UREA AND URIC ACID ADSORPTION BY NANOPOROUS BIOMATERIALS

by

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DEDICATION

I would like to dedicate this thesis to

my wonderful family...

my father,

my mother,

my sisters

and my grandparents who almost got to see me graduate.

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LIST OF ABBREVIATIONS

AC	: Activated carbon
ACF	: Activated carbon fibre
AEAPTS	: N-(2-aminoethyl)-3-aminopropyltrimethoxysilane
APTES	: (3-Aminopropyl)triethoxysilane
ARF	: Acute renal failure
BET	: Brunauer–Emmett–Teller
BJH	: Barrett-Joyner-Halenda
Ca^{+}	: Calcium ion
CAC	: Commercial activated carbon
CKD	: Chronic kidney disease
C_{MAX}	: Maximum uremic concentration
C_N	: Normal / healthy uremic concentration
CTAB	: Cetrimonium bromide
C_U	: Uremic concentration, concentration of ESRD patients
DI	: Deionised
DFT	: Density Functional Theory
EDX	: Energy dispersive x-ray spectroscopy
EFB	: Empty fruit bunch
ESAO	: European Society of Artificial Organs
ESRD	: End stage renal disease
EUTox	: European Uremic Toxin Workgroup
FESEM	: Field emission scanning electron microscope
FSM-16	: Folded Sheets Mesoporous Materials No. 16

FTIR	: Fourier transform infrared
GAC	: Granular activated carbon
HAp	: Hydroxyapatite
HCl	: Hydrochloric acid
HD	: Hemodialysis
HP	: Hemoperfusion
HRTEM	: High resolution transmission electron microscope
IUPAC	: International Union of Pure and Applied Chemistry
KBr	: Potassium bromide
MCM-41	: Mobil Composition of Matter No. 41
MS	: Mesoporous silica
N_2	: Nitrogen gas
PAC	: Powdered activated carbon
PO_4^{3-}	: Phosphate ion
RRT	: Renal replacement therapy
SBA-15	: Santa Barbara-15
TEM	: Transmission electron microscopy
TEOS	: Tetraethyl orthosilicate
TGA	: Thermogravimetric analysis
TMOS	: Tetramethyl orthosilicate
TPOS	: Tetrapropyl orthosilicate
WAK	: Wearable artificial kidney
XRD	: X-ray diffraction

LIST OF SYMBOLS

C	: concentration (mol/L)
C_i	: initial concentration (mg/L)
C_t	: final concentration (mg/L)
m	: mass (g)
M	: molarity (mol/L),
MW	: molecular weight (g/mol).
Q	: amount of adsorbed (mg/g)
V	: volume of the solution (L)
y	: adsorbed at time (t)
A	: apparent adsorption capacity (mg/g)
k	: nominal rate constant (mg/g/s)
M/a	: normalised surface area adsorption capacity (molecules/nm^2)
A1	: adsorption capacity (mg/g)
k1	: nominal rate constant of adsorption (mg/g/s)
A2	: desorption capacity (mg/g)
k2	: nominal rate constant of desorption (mg/g/s)

PENJERAPAN UREA DAN ASID URIK OLEH

BAHAN BIO BERLIANG NANO

ABSTRAK

Kelemahan hemodialisis zaman ini telah menjadi punca penyelidikan dan pembangunan beberapa prototaip ginjal buatan mudah alih. Komponen utama model mudah alih ini (berbanding hemodialisis) ialah sistem dialisat tertutup. Secara tipikalnya, model-model ini biasanya menggunakan karbon teraktif sebagai bahan penjerap. Penggunaan bahan penjerap yang lebih unggul, jumlah dialisat yang diperlukan untuk penyingkiran toksin uremik boleh dikurangkan dan dikitar semula. Motivasi utama bagi penyelidikan ini ialah kekurangan pemilihan bahan untuk bahan penjerap dalam model-model ini. Oleh itu, objektif projek ini ialah sintesis dan penilaian tiga jenis biobahan berliang nano yang baru, iaitu gentian karbon teraktif berongga (ACF), silika berliang meso (MS) dan hidroksiapatit berliang meso, dengan sasaran untuk penyingkiran konstituen utama toksin uremik, iaitu urea dan asid urik. ACF telah diperolehi melalui kaedah pengaktifan asid dengan menggunakan asid-asid yang berbeza; asid bukan organic sulfurik, nitrik dan fosforik; asid organik asetik dan sitrik. MS dan HAp telah disintesis melalui kaedah templat lembut dengan surfaktan Pluronics. Keputusan awal penjerapan urea telah menunjukkan bahawa MS dengan kumpulan berfungsi amina dan ACF terawat asid sulfuric adalah sangat baik (550 mg/g) berbanding dengan karbon teraktif komersil (CAC). Ujian kinetik penjerapan urea telah mendedahkan mekanisma penjerapan urea oleh ACF (jerapan fizikal) dan MS (jerapan kimia). MS amina dan diamina menjerap lebih daripada 30 molekul per nm^2 (jerapan kimia yang kuat) berbanding MS biasa, CAC dan pelbagai

