer that is ethylene glycol dimethacrylate, an initiator, a porogen and optionally a second monomer that is a hydrophilic monomer;
- (ii) conducting a polymerisation reaction to form a bulk porous material; and
- (iii) grinding or pulverising the bulk porous material to provide a particulate porous material.

The term "initiator" refers to a chemical species that reacts with a monomer to initiate a polymerisation reaction. Examples of initiators include, but are not limited to, 2'-azobis(2-methylpropionitrile) (AIBN), 4,4'-azobis(4-cyanovaleric acid) (ACVA), benzoyl peroxide, and 1,1'-azobis(cyclohexanecarbonitrile).

When used herein, the term "porogen" refers to a material additive that can influence the pore structures of the particulate porous material. Examples of porogens include, but are not limited to, toluene, chloroform, acetone, dimethylformamide, dimethylsulfoxide, dioxane, ethyl acetate, and acetonitrile.

In some embodiments of the sixth aspect of the invention that may be mentioned herein, the porogen may be an organic solvent selected from the group consisting of toluene, chloroform, acetone, dimethylformamide, dimethylsulfoxide, dioxane, ethyl acetate, and acetonitrile.

Grinding or pulverising of the bulk porous material may be carried out by one or more of the following procedures:

1. manual grinding with a mortar and a pestle;
2. milling using an analytical or industrial mill; and
3. cryomilling, which involves milling of a sample chilled to 77 K.

The method of preparing a particulate porous material as defined above may further comprise washing the particulate porous material to remove the porogen. For example, the particulate porous material may be washed by sonicating the particulate porous material in a solvent, centrifuging the particulate porous material in the solvent, and decanting the supernatant solvent, to remove the porogen. As will be appreciated, the solvent may be selected from one or more of methanol, acetic acid, ethanol, deionised (DI) water, acetone, and sulfuric acid.

In some embodiments of the sixth aspect of the invention that may be mentioned herein, the porogen may be an organic solvent having a Hansen distance (R_a) of up to 10 MPa^{1/2}, where $D_{\text{polymer}} = 17.0$, $P_{\text{polymer}} = 9.7$ and $H_{\text{polymer}} = 4.9$.

5

The Hansen distance (R_a) may be calculated by applying the following equation.

$$R_a = \sqrt{4(D_{\text{solv}} - D_{\text{polymer}})^2 + (P_{\text{solv}} - P_{\text{polymer}})^2 + (H_{\text{solv}} + H_{\text{polymer}})^2}$$

The values of D_{polymer} , P_{polymer} and H_{polymer} may be determined by conducting Solubility Sphere Analysis according to the Hansen method. Specific details are provided in the following examples.

10

In some embodiments of the sixth aspect of the invention that may be mentioned herein, step (ii) may be performed at a temperature of from 50°C to 70°C.

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As will be appreciated, the present invention provides the following advantages.

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- **Simplified synthesis:** the present invention is the only B2M sorbent that can be synthesised through bulk polymerisation in a single step, from only three reagents. This allows for efficient and scalable production. Moreover, it uses cost-effective raw materials, making it economically viable for large-scale production.
- **Novel design:** novel in terms of chemical structure and microstructure, using a combination of pore size, porosity and hydrophilic/hydrophobic interactions to trap and adsorb B2M. There is no need for inactive carrier material, the polymer matrix is the adsorbent. This leads to reduced wastage of material and enables manufacturing of smaller devices.
- **Novel, tunable structure:** synthesis of porous sorbents from pure EGDMA appears to be a novel approach. Additionally, in the present disclosure, we have demonstrated the tunability of porosity and pore size of this novel sorbent by varying porogen type and porogen ratio. Tunability of functionality was demonstrated by incorporation of various comonomers while maintaining B2M adsorption capability (see Example 8 below).
- **Desirable handling properties:** the present invention is essentially a polyester powder. It is stable at room temperature and is inert under physiological conditions.
- **High capacity:** the present invention demonstrates exceptional performance, exhibiting high binding capacity even at low B2M concentrations, for example in

dialysis fluid. Notably, it maintains its selectivity for B2M even in the presence of high glucose and salt concentrations, and other proteins commonly found in biological fluids.

- **Fast binding kinetics:** the kinetic properties of the present invention are also favourable, achieving rapid adsorption of significant quantities of B2M in both static and dynamic flow environments.
- **Sterilisability:** additionally, the present invention is sterilizable, ensuring its suitability for medical applications without compromising its adsorption capabilities.
- The following examples provides evidence of its performance in terms of binding capacity, selectivity, and kinetic properties, highlighting its potential impact in improving peritoneal dialysis outcomes.

Accordingly, the present invention may find applications in the following.

- **Hemoperfusion:** the primary application of B2M sorbent materials is as an active ingredient in hemoperfusion sorbent cartridges. In this type of application, the blood is passed through the sorbent cartridge. The sorbent interacts directly with the blood and filters it by removing unwanted B2M proteins.
- **Peritoneal dialysis:** the sorbent material may be used in a sorbent cartridge for dialysate regeneration in sorbent peritoneal dialysis (PD). For example, Poly-EGDMA could partially replace AC in an existing sorbent design, saving space and reducing overall device weight. The cartridge would also be more selective, with less glucose adsorption caused by AC.
- **Hemodialysis:** similarly, the sorbent could be of benefit when used in sorbent hemodialysis, allowing the manufacture of an optimised HD sorbent cartridge.
- **General:** Poly-EGDMA can be used to selectively remove B2M from biological fluids in general. For example, Poly-EGDMA may be used for selective removal of B2M from a research sample or a blood sample.

Further aspects and embodiments of the invention will now be discussed by reference to the following non-limiting examples.

Examples

Materials

All reagents and solvents were purchased from Sigma-Aldrich or TCI and used as received.

Analytical techniques

SEM

Samples were coated with Pt and analysed using FESEM JEOL JSM-7800F PRIME.

Example 1. Crosslinker Evaluation

5

The main criteria for a suitable sorbent are:

- high adsorption capacity, >1 mg/mL for beads or >1 mg/g for powder (as per application);
- high selectivity, minimal albumin removal (<10%); and
- biocompatibility (minimal binding of platelets and leucocytes, minimal increase in inflammatory markers).

10

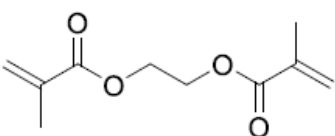
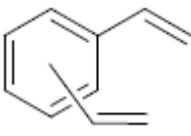
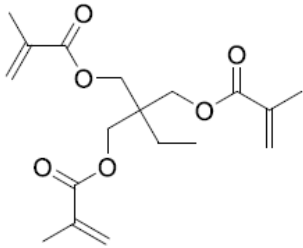
Selection of crosslinking monomers was evaluated in order to identify suitable polymer matrix with affinity for the B2M molecule in PD solution. In total, 5 crosslinkers with varying structure, molecular weight, and hydrophobicity were screened. Each crosslinker was polymerized under similar conditions via free-radical polymerization. Resultant polymers were characterized and analysed for B2M adsorption properties.

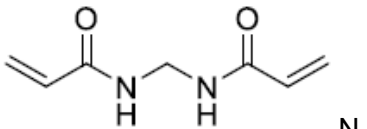
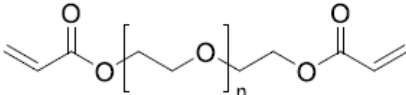
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Results and discussion

Table 1. Crosslinker evaluation.

