SORBENTS

FIELD OF INVENTION

The present invention relates to an activated carbon adsorbent for oral administration and compositions comprising the activated carbon adsorbent. The activated carbon adsorbent and compositions of the invention find utility in the treatment of a renal disease or a liver disease. The present invention also relates to an *in vitro* method of removing one or more uremic toxins from a fluid, and a method of manufacturing the activated carbon adsorbent of the present invention.

BACKGROUND

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.

Chronic kidney disease (CKD) is a significant global health issue, characterized by kidney damage or a glomerular filtration rate (GFR) below 60 mL/min per 1.73 m^2 for more than three months. By 2030, it is estimated that 5.4 million people will require dialysis, underscoring the importance of early CKD management and risk factors such as high blood pressure, diabetes, and proteinuria control before leading to end-stage renal disease (ESRD). CKD is associated with an increased risk of cardiovascular events, hospitalization, and mortality.

A meta-analysis across 40 countries and regions revealed an overall prevalence of CKD stages 3-5 of 6.3%. The ESRD has been on the rise, with approximately 1870 cases per million population reported in 2010 compared to 1355 per million in 2000, highlighting the urgency for treatments to slow or prevent CKD progression. Early management of CKD is crucial to reduce cardiovascular events, improve quality of life, and prolong survival. Current guidelines emphasize managing factors like hypertension and diabetes, although many patients still require dialysis or transplantation.

Decreased GFR in CKD is associated with elevated levels of uremic toxins such as creatinine (Cr), hippuric acid (HA) and protein bound uremic toxin (PBUT) like indoxyl sulfate (IS) and p-cresyl sulfate (PCS) in plasma and tissues, which have been linked to increased morbidity, mortality and cardiovascular disease, supported by both animal and human studies. IS is synthesized in the liver from indole, a tryptophan metabolite produced by intestinal bacteria, such as *E. coli*. In CKD patients with serum creatinine (sCr) levels of 3.0 mg/dL or higher, serum IS (sIS) levels are often elevated, typically exceeding 0.8–1.0 mg/dL. The accumulation of these toxins in blood circulation in CKD patients appears to accelerate disease progression by involving multiple mechanisms, including oxidative stress, stimulation of pro-inflammatory

and/or pro-fibrotic factors, endoplasmic reticulum stress, increased oxygen consumption in proximal tubules, and disruption of tubular hypoxic response.

Coronary artery calcification (CAC) in CKD accelerates arterial stiffness and compromises coronary flow reserve, making it a robust predictor of cardiovascular events and mortality compared to other subclinical atherosclerosis indicators. The association between uremic toxins, such as IS, and vascular calcification suggests that reducing IS levels could potentially prevent CAC progression. Additionally, CKD and ESRD patients commonly experience sarcopenia, characterized by muscle loss and weakness, particularly as kidney function declines. This muscle wasting is exacerbated by chronic inflammation associated with CKD, leading to reduced exercise capacity and progressive weight loss. Uremic toxins like IS contribute to muscle wasting by entering muscle cells through specific transporters, inducing oxidative stress and inflammation, while also down-regulating Klotho expression, a protein crucial for maintaining muscle health.

In renal care, activated carbon powder (ACP) and activated carbon beads (ACB) have emerged as prominent adsorbents, albeit with limited success. Conventional ACP, typically derived from coal, lignite, peat, petroleum residue, or wood, poses challenges due to its high cost and non-renewable nature, leading to significant environmental impacts. To address these concerns, researchers have compared the environmental impacts of alternative biomass-derived activated carbons with conventional ACP using life cycle assessment (LCA) methodologies (Hjaila *et al*., 2013, Journal of environmental management, 130, pp.242-247; Joseph *et al*., 2020, Environmental Research Letters, 15(6), p.064023; Kim *et al*., 2019, Environmental Engineering Research, 24(1), pp.117-126; Loya-González *et al*., 2019, Journal of cleaner production, 219, pp.316-325; Sepúlveda-Cervantes *et al*., 2018, Waste Management & Research, 36(2), pp.121-130).

Utilizing the CML 2001 characterization method, potential environmental impacts were assessed across 12 categories, revealing that direct emissions from ACP and electricity production were primary contributors (Vilén *et al*., 2022, *Journal of Environmental Management*, *324*, p.116356). Coal-based ACP exhibited the highest impact across ten of the twelve categories, with its Global Warming Potential (GWP) further exacerbated by biogenic carbon emissions. Despite environmental concerns, the suitability of alternative activated carbons for dialysis remains limited due to factors such as inadequate pore sizes for effective removal of both small and medium size toxins. Moreover, conventional ACP presents its own set of limitations, including issues related to morphology, size, and porosity. The tight packing induced by minute and irregular particle size also impedes its application as a sorbent for sorbent-based dialysis due to high back pressure.

Thus, there remains a need for improved and/or alternative sorbents suitable for use as oral sorbents and as sorbents in sorbent-based dialysis.

SUMMARY OF INVENTION

The present invention provides an activated carbon adsorbent for oral administration, wherein the activated carbon adsorbent is in the form of particles obtained by carbonization and subsequent activation of poly(styrene-co-divinylbenzene) particles, wherein the poly(styrene-co-divinylbenzene) particles are macroporous and have a moisture content of from about 5 wt% to about 60 wt% relative to the total mass of the poly(styrene-co-divinylbenzene) particles.

As described herein, the present inventors have found that carbonization and activation of poly(styrene-co-divinylbenzene) particles that are macroporous and have a moisture content of from about 5 wt% to about 60 wt% provides an activated carbon adsorbent that is surprisingly suitable for use as an oral adsorbent, which also has utility as an adsorbent for sorbent-based dialysis. For example, the present inventors found that the resulting activated carbon adsorbent, when used in a 50 g flash column, was surprisingly effective at adsorbing creatinine and β2-microglobulin (B2M). The activated carbon adsorbent also displayed superior adsorption of creatinine, p-cresyl sulfate (PCS), indoxyl sulfate (IS), and hippuric acid (HA) under conditions that simulate the GI tract compared to AST-120, which is approved as an oral sorbent for dialysis patients. The activated carbon adsorbent was also found to adsorb indole under conditions that simulate the GI tract.

The present invention also provides an activated carbon adsorbent for oral administration, wherein the activated carbon adsorbent is in the form of particles, comprises micropores and mesopores, and has a total surface area of at least about 1000 m^2/g and a total pore volume of at least about 1 cm^3/g, wherein at least about 50% of the total pore volume is formed by the micropores, and at least about 20% of the total pore volume is formed by the mesopores. When the activated carbon adsorbent of the present invention displays this specific combination of surface area, total pore volume, and pore size distribution, the activated carbon adsorbent is surprisingly effective at adsorbing both creatinine and B2M.

The present invention also provides a pharmaceutical composition comprising the activated carbon adsorbent of the present invention and a pharmaceutically acceptable binder, carrier or excipient.

The present invention also provides a use of the activated carbon adsorbent of the invention in the manufacture of a medicament for the treatment of a renal disease or a liver disease.

The present invention also provides a sorbent cartridge containing the activated carbon adsorbent of the invention, and an *in vitro* method of removing one or more uremic toxins from

a fluid, the method comprising providing a fluid comprising one or more uremic toxins, and contacting the fluid with the activated carbon adsorbent of the invention.

Also provided herein is a method of manufacturing the activated carbon adsorbent of the present invention, the method comprising:

a) providing poly(styrene-co-divinylbenzene) particles;
b) subjecting the poly(styrene-co-divinylbenzene) particles to a carbonization process comprising increasing the temperature of the poly(styrene-co-divinylbenzene) particles from about room temperature to about 900 °C over about 6 hours under an inert atmosphere, thereby providing a carbonized intermediate product; and
c) subjecting the carbonized intermediate product obtained from step (b) to an activation process comprising heating the carbonized intermediate product at a temperature of about 900 °C for 10 h under CO_2 gas flow, thereby providing the activated carbon adsorbent of the present invention.

Preferred but optional features are set out in the dependent claims. Additional aspects and embodiments of the activated carbon adsorbent, compositions, sorbent cartridge, methods and uses of the present invention will be apparent from the following description, figures and claims. As can be appreciated from the foregoing and following description, each and every feature described herein, and each and every combination of two or more such features, is included within the scope of the present disclosure provided that the features included in such a combination are not mutually inconsistent. In addition, any feature or combination of features may be specifically excluded from any embodiment.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 shows a graph of the thermogravimetric analysis (TGA) generated from screening various cationic and anionic polymer resins following carbonization.

Fig. 2 shows the adsorption efficiency of ACBs (Batch 36 and 39) of the present invention for creatinine and B2M. A) Structures of creatinine and B2M. B) Description of full-scale 50 g column for C) Creatinine and D) B2M.

Fig. 3 shows field emission scanning electron microscopy (FESEM) images of Batch 36 showing the size, shape and porosity of the inner and outer surface of the ACB.

Fig. 4 shows transmission electron microscopy (TEM) images for surface morphology comparison of reference samples (Calgon ACP and AST-120) with ACB (Batch 36 and 39) of the invention. A) Calgon ACP. B) AST-120. C) Batch 36. D) Batch 39.

Fig. 5 shows the pore size distribution graph for poly(styrene-co-divinylbenzene) particles, reference samples, and ACBs of the present invention. A) Amberlite HPR-2900H; B) Calgon ACP; C) AST-120; D) Batch 36; and E) Batch 39.

Fig. 6 shows the results from preliminary studies for ACBs (Batch 36) of the present invention for creatinine adsorption at various concentrations. Each sample contained 300 mg ACB in 20 mL of deionized (DI) water, and was mixed at 250 rpm at room temperature (RT) for 2 h.

