

Supporting Information

Removal of Natural Steroid Hormones from Wastewater Using Membrane Contactor Processes

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Materials and Methods

Solution Chemistry – Wastewater Ersatz

The humidity condensate and urine simulant solutions used in the current investigation correspond to recipe 1A for Transit Mission Wastewater Ersatz (19) and the hygiene wastewater simulant solution corresponds to recipe 2A for Early Planetary Base Wastewater Ersatz (19).

Table S1. Wastewater Ersatzs (all purities > 99% unless otherwise noted)

| | Formula | MW | Concentrate (g) | Concentrate (ml) |
|--------------------------------------------------|-------------------------------------------------|--------|-----------------|------------------|
| Concentrate 1 (10x): Urine 1 – Organics | | | | |
| Urea | NH_2CONH_2 | 60.06 | 52.021 | - |
| Creatinine (98%) | $\text{C}_4\text{H}_7\text{N}_3\text{O}$ | 113.10 | 5.221 | - |
| Histidine, soluble (98%) | $\text{C}_6\text{H}_9\text{N}_3\text{O}_2$ | 155.20 | 0.958 | - |
| Taurine | $\text{C}_2\text{H}_5\text{NSO}_3$ | 125.10 | 0.556 | - |
| Glutamic acid | $\text{C}_5\text{H}_9\text{NO}_4$ | 147.10 | 1.660 | - |
| Glucose (96%) | $\text{C}_6\text{H}_{12}\text{O}_6$ | 390.40 | 2.636 | - |
| Ammonium citrate (99%) | $(\text{NH}_4)_2\text{C}_6\text{H}_5\text{O}_7$ | 226.20 | 12.340 | - |
| Ammonium formate (97%) | NH_4HCO_2 | 63.10 | 1.466 | - |
| Ammonium oxalate monohydrate | $(\text{NH}_4)_2\text{C}_2\text{O}_4$ | 142.10 | 0.665 | - |
| | | | | |
| Concentrate 2 (10x): Urine 2 – Inorganics | | | | |
| Sodium Chloride | NaCl | 58.40 | 23.126 | - |
| Magnesium chloride hexahydrate | $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ | 203.31 | 5.483 | - |
| Potassium bicarbonate | KHCO_3 | 100.10 | 2.197 | - |
| Potassium carbonate | K_2CO_3 | 138.21 | 0.474 | - |
| Potassium monobasic phosphate | KH_2PO_4 | 136.09 | 1.069 | - |

| | | | | |
|--------------------|---------------------------------|--------|-------|---|
| Potassium Chloride | KCl | 74.60 | 5.436 | - |
| Potassium sulfate | K ₂ SO ₄ | 174.29 | 7.424 | - |
| Calcium chloride | CaCl ₂ | 110.99 | 0.221 | - |
| Sodium sulfate | Na ₂ SO ₄ | 142.00 | 4.144 | - |

| | Formula | MW | Concentrate (g) | Concentrate (ml) |
|-------------------------------------------------|-----------------------------------------------------------------------|--------|-----------------|------------------|
| Concentrate 3 (10x): Humidity Condensate | | | | |
| Acetic acid | CH ₃ CO ₂ H | 60.05 | - | 0.441 |
| Benzoic acid | C ₆ H ₅ CO ₂ H | 122.20 | 0.046 | - |
| Benzyl alcohol | C ₆ H ₅ CH ₂ OH | 108.14 | - | 0.259 |
| Ethanol | C ₂ H ₆ O | 46.07 | - | 1.506 |
| Acetone | CH ₃ COCH ₃ | 58.08 | - | 0.030 |
| Caprolactam | C ₆ H ₁₁ NO | 113.16 | 0.191 | - |
| Phenol | C ₆ H ₅ OH | 94.11 | 0.027 | - |
| N,N-Dimethylformamide | HCON(CH ₃) ₂ | 73.10 | - | 0.035 |
| Ethylene glycol | HOCH ₂ CH ₂ OH | 62.07 | - | 0.157 |
| 4-Ethyl morpholine | C ₆ H ₁₃ NO | 115.18 | - | 0.072 |
| Formaldehyde (37%) | HCHO | 30.03 | - | 0.461 |
| Formic acid (96%) | HCO ₂ H | 46.03 | - | 0.208 |
| Lactic acid | CH ₃ CH(OH)CO ₂ H | 90.08 | - | 0.187 |
| Methanol | CH ₃ OH | 32.04 | - | 0.218 |
| 1,2-Propanediol | C ₃ H ₈ O ₂ | 76.09 | - | 0.013 |
| 2-Propanol | (CH ₃) ₂ CHOH | 60.10 | - | 0.042 |
| Propionic acid | CH ₃ CH ₂ CO ₂ H | 74.08 | - | 0.042 |
| Urea | NH ₂ CONH ₂ | 60.06 | 0.101 | - |
| Concentrate 4 (20x): Hygiene Wastewater | | | | |
| NASA Whole Body Shower Soap (40%) ¹ | | | 30.076 | |
| Acetic acid | CH ₃ CO ₂ H | 60.05 | - | 0.681 |
| Urea | NH ₂ CONH ₂ | 60.06 | 0.180 | - |
| Ethanol | C ₂ H ₆ O | 46.07 | - | 0.130 |
| Lactic acid | CH ₃ CH(OH)CO ₂ H | 90.08 | 1.627 | - |
| Methanol | CH ₃ OH | 32.04 | - | 0.060 |
| Propionic acid | CH ₃ CH ₂ CO ₂ H | 74.08 | - | 0.246 |
| Chemical Pretreatment | | | | |
| Oxone | 2KHSO ₅ ·KHSO ₄ ·K ₂ SO ₄ | 614.80 | 1.671 | - |
| Potassium benzoate | K(C ₆ H ₅ COO) | 160.22 | 0.334 | - |
| Sulfuric acid (96%, 36N) | H ₂ SO ₄ | 98.08 | - | 0.615 |

¹ Geropone TC42 (formerly Igepon TC-42) is manufactured by Rhodia North America Chemicals and is approximately 60% water.