20

 ethylene glycol dimethacrylate	 divinylbenzene	 trimethylolpropane trimethacrylate
Acronym: EGDMA CAS Number: 97-90-5 Molecular Weight: 198.2 Formula: C₁₀H₁₄O₄ logP: 2.8 (est), hydrophobic (log P > 0) Solubility in water: 580.5 mg/L @ 25 °C (est) (sparingly soluble)	Acronym: DVB CAS Number: 1321-74-0 Molecular Weight: 130.2 Formula: C₁₀H₁₀ logP: 3.0-3.2 (est) Solubility in water: 52.5 mg/L @ 25 °C (est) H-bond donor: none H-bond acceptor: none	Acronym: TRIM CAS Number: 3290-92-4 Molecular Weight: 338.4 Formula: C₁₈H₂₆O₆ logP: 3.2 (est) Solubility in water: 13 mg/L @ 25 °C (exp) H-bond donor: none

H-bond donor: none H-bond acceptor: 4 Density: 1.051 g/mL Melting point: -40 °C (-40 °F; 233 K) Boiling point: 98 to 100 °C (208 to 212 °F; 371 to 373 K) (5 mmHg)		H-bond acceptor: 6
 N'-methylenebisacrylamide	 polyethylene glycol diacrylate PEGDA 575, M_n 575 g/mol	
Acronym: BA CAS Number: 110-26-9 Molecular Weight: 154.2 Formula: $C_7H_{10}N_2O_2$ logP (o/w): -1.4 (est) Solubility in water: 20 g/L @ 20 °C H-bond donor: 2 H-bond acceptor: 2	Acronym: PEGDA Values for n = 10 (decaethylene glycol diacrylate) CAS Number: N/A Molecular Weight: 566.6 Formula: $C_{26}H_{46}O_{13}$ logP (o/w): -0.1 (est) Solubility in water: Soluble H-bond donor: 0 H-bond acceptor: 13	

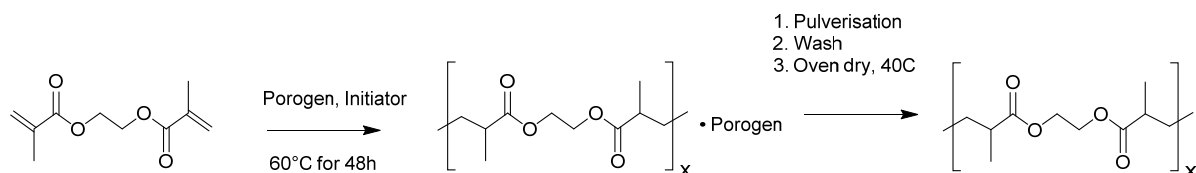
Hydrophobicity (logP) : DVB $\approx$ EGDMA > TRIM > PEGDA > BA

Molecular weight: PEGDA > TRIM > EGDMA > DVB > BA

Crosslinkable groups: TRIM > DVB = EGDMA = BA = PEGDA

5

Example 2. Synthetic method for polymers



Each crosslinker discussed in Example 1 was polymerized under similar, free-radical bulk polymerization conditions (FIG. 1). Allowance was made for variation in crosslinker solubility by adjusting porogen and washing conditions.

10

In a 20 mL glass vial, the crosslinking monomer and initiator (ACVA), 30 mg, 0.11 mmol) were added to a porogen (6 mL) and sonicated for 30 s. The vial was purged with N₂ for 2 min and sealed with a rubber lined cap. The mixture was polymerized by placing the vial in an oven at 60 °C for 48 h.

5

The resultant polymeric solid was divided into ~1 cm pieces with a spatula and transferred to a mortar and pestle. The polymer pieces were processed in two steps:

1) the transferred pieces were initially pulverized into particles smaller than approximately 3 mm to allow for efficient grinding; and

10 2) the polymer was ground at approximately 1 cycle per second for 60 s.

The polymer particles were then collected in a 50 mL falcon tube and subjected to sequential washing steps consisting of:

a) sonication for 15 min in 30 mL of solvent; and

15 b) centrifugation for 10 min at 5000 rpm and decantation of supernatant solvent.

The resultant powders were oven dried at 40 °C until no weight change was detected, typically 48 h.

20 The washing step for each polymer is provided in the table below.

Table 2. Washing steps.

Sample	Crosslinker	Porogen	Washing Steps	Yield / Product
1A	EGDMA 9mmol	Chloroform	1. Methanol (MeOH)/5% Acetic acid 2. Ethanol (EtOH) 3. DI H ₂ O 4. Acetone	1.63 g White, free- flowing powder
1B	DVB 9mmol	Chloroform	1. MeOH/5% Acetic acid 2. MeOH	1.05 g White, free- flowing powder
1C	TRIM 9mmol (1:1 molar equivalent	Chloroform	1. MeOH/5% Acetic acid 2. EtOH 3. DI H ₂ O 4. Acetone	2.74 g White, free- flowing powder

	to Ex 1A)			
1D	BA 12mmol	DMSO	1-4. 0.1 M H ₂ SO ₄ 5. DI H ₂ O 6. EtOH	1.62 g Translucent gelatinous solid
1E	PEGDA 3.4mmol (1:1 volume equivalent to Ex 1A)	Acetonitrile	1-5. 0.1 M H ₂ SO ₄ 6. DI H ₂ O 7. EtOH	1.71 g Translucent gelatinous solid

Specifically, polymer samples (1A-1C) were prepared with 3 different crosslinkers, under similar conditions:

1. crosslinker 9 mmol, ACVA 0.04 mmol, porogen 6 mL;
2. polymerised at 60 °C for 48 h, pulverised < 3 mm and ground 60 s;
3. washed as depicted in Table 2 above; and
4. vacuum dried in a vacuum drying oven at 40 °C for 48 h.

Example 3. Static Adsorption Test

Samples 1A-1E prepared in Example 2 were taken for static adsorption test.

Static test method

50 mg sample was incubated with 100 mL PD fluid (typically 1000-3000 µg B2M/L, total protein 0.5-1.0g/L. More specifically, 2108-2812 µg B2M/L, total protein 0.7-0.9 g/L), and incubated for 2 h at 23 °C, 150-250 rpm agitation rate. 50 mg AC tested for comparison.

B2M adsorption calculation:

$$\text{Mass of B2M adsorbed per gram (mg/g)} = (C_{\text{pre}} - C_{\text{post}}) * V_{\text{sample}} / M_{\text{sample}}$$

where C_{pre} = starting concentration of B2M in µg/L, C_{post} = final concentration of B2M in µg/L, V_{sample} = volume of PD fluid, and M_{sample} = mass of polymer/AC sample in grams.

B2M assay

B2M assay was performed by SGH Clinical Pathology lab. β2-microglobulin was tested through Immuno-turbidimetry method performed on Roche Cobas c502 chemistry analyser (Brand/Part Number: Tina-quant β2-Microglobulin (B2MG) (08047430190). Assay kit manufacturer: Roche), according to the manufacturer's protocol.

Protein Loss - Static Test Method

Test sample was prepared in same manner as in B2M assay.

5 Protein loss calculation:

$$\text{Estimated protein loss (g/L)} = C_{\text{pre}} - C_{\text{post}}$$

Where C_{pre} = starting protein concentration in g/L, and C_{post} = final protein concentration in g/L.

Protein estimation was determined by Bradford assay.

10

Bradford assay

In a 96-well plate, sample solutions (5 μL) were incubated with Bradford Reagent (250 μL ; TCI Chemicals, Product No B5702) for 20 min, along with a blank solution (5 μL Milli-Q water) and standard solution (1.0g/L Bovine Serum Albumin solution). The absorbance at 595 nm was measured using a UV-vis well plate reader. A blank correction was performed on the samples and standard by subtracting the absorbance attributed the blank solution. The absorbance value from the 1 g/L BSA solution was used to create a single point calibration curve (absorbance vs concentration). The calibration curve was used to determine the estimated protein content of samples based on their absorbance values.

20

Results and discussion

Table 3. Static adsorption test results.

Sample No	Material	B2M Adsorbed($\mu\text{g/g}$)	Protein loss (g/L)
1A	Poly-EGDMA	2232.2	<0.1g/L
1B	Poly-DVB	0.0	<0.1g/L
1C	Poly-TRIM	25.2	<0.1g/L
1D	Poly-BA	93.5	<0.1g/L
1E	PEGDA	13.3	<0.1g/L
1F	Activated Carbon	66.1	<0.1g/L

Poly-EGDMA (1A) exhibited unexpectedly strong, selective adsorption behaviour toward B2M in comparison to the other materials tested. This B2M selectivity is observed amongst a mixture of different proteins and solutes being present in PD fluid.

25

Example 4. Nitrogen Porosimetry

Samples 1A, 1B, 1C and 1F prepared in Example 2 were taken for nitrogen porosimetry study.

Surface area, pore volume and pore size distribution

Dinitrogen adsorption-desorption experiments were carried out by static manometric method (Standardization, I.O.f., Determination of the specific surface area of solids by gas adsorption — BET method. **2022**, ISO 9277:2022). Measurements were performed at 77.3 K on a Micrometrics ASAP model 2020 porosimeter. Equilibration time was set to 10 s and free space was measured by the instrument.