Fig. 7 shows concentration-dependent creatinine adsorption in gut simulation by the ACBs (Batch 36) of the present invention. A). Description of experimental conditions and creatinine adsorption after 6 h. Each sample contained 1 g ACB in synthetic dialysate (SD) containing creatinine (14.2-87.4 mmol), and was mixed at 250 rpm at RT for 6 h. B) Adsorption graph.

Fig. 8 shows the adsorption of creatinine by ACBs (Batch 36) of the present invention in gut simulation over time. A) Each sample contained 1 g ACB in synthetic dialysate (SD) containing creatinine (14.2-87.4 mmol), and was mixed at 250 rpm at RT for 2-24 h. B) Graph showing creatinine adsorption by the ACBs over the 2-24 h period. C) Graph showing the % increase in creatinine adsorption over time.

Fig. 9 shows the effect of pH of reaction mixture on creatinine adsorption in gut simulation by the ACBs (Batch 36) of the present invention. A) Description of experimental conditions. Each sample contained 1 g ACB and creatinine (79.9-80.4 mmol) in SD, and was mixed at 250 rpm at RT for 1-6 h. B) Graph showing the adsorption of creatinine by the ACBs at pH 1, 3 or 5.

Fig. 10 shows a comparison of the adsorption of creatinine by AST-120, ACBs (Batch 36) of the present invention, and Ambersorb 560. Each sample contained 1 g ACB (AST-120, Batch 36, or Ambersorb 560) and 300 mg creatinine in 1 L DI water, and was mixed at 250 rpm at RT for 1-6 h.

Fig. 11 depicts the adsorption of uremic toxins by ACBs (Batch 36) of the present invention. A) Chemical structures of indoxyl sulfate (IS), *p*-cresyl sulfate (PCS) and hippuric acid (HA). B) Table with experimental condition, and the amount of each toxin adsorbed by the ACBs. Each sample contained a set amount of the ACB and 20 mg toxin in 20 mL DI water (simulated to 1000 mg/L), and was mixed at 250 rpm at RT for 2 h across a pH range of 7 to 1.

Fig. 12 shows the adsorption of a uremic toxin mixture by ACBs (Batch 36) of the present invention. Each sample contained 1 g ACB and 40 mg of each of creatinine, IS, PCS and HA in 500 mL DI water at a pH 7.2, and was mixed at 250 rpm at RT for 2 h.

Fig. 13 shows the adsorption of uremic toxin mixture by ACBs (Batch 36) of the present invention at pH 3.3 or 7.2. Each sample contained 1 g ACB, 300 mg creatinine, and 40 mg

each of IS, PCS and HA in 500 mL DI water, each sample was mixed at 250 rpm at RT for 2 h. A) Adsorption of creatinine. B) Adsorption of mixture of IS, PCS and HA.

Fig. 14 shows a comparison of the adsorption of uremic toxin mixture by ACBs (Batch 36) of the present invention and AST-120. Each sample contained 1 g of ACB (Batch 36 or AST-120), 300 mg creatinine, and; 40 mg each of IS, PCS and HA in 500 mL DI water, and each sample was mixed at 250 rpm at RT for 2 h. Each sample has a pH of 7.2. A) Adsorption of creatinine. B) Adsorption of toxin mixture of IS, PCS and HA.

Fig. 15 shows the adsorption of toxin precursor indole by ACBs (Batch 36) of the present invention. Each sample contained 1 g ACB and a set amount of indole (i.e. 1) 40 mg indole; 2) 300 mg of indole; and 3) 460 mg of indole) in 500 mL DI water, and was mixed at 250 rpm at RT for 2 h. Each sample has a pH of 7.2.

DESCRIPTION

As described below in detail, the present inventors have developed an activated carbon adsorbent that is suitable for oral administration and is surprisingly effective at adsorbing a wide range of toxins, in particular uremic toxins such as creatinine, PCS, IS, HA, and B2M. The activated carbon adsorbent is also surprisingly effective at adsorbing uremic toxin precursors, such as indole. The adsorption properties, together with the physical properties of the activated carbon adsorbent, make the activated carbon adsorbent particularly suitable for use as an oral sorbent for treating renal and/or liver diseases. In certain embodiments, the activated carbon adsorbent of the invention is also suitable for use in an *in vitro* method of removing uremic toxins from a fluid, such as waste dialysis fluid.

The activated carbon adsorbent of the present invention is in the form of particles. As such, the activated carbon adsorbent of the present invention may also be referred to herein using the terms “activated carbon beads” or “ACBs”.

As used herein, the terms “toxin” or “toxins” refer to organic compounds which accumulate in the bloodstream and cannot be eliminated from the body. The terms encompass uremic toxins and protein bound uremic toxins (PBUT). The term “uremic toxins’ includes, but is not limited to, urea, uric acid, creatinine, PCS, IS, B2M, and inorganic phosphate. 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-II (major histocompatibility-II 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*., Kidney, 360, 2020, 1, 1447-1455).

The term “toxin precursor” as used herein refers to a substance that can be transformed into a toxic compound by the body. An example of a toxin precursor is indole, which is a precursor to indoxyl sulfate. Indole is generated from tryptophan, a natural amino acid sourced from protein diets, and metabolized by intestinal bacteria, such as *E. coli*, in the gut. Subsequently, it is further metabolized into indoxyl sulfate in the liver, transferred into the bloodstream, and excreted by the kidneys in healthy individuals.

The word “comprising” as used herein 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 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.

The term “about” as used herein can allow for a degree of variability in a value or range, for example, within 10%, within 5%, within 1%, within 0.5%, within 0.1%, within 0.05%, within 0.01%, within 0.005%, or within 0.001% of a stated value or of a stated limit of a range, and includes the exact stated value or range.

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.

As disclosed herein, the activated carbon adsorbent of the present invention is obtained by carbonization and subsequent activation of poly(styrene-co-divinylbenzene) particles. Carbonization is the process of converting a carbonaceous starting polymer to carbon. That is to say that the carbonaceous starting material is carbonized. Carbonization is also know by the term “pyrolysis”. Typically, the carbonization is carried out under an inert atmosphere (for example nitrogen or argon). Typically, carbonization is carried out by increasing the temperature of the poly(styrene-co-divinylbenzene) particles defined herein from room temperature (i.e. about 20 °C to about 25 °C) to a temperature of about 900 °C under an inert atmosphere. The total time for the carbonization is usually from about 30 minutes to about 6 hours. In certain embodiments, carbonization of the poly(styrene-co-divinylbenzene) particles is carried out by increasing the temperature of the particles from room temperature (i.e. about 20 °C to about 25 °C) to a temperature of about 900 °C over about 6 hours under an inert atmosphere.

The term “inert atmosphere” as used herein refers to an environment where the concentration of reactive gases, such as oxygen and moisture, is reduced or eliminated. Typically, this is achieved by introducing inert gases like nitrogen, argon, or carbon dioxide into a sealed or controlled environment. The poly(styrene-co-divinylbenzene) particles disclosed herein may undergo carbonization under any suitable inert atmosphere, for example under a nitrogen atmosphere or an argon atmosphere. For example, the poly(styrene-co-divinylbenzene) particles may undergo carbonization by increasing the temperature of the poly(styrene-co-divinylbenzene) particles from room temperature (i.e. about 20 °C to about 25 °C) to a temperature of about 900 °C over about 6 hours under an argon atmosphere.

Following carbonization, the carbonized intermediate product is subjected to an activation process, to produce the activated carbon adsorbent of the present invention. The activation process partially oxidizes and degrades the carbon structure formed during carbonization, creating a network of pores within the carbon. As a result, activation also causes some loss of material from the carbonized intermediate product. Typically, activation is carried out at a temperature of about 900 °C for 10 h under CO_2 gas flow.

Thus, also provided herein is a method of manufacturing the activated carbon adsorbent of the present invention, the method comprising:

a) providing poly(styrene-co-divinylbenzene) particles disclosed herein;
b) subjecting the poly(styrene-co-divinylbenzene) particles to a carbonization process comprising increasing the temperature of the poly(styrene-co-divinylbenzene) particles from about room temperature to about 900 °C over about 6 hours under an inert atmosphere, thereby providing a carbonized intermediate product; and

c) subjecting the carbonized intermediate product obtained from step (b) to an activation process comprising heating the carbonized poly(styrene-co-divinylbenzene) particles at a temperature of about 900 °C for 10 h under CO_2 gas flow, thereby proving the activated carbon adsorbent of the present invention.

In embodiments, the poly(styrene-co-divinylbenzene) particles subjected to carbonization and activation are macroporous and have a moisture content of from about 5 wt% to about 60 wt% relative to the total mass of the poly(styrene-co-divinylbenzene) particles. It has been found that the water content of the poly(styrene-co-divinylbenzene) particles, which are subject to carbonization and activation, affects the adsorption properties of the resulting activated activated carbon adsorbent, in particular its ability to absorb B2M. As demonstrated in the Examples disclosed herein, carbonization and activation of poly(styrene-co-divinylbenzene) particles with a moisture content of from about 40 wt% to about 60 wt% (e.g. about 50 wt%) provided an activated carbon adsorbent that displayed superior B2M adsorption compared to activated carbon adsorbent prepared using poly(styrene-co-divinylbenzene) particles with a lower water content. Thus, in certain embodiments, the poly(styrene-co-divinylbenzene) particles have a moisture content of from about 40 wt% to about 60 wt% (e.g. about 50 wt%) relative to the total mass of the poly(styrene-co-divinylbenzene) particles.

The term “moisture content” as used herein refers to the total amount of water in a material relative to the total mass of the material. The moisture content may be expressed as a wt% relative to the total mass of the material. Thus, for example, poly(styrene-co-divinylbenzene) particles with a moisture content of about 5 wt% may contain about 5 g of water in every 100 g of the poly(styrene-co-divinylbenzene) particles.

