Review

Urea removal strategies for dialysate regeneration in a wearable artificial kidney

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ARTICLE INFO

Keywords:
Artificial kidney
Urea
Sorbent
Urease
Electro oxidation
Hemodialysis

ABSTRACT

The availability of a wearable artificial kidney (WAK) that provides dialysis outside the hospital would be an important advancement for dialysis patients. The concept of a WAK is based on regeneration of a small volume of dialysate in a closed-loop. Removal of urea, the primary waste product of nitrogen metabolism, is the major challenge for the realization of a WAK since it is a molecule with low reactivity that is difficult to adsorb while it is the waste solute with the highest daily molar production. Currently, no efficient urea removal technology is available that allows for miniaturization of the WAK to a size and weight that is acceptable for patients to carry. Several urea removal strategies have been explored, including enzymatic hydrolysis by urease, electro-oxidation and sorbent systems. However, thus far, these methods have toxic side effects, limited removal capacity or slow removal kinetics. This review discusses different urea removal strategies for application in a wearable dialysis device, from both a chemical and a medical perspective.

1. Introduction

End stage kidney disease (ESKD) is a severe life-threatening disease that affects approximately 3 million patients worldwide [1]. Kidney transplantation is the optimal treatment, but the median wait time for a first kidney transplant is 3.6 years in the US [2], graft failure rate is high [3] and not every patient is eligible for kidney transplantation. Thus, the majority of patients with ESKD relies on hemodialysis (HD) or peritoneal dialysis (PD) to replace kidney function for a certain period of time (Fig. 1). The quality of life of dialysis patients is poor due to a high morbidity and high treatment burden [4]. The vast majority of patients (88% [5]) is treated with intermittent in-center HD (3 × 4 h per week), resulting in inadequate removal of waste solutes and excess water, which are normally excreted continuously by the healthy kidneys via the urine. Accumulation of waste solutes causes uremic symptoms, such as nausea and pruritus (itchy skin) that markedly decrease patients' well-being. Importantly, retained waste solutes exert toxic effects to multiple organs, particularly those of the cardiovascular system [6,7]. Fluid overload is associated with hypertension, heart failure and mortality [8]. To limit accumulation of water and waste solutes, dialysis patients have to adhere to strict fluid and dietary restrictions, which further compromise their quality of life [9,10]. In addition, the time spent travelling to the dialysis center and dialysis procedure, significantly limits patients' freedom and autonomy. PD provides more continuous renal replacement therapy outside the hospital. However, the level of blood purification (or clearance, i.e. the volume of body water that is cleared of a solute per time unit) is relatively low for patients that undergo this treatment [11]. Moreover, most patients can be treated with PD for only a limited period of time due to functional decline of the peritoneal membrane, primarily caused by exposure to toxic high glucose concentrations in PD solutions, and recurrent infection of the peritoneal membrane. As a consequence, patients that are treated with PD have to switch to HD after a median of 3.7 years [12].

A wearable artificial kidney (WAK) that could provide more frequent (up to permanent) high toxin clearance outside the hospital
would be a considerable improvement for both HD and PD patients. Such a small dialysis device, preferably less than 2.0 kg when worn on the body [13] or less than 11 kg when used as a table device (i.e. the advised maximum weight to lift for PD patients [14]), will substantially increase patients’ mobility, freedom and ability to engage in social and economic life. Importantly, more frequent or continuous treatment with a WAK will possibly reduce waste solute concentrations and fluctuations of patients’ internal environment and fluid status and may therefore improve clinical outcomes, in terms of quality of life, survival, hospitalization rates, left ventricular mass, blood pressure, bone mineral metabolism and medication burden, as observed with more frequent or prolonged HD [15–19].

The key for the development of a WAK is efficient regeneration of dialysate from patients (i.e. spent dialysate) in a closed-loop system (Figs. 1 and 2). Regeneration of spent dialysate will allow for the use of a small volume of dialysate (ideally less than 0.5 L), whereas conventional single-pass HD uses a large volume of dialysate (approximately 120 L per 4-h HD session) typically produced by a stationary water treatment system. Traditional PD requires a considerable volume of bagged dialysis fluids (8–12 L per day) to be stored at the patients’ home. Dialysate regeneration entails removal of uremic solutes from spent dialysate and maintenance of stable dialysate pH and electrolyte concentrations. The solutes that need to be removed to regenerate the dialysate are uremic waste solutes that are normally excreted by the healthy kidneys and are transported to the dialysate during dialysis. This includes ions, such as phosphate and potassium which accumulation leads to vascular calcification and (lethal) arrhythmia, respectively, and organic solutes, such as small nitrogenous waste products (e.g. urea, creatinine) and low molecular weight proteins (e.g. β2-microglobulin, a protein associated with dialysis related amyloidosis). Of note, highly protein bound uremic toxins are hardly removed by dialysis in general since these solutes are primarily bound to albumin (66 kDa) that does not pass the semipermeable membrane separating the dialysate and the blood. Phosphate and potassium ions are removed from spent dialysate by ion-exchangers and most organic waste solutes can efficiently be removed by activated carbon (AC) [20,21]. Importantly, the affinity of AC for urea is relatively low (0.1–0.2 mmol/g, see paragraph 5) [22,23] while daily molar production of urea is higher than that of other waste solutes (240–470 mmol per day) [24,25]. As a consequence, the greatest challenge for efficient dialysate regeneration is urea removal [26]. The aim of this review is to discuss strategies to remove urea from dialysate that can be applied in a WAK (i.e. enzymatic hydrolysis, electrochemical oxidation (EO), chemisorption and physisorption) and the advantages, disadvantages and possible improvements of these methods both from a chemical and clinical perspective.

2. Urea

In the human body, proteins and other nitrogen-containing nutrients are metabolized into ammonium, which is converted in the liver into urea via the urea cycle [27]. Urea is transported to the kidneys through the bloodstream, where it is excreted in the urine [28]. Urea is the main circulating non-protein nitrogen compound and accounts...
cyanate with H₂O is very slow and ammonium and cyanate fragments remain present to a minor extent. However, under physiological conditions, the reaction of cyanate with H₂O under acidic conditions to form CO₂ and NH₃ and cyanate (0.1–0.8%) (reaction (1)) [31]. In principle, cyanate can be in equilibrium with traces of the fragmentation products ammonium and bicarbonate and/or acetate anions. Finally, calcium, magnesium and potassium ions (in the form of their chloride salts) are infused to reconstitute the dialysate.

In general, urea is a very unreactive metabolite: urea is uncharged at physiological pH and is neither very nucleophilic nor electrophilic. To enable urea to act as a nucleophile, a very strong electrophile is needed (as discussed in paragraph 5). The spontaneous hydrolysis of urea (thus acting as an electrophile) in aqueous solution is very slow unless catalyzed by urease (as discussed in paragraph 3). In aqueous solution urea is in equilibrium with traces of the fragmentation products ammonium and cyanate (0.1–0.8%) (reaction (1)) [31]. In principle, cyanate can further react with H₂O under acidic conditions to form CO₂ and NH₃ (reaction (2)). However, under physiological conditions, the reaction of cyanate with H₂O is very slow and ammonium and cyanate fragmentation products remain present to a minor extent.

\[
\begin{align*}
\text{H}_2\text{N}^+\text{NH}_2 &\rightarrow \text{NH}_4^+ + \text{N}^\ominus\text{C}^\ominus\text{O} & (1) \\
\text{H}_2\text{O} + \text{N}^\ominus\text{C}^\ominus\text{O} &\rightarrow \text{CO}_2 + \text{NH}_3 & (2)
\end{align*}
\]

Urea is a chaotropic agent that can disrupt the globular structure of proteins by breaking hydrogen bonds, thereby altering protein and enzyme function [29,32]. Although mildly elevated urea concentrations in the range of 10–20 mM are well tolerated, uremic concentrations above 20 mM have been associated with toxicity, including insulin resistance, disruption of the gastrointestinal barrier which may result in leakage of pro-inflammatory mediators from the gut into the bloodstream, production of radical oxygen species, induction of apoptosis and death of smooth muscle cells, and endothelial changes promoting atherosclerosis [29]. These effects are either caused by urea directly, by cyanate or ammonium (reactions (1) and (2)), or as a result of the reaction of isocyanic acid (conjugated acid of cyanate) with arginine or lysine residues in proteins (carbamylation) [33], as shown in reaction (3).

\[
\begin{align*}
\text{Protein-}\text{NH}_2 + \text{HN}^+\text{C}^\ominus\text{O} &\rightarrow \text{Protein-}\text{NH}_2 + \text{CO}_2 + \text{NH}_3 & (3)
\end{align*}
\]

Carbamylated proteins cause a variety of biochemical alterations, including transition of mesangial cells into a profibrogenic cell type, altered leukocyte response (caused by carbamylated collagen), vascular damage (caused by carbamylated low-density lipoprotein) and abnormal erythropoietin response (caused by carbamylated erythropoietin). Therefore, high urea concentrations should be avoided [29]. To remove the daily urea production of 240–470 mmol [24,25], while keeping the urea concentration below 20 mM with a daily 8-h dialysis regime, urea clearance during dialysis should be at least 25–49 mL/min, respectively, resulting in a 24-h time-averaged clearance of 8–16 mL/min.