10 Samples (50-100 mg) were degassed at 40 °C under vacuum for 16 h prior to data collection.

Surface area, pore volume and pore size distribution were calculated using methods provided by Micromeritics MicroActive software version 5.02.

15 Multipoint surface area measurements utilized an 8-point adsorption isotherm collected over 0.05 to 0.20 p/p⁰ and were analyzed using BET theory (Brunauer, S., Emmett, P. H. & Teller, E., *J. Am. Chem. Soc.*, **1938**, 60, 309–319).

20 The single point total pore volume was calculated and reported by Micromeritics MicroActive software. The single point total pore volume was calculated at a single point approaching saturation pressure p/p⁰ >0.95, which was an application of the Gurvich rule (Rouquerol, J., *et al.*, *Adsorption by powders and porous solids: principles, methodology and applications*. **2013**: Academic press).

25 BJH adsorption pore size distributions utilized a 33-point adsorption isotherm and were analyzed via the BJH method provided by Micromeritics software, using the Halsey-Faas correction option (Barrett, E. P., Joyner, L. G. & Halenda, P. P., *J. Am. Chem. Soc.*, **1951**, 73, 373–380). Pore size ranges and isotherm types were classified according to IUPAC recommendations (Rouquerol, J., *et al.*, *Pure Appl. Chem.*, **1994**, 66, 1739–1758; and
30 Thommes, M., *et al.*, *Pure Appl. Chem.*, **2015**, 87, 1051–1069).

The Brunauer-Emmett-Teller (BET) Adsorption Average Pore Size refers to an assumed cylindrical-shaped pore width calculated via the Gurvich rule and BET method:

$$D = 4V/S$$

35 Where D = average pore diameter, V = single point total pore volume and S = specific surface area determined by BET model. The data were cited as calculated and reported by the MicroActive software.

Porosity was calculated via the below equation:

$$\text{Porosity (\%)} = \text{single point total pore volume (cm}^3\text{/g)} * \text{tapped density (g/cm}^3\text{)} \times 100$$

- 5 Tapped density was measured by mechanically tapping the dry polymer samples in a 5 mL measuring cylinder, and allowing the sample to settle to a final volume under its own weight. The tapped density was calculated as follows:

$$\text{Tapped density} = \text{Sample mass (g)} / \text{Final Volume (cm}^3\text{)}$$

- 10 The sample (1 g) was loaded into the measuring cylinder. The sample was then tapped for 60 s at 1 tap per second on the lab bench and volume measured. The sample was then tapped again for further rounds of 60 s until no further change was observed in the sample volume. This value was then taken as the final volume.

15 Results and discussion

FIG. 2 depicts the nitrogen porosimetry results.

Table 4. Nitrogen porosimetry results.

Sample	1A	1B	1C	1F
Material	Poly-EGDMA	Poly-DVB	Poly-TRIM	Calgon AC
B2M Adsorption ($\mu\text{g B2M/g sample}$)	2232.2	0.0	25.2	66.1
Surface Properties				
Isotherm type (Hysteresis)	IVa (H2/H4)	IVa (H2/H4)	IVa (H2/H4)	Ia
Interpretation	Mixed porosity Mesopore dominant Constricted pores	Mixed porosity Mesopore dominant Constricted pores	Mixed porosity Micro- mesoporous	Pure Microporous
BET Surface Area: $\text{m}^2\text{/g}$	405.89	975.44	116.1	776.82
Single point total pore volume: $\text{cm}^3\text{/g}$	0.36	1.09	0.07	0.38

BET Adsorption average pore size: Å	35.81	44.69	25.61	20.01
BJH Adsorption average pore size: Å	54.47	50.76	40.00	40.55
Bulk properties				
Porosity	21.77%	36.27%	4.45%	N/A

N₂ adsorption-desorption experiments revealed that the synthesised polymers were mesoporous with average pore size in similar range of 2.9-4.5 nm according to BET model. 1A had much higher affinity and capacity for B2M than the other synthesised mesoporous polymers 1B and 1C. This is in spite of the finding that 1B had almost twice the porosity, surface area and total pore volume of 1A. 1C had a very low degree of porosity, therefore low B2M capacity was not surprising. AC had high degree of microporosity and surface area but this did not translate into significant B2M adsorption. Purely microporous materials appear unsuitable for B2M adsorption, which could be due to restrictive pore size vs B2M hydrodynamic radius (2.0 nm vs 1.6 nm). In this case, molecular structure and functional properties dominated over surface properties as factors influencing B2M adsorption. This could imply that Poly-EGDMA exhibits chemical properties which are particularly suitable towards selective adsorption of B2M. Due to lack of porosity, gelatinous nature and low B2M binding, hydrophilic polymers 1D and 1E were not further characterised.

Poly-EGDMA is a novel porous adsorbent material developed for the highly selective and efficient removal of B2M from PD fluid (FIG. 3). Porous poly-EGDMA particles have an abundance of micro/mesopores (FIG. 3), are hydrophobic, and have simple synthesis. They are able to bind B2M in PD fluid in high efficiency, high selectivity, and high stability.

As such, a new B2M adsorbent material has been prepared from cross-linked Poly-EGDMA. EGDMA is a difunctional monomer which is commonly used as a crosslinker in free-radical polymerisation reactions. It is normally used in small amounts (1-2% of total monomer content) and copolymerised with other monofunctional monomers like styrene and methyl methacrylate to form crosslinked polystyrene (PS) or polymethylmethacrylate (PMMA). EGDMA itself is polymerised to poly-EGDMA, a heavily crosslinked network polymer with high chemical and physical stability.

Example 5. Proposed Poly-EGDMA adsorption behaviour

Optimal structure and porosity

- 5 • Poly-EGDMA: conglomerates of irregular shaped nodules
- Pores: free space between conglomerates
- Micropores and small mesopores – size selectivity and B2M sieving effect
- Macropores – efficient fluid transport channel into microparticle

10 *IUPAC Classification*

Macropore: >50nm

Mesopore: 2-50 nm

Micropore: < 2nm

15 *Pore diameter determination*

The pore diameter data was derived from the 33-datapoint adsorption branch of the N₂ isotherm. Miromeritics MicroActive software version 5.02 was used to analyse the data according to the Barrett-Joyner-Halenda model with Halsey-Faas correction, in order to derive the pore size distribution and average pore diameter data.

20

Results and discussion

B2M has a globular, compact structure, and a hydrodynamic radius of ~1.6 nm, thus it can fit into large micropores and small mesopores.

- 25 FIG. 4 shows a SEM image of the poly-EGDMA microparticle. Poly-EGDMA has a high proportion of large micropores and small mesopores. The average pore diameter was 6.0 nm (FIG. 5).

Literature simulations (C. J. Dongmo Fomthum *et al.*, *Mol. Biosyst.* **2017**, *13*, 2625-2637)

30 have shown that B2M binds quickly and irreversibly to hydrophobic surfaces via a 2-step process:

1. initial adsorption by polar and charged groups; and
2. partial unfolding occurs, maximizing adhesion of the protein to the hydrophobic surface (accentuated interaction of hydrophobic groups).

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Poly-EGDMA is hydrophobic overall (EGDMA water/octanol partition coefficient $\rightarrow \log P = 2.8$, lipophilic > 0). Ester groups may serve as polar groups for initial adsorption step.

Example 6. Effect of Porogen Type

5

Attempt was made to prepare Poly-EGDMA with different pore sizes, by varying porogen type. This was done in order to characterize the relationship between pore size and B2M sorption.

Synthesis of Poly-EGDMA (FIG. 6)

- 10 Four samples were polymerized under similar conditions to Sample 1A in Example 2 except that the initiator used was AIBN and the porogen was varied.

Table 5. Synthetic method for 2A-2L.

