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Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

# Oxalic acid complexes: promising draw solutes for forward osmosis (FO) in protein enrichment

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Received (in XXX, XXX) Xth XXXXXXXXXX 20XX, Accepted Xth XXXXXXXXXX 20XX

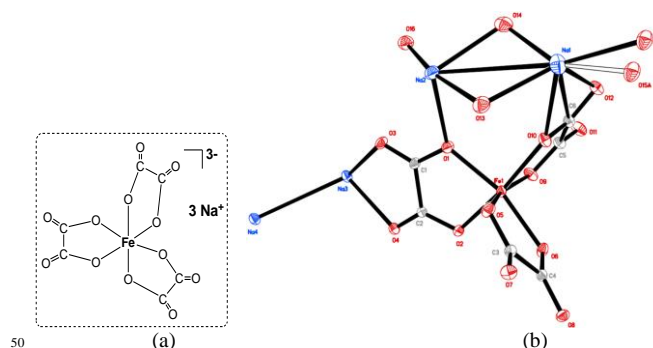
DOI: 10.1039/b000000x

Highly soluble oxalic acid complexes (OACs) were synthesized through a one-pot reaction. The OACs exhibit excellent performance as draw solutes in FO processes with high water fluxes and negligible reverse solute fluxes. Efficient protein enrichment was achieved. The diluted OACs can be recycled via nanofiltration and are promising as draw solutes.

The demand for proteins is steadily increasing, effective protein production is needed. Protein enrichment is an essential step in protein production. As most proteins are labile and heat sensitive, athermal enrichment technologies are preferred. Membrane technology has gained importance in biotechnology due to its mild operating conditions and superior separation ability.<sup>1</sup> Microfiltration, ultrafiltration and nanofiltration (NF) have been extensively used for protein enrichment. However, these pressure-driven processes are usually energy intensive and severe membrane fouling is often encountered. In contrast, forward osmosis (FO) employs the osmotic pressure difference to induce water transport. Not only does it consume less energy but also results in lower fouling because of no hydraulic pressure involved.<sup>2-5</sup> These features make FO very attractive in protein enrichment.<sup>6,7</sup>

As the osmotic pressure gradient across the membrane is the driving force for FO processes, draw solutes with characteristics of high osmotic pressures, low reverse fluxes and easy regeneration are crucial to fulfil protein enrichment. Most conventional draw solutes of inorganic salts are not suitable for protein enrichment because they have severe reverse salt fluxes.<sup>6,8</sup> The permeated salts may denature the feed proteins.<sup>7</sup> A variety of novel draw solutes have been proposed recently.<sup>9-15</sup> However, most novel draw solutes either undergo complicated synthesis procedures<sup>10,15</sup> or have relatively low water fluxes.<sup>9,11,14</sup> To satisfy the aforementioned requirements, Fe<sup>3+</sup> and Cr<sup>3+</sup> complexes with oxalic acid (OA) as ligands and Na<sup>+</sup> as counterions (denoted by Na-Fe-OA and Na-Cr-OA, respectively) were investigated as draw solutes in this work. Such substances possess lots of hydrophilic groups and are ionizable in water which make them desirable as draw solutes.

Na-Fe-OA and Na-Cr-OA were synthesized according to a modified method.<sup>16</sup> Quantitative yields were achieved in their syntheses. Fig. 1 (a) shows the proposed structure of Na-Fe-OA. Na-Cr-OA is structurally similar to Na-Fe-OA (Fig. S1 (a)). The proposed structures were confirmed by their single-crystal X-ray crystallography (Fig. 1(b) and Fig. S1(b); Tables S1 and S2).



