

Removal of Natural Estrogens and Synthetic Compounds Considered to be Endocrine Disrupting Substances (EDs) by Coagulation and Nanofiltration

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Abstract

Our paper presents the evaluation of retention coefficient of such natural estrogens as estrone (E1), estradiol (E2) and estriol (E3) as well as synthetic compounds considered to be endocrine disrupting substances (EDs) – ethynylestradiol (EE2), mestranol (MeEE2) and diethylstilbestrol (DES) in water treatment processes, i.e. coagulation and nanofiltration. Estrogens were retained in effect of the adsorption on the particles of humic acids and on flocules of $\text{Al}(\text{OH})_3$ and $\text{Fe}(\text{OH})_3$. The efficiency of coagulation process was not good enough to remove these compounds completely from water. As a second stage of water treatment, nanofiltration was proposed. In this process, estrogens were adsorbed on membranes in their pores ($J_w/J_w < 1$), and their retention was dependent on hydrophobicity of micropollutants ($\log K_{ow}$). The best treatment results were obtained for polyamide membrane (NF-DS5DK) within the range 93.5-99.9%.

Keywords: natural and synthetic estrogens, coagulation, membranes, nanofiltration

Introduction

During the last decade, there has been growing concern about environmental chemicals that disrupt normal endocrine functions in living organisms, including humans. These chemicals include naturally occurring plant products (phytoestrogens), natural hormones of human origin such as the primary estrogenic steroid of vertebrates and pharmaceutical hormones such as those in the human contraceptive pill, pesticides, polycyclic aromatic hydrocarbons, phthalates plasticizers and alkylphenols [1-5].

The substances of that kind are defined as “Endocrine disrupting substances – EDs”. In the document published in 1999 (and adopted in the year 2000) the European Union agreed on

the purposefulness and necessity to develop appropriate tools in the natural environment to monitor pollution having this biological impact, and suggested that they should be treated as priority substances which have to be monitored [6].

The presence of chemical compounds of estrogenic activity in the natural environment has raised interest worldwide since mixtures of these compounds are excreted by women, man and also by animals and they have always been present in the environment [1]. What is more, their physicochemical properties ensure high bioavailability from hazard sources and enable cumulation mainly in fat tissue of living organisms [4]. Recently there has been a tendency to investigate the hazard posed to human health by these substances.

Sexual hormones – female estrogens belong to steroid derivatives derived from the structure of 1,3,5,(10)-estratrien. In the organism of mammals three main hormones

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Table 1. Characteristics of the applied coagulants (according to data provided by manufacturer).

Parameter	PIX-113	PAX-18
Iron (Fe ²⁺) / Al ³⁺ , %	0.2-0.7	9.0±0.3
Fe ₂ (SO ₄) ₃ / Al ₂ O ₃ , %	40-42	17.0±0.5
Free sulfuric acid / Chlorides (Cl ⁻), %	from -5 to +1	21.0±2.0
Density, g/cm ³ (at 20°C)	1.5-1.6	1.36±0.01%
Index of dynamic viscosity, mPa·s (at 20°C)	60	ok. 30
Freezing temperature, °C	about 30	-20
pH	below 1	1.0±0.2%
Form	dark brown solution	light yellow solution

from the estrogen group are produced, i.e. the most active estradiol (E2), estrone (E1) and the weakest estriol (E3) which has only 10% of estradiol's effect. We should also remember about chemical compounds produced outside the organism and defined as exogenic hormonomimetic substances. They are principally semisynthetic analogues of estradiol of the preserved estrogenic function, e.g. ethynylestradiol (EE2) and mestranol (MeEE2), used as components of oral contraceptives and in hormonal substitute therapy as well as in veterinary treatment of domestic or breed animals. Surprisingly, it has turned out that a considerable affinity to estrogenic receptors is also exhibited by non-steroid compounds of rather loose structural analogy to estradiol, e.g. synthetic diethylstilbestrol (DES) [1]. This compound was applied pharmacologically in the 1940s to 1970s as a contraceptive and it is an extreme example for specific effects demonstrated by a substance of estrogenic activity and for the range of toxic effects evoked in test animals. After decades of its application there was an increased number of incidents where some irregularities were found connected with reproductive system in people who were subjected to its harmful activity during uterine life.

