Mechanistic investigation of solid-phase sorbent failure during the processing of equine urine

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Introduction

SPE methods for the analysis of horse and other herbivore urine suffer a high rate of blockages during use with failures often attributed to sample viscosity or suspended materials. We describe a study of C18 sorbent performance with both equine urine and viscous surrogates to derive a mechanistic understanding of sorbent blocking.

Experimental

Horse urine (8 mL) was added to dry Sephadex LH-20 powder (4 g), allowed to stand for 2 hours and stripped urine collected from the bed under gravity. Flowrates were measured using a vacuum manifold (Vac Elut SPS 24, Varian, USA). Columns were Bond Elut C18 columns (200 mg, 3 mL, Lot Number 070982, Varian), graduated in 0.25 mL intervals and conditioned with methanol (3 mL at 1 mL/min) and water (3 mL at 1 mL/min) immediately prior to use. The time elapsed was measured for every 0.25 mL of sample passed through the sorbent at 17 kPa vacuum. Instant flow rate (Instant flowrate$_n$ = $V_n - V_{n-1}$ / $t_n - t_{n-1}$) was considered equivalent to instant relative fluidity of the sample.

Results and discussion

In SPE, the interaction of sample and sorbent may result in a change in the sample viscosity while it is in contact with the sorbent. To measure this effect on flow rate, a SPE device may be used as a surface active viscometer and flowrate related to viscosity by the Poiseuille Equation.

$$\text{Volume rate of flow} = \pi\ (P_{entrance}-P_{exit})\ r^4 / 8l\eta$$

Glycerol and gelatin solutions were used as urine surrogates to explore the influence of hydrogen bonding and macromolecular content on the on-column fluidity. Glycerol is a useful model to investigation sample viscosity based on intermolecular hydrogen bonding and penetration of the sorbent bonded phase by small molecules. Gelatin exhibits strong intermolecular (and intra-molecular) hydrogen bonding and was selected to model the contribution of these forces by the proteoglycans found in urine. The gelatin molecules are excluded from significant penetration of the fine pores of the sorbent by virtue of their high molecular weight and their influence on the flow is likely to be exerted on the interparticle void.

The fluidity plots for glycerol and gelatin solutions on C18 columns are shown in Figure 1. Neither compound blocked the sorbent with full flow restored after the solution had passed through the sorbent. In contrast, diluted equine urine showed a volume dependent decrease in flowrate and extrapolation of the measured flowrate predicted the failure of the sorbent after passage of 10 mL of sample (equivalent to 3 mL of undiluted urine).

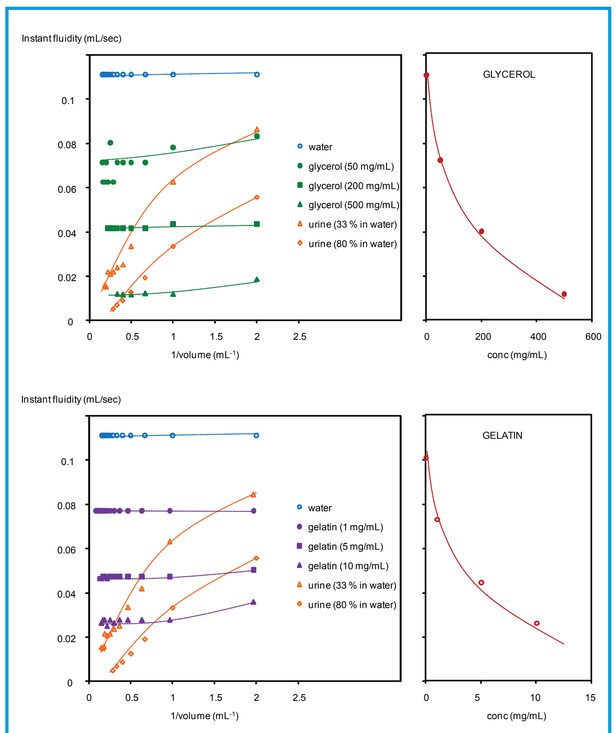

Figure 1. The fluidity and limiting fluidity plots for solutions of glycerol (top) and gelatin (bottom) on C18 columns compared with equine urine samples diluted with water.

The fluidity of another horse urine sample, diluted to different concentrations with water is shown in Figure 2. Correcting the relative fluidity for dilution shows that while an initial dilution causes a marked increase in the equivalent volume of urine that can be passed through the sorbent, larger dilutions do not.

