Detection of estrogens in water using novel reverse osmosis enrichment device and *in vitro* bioassay

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Introduction and objectives

A significant number of constituents in water are suspected to be Endocrine Disrupting Chemicals (EDCs), such as estrogens or androgens that pose risk to water organisms even at very low doses. However, levels of EDCs in water are below the detection limit of analytical methods, so the samples must be enriched prior to detection. We suggest to use Reverse Osmosis (RO) process for the enrichment of estrogens in water, since RO is well established separation method in the different areas such as water desalination, separation of pollutants, or in food industry for concentration of liquid products.

The objectives of our study are:

I. To construct benchtop RO device for enrichment of trace levels of EDCs in the water.

II. To assess the performance of constructed RO device for enrichment of estrogens in water for their subsequent *in vitro* analysis.

Experimental Design and Procedure

The experiment was carried out in three major steps:

1) Preparation of Spiked Samples: Mixing estrogens from stock solution in MeOH with tap water to reach certain concentration.

2) Concentration of Estrogens using the RO Device: The feed water, concentrate, and permeate were collected for *in vitro* analysis after the RO process.

3) In vitro Analysis: HeLa9903 cell line was used for the assessment of estrogenicity of water samples. Estrogens bind to the estrogen receptor which allows the transactivation of a firefly luciferase reporter gene, resulting in expression of luciferase activity which is measured using a luminometer and quantified as 17β-Estradiol Equivalents (EEQs) [1].

4) The water samples were directly assessed by optimized HeLa9903 bioassay that can test aqueous samples only 1.5x diluted by test media (instead of usual 200x). Reliability of the optimized biotest was not affected (Fig. 3).

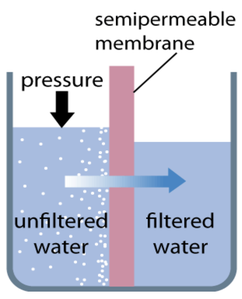

Figure 1. Principle of water sample enrichment in RO process.

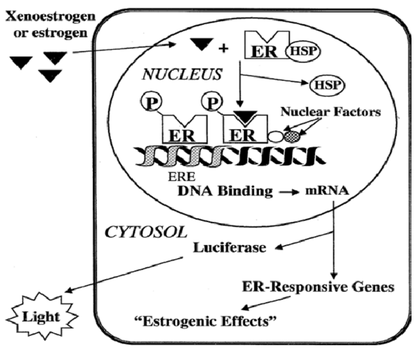

Figure 2. Mechanism of ER mediated receptor response in HeLa9903 biotest [2]

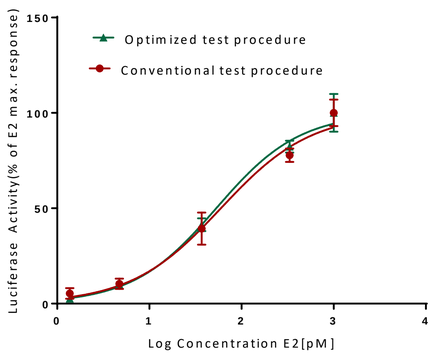

Figure 3. Dose Response curves of reference compound 17β-estradiol. The means ± SD of triplicate determinations are depicted.

Results

Summary of performance of the RO device with BW30LE membrane treated with 50 % EtOH:

1) At retentate flow rate 25 $L \cdot h^{-1}$, and pressure 4 bars, the permeate flow rate was 19.8 $L \cdot h^{-1}m^{-2}$, and increased to 48.7 $L \cdot h^{-1}m^{-2}$ at 10 bars with the same retentate flow rate 25 $L \cdot h^{-1}$ (Fig. 5)

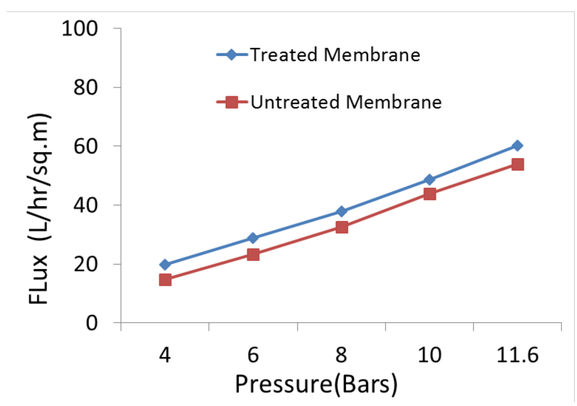

Figure 5. Comparison of permeate flow through treated (in 50 % EtOH [3]) and untreated membrane.

Table shows the theoretical and measured concentration factors for estrogens in the water samples and the final enrichment recovery values.