3. Enzymatic hydrolysis of urea

3.1. Ureases

Krajewska published extensive reviews on ureases, their binding pockets, catalytic properties and their inhibitors [34,35]. Most ureases found in plants and fungi are present as a trimer or hexamer of subunits with a molecular weight of ca. 90 kDa per subunit and have a hydrodynamic diameter of ca. 14 nm [34,36]. Ureases use a zinc-ion to coordinate the carbonyl group of urea, making the carbonyl group more electrophilic. While urea is very stable in physiological aqueous solutions towards chemical hydrolysis (pH 7.4 and 37 °C) [34], urease-catalyzed hydrolysis of urea into ammonium and bicarbonate (reaction (4)) is very efficient. Urease derived from jack beans (Canavalia ensiformis) is one of the most active ureases. This enzyme follows Michaelis-Menten kinetics, has a relatively low Kᵢ of 2.9–3.6 mM, i.e. the substrate concentration at which urease activity is 50% of the maximum activity, and is most active at a pH of 7.0–7.5 [34]. The turnover rate of urease is up to 5.9 × 10³ s⁻¹ [37–39], while the rate of uncatalyzed urea hydrolysis is too slow to determine experimentally and independent of the pH in the range of 4–10 [40,41]. Since the dialysate urea concentration is ~10–30 mM (thus, far above the Kᵢ) and pH of the dialysate is around 7.4, the activity of urease is close to Vmax in dialysate. Based on these values, in theory, only ~3–5 mg of active urease is sufficient for complete removal of urea from dialysate during a 4-h dialysis session at dialysate flow rates up to 300 mL/min and urea concentrations ranging from 2 to 40 mM. However, it was found empirically that ~30–50 g of immobilized urease (including matrix) is needed to accomplish this (unpublished data). Therefore, enzymatic urea hydrolysis would be a very attractive approach to remove urea from dialysate [42,43]. However, the formed ammonium is much more toxic than urea itself. Therefore, a urea removal strategy based on urease should be complemented with a strategy to remove ammonium, as discussed in the “the REDY sorbent system” section.

\[
\begin{align*}
\text{H}_2\text{N}^+\text{NH}_2 + 2\text{H}_2\text{O} + \text{H}^+ &\rightarrow \text{HO}^\ominus\text{O} & (4)
\end{align*}
\]

3.2. Urease immobilization

Immobilization of urease onto a solid support is essential for the development of a urease-based WAK. Urease immobilization has been reviewed by Krajewska [35] and can be achieved by chemical (covalent bonding) or physical immobilization (non-covalent bonding). In general, immobilization reduces Vmax and increases Kᵢ. A higher urease activity level is usually maintained by physical immobilization as
compared to chemical linkage to a carrier. Urease has been chemically immobilized on porous chitosan/glutaraldehyde beads [44], poly(N-isopropylacrylamide-co-N-acryloxsuccinimide-co-2-hydroxyethyl methacrylate) hydrogels [45], polyacrylonitrile hollow fibers [46,47], beads based on poly(acrylamide-co-acrylic acid) [48], polyvinylalcohol thiacrylate) hydrogels [45], gelatin [50], microporous epoxy-functionalized methacrylamide copolymer (Eupergit®) [51] and cellulose [52]. In addition, it has been physically immobilized on AC, Al₂O₃ and zirconium phosphate [53,54]. The different immobilization methods are associated with varying strengths of the bond between support material and enzyme. Physical immobilization is weakest, and is considered easily reversible. Chemical immobilization is generally better, although certain covalent bonds used for chemical immobilization, e.g. the imine-linkages formed by the reaction of glutaraldehyde with amino-groups of urease, are hydrolyzable (i.e. reversible) under the conditions found in dialysate. The preferred option for application in a wearable artificial kidney is therefore immobilization through non-hydrolyzable chemical linkers, for example through amine or ether bonds formed between urease and epoxy-substituted support materials.

Depending on the reaction conditions, epoxy-substituted support materials can form covalent bonds with carboxylic acid-, thiol-, amino- or phenolic groups of the enzyme. Bortone et al. reported that single-point immobilization of urease on Eupergit® by epoxy coupling slightly reduced the enzyme’s binding constant (Michaelis constant) $K_M$ for urea from 3 mM to 5 mM [34,51] while the constant for the conversion of urea to ammonium ($k_{cat}$) remained approximately unchanged. The activity of the enzyme was reduced by factor 2 upon immobilization as a result of limited diffusion of urea (and therefore a local urea concentration lower than $K_M$ in the sorbent).

### 3.3. The REDY sorbent system

Until now, the REcirculation DialYSIS (REDY) sorbent system [55], which contains urease (derived from jack beans and physically immobilized on aluminum oxide (Al₂O₃)) for enzymatic conversion of urea, is the only dialysate regeneration system that has been marketed. From 1973 to 1994, more than six million treatments were successfully performed with this transportable (approximately 20 kg) dialysis system, demonstrating the clinical feasibility of HD with dialysate regeneration [56]. However, manufacturing of the REDY sorbent system was discontinued in 1994. The relatively high costs of the disposable sorbent cartridges, inferior treatment adequacy compared to single-pass dialysis as a result of limited dialysate flow rates (max. 250 mL/min) and concerns about aluminum-induced osteomalacia (bone softening) and dementia may have contributed to this [57-61].

Dialysate regeneration with the REDY sorbent system has been described in detail by Agar [20], Ash [21] and others [62-64]. In brief, dialysate passes several sorbent layers, starting with AC which adsorbs non-urea organic compounds. Next, urea is hydrolyzed by immobilized urease into ammonium and (bi)carbonate. Subsequently, ammonium is exchanged for sodium or hydrogen cations by a zirconium phosphate ion-exchanger [65]. Finally, zirconium oxide and zirconium carbonate adsorb phosphate in exchange for hydroxide, bicarbonate and acetate. Even though urease (hydrodynamic diameter ca. 14 nm [34,36]) cannot pass the dialysis membrane (cut off 5-8 nm [66]), it is important that urease is immobilized upstream of the zirconium phosphate layer to prevent ammonium release into the patient. A schematic overview of the REDY sorbent system is shown in the review by Roberts [42].

The REDY system, however, has several drawbacks. First, following reaction (1), for every equivalent of urea, two equivalents of ammonium are formed. Thus, the daily urea production of 240–470 mmol [24,25,67] is converted into 480–940 mmol of ammonium. Moreover, ammonium is more toxic than urea [68] and a relatively large amount of zirconium phosphate (~0.5–1 kg) is required to (almost) completely bind this amount of ammonium. Second, zirconium phosphate does not only bind ammonium, but also calcium, magnesium and (too much) potassium ions, resulting in the necessity to re-infuse these adsorbed cations from a separate reservoir [69]. Although this allows for a personalized dialysate prescription by adjustment of the calcium, magnesium and potassium ion concentrations to the patients’ need, the re-infusion reservoir further increases the size and weight of the device. Third, the adsorbed cations are exchanged for hydrogen and sodium cations. The released protons may (partially) react with bicarbonate generated during urea hydrolysis to form water and carbon dioxide, which can be effectively removed from the dialysate circuit via a degasser [70]. Bicarbonate release into the spent dialysate is in fact favorable as it may correct for metabolic acidity, a common complication of kidney failure due to impaired excretion of non-volatile acid.

However, sodium release is a major concern as higher dialysate sodium concentrations are associated with weight gain between dialysis sessions and related complications such as hypertension [71]. To prevent a rise in dialysate sodium concentration, a sodium-free dialysate reservoir could be used to dilute the released sodium ions, although this would be at the expense of miniaturization.

In addition to the miniaturization issues, long-term treatment with the REDY system has been associated with a severe form of fracturing osteomalacia and encephalopathy (brain injury) due to leaching of aluminum ions from AC that was often contaminated with this metal [58,59,61,72,73]. In 1982, cartridges became available with reduced aluminum content that did not show aluminum release above the maximum allowable level of 0.37 μg in water for dialysate preparation according to the Association for the Advancement of Medical Instrumentation (AAMI) [74,75]. Still, urease physically immobilized onto Al₂O₃ is considered a potential hazard for chronic dialysis patients. These drawbacks and non-cost competitiveness with single-pass HD, resulted in the disappearance of the REDY system from clinical practice in 1994 [42].

### 3.4. “Second generation” urease-based wearable artificial kidney devices

Several research groups are currently working on REDY-like sorbent system for the development of miniature dialysis systems for both HD and PD using disposable cartridges [76]. These second-generation urease-based systems are characterized by a more stable sodium and acid-base profile compared with the original REDY sorbent system. Best known is the WAK as developed by Gura et al., which has been evaluated during two pilot clinical trials [69,77]. In the most recent trial in 2016, seven HD patients were treated with the WAK up to 24 h while being mobile [69]. The device weighed approximately 5 kg, including dialysate and sorbents (65 g physically immobilized urease on Al₂O₃, 600 g zirconium phosphate, 51 g hydrous zirconium oxide and 153 g AC). Mean plasma clearances of urea, creatinine, phosphate and β₂-microglobulin per 24-h treatment compared favorably to time-averaged plasma clearances of intermittent HD [11,78]. Importantly, no serious adverse events were observed. However, several urease related technical problems were reported. In one patient after 11 h of treatment, dialysate ammonium concentration exceeded 2.9 mM, which was set as the maximum allowable concentration, indicating zirconium phosphate saturation. Plasma sodium concentration remained stable during the first 16 h of treatment (~130 mM) and tended to increase to 135 ± 4 mM (p = 0.13) after 24 h of treatment (sodium balance was not reported). Other encountered problems were excessive carbon dioxide bubbles in the dialysate circuit exceeding degassing capacity, formation of blood clots in the blood circuit and technical issues such as tubing kinking and early battery failure, which prompted early termination of the trial [11,78].