Sample	Crosslinker/ Initiator	Porogen	Washing Steps	Yield / Intermediate product
2A	EGDMA 9mmol ACVA 0.11mmol	Toluene	1. MeOH/5% Acetic acid 2. EtOH 3. DI H ₂ O 4. Acetone	1.61 g White monolithic solid
2B	EGDMA 9mmol AIBN 0.04mmol	Chloroform	1. MeOH/5% Acetic acid 2. MeOH 3. EtOH 4. MeOH	1.57 g White monolithic solid
2C	EGDMA 9mmol AIBN 0.04mmol	n-Hexane	1. MeOH/5% Acetic acid 2-4. MeOHx3	1.48 g White, free-flowing powder
2D	EGDMA 9mmol AIBN 0.04mmol	Acetone	1. MeOH/5% Acetic acid 2-4. MeOHx2	1.67 g White monolithic solid
2E	EGDMA 9mmol AIBN 0.04mmol	Methanol	1. MeOH/5% Acetic acid 2-3. MeOHx2	1.55 g White, free-flowing powder

2F	EGDMA 9mmol AIBN 0.04mmol	DMF	1. DI H ₂ O 2. MeOH/5% Acetic acid 3-4. MeOHx2	1.68 g White monolithic solid
2G	EGDMA 9mmol AIBN 0.04mmol	DMSO	1. DI H ₂ O 2. MeOH/5% Acetic acid 3-4. MeOHx2	1.70 g White monolithic solid
2H	EGDMA 9mmol AIBN 0.04mmol	Dioxane	1. MeOH/5% Acetic acid 2-3. MeOHx2	1.69 g White monolithic solid
2I	EGDMA 9mmol AIBN 0.04mmol	Ethyl Acetate	1. MeOH/5% Acetic acid 2-3. MeOHx2	1.70 g White monolithic solid
2J	EGDMA 9mmol AIBN 0.04mmol	iso- Propanol	1. MeOH/5% Acetic acid 2-3. MeOHx2	1.62 g White, free-flowing powder
2K	EGDMA 9mmol AIBN 0.04mmol	Ethanol	1. MeOH/5% Acetic acid 2-3. MeOHx2	1.29 g White, free-flowing powder
2L	EGDMA 9mmol AIBN 0.04mmol	Acetonitrile	1. MeOH/5% Acetic acid 2-3. MeOHx2	1.69 g White monolithic solid

This synthetic method is a free radical polymerisation process. It is a simple 1-step synthesis + washing, sieving and drying. It gives high yield and is scalable.

- 5 *Hansen solubility parameter system (Chesnokov, S.A., et al., Macromol. Biosci., 2021, 21, 2000402.)*

There are number of different systems to classify solvents and their molecular properties (dielectric constant, polarity index, dipole moment, etc.). The Hansen solubility parameter

system attempts to classify and rank solvents according their propensity to interact via dispersion, polar and H-bonding forces. Hansen solubility parameters, Hansen distance and sphere radius were calculated according to Hansen theory (Hansen, C. M., *Hansen Solubility Parameters*. 2007) by means of multiresponse optimization, utilizing the Excel Solver and DATAFIT functions (Díaz De Los Ríos, M. & E. Hernández Ramos, *SN Applied Sciences*, 2020, 2).

By theory, the closer the Hansen parameters between a solvent and a given monomer, the better suited the solvent is to solublise that monomer and its polymeric derivatives (good vs bad solvent). The closer the solubility parameters between the polymer and the porogenic agent, the greater the thermodynamic affinity and lesser effect of phase separation during polymerisation. This affinity has a profound effect on polymer pore size. For example, literature studies found that the closer the value of solubility parameters are between EGDMA and its solvent, the smaller the resultant pore size in its polymer products.

Table 6. Hansen solubility parameters.

	Dispersion	Polar	H-bonding
EGDMA monomer	16.4	5.2	8.6
n-Hexane	14.9	0	0
Benzene	18.4	0	2
Toluene	18	1.4	2
Diethyl ether	14.5	2.9	4.6
Chloroform	17.8	3.1	5.7
1,4-Dioxane	17.5	1.8	9
Ethyl acetate	15.8	5.3	7.2
Tetrahydrofuran (THF)	16.8	5.7	8
Dichloromethane	17	7.3	7.1
Acetone	15.5	10.4	7
Acetonitrile (MeCN)	15.3	18	6.1
Dimethylformamide (DMF)	17.4	13.7	11.3
Dimethyl sulfoxide (DMSO)	18.4	16.4	10.2
Acetic acid	14.5	8	13.5
n-Butanol	16	5.7	15.8
Isopropanol	15.8	6.1	16.4
n-Propanol	16	6.8	17.4

Ethanol	15.8	8.8	19.4
Methanol	14.7	12.3	22.3
Formic acid	14.6	10	14
Water	15.5	16	42.3

The polymers prepared were imaged with SEM, taken for static adsorption test as described in Example 3, and taken for nitrogen porosimetry study as described in Example 4.

5 Results and discussion

For 2A, 2B, 2D, 2F, 2G, 2H, 2I and 2L

- Bulk monolith formed.
- Slow/reduced phase separation during polymerisation process.
- Good solvent-polymer affinity.
- Typically porous due to presence of porogen in polymer lattice.

For 2C, 2E, 2J and 2K

- Particulate product formed directly (without pulverisation).
- Indicative of fast, significant phase separation during polymerisation stage.
- Poor solvent-polymer affinity.
- Typically non-porous due to early separation of porogen and polymer phases.

FIG. 7 depicts the SEM images of monolithic polymers. FIG. 8 depicts the SEM images of particulate polymers. The N₂ adsorption-desorption isotherms are depicted in FIG. 9.

Table 7. Static adsorption test of 2A-2L.

Sample No	Porogen	Product	B2M Adsorbed (µg/g)	Protein loss (g/L)
2A	Toluene	Monolith	2991.7	N/A
2B*	Chloroform	Monolith	4403.0	0.1
2C*	n-Hexane	Particulate	0.0	N/A
2D	Acetone	Monolith	3471.7	0.1
2E	Methanol	Particulate	90.5	<0.1
2F*	DMF	Monolith	2282.6	<0.1

2G	DMSO	Monolith	3154.7	<0.1
2H	Dioxane	Monolith	3279.8	<0.1
2I	Ethyl Acetate	Monolith	3513.1	<0.1
2J	IPA	Particulate	480.9	<0.1
2K	Ethanol	Particulate	6.9	<0.1
2L*	Acetonitrile	Monolith	2432.5	<0.1

*Selected for further analysis.

Monolithic Poly-EGDMA had much high B2M capacity than particulate Poly-EGDMA. Highest performing monolith (2B) was selected for analysis by N₂ adsorption and compared with below average monoliths (2F, 2L) and particles (2C).

Table 8. Nitrogen porosimetry results of 2B, 2C, 2F and 2L.

Sample	2B	2C	2F	2L
Isotherm type (Hysteresis type)	IVa (H2/H4)	II	II	II
Interpretation	Mixed porosity Mesopore dominant Constricted pore entrance	Non-porous	Mixed porosity Macropore dominant	Mixed porosity Macropore dominant

The pore size distribution according to BJH model is depicted in FIG. 10.

Table 9. Surface properties of 2B, 2C, 2F and 2L.

Sample	2B	2C	2F	2L
Porogen	CHCl ₃	Hexane	DMF	MeCN
B2M Adsorbed (µg B2M/g sample)	4403.00	0.00	2282.60	2432.50
Surface Properties				

BET Surface Area: m ² /g	449.03	7.10	542.09	639.84
Single point total pore volume: cm ³ /g	0.45	0.01	0.77	0.73
BET Adsorption average pore size: Å	40.51	84.41	56.52	45.89
BJH Adsorption average pore size: Å	59.90	384.83	117.38	104.28
Bulk Properties				
Particle Size, D50	68.0	20.7	64.3	96.2
Porosity	24%	0.46%	20%	16%

Porosity

From Samples 2C, 2E, 2J and 2K, it is clear that the presence of crosslinked EGDMA is not sufficient to guarantee B2M adsorption. Good porogen and sufficient porosity in the final polymer are required to allow adsorption process to occur.

Pore size

From Samples 2B, 2F and 2L, average pore size and pore size distribution may play influential role towards B2M adsorption. Other factors such as surface area are less important.

Comparing B2M adsorption and surface properties, it appears that mesopore dominant poly-EGDMA (i.e., 2B) with narrower pore size distribution is preferred. Poly-EGDMA with broad pore size distribution and macropore dominant characteristics (i.e., 2F and 2L) is less preferable.

Porogen suitability

Good porogen → porous monolith → Chloroform, DMSO, Acetone, Dioxane, Ethyl Acetate, MeCN, DMF, toluene.

Poor porogen → non-porous particles → MeOH, hexane, IPA, ethanol.

Example 7. Effect of porogen affinity

We aimed to characterise the relationship between porogen affinity, polymer properties and B2M adsorption.

Example 6 revealed that porogen affinity has substantial effect on final polymer properties. Polymerisation was attempted in mixed porogen environment. According to Hansen solubility theory, a mixed porogen exhibits properties according to the weighted average of the parent porogens. This was done in order to characterize the relationship between pore size and B2M sorption, and to find optimal porosity.