Hormone	Concentration in feed water sample [pM EEQ]	Concentration in RO-enriched sample [pM EEQ]	Theoretical enrichment factor	Measured enrichment factor	**Recovery [%]**
17β-Estradiol	3.58	38.76	9.71	10.83	**111.52**
Ethinyl estradiol	14.02	91.33	8.00	6.52	**81.45**
Estrone	12.86	24.10	8.83	1.87	**21.22**
Estriol	6.25	95.45	7.48	15.27	**204.26**
Genistein	7.48	67.79	10.53	9.07	**86.11**

Benchtop RO device characterization

RO device can be used with both flatsheet and spiral wound membranes in both crossflow and dead-end filtration mode (Fig. 4).

Device is highly versatile, allowing to work with different types of RO membranes, or manipulation of pressure and flowrate for optimal working conditions.

Majority of device compartments are made of inert materials such as PTFE or stainless steel.

The comparison of both RO systems are summarized in the table below.

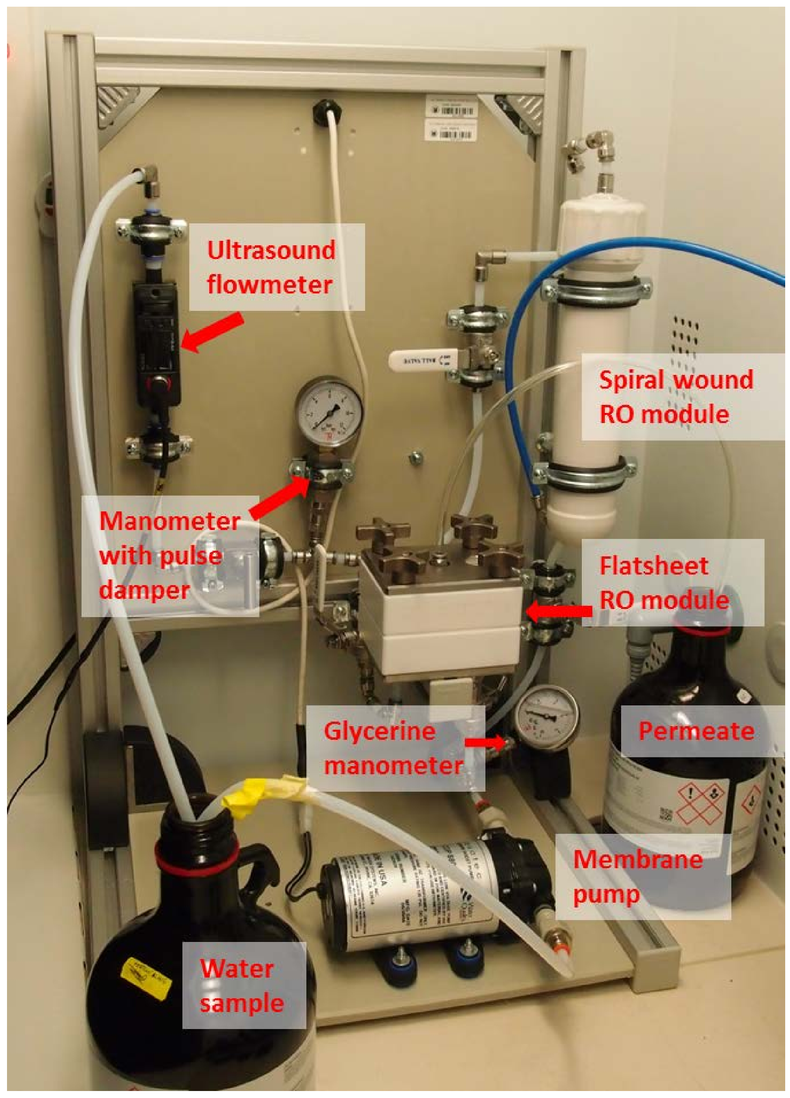

Figure 4. Benchtop RO device configuration.

Operational parameters of experimental spiral membrane and flat sheet membrane reverse osmosis systems.		
Parameters	Spiral Membrane System	Flat sheet Membrane System
Membrane area	cca 0.5 m^2	0.0042 m^2
Maximum Applied Pressure	3.4 bars	12 bars
Regulation of Flow rate & Pressure	Pressure and flow rate can be adjusted to obtain the required flux (up to 3.4 bars)	Pressure and flow rate can be adjusted to obtain the required flux (up to 12 bars)
Dead Volume	150 ml	80 ml
Permeate Flow rate	Variable upt to 10 L/hr	Variable up to 0.2 L/hr

Conclusions

1) Our preliminary results suggest that RO process can be successfully employed to enrich the estrogens in water samples.
2) The enrichment recovery values of various estrogens varied from 21 to 204 % using flat sheet RO membrane type BW30LE.
3) High variability in recovery values suggest necessity of further optimization of both RO-enrichment process and subsequent *in vitro* assessment.

References

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Acknowledgment

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