**Fig. 1** (a) Proposed and (b) X-ray single crystal structure of Na-Fe-OA. Both complexes consist of an octahedral anion framework and three Na<sup>+</sup> (Na<sub>3</sub> and Na<sub>4</sub> in Na-Fe-OA occupy special positions with 50% occupancy for each atom. The same situation occurred in Na<sub>1</sub> and Na<sub>2</sub> of Na-Cr-OA). The octahedral framework contains a metal core and three five-membered rings. This is a desirable scaffold as it not only contributes to the stability of OACs, but also reduces their reverse flux in FO and makes OACs easily being recycled. Despite the configural similarity, Fe<sup>3+</sup> and Cr<sup>3+</sup> complexes are different in their crystal structures owing to their differences in metal electronic structures. The distances between the metal core and its directly connected oxygens (M-O) are shorter in Na-Cr-OA than in Na-Fe-OA, indicating a stronger interaction of Cr-O than Fe-O. Meanwhile, the distance between carbon and the terminal oxygen is generally shorter in Na-Cr-OA than in Na-Fe-OA. These data imply a stronger dissociation ability of Na-Cr-OA than Na-Fe-OA. Hence, the former has a higher osmotic pressure than the latter at the same concentration, as confirmed by the experimental results (Fig. S5).

The coordination between OA and Fe<sup>3+</sup> or Cr<sup>3+</sup> was confirmed by FTIR (Fig. S2). In both complexes, the peak at ~ 3500 comes from the O-H groups of the crystal water. The absorptions at 1648 ~ 1690 cm<sup>-1</sup> and 1252 ~ 1265 cm<sup>-1</sup> correspond to C=O and C-O groups, respectively, indicative of the presence of carboxylic groups.<sup>17,18</sup> The lower stretching frequencies of C=O ranging from OA (1690 cm<sup>-1</sup>) to OACs (1660 ~ 1670 cm<sup>-1</sup>) are a result of perturbation at the hydroxyl site leading to the weakening of C=O through conjugation. The absorption at ~ 560 cm<sup>-1</sup> in OACs is from the metal-oxygen bond (M-O), as verified in the similar complexes.<sup>19</sup> All these results manifest the successful coordination between OA ligands and the metal cores.

The detailed compositions of Na-Fe-OA and Na-Cr-OA were determined by TGA measurements (Fig. S3). The thermal decomposition of both OACs takes place in two stages. The weight loss in the first stage occurring at 45 ~ 150 °C is associated with the loss of the crystal water. With a further increase in temperature, the dehydrated intermediates undergo the second-stage decomposition (240 ~ 480 °C) where the organic components are evaporated in the forms of CO and CO<sub>2</sub> with metal oxides left. Na-Cr-OA exhibits higher thermal stability than Na-Fe-OA. The OACs have a similar thermal decomposition mechanism as those with similar structures.<sup>18,20,21</sup> The TGA study shows that the observed weight losses are very close to the calculated ones for both OACs (Table S3).

The relative viscosities ( $\eta_r$ ) of Na-Fe-OA, Na-Cr-OA and OA were recorded in Fig. S4(a). The highest concentration of 1.5 M was used for Na-Fe-OA and OA due to their limited solubility.  $\eta_r$  increases with an increase in draw solute concentration for all compounds, and the increment is larger at higher concentrations. OACs with Na-Fe-OA have the largest  $\eta_r$  at the same concentration. As the viscosity of a draw solution has a negative effect on water flux in FO processes, Na-Cr-OA may perform better than Na-Fe-OA when other conditions are the same. However, the viscosity effect on FO performance may be insignificant for these OACs because they have much lower  $\eta_r$  than other draw solutes such as Na-Fe-CA<sup>18</sup> and PAA-Na compounds<sup>22</sup> as shown in Fig. S4(b). Even at their highest studied concentrations, OACs exhibit much lower  $\eta_r$ . This is possibly due to the fact that OACs have smaller molecular sizes than Na-Fe-CA<sup>18</sup> and PAA-Na compounds.<sup>22</sup>

Fig. S5 displays the relationship between osmotic pressure and draw solution concentration for these two OACs using OA as a reference. Due to the colligative property, the osmotic pressure increases with increasing concentration of both OACs and OA. Among them, Na-Cr-OA has the highest osmotic pressure, followed by Na-Fe-OA and then OA at the same concentration. This could be ascribed to the higher dissociation degree of Na-Cr-OA than Na-Fe-OA, as presented in the X-ray single-crystal structural studies. More ionic species may be present in the OACs solutions than in the OA solution. In addition, the increment of osmotic pressure is not proportional to the increment of concentration possibly due to the reduced ionic dissociation at higher draw solution concentrations.