The term “macroporous” as used herein refers to a porous material that comprises predominantly macropores, wherein a macropore is a pore with a size of greater than 50 nm. In this context, the term “predominately” as used herein means that greater than 50% of the total pore volume of the porous material is formed by macropores.

The poly(styrene-co-divinylbenzene) particles may also have an average particle size of from about 400 µm to about 800 µm, for example from about 500 µm to about 650 µm, for example about 575 µm. By using poly(styrene-co-divinylbenzene) particles with this size, the resulting activated carbon adsorbent may have a particle size that is particularly suitable for use as an oral sorbent and a sorbent for sorbent-based dialysis.

In certain embodiments, the poly(styrene-co-divinylbenzene) particles comprise poly(styrene-co-divinylbenzene) with a degree of crosslinking of greater than about 80%. For example, greater than about 85%, greater than about 90%, or greater than about 95%. As used herein, the term “degree of crosslinking” refers to the percentage of the poly(styrene-co-

divinylbenzene) chains that are connected by crosslinks formed by the divinylbenzene component of the polymer. Carbonization and activation of poly(styrene-co-divinylbenzene) with a degree of crosslinking of greater than about 80% has been found to be particularly effective at producing an activated carbon adsorbent that is suitable for use as an oral sorbent and a sorbent for sorbent-based dialysis. The poly(styrene-co-divinylbenzene) particles may comprise poly(styrene-co-divinylbenzene) that is functionalized by an acidic or basic group. For example, the poly(styrene-co-divinylbenzene) particles described herein may comprise poly(styrene-co-divinylbenzene) that is sulfonated.

In certain embodiments, the poly(styrene-co-divinylbenzene) particles, which are subjected to carbonization and activation, have a total pore volume of at least about 0.4 cm^3/g when in a dry form. For example, the poly(styrene-co-divinylbenzene) particles when in a dry form may have a total pore volume of from about 0.4 cm^3/g to about 0.6 cm^3/g.

The term “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. Methods for determining the total pore volume of a material are known in the art. For example, the total pore volumes disclosed herein may be determined from a single point of an N_2 adsorption isotherm at 77.3 K with a saturation pressure of p/p0>0.99, using the Gurvich rule (Rouquerol, J., Rouquerol, F., Llewellyn, P., Maurin, G. and Sing, K., 2013. Adsorption by powders and porous solids: principles, methodology and applications. Academic press).

In certain embodiments, the poly(styrene-co-divinylbenzene) particles have a total pore volume of at least about 0.4 cm^3/g when in a dry form, wherein at least 50% of the total pore volume is formed by the mesopores, less than about 30% of the total pore volume is formed by the micropores, and less than 20% of the total pore volume is formed by the macropores. Without wishing to be bound by theory, the present inventors believe that the pore size distribution of the poly(styrene-co-divinylbenzene) particles affects the pore size distribution of the resulting activated carbon adsorbent following carbonization and activation, thus enabling the provision of an activated carbon adsorbent with desirable uremic toxin adsorption characteristics. In certain embodiments, the poly(styrene-co-divinylbenzene) particles have a total pore volume of at least about 0.4 cm^3/g when in a dry form, wherein from about 50% to about 70% of the total pore volume of the poly(styrene-co-divinylbenzene) particles when in a dry form is formed by the mesopores. In certain embodiments, from about 22% to about 28% of the total pore volume of the poly(styrene-co-divinylbenzene) particles may be formed by the micropores, and from about 5% to about 15% of the total pore volume of the poly(styrene-co-divinylbenzene) particles may be formed by the macropores.

The pore size distribution of the poly(styrene-co-divinylbenzene) particles when in a dry form, as disclosed herein, may be determined from N_2 adsorption isotherms at 77.3 K and CO_2 adsorption isotherms at 273.15 K, using a 2D nonlocal density functional theory (2D-NLDFT) model to generate a pore size distribution plot (Ravikovitch, P. I., & Neimark, A. V. (2002). "Density functional theory model of adsorption on amorphous and microporous silica materials." Langmuir, 18(17), 1550-1560). For the avoidance of doubt, the pore size distribution plot is a plot of cumulative pore volume against pore size for a material.

In certain embodiment, the poly(styrene-co-divinylbenzene) particles when in a dry form have a total surface area of from about 200 m^2/g to about 500 m^2/g. For example, the poly(styrene-co-divinylbenzene) particles when in a dry form may have a total surface area of about 400 m^2/g. The total surface area is the sum of the total external surface area and the total pore area of the particles, where the external surface area may be determined from N_2 adsorption isotherms at 77.3 K, using the T-plot method (Lippens, B.C. and De Boer, J.H., 1965. Studies on pore systems in catalysts: V. The t method. Journal of Catalysis, 4(3), pp.319-323), and the total pore area was determined using the pore size distribution of the poly(styrene-co-divinylbenzene) particles, as determined using the 2D-NLDFT method described above.

N_2 adsorption-desorption measurements may be performed at 77.3 K using a gas adsorption analyzer or porosimeter. CO_2 adsorption-desorption measurements can be performed at 273.15 K using a gas adsorption analyzer or porosimeter. A suitable porosimeter is a Micrometrics porosimeter, such as a Micrometrics 3Flex model porosimeter. Specific details are provided below and in the examples. The N_2 and CO_2 adsorption-desorption measurements are performed on the poly(styrene-co-divinylbenzene) particles and adsorbents disclosed herein when they are in a dry form, and specifically after the particles and adsorbents have been subjected to a temperature of about 300 °C for about 6 hours.

When the poly(styrene-co-divinylbenzene) particles are in a dry form, they may have a moisture content of less than about 5 wt%, for example, less than 1 wt% (e.g. 0 wt%). As will be understood by one skilled in the art, the poly(styrene-co-divinylbenzene) particles must be first dried to enable N_2 and CO_2 adsorption-desorption measurements. The process of drying the poly(styrene-co-divinylbenzene) particles may result in shrinkage of the particle size and pore sizes of the poly(styrene-co-divinylbenzene) particles.

The poly(styrene-co-divinylbenzene) particles disclosed herein may be prepared using methods known to those skilled in the art of organic chemistry. For example, poly(styrene-co-divinylbenzene) particles may be formed by the polymerisation of styrene and divinylbenzene in the presence of azobisisobutyronitrile (AIBN). Specific methods for preparing poly(styrene-co-divinylbenzene) particles are disclosed by Shim *et al*. Journal of Polymer Science Part A:

Polymer Chemistry 42.4 (2004): 835-845, which is incorporated herein by reference. Suitable poly(styrene-co-divinylbenzene) particles may also be purchased for commercial sources.

In certain embodiments, the activated carbon adsorbent of the present invention has a total surface area of at least about 1000 m^2/g when in a dry form. In certain embodiments, the activated carbon adsorbent of the present invention has a total surface area of from about 1000 m^2/g to about 3000 m^2/g when in a dry form. For example, the activated carbon adsorbent, when in a dry form, may have a total surface area of from about 1500 m^2/g to about 3000 m^2/g, or from about 2000 m^2/g to about 2700 m^2/g, for example, from about 2200 m^2/g to about 2600 m^2/g.

The total surface area of the activated carbon adsorbent of the present invention may be determined using the same method as described herein for the poly(styrene-co-divinylbenzene) particles. Thus, for the avoidance of doubt, in certain embodiments, the activated carbon adsorbent of the present invention has a total surface area of at least about 1000 m^2/g, wherein the total surface area is the sum of the total external surface area and the total pore area of the activated carbon adsorbent, where the total external surface area is determined from N_2 adsorption isotherms at 77.3 K using the T-plot method, and the total pore area is determined from N_2 adsorption isotherms at 77.3 K and CO_2 adsorption isotherms at 273.15 K using a 2D nonlocal density functional theory (2D-NLDFT) model. The activated carbon adsorbent undergoes heating (e.g. heating at about 300 °C for about 6 hours) prior to analysis and is therefore in a dry form. That is to say that the activated carbon adsorbent that is analyzed has a moisture content of less than about 10 wt%, or less than about 5 wt%, for example, less than about 1 wt% (e.g. 0 wt%). Thus, the total surface area, total pore volume, and pore size distribution values disclosed herein for the activated carbon adsorbent relate to activated carbon adsorbent when in a dry form.

The activated carbon adsorbent of the present invention has a moisture content following carbonization and activation of less than about 10 wt%, for example from about 5 wt% to about 10 wt%. Thus, in certain embodiments, the activated carbon adsorbent of the present invention has a moisture content following carbonization and activation of less than about 10 wt%, for example from about 5 wt% to about 10 wt%. The total surface area, total pore volume, and pore size distribution values disclosed herein for the activated carbon adsorbent are therefore expected to be similar, or the same, as those for the activated carbon adsorbent obtained directly from carbonization and activation (i.e. when it has a moisture content of less than about 10 wt%).

In certain embodiments, the activated carbon adsorbent of the present invention has a total pore volume of at least about 1 cm^3/g. For example, the activated carbon adsorbent may have

a total pore volume from about 1 cm^3/g to about 1.5 cm^3/g. For example, a total pore volume of about 1.3 cm^3/g.

The total pore volume of the activated carbon adsorbent may be determined using the same method as described herein for the poly(styrene-co-divinylbenzene) particles. Thus, for the avoidance of doubt, in certain embodiments, the activated carbon adsorbent of the present invention may have a total pore volume of at least about 1 cm^3/g, as determined from a single point of an N_2 adsorption isotherm at 77.3 K with a saturation pressure of p/p0>0.99, using the Gurvich rule. Without wishing to be bound by theory, the present inventors believe that the presence of micropores enables the adsorption of toxins such as creatinine, PCS, HA and IS, and precursor toxins such as indole, while the presence of mesopores enables the adsorption of larger toxins such as B2M. In certain embodiments, substantially all of the total pore volume of the activated carbon adsorbent is formed from micropores, mesopores, or a combination of micropores and mesopores. For example, at least about 60% (e.g. at least about 70%, at least about 80%, at least about 90%, at least about 95%, or 100%) of the total pore volume of the activated carbon adsorbent is formed by micropores, mesopores, or a combination of micropores and mesopores.