According to literature data, estrogenic sexual hormones are present in water environment in very low concentrations, about 1 ng/l (yet for organisms being physiologically active) [7]. The problem involving routine analysis of hormones in surface and polluted water is caused by the fact that there are no good procedures to determine these compounds in such low concentrations [7]. Frequently the concentrations of hormones in the sampled specimens are below detection limits, i.e. below detection capabilities of the applied methods [7]. The difficulty involving the analysis of hormonal compounds present in the environment is also caused by a complex matrix of environmental samples. Therefore the analytical procedures elaborated to date involve a complex, to a different degree, preparation stage of the sample, covering filtration, extraction, treatment, derivation and evaporation. In analytical procedures for qualitative-quantitative determination, chromatographic techniques are applied.

Nanofiltration is a promising membrane technique with a large number of applications in the field of process water, drinking water or wastewater treatment. NF membranes can separate small organic micropollutants and multivalent ions from liquid streams [8-9]. In our previous work [10], we examined the rejection properties of NF membranes for trihalomethanes (THMs), polycyclic aromatic hydrocarbons (PAHs) and phthalates, and it was found that higher desalting membranes can reject effectively many hazardous organic pollutants.

The objective of the present paper is to evaluate the possibility of removing from water some selected estrogens in the process of coagulation and nanofiltration. Sorption of estrogens on humic acid (HAs) was considered as well as their removal in a unit process – coagulation, and in a hybrid process – coagulation, nanofiltration.

Materials and Methods

Coagulants

In the investigation we applied as coagulants ferric sulfate PIX-113 and polyaluminum chloride PAX-18 produced by Kemipol Ltd. (Poland). Their characteristics are presented in Table 1.

Procedure for the Determination of Estrogens in Water Samples

Particular estrogens were determined using the solid phase extraction (SPE) method for their separation from the water matrix and capillary gas chromatography with mass detector (GC-MS). We applied the chromatograph Varian, model Saturn 2100 equipped with a CP-Sil 8 column of dimensions 30m x 0.25mm x 0.25µm (film thickness) with phenyldimethylpolysiloxane phase manufactured by Varian.

SPE were carried out in columns with octadecylsilane filling (C₁₈), J.T. Baker from water sample (1 liter), which had been cleaned on the glass fiber screen of the pore diameter

of 0.45 μ m (Millipore) and acidified to pH 3. The separated estrogens were subjected to silylation at 60°C using a mixture of three components: N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA), trimethylsilyliodide (TMIS), and dithioerythritol (DTE) manufactured by Sigma-Aldrich, in proportions (1000:4:2; v/v/w). The reaction yielded the derivatives of trimethylsilylethers which were subjected to chromatographic qualitative-quantitative analysis. The qualitative determination of estrogens was carried out comparing the retention times from chromatograph standards and samples and their mass spectra. The quantitative analysis was carried out using the method of internal standard, adding, before the GC-MS analysis, mirex – the standard of Sigma-Aldrich. In the quantitative analysis, selected ions SIM (Single Ion Monitoring) were recorded. The recorded ions using SIM method – m/z E1:342, 257, E2: 416, 258, E3: 504, 386, EE2: 425, 285, MeEE2: 367, 227, DES: 412, 383.

Model Waters

The investigation studies involved model waters prepared on the basis of deionized water with the addition of humic acids (HAs, mg/l) as well natural and synthetic estrogens of the concentration of 1 μ g/l of each standard of the firm Sigma-Aldrich (Fig.1). Natural organic matter (NOM) was determined in the investigated water based on the measurement of organic carbon, using for this purpose the analyzer of carbon and nitrate “multi N/C” of the firm Analytic Jena. The characteristic of model waters is presented in Table 2.

Membranes and Apparatus

We applied in the investigation studies two types of flat membranes for nonfiltration (NF) of the firm Osmonics Inc. (USA) of 155cm² effective area, that is:

- NF polyamide membrane type DS5DK SEPA CF (Osmonics Inc.) - of the cut-off 200-300 Da,
- NF cellulose membrane type SF10 SEPA CF (Osmonics Inc.) - of the cut-off 150-300 Da.

