Supplementary Files

Screening of Potential Non-Systemic Treatments for Hyperammonemia

Brad Nicklas,[1] Simon Velasquez Morales,[1,2,3], Jian Qian,[4] Kyle J. Stephens,[1,2,3] David R. Corbin,[3] Mark B. Shiflett,[1,3] Cory J. Berkland[1,4], Alan M. Allgeier*[1,2,3]

[1]Department of Chemical & Petroleum Engineering, University of Kansas, Lawrence, Kansas 66045, United States

[2]Center for Environmentally Beneficial Catalysis, University of Kansas, Lawrence, Kansas 66045, United States

[3]Institute for Sustainable Engineering, University of Kansas, Lawrence, Kansas 66045, United States

[4]Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, Kansas 66047, United States

Approximate dosage calculation:

Estimation of relevant colonic ammonia concentration: The acceptable blood concentration [1] is estimated at 50 µM [1] and acute hyperammonemia is acknowledged at a concentration of 300 µM [2]. Assuming 100-fold increase over normal concentration allows a conservative assessment of therapeutic dose and acknowledges that equilibrium adsorption capacity at lower colon concentrations is expected to be lower. The dose quantity is expected to be required once per colonic transit time, assumed conservatively as 24 h.

Estimate of colon volume: 6.95 L

$$5\frac{\boldsymbol{mmol}}{\boldsymbol{L}} * \mathbf{6.95}\boldsymbol{L} * \frac{\mathbf{17}\boldsymbol{mg}}{\boldsymbol{mmol}} = \mathbf{590.8}\ \boldsymbol{mg\ NH_3}$$

$$\boldsymbol{conservative\ daily\ dosage\ estimate} = \frac{\mathbf{590.8}\ \boldsymbol{mg\ NH_3}}{\boldsymbol{adsorption\ capacity}\ (\frac{\boldsymbol{mg}}{\boldsymbol{g}})}$$

Characterization Data:

Table S1. Elemental Composition of Cu-PAA

Element	Mass %
C	47.8
O	43.4
H	5.35
Cu	3.34
P	0.0795
Ca	0.0265
Si	0.0109
Cr	0.00355
S	0.00338
Pb	0.00222

Table S2. Elemental Composition of Cu-C100EH

Element	Mass %
C	81.8
H	6.87
O	5.29
S	2.98
Cu	2.9
P	0.0468
Na	0.0361
Ca	0.0199
Cl	0.0151
Si	0.0139
Al	0.00663
Fe	0.00345
Th	0.00188

Table S3. Elemental Composition of Fe-PAA

Element	Mass %
C	45.2
O	43.4
Fe	5.87
H	5.06
S	0.391
P	0.074
Ca	0.024
Mg	0.016
Si	0.014
Co	0.009

Table S4. Elemental Composition of Fe-C100EH

Element	Mass %
C	75.4
O	8.78
H	6.33
Fe	5.01
S	4.37
P	0.047
Ca	0.023
Cl	0.013
Si	0.009
Mn	0.008

Poly(acrylic acid) cross-linking

Since PAA (1800 M_w) is soluble in water at room temperature a series of cross-linked materials using N-N'-methylbisacrylamide was synthesized as reported in the Experimental Section. The ammonia binding capacity unaffected by cross-linking (Figure S1).