In certain embodiments, the activated carbon adsorbent of the present invention comprises micropores and mesopores. The present inventors have found that the presence of micropores and mesopores in the activated carbon adsorbent of the present invention makes it surprisingly effective at adsorbing a wide range of toxins, in particular uremic toxins such as creatinine, PCS, HA, IS, and B2M. This adsorption profile is lacking in established carbon based oral adsorbents, such as AST-120 (Kureha Corporation, Tokyo, Japan), also known as Kremezin. Thus, in certain preferred embodiments, the activated carbon adsorbent of the present invention is capable of adsorbing one or more of B2M, creatinine, PCS, HA, IS, and indole. The ability of the activated carbon adsorbent of the present invention to adsorb B2M makes it particularly useful as a sorbent for sorbent-based dialysis, and the ability of the activated carbon adsorbent of the present invention to adsorb indole, together with toxins such as creatinine, PCS, HA and IS, makes it particularly useful as an oral sorbent.

The activated carbon adsorbent of the present invention has been found to be especially effective at adsorbing B2M and creatinine when it comprises micropores and mesopores, and has a total surface area of at least about 1000 m^2/g and a total pore volume of at least about 1 cm^3/g, wherein at least about 50% of the total pore volume is formed by the micropores, and at least about 20% of the total pore volume is formed by the mesopores.

Thus, the present invention also provides an activated carbon adsorbent for oral administration, wherein the activated carbon adsorbent is in the form of particles, comprises micropores and

mesopores, and has a total surface area of at least about 1000 m^2/g and a total pore volume of at least about 1 cm^3/g, wherein at least about 50% of the total pore volume is formed by the micropores, and at least about 20% of the total pore volume is formed by the mesopores.

In certain embodiments, from about 50% to about 80% of the total pore volume of the activated carbon adsorbent of the present invention is formed by the micropores, and from about 20% to about 40% of the total pore volume of the activated carbon adsorbent is formed by mesopores. In certain exemplary embodiments, the activated carbon adsorbent of the present invention may have a total pore volume of from about 1.1 cm^3/g to about 1.4 cm^3/g (e.g. about 1.3 cm^3/g), wherein from about 50% to about 80% of the total pore volume of the activated carbon adsorbent of the present invention is formed by the micropores, and from about 20% to about 40% of the total pore volume of the activated carbon adsorbent is formed by the mesopores.

The term “micropore” as used herein refers to a pore with a pore diameter of less than about 2 nm, the term “mesopore” as used herein refers to a pore with a pore diameter of from about 2 nm to about 50 nm. The term “macropore” as used herein refers to a pore with a pore diameter of greater than about 50 nm.

The activated carbon adsorbent of the present invention may have an average pore size of from about 1 nm to about 10 nm, for example, from about 1 nm to about 8 nm, or from about 1 nm to about 4 nm (e.g. about 2 nm). As disclosed herein, the average pore size (nm) of the activated carbon adsorbent may be calculated using the formula below with assumption of pores of cylindrical shape,

$$D = \frac{4V}{S} \quad (1)$$

where, D=average pore diameter, V= total pore volume at $p/p_{\circ}>0.99$, and S= Total Surface area (as determined using the method described above).

In certain embodiments, the activated carbon adsorbent of the present invention has a density of from about 0.2 mL/g to about 0.5 mL/g, for example about 0.4 mL/g. The density of the activated carbon adsorbent may influence the activated carbon adsorbent’s ability to adsorb toxins. Without wishing to be bound by theory, activated carbon adsorbent with a lower density generally suggests that the activated carbon adsorbent has a higher volume of void spaces, which translates to greater porosity. The density of the activated carbon adsorbent may be determined using any suitable method known in the art. The density of the activated carbon adsorbent disclosed herein refers to the tapped density of the activated carbon adsorbent. The tapped density of the activated carbon adsorbent may be measured by mechanically tapping a dry sample of the activated carbon adsorbent in a measuring cylinder (e.g. 5 ml

measuring cylinder), and allowing the sample to settle to a final volume under its own weight. The tapped density can then be calculated as follows: Tapped density = Sample mass (g) / Final Volume (cm^3).

In certain embodiments, the activated carbon adsorbent of the present invention has an average particle size of from about 300 μm to about 350 μm. For example, an average particle size of about 300 μm. As used herein, the term “average particle size” refers to the mean particle size of a material based on the volume-based particle size distribution, often measured by the laser diffraction or light scattering particle size distribution measurements. Particle size can also be determined using Field Emission Scanning Electron Microscopy (FESEM), as described in the Examples section herein. In particular, high-resolution FESEM images of the activated carbon adsorbent were analysed for particle morphology. By analyzing the FESEM images, precise measurements of particle dimensions of the activated carbon adsorbent were obtained.

Typically, the activated carbon adsorbent of the present invention is in the form of spherical particles. The spherical shape of the the activated carbon adsorbent enables higher packing density compared to particles with an irregular shape. Higher packing density can enhance the flow of fluids through an adsorbent bed containing the activated carbon adsorbent, which can reduce pressure drop and improve mass transfer rates. Such beneficial properties make the the activated carbon adsorbent of the present invention particularly useful as an *in vitro* sorbent, for example as a sorbent for sorbent-based dialysis. Additionally, the spherical form of the the activated carbon adsorbent provides a higher surface area-to-volume ratio, which can increase the efficiency of the adsorption process. The shape can also facilitates easier handling and processing in various industrial applications.

Compositions and Utility:

The present invention also provides a composition comprising the activated carbon adsorbent of the present invention and at least one binder, carrier or excipient. Preferably, the composition of the invention is a pharmaceutical composition. For example, the composition may be a pharmaceutical composition comprising the activated carbon adsorbent according to the present invention and at least one pharmaceutically acceptable binder, carrier or excipient. Preferably, the composition of the invention is a pharmaceutical composition suitable for oral administration.

Pharmaceutical compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the activated carbon adsorbent; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water

liquid emulsion or a water-in-oil liquid emulsion. The activated carbon adsorbent may also be presented as a bolus, electuary or paste. Suitable pharmaceutically acceptable binders, carriers or excipients for including in the pharmaceutical formulation of the present invention may be selected with due regard to the intended route of administration and standard pharmaceutical practice. Suitable pharmaceutical formulations may be found in, for example, Remington The Science and Practice of Pharmacy, 19th ed., Mack Printing Company, Easton, Pennsylvania (1995). Otherwise, the preparation of suitable formulations may be achieved routinely by the skilled person using routine techniques and/or in accordance with standard and/or accepted pharmaceutical practice.

As will be appreciated from the disclosure herein, the activated carbon adsorbent and pharmaceutical composition of the present invention may find utility as a medicament. The ability of the activated carbon adsorbent of the present invention to adsorb a wide range of uremic toxins, in particular creatinine, PCS, HA, IS, and B2M, and the toxin precursor indole, makes it a promising oral adsorbent for the treatment of a renal disease or a liver disease. Thus, the activated carbon adsorbent and pharmaceutical composition may find use as an oral sorbent, for example for the treatment of a renal disease or a liver disease, for example chronic kidney disease (CKD) or chronic liver disease (CLD). Reducing the build-up of toxins in patients suffering from a renal disease or a liver disease may prevent or delay the progression of kidney failure or liver failure. As such, the activated carbon adsorbent and pharmaceutical composition of the present invention may also find utility in preventing or delaying kidney failure or liver failure.

Also provided herein is a method of treating a renal disease or a liver disease in a patient, said method comprising administering a pharmaceutically effective amount of the activated carbon adsorbent to the patient. The present invention also provide a use of the activated carbon adsorbent of the present invention in the manufacture of a medicament for the treatment of a renal disease or for the treatment of a liver disease. The medicament comprising the activated carbon adsorbent is to be administered orally.

The amount of the activated carbon adsorbent or pharmaceutical composition of the present invention required to achieve a therapeutic effect will vary with the characteristics of the subject under treatment, for example the species, age, weight, sex, medical conditions, the particular disease (e.g. renal disease or liver disease) and its severity, and other relevant medical and physical factors. An ordinarily skilled physician can readily determine and administer an effective amount of the activated carbon adsorbent or pharmaceutical composition required for treatment of the liver disease or renal disease.

The activated carbon adsorbent of the invention may be administered daily (including several times daily), every second or third day, weekly, every second, third or fourth week or even as a high single dose depending on the subject and disease to be treated.

As used herein the terms "subject" or "patient" are well-recognized in the art, and, are used interchangeably herein to refer to a mammal, including dog, cat, rat, mouse, monkey, cow, horse, goat, sheep, pig, camel, and, most preferably, a human. In some embodiments, the subject is a subject in need of treatment or a subject with a disease or disorder. However, in other embodiments, the subject can be a normal subject. The term does not denote a particular age or sex. Thus, adult and newborn subjects, whether male or female, are intended to be covered.

In vitro methods:

As described herein, the activated carbon adsorbent of the present invention is surprisingly effective at adsorbing a wide range of toxins, in particular uremic toxins such as creatinine, PCS, HA, IS, and B2M. The ability of the activated carbon adsorbent to adsorb B2M makes it particularly useful as a sorbent *in vitro*, for example as a sorbent for sorbent-based dialysis. Thus, the activated carbon adsorbent finds utility in an *in vitro* method of removing uremic toxins from a fluid, such as waste dialysis fluid. For example, removing uremic toxins from waste dialysis fluid obtained from a patient undergoing peritoneal dialysis.

