A regenerable potassium and phosphate sorbent system to enhance dialysis efficacy and device portability: an in vitro study

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ABSTRACT

Background. Continuous dialysis could provide benefit by constant removal of potassium and phosphate. This study investigates the suitability of specific potassium and phosphate sorbents for incorporation in an extracorporeal device by capacity and regenerability testing.

Methods. Capacity testing was performed in uraemic plasma. Regenerability was tested for potassium sorbents, with adsorption based on cationic exchange for sodium, with 0.1 M and 1.0 M NaCl. To regenerate phosphate sorbents, with adsorption based on anionic exchange, 0.1 M and 1.0 M NaHCO₃ and NaOH were used. Subsequently, sodium polystyrene divinylbenzene sulphonate (RES-A) and iron oxide hydroxide (FeOOH) beads were incorporated in a cartridge for testing in bovine blood using a recirculating blood circuit and a dialysis circuit separated by a high-flux dialyzer (dynamic setup). Preloading was tested to assess whether this could limit calcium and magnesium adsorption.

Results. In the batch-binding assays, zirconium phosphate most potently adsorbed potassium (0.44 ± 0.05 mmol/g) and RES-A was the best regenerable potassium sorbent (92.9 ± 5.7% with 0.1 M NaCl). Zirconium oxide hydroxide (ZIR-hydr) most potently adsorbed phosphate (0.23 ± 0.05 mmol/g) and the polymeric amine sevelamer carbonate was the best regenerable sorbent (85.7 ± 5.2% with 0.1 M NaHCO₃). In the dynamic setup, a potassium adsorption of 10.72 ± 2.06 mmol in 3 h was achieved using 111 g of RES-A and a phosphate adsorption of 4.73 ± 0.53 mmol in 3 h using 55 g of FeOOH. Calcium and magnesium preloading was shown to reduce the net adsorption in 3 h from 3.57 ± 0.91 to −0.29 ± 1.85 and 1.02 ± 0.05 to −0.31 ± 0.18 mmol, respectively.

Conclusion. RES-A and FeOOH are suitable, regenerizable sorbents for potassium and phosphate removal in dialysate regeneration. Use of zirconium carbonate and ZIR-hydr may further increase phosphate adsorption, but may compromise sorbent regenerability. Use of polymeric amines for phosphate adsorption may enhance sorbent regenerability. Calcium and magnesium preloading considerably reduced net adsorption of these ions.

INTRODUCTION

Conventional haemodialysis and peritoneal dialysis have major shortcomings. The quality of life on dialysis is low due to inadequate removal of uraemic waste molecules and excess fluid [1–3], and morbidity and mortality in dialysis patients are high [3, 4]. Increasing the dialysis dose would be a major improvement in renal replacement therapy [3, 5–9]. This could be achieved with a wearable artificial kidney [10]. In addition, a miniaturized design that is independent of a fixed water supply because of dialysate regeneration will offer more freedom and autonomy to the patient.

In designing such a portable device, effective removal strategies have to be developed for all uraemic waste products and...
excess sodium and water. Among the substances that have to be removed by a wearable device are potassium and phosphate. In this paper, we explored ion exchange as removal strategy for these ions. Ideally, ion exchangers should have a high binding capacity and selectivity for the ions to be removed and should not release unwanted substances into the fluid to be treated. Furthermore, regenerability of ion exchangers is an important topic. Regenerating instead of replacing exchangers several times daily, as applied in other sorbent-based artificial kidneys [10–17], will substantially limit the costs of the wearable system. We, therefore, investigated the binding capacity, selectivity and regenerability of several ion exchangers for potassium and phosphate in short batch-binding assays. Furthermore, we incorporated a selected cation and anion exchanger in a prototype of a miniature artificial kidney device and used fresh bovine blood spiked with potassium and phosphate to simulate the in vivo situation.

MATERIALS AND METHODS

Materials

In batch-binding assays, zeolite (ZEO, Na$_{12}$[AlO$_2$]$_{12}$(SiO$_2$)$_{12}$, xH$_2$O, Sigma Zwijndrecht, The Netherlands), a cationic sodium polystyrene/divinylbenzene sulphonate exchanger (RES-A, Resonium A, Sanofi-Aventis Gouda, The Netherlands) and zirconium phosphate (ZIR-phos, Zr(HPO$_4$)$_2$, Sigma) were tested for potassium adsorption. For phosphate adsorption, zirconium oxide hydroxide (ZIR-hydr, ZrO(OH)$_2$, Sigma), zirconium carbonate (ZIR-car, Zr(CO$_3$)$_2$, Sigma), iron oxide hydroxide (FeOOH, Sigma) and sevelamer carbonate (SEV-car, Renvela, Sanofi-Aventis) were tested. To allow comparison of the sorbents, all were tested in powder form.