Synthesis of Poly-EGDMA-co-AA (FIG. 1)

Poly-EGDMA-co-AA was prepared by following the protocol in Example 2 except an additional monomer is added in the first step (AA) and a mixture of two porogens is used instead of a single porogen. More specifically, EGDMA (75%) and acrylic acid (AA, 25%) were copolymerized in a mixture of good porogen (CHCl_3) and poor porogen (MeOH).

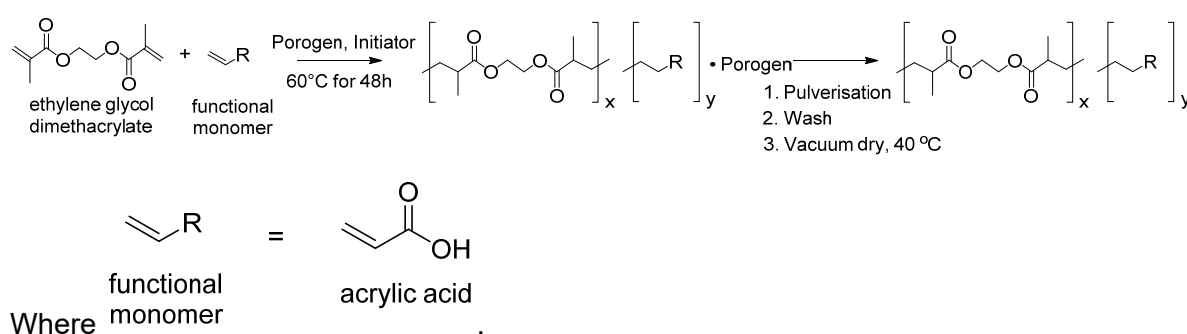


Table 10. Synthetic method for 3A-3E.

Sample	Crosslinker / Monomer / Initiator	Porogen	Washing Steps	Yield / Intermediate product
3A	EGDMA 9mmol AA 3mmol ACVA 0.11mmol	6mL MeOH	1. 0.1 M H_2SO_4 2-5. DI H_2O 6. EtOH	1.51 g White, free-flowing powder
3B	EGDMA 9mmol AA 3mmol ACVA 0.11mmol	5mL MeOH 1mL CHCl_3	1. 0.1 M H_2SO_4 2-5. DI H_2O 6. EtOH	1.56 g Mixture of white powder / monolith
3C	EGDMA 9mmol AA 3mmol	4mL MeOH 2mL CHCl_3	1. 0.1 M H_2SO_4 2-5. DI H_2O 6. EtOH	1.89 g Mixture of white powder / monolith

	ACVA 0.11mmol			
3D	EGDMA 9mmol AA 3mmol ACVA 0.11mmol	3mL MeOH 3mL CHCl ₃	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	2.23 g White monolithic solid
3E	EGDMA 9mmol AA 3mmol AIBN 0.04mmol	6mL CHCl ₃	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	2.30 g White monolithic solid

The polymers prepared were taken for static adsorption test as described in Example 3.

Results and discussion

- 5 Based on outcome of 3A and 3E, EGDMA-AA had similar porogen affinity to pure EGDMA. Both encountered early phase separation in poor porogen, and vice versa.

Table 11. Static adsorption test of 3A-3E.

Sample	3A	3B	3C	3D	3E
Good Porogen %	0%	16.7%	33.3%	50%	100%
B2M Adsorbed (mg B2M/g sample) 3A-3D 50mg/25mL PD fluid (1110ug/mL) 3E: 25mg /10mL PD fluid (2304ug/mL)	2.07	9.39	339.44	406.41	883.08
Surface Properties					
Isotherm type / interpretation	Non-porous	Non-porous	Type II Macroporous	Type II Macroporous	Type II Macroporous
BET Surface Area: m ² /g	0.11	23.73	265.09	381.99	298.43

Single point total pore volume: cm ³ /g		0.01	0.36	0.56	0.70
BET Adsorption average pore size: Å		18.35	54.86	59.14	93.89
BJH Adsorption average pore size: Å		79.24	134.63	122.47	180.20
Bulk Properties					
Density	0.34	0.42	0.23	0.30	N/A
Porosity	0.00%	0.46%	8.29%	16.75%	N/A

Based on result 3C-3E, there appears to be a minimum requirement of ~30% good porogen to be present in order to produce an active polymer. From 3C, it appears that at least 8% porosity and 0.36 cm³/g total pore volume may be required for substantial B2M adsorption to occur.

Example 8. Effect of copolymerisation

We attempted to characterise the relationship between polymer functionalisation, polymer properties and B2M adsorption, and to expand on available intermolecular interactions beyond those provided by EGDMA. The hydrophobic and hydrophilic monomers are depicted in FIG. 11.

Table 12. Synthesis and static adsorption test.

Sample	Monomer / Initiator	Porogen	Washing Steps	Yield	B2M Test Protocol	B2M Adsorbed (µg/g)
4A	N/A AIBN 0.04mmol	6mL MeCN	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	1.74g	25mg / 10mL PD fluid (2304µg B2M /mL) 50mg / 100mL PD fluid (2116-2175µg B2M /mL)	787.3* 1907.0

4B	AA 3mmol AIBN 0.04mmol	6mL MeCN	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	1.88g	25mg / 10mL	576.9
4C	MAA 3mmol AIBN 0.04mmol	6mL MeCN	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	1.83g	25mg / 10mL	597.6
4D	HEMA 3mmol AIBN 0.04mmol	6mL MeCN	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	2.07g	25mg / 10mL	624.7
4E	IA 3mmol AIBN 0.04mmol	6mL MeCN	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	1.91g	25mg / 10mL	823.0*
4F	AU 3mmol AIBN 0.04mmol	6mL MeCN	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	1.80g	25mg / 10mL	848.2*
4G	TFMAA 3mmol AIBN 0.04mmol	6mL MeCN	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	1.95g	50mg/ 100mL	1533.2
4H	AAm 3mmol ACVA 0.11mmol	6mL MeCN	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	1.91g	25mg / 10mL	419.1
4J	DMAEA 3mmol ACVA 0.11mmol	6mL MeCN	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	2.70g	25mg / 10mL	330.2
4K	DA 3mmol ACVA 0.11mmol	6mL CHCl ₃	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O	2.39g	50mg/ 100mL	30.5

			6. EtOH			
4L	DMAPS 3mmol ACVA 0.11mmol	6mL DMSO	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	3.06g	50mg/ 100mL	517.7
4M	OA 3mmol ACVA 0.11mmol	6mL CHCl ₃	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	2.69g	50mg/ 100mL	77.7
4N	5-VT 3mmol ACVA 0.11mmol	6mL MeCN	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	2.24g	50mg/ 100mL	474.6
4O	1-VIM 3mmol ACVA 0.11mmol	6mL MeCN	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	2.25g	50mg/ 100mL	202.2
4P	4-VPY 3mmol ACVA 0.11mmol	6mL MeCN	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	2.22g	50mg/ 100mL	523.9
4Q	APMA 3mmol ACVA 0.11mmol	6mL DMF	1. 0.1 M H ₂ SO ₄ 2-5. DI H ₂ O 6. EtOH	2.44g	50mg/ 100mL	269.7

*100% clearance.

EGDMA (9 mmol, 75% monomer content) was successfully copolymerised in the presence of functional monomers (3 mmol, 25% monomer content). This provided capability to introduce additional functionality for different applications. Copolymers retained B2M adsorption behaviour to varying extent, but did not exceed capacity of patent polymer. Carboxylate, amine/amide and alcohol containing copolymers retained B2M activity to a certain extent. Best performance was observed for IA, AU and TFMAA. Inclusion C12 and C18 alkyl chains reduced adsorption capacity significantly.

Example 9. Equilibrium adsorption isotherm

Poly-EGDMA was prepared as described in Example 2 (MeCN porogen), and taken for static adsorption test as described in Example 3. Specifically, samples (mass = 82, 103, 128, and 151 mg) were incubated with 100 mL PD fluid (2135 μg B2M/L) for 2 h at 23 °C, 250 rpm. B2M adsorption was calculated as in Example 3.