Accordingly, the present invention also provides an *in vitro* method of removing one or more uremic toxins from a fluid, the method comprising providing a fluid comprising the one or more uremic toxins, and contacting the fluid with the activated carbon adsorbent of the present invention. In certain embodiments, the one or more uremic toxins are selected from the group consisting of creatinine, PCS, HA, IS, B2M, and a combination of two or more thereof (e.g. creatinine, B2M, or a combination of creatinine and B2M). Typically, the one or more uremic toxins is removed from a waste dialysis fluid obtained from a patient undergoing peritoneal dialysis.

When the activated carbon adsorbent is used in an *in vitro* method of removing one or more uremic toxins from a fluid, it may be housed within a sorbent cartridge. For example, 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). Thus, the present invention also provide a sorbent cartridge containing the activated carbon adsorbent as disclosed herein.

The sorbent cartridge of the present invention may further comprise one or more of:

cation exchange particles (e.g. zirconium phosphate);

anion exchange particles (e.g. hydrous zirconium oxide);

a buffer (e.g. calcium carbonate); and

a urease.

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

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^+).

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 about 10 μm to about 1000 μm, about 100 μm to about 900 μm, about 200 μm to about 900 μm, about 300 μm to about 800 μm, about 400 μm to about 700, 500 μm to about 600 μm, about 25 μm to about 200 μm, about 25 μm to about 150 μm, about 25 μm to about 80 μm, about 25 μm to about 50 μm, about 50 μm to about 100 μm, about 125 μm to about 200 μm, about 150 μm to about 200 μm, about 100 μm to about 175 μm, about 100 μm to about 150 μm,about 150 μm to about 500 μm, or about 250 μm to about 1000 μm.

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 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. When in the form of particles, the zirconium oxide particles may have an average particle size in the range of from about 10 μm to about 1000 μm, about 100 μm to about 900 μm, about 200 μm to about 900 μm, about 300 μm to about 800 μm, about 400 μm to about 700, about 500 μm to about 600 μm, about 10 μm to about 200 μm, about 10 μm to about 100 μm, about

10 μm to about 30 μm, about 10 μm to about 20 μm, about 20 μm to about 50 μm, about 25 μm to about 50 μm, about 30 μm to about 50 μm, about 40 μm to about 150 μm or from about 80 μm to about 120 μm or from about 160 μm to about 180 μm, about 25 μm to about 250 μm, about 250 μm to about 500 μm, about 250 μm to about 1000 μm.

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 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 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” and both refer to an enzyme able to react with a uremic toxin as a substrate. For example, the uremic toxin-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 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).

The contents of the articles, patents, and patent applications, and all other documents and electronically available information mentioned or cited herein, are hereby incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference. The applicant reserves the right physically to incorporate into this application any and all materials and information from any such articles, patents, patent applications, or other physical and electronic documents.

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

EXAMPLES

***Materials*:**

Chemicals including creatinine, IS, PCS, HA, indole, and B2M were procured from Sigma Aldrich, and used as received unless otherwise specified.

Table 1: Polymer particles used in the present examples

Polymer particles	Polymer type	Particle size	Supplier
Merrifield peptide resin	Styrene-divinylbenzene. Functionalised by chloromethyl group. >1% cross-linked.	74-152 µm (100-200 mesh size)	Merck
AmberLite™ HPR2900	Styrene-divinylbenzene. Functionalised by sulfonic acid. >95% cross-linked. Approx. 50 wt% moisture content. Macroporous.	575 ± 50 µm	Dupont
Dowex 50Wx8	Styrene divinylbenzene (gel). Functionalised by sulfonic acid. >8% cross-linked. 50-58 wt% moisture content.	152-279 µm (50-100 mesh size)	DuPont Water Solutions
Diaion SMNUPB	Mixed styrene divinylbenzene (gel). Functionalised by sulfonic acid / Type I. (trimethyl ammonium groups), 1:1 ratio. 60-70 wt% moisture content.	700-720 µm	Mitsubishi Chemical
AmberChrom™ 50WX4 50-100H	Styrene divinylbenzene (gel). Functionalised by sulfonic acid. 8% cross-linked. 64-72 wt% moisture content.	74-152 µm (100-200 mesh size)	DuPont Water Solutions
Amberlite™ IRN78 OH hydroxide form	Styrene-divinylbenzene. Functionalised by trimethylammonium. 8% cross-linked. 54-60 wt% moisture content.	580-680 µm	DuPont Water Solutions
Relite JA310	Styrene-divinylbenzene. Functionalised by tertiary amine. 51-61 wt% moisture content.	710 µm	Mitsubishi Chemical

Dowex MAC-3	Polyacrylic. Functionalised by carboxylic acid. 44-52 wt% moisture content.	300-1200 µm	Sigma-Aldrich
Diaion UBA200P	Styrene-divinylbenzene. Functonalised by type II (dimethylethanol ammonium groups 45-51 wt% moisture content.	575 ± 50 µm	Mitsubishi Chemical
Diaion PA312LOH	Styrene divinylbenzene (gel). Functionalised by sulfonic acid. 58-68 wt% moisture content.	700 µm	Mitsubishi Chemical

General methods:

Carbonization and activation:

Carbonization and activation of the polymer particles were conducted using a pyrolysis instrument (Carbolite Gero, Lindberg/Blue M, Thermolyne) with a maximum temperature capacity of 1200 °C.

Field Emission Scanning electron microscopy (FESEM):

Field Emission Scanning electron microscopy (FESEM) measurements were performed using a JEOL JSM-7600F instrument. The particle dispersion was deposited and dried on a silica substrate supported by carbon adhesive tape, and examination was carried out at an acceleration voltage of 2-5 kV.

Transmission electron microscopy (TEM):

Transmission electron microscopy (TEM) was conducted on specific samples utilizing a JEOL 2200FS instrument. The instrument was operated at an acceleration voltage of 200 kV.

Characterization of polymer particles and ACB surface area:

Polymer particles and ACB samples (33-105 mg) were degassed at 300 °C under vacuum for 6 h prior to data collection. This degassing protocol does not alter the morphology of the ACB as they were originally synthesized at high temperatures (~900 °C). However, for the precursor Amberlite HPR 2900 particles, which contain approximately 50% moisture, this process resulted in significant shrinkage of the particle size due to moisture removal at 300 °C for 6 h. As a result, the analysis performed on this sample reflects the properties of the shrunken particles rather than the particles in their original form (i.e. particles with a moisture content of from about 5 wt% to about 60 wt%).

Nitrogen adsorption-desorption experiments were carried out by static manometric method (Determination of the specific surface area of solids by gas adsorption - BET method. 2022, ISO 9277:2022(en)). Measurements were performed at 77.3 K on a Micromeritics 3Flex model.

Equilibration time was set to 10-30s for samples Calgon ACP, AST-120, Batch 36 and Batch 39; and was set to 5s for Amberlite HPR 2900H. The free space was measured by the instrument.

Similarly, CO_2 adsorption experiments were carried out by static manometric method. Measurements were performed at 273.15 K on a Micromeritics 3Flex model. Equilibrium time was set to 10s for all the samples.

Surface area referred to as Total Surface Area was calculated as the sum of the t-Plot External Surface Area and the Total Area in Pores. These values were obtained from the report generated by using the Micromeritics Microactive software Version 6.0.

To further elaborate, the t-plot external surface area was determined using the T-plot method from nitrogen adsorption isotherms measured at 77.3 K (Lippens, B.C. and De Boer, J.H., 1965. Studies on pore systems in catalysts: V. The t method. Journal of Catalysis, 4(3), pp.319-323). Likewise, the Total Area in Pores was determined using the 2D Non-Local Density Functional Theory (NLDFT) model (Neimark, A. V., Ravikovitch, P. I., Vishnyakov, A. (2000). "Adsorption hysteresis in nanopores: Theory and experiment." Physical Review E, 62(2), R1493-R1496, Ravikovitch, P. I., & Neimark, A. V. (2001). "Characterization of Micro- and Mesoporosity in SBA-15 Materials from Adsorption Data by the NLDFT Method." Journal of Physical Chemistry B, 105(29), 6817-6823). By fitting the N_2 and CO_2 adsorption data to this model, the distribution of pore sizes and corresponding surface areas are obtained. The total pore area is then calculated by integrating the surface contributions from all identified pore sizes, providing a comprehensive measure of the surface area within the material's porous structure.

Characterization of polymer particles and ACB pore volume and size:

The total pore volume was obtained from the report generated using the Micromeritics Microactive software 6.0. The single point total pore volume was calculated at a single point approaching saturation pressure p/p0>0.99, which was an application of the Gurvich rule (Rouquerol, J., Rouquerol, F., Llewellyn, P., Maurin, G. and Sing, K., 2013. Adsorption by powders and porous solids: principles, methodology and applications. Academic press.)

The micropore and mesopore volumes were determined from the pore size distribution plot (cumulative pore volume vs. pore size) generated using the 2D-NLDFT model (Ravikovitch, P. I., & Neimark, A. V. (2002). "Density functional theory model of adsorption on amorphous and microporous silica materials." Langmuir, 18(17), 1550-1560). The micropore volume was identified as the cumulative volume at a pore width of 2 nm. The mesopore volume was then calculated by subtracting this micropore volume from the cumulative volume at a pore width of 50 nm.

Macropore volume was obtained as the difference of the single point total pore volume and the cumulative pore volume at 50 nm obtained from the 2D-NLDFT pore size distribution plot.

The average pore size (nm) was calculated using the formula below with assumption of pores of cylindrical shape,

$$D = \frac{4V}{S} \quad (1)$$

(Rouquerol, F., Rouquerol, J., Sing, K. S. W., Llewellyn, P., & Maurin, G. (2013). Adsorption by Powders and Porous Solids: Principles, Methodology and Applications (2nd ed.). Academic Press.)

where, D=average pore diameter, V= single point total pore volume at $p/p_{\circ}$>0.99, and S= Total Surface area (as determined using the method described above).