ACF, yang telah menjerap kurang daripada 10 molekul per nm^2 (jerapan fizikal). Faktor-faktor utama yang mempengaruhi kapasiti jerapan ialah keliangan dan kimia permukaan yang sesuai, yang mana kedua-duanya dipunyai oleh MS amina dan diamina. Kebolehubahan permukaan berfungsi bagi MS merupakan asas untuk ujian jerapan urik asid seterusnya. Satu hipotesis peningkatan jerapan asid urik oleh MS terfungsi amina merupakan melalui tindak balas kimia asid-amina. Jerapan asid urik oleh MS tidak mengikut keluk jerapan teori. Analisa yang mendalam dengan menggunakan aplikasi MATLAB menunjukkan bahawa MS telah menjalani jerapan dan nyahrepan serentak, dengan kadar jerapan permulaan setinggi 20.3 mg/g/s berbanding gel silica dengan kadar jerapan hanya 0.39 mg/g/s. Sebagai kesimpulan, secara keseluruhannya, keputusan jerapan urea dan asid urik MS dan ACF lebih baik berbanding CAC dan gel silika komersil.

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ABSTRACT

The limitations of the present hemodialysis have led towards the research and development of several wearable artificial kidney prototypes. The most important component of the miniaturised model is the closed-system dialysate, achieved through the utilisation of solid activated carbon as adsorbents. With the application of superior alternative adsorbents, the amount of dialysate required could be reduced due to efficient regeneration. The main motivation for this project is the lack of adsorbent materials selection. Thus, this project aims to synthesise and evaluate three emerging nanoporous biomaterials, i.e. hollow activated carbon fibre (ACF), mesoporous silica (MS) and mesoporous hydroxyapatite (HAp), targeting major uremic toxin constituent urea and uric acid. ACF was obtained though the acid activation route, with variation in acid used; inorganic acids sulphuric, nitric and phosphoric; organic acids acetic and citric. MS and HAp was synthesised through soft templating route using Pluronics surfactant. Results show that amine functionalised MS and sulphuric acid treated ACF performed well in the preliminary urea adsorption capacity evaluation (550 mg/g), as compared to the control commercial activated carbon (CAC) (350 mg/g). Subsequent urea kinetics study revealed better understanding of urea adsorption mechanism by ACF and MS, whereby ACF and MS operate through physisorption and chemisorption respectively. Amine and diamine MS adsorbed more than 30 molecules per nm^2 (strong chemisorption interaction) compared to bare MS, CAC and various ACF, which adsorbed less than 10 molecules per nm^2 (physisorption). The most important factors which govern adsorption capacity are porosity and suitable surface chemistry,

both which are possessed by amine and diamine MS. The flexibility of surface functionalisation of MS is the basis of subsequent uric acid adsorption kinetics test. Amine functionalised MS is hypothesised to improve uric acid adsorption through acid-amine reaction. Uric acid adsorption by MS did not follow theoretical adsorption curve. Further analysis using MATLAB curve fit revealed that MS underwent simultaneous adsorption-desorption, with initial adsorption rates as high as 20.3 mg/g/s compared to commercial silica gel, with initial adsorption rate of 0.39 mg/g/s. As a conclusion, on a whole, MS and ACF performed better than the benchmarked CAC and commercial silica gel in terms of urea and uric acid adsorption.

CHAPTER 1: INTRODUCTION

1.1 Research Background

Currently, the commonly accepted treatment for patients' who suffer from kidney failure is hemodialysis. Nevertheless, due to the size of hemodialysis machines, patients would have to endure mobility restraint. Kidney transplant is by far the most ideal renal replacement treatment option but the number of patients usually greatly outnumbers the donors. The limited supply of available kidneys is the main reason kidney transplants are close to impossible, not to mention the donor-patient compatibility of the organ which reduces the chance for a patient to accept the treatment. Peritoneal dialysis is a smaller renal replacement setup using the patient's peritoneum in the abdomen as a membrane for dialysis, which offers better mobility and flexibility for uremic toxin removal. However, the patients are faced with risks for future complications, such as infection in the peritoneum. Patients undergoing peritoneal dialysis have better mobility as compared to hemodialysis (Diaz-Buxo et al., 1981; Olcott IV et al., 1983; Kooman et al., 2015). Though peritoneal dialysis shows better survival rates during initial stages of dialysis, the survival rates drop significantly over a longer treatment period compared to hemodialysis. Complications such as infection or peritonitis ultimately highlight hemodialysis as a better option (Sinnakirouchenan and Holley, 2011). Thus, despite all the disadvantages, hemodialysis still remains the most feasible option in terms of safety, cost and treatment efficiency.