Freundlich and Langmuir parameters

Langmuir parameters q_{max} and b were obtained by linear regression, by plotting $1/Q_{\text{eq}}$ vs $1/C_{\text{eq}}$, to yield a straight line with y-intercept $1/q_{\text{max}}$ and slope $1/(b \times q_{\text{max}})$.

Freundlich parameters K_F and $1/n$ were obtained by linear regression, by plotting $\log Q_{\text{eq}}$ vs $\log C_{\text{eq}}$ to yield a straight line with y-intercept $\log K_F$ and slope $1/n$.

Results and discussion

FIG. 12 depicts the equilibrium adsorption isotherm.

Table 13. Equilibrium adsorption isotherm results.

Material (g)	Total Volume (mL)	[B2M] $\mu\text{g/L}$ initial	$C_{\text{eq}} = [\text{B2M}]$ $\mu\text{g/L}$ final	$Q_{\text{eq}} = [\text{B2M}]$ adsorbed $\mu\text{g/g}$	Q_F ($\mu\text{g/g}$)	Q_L ($\mu\text{g/g}$)
0.151	100.0	2135.0	200.0	1282.1	1301.3	1283.7
0.128	100.0	2135.0	335.0	1408.7	1386.8	1407.6
0.103	100.0	2135.0	579.0	1508.5	1483.6	1497.9
0.082	100.0	2135.0	873.0	1533.6	1560.7	1543.7

Table 14. Summary of physical properties and isotherm parameters.

Summary of Physical Properties and Isotherm Parameters						
Sorbent	Freundlich			Langmuir		
	K_F	$1/n$	R^2	q_{max}	b	R^2
Poly-EGDMA	676.96	0.123	0.9473	1642.5	0.0179	0.9959

Example 10. Device Test

The B2M clearance by Poly-EGDMA in dynamic flow environment was evaluated.

Synthesis

Poly-EGDMA was prepared in the same manner as Sample 1B in Example 2 except at 60 g scale. Fine particles were removed by 3x sedimentation in EtOH (500 mL).

Cartridge test

- 5 A sorbent cartridge containing Poly-EGDMA was prepared and the B2M adsorption was evaluated.

10 Poly-EGDMA, ZP, HZO and CaCO₃ were packed into a reusable flash column. Spent PD fluid was heated to 37 °C and passed through the column, and 1 L samples were collected consecutively.

Pre and post B2M concentration were measured, along with volume cleared and pressure. B2M adsorption calculation:

$$\text{Mass B2M adsorbed} = \Sigma (C_{\text{pre}} - C_{\text{post}}) * V_{\text{sample}}$$

- 15 Where C_{pre} = starting concentration of B2M in µg/L, C_{post} = final concentration of B2M in µg/L, and V_{sample} = volume of collected PD fluid in L.

20 The device set up is shown in FIG. 13. As shown in FIG. 13, the device set up 1300 includes a pre sample 1301, an ex-vivo PD fluid (37 °C) 1302, a peristaltic pump 1303 (e.g. WT600F Intelligent Dispensing Peristaltic Pump), a pressure sensor 1304, a sorbent cartridge 1305, and a post sample 1306.

Table 15. Cartridge composition and test parameters.

Example	Poly-EGDMA	ZP	HZO	CaCO ₃	Flow Rate	Pressure	Treatment volume litre
	g	g	g	g	mL/min	mbar	
10	12	187	153	6	25	550-980	6.5 (1x Automated Peritoneal Dialysis (APD) *.)

* The volume of fluid purified by the cartridge consisted of the total spent dialysate from a single APD therapy.

Results and discussion

5 **Table 16.** Sample composition.

Sample #	Pre [B2M] µg/L	[B2M] µg/L	Volume (L)	Cumulative Volume (L)	B2M adsorbed µg	Cumulative B2M adsorbed µg
PRE	787		0.00	0.0	0	0
1	787	200	1.00	1.0	587	587
2	787	200	1.06	2.1	622	1209
3	787	200	1.02	3.1	599	1808
4	787	200	1.10	4.2	646	2454
5	787	200	1.00	5.2	587	3041
6	787	200	1.02	6.2	599	3639
7	787	200	0.34	6.5	200	3839

Cartridge test passed as B2M was reduced to undetectable level and pressure drop was < 1 bar. B2M clearance was >75% and at least 3839 µg.

10 **Example 11. Particle size**

The effect of particle size on B2M adsorption in static environment was evaluated.

Synthesis

15 Poly-EGDMA was prepared in the same manner as Sample 1B in Example 2 except at 60 g scale.

Particle size distribution analysis

20 The particle size distribution of pre-sieved material was analysed by laser diffraction using Horiba LA-960 Laser Scattering Particle Size Distribution Analyser via wet measurement method. The method parameters are listed in Table 17 below. After pulverization, washing and drying, the powder was sieved into four fractions.

Table 17. Laser scattering particle size distribution analyser LA-960 parameters.

Median size:	162.06665 (µm)	Mean size:	212.79991 (µm)
Transmittance (R):	82.1 (%)	St. Dev.:	183.1436 (µm)
Transmittance (B):	85.9 (%)	Geo. mean size:	131.5126 (µm)
Circulation speed:	5	Geo. St. Dev.:	3.0532 (µm)
Agitation speed:	2	Mode size:	366.8370 (µm)
Ultrasound:	01:32 (7)	D10:	26.97831 (µm)
Distribution base:	Volume	D90:	475.04153 (µm)
Material:	Poly-EGDMA	D50:	162.06665 (µm)
Refractive index (R):	1.510		

Static adsorption test

B2M adsorption capacity was determined in a similar manner to Example 3. Pre-B2M concentration: 2606 µg/L. 50 mg polymer/100 mL PD fluid.

5

Results and discussion

FIG. 14 depicts the particle size distribution data.

Table 18. Parameter and particle size.

Parameter	Particle size
D10	27.0
D50	162.1
D90	475.0

10

Table 19. Particle size and B2M adsorbed.

Sample	Fraction	B2M Adsorbed (µg /g)
7A	Unsieved	3073.3
7B	<106 µm	4827.1
7C	106-150 µm	3659.6
7D	150-212 µm	3137.8
7E	>212 µm	1225.6

Based on the D10 and D90 values of the parent material 7A, it would be fair to assume that the undersize fraction 7B is mostly 27-106 µm while the oversize fraction 7E is mostly 212-475 µm. All fractions isolated had significant B2M adsorption capacity. Capacity increased

15

dramatically as particle size range reduced from 7E to 7B. This makes sense as smaller particles have a higher amount of exposed surface area per unit mass, allowing for greater availability of surface adsorption sites.

5 Example 12. Hansen Solubility Parameter

We aimed to define what is a good porogen for Poly-EGDMA according to HSP theory and to calculate HSP range for good porogen.

10 Hansen Solubility Parameter (HSP) determination for good porogen

Firstly, the HSP of Poly-EGDMA was approximated by conducting Solubility Sphere Analysis according to the Hansen method. In brief:

- a. each porogen was assigned a score (1 or 0) based on the experimental outcome of Example 6 (good porogen = 1, bad porogen = 0);
- 15 b. a 3D spherical function was fitted to encompass the 2xD, P and H values of all porogens with good score, while excluding porogens with bad score;
- c. the radius of the sphere was optimized to smallest possible value while maintaining a goodness of fit (A_i) of 1; and
- d. the HSP of Poly-EGDMA was estimated to be the origin of the sphere (according to
- 20 Hansen method), with $D_{\text{polymer}} = 17.0$, $P_{\text{polymer}} = 9.7$ and $H_{\text{polymer}} = 4.9$. The radius of interaction for Poly-EGDMA was also estimated, where $R_0 = 9.1$.

Secondly, HSP of good porogen was estimated to be all porogens within a Hansen Distance (R_a) less than or equal to R_0 , where $R_a \leq R_0$, i.e., $R_a \leq 9.1$.

$$25 \quad R_a = \sqrt{4(D_{\text{solv}} - D_{\text{polymer}})^2 + (P_{\text{solv}} - P_{\text{polymer}})^2 + (H_{\text{solv}} + H_{\text{polymer}})^2}$$

Results and discussion

Table 20. HSP values and fitted dataset.