Most other miniature dialysis systems that are currently under development make use of a modified REDY-type sorbent system for dialysate regeneration [76]. AWAK Pte Ltd (Singapore and Burbank, CA) recently evaluated an automated wearable artificial kidney (AWAK PD™, <2 kg [79]) for PD during a First-In-Human clinical trial in 14 PD patients [80]. AWAK treatment for >10.5 h per day up to three days
resulted in a significant decrease in urea, creatinine and phosphate plasma concentrations from 20.8 to 14.9 mM (p = 0.001), 976 to 668 μM (p = 0.001) and 1.7 to 1.5 mM (p = 0.03), respectively. A weekly peritoneal Kt/Vurea (the volume cleared of urea over the total urea distribution volume) of >1.7 was achieved, which is the minimum Kt/Vurea in anuric patients recommended by the International Society of Peritoneal Dialysis guidelines [81]. Although no serious adverse events occurred, 73% of subjects experienced temporary abdominal discomfort, which resolved spontaneously after dialysate drainage or bowel movement. Importantly, plasma sodium, potassium and bicarbonate concentrations were stable, although systemic ammonium concentrations were not reported.

A schematic overview of a urease-based sorbent purification system is shown in Fig. 2.

4. Electrochemical decomposition of urea

In 1966, Tuwiner published a report on a water reclamation system for manned space vehicles that used electrochemical treatment to remove urea from urine [87]. This report initiated research on electro-oxidation (EO) of urea for dialysate regeneration (see Table 1 for an overview of studies). A schematic representation of an EO-based purification unit is shown in Fig. 3, in which AC is placed upstream of the EO-unit to remove most organic waste solutes. EO is in principle an attractive technique for a WAK because it converts urea into gaseous products (nitrogen, hydrogen and carbon dioxide) that can easily be removed from dialysate by a bubble trap, and, importantly, EO-modules are small in size, lightweight, have a long life time and are relatively inexpensive and potentially reusable. Evaluation of an EO-based WAK (6 A/h) in uremic goats showed that 24-h treatment would suffice to remove the daily urea production [88], which requires ~100 g of battery that delivers 25 W per hour (or, for example, 4 batteries of 600 g per day). However, the challenge for EO is control of the exact reactions and formed products, because besides the mentioned gasses also other, mainly toxic, compounds are generated, such as active chlorine species and chloramines that need to be removed by placing AC downstream of the EO-unit (Fig. 3).

4.1. Reactions and products

Direct EO of urea converts urea into nitrogen gas (N₂), carbon dioxide gas (CO₂) and hydrogen gas (H₂) (reaction (5)). This reaction is the net result of urea oxidation at the anode and simultaneous reduction of the reduced species at the cathode. In Table 1, an overview of studies is given. The current density and applied potential indicate anodic and cathodic reactions, respectively. The EO-modules are small in size, lightweight, have a long life time and are relatively inexpensive and potentially reusable.

Table 1

| Anode material | [Urea] (mM) | Applied current density (mA cm⁻²) or potential (V) | Urea removal per unit area (mmol/h/m²) | In vitro (S,D, U, aU), or in vivo (V) | Reference |
|----------------|------------|--------------------------------------------------|----------------------------------------|-------------------------------------|----------|
| Pt 8.7         | 0.5 mA cm⁻²| 2.4–5.3                                          | S                                      | [99] |
| Pt 8.3         | 6–18 V     | 4.210⁻¹⁷–7.310³                                  | D                                      | [100] |
| Pt 10 a) 0.11 mA cm⁻² b) 0.22 mA cm⁻² e) 0.8 V d) 1.0 V e) none | 4.6 b) 8.3 c) 8.3 d) 9.1 e) 2.9 | a) 7.4 S b) 4.6 c) 8.3 d) 9.1 e) 2.9 | [98] |
| Pt 3.3         | Not specified | 1.110⁻¹⁴–1.910²                                  | D                                      | [100] |
| Pt 33          | 7–20 mA cm⁻² | 5.110⁻¹⁰                                        | D                                      | [97] |
| C foil 42      | 25.2–39.1 mA cm⁻² 8.5–11.5 V | 1.410⁻¹⁴–1.710³                          | –                                      | [108] |
| Pt 9–33; a) 0.88 mA cm⁻² b) 1.2 V; c) 0.64 mA cm⁻²; e) -1.0 V | 1.110⁻¹⁴–1.910² a) 17–37 b) 1.4–1.5 c) 16–5.310² d) S e) 17–37 | a) S b) D c) U d) aU e) aU | [96] |
| Ru–Ti–Sn–O (RTTO) 0–500 | 4–61 mA cm⁻² | 7.10⁻¹⁰                                          | S                                      | [90] |
| Pt 12.9        | 10; 20; 30; 49; 59 mA cm⁻² | 1.910⁻¹⁰–5.110²                                  | D                                      | [95] |
| Pt 29          | 5 mA cm⁻²                                           | a) 6.10⁻¹⁰                                     | D                                      | [97] |
| Pt a) 71.4; b) Fe and Pt b) NaN | 40 mA cm⁻² | 1.410⁻¹⁴–3.410³                                   | –                                      | [109] |
| (Pt–Ir)70:30 | 0–167      | 20–100 mA cm⁻²                                   | S                                      | [110] |
| (Pt–Ir)70:30 | 2–10 mA cm⁻² | 1.410⁻¹⁴–4.810³                                  | D                                      | [105] |
| Ti/IrO₂ 266   | 15 ± 0.3 mA cm⁻²                                   | 4.410⁻¹⁰–7.110²                                 | U                                      | [112] |
| BiO³–TiO₂ 41.6 | 2.0 V     | 0.53                                              | S                                      | [113] |
| Pt a) 20; b) RuO₂ | 2.8 V   | 4.7–8.9                                          | a) 7.310²                                 | [89] |
| Pt 5–30       | 6.8–17 mA cm⁻²                                   | a) 4.510²                                     | b) S                                    | [89] |
| Graphite d) –20 | 10 mA cm⁻²                                       | a) 3.210²                                     | D                                      | [105] |
| Pt 0.03       | 5–20 mA cm⁻²                                      | a) 32–4.210²                                   | S                                      | [107] |
| Pt 17–167     | 2–10 mA cm⁻²                                      | b) –                                           | c) 61–1.010³                             | [89] |
| Ti–RuO₂ 266   | 15 ± 0.3 mA cm⁻²                                   | d) 1.710⁻¹⁴–4.810²                             | c) S                                    | [89] |
| SnO₂–Sb₂O₅ c) BDD |               | d) 3.210²                                     | D                                      | [105] |
| a) BDD 200    | 40 mA cm⁻²                                        | a) 700                                         | aU                                     | [114] |
| b) BiO₂ 30    | 5 mA cm⁻²                                         | a) 2.510²                                     | S                                      | [115] |
| c) Graphite 30 | 10 mA cm⁻²                                        | a) 2.510²                                     | D                                      | [115] |
| Graphite 7–14 | 10 mA cm⁻²                                        | b) –                                           | c) 2.510²                               | [115] |

* S, salt solution; D, unused dialysate with added urea; sD, spent dialysate; U, urine; aU, artificial urine.
of water at the cathode. When chloride ions (Cl\(^{-}\)) are present in the solution, as is the case in dialysate, the oxidation of urea can occur via a second indirect route, mediated by anodically generated active chlorine species such as hypochlorite (OCl\(^{-}\)) [89,90].

\[
\begin{align*}
\text{H}_2\text{N} &\text{NH}_2 + \text{H}_2\text{O} \rightarrow \text{N}_2 + \text{CO}_2 + 3 \text{H}_2 \\
6 \text{Cl}^{-} \rightarrow 3 \text{Cl}_2 + 6e^{-} \\
3 \text{Cl}_2 + 3 \text{H}_2\text{O} \rightarrow 3 \text{HOCl} + 3 \text{H}^{+} + 3 \text{Cl}^{-} \\
\text{H}_2\text{N} &\text{NH}_2 + 3 \text{HOCl} \rightarrow \text{N}_2 + \text{CO}_2 + 3 \text{H}^{+} + 3 \text{Cl}^{-} + 2 \text{H}_2\text{O}
\end{align*}
\]

Ideally, the indirect reactions (6)–(8) result in the same products as the direct route, i.e. nitrogen, carbon dioxide and hydrogen gas. However, in most cases, at least trace amounts of toxic side products such as nitrate (NO\(_3^{-}\)), nitrite (NO\(_2^{-}\)), ammonia (NH\(_3\)), chloramines (NH\(_4\)Cl\(_2\)) and active chlorine species are formed. Nitrate may cause hazardous products. An example is oxidation of glucose into aldehydes, which occurs more readily than oxidation of urea, as observed in a number of studies [88,95–100]. For PD, aldehydes are associated with pathological changes of the peritoneal membrane [101,102]. Consequently, to render EO suitable for dialysate regeneration, control over reactions and their products is paramount. Key parameters which can be used to control the reactions and products are the electrode material, applied potential, and applied current density. In the following paragraphs we will discuss these parameters and how they affect the formation of unwanted products. Subsequently, the efficacy of urea removal and the current efficiency will be discussed.