The current hemodialysis setup which uses diffusion-based membrane technology is not efficient since uremic toxins with high molecular mass could not be removed (Vanholder et al., 2003). The residual uremic toxins which were not

removed would gradually build up in the patients' body. The retention of uremic toxin similar to a slow and gradual poisoning (uremia). Eventually, life span is shortened as the suffering period is prolonged. Each individual uremic toxin build-up in a chronic kidney disease (CKD) patient would cause its own complications. For instance, uric acid retention would cause serious problems such as gout, diabetes and leukemia (Kang, 2014). Present hemodialysis is not a perfect solution; the period of initial uremic toxin poisoning leading up until the upper concentration threshold which the human body could withstand, is merely prolonged. One other inherent problem of hemodialysis is that it is not a continuous uremic toxin removal process. High and low uremic toxin cyclic levels during treatment and non-treatment periods would cause a state of shock on a patient's body (Figure 1.1).

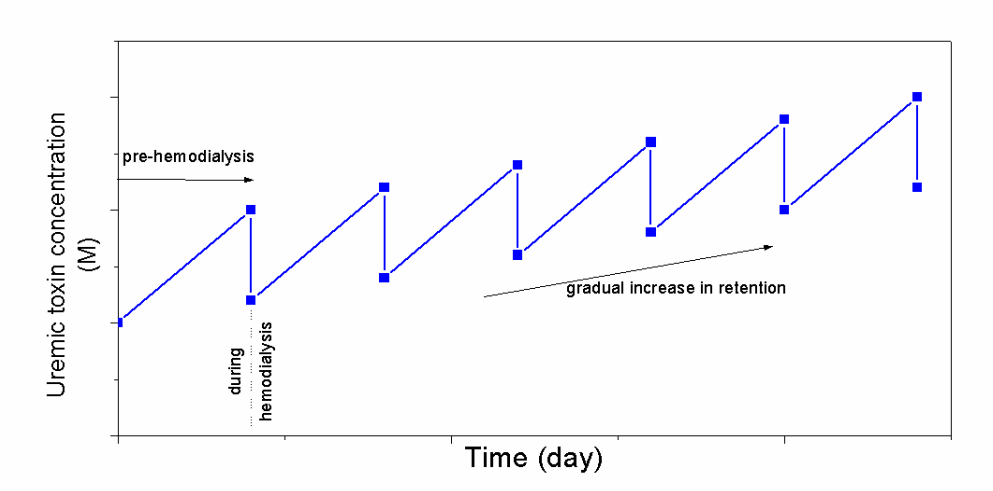

Figure 1.1: Uremic toxin retention cycle pre- and during hemodialysis

Furthermore, the mobility of patients is hindered due to their dependency on hemodialysis. Patients would have to travel to hemodialysis centres for treatment on alternate days during a week (3 times), and undergo hemodialysis treatment for 4 hours during each session, excluding travel (Dhondt et al., 2000). Patients would face difficulty in their job commitments due to constant absence from work during

treatment periods. Patients without a flexible working hour job might lose their source of income once the long-term treatment begins, which would directly cause the country to lose its workforce. They would not only become a liability to the country, but also a long-term financial burden for themselves and to the national healthcare system. In 2013, the total number of end stage renal disease (ESRD) patients in Malaysia amounts to 26,159 and is estimated to increase 4,000 yearly. Statistically, 14 out of 100,000 people suffers from kidney failure (Cheng, 2013).

The dialysis process itself is a long-term painful and expensive process, not only does CKD cause physically difficulty, but also mental stress. Hemodialysis takes a serious toll on patients, as side effects include shortness of breath, nausea and vomiting (Cukor et al., 2013; Vasilopoulou et al., 2015). Patients are also prone to mental side effects as depression since most of them have to make drastic changes in their home or work life and even to the extent of giving up certain responsibilities or activities. Patients no longer have the ability being away from designated dialysis centres, let alone the luxury to travel. Due to its bulky size and imperfect uremic toxin removal, hemodialysis is merely a life support once a patient's kidneys lose their functionality.