Pilot studies have shown that powdered sorbents will cause obstruction of the dialysate circuit, so dynamic tests were performed using sorbent beads to allow incorporation in the prototype miniature artificial kidney. For potassium adsorption, RES-A beads (diameter 0.6–0.8 mm) were available and, for phosphate adsorption, polystyrene/divinylbenzene (PS/DVB) beads loaded with FeOOH (FeOOH beads, diameter 0.3–0.4 mm). The RES-A beads wholly consist of potassium sorbent, whereas the PS/DVB beads served as a non-adsorptive holder for phosphate adsorbing FeOOH. The bead diameter of 0.3–0.8 mm, as used, was a compromise between flow resistance and performance. Beads were provided by Nanodialysis, The Netherlands.

Batch-binding assays

Batch-binding assays were performed at room temperature with plasma obtained by plasmapheresis from three patients with Goodpasture syndrome.

Capacity testing was performed by the following steps:

1. Four millilitre fresh plasma was added to 100 mg sorbent.
2. The resulting mixture of plasma and sorbent was placed on a shaker and centrifuged after 1 h.
3. The supernatant was stored for analyses.
4. Steps 1–3 were repeated twice with the used sorbents.

The amount of adsorbed potassium (mmol/g sorbent), phosphate and calcium was calculated as follows:

Amount adsorbed

\[
\text{Amount adsorbed} = \frac{[ (C_b - C_{s1}) + (C_b - C_{s2}) + (C_b - C_{s3}) ] \times V}{W} \]

where \( C_b \) is the concentration (mmol/L) in the blank plasma, \( C_{s1} \) the concentration (mmol/L) in the plasma after contact with the sorbents with \( n = 1, 2 \) and 3 for the consecutive plasma, \( V \) the volume (L) of plasma and \( W \) is the mass (g) of the added sorbent. Because ion exchangers may also release substances, similar calculations were used to measure the release of sodium by RES-A and iron by the FeOOH beads into the supernatant. Magnesium concentrations were not measured in the batch-binding assays, as the divalent cations magnesium and calcium are known to show similar binding characteristics [18].

Subsequently, regenerability of the sorbents was tested by adding regeneration solutions to the potassium- and phosphate-loaded sorbents. Adsorption by the selected potassium sorbents is based on cationic exchange for sodium, so 0.1 and 1.0 M NaCl were used to regenerate the potassium sorbents. To regenerate the phosphate sorbents, with adsorption based on anionic exchange for OH$^-$ or CO$_3^{2-}$, 0.1 and 1.0 M NaHCO$_3$ and 0.1 and 1.0 M NaOH were used. Regenerability testing was performed by the following steps:

1. Four millilitre regeneration solution was added to the potassium- and phosphate-loaded sorbents, used in capacity testing.
2. The resulting mixture of regeneration solution and sorbent was placed on a shaker and centrifuged after 20 min.
3. The supernatant was stored for analyses.
4. Steps 1–3 were repeated twice with the used sorbents.

The amount of desorbed potassium and phosphate was calculated as follows:

Amount desorbed

\[
\text{Amount desorbed} = (C_r_1 + C_r_2 + C_r_3) \times V
\]

where \( C_r \) is the concentration (mmol/L) in the regeneration solution after contact with the sorbents with \( n = 1, 2 \) and 3 for the consecutive solutions and \( V \) is the volume (L) of regeneration solution.

A desorption/adsorption ratio was calculated by dividing the total amount that was desorbed by the total amount that was adsorbed:

Desorption/adsorption ratio

\[
\frac{C_r_1 + C_r_2 + C_r_3}{(C_b - C_{s1}) + (C_b - C_{s2}) + (C_b - C_{s3})}
\]

A desorption/adsorption ratio of 1.0 would mean complete regenerability of the sorbent.

Dynamic tests with RES-A beads and FeOOH beads

An adsorption unit was provided by Nanodialysis BV (Oirschot, The Netherlands). It consists of a polyoxymethylene
housing (Nanodialysis BV), with a mixture of 111 and 55 g of the selected potassium and phosphate sorbents, respectively. These amounts were derived from pilot experiments.