Surface area analysis using the Brunauer-Emmett-Teller (BET) method:

Where specified, the surface area of certain samples disclosed herein was determined using the BET method. In brief, surface area was determined using the BET equation on the linear zone of a BET plot generated using a Micromeritics 3FLEX instrument. Nitrogen adsorption was assessed at liquid nitrogen temperature (77 K) following adequate thermal treatment of each sample at 300 °C.

Uremic toxin level measurements:

Creatinine levels in samples were analyzed using a Vitros XT3400 instrument (Ortho Clinical Diagnostics, Raritan, NJ, USA), with a limit of detection for VITROS creatinine slides set at 13-1268 µmol/L.

For B2M analysis, samples were sent for immunoassay analysis by an external test provider at the SEMC Academia Clinical Chemistry division, Singapore General Hospital. The analysis and quantification of toxins (IS, PCS, and HA) were determined using an LC-MS/MS approach by an external test provider, CCIC Singapore (Shu, C. *et al.*, Biomed. Chromatogr. 2016, *30*(11), 1782-1788). For this, HPLC analysis was performed using an Agilent 1200 HPLC system (Agilent Inc., MA, USA), comprising a G1311A quaternary pump, a G1322A vacuum degasser, a G1329A autosampler, and a G1316A thermostatic column. Separation was carried out on an Agilent Zorbax SB-C18 column (3.5 µm, 2.1 × 100 mm) with the column temperature maintained at 30 °C. The mobile phase consisted of a mixture of acetonitrile and 10 mM ammonium acetate buffer (10:90, v/v), employing an isocratic elution at a flow rate of 0.3 mL/min. The injection volume was 5 µL, and the analysis time for each injection was 5 min. Quantification was performed using electrospray ionization in the negative mode with the spray voltage set at 4000 V. Nitrogen was used as the nebulizer gas, with a nebulizer pressure

of 40 psi and a source temperature of 105 °C. Desolvation gas (nitrogen) was heated to 350 °C and delivered at a flow rate of 10 L/min.

General procedure for carbonization and activation of polymer particles:

The required number of 50 g batches of poly(styrene-co-divinylbenzene) particles were placed into five alumina boats and sequentially positioned in the furnace tube. The temperature was gradually increased from 30°C to 900°C over 6 h at a ramp rate of 2.5 °C/min under optimal argon gas flow (1000 cc/min). Subsequently, activation was conducted at 900 °C with a dwell duration of 10 h under optimal CO_2 gas flow (1000 cc/min). To monitor the gas flow and manage the release of volatile organic residues, the gas outlet was submerged in water and connected to an exhaust system designed to absorb the released gases. Following the activation step, the temperature was gradually lowered to 30 °C over 9-10 h. The activated carbon beads (ACB) were then collected and subjected to a water wash, with the pH adjusted to 6-8. They were then dried in an oven at 100°C for 4-6 h to achieve a loss on drying (LOD) of 3-10 wt%. The resulting final ACB were stored in glass bottles for further use in peritoneal dialysis (PD) device and oral sorbent applications.

Representative procedure for evaluating creatinine adsorption capacity of ACBs at 1.5 g scale under dynamic conditions:

1.5 g of the ACBs were loaded into a 9 mL chromatography flex column measuring 50 mm in length and 15 mm in diameter. A synthetic dialysate (SD) mixture containing creatinine at a concentration of 500 µmol/L was passed through the column using a peristaltic pump at a flow rate of 5 mL/min. Initial samples were collected at 10 min (50 mL) and 30 min (100 mL) time points. Subsequent samples were collected at 30 min intervals (150 mL) so on until the ACB reached a point of exhaustion, indicated by a creatinine concentration of >50 µmol/L (leaching of creatinine). The collected samples were analyzed using a Vitros XT3400 instrument to measure the leakage of creatinine.

Representative procedure for evaluating creatinine and B2M adsorption capacity of the ACBs at full-scale 50 g column under dynamic conditions:

50 g of ACB was loaded into a 377 mL flash column from Hawach Scientific, measuring 218.5 mm in length and 60.6 mm in diameter. SD mixture containing creatinine at approximately 500 µmol/L or ex-vivo fluid from a peritoneal dialysis (PD) patient with B2M input of approximately 2000 µg/L was passed through the column using a peristaltic pump at a flow rate of 50 mL/min. Pre and subsequent samples were taken and analyzed for creatinine levels at every 2 L interval (40 min) up to 14 L (280 min). The samples were analyzed for creatinine levels using the Vitros XT3400 instrument. For B2M analysis, pre and post (14 L) samples were taken and analyzed by an external test provider at the SEMC Academia Clinical Chemistry division, Singapore General Hospital.

Representative procedure for evaluating B2M adsorption capacity of ACBs under static conditions:

300 mg of ACB was loaded into a 50 mL Falcon tube (measuring 115 mm in length and 30 mm in diameter, Fisher Scientific) containing 20 mL of ex-vivo fluid from a PD patient with B2M input approximately 2000 µg/L. The tube was then subjected to shaking on a shaker at 250 rpm for 2 h. Pre- and post-treatment samples were collected and analyzed for B2M levels by an external test provider at the SEMC Academia Clinical Chemistry division, Singapore General Hospital.

Representative procedure for evaluating creatinine, IS, PCS and HA adsorption capacity of ACB under static conditions:

1 g of ACB was loaded into a 1000 mL blue cap glass bottle. The bottle contained 500 mL of deionized (DI) water, along with 300 mg of creatinine, 40 mg each of IS, PCS, and HA. Subsequently, the bottle underwent shaking on a shaker at 250 rpm for 2 h. Pre- and post-treatment samples were collected and analyzed using Vitros XT3400 and Agilent 1200 HPLC instruments.

Example 1: Development of an activated carbon adsorbent

Various cationic and anionic polymer particles were subjected to pyrolysis and activation to provide activated carbon beads. The polymer particles used were Amberchrom, Amberlite, Dowex, Merrifield peptide, Relite, and Diaion (See Table 1 above). The resulting beads were analyzed by TGA to evaluate carbonization yield. The analysis revealed that Amberlite HPR 2900H exhibited significant carbonization (~30%), alongside Merrifield peptide (as depicted in Figure 2), attributed to its high cross-linking (>95%).

The Amberlite HPR 2900H was selected for further development because TGA analysis showed that Amberlite HPR 2900H demonstrated a high carbonization yield of approximately 30% and exhibited a lower loss of mass compared to the other resins tested. Although the Merrifield resin showed a similar yield, Amberlite HPR 2900H was selected for further studies due to its particle size, strength and porosity. Table 2 shows the surface area and porosity characterization of Amberlite HPR 2900H.

Table 2: Characterization of Amberlite HPR 2900.

Parameter	**Amberlite HPR2900 H**
Total surface area (m^2/g)[a]	409
Total pore volume (cm^3/g)[b]	0.53
Micropore volume (cm^3/g)[c]	0.14
Mesopore volume (cm^3/g)[d]	0.34
Macropore volume (cm^3/g)[e]	0.05

Microporosity (%)[f]	25.52
Mesoporosity (%)[f]	63.33
Macroporosity (%)[f]	11.15
Average Pore size (nm)[g]	5.18

[a] Total Surface Area (m^2/g): The total surface area of the sample is calculated as the sum of the external surface area (derived from the T-Plot method) and the total area in pores (obtained from 2D-NLDFT analysis). [b] Total Pore Volume (cm^3/g): The total pore volume represents the combined volume of all pores within the sample, spanning micropores to macropores. This value is obtained from the single-point adsorption at relative pressure (p/p_0) close to 1, reflecting the total pore volume. [c] Micropore Volume (cm^3/g): The micropore volume is calculated from the cumulative pore volume up to a pore size of 2 nm, based on the porosity distribution analysis using the 2D-NLDFT model. [d] Mesopore Volume (cm^3/g): The mesopore volume is determined by subtracting the micropore volume from the cumulative pore volume up to 50 nm, using the porosity distribution data from the 2D-NLDFT model. [e] Macropore Volume (cm^3/g): macropore volume was obtained by subtracting the cumulative pore volume at 50 nm from the total pore volume. [f] Macroporosity, mesoporosity, and microporosity are calculated as the respective percentages of the total pore volume based on their corresponding values. [g] Average pore size (nm): calculated using the formula D=4V/S assuming cylindrical pore structure, where D is average pore diameter, V is the single point total pore volume at $p/p_{\circ}>0.99$ and S is the total surface area obtained as the sum of t-plot external surface area and total area in pores obtained by 2D-NLDFT analysis.

Example 2: Development of the activated carbon adsorbent of the present invention

Further optimization of the carbonization and activation conditions using the Amberlite HPR 2900H polymer particles was carried out (see Table 3). The application of the resulting ACBs as a sorbent for a peritoneal dialysis (PD) was also assessed.

Polymer particles, initially at 50 wt% moisture content as purchased, were loaded into an alumina boat and placed in the furnace tube. Carbonization was carried out at various temperatures and durations with a gradual temperature increase starting from 30 °C under argon gas flow, as specified in Table 3. Subsequently, the carbonized beads underwent activation at different temperatures and durations, also indicated in Table 3, under CO_2 gas flow.

The adsorption profile and morphological characteristics of the resulting material were assessed using the methods described in the material and methods section above.

Results:

Observations from the pyrolysis and activation optimization experiments are shown in Table 3.

Table 3: Optimization for pyrolysis and activation conditions.