Solid Phase Extraction Procedure

Sep-Pak C₁₈ columns (WAT036905, Waters Corp., Milford, MA) were sequentially conditioned with 4 mL of HPLC-grade methanol and 6 mL of DDW. Depending on the type of sample, either 3, 6, 12, or 120 mL of sample were passed through the columns at a rate of 10 mL/min. 15 mL of DDW were then passed through the columns to remove any loosely-bound components. The columns were dried for 10 min under vacuum before being eluted with 5 mL of HPLC-grade methanol. The methanol was evaporated under a gentle stream of nitrogen to a volume of less than 0.2 mL. Buffer provided by the immunoassay kit manufacturers was then added to the samples to produce a total sample volume of 12 mL, which could be analyzed using the immunoassay kits. Depending on the initial volume of sample added to the columns, the final samples (following preparation) were at a dilution/concentration of either 1:4, 1:2, 1:1, or 10:1. Samples had to be diluted/concentrated to ensure that they would fall in the linear range of the standard curves produced by the kits. An extraction efficiency test was performed to ensure that a high percentage of hormones were recovered from the initial samples using the extraction procedure.

Extraction Efficiencies of Estrone and Estradiol

The extraction procedure was found to remove approximately 85% of the EIA kit interference experienced when samples were not extracted. The remaining 15% of the interference was not taken into account for rejection calculations because interference was found to cause the estrone and estradiol concentrations in the permeate samples to be overstated. Therefore, interference experienced during the EIA kit analyses, if anything, would have led to reporting of conservative rejection results. An extraction efficiency test was performed on

samples containing estradiol. Based on results summarized in Table S1, an average extraction efficiency of 80% was used for rejection calculations. The recoveries summarized in Table S1 were taken from three different extraction tests. The procedure was refined for each extraction test, and the extraction procedure used in the analysis of the samples reported in this paper correspond to the procedure with 96.8% recovery. Therefore, using an extraction efficiency of 80% substantiates that conservative rejection values were calculated.

Table S2. Recovery of estradiol from water samples using solid-phase extraction.

| Concentration Before Extraction ng/L | Concentration After Extraction ng/L | % Recovery |
|-----------------------------------------------------|----------------------------------------------------|-------------------|
| 1,100 | 790 | 71.8 |
| 840 | 650 | 77.4 |
| 950 | 920 | 96.8 |

EIA Kit Analysis for Estrone and Estradiol

Detection limits of 10 ng/L and 8 ng/L for estrone and estradiol, respectively, were given by the manufacturers and verified independently. Permeate samples were concentrated 10:1 as described above so that concentrations in the range of 1 ng/L could be detected. The estrone EIA kit manufacturer reported a 2.2% cross-reactivity with estradiol, and the estradiol EIA kit manufacturer reported a 4% cross-reactivity with estrone. Cross-reactivity was verified independently to be negligible for both kits. Both manufacturers reported an intra-assay coefficient of variation (CV) of 10% which was verified independently.

Results and Discussion

Chemical Pretreatment of Waste Streams

Versostko et al. (19) provides a chemical pretreatment procedure for feed solutions containing urine. The pretreatment is achieved by addition of strong oxidants (potassium monopersulfate (oxone), potassium benzoate, and sulfuric acid) to the feed solution. The purpose of the pretreatment is to oxidize organic compounds in the urine and to maintain low pH levels in the wastewater to prevent the release of ammonia. This is particularly important for the DCMD process because otherwise, ammonia will evaporate with the water and not be rejected.

In the current investigation, Verostko's pretreatment was not used because it would likely cause oxidation of the estrone and estradiol. Therefore, for the feed solutions used in the DCMD experiments, only sulfuric acid was added to adjust the pH to 3. For the feed solutions used in the FO experiments, only NaOH was added to adjust the pH to 6.5 to prevent oxidation of the CTA membrane.

Although Verostko's pretreatment was not performed on any of the feed solutions used in the DCMD and FO experiments, it was important to understand the extent of estrone and estradiol degradation during the chemical pretreatment. The estrone and estradiol concentrations were measured before and after chemical pretreatment (i.e. potassium monopersulfate (oxone) and potassium benzoate addition) of a wastewater feed solution containing humidity condensate and urine components. After chemical pretreatment, the estradiol concentration decreased by approximately 48% and the estrone concentration increased by approximately 12%. The increase in estrone concentration can be attributed to the fact that estrone is a degradation byproduct of estradiol (24). Therefore, it is estimated that the chemical pretreatment was able to degrade approximately 36% of hormones overall. The use of this pretreatment for direct potable

use applications in advanced life support system would help lower concentrations of hormones having to be removed by the main treatment process (e.g., DCMD or FO).