Electronic Supplementary Material (ESI) for ChemComm.

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Ubiquitous Aluminium Contamination in Water and Amyloid Hybrid Membranes as a Sustainable Possible Solution

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Content

1. Supporting Materials and Methods

Material

β-lactoglobulin protein was purified from whey protein isolate (Fonterra, New Zealand) and used for amyloid fibrils preparation. The membranes were prepared by assembling of an aqueous mixture of amyloid fibrils on 0.22 μm microfiltration (MF) cellulose filter support, supplied from Merck Millipore. Also, the commercial hybrid membranes were kindly received from BluAct Technologies GmbH. Aluminium aqueous solutions were prepared by dissolving proper amount of aluminium chloride hexahydrate ($AlCl_3.6H_2O$), aluminium nitrate nanohydrate ($Al(NO_3)_3.9H_2O$) and aluminium sulfate octadecahydrate ($Al_2(SO_4)_3.18H_2O$), which were all purchased from Sigma Aldrich.

Methods

β-lactoglobulin amyloid fibrils preparation

β-lactoglobulin protein was purified from whey according to previous procedures [1]. In order to prepare amyloid fibrils, proper amounts of purified monomer powder were dissolved in Milli-Q water to obtain 2 wt.% solution. The solution was gently stirred, and its pH adjusted to 2.0. Then, the solution was transferred to a 90°C bath with a magnetic stirring rate of 200-300 rpm. During the incubation, *β*-lactoglobulin monomers unfold, hydrolyse and assemble into *β*-lactoglobulin amyloid fibrils. After five hours, the laboratory glass bottle was quenched in ice bath to stop the fibrillation. The obtained fibril solution was stored in a 4°C fridge. More details about β-lactoglobulin amyloid fibrils preparation can be found in our previous paper [2].

Amyloid fibrils membrane preparation

Amyloid fibrils membrane was prepared by vacuum filtration of 1 ml of aqueous solution of 0.2 wt. % β-lactoglobulin fibril solution on the surface of cellulose filters (diameter of 25 mm).

The obtained membrane film was further washed with Milli-Q water to remove protein monomers. The pristine activated carbon membrane was fabricated by using aqueous solution of it (10 wt. %) and the same method as pure amyloid fibrils membrane.

Binding Isotherms

In order to evaluate binding isotherms, fixed amounts of amyloid fibrils (10 mg) were titrated by various aluminium concentration ranges, having a final volume of 4 ml. After 48 h, the solutions were filtered by 0.22 μm MF cellulose. After measuring the concentration and calculating adsorbed amounts, the approach of Swillens [3] and Motulsky [4] was applied to fit the binding isotherms. A single binding metal-ligand pair with a single, average, binding constant are assumed in this approach:

$$[M \cdot L] = \frac{1}{2}\left([M_0] + [L_0] + \frac{1}{K_a}\right) - \frac{1}{2}\sqrt{\left([M_0] + [L_0] + \frac{1}{K_a}\right)^2 - 4[M_0][L_0]} \quad (3)$$

in which $[M]$ and $[L]$ are the bound metal and ligand concentration (identical to [M · L]), $[M_0]$ and $[L_0]$ are the initial total metal and ligand concentration, respectively and K_a is the binding constant.

Isothermal titration calorimetry measurements

The ITC experiments were performed at constant pressure and different temperatures of 5, 25 and 75 °C, by separated titration of aluminium sulfate octadecahydrate aqueous solutions (in syringe) into aqueous solutions of β-lactoglobulin monomers and fibrils (in cell) at pH of 3; using a Malvern MicroCal™ PEAQ-ITC. The concentration of β-lactoglobulin was kept constant at 0.05 mM and according to adsorption capacities, concentration of $Al_2(SO_4)_3$ was chosen as 3 mM. In each injection, 2 μL of aluminium solution was titrated into the β-lactoglobulin monomer solution with total volume of 280 μL and the heat change in the sample cell was measured and compared to the reference. Total reaction heat was obtained by

integrating the peak after each injection. The calorimetric binding enthalpy, adsorption constant, number of adsorption sites and changes in entropy were calculated based on classical thermodynamic relations using the source software provided by the manufacturer. During ITC measurement, dilution effects for all solute species were considered and corresponded enthalpies were subtracted from total enthalpy to obtain the net enthalpy of binding. Enthalpy of dilution for ligand and protein was measured by titrating aluminium solution in the syringe to water in sample cell and titrating water in the syringe to protein in sample cell, respectively. In ITC figures the upper panels show the heat traces as a function of time, and the lower panels show the enthalpy changes as a function of the molar ratio of aluminium to amyloid fibrils equivalent (with respect to β-lactoglobulin monomers).