In the recent decade, wearable artificial kidney models (based on present hemodialysis design) are developed to solve the shortcomings of hemodialysis. At present, the frontrunner model developed by Gura and co-workers are undergoing clinical trials since early 2010 (Gura et al., 2009; Gura and Rambod, 2010; Davenport, 2015). However, the Gura prototype is still under research and mainly utilises ordinary activated carbon as the adsorbent. These miniaturised models are made possible due to the key component designed in these models, i.e. dialysate regenerative system. The bulk amount of dialysate fluid is regenerated using only

activated carbon as a solid sorbent. Without a deep understanding of biomaterials engineering and adsorbent–adsorbate chemistry, the load/capacity, efficiency and effectiveness of the mentioned prototype is limited and rigid, i.e. degree of miniaturisation is inhibited by the adsorbent design.

1.2 Problem Statement

Firstly, the limitations of the conventional hemodialysis, based on brief literature covered in the previous section (Section 1.1 Research Background), i.e. bulky device which hinders patient mobility, could be overcome by the introduction of wearable artificial kidney models. Table 1.1 shows the comparison between the hemodialysis and an ideal artificial kidney. Hemodialysis based wearable artificial kidney systems are still at clinical trial stages at best. The introduction of nanoporous adsorbent in the form of activated carbon could effectively reduce the amount of dialysate fluid through active and continuous adsorption of uremic toxins from the dialysate fluid.

Table 1.1: Hemodialysis and ideal artificial kidney

Hemodialysis	Ideal artificial kidney
Intermittent	Continuous
Retention of middle molecules	Indiscriminate removal of uremic toxins
Retention of protein bound uremic toxins	
Bulky	Portable
	Light weight

Secondly, the materials selection for the choice of adsorbent used for dialysate regeneration has been limited to only activated carbons, as evidently employed in all reviewed patents. The only alternative nanoporous adsorbent studied,

aside from activated carbon (Gura et al., 2009), is zeolite (Wernert et al., 2006; Wernert et al., 2005). These reports indicate that zeolite is better suited for the adsorption of middle molecules such as creatinine and p-cresol. The uremic toxins targeted in this study are urea and uric acid, which belong to the uremic toxin group of small molecules. The study on alternative nanoporous biomaterials which could produce better uremic toxin adsorption results would be highly valuable as the wearable artificial kidney models could be further miniaturised through reduced dialysate amount used in such systems. The amount of adsorbent materials for the dialysate regenerative system could also be reduced, thus a significant reduction in size and weight of the wearable device. The indication on the adsorption performance of a material used in this project is the mass of uremic toxin removed by a fixed amount of adsorbent, expressed in the form of mg/g. A higher number in such case would indicate more uremic toxin removed by the same amount of adsorbent. Apart from scalability size-wise, the duration of use for these higher adsorption nanoporous materials could be extended, i.e. the frequency of change of adsorbent materials (in cartridge form) is prolonged for the same amount of material. This project is focused on the development of improved alternative nanoporous adsorbents, i.e hollow activated carbon fibre, mesoporous silica and mesoporous hydroxyapatite, for the intended application of uremic toxin adsorption for wearable artificial kidney systems. Each of the three nanoporous biomaterials were selected based on the individual improvements in terms of physical properties (surface area) and chemical functionality (surface functional group and biocompatibility).

Hollow activated carbon fibre was selected as an alternative nanoporous adsorbent material due to its higher surface area and shorter diffusion length. The hollow macropore channels enables shorter access of flowing adsorbates into the

micropores of this activated carbon, i.e. better adsorption kinetics (Bandosz, 2006). Access to micropores for conventional powdered or granular activated carbon are more restricted in comparison due to the lack of macropore (only mesopores). Mesoporous silica on the other hand, was selected due to the fact that the flexibility of surface functionalisation (Matsumoto et al., 2006). Active functional groups could be grafted and anchored on the surface of mesoporous silica to selectively target identified functional groups of uremic toxins urea (amine group) and uric acid (carbonyl group). The third nanoporous biomaterial, mesoporous hydroxyapatite, was selected due to its excellent biocompatibility (Akao et al., 1981; Selvakumar et al., 2015) and good protein adsorption (Shen et al., 2008).

1.3 Research Objectives

The purpose of this research is to explore potential alternative biomaterials for uremic toxin adsorption. Material selection for this application is limited to activated carbon as the sole adsorbent on this list. Research objectives for this project are:

1. To synthesise and characterise three nanoporous biomaterials, i.e. activated carbon fibre, mesoporous silica and mesopores hydroxyapatite for urea and uric acid adsorption.
2. To evaluate the urea adsorption capacity of activated carbon fibre, mesoporous silica and mesopores hydroxyapatite.
3. To study the kinetics and mechanisms of urea and uric acid adsorption by activated carbon fibre, mesoporous silica and mesopores hydroxyapatite.