HSP values	Dispersion	Polar	H- bonding	R_0
EGDMA monomer	16.4	5.2	8.6	
poly-EGDMA (estimated)	17.0	9.7	4.9	9.1

<i>Fitted dataset</i>					<i>Porogen Score</i>		
Porogen	Dispersion	Polar	H-bonding	R _a	Experimental	Calculated	A _i
n-Hexane	14.9	0	0	11.6	0	0	1.000
Toluene	18	1.4	2	9.0	1	1	1.000
Chloroform	17.8	3.1	5.7	6.9	1	1	1.000
Acetone	15.5	10.4	7	3.7	1	1	1.000
Acetonitrile	15.3	18	6.1	9.0	1	1	1.000
Dimethylformamide	17.4	13.7	11.3	7.6	1	1	1.000
Dimethyl sulfoxide	18.4	16.4	10.2	9.0	1	1	1.000
1,4-Dioxane	17.5	1.8	9	9.0	1	1	1.000
Ethyl acetate	15.8	5.3	7.2	5.5	1	1	1.000
Methanol	14.7	12.3	22.3	18.2	0	0	1.000
Isopropanol	15.8	6.1	16.4	12.3	0	0	1.000
Ethanol	15.8	8.8	19.4	14.8	0	0	1.000
Water	15.5	16	42.3	38.1	0	0	1.000

D, P, H, R₀, R_a units = MPa^{1/2}

FIG. 15 depicts the Hansen Solubility Sphere (experimental). FIG. 16 depicts the Hansen Solubility Sphere (predicted).

5

Table 21. Predicted dataset.

<i>Predicted dataset</i>						<i>Porogen ranking</i>		
Porogen	Abbrev	Dispersion	Polar	H-bonding	R _a	R _{ED}	Exp	Pred
Benzene	BEN	18.4	0	2	10.5	1.2	N/A	0
Diethyl ether	Et ₂ O	14.5	2.9	4.6	8.5	0.9	N/A	1
Tetrahydrofuran	THF	16.8	5.7	8	5.1	0.6	N/A	1
Dichloromethane	DCM	17	7.3	7.1	3.3	0.4	N/A	1
n-Butanol	n-BuOH	16	5.7	15.8	11.8	1.3	N/A	0

n-Propanol	n-PrOH	16	6.8	17.4	13.0	1.4	N/A	0
Benzonitrile	PhCN	17.4	9	3.3	1.9	0.2	N/A	1
2-Butanone	MEK	16	9	5.1	2.1	0.2	N/A	1
2-Heptanone	MAK	16.2	5.7	4.1	4.4	0.5	N/A	1
Cyclohexanone	CH	17.8	6.3	5.1	3.8	0.4	N/A	1

Prediction of Good Porogen

The literature DPH value of untested porogen can be used to calculate R_a and relative energy difference (RED), where $RED = R_0/R_a$. For porogens with $RED \leq 1$, their DPH will lie within the solubility sphere and are thus likely to be good porogens. Conversely, porogen with $RED > 1$ are likely to be poor porogens.

Hansen Solubility Parameter

Hansen theory utilises the principle of “like dissolves like” to describe the interaction of compounds and solvents. Based on the experimental polymerization results, solvents were categorized as good or poor porogen for Poly-EGDMA. By fitting good porogens into and excluding poor porogens from a spherical function, the HSP of Poly-EGDMA was estimated to be $D = 17.0$, $P = 9.7$ and $H = 4.9$. This is somewhat different to the literature values published for the monomer ($D = 16.4$, $P = 5.2$, $H = 8.6$, T. Renkecz *et al.*, Molecular Imprinting **2014**, 2, 1-17).

The radius of interaction for Poly-EGDMA was also estimated, where $R_0 = 9.1$. The HSP of good porogen was estimated to be all porogens with a Hansen Distance (R_a) less than or equal to R_0 , where $R_a \leq R_0$, i.e. $R_a \leq 9.1$. Alternatively, porogens with $R_{ED} \leq 1$ are likely to be good porogens, and porogen with $R_{ED} > 1$ are likely to be poor porogens, where $R_{ED} = R_0/R_a$.

Table 22. Scope of the invention.

Parameter	Range	Supporting Examples	
Polymer Composition			
EGDMA	75-100%	75%: Example 8	100%: Examples 1-7

Initiator	AIBN/ACVA (Approx 1mol%)	AIBN: Example 6	ACVA: Example 2
Comonomer	25% Acrylic, allyl, vinyl monomers	Example 8	
Porogen volume: % (v/v)	70-82%	Example 7	
Porogen type:	Good porogen: Acetone, CHCl ₃ , MeCN, Toluene, Ethyl Acetate, etc.	Example 6	
	Good porogen in Hansen space: $R_a \leq 9.1$, where $D_{\text{polymer}} = 17.0$, $P_{\text{polymer}} = 9.7$ and $H_{\text{polymer}} = 4.9$	Example 12	
Surface properties			
BET Surface area (m ² /g)	265-640	265: Sample 3C	640: Sample 2L
BET Total pore volume: cm ³ /g	0.36-0.77	0.36: Sample 3C	0.77: Sample 2F
BET Adsorption average pore size: Å	36-94	36: Sample 1A	94: Sample 3E

BJH Adsorption average pore size: Å	54-180	54: Sample 1A	180: Sample 3E
Bulk properties			
Porosity: % (v/v)	8.3-28	8.3: Sample 3C	28: Sample 3D
Particle size: mm	27-475	Samples 7B-7E	

Therefore, our sorbent is novel in terms of chemical structure and microstructure, using a combination of desired pore size, porosity and hydrophilic/hydrophobic interactions to trap and adsorb B2M.

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Tailored pore size range: B2M adopts a globular structure in solution, with a reported hydrodynamic radius of 1.6 nm.

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Poly-EGDMA is a highly porous material. Through the bulk polymerisation process in presence of a porogen, the polymer precipitates in the form of irregular shaped nodules, which further crosslink and join up to form conglomerates. The porogen forms a separate phase in between conglomerates, leading to the formation of pores and eventual construction a highly porous network.

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- Micropores & small mesopores – size selectivity and B2M sieving effect
- Macropores – efficient fluid transport channel into microparticle

The unique pore size distribution of our material may contribute to the high selectivity and adsorption capacity for B2M.

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As such, in the present disclosure, we have surprisingly generated a material with high selectivity, without presence of template and functional monomer. This is advantageous as it would be very difficult to introduce B2M as a template molecule for a number of reasons.

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1. Expensive cost of B2M.
2. Insolubility and instability of B2M in organic porogens.
3. Difficulty in removing B2M template to generate the cavities. The lack of necessity for a functional monomer simplifies and speeds up the entire synthetic process.

Comparative Example 1

Sorbent-based devices have been previously developed to aid the removal of additional B2M in hemodialysis. The devices are hemoperfusion cartridges, which are intended to be placed directly in the blood line of the HD circuit, either before or after the dialyser. Most concepts contain porous polymeric beads. Adsorption of B2M typically takes place by sieving/size-exclusion effect through a specific pore size, and through intermolecular forces such as hydrophobic interactions.

For example, Kaneka Corporation (Japan) has developed Lixelle (EP0647470 and Suzuki, K., Shimazaki, M. and Kutsuki, H., *Ther. Apher. Dial.*, **2003**, 7, 104-107), an FDA approved hemosorbent product for the treatment of DRA. Cytosorbents have also developed a corresponding product (WO1999006098) which has undergone clinical trial, but not commercialised.

Although the addition of hemoperfusion column enhanced B2M removal by 1.5-2x, numerous limitations affect the clinical acceptability and commercialisability of these solutions.

- **Cost:** Lixelle is only available in Japan, reportedly due to high cost of the device. Cytosorb's product is not available in the market, 20 years after research was published.

- **Biocompatibility:**

- Several adverse effects were reported during Lixelle clinical trials, including anemia and hypotension. These are associated with the large extracorporeal volume of the device (150-350 mL).
- Leucocyte activation was also indicated in some users, associated with bioincompatibility.
- There is limited data available on Betasorb/cytosorb device.

- **Adsorption capacity:**

- Lixelle appears to have insufficient B2M capacity for present application, since extracorporeal volume is large enough to cause adverse effects.
- There is limited data available on Cytosorb device. Davankov research paper (Davankov, V., *et al.*, *J. Chromatogr. B: Biomed. Sci. Appl.*, **2000**, 739, 73-80) indicates similar adsorption capacity to Lixelle in serum.

- **Applicability to Sorbent PD:**

- From Lixelle research paper (S. Furuyoshi *et al.*, *Ther. Apher.* **1998**, 2, 13-17), B2M capacity appears very low when B2M concentration is low.