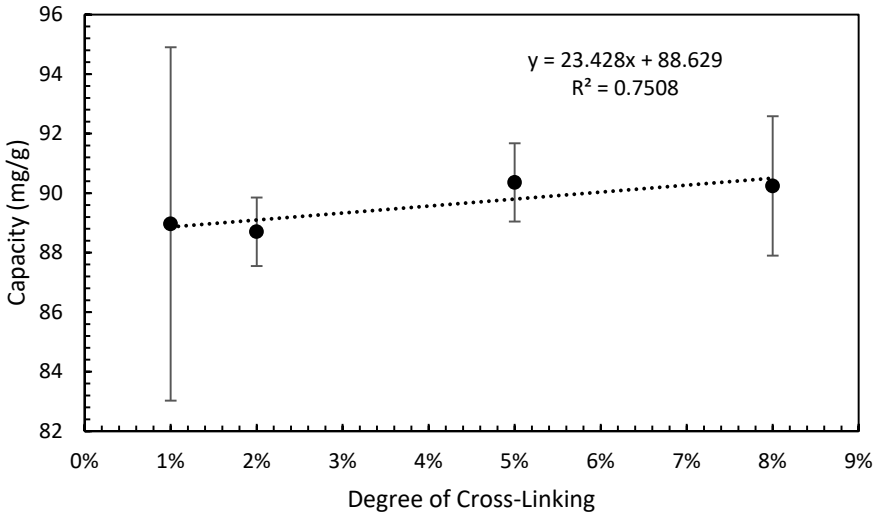

Figure S1. *Ammonia equilibrium capacity as a function of cross-linking of PAA*

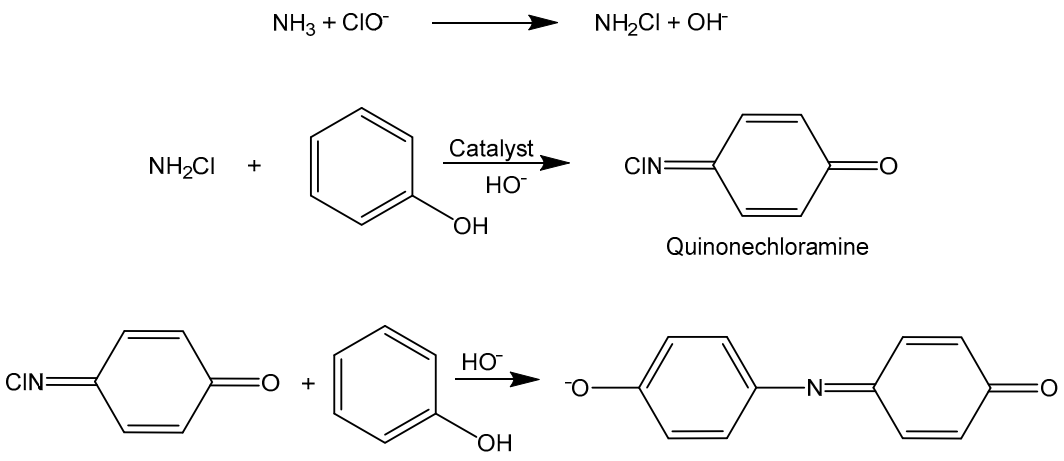

Figure S2. *Berthelot reaction [3].*

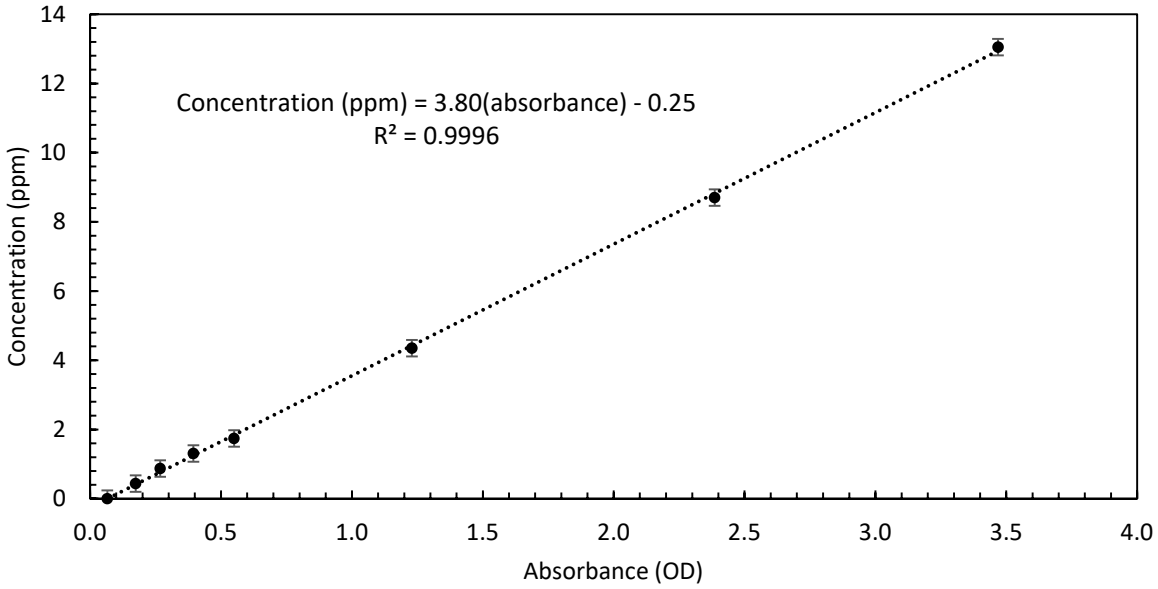

Figure S3. *Standard curve for ammonia concentration using Berthelot's reaction.*

Poly(vinyl sulfonic acid) (PVSA) was evaluated in parallel assays to C100EH (polystyrene sulfonic acid). PVSA demonstrated the highest acidification effect of any material tested and had the highest