The suitability of OACs as draw solutes was determined via FO. Fig. 2 shows the FO performance of 1 M OACs via both TFC-PES<sub>1</sub> and TFC-PES<sub>2</sub> hollow fiber membranes under the PRO mode (draw solution facing the selective layer) using DI water as the feed. The two membranes were prepared using different dope formulations and spinning conditions as described elsewhere.<sup>23,24</sup> Both complexes exhibit good performance in terms of high water fluxes and insignificant reverse solute fluxes. Na-Cr-OA outperforms Na-Fe-OA, consistent with the observations in both osmotic pressure and X-ray single crystal structure analyses. TFC-PES<sub>2</sub> membrane surpasses TFC-PES<sub>1</sub> membrane at the same conditions in FO. Better performance may be obtained if a more ideal FO membrane is available. In every condition, the amount of OACs loss when treating a 1 L feed solution is negligible with  $J_s/J_w \approx 0$ . This reveals the advantage of using OACs as draw solutes. To assess the effect of concentration on FO performance, Na-Cr-OA

at various concentrations was studied via TFC-PES<sub>2</sub> membrane. NaCl was also included as a benchmark (Fig. 3). Under all conditions, water flux increases with draw solute concentration increase, in line with the change of osmotic pressure (Fig. S5). The PRO mode consistently outperforms the FO mode (draw solution facing the support layer) in terms of water flux. Interestingly, Na-Cr-OA outperforms NaCl under the PRO mode but is inferior to NaCl under the FO mode at high concentrations. This is due to the low diffusion coefficient of Na-Cr-OA because of its large structure. Thus, it has more severe internal concentration polarization than NaCl. However, Na-Cr-OA has an insignificant reverse flux compared to NaCl. As a consequence, the replenish cost for Na-Cr-OA as draw solutes is minimal and the feed contamination can be significantly avoided. These unique characteristics are essential for protein enrichment because proteins can be denatured easily by salts.<sup>6</sup>

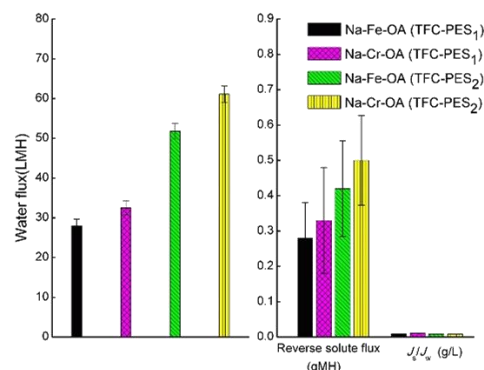


Fig. 2 FO performance of 1 M Na-Fe-OA and Na-Cr-OA via TFC-PES<sub>1</sub> and TFC-PES<sub>2</sub> membranes (Feed: DI water, PRO mode).

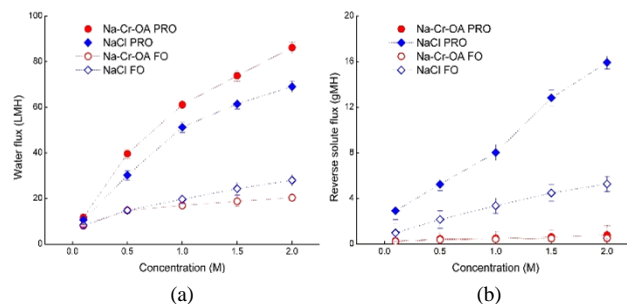


Fig. 3 Comparison of FO performance between Na-Cr-OA and NaCl: (a) water flux, (b) reverse flux (TFC-PES<sub>2</sub> membrane, Feed: DI water).