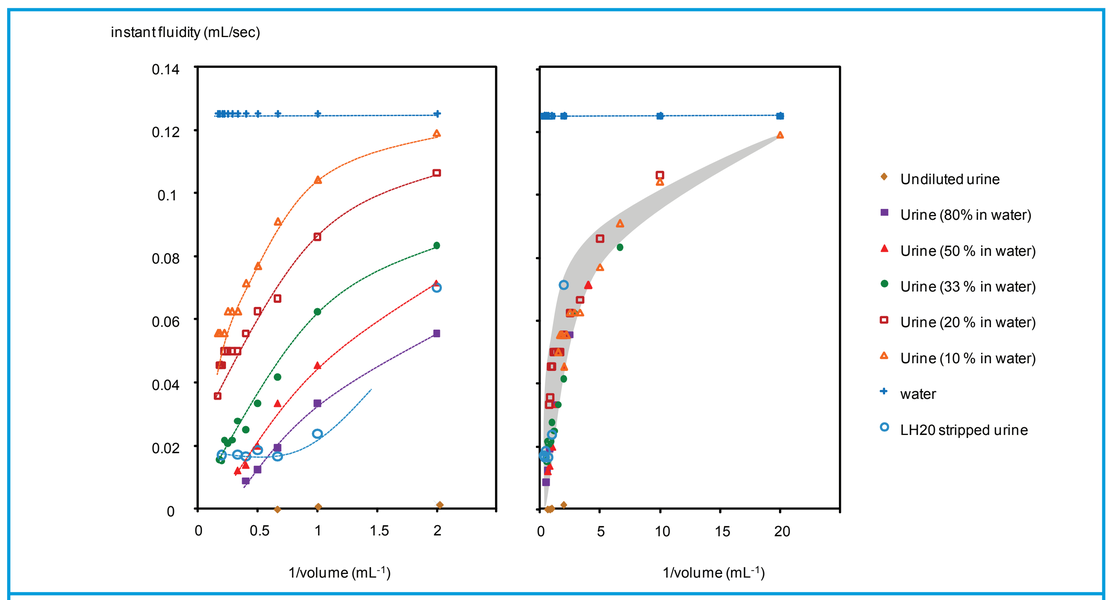

Figure 2. The fluidity plots for an equine urine sample diluted at varying rates with water and the same urine depleted with LH-20 resin (left) and the same fluidity data corrected for the actual volume of urine processed (right).

When LH-20 was used to deplete equine urine of small molecules (supporting GCMS analysis not shown), the LH-20 fraction showed a significant change in fluidity relative to the same urine sample diluted with water. The experimental data suggested that the depleted urine was unlikely to block the sorbent even for very large sample volumes.

In combination, these results suggest that either the sorbent is obstructed mechanically (for example by particulates) and that the top frit plays a significant role in flow failure or that reduced fluidity is the result of complex interactions leading to a coagulation of the matrix in the interparticle void.

To test the effect on the frit three urine samples were diluted and passed through a column of frit material. The fluidity plot for the passage of urine and water wash is shown in Figure 3.