### 4.2. Electrode material

The electrode material has to meet several requirements. First, it needs to be chemically stable to ensure its continuous operation for at least several days. Hence, corrosion or degradation is highly undesirable. Second, the material should not leach toxic metals, as has been observed for platinum and ruthenium electrodes [89,98,103]. Third, the ideal electrode material should be selective, favoring the desired reaction and hardly catalyze undesired reactions. The electrode material determines to some extent which reactions can take place. Often, reagents need to bind to the electrode surface in a specific configuration before electron transfer between the reagent and electrode can take place. Furthermore, the potential at which reactions occur is electrode material dependent which means that the order in which reagents react can vary with the electrode material. For example, platinum is a known catalyst of many reactions, among which chloride and water oxidation which occur at lower potentials on platinum electrodes than on non-catalytic materials. Water oxidation in the WAK is undesirable, because this will greatly reduce the current efficiency (see below) and results in formation of oxygen bubbles that can block the anode. Conversely, boron-doped diamond electrodes do not catalyze water oxidation, but generate hydroxyl radicals with high efficiency [104]. Hydroxyl radicals in turn can act as mediators in the indirect oxidation of urea, just like active chlorine species [105]. Wester et al. compared platinum, ruthenium and graphite electrodes and found graphite to be the most favorable because of its acceptable urea removal rate with limited chlorine formation [89]. Researchers from Lockheed Inc. investigated over 50 anode materials for their corrosion resistance in electrochemical treatment of urine. They found that 10% rhodium-containing platinum was the optimal electrode material based on corrosion resistance and current efficiency [103]. In addition, various cathode materials were studied for urine pretreatment and platinum was selected as the best, because of its low overpotential for hydrogen evolution [103]. This means that closing the current loop by reactions at the counter electrode will readily occur through hydrogen evolution from water reduction, making it possible for this electrode to sustain large current densities without undesired side reactions taking place. Other materials that have been investigated for their applicability as anode for EO of urea, for either dialysate regeneration or waste water treatment, are listed in Table 1. Publications on waste water regeneration under physiological conditions are included, since urea removal from urine is comparable to dialysate regeneration. Recently,
nickel-based electrodes have received a lot of attention in waste water treatment due to their efficient urea oxidation capacity [106]. However, these electrodes only function at or above pH 9, and are therefore unsuitable for dialysate regeneration at physiological conditions (pH 7.4–8.0). Besides the electrode material, functionalization of the electrode surface with catalytic groups or incorporation of selective membranes may increase selectivity of the reaction at the electrode.

4.3. Oxidation potential of urea

Whether or not a species such as urea can be oxidized at the anode is primarily dependent on the applied potential, which has to be high enough to overcome the oxidation potential of urea. However, the exact oxidation potential is unknown, as discussed below. Cyclic voltammetry (CV) is routinely used to determine the oxidation potential of compounds. During CV a potential scan is performed and the resulting current is measured. When the oxidation potential is approached, oxidation of the tested compound occurs, which in turn results in an increase of the current. At higher potentials, all available molecules of the oxidizable species near the electrode surface are instantly depleted and oxidation becomes diffusion limited resulting in a decrease of the current. CV diagrams therefore often have distinctive shapes, with clear oxidation peaks, see Fig. 4A. Unfortunately, urea does not produce such a sharp oxidation peak in the CV diagram, but a small increase in current over a wide range of potentials is observed, see Fig. 4B for an example [89,91,99,110,111,112].

For platinum electrodes, changes in currents for urea were reported between 0.5 and 0.9 V vs Ag/AgCl [96,98,110,116,117]. Since chloride was present in the urea solutions and the standard potential of chloride oxidation is in the same range (0.6–1.2 V) [118], the increased currents could be due to oxidation of chloride, resulting in indirect oxidation of urea (reactions (6)–(8)). Formation of hypochlorite was not detected below 1.2 V, but this may be due to complete consumption of hypochlorite by (indirect) urea oxidation at lower potentials. Reports of CV in the absence of chloride are rare, likely because chloride is unavoidable in reported applications of EO for urea removal such as dialysis regeneration and waste water treatment. Hernandez et al. performed CV in a sodium perchlorate solution and reported increased currents on platinum electrodes between 0.7 and 1.2 V, suggesting that the oxidation potential of urea lies within this range for platinum electrodes [105]. On the other hand, using Ti–RuO2-electrodes at the same voltages, only urea adsorption and no urea oxidation was observed [105]. On boron doped diamond (BDD) and SnOx–SbO5 electrodes neither direct urea oxidation, nor adsorption of urea was observed, but water oxidation occurred, suggesting indirect oxidation of urea by the formed hydroxyl radicals [105]. The observation that urea can be oxidized by active chlorine and hydroxyl radicals suggests that the oxidation potential of urea is lower than that of chloride (0.6–1.2 V) and water (1.0 V). Nevertheless, direct oxidation of urea does not readily occur on the investigated materials under physiological conditions. This may be due to blocking of the electrode surface by strongly adsorbed urea on materials such as platinum, and lack of adsorption of urea and therefore lack of interaction with the electrode surface for other materials. Taken together, due to the presence of chloride in the dialysate and no or minimal direct oxidation of urea below chloride oxidation potentials with the electrode materials tested thus far, the best strategy for electrochemical dialysate regeneration needs to be focused on indirect urea oxidation. However, if a material would be found, which enables direct oxidation of urea, this could theoretically increase the efficiency and safety of the process. Unfortunately, such a material has not been identified so far.

4.4. Current driven and potential driven mode

Electro-oxidation can be performed in a current or a potential driven mode. This means that either the current or the potential at the anode can be set, and the other follows as a result of the setting and therefore cannot be independently selected. The magnitude of the potential difference between anode and cathode determines which reactions take place and only (combinations of) reactions with an oxidation potential lower than the potential difference between the electrodes occur.

4.5. Current driven mode and current density

In most publications a current driven mode is used, probably because this is the easiest to implement. At the electrode-electrolyte interface electrons have to be transferred to molecules or ions through a redox reaction resulting in oxidation of molecules at the anode and simultaneous reduction at the cathode. First, the most reactive species, i.e., the species with the lowest oxidation potentials, will participate in this reaction. When these species are depleted, the potential difference between the electrodes increases resulting in oxidation of less reactive species. This gives little control over the exact reactions and carries a high risk of undesired side reactions such as electrolysis of water. Since electron transfer occurs at the electrode surface, the surface area of the electrode is an important determinant of the reactions.

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1 All potentials in this review are given relative to the Ag/AgCl reference electrode (EAg/AgCl, sat KCl = 0.197 V vs standard hydrogen electrode, SHE). Where literature mentions Ag/AgCl without giving the chloride concentration, saturated KCl is assumed.
When the same current can be divided over twice the surface area, twice as many molecules of the reactive species are available for reaction. The applied current is therefore normally expressed as **current density**: the current per unit electrode area. Thus, there are two ways to decrease undesired side reactions: (1) increasing the concentration of the desired reagent, or (2) decreasing the current density. Of note, increasing the concentration at or in the vicinity of the electrode surface can also be achieved by active convection such as stirring or application of flow over the electrode surface.

In the case of indirect oxidation of urea by active chlorine species, the oxidation of chloride is desired yielding active chlorine species which in turn decompose urea, but also result in the formation of unwanted by-products such as chloramines. Controlling the ratio of urea supply vs current density is therefore of utmost importance. When the current density is too low, insufficient amounts of active chlorine will be formed, which leads to incomplete urea oxidation and accumulation of intermediate products such as chloramines. When the current density is too high, this results in excess formation of active chlorine, which increases the concentration of undesired active chlorine in the effluent. Thus, there is a delicate balance between incomplete oxidation, complete oxidation and over-chlorination. The challenge is to determine the point at which chlorine levels exceed the oxidant demand and free chlorine starts to build up in the dialysate, which is called break-point chlorination.

### 4.6. Potential driven mode and pulsed-potential techniques

In the potential driven mode, a fixed potential is applied at the anode which is in contrast with the current driven mode where there is no control over the potential difference that develops between the electrodes. The potential driven mode has been less widely used in literature, likely because it depends on a three electrode system, in which besides a cathode and anode also a reference electrode is used. In potential driven oxidation, the potential at the anode relative to the reference electrode can be used to tailor which reactions occur, since no reaction with an oxidation potential higher than the applied potential will take place. The potential at the cathode changes to accommodate the current needed to sustain the anodic potential but the potential at the cathode is otherwise not controlled. For removal of urea, the anode potential should be set at such a value that urea oxidation occurs, but not higher to prevent side reactions such as water oxidation. However, the potential at which urea oxidation occurs is relatively high (>0.7 V), which implies that not all side reactions can be avoided.