To study protein enrichment via FO, both Na-Cr-OA and NaCl at 1.0 M were used to concentrate bovine serum albumin (BSA) solutions under the PRO mode. Fig. S6 compares the changes of BSA concentration over time. After 3 hours, the enrichment percentages are 52.5% and 32.5% for Na-Cr-OA and NaCl, respectively, consistent with the observation when using DI water as the feed (Fig. 3), Na-Cr-OA is more efficient than NaCl to enrich the protein solution. The possible structural change of BSA after FO was determined by CD measurements (Fig. 4). The CD spectra show no significant difference between the original and the concentrated BSA enriched by Na-Cr-OA, demonstrating that BSA remains intact in its configuration. In contrast, there is a noticeable detour in the CD spectra of BSA concentrated by NaCl. The BSA undergoes significant conformation changes after the FO process due to the effects of severe reverse NaCl flux.<sup>6</sup> Therefore,

Na-Cr-OA exhibits its another advantage as a draw solute in protein enrichment.

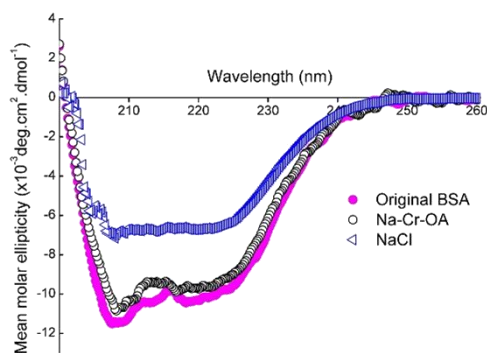


Fig.4 CD spectra of original and concentrated BSA via FO.

Fig. 5 displays the water flux and solute rejection as a function of Na-Cr-OA concentration via an NF process. The details of the pressure-driven NF process has been depicted elsewhere.<sup>18,21</sup> Both water production and rejection decrease with increasing feed concentration as a result of reduced effective driving forces. Concentration polarization and membrane fouling may also contribute to the reduction. Nevertheless, a solute rejection of higher than 97% is achieved when the Na-Cr-OA concentration increases from 0.05 to 0.19 M over 3.6 hours. The recycled OACs have been reused and shown the same FO performance. To further concentrate the draw solution and improve the water productivity, one may explore other suitable NF membranes and study other membrane processes such as membrane distillation.<sup>7,10</sup>

In summary, OACs were synthesized via a facile but efficient one-pot reaction. Their unique features of expanded structure, highly soluble and ionizable in water enable them to perform as draw solutes with excellent FO performance superior to the conventional NaCl draw solute. OACs are especially efficient for protein enrichment via FO without denaturing proteins. They can be easily regenerated by an NF process. Their performance can be further improved if a more ideal FO membrane is available. This encouraging outcome demonstrates the suitability of OACs as draw solutes for FO processes and inspire future exploration of novel draw solutes for protein enrichment.

This research is supported by the National Research Foundation, Prime Minister's Office, Singapore under its Competitive Research Program entitled, "Advanced FO Membranes and Membrane Systems for Wastewater Treatment, Water Reuse and Seawater Desalination" (grant number: R-279-000-336-281 and R-278-000-339-281). Special thanks are also given to Mr. Chunfeng Wan for his valuable help.

#### Notes and references

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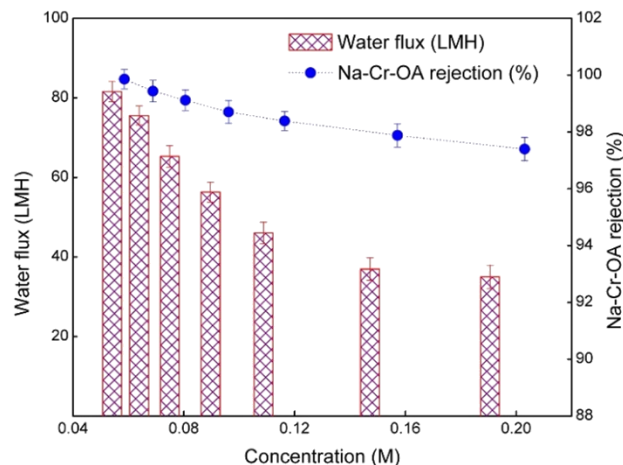


Fig.5 Water flux and rejection during Na-Cr-OA re-concentration

† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

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