For the testing of membrane filtration process we applied the apparatus whose main element was a module

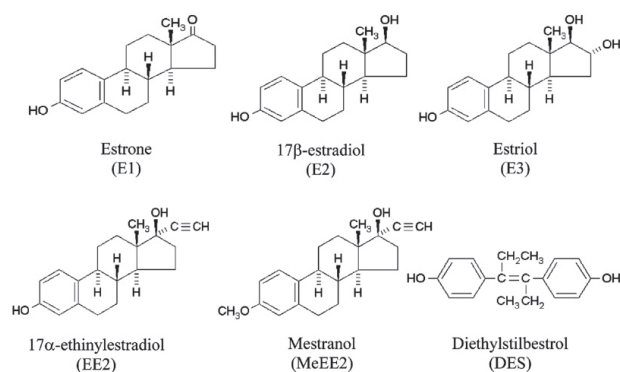


Fig. 1. Molecular structure of natural and synthetic estrogens.

Table 2. Characteristic of model waters.

Parameter	Quantity
pH	6.8 - 7.2
Temperature, °C	20
Absorbance UV ₂₅₄ , cm ⁻¹	0.605
Dissolved Organic Carbon DOC, mg/l	5.5
HAs, mg/l	10.2
Turbidity, NTU	3.5

produced by the American firm Osmonics Inc., type SEPA CF-HP, which enabled us to carry out the process in cross-flow system. The diagram is presented in Fig.2.

Results and Discussion

Operational Parameters of Coagulation

As the first step we determined the influence of coagulant dosage on the obtained results involving the removal of organic compounds being the matrix of water (HAs), following classical stages for this process, i.e. coagulation (stirring rate 110s⁻¹ over 1min), flocculation (stirring rate 25 s⁻¹ over 15min) and sedimentation (20min). The obtained results of water treatment are presented in Fig.3 – a for PAX-18 and b for PIX-113.

Membrane Filtration

As a second step, the water pretreated in the coagulation process was filtered on nanofiltration membranes in constant process conditions (i.e. under transmembrane pressure of 2.0 MPa, velocity of water flow over the membrane surface of 0.80 m/s at 20 \pm 2°C).

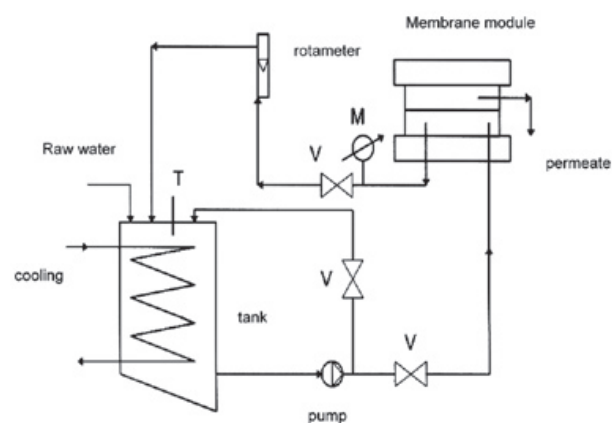


Fig. 2. Diagram of the apparatus.

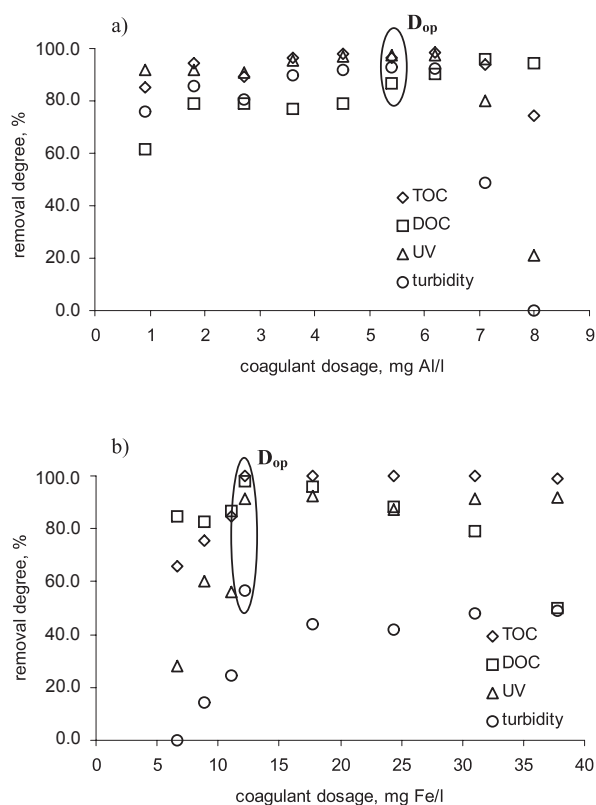


Fig. 3. Influence of coagulant dosage - a) PAX - 18 and b) PIX - 113 - on the removal degree of organic compounds during classical coagulation with pH 7 (D_{op} – optimal dosage, TOC – Total Organic Carbon, DOC – Dissolved Organic Carbon, UV₂₅₄, absorbance at 254 nm).