ammonia capacity in all solutions (Figures S4 and S5). However, PVSA had a large capacity for potassium, which would likely cause electrolyte imbalances within the body (Figure S6).

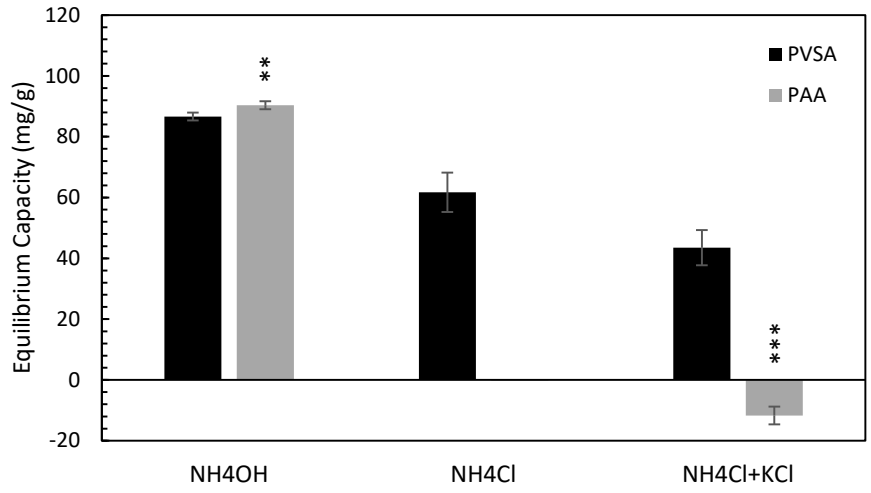

Figure S4. *Ammonia equilibrium capacities of PVSA and PAA (50 mg adsorbent, 50 mM aqueous solutions (ammonia basis), room temperature, 48 hours with stirring. In the last case: 50mM NH_4Cl and 50 mM KCl.)* *** $p < 0.001$; ** $0.001 \leq p \leq 0.05$; * $p > 0.05$ student's t-test two-tailed homoscedastic.