Membrane filtration measurements

The as-prepared pure and commercial hybrid membranes with the surface of 1.77 cm^2 t were used for aluminium removal from aqueous solutions by bench scale dead end vacuum filtration setup. In each filtration experiment 10 ml of aqueous aluminium solutions with different concentrations, were used. Furthermore, in order to determine the individual role of amyloid and activated carbon, filtration of aqueous solution of the aluminium through the pure amyloid and activated carbon membranes was performed. Aluminium cconcentrations in membrane feed and permeate streams were measured by atomic absorption spectroscopy (AAS). All samples were analyzed in triplicates for total aluminium content. For aluminium concentrations below 500 ppb, an AA240Z graphite-furnace (GFAAS) equipped with a GTA 120 graphite tube atomizer and PDS 120 programmable sample dispenser was used. In addition, for aluminium concentrations higher than 1 ppm, an AA240FS fast sequential atomic absorption spectrometer (flame AAS) from Variant was used. Then, membrane efficiency (E, %) was calculated by equation (1):

$$E = (1 - \frac{C_p}{C_f}) \times 100 \quad (1)$$

in which C_p and C_f stand for aluminium concentrations in membrane permeate and feed streams ($\mu g\ L^{-1}$), respectively [5]

2. Supporting Figures

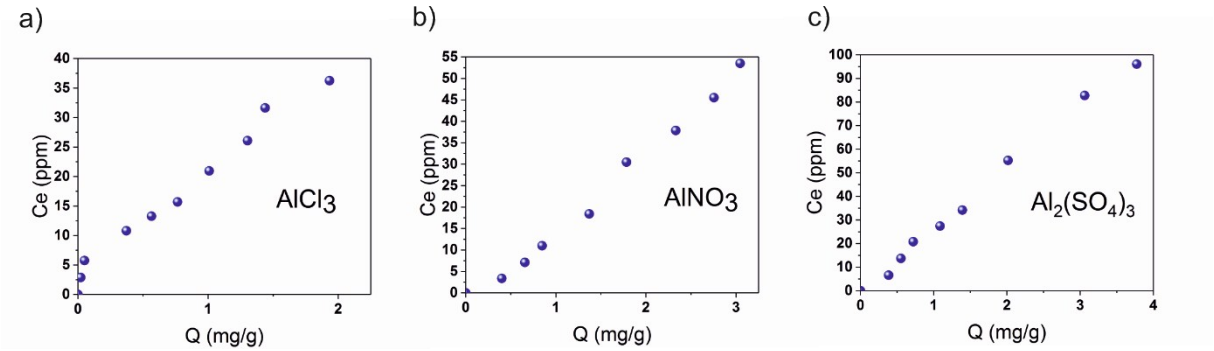

Figure S1. Relation between equilibrium concentration and adsorption capacity.

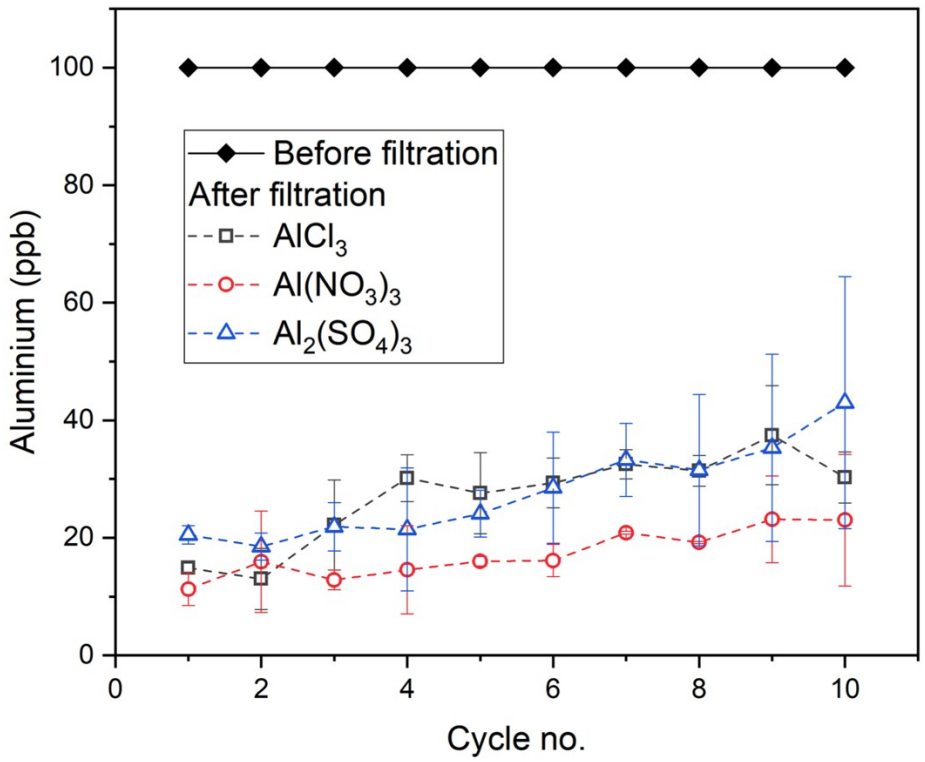

Figure S2. Stability of pure amyloid membrane: (Concentration of $AlCl_3$, $Al(NO_3)_3$ and $Al_2(SO_4)_3$ before and after filtration through the same 2 mg amyloid fibrils membrane for 10 cycles).

3. Supporting References

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