Commercially available high-flux dialyzers (Gambro 2H 0.2 m², cut-off 50 kDa; polymer blend of polyamide, polyarylether-sulfone and polyvinylpyrrolidone; Gambro Dialsatoren GmbH Hechingen, Germany) were used to separate blood and dialysate (Figure 1) to prevent proteins from entering the adsorption unit. Commercially available Thomas pumps (Gardner Denver Thomas, Sheboygan, WI, USA) were used.

Three batches of fresh bovine blood (2.0–3.0 L, collected at an abattoir) were re-circulated over the dialyzer with an average flow rate of 120 mL/min for 3 h after rinsing the sorbents with 500 mL of an isotonic solution containing 154 mM NaCl. Because the selected sorbents also bind calcium and magnesium to some extent, CaCl₂ (2.25 mM) and MgCl₂ (0.84 mM) were added to this isotonic solution to preload the sorbents with calcium and magnesium and prevent net calcium and magnesium adsorption during dialysis. The reservoir was stirred continuously with a magnetic stirrer. The isotonic solution was pumped through the adsorption unit and the dialyzer in counter current direction with an average flow rate of 22 mL/min. Samples were withdrawn directly from the reservoir. At 0, 60 and 120 min potassium phosphate (K₂PO₄ · 3H₂O) was added to the reservoir to maintain the potassium and phosphate concentrations, mimicking inter-compartmental transport of potassium and phosphate from tissue compartments to the plasma. The total amounts of potassium, phosphate, calcium and magnesium adsorbed per hour were calculated as follows:

\[ \text{Amount adsorbed (mmol)} = (C_b - C_s) \times V \]

where \( C_b \) and \( C_s \) are the concentrations (mmol/L) in the reservoir at the start and the end, respectively, of each hour during the experiment and \( V \) is the batch volume (L). Similar calculations were used to measure the release of sodium.

**RESULTS**

**Batch-binding assays with potassium sorbents**

All sorbents showed significant adsorption of potassium (Figure 2A). ZIR-phos appeared to be the most potent sorbent of potassium (0.44 ± 0.05 mmol/g). Interestingly, the least effective potassium sorbents, ZEO and Resonium A, showed considerable calcium adsorption (Figure 2B). ZIR-phos appeared to be the most potent sorbent (0.16 ± 0.04 and 0.15 ± 0.03 mmol/g respectively), whereas calcium adsorption by ZIR-phos was limited (0.04 ± 0.02 mmol/g). Figure 2C underlines that the ratio of calcium versus potassium adsorption is most favourable for ZIR-phos. This implies that this sorbent would necessitate the lowest levels of calcium infusion in a dynamic setup to compensate for undesired calcium adsorption.

ZEO and RES-A released sodium (0.94 ± 0.19 and 1.51 ± 0.25 mmol/g, respectively, \( P < 0.001 \) versus blank) while ZIR-phos adsorbed sodium (2.01 ± 0.19 mmol/g, \( P < 0.001 \) versus blank). There was a marked acidifying effect of ZIR-phos (pH 4.80 ± 0.23 versus 8.13 ± 0.07 in the blank plasma, \( P < 0.001 \)), and a mild alkalizing effect of ZEO (pH 8.68 ± 0.10, \( P \leq 0.005 \)), with only RES-A not affecting pH (pH 8.20 ± 0.05).

Regenerability tests showed full regenerability of RES-A and ZEO with 1.0 M NaCl, whereas ZIR-phos reached less than half regenerability (desorption/adsorption ratio 0.46 ± 0.17) with the concentrated NaCl solution (Figure 3). Only RES-A could be regenerated to a large extent (desorption/adsorption ratio 0.93 ± 0.06) under mild conditions using 0.1 M NaCl (Figure 3).

**Batch-binding assays with phosphate sorbents**

All sorbents showed significant adsorption of phosphate (Figure 4A). ZIR-car and ZIR-hydr appeared to be the most powerful sorbents (0.22 ± 0.04 and 0.23 ± 0.05 mmol/g, respectively). The most powerful phosphate sorbents also showed more calcium adsorption (Figure 4B). However, there

**Fig. 1**: Dialysis circuit consisting of a 3 L blood reservoir, 2 Thomas pumps (speed of pump from reservoir 120 mL/min and from dialyzer 22 mL/min), a dialyzer (0.2 m² Gambro 2H high-flux dialysis membrane, a polyarylether-sulfone polymer) and an adsorption unit (a polyoxymethylene housing) containing a mixture of 111 g of RES-A beads and 55 g of FeOOH beads.
was no difference in the ratio adsorbed calcium/phosphate between the phosphate sorbents (Figure 4C). ZIR-car had an acidifying effect and SEV-car a marked alkalizing effect (pH 7.59 ± 0.09 and 10.11 ± 0.04, respectively, versus 8.13 ± 0.07 in blank plasma, P < 0.001). ZIR-hydr (pH 8.19 ± 0.10) and FeOOH (pH 8.25 ± 0.08) had little effect on pH.