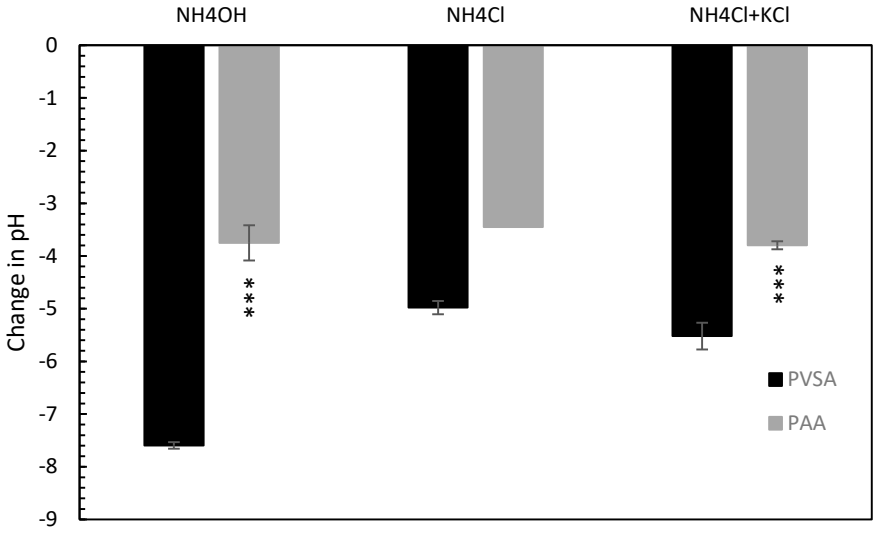

Figure S5. *Changes in pH caused by PVSA and PAA in aqueous ammonia solutions (50 mg adsorbent, 50 mM aqueous solutions (ammonia basis), room temperature, 48 hours with stirring. In the last case: 50mM NH_4Cl and 50 mM KCl.)* *** $p < 0.001$; ** $0.001 \leq p \leq 0.05$; * $p > 0.05$ student's t-test two-tailed homoscedastic.

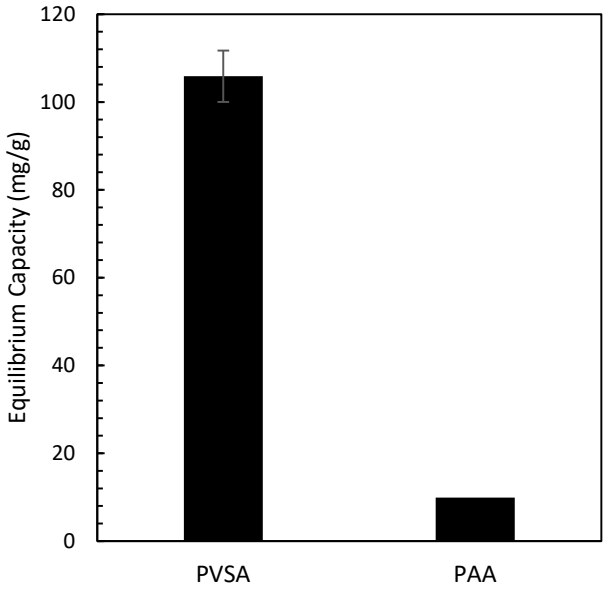

Figure S6. *Potassium equilibrium capacities of PVSA and PAA in 50 mM NH_4Cl, 50 mM KCl solution (*(50 mg adsorbent, room temperature, 48 hours with stirring.)

References

1. Liu, J.; Lkhagva, E.; Chung, H.-J.; Kim, H.-J.; Hong, S.-T. The Pharmabiotic Approach to Treat Hyperammonemia. *Nutrients* **2018**, *10* (2), 140. https://doi.org/10.3390/nu10020140 .
2. Savy, N.; Brossier, D.; Brunel-Guitton, C.; Ducharme-Crevier, L.; Du Pont-Thibodeau, G.; Jouvet, P. Acute pediatric hyperammonemia: current diagnosis and management strategies. *Hepat Med*. **2018**, *10,* 105. https://doi.org/10.2147/HMER.S140711.
3. Zhu, Y.; Chen, J.; Yuan, D.; Yang, Z.; Shi, X.; Li, H.; Jin, H.; Ran, L. Development of Analytical Methods for Ammonium Determination in Seawater over the Last Two Decades. *TrAC Trends in Analytical Chemistry* **2019**, *119*, 115627. https://doi.org/10.1016/j.trac.2019.115627.