Batch	**Initial Weight of Amberlite HPR 2900H (g)**	**Pyrolysis**	**Activation**	**Final Weight of ACB (g)**	**Yield (%)**	**Remarks**
3	3	ramp: 700 ˚C in 45 min	soak: 700 ˚C for 1hr	0.52	17.3	Successfully produced carbonized beads. Free flowing. Shape retained. However, not obtained good adsorption results.
9	5	ramp: 850 ˚C in 2hr	soak:850 ˚C for 3hr	0.48	9.6	
13	30[a]	ramp: 900 ˚C in 2hr	soak:900 ˚C for 3hr	1.6+1.9	11.9	Two 15 g boats were kept in a row in the tube furnace. Obtained good adsorption results.
21	15	ramp: 900 ˚C in 2hr	soak:900 ˚C for 3hr	1.68	11.2	Obtained good adsorption results.
25	50	ramp: 900 ˚C in 2hr	soak:900 ˚C for 3hr	5.74	11.5	Not obtained good adsorption results.
33	150[b]	ramp:900 ˚C in 6hr	soak:900 ˚C for 15hr	2.4+2.5+2.5	5.0	Obtained good adsorption results. Surface area 2500 m^2/g by BET analysis.

35	250[c]	ramp:900 ˚C in 6hr	soak:900 ˚C for 10hr	23	9.2	Obtained good adsorption results as batch 33
36	250[c]	ramp:900 ˚C in 6hr	soak:900 ˚C for 10hr	19.6	7.9	Very efficient adsorption results were obtained. Surface area 2860 m^2/g by BET analysis.
39	250[c]	ramp:900 ˚C in 6hr	soak:900 ˚C for 10hr	52	21	Pre-dried at 60 ˚C in oven for 2 days before subjecting to pyrolysis. Obtained good adsorption results.

[a]Equally distributed into 2 alumina boats. [a]Equally distributed into 3 alumina boats. [a]Equally distributed into 5 alumina boats.

In this initial screening, batches 3 and 9 failed to yield satisfactory adsorption results for creatinine in synthetic dialysate (SD). This could be attributed to inadequate porosity and surface area at carbonization and activation temperatures ranging from 700-850 °C. However, upon increasing both carbonization and activation conditions to 900 °C for 2 h each, batches 13 and 21 exhibited improved adsorption results.

Further extension of carbonization and activation times to 6 and 10 h, respectively, for batches 33, 35, 36, and 39 (except for Batch 33 with an activation time of 15 h), yielded superior creatinine adsorption compared to batches 13 and 21. This improvement can be attributed to the higher porosity and surface area of the resulting ACB under these conditions (see Figure 4 for internal and external surface porosity).

Notably, Batch 36 outperformed compared to the remaining batches (13, 21, 33, 35, and 39) and reference samples (Calgon ACP and AST-120). This superiority is likely attributed to its low density (0.39 mL/g), high surface area (2439 m^2/g), high total pore volume (1.28 cm^3/g), and a combination of micropores (about 0.7 cm^3/g by volume) and mesopores (about 0.4 cm^3/g by volume), along with an appropriate average pore size of about 2 nm (refer to Figure 5 and Tables 4 and 5). Additionally, as a control experiment, precursor (i.e. the Amberlite HPR 2900H) for Batch 39 beads were pre-dried at 60 °C in an oven before pyrolysis to assess any differences in adsorption of the resulting ACB. From this experiment, it was concluded that the B2M adsorption efficacy is compromised when pyrolyzing pre-dried beads rather than wet beads, albeit with a significant improvement in yield (see Table 3). For the pre-dried precursor for Batch 39 the moisture content of the precursor was from about 5 wt% to about 10 wt% relative to the total mass of the precursor. In contrast, the precursor for Batch 36 has a moisture content of about 50 wt% relative to the total mass of the precursor.

As shown in Table 4, using Batch 21, dynamic testing with 1.5 g of ACB and shake test with 300 mg of ACB, resulted in the adsorption of 640 mg of creatinine and 6.5 mg of B2M, respectively (see experimental section for methods). Subsequently, dynamic testing (simulated conditions similar to our PD device) with Batch 36 was conducted using a full-scale 50 g column test for both creatinine and B2M, resulting in the adsorption of 808 mg and 13.4 mg of creatinine and B2M, respectively. Similarly, Batch 39 yielded nearly close results to Batch 36, comparable to the outcomes of a PD device using Calgon ACP. In comparison to reference AST-120, we also conducted dynamic and shake tests with 1.5 g and 300 mg of AST-120, respectively, resulting in lower adsorption efficiency for creatinine and B2M. The structures of creatinine and B2M, description of the full-scale 50 g column, experimental conditions, and adsorption calculations are depicted in Fig. 3.

Table 4: Binding or adsorption capacity of creatinine and B2M of the activated carbon adsorbents.

Batch	Density (g/mL)	Creatinine binding capacity	B2M binding capacity	Remarks
21	0.45	640 mg cleared[a]	6.5 mg cleared[b]	
36	0.39	808 mg cleared[c]	13.4 mg cleared[d]	Conducted in full-scale 50 g column
39	0.49	913 mg cleared[e]	11 mg cleared[f]	
Calgon ACP	0.60	791 mg cleared[g]	15 mg cleared[h]	
AST-120	0.46	753 mg cleared[i]	4 mg cleared[b]	Commercialized oral sorbent by Kureha, Japan

[a]Based on 1.5 g scale column (prorated to full-scale 50 g column) with 500 µmol/L (pre). [b]Conducted by shake test on a shaker: 300 mg of ACB in 20 mL ex-vivo dialysis fluid (i.e. DIANEAL following drainage from a patient's peritoneal cavity). [c]511 µmol/L (pre SD). [d]1748 µg/L (pre ex-vivo) and 959 µg/L (post ex-vivo). [e]577 µmol/L (pre SD). [f]2832 µg/L (pre ex-vivo) and 2392 µg/L (post ex-vivo). [g]550 µmol/L (pre SD). [h]Product requirement specification. [i]Based on 1.5 g scale column (prorated to full-scale 50 g column) with 581 µmol/L (pre). SD = Synthetic dialysate. pre = the solution of SD before adsorption. post = the solution of SD after adsorption. Pre ex-vivo = ex-vivo dialysis fluid before treatment with ACB. Post ex-vivo = ex-vivo dialysis fluid after treatment with ACB.

Morphological characterisation of the ACBs:

The ACBs were characterized using field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), and using N_2 and/or CO_2 gas adsorption to assess porosity and surface area (refer to Figs. 3-5).

FESEM analysis revealed that the ACBs were uniformly spherical in size (300 µm) and displayed consistent reproducibility in porosity across samples (as depicted in Fig. 3).

TEM analysis offered insights into potential porosity channels across the ACB surface, compared to reference samples Calgon ACP and AST-120, which is crucial for experimental design (Fig. 4).

Additionally, N_2 and/or CO_2 gas adsorption analysis was performed to determine surface area and pore size, referencing Calgon ACP and AST-120 (Fig. 5, and Table 5). Notably, representative samples from batches 36 and 39 exhibited comparable or superior surface area and pore size to the reference samples.

Table 5: Characterization of ACBs.

Parameter	Calgon ACP	AST-120	Batch-36	Batch-39
Total surface area (m^2/g)[a]	1072	1445	2439	1525
Total pore volume (cm^3/g)[b]	0.58	0.77	1.28	0.76
Micropore volume (cm^3/g)[c]	0.34	0.64	0.72	0.54
Mesopore volume (cm^3/g)[d]	0.2	0.09	0.42	0.19
Macropore volume (cm^3/g)[e]	0.04	0.04	0.14	0.03
Microporosity (%)[f]	58.13	83.2	56.3	70.29
Mesoporosity (%)[f]	35.12	11.11	32.97	24.35
Macroporosity (%)[f]	6.75	5.68	10.73	5.37
Average Pore size (nm)[g]	2.16	2.13	2.09	2

[a-f] are the same [a-f] in Table 2.

Example 3: Evaluation of the ACBs for use as an oral sorbent.

The suitability of Batch 36 as an oral sorbent was investigated. Oral sorbents adsorb uremic toxins and their precursors within the GI tract, facilitating their excretion in feces and thereby retarding the progression of renal disease. Uremic toxins, including creatinine, IS, PCS, and HA, play pivotal roles in the pathogenesis of CKD by exacerbating glomerulosclerosis, interstitial fibrosis, and vascular damage. While AST-120 and DW-7202 are recognized oral sorbents capable of adsorbing small-sized toxins at high cost, an objective of the present invention is to provide an ACB with enhanced adsorption efficiency.

To initially test the adsorption of creatinine, several experiments were designed using various concentrations of creatinine in SD to simulate conditions akin to those experienced by CKD patients at different stages of kidney function (Fig. 6). Each experiment involved 300 mg of ACB in 20 mL of DI water with varying initial concentrations of creatinine (pre), which were then shaken on a shaker at 250 rpm at room temperature (RT) for 2 h. From these preliminary studies, it was observed that creatinine was effectively adsorbed within the initial concentration range of 540-1508 µmol/L, providing valuable insights into the examination of patients' creatinine levels at various stages of kidney function.

In the subsequent experiments, we simulated gut concentrations with a high input of creatinine (500 mg). By varying the reaction volume from 50 to 300 mL and adjusting the initial creatinine concentrations to approximately 14-87 mmol, we observed that higher creatinine concentrations led to increased adsorption by the ACB (Fig. 7A). Specifically, it was noted that a gradual increase in adsorption from experiments with initial creatinine concentrations ranging from 14 to 56 mmol, reaching saturation at concentrations between 56 and 87 mmol

(Fig. 7B). Therefore, it is observed that the removal or adsorption of creatinine increases with increasing concentration until the beads become saturated.