An alternative method, which may improve the selectivity of EO for urea in dialysate is the application of pulsed potentials [119,120]. For lidocaine it was found that oxidation by potential pulses (of 3.0 V vs Ag pseudo-reference electrode) considerably enhanced the yield of the reaction products as compared to a continuous potential (of 3.0 V vs Ag). Tuning of the cycle time modulated the selectivity of the oxidation reaction (more 4-hydroxylation product at cycle times of 0.2–12 s and more N-dealkylation product at cycle times <0.2 s) [119]. The increased selectivity for certain reaction paths is likely a result of a more active electrode surface, since the surface is rapidly passivated under oxidative conditions during the pulse, but recovers between the pulses at the lower potential. The same may hold for urea oxidation, where adsorption and deactivation of the platinum electrode surface is a known phenomenon [79]. In an attempt to overcome this, Yao et al. switched the potentials of anode and cathode every 20 s to repel adsorbed species from the anode, while limiting the anode potential to maximum 1.2 V and the cathode potential to minimum −1.0 V to prevent side reactions. However, although they mention that switching “provides a certain degree of selectivity”, unwanted oxidation of glucose, creatinine and uric acid still occurred [96]. Optimization of the pulse time and applied potentials may further improve selectivity.

### 4.7. Urea removal efficacy

Besides urea removal with minimal formation of unwanted toxic side products, effective urea removal is important to enable miniaturization of the WAK device. A large range of urea removal rates has been reported in literature ranging from 0.53 to 7.310^3 mmol/h/m^2 (median 3.810^2 mmol/h/m^2, interquartile range 1.310^2 to 1.110^2 mmol/h/m^2), see Table 1. Therefore, to remove a daily urea production of 240–470 mmol with a daily 8-h dialysis scheme, 0.2–3.6 m^2 of electrode surface is required. By using mesh or nano-structured electrodes, or by folding, dividing or stacking the electrodes, such electrode areas can be easily incorporated into a WAK. Moreover, nano-structuring the electrodes may even improve the catalytic activity of the electrodes [121].

The urea removal rate depends on various parameters. First, it increases with increasing current density since more species in the solution are activated [89,105,110,111]. Second, it increases with increasing urea concentration which results in a faster supply of urea to the electrodes. However, a maximum urea removal rate is reached when urea removal is limited by generation of active chlorine [88,89,110,111]. Third, urea removal is higher at higher temperature, possibly as a result of faster diffusion and thereby faster mixing of urea and active chlorine [108] or due to the increased number of molecules with a kinetic energy higher than the activation energy of the reaction. Keller et al. report 22% lower urea removal at 25 °C vs 37 °C [98,100] and others found complete urea removal only above 55 °C [108]. Finally, the cell configuration (electrode pairs in parallel or in series) affects urea removal rates. Koster et al. reported higher removal rates for a parallel configuration of four EO units than for a series configuration with equal net flow, suggesting that a longer residence time within a single EO unit is more efficient than multiple short residences on the same electrodes [92].

### 4.8. Current efficiency

For application of EO in a WAK, it is important to consider the current efficiency of urea oxidation, because it determines the size and weight of the battery. Current efficiency is defined as the ratio of the current used for urea removal to the total current through the cell. Simka et al. [110,111] observed that higher current densities resulted in lower current efficiencies, probably because at high current density supply of reagents cannot keep up with the current which is consequently wasted on side reactions. On the other hand, higher urea concentrations led to more efficient use of the active chlorine due to a higher probability to react with urea resulting in a higher current efficiency. In principle, there is an optimum current density, where supply of reagent and current are in balance. To enhance supply of urea and improve current efficiency, forced convection near the electrode surface may be applied, for example through stirring or increased flow rates, although this may require additional or larger pumps and higher power consumption, necessitating larger batteries, compromising miniaturization.

### 5. Urea sorbents

A sorbent that can specifically and efficiently bind urea would be an attractive material for a WAK because, unlike with enzymatic- and electrochemical degradation, no potentially harmful side-products are generated. Urea sorption relies on hydrogen bond formation and dipole interactions or the formation of a covalent bond with urea acting as the nucleophile. Therefore, water, hydrophilic compounds and nitrogen-containing solutes (e.g. creatinine and amino acids) compete for the binding sites of the sorbent. The competition of water molecules cannot be avoided. However, competition of other solutes can be circumvented by placing AC upstream of the urea sorbent, as AC can remove these competing solutes from dialysate. Recent developments have shown...
Sorbents can remove urea from dialysate either by forming covalent or coordination bonds (chemisorption) or by non-covalent bonds (van der Waals forces, dipole interactions and hydrogen bonds, i.e. physisorption). Since urea is uncharged under physiological conditions it is unable to form ionic bonds. In order to remove the daily urea production (240–470 mmol/day) during a 4- to 8-h dialysis session with a reasonable amount of sorbent (<500 g), both high binding capacity and fast sorption kinetics are required. However, urea sorption from dialysate is difficult because urea and water are both small, polar and weakly nucleophilic molecules [26]. Therefore, a sorbent with affinity for urea based on hydrogen bonds, dipole interactions or electrophilicity, most likely also has affinity for water which is present in the dialysate in a huge molar excess (55 M versus 60 mM for urea at most).

In general, chemisorption is an exothermic and thermally activated process in which non-reversible covalent or coordination bonds are formed with specific functional groups or metal ions present in e.g. a polymeric matrix. However, the kinetics of urea binding to such matrices are relatively slow. In contrast to chemisorption, physisorption is very fast and reversible [122]. Because urea is a very polar molecule, physisorption primarily occurs via hydrogen bonding and dipole interactions, resulting in mono- or multilayers on the sorbent’s surface [123]. A disadvantage of non-covalent bonding is that sorbent-bound urea is in equilibrium with urea dissolved in the dialysate. The relation between the adsorbed amount and the concentration of urea in solution can be described by the so-called Langmuir isotherm. Since the urea concentration of the dialysate decreases during dialysis, the amount of urea bound per time unit (and thus removed from dialysate) decreases in time. Depending on the type of binding, regeneration of urea sorbents is possible, allowing for re-use of materials.

Urea sorbents are potentially bio-incompatible. For example, AC is hemoincompatible as direct contact with blood, as during charcoal hemoperfusion, is associated with platelet activation and a decrease in platelet and white blood cell count [124,125]. In addition, many urea sorbents have reactive (e.g. carbonyl) groups or may contain toxic compounds (e.g. aluminum in zeolites) as leaching of compounds with aldehydes/carbonyl groups in patients may cause oxidative stress and aluminum may cause bone disease (osteomalacia) [58,59], microcystic anemia [126] and brain damage (encephalopathy) [59]. Hemoincompatibility can be prevented by placing urea sorbents in a dialysate circuit that is separated from blood by a semipermeable membrane that is impermeable for sorbent particles but not for urea. In addition, the biocompatibility of novel urea sorbents has to be evaluated extensively in vitro and in vivo, including an evaluation of leachables and degradation products, prior to use in humans. To prevent leaching, it is necessary that the sorbent is stable under dialysis conditions and properly washed using validated protocols.

A frequently studied sorbent for urea is activated carbon (AC), a carbonized and chemically activated material with a surface area...
commonly in the range of 500–1500 m²/g [127]. Its urea binding capacity has been evaluated under both static (sorbent suspended in an aqueous urea solution) and dynamic (urea solution pumped through a sorbent cartridge) conditions, as summarized in Table 2. To compare binding capacities of different materials, we calculated the amount of urea adsorbed per gram of sorbent \( q_{\text{adsorbed}} \) (mmol/g) at a dialysate urea concentration of 20 mM (Table 2), a concentration representative for dialysis patients [128]. The amount of urea adsorbed per gram of sorbent was calculated based on the Langmuir adsorption model (equation (1)) [129] or linear correlation [130–132], as appropriate. Studies were excluded if \( q_{\text{adsorbed}} \) at 20 mM could not be estimated from the available data [22,123,133–138].

\[
q_{\text{adsorbed}} = q_{\text{max}} \frac{K_L [\text{urea}]}{1 + K_L [\text{urea}]}
\]

(1)

Equation (1). \( K_L \) = Langmuir constant, \( q_{\text{max}} \) = maximum binding capacity.

As shown in Table 2, most types of AC have a urea binding capacity of approximately 0.2 mmol/g at equilibrium urea concentration of 20 mM [122,130,138]. Equilibrium is reached within 2 h [122,135,139]. Kim, Lehmann and Giordano showed that urea adsorption by AC increased during “cold dialysis”, when the dialysate was cooled to 0–5 °C [130–132,136]. Since adsorption of urea onto AC is exothermic, the desorption of urea from AC is endothermic. Therefore, at lower temperatures the adsorption-desorption equilibrium shifts towards adsorption and \( q_{\text{adsorbed}} \) thus increased. Another strategy to increase the affinity of AC for urea is to increase the number of oxide functional groups which has been shown to increase the affinity for urea due to H-bonding with the NH₂ group of urea (~0.20 mmol/g versus ~0.07 mmol/g for the un-oxidized AC tested at an equilibrium urea concentration of 40 mM) [141]. However, the urea binding capacity reported was still low (0.20 mmol/g).