- This may cause complications in the use of this sorbent in sorbent PD, where B2M level in dialysate is 10-20x less than serum.

Sabic (WO2018211389A1) and Tharpa *et al.* (K. Tharpa *et al.*, *J. Artif. Organs* **2020**, 23, 47-53) disclose the preparation of a family urea-MIPs for dialysis purposes, by polymerisation of EGDMA and other crosslinkers, in the presence of hydrophilic monomers and urea. Thus, all of these products contain a comonomer, which suggest complicated synthetic processes.

In contrast, in our process, the bulk polymerisation takes place in a single step. The two main reagents (EGDMA and initiator) are used up in the polymerisation process, essentially reaching quantitative yield, after which only the porogen needs to be removed. The resultant polymer can be ground and sieved to any preferred size or particle size distribution.

In the case of Lixelle beads, their product first requires preparation of crosslinked cellulose beads with correct pore size distribution and particle size. Then, several chemical reaction steps are required for the linking of the hydrophobic ligand. With hexadecylamine for example, typically the cellulose surface would need to be activated with sodium hydroxide, followed by epoxidation and covalent coupling via amide bond.

In the case of cytosorbents (PCT/US1998/016118 and Davankov, V., *et al.*, *J. Chromatogr. B: Biomed. Sci. Appl.*, **2000**, 739, 73-80), the invention involves formation of PS-DVB beads with hydrophilic coating. The synthesis of DVB beads is a comparatively lengthy process, requiring a more complex form of polymerisation such as emulsion or suspension polymerisation. These processes require addition of surfactant or stabilising agent, with many subsequent washing steps needed to remove the agent.

Comparative Example 2

Poly-EGDMA is a highly micro/mesoporous material, with a high proportion of large micropores and small mesopores, with an average pore size of 3.6-9.4 nm. These smaller pores are believed to provide size selectivity and B2M sieving effect. A small number of macropores present may allow for better fluid penetration into the interior of the particle, allowing for efficient mass transfer.

In contrast, other sorbents appear not to have not given priority to pore size (Lixelle, Kaneka Corporation (Japan)) or have focused on larger average pore size (Cytosorbents: 15-26 nm).

Comparative Example 3

Integrated binding sites within Polymer Matrix

B2M is a polypeptide is made up of 99 amino acid residues. B2M has both hydrophobic and hydrophilic residues on its surface. Literature simulations have shown that B2M binds quickly and irreversibly to hydrophobic surfaces via a 2-step process:

- initial adsorption by polar and charged groups; and
- partial unfolding occurs, maximizing adhesion of the protein to the hydrophobic surface (accentuated interaction of hydrophobic groups).

Poly-EGDMA is hydrophobic overall, based on EGDMA molecular properties ($\log P = 2.8$, lipophilic > 0). Ester groups may serve as polar groups for initial adsorption step, while alkyl groups may provide hydrophobicity required for irreversible binding. The enhanced binding capacity of Poly-EGDMA may be due to utilisation of the entire polymer matrix for surface adsorption, whereby a larger surface area is available for B2M adsorption, allowing for more efficient and effective adsorption. The structure may allow for higher density of binding sites, compared to a design such as Lixelle, where only surface ligands are available for protein binding. This leads to increased binding affinity, as multiple interactions can occur simultaneously between B2M and the polymer matrix, enhancing overall binding strength.

Comparative Example 4

No inactive carrier material

In the case of Poly-EGDMA, the polymer matrix is the adsorbent. There is no need for an inactive solid support as in the cellulose beads used in Lixelle sorbent. This avoids wastage of space and enables manufacturing of smaller and lighter device.

Comparative Example 5

When considering the suitability of sorbent materials for a sorbent cartridge device in dynamic flow applications, the behavior and adsorption characteristics play a crucial role. Poly-EGDMA demonstrates rapid uptake and reaches saturation adsorption even at low B2M concentrations, indicating a strong affinity and efficient adsorption relationship with B2M. Therefore, Poly-EGDMA has high capacity and affinity for B2M.

In contrast, Lixelle beads exhibit a linear relationship between solute concentration and the quantity adsorbed, suggesting a weaker affinity or limited adsorption capacity even at high

serum B2M concentrations. Further, large amount of beads are required to achieve significant binding and thus, they are likely to be ineffective for sorbent dialysis application where dialysate [B2M] is 1/60th of serum level.

5 **Table 23.** Comparative data.

	Kaneka Corp (S. Furuyoshi et al., <i>Ther. Apher.</i> 1998, 2, 13-17)	Akizawa, T., et al., <i>ASAIO Journal</i>, 1987, 33, 532-537	Sorbent in the present invention
Product	Hexadecylamine cellulose beads	Methacrylic acid (MMA)-DVB beads	Poly-EGDMA powder
Solution Type	Serum	Serum	PD Fluid
Initial B2M	10 mg/L	20 mg/L	2.1 mg/L
Sorbent to Solution Ratio (lower ratio is better)	1 mL beads : 36 mL serum	1 mL beads : 12 mL serum	1 g powder : 2000 mL PD fluid
B2M Adsorbed	≈150 µg/mL	≈ 200 µg/mL	2232.2 µg/g (Sample 1A)

Therefore, we have disclosed a porous polymeric sorbent based on crosslinked Poly-EGDMA, which unexpectedly has high adsorption capacity and selectivity for B2M, without templating. In comparison with the prior art, it can be produced cheaply, in addition to high chemical and physical stability. Additionally, new functionalities such as hydrophilic moieties for improved biocompatibility can be easily incorporated into the synthesis and final product.

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Claims

1. Use of a particulate porous material to adsorb beta-2 microglobulin, said particulate porous material comprising a crosslinked polymeric matrix including a first set of constitutional units formed from ethylene glycol dimethacrylate monomers

wherein:

the particulate porous material comprises a plurality of pores having a pore diameter of from 1.6 nm to 50 nm;

the particulate porous material has a porosity of at least 3% (v/v) and a single point total pore volume of at least 0.15 cm³/g; and

wherein the crosslinked polymeric matrix is a homopolymer consisting of the first set of constitutional units or the crosslinked polymeric matrix is a copolymer consisting of the first set of constitutional units and up to 30 mol % of a second set of constitutional units formed from a hydrophilic monomer.

2. The use according to Claim 1, wherein:

(a) the particulate porous material has a Brunauer-Emmett-Teller adsorption average pore size of from 3 to 15 nm, such as from 3.5 to 10 nm (e.g. 3.6 to 9.4 nm); and/or

(b) the particulate porous material has a porosity of at least 6% (v/v) and a single point total pore volume of at least 0.3 cm³/g.

3. The use according to Claim 1 or 2, wherein: (a) the hydrophilic monomer is selected from one or more of the group consisting of itaconic acid, 2-(trifluoromethyl)acrylic acid, and allylurea, and/or

(b) the second set of constitutional units are present in an amount of from 2 to 25 mol % of the crosslinked polymeric matrix.

4. The use according to any one of the preceding claims, wherein the particulate porous material has a porosity of from 8 to 30% (v/v), such as from 10 to 28% (v/v) (e.g. from 14 to 24% (v/v)).

5. The use according to any one of the preceding claims, wherein the particulate porous material has a single point total pore volume of from 0.36 to 0.85 cm³/g, such as from 0.4 to 0.8 cm³/g (e.g. from 0.45 to 0.75 cm³/g).

6. The use according to any one of the preceding claims, wherein the particulate porous material has a porosity of from 8 to 30% (v/v) and a single point total pore volume of from 0.36 to 0.85 cm³/g;

optionally a porosity of from 10 to 28% (v/v) and a single point total pore volume of from 0.4 to 0.8 cm³/g.

7. The use according to any one of the preceding claims, wherein the particulate porous material comprises a plurality of pores having a pore diameter of from 1.6 nm to 10 nm.

8. The use according to any one of the preceding claims, wherein the particulate porous material has a nitrogen adsorption isotherm that is Type II or Type IV.

9. The use according to any one of the preceding claims, wherein the particulate porous material has a D50 of less than 200 µm.

10. The use according to any one of the preceding claims, wherein the particulate porous material is contained within a sorbent cartridge.

11. The use according Claim 10, wherein the sorbent cartridge further contains one or more of:

cation exchange particles (e.g. zirconium phosphate);
anion exchange particles (e.g. hydrous zirconium oxide);
activated carbon;
a buffer (e.g. calcium carbonate); and
a urease.