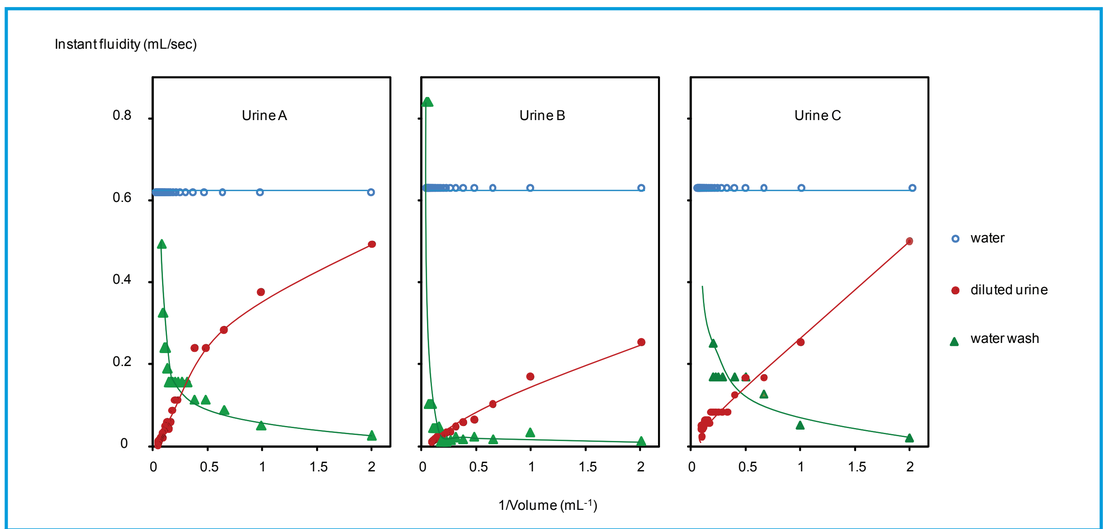

Figure 3. The instant relative fluidity plots for a column composed of six frits through which was passed equine urine diluted with distilled water and water as a post-sample application wash solvent.

Although flow reduction shows that the frit contributes to failure of the columns, restoring flow with a wash of water suggests failure is due to retained soluble species ("adsorptive blocking") rather than by mechanical blocking by particulates. Where blockage was initiated by chemical interactions, water must initially penetrate the sorbent bed and disrupt the interaction of matrix and sorbent before flushing weakly retained species away.

To test the frit further, a C18 column was modified with a side vent valve and urine was passed through the sorbent until it was almost blocked. Removal of the top frit did not restore the flow but if the sample was replaced with ammonium acetate (0.5 M), saline or with methanol and allowed to stand for 10 – 30 minutes, flow through the sorbent could be restarted. Partial flow was also restored to the column with the removal of the top 0.5 mm of sorbent and full flow was restored by the removal of up to a maximum of 1.5 mm (30 % of the sorbent volume). After restoring the flow, the column again passed approximately the same volume of urine before becoming blocked again.

These findings support a mechanism of sorbent blocking that involves the retention of compounds from the urine and that the process of retention acts to change the remaining matrix occupying the interparticle volume to the point of immobility.

We propose that the strong retention of conjugated organic compounds in the first few millimetres of sorbent effectively alters the nature of the sorbent surface and leads to a dramatic increase in its hydrophilicity (Figure 4. top). This change allows significantly interaction with long chain proteoglycans resulting in their immobilisation by a network of hydrogen bonding (Figure 4. bottom).

The progression of this process into the first few millimetres of the sorbent may lead to such an increase in viscosity that the sorbent becomes effectively blocked to fluid flow. Flow may be restored to the column by diffusion into the bed of modifiers such as ammonium acetate, methanol and other reagents that are capable of disrupting the ionic interactions between the retained phenolics and the proteoglycans or disrupting the retention of the phenolic species by the C18 sorbent.

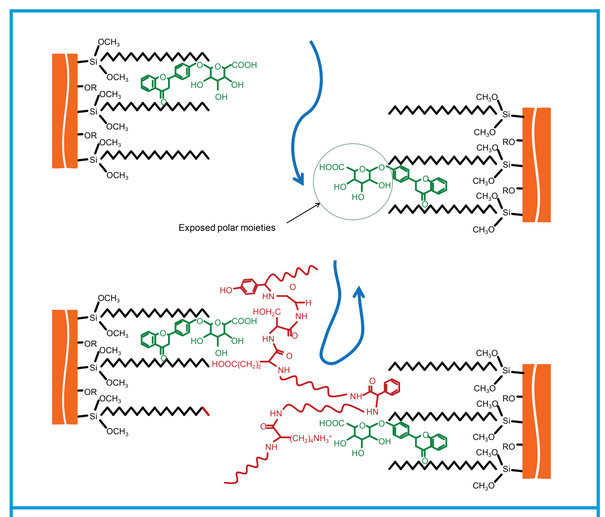

Figure 4. The mechanism responsible for equine urine blocking reversed-phase solid-phase sorbents is proposed to involve the concerted binding of small polar organic compounds by primary interactions (top) and the subsequent clotting of the macromolecular fraction by hydrogen-bonding and weak primary interactions (bottom).

Conclusion

SPE blockage by equine urine is proposed to result from the coagulation or clotting of the proteoglycan fraction through a hydrogen-bonded network that is seeded from the sorbent surface by retained polar organic compounds. The same small molecules may also play a role in propagation of the clot throughout the sample. Blocking of SPE phases can be avoided if sample preparation is undertaken to break-up the urine matrix by stripping or modification.

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