Silicon dioxide (SiO₂), also known as silica, has been used as a sorbent for both organic and inorganic compounds. Some forms of silica such as mesoporous SBA-15 (Santa Barbara Amorphous-15) and MCM-41 (Mobil Composition Matter-41) have a high surface area (generally 400–900 m²/g [142]) and small pores (<10 nm) which make them attractive materials for applications such as waste water treatment and drug delivery [143,144]. Cheah et al. reported a very high \( q_{\text{adsorbed}} \) for SBA-15 and amine-functionalized SBA-15 (38 mM urea solution in distilled water) of 7.9 and 8.7 mmol/g, respectively [139]. However, the reported \( q_{\text{adsorbed}} \) for commercial AC that they used as a reference (i.e. 5.4 mmol/g at 38 mM urea in distilled water) was much higher than found in other publications, which might be explained by the use of an unvalidated method for urea concentration determination. Although the authors found that functionalization of the mesoporous silica with amines reduced the surface area, \( q_{\text{max}} \) and \( q_{\text{adsorbed}} \) increased, possibly because the introduced (protonated) amino groups allowed a better packing of urea molecules on the silica surface via hydrogen bonding and/or dipole interactions.

Zeolites are nano porous and crystalline materials mainly consisting of silicium and aluminum oxides. These aluminosilicate networks have an overall negative charge, which is counterbalanced by cations such as Na⁺ and K⁺ in the lattice. Zeolites are widely used as sorbents and ion-exchangers [145–147]. Cheng et al. investigated Zeolite ZSM-5 for urea sorption and found that \( q_{\text{max}} \) (0.70 mmol/g) was higher than that of AC (0.41 mmol/g) and graphene oxide (0.45 mmol/g) while \( q_{\text{adsorbed}} \) was comparable with that of AC and graphene oxide because of the relatively low \( K_L \) (0.019 L/mmol compared to 0.057 L/mmol for AC) for ZSM-5 [122]. Wernert et al. tested uremic toxin binding of several zeolites (Linde type A, stilbite, silicalite, mordenite and fausjinite) with different physical and chemical properties, among which pore sizes and counter cations [134]. It was found that a smaller pore size did not increase the affinity for urea, but decreased the affinity for other (bigger) solutes instead. Stilbite (X₉(Si₂₇Al₉)O₇₂·28(H₂O)) with Na⁺ counter ions (\( q_{\text{max}} \) = Na₉) showed higher affinity for urea than for other solutes than stilbite with K⁺ or Ca²⁺ as counter ions although urea binding capacity of STI-Na (1.1–1.2 mmol/g) was comparable to the binding capacity of AC (1.2 mmol/g) reported in this study. Importantly, aluminum leaching from aluminum-containing zeolites is a potential hazard [122]. The aluminum-free silicalite would therefore be a safer option for application in a WAK, but its urea binding capacity was slightly lower than the binding capacity of AC (1.0 vs 1.1–1.2 mmol/g) reported in this study [134]. Overall, mesoporous silicas and zeolites seem to be attractive urea sorbents, although two studies also reported an unlikely high urea sorption capacity for AC (1.0–5.1 mmol/g at a urea concentration of 20 mM) [134,139], putting the reported high values for these sorbents into question.

Recently, two-dimensional (single layer) transition metal carbides with O-, OH- and F- surface terminations (MXenes) have been reported as novel urea sorbents for dialysate regeneration [140]. MXenes are stacked nanosheets with a thickness of ~1 nm per layer and have a size of 1–4 μm. The general formula of these materials is TiₓCᵧTx in which Tₓ represents surface groups such as O-, OH- or F- that bind urea via hydrogen bonds and dipole interactions. Even though the binding capacity of the reported MXenes for urea is low at 37 °C (\( q_{\text{adsorbed}} = 0.12 \text{ mmol/g} \) the authors state that the fact that these materials bind urea is very promising because MXenes represent a large family of materials with different compositions which can be further explored to identify the best urea sorbent.

5.1. Chitosan-based urea sorbents

Chitosan (CS, Fig. 6), a partially deacetylated polymer of chitin (deacetylation > 50%), is a linear polysaccharide composed of randomly distributed β-1,4-linked β-glucosamine and N-acetyl-β-glucosamine units [148–150]. The amino groups in CS accounts for its sorption capacity due to hydrogen bonds and dipole-interactions with various biomolecules including urea, proteins [151], nucleic acids [152] and cholesterol [153]. CS and its derivatives have many pharmaceutical and biomedical applications, including use in drug delivery systems, tissue engineering, wound dressings and vaccine delivery [154,155].

Table 3 shows the urea binding capacity of CS based urea sorbents. As indicated, the conditions under which the urea binding has been assessed greatly vary between studies, complicating the comparison of chitosan-based materials. Jing et al. utilized CS to stabilize oxycellulose based urea sorbents and developed a membrane consisting of a 90% oxycellulose core and a 10% CS coating with urea binding capacity of 0.14 mmol/g [156]. Even though CS is capable of binding urea via hydrogen bonds, the binding capacity is too low for application in a WAK. Therefore, several attempts have been made to improve its binding efficiency [158–162,165,166]. The most frequently studied approach is complexation of CS with metal ions via coordinate bonds with the amino groups of CS. It has been shown that urea binds to the unoccupied d-orbital of metal ions, among which Cu²⁺ and Zn²⁺ [164],
by available amino groups for Cu²⁺ binding. When the accessibility of Cu²⁺ and urea (than that between Fe²⁺ and Fe³⁺ and urea) [161].

Cross-linked CS/Cu²⁺ copolymer

30

pH 7, RT, 12 h

4.4

[162,163]

Cross-linked CS/Zn²⁺ particle

34.2

Deionized water, RT, 12 h

0.8

[164]

via its oxygen atom (Fig. 6) [167,168]. The coordinate bond is generally an order of magnitude stronger than the hydrogen bond with water, and therefore urea preferentially binds to metal ions. Cu²⁺ has been studied most extensively for urea binding, because it has a relatively high affinity for CS compared with other metal ions (Cu²⁺ ≫ Hg²⁺ ≫ Zn²⁺ ≫ Cd²⁺ ≫ Ni²⁺ ≫ Co²⁺ ≈ Ca²⁺) and, importantly, fabrication of CS/metal ion complexes is a simple process which can be achieved by immersing CS into aqueous solution containing metal ions [149]. Key to obtain a high binding capacity of CS/Cu²⁺ complexes for urea is to increase the Cu²⁺ loading content of CS, which can be achieved by improving accessibility (by applying macroporosity or smaller particles) or by increasing availability of amino groups (by using a crosslinker, see next paragraph). Chen et al. prepared Cu²⁺ loaded-CS-fibroin blend membranes and observed a urea binding capacity of 0.3 mmol/g at a urea concentration of 22 mM [157]. The fibroin blend membrane was rather dense, which limited the accessibility of amino groups for Cu²⁺ binding. When the accessibility of amino groups was improved by fabricating a macroporous CS membrane with pores of 25–35 μm, the amount of Cu²⁺ loaded in CS increased as compared to the fibroin blend membranes, which resulted in a substantial increase of urea binding capacity from 0.3 to 1.3 mmol/g [158]. In addition to CS/Cu²⁺ complex membranes, CS/Cu²⁺ sorbent particles have also been explored for urea binding [159-161,165]. Zhou et al. [159,165] synthesized CS/Cu²⁺ particles (size not reported) with a pore size of 200 nm and observed a urea binding capacity of 2.0 mmol/g at a urea concentration of 22 mM. Pathak et al. compared urea binding capacities of CS/Cu²⁺ membranes and particles, and found that the urea binding capacities of membranes was slightly lower than that of particles [160]. Furthermore, urea sorption increased with decreasing particle size (0.1, 0.2 and 0.4 mmol/g for particles with diameters of 710, 320 and 297 μm, respectively), which is likely due to the increased surface area and therefore the accessibility of the functional groups in the smaller particles. In another paper, Pathak et al. prepared CS-magnetite (CS-Fe₃O₄) nanocomposite particles with a size of 12–33 nm, by coprecipitation of Fe²⁺ and Fe³⁺ with NaOH in the presence of CS, followed by hydrothermal treatment of the aqueous dispersion from 90 °C to 80 °C for 2 h. The urea binding capacity of the CS-Fe₃O₄ nanoparticles only slightly increased as compared to the larger CS/Cu²⁺ particles (size 297 μm) (0.5 mmol/g vs 0.4 mmol/g), probably because the advantage of the larger surface area of the nanoparticles hardly outweighed that of the stronger interaction between Cu²⁺ and urea (than that between Fe²⁺ and Fe³⁺ and urea) [161].

Although the aforementioned studies show that urea binding capacity of CS can be increased by complexation with metal ions, the ability of CS to form complexes with metal ions in water is limited, since most of the amino groups and hydroxyl groups of CS form hydrogen bonds with each other and water molecules, thereby decreasing the number of amino groups available for metal ion complex formation and thus for urea adsorption [157,169]. Chen et al. [170] showed that the capacity to form CS-metal ion complexes increased by partially cross-linking the amino groups of CS with glutaraldehyde. Even though part of the amino groups is consumed by the reaction with glutaraldehyde, the cross-linking process prevents remaining amino groups from forming hydrogen bonds with hydroxyl groups, resulting in an overall higher number of available amino groups for CS-metal ion complex formation [162,164]. Wilson et al. [162] hypothesized that, in addition to the unreacted amino groups of CS, the aldehyde groups originating from glutaraldehyde can also complex Cu²⁺ ions, and they prepared CS/Cu²⁺ complexes by incubating Cu²⁺ ions with glutaraldehyde cross-linked CS. A high urea binding capacity of 4.4 mmol/g was found at a urea concentration of 1–30 mM, however the CS:urea ratio was not specified [162]. This material showed fast urea sorption kinetics at 10 mM urea concentration as equilibrium was reached within 20 min [163]. Of note, the molar ratio of amino groups of CS and aldehydes of glutaraldehyde was 1:2, which means that theoretically all amino groups can be converted into imines.