Regenerability tests (Figure 5) showed that all phosphate sorbents could be more or less completely regenerated using 1.0 M NaOH. Only SEV-car could be regenerated under mild conditions using 0.1 M NaHCO₃ (desorption/adsorption ratio 0.86 ± 0.05).

**Sorbents incorporated in our prototype of a wearable artificial kidney device**

Further testing was performed using RES-A beads and FeOOH beads since these ion exchangers were available to us in a spherical form that could be incorporated in a dialysis device. Noteworthy is that the calcium/phosphate adsorption ratio of the FeOOH beads was much lower than that of FeOOH in powder form (0.25 ± 0.11 versus 0.66 ± 0.14 mmol/mmol, respectively, P < 0.001), presumably because of reduced accessibility of the adsorbed phosphate groups in the porous structure for calcium binding due to steric hindrance. Release of iron by the FeOOH beads was not detected.

**Dynamic testing with bovine blood**

Cumulative potassium adsorption (Figure 6A) from bovine blood amounted to 10.72 ± 2.06 mmol in 3 h. Adsorption in the first hour was significantly more than in the third hour (5.85 ± 1.73 versus 2.67 ± 0.22 mmol, respectively, P < 0.05). Phosphate adsorption showed a similar pattern (Figure 6B). Cumulative adsorption after 3 h was 4.73 ± 0.53 mmol. There was a decline in phosphate adsorption during the experiment, but this did not reach statistical significance (2.15 ± 0.54 mmol in the first hour versus 1.44 ± 0.07 mmol in the third hour, P = 0.09). In exchange for potassium adsorption, sodium was released by the RES-A beads resulting in cumulative sodium release of 10.67 ± 4.16 mmol in 3 h.
The experiment with the highest starting concentrations of potassium and phosphate (Experiment 1, [K+] 6.29 mmol/L and [PO4 3−] 2.40 mmol/L) showed the highest cumulative adsorption of both electrolytes, whereas Experiment 2, with the lowest starting concentrations ([K+] 4.05 mmol/L and [PO4 3−] 1.99 mmol/L), showed the lowest cumulative adsorption. These observations indicate concentration dependency of adsorption by the sorbents.

Considerable calcium and magnesium adsorption was observed. This could be prevented by preloading the sorbents with calcium and magnesium chloride without affecting potassium and phosphate adsorption (Figure 7A and B).

**DISCUSSION**

In the present study, we screened a number of ion-exchange sorbents with respect to their potential to bind potassium and phosphate, respectively. In addition, we studied the possible release of unwanted substances as well as sorbent regenerability. The latter is an important aspect for the future application of these binders in miniaturized wearable or portable artificial kidney devices.

As summarized in the semi-quantitative overview of our key potassium results in Table 1, ZIR-phos was the most potent potassium sorbent with the advantage that adsorption is based on exchange for sodium as well as hydrogen [11, 19]. This even resulted in some net sodium removal, but a potential disadvantage is the induction of metabolic acidosis due to proton release. ZIR-phos showed limited regenerability and, therefore, appears mainly suitable for application in a non-regenerable system that uses replaceable cartridges. This approach has been successfully applied in the REDY system [11, 12]. However, economically a regenerable system is preferable to a non-regenerable system [20]. In this respect, both ZEO and RES-A are more promising. ZEO showed ∼50% regenerability with 0.1 M sodium regeneration fluid, but was a somewhat less potent potassium sorbent than ZIR-phos. In contrast to ZIR-phos, ZEO released some sodium in exchange...
for potassium, but there was a small and theoretically favourable increasing effect on pH. A potential disadvantage of ZEO, which is an aluminosilicate mineral, is the release of aluminium (data not shown). Under the conditions of the batch studies, RES-A was the least effective potassium sorbent, albeit with neutral pH effects and good regenerability, even with a 0.1 M NaCl solution. A disadvantage is the considerable sodium release in exchange for potassium, which may complicate patient fluid management. However, downward adjustments of the sodium content of the dialysate could be applied to prevent sodium loading of patients. Finally, all sorbents showed some degree of calcium adsorption, probably necessitating some form of calcium loading of the sorbents in clinical applications.