Next, the effect of duration on creatinine adsorption levels was assessed. This was to assess dose mapping in gut (refer to Fig. 8). Similar to previous experiments (as shown in Fig. 7), experiments were conducted with creatinine concentrations ranging from 14 to 87 mmol/L in volumes of 50 to 300 mL. These experiments extended up to 24 h, with sample analyses performed at 2-h, 6-h, and 24-h intervals (Fig. 8A). Overall, it was observed that adsorption increased from 2 to 24 h (Fig. 8B), with a rapid increase observed from 2 to 6 h, showing an average 40.1% increase, followed by a slower increase of 5.7% from 6 to 24 h (Fig. 8C). In conclusion, it is noteworthy that the adsorption efficiency of the ACB is significantly higher from 2 to 6 h, likely due to all pores being open and available for rapid adsorption. Subsequently, once the pores reach maximum occupancy, the adsorption efficiency slows down.

To understand how pH affects creatinine adsorption, mimicking gut conditions, experiments at different pH levels: pH 1, pH 3, and pH 5 were conducted (refer to Fig. 9). It is important to note that in previous experiments, which are detailed in Figs. 7-9, a neutral pH of 7.2 was used. When analyzing the results of the experiments conducted at lower pH levels, a decrease in creatinine adsorption over time was observed. There are several potential explanations for this phenomenon: firstly, at lower pH levels, there may be a higher likelihood of creatinine decomposition, leading to reduced adsorption. Additionally, changes in the properties of the pores within the ACB, such as alterations in pore size or volume, may occur under acidic conditions, impacting their ability to effectively adsorb creatinine molecules. These findings highlight the importance of considering pH as a critical factor influencing the adsorption capabilities of activated carbon, particularly in applications aimed at mimicking GI conditions.

The design of the experiments detailed in Figs. 6-9 revealed that Batch 36 provided good adsorption for creatinine.

The adsorption profile of Batch 36 was compared with well-known reference samples, AST-120 and commercial Ambersorb 560, to ascertain its efficacy at pH 7.2. Upon comparison, the efficiency of Batch 36 was found to be superior to that of AST-120 and Ambersorb 560 when tested with 1L of SD containing 300 mg of creatinine input (refer to Fig. 10). This result demonstrates the applicability of Batch 36 as an alternative oral sorbent for creatinine adsorption.

For our next investigation into other uremic toxins, namely IS, PCS, and HA (refer to Fig. 11A for chemical structures), we screened the conditions for individual toxin adsorption using Batch 36, as detailed in Fig. 11B. In the first set of experiments, we varied the concentration of beads

(Entries 1-4, Fig. 11B) at pH 7, while in the second set of experiments, we assessed the effect of pH from 7 to 1 using 1000 mg of beads (Entries 5-8, Fig. 11B). Each experiment utilized 20 mg of toxin in 20 mL of SD, simulating an initial concentration of 1000 mg/L. By gradually increasing the concentration of beads (ranging from 200 to 1000 mg) and adjusting the pH (from 7 to 1), we observed higher adsorption efficiency for IS and PCS. Interestingly, for HA, effective adsorption was observed even at lower bead concentrations and across all pH conditions.

After observing efficient adsorption of individual toxins such as creatinine, IS, PCS, and HA using 1000 mg of Batch 36, we proceeded to design conditions for the mixture of these toxins to simulate gut conditions (refer to Fig. 12). A solution containing 40 mg of each toxin in 500 mL of deionized (DI) water was prepared. Subsequently, 1 g of Batch 36 was added to the prepared solution. The experiment was conducted at RT for 2 h with agitation at 250 rpm at pH 7.2. We observed that the adsorbance of the toxins ranged from 27 to 36 mg, compared to the initial concentration of 40 mg for each toxin. Our findings revealed that the efficiency of Batch 36 was not compromised when exposed to a mixture of toxins resembling those present in the gut.

We next replicated the experiment described in Fig. 12 increasing the creatinine input to 300 mg while maintaining the same concentration of other toxins (40 mg each). Two experiments with this composition were conducted, one at pH 7.2 and the other at pH 3.3, to compare adsorption efficiency of Batch 36 under varied pH conditions (refer to Fig. 13). Interestingly, we observed highly efficient adsorption of IS, PCS, and HA at pH 3.3. However, a lower adsorption of creatinine was observed at pH 3.3 compared to pH 7.2.

For the subsequent experiment, aimed at assessing the adsorption efficiency of Batch 36 compared to AST-120, we repeated the experiment described in Fig. 13. However, in this iteration, 1 g of AST-120 was used instead of Batch 36 at pH 7.2 (refer to Fig. 14). This experiment was then compared with the experiment described in Fig. 13, where Batch 36 was utilized under the same pH conditions. Our observations highlighted that the efficiency of Batch 36 exceeded that of AST-120 for all toxins, including creatinine, IS, PCS, and HA. Overall, the results from Figs. 11-14, demonstrate the versatility and effectiveness of Batch 36 in adsorbing a range of uremic toxins under varying experimental conditions. Such insights are crucial for optimizing the design and application of ACB-based therapies for the treatment of CKD and other related conditions.

The ability of the ACB to adsorb indole was also investigated. Indole is a precursor to the IS toxin. Indole is generated from tryptophan, a natural amino acid sourced from protein diets, and metabolized by intestinal bacteria, such as *E. coli*, in the gut. Subsequently, it is further

metabolized into IS in the liver, transferred into the bloodstream, and excreted by the kidneys in healthy individuals. However, in CKD patients, IS accumulates in the blood, exacerbating kidney function. Therefore, it is imperative to eliminate indole at its onset in the gut. In these experiments, varying concentrations of indole (40, 300, and 460 mg) were prepared in 500 mL of DI water and exposed to 1 g of ACB (Fig. 15). The experiments were conducted at RT for 2 h with agitation at 250 rpm and pH 7.2. Remarkably, almost complete adsorption of indole was observed in all experiments. These findings underscore the superior efficiency of Batch 36 in capturing the precursor IS toxin.

Summary

In the present disclosure, systematic design and fabrication of ACBs are described, together with the application of the ACBs as a sorbent for use in a dialysis device and as an oral sorbent. Firstly, an appropriate raw material, poly(styrene-*co*-divinylbenzene) with 95% cross-linking, was identified. Secondly, a rigorous screening to optimize conditions for the carbonization and activation processes was conducted with the aim of providing ACBs with the desirable properties. Subsequently, the resulting ACBs were characterized by FESEM and BET analysis to assess their suitability for the intended application in CKD. Thirdly, the applicability of the ACB as an alternative material to the current ACP used in a PD device was assessed. This involved scrutinizing adsorption studies using both small-sized (e.g., creatinine) and medium-sized (e.g., B2M) toxins in dynamic tests, including 1.5 g and full-scale 50 g columns, as well as static tests such as shaking on a shaker.

Finally, the applicability of the ACB as an oral sorbent for the adsorption of small-sized uremic toxins (e.g., creatinine, indole, IS, PCS, and HA), as an alternative material to the existing commercial AST-120 was investigated. This involved conducting various experiments to study the influence of toxin concentrations (either individual or mixture), ACB concentration, duration or dose of ACB, percentage increase in adsorbance, and the effect of pH on adsorbance. The efficiency of the ACB of the present invention was also compared with other available reference samples such as Calgon ACP, AST-120, and Ambersorb 560, where the ACBs of the present invention were found to be superior. Overall, our experimental designs and results highlight the versatility and effectiveness of the ACB of the present invention in adsorbing a range of uremic toxins under varying experimental conditions. Such insights are crucial for optimizing the design and application of ACB-based therapies for the treatment of CKD. These findings also underscore the importance of exploring diverse treatment options and optimizing approaches to address the multifaceted challenges faced by CKD patients. Table 5 provides a comparative overview of previous ACB productions alongside the ACB of the present invention.

Table 5: Production and characteristics of previous ACBs and comparison with the ACBs of the present invention.

ACB	Starting Material	Production Method	Characteristics
AST-120 (Kremezine)	Petroleum-based pitch prepared from naphthalene	Pyrolysis of Petroleum pitch at 600-1000 °C, N_2. Activation: Steam activated at 850-1000 °C. ACB is produced by two methods: The first method is, for example, Japanese Patent Publication No. 51-76. U.S. Pat. No. 3,917,806 and Japanese Unexamined Patent Publication No. 54-89010 (U.S. Pat. No. 4,761,284). First, the pitches in the molten state in the form of small spherical particles are infusibilized with oxygen, and then, It is carbonized by heating at a temperature of 600 to 1000 ° C. in an inert atmosphere, and then 850 to 100 in a steam atmosphere. This is a method of activation at a temperature of 0 ° C. The second method is, for example, Japanese Patent Publication No. 59-10930 (US Pat. No. 442). No. 0433), first, crush the string-like pitches in a molten state, put them in hot water to make them spherical, and then make them infusible with oxygen, and then It is a method of carbonizing and activating under the same conditions as the first method.	Size: 50-2000 μm (esp. 100-1000 mm) specific surface area of 500-2000 (esp. 700-1500) m^2/g Content of voids each having 100-75000 Å pore radius of 0.01-1 (esp. 0.05-0.8) ml/g Absorb only small molecules like creatinine, indole, *p*-cresol sulfate etc. It does <u>not</u> bind β2-microglobulin (B2M)
DW-7202 (Renamezine)	Furan resin-based formula	Not known	Size: 200-500 μm
Ambersorb 560	Not Known	Not known	Size: 300-710 μm Density: 0.53 Surface are: ≥ 450 m^2/g

ACBs of present invention	Poly(styrene-co-DVB) with surface functionalized with sulfonic acid.	Pyrolysis of PS bead at 900 °C, 6 h, argon Activation: CO_2 activated at 900 °C, 10 h	Size: 300-350 μm Density: 0.35-0.5 Surface are: 1300-2800 m^2/g. Absorb small molecules like creatinine, indole, *p*-cresol sulfate, and also proteins such as beta-2-microglobulin.