In conclusion, CS/Cu²⁺ complex sorbents demonstrate high urea binding capacities, especially when glutaraldehyde cross-linking is performed. However, for application in a WAK, potential copper leaching is a major concern. In addition, glutaraldehyde leaching is another safety concern since the imine formed in the reaction between the amino group of CS and glutaraldehyde can be hydrolyzed depending on the pH. Acute and/or chronic toxicity due to Cu²⁺ release may manifest as gastrointestinal symptoms, hemolytic anemia and/or hepatoto-, neuro- and renal toxicity [171]. Although Zhou et al. [159] and Pathak and Bajpai [160] did not detect Cu²⁺ desorption from CS/ Cu²⁺ complex sorbents, safety concerns are an issue for copper ion based urea sorbents.

5.2. Molecular imprinting-based urea sorbents

Molecular imprinting is a relatively novel technique to synthesize a polymer matrix with binding sites complementary to the template molecule (e.g. urea) in terms of shape, size and location of binding units [172–174]. Scheme 1 shows a schematic preparation procedure of a Molecularly Imprinted Polymer (MIP) with specific recognition for the template molecule. Technically, MIP production is rather simple and easy to modulate. Neither complex organic synthesis nor molecule design are required. In general, MIPs show excellent chemical and thermal stability, regenerability, and solvent resistance compared with natural counterparts that also possess specific recognition abilities, such as antibodies [175]. Specific urea recognition is advantageous for a urea sorbent in a WAK to avoid competition by other nitrogenous solutes and prevent adsorption of other beneficial molecules such as amino acids. MIPs are widely used for various applications, such as chromatographic separation [176], sensing [177], drug delivery [178] and catalysis [179].

For the preparation of a MIP (Scheme 1), a reversible complex is first formed between the template and complementary functional monomers via covalent and/or non-covalent binding. Subsequently, the complex is co-polymerized with an excess amount of cross-linker, resulting in fixation of the complex in a solid polymer matrix. When the template is removed from the polymerized complex, the geometry and position of the remaining functional groups will be complementary to the template. The imprinting factor is a measure of the imprinting quality, and is defined as the ratio of the binding capacity of imprinted...
Several attempts have been made to synthesize a urea-imprinted MIP for urea detection or removal [180–188], in which the template recognition is based on hydrogen bonding. Alizadeh et al. synthesized a urea-imprinted MIP using methacrylic acid as functional monomer and ethylene glycol di-methacrylate as cross-linker, all dissolved in acetonitrile. The authors reported a urea binding capacity of 6.3 mmol/g and an imprinting factor of 12.6 in aqueous solution at an unspecified urea concentration [186]. This is a remarkable but questionable finding because the theoretical maximum urea binding capacity of this MIP is only 0.28 mmol/g (assuming that each template molecule added to the mixture provides one imprinting site). Additionally, the carboxylic acid groups, which imprint the sorbent in acetonitrile during the synthesis, are likely protonated during the binding experiment in unbuffered water, but will be deprotonated in a buffered solution (such as dialysate), thereby changing the specific binding. The authors do not comment on both issues, which questions the credibility of this paper.

Macromolecules with suitable functional groups have also been employed to prepare MIPs for urea, even without the addition of a cross-linker [180–185,188]. Huang et al. [188] and Lee et al. [184] evaporated DMSO in which poly(ethylene-co-vinyl alcohol) and urea were dissolved, followed by removal of urea by extraction with ethanol and water, which resulted in the formation of a urea-imprinted MIP membrane. Despite the absence of a cross-linker which normally maintains imprinting site integrity during processing, the MIP membrane demonstrated good imprinting quality with an imprinting factor of 2.3 and a urea binding capacity of 0.4 mmol/g at a urea concentration of 16.7 mM. Also chitosan has been used for the design of a urea-imprinted MIP [180,182,183]. For example, an electrochemical urea sensor was constructed based on MIP films which were prepared by potentiostatic electrodeposition of chitosan onto an Au disk electrode. The authors reported a urea binding capacity of 6.3 mmol/g but has a very limited urea binding capacity.

### 5.3. Carbonyl-type sorbents

Carbonyl-type sorbents possess high urea binding capacities and have the ability to form one or two irreversible covalent bonds between their electrophilic carbonyl groups and weakly nucleophilic urea molecules. The electron pair on the nitrogen atom can undergo a nucleophilic attack on the carbonyl group, resulting in a hemi-aminal or imine group (Scheme 2) [189,190]. Because urea is a weak nucleophile, these sorbents lack the selectivity of MIPs and zeolites as the carbonyl groups can also react with other (more reactive) nitrogen containing solutes present in the dialysate. However, since the urea concentration in the dialysate is much higher than the concentration of competing solutes, such as creatinine and amino acids, and because the sorbents contain urea-reactive groups in excess, the competing solutes may not substantially limit urea removal.

Several urea sorbents that are composed of (crosslinked) polymers with urea-reactive carbonyl groups have been reported in literature such as aldehydes, glyoxaldehydes, ketoesters and ninhydrins (Fig. 7). Carbonyl-based urea sorbents and their binding capacity are shown in Table 4.

Oxidation of alcohol groups in biopolymers such as starch and cellulose using oxidizing agents such as hydrogen peroxide [192], results in aldehyde-containing sorbents with a theoretical maximum aldehyde content and thus urea binding capacity of 4.2 mmol/g if all alcohol groups were oxidized and the aldehyde and urea react in a 1:1 ratio [191]. However, as shown in Table 4, the observed binding capacities of these materials are relatively low: 0.14–0.22 mmol/g [133,191]. This might be due to the fact that urea does not react with the aldehyde, as was observed in a study with 14C-labeled urea [201] and in our own study on the kinetics of urea with a variety of carbonyl groups including aldehydes [202]. In this study, the authors propose that the urea-ammonium/cyanate equilibrium (reaction (1)) shifts when ammonia is bound by the aldehydes in the sorbent and thus urea in fact decomposes [201]. In this case, two aldehyde groups are needed to remove one urea molecule. Moreover, the low urea binding capacities can also be attributed to the slow kinetics of the spontaneous formation of ammonium from urea (reaction (1)). Besides, some oxycelluloses are reported to be unstable in dialysate, as small fragments of the sorbent were formed, which carries the risk of passage through the dialysis membrane into the bloodstream [203]. The low binding capacity and poor stability in dialysate, render these aldehyde-based materials unsuitable as sorbents in a WAK. Recently, Abidin et al. reported oxidized starch nanoparticles containing aldehyde groups with a urea binding capacity of 3.0 mmol/g [192]. The authors proposed that urea forms covalent bonds with the aldehyde groups, but according to the IR spectrum presented and carbonyl content determination assay, the number of carbonyl groups on the oxystarch is too low (0.068 carbonyl groups per 100 glucose units) to remove urea based on covalent binding alone. Similarly, Bing-Lin et al. reported that oxidized epichlorohydrin-crosslinked cyclodextrin reported covalent bonds with urea [193].

![Scheme 1](image1.png)

**Scheme 1.** The formation of template-specific gaps in the polymeric matrix of a molecularly imprinted polymer (MIP).

![Scheme 2](image2.png)

**Scheme 2.** General reaction mechanism of urea nitrogen with a carbonyl group.
the urea binding capacity increased with an increasing urea concentration. Therefore, we conclude that urea is most likely bound by physisorption [192]. Finally, Eisen filed a patent in which modified poly-vinylalcohol sorbents are claimed containing ketoester moieties with very high and fast urea binding (4.2 mmol/g in 1 h) in a concentrated urea solution (420 mM) at room temperature. However urea binding is not reported at concentrations more relevant to dialysate purification [194].

5.4. Carbonyl-hydrate sorbents

A sorbent with high reactivity towards urea should favorably be very electrophilic. However, highly electrophilic carbonyl groups tend to react with water as a competing nucleophile to form a hydrate. Usually, the carbonyl and its hydrate are in equilibrium in an aqueous environment. However, water has only one nucleophilic site (its oxygen atom), while urea has two (its nitrogen atoms). Therefore, if two or three carbonyl groups are adjacent, for example in indanetrione (11) that is in equilibrium with its hydrate ninhydrin (10), urea reacts first with the central carbonyl of the triketone (to form intermediate 12), and then the second nitrogen will form a second intramolecular covalent bond with one of the adjacent carbonyl groups. This results in the formation of a favorable 5-membered ring (13), completing the reaction (Scheme 3) [204]. In short, the reaction between water and the carbonyl groups is an equilibrium, whereas the reaction between urea and the carbonyl groups is not and therefore urea will be effectively removed from dialysate.

In a recent publication we reported rate constants (k2) of urea with low molecular weight hydrated carbonyl compounds under physiological conditions. We found that phenyl glyoxaldehyde and ninhydrin are among the compounds that react fastest with urea (k2 = 4.1 and 6.8 M−1 h−1, respectively). Triformylmethane reacted even faster (factor ~23 faster than ninhydrin). Therefore a sorbent in which trifromylmethane is incorporated may be very interesting for urea removal from spent dialysate [202].