The summary of our key phosphate results in Table 2 shows that ZIR-car and ZIR-hydr were the most potent phosphate sorbents. Regenerability was similar to that of FeOOH, which also showed reasonable phosphate adsorption. By far, the best regenerability was demonstrated by SEV-car which, despite being an effective oral phosphate binder in clinical practice, was the least effective phosphate binder in the setting of our batch experiments. Remarkably, all phosphate sorbents showed some calcium binding that was proportional to phosphate adsorption, again probably necessitating some form of calcium loading of the sorbents in clinical applications. We presume that this is possibly due to calcium binding to adsorbed phosphate via charge interaction. Whereas most phosphate binders had minimal effects on pH, SEV-car had a considerable alkalizing effect. This might be of use to compensate for the acidifying effects of some potassium binders, such as ZIR-phos, by combining these sorbents.

For dynamic testing, sorbent beads instead of powders have to be used because the resistance to fluid flow in the sorbent cartridge is inversely related to particle size. Since only RES-A and FeOOH were available to us as beads at the time of the experiments, these sorbents were used for the proof of principle experiments. The sorbent cartridge, containing 111 g of RES-A and 55 g of FeOOH beads, was remarkably effective during the 3-h dynamic experiments using a miniaturized dialysis module. In this period, the cartridge removed ∼10 and 5 mmol of potassium and phosphate, respectively. These preliminary findings clearly show that it will become feasible to remove a very considerable part of the daily load of potassium and phosphate by sorbent technology incorporated in miniaturized dialysis devices. However, additional investigation is required into optimal sorbent content of the cartridge, its rate of saturation and the frequency of regeneration dictated by this.

Notably, a cartridge containing RES-A combined with FeOOH not only appears to be surprisingly effective, but may also be more readily acceptable to clinicians and regulatory agencies as far as safety and toxicology aspects are concerned compared with the other sorbents investigated in the present study. RES-A has been applied for decades as oral potassium sorbent and safety risks seem limited [21]. Furthermore,
although iron release was not detected using the FeOOH beads, release of small amounts of iron may not harm dialysis patients as many of them suffer from iron deficiency [22].

In the batch-binding studies, calcium was adsorbed by all potassium and phosphate exchangers. Net calcium and magnesium adsorption was successfully eliminated in our dynamic setup by preloading the sorbents with these cations. This might abolish the need for post-cartridge infusion of these electrolytes, as applied in the well-known (modified) REDY sorbent system [10, 12, 14, 23], containing activated carbon, urease, ZIR-phos, zirconium oxide and ZIR-car. However, to control for calcium and magnesium levels in the wearable device, implementation of online sensors for both electrolytes shall probably be necessary.

The zirconium compounds were not available to us in bead form that could be incorporated into the prototype. Hypothetically, even less sorbent would be required using zirconium compounds because of their high adsorptive capacity for both potassium (ZIR-phos) and phosphate (ZIR-car and ZIR-hydr). Zirconium has been safely incorporated in all kinds of (bio)medical applications [24]. It is known for its high chemical stability supporting its use in dialysis patients [25], although an increase in oxidative metabolism has been reported after long-term exposure [26]. SEV-car, which was also not available to us in bead form, is an interesting compound because of its excellent regenerability and the fact that it has a well-known safety profile in humans. Analogous to RES-A and FeOOH, this may enhance the acceptance of incorporation of this compound in dialysis devices by clinicians and regulatory agencies.

**CONCLUSIONS**

Adequate potassium and phosphate adsorption from dialysate can be achieved by the use of modest amounts of RES-A and FeOOH. RES-A shows excellent and FeOOH acceptable regenerability under mild conditions. Use of ZIR-car and ZIR-hydr may further increase phosphate adsorption but may compromise sorbent regenerability. Use of polymeric amines for phosphate adsorption may enhance sorbent regenerability. Calcium and magnesium adsorption can be limited by preloading these sorbents with these electrolytes. Future research should elucidate whether these sorbents can be applied safely in *in vivo* conditions, e.g. in a wearable artificial kidney device for continuous dialysis.

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**CONFLICT OF INTEREST STATEMENT**

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