A drawback of carbonyl-hydrate based sorbents is their relative complex synthesis. Nevertheless, several carbonyl-hydrate sorbents have been reported. Wong reported a zirconium-glyoxaldehyde complex with a urea binding capacity of 0.45 mmol/g [195]. Kuntz et al. reported a three-step modification of polystyrene to obtain ketoaldehyde hydrate groups [196,197]. Similarly, Poss et al. chemically modified polystyrene to form ketoaldehyde hydrate groups in only two reaction steps, and they reported higher urea binding than the material described by Kuntz (1.5 vs 0.52 mmol/g) (Scheme 4) [198]. Under physiological conditions, ketoaldehyde hydrates are in equilibrium with water and the dehydrated ketoaldehyde group that can form covalent bonds with urea [198].

Similarly, Smakman and Van Doorn modified polystyrene in five steps to form a resin with ninhydrin groups (Scheme 5) [200]. Analogous to the ketoaldehyde-type sorbent presented above, the urea reactive group, indanetrione, is in equilibrium with the ninhydrin group [204]. This ninhydrin-type sorbent showed a maximum binding capacity of 2.0 mmol/g. They also reported that this sorbent bound 1.2 mmol/g from simulated dialysate with a urea concentration of 25–33 mM at 37 °C in 8 h. Furthermore, the material could be sterilized by γ-radiation and did not leach any compounds into the dialysate [199].

Table 4
Covalent urea sorbents reported in literature.

| Polymer matrix | Appearance | Urea-reactive group | Urea-reactive groups available (mmol/g) | Reported urea binding capacity (mmol/g) | Reference |
|----------------|------------|---------------------|----------------------------------------|----------------------------------------|-----------|
| Oxystarch      | Gel        | Aldehyde            | 0.62–4.2                               | 0.14–0.20                              | [191]     |
| Oxystarch      | Gel        | Aldehyde            | n.a.                                   | 0.13–0.22                              | [133]     |
| Oxystarch      | Particles  | Aldehyde            | n.a.                                   | 3.0                                    | [192]     |
| Oxidized crosslinked cyclodextran | Particles | Aldehyde            | 4.5                                    | 1.1                                    | [193]     |
| Glyoxalic ester-PVA | Gum     | Kettoester          | n.a.                                   | 0.01–4.2                               | [194]     |
| Zr/glyoxal complex | Immobilized on AC or on Zirconium sorbent | Glyoxaldehyde (hydrate) | 0.52–1.1                               | 0.20–0.45                              | [195]     |
| Modified PS    | Linear polymer | Glyoxaldehyde (hydrate) | 3.8–4.0                               | 0.52                                    | [196,197] |
| Modified PS-DVB copolymer | Macroporous beads | Glyoxaldehyde (hydrate) | 3.1–3.6                               | 1.5                                    | [198]     |
| Modified PS-DVB copolymer | Macroporous beads | Indanetrione (ninhydrin) | >2.7 [199]                             | 1.1–2.0                                 | [200]     |

* Over the first 24 h.
in a WAK [199]. In addition, carbonyl-based sorbents are not selective towards urea because they will react faster with more nucleophilic compounds present in the dialysate (such as creatinine and amino acids). These solutes can be prevented from entering the urea-sorbent column by placing AC upstream of the urea sorbent column.

6. Conclusions and perspectives

The greatest challenge in the development of a WAK is the development of an efficient urea removal technology. Enzymatic hydrolysis, electrochemical decomposition, physisorption and chemisorption have been explored, but all of these methods have disadvantages. A summary of the urea removal strategies and their advantages and disadvantages is given in Table 5.

Urease for enzymatic hydrolysis of urea followed by ammonium adsorption by a cation-exchanger is currently the most efficient and effective method to remove urea from dialysate. A relatively small amount of enzyme immobilized on a substrate (30–50 g) is required to convert the daily urea production into ammonium and bicarbonate. Even though bicarbonate formation is an advantage as it may correct for metabolic acidosis, a urease-based urea removal system is associated with some major disadvantages: (1) the need for a relatively large amount of zirconium phosphate to remove ammonium ions, (2) risk of early sorbent exhaustion resulting in release of toxic ammonium into the patient (3) binding of other cations (i.e. calcium, magnesium and potassium), requiring replenishment of these cations from a separate reservoir, and (4) sodium release which complicates fluid management. Overall, urea removal by enzymatic hydrolysis is effective and offers prospects for a portable artificial kidney, but all elements required to overcome the existing disadvantages of this method make it very difficult to reduce the size of the device to wearable proportions without the need for frequent change of disposables.

Urea removal by electro-oxidation seems promising, but significant challenges remain. First of all, the selectivity has to be ensured to minimize generation of harmful side-products and undesired oxidation of important dialysate components. Options for improving selectivity include optimization of the electrode material, functionalization of the electrode with catalytic groups or selective membranes and pulsed-potential techniques. Furthermore, a trade-off between efficacy and current efficiency (the fraction of the current used for urea removal) will have to be made. Increasing the electrode area and/or area to volume ratio will have positive effects on both the efficacy and the efficiency. Moreover, forced convection may further improve both. Increased current density to improve efficacy is not a good approach, as this increases formation of toxic side-products and reduces efficiency. Alternatively, a potential driven approach can be implemented, which should reduce side-products and increase efficiency. However, complete avoidance of side-products will be challenging due to the relatively high oxidation potential of urea and primary dependence on indirect oxidation. This will require strategies to reduce the supply of other readily oxidizable substances to the electrodes and to remove side-products downstream of the electrodes (e.g. by applying activated carbon up- and downstream of the electrodes, respectively).

An applicable urea sorbent with high specificity, high binding capacity and fast kinetics that does not release toxic side products, is not yet available. The affinity for urea of AC and zeolites is still too low to allow for application in a wearable device. Future studies might yield urea-specific zeolites or modified AC with high affinity and binding capacity. Urea sorption on silica shows high urea binding capacities, but needs to be validated to evaluate the true potential of this material for incorporation in a WAK.

(Gluteraldehyde-crosslinked) CS/Cu2+ complex sorbents show high urea binding capacity. However, potential copper leaching is a substantial concern for application in a WAK.

A urea-imprinted MIP seems appealing because of its potential specificity for urea. However, molecular imprinting technology for the synthesis of a urea sorbent is still at a very early stage. MIP-based urea sorbents have been developed for urea detection, but only a few studies focused on urea binding for dialysate regeneration. Furthermore, available urea-imprinted MIPs are based on hydrogen bond interaction, which poses the problem of competitive binding by water. Another possible drawback of MIP based urea sorbents is that a substantial
amount of “nonfunctional” cross-linker is required to maintain the MIP structure, which decreases the maximum binding capacity. Alternative imprinting strategies such as covalent or coordination imprinting are potentially suitable and need further exploration.

Carboxylate-hydrate urea sorbents seem promising for application in a WAK as (1) they are capable of removing urea from dialysate without releasing potentially harmful side-products and (2) a relatively small amount of sorbent (300–600 g of ninhydrin-type sorbents) would suffice to remove the daily urea production of 240–470 mmol. However, a limitation of carboxyl-type sorbents is the slow adsorption kinetics under physiological conditions. Urea sorption is fastest within the first hours but insufficient (max. 0.75 mmol/g in 4 h), which would significantly increase the amount of sorbent beyond the acceptable limit for a wearable device. Further research should focus on a covalent urea sorbent with a higher binding capacity and faster kinetics. Another issue of the carboxyl-hydrate sorbent is the low specific activity for urea. Urea is a very weak nucleophile, so stronger nucleophiles such as amino acids and creatinine would most likely also react with these sorbents, thereby reducing the number of potential binding sites for urea. This might be circumvented by placing AC upstream of the sorbent to remove these components before they pass the urea sorbent. In conclusion, none of the currently available urea removal methods make a truly wearable dialysis devices feasible at present. However, a portable dialysate regeneration system seems within reach in the near future. Several technological developments in the different urea removal strategies may enable realization of a light-weight WAK in the more distant future.

Data availability

The data reported in this review can be retrieved from the original papers as cited in the text.

Acknowledgements

The Dutch Kidney Foundation (grant NT 12.05) and the European Commission (WEAKID, Horizon 2020 research and innovation program, grant agreement no. 733169) supported the work of M.K. van Gelder and K.G.F. Gerritsen. J.A.W. Jong and Y. Guo are supported by the Dutch organization for Scientific Research (NWO-TTW, project 14433) and the Dutch Kidney Foundation. All authors acknowledge the financial support of the strategic alliance of the University of Twente, University of Utrecht and University Medical Center Utrecht. J.A.W. Jong, Y. Guo, W.E. Hennink, C.F. van Nostrum and K.G.F. Gerritsen are currently working on new covalent urea sorbents and have recently filed a patent on this topic (WO2019110557). M.K. van Gelder, K.G.F. Gerritsen and C. Blüchel are involved in the development of urease-based portable artificial kidney devices supported by NeoKidney – an initiative of the Dutch Kidney Foundation. M.K. van Gelder, L.Folkertsm, M. Odijk and K.G.F. Gerritsen are working on an electro-oxidation based urea removal system.

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Biomaterials 234 (2020) 119735