During the nanofiltration of model waters pretreated in the coagulation process the permeate flux (J_v) was decreasing, and the obtained results were lower than in the case of deionized water flux (J_w), which was determined for a new membrane. The ratio of volumetric permeate flux to the flux of deionized water (J_v/J_w) for both membranes was about 0.68, with initial preparation of water with coagulant PIX-113. Slightly worse results of water pretreatment and higher intensification of pore blocking mechanism ($J_v/J_w=0.56$) were obtained using the coagulant PAX-18 as the first step. The results involving the removal of organic substances using coagulation with PAX-18 and PIX-113 as well as in the nanofiltration process are presented in Fig.4.

Coagulation removes organic matter from water in the range of 91-97% (DOC). Better results were observed during coagulation of the water using PAX-18. Application of nanofiltration in the second step of the water treatment increased organic matter removal to almost 100%. The same results were observed for the nanofiltration membrane made from polyamide or cellulose.

The carried out modeling of nanofiltration process in work [11] defined the mechanism of pore blocking in membranes for nanofiltration as complete. We applied here a mathematical model based on time changes of mass

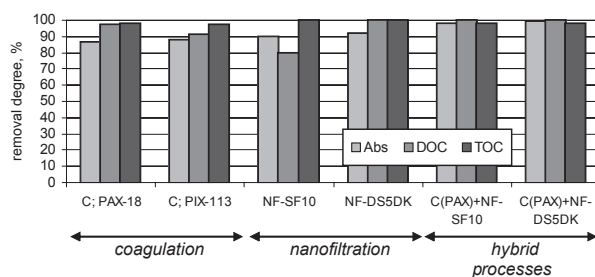


Fig. 4. Obtained effect involving the removal of organic matter in the investigated processes.

transport resistance and a filtration model in constant pressure conditions (model of J.Hermia). Mutual reaction of the membrane material and the particles being removed as well as the adsorption on the walls of membrane pores has influence on adsorptive fouling of the membrane and is the basis for the complete blocking mechanism of its pores. This mechanism is responsible for the observed considerable drop of volumetric permeate flux during nanofiltration.

Removal of Estrogens

The obtained values of retention coefficients of estrogens as a result of sorption on humic acids in coagulation and hybrid processes are presented in Table 3.

We should admit that the processes taking place in effect of the presence of natural organic matter (NOM), being the matrix of this water, which most frequently result in adsorption of micropollutants and lower their concentration in dissolved phase, must be treated as an important factor influencing the concentration of organic micropollution in surface waters.

The presence of humic acids (HAs) in treated water caused the drop in the density of estrogens from 38.5% (E3) to 51.6% (DES). It was stated in the work [12] that from 15 to 50% of estrogens and estrogenic compounds in water occur in the absorbed form on humic acids.

The coagulation process applied in the first step turned out to be inefficient for the removal of this group of micropollutants, with the higher values of retention coefficient being determined during coagulation with aluminum coagulant (PAX-18). Estrogens were removed in this case within the range from 68.8% (E3) to 91.5% (DES).

According to some authors, hydrophobicity of the compounds expressed as the partition coefficient between n-octanol phase and water (K_{ow}) is one of the key parameters taken into consideration when predicting the retention coefficient of organic micropollutants in membrane processes [12-13]. The dependence of $\log K_{ow}$ on the obtained retention coefficient of the investigated compounds in the nanofiltration process is presented in Fig.5.

Nanofiltration turned out to be an efficient method for the removal of estrogens from waters. The obtained values

Table 3. Obtained values of retention coefficients of estrogens in water treatment processes.

Estrogen	Molecular mass g/mol	Solubility in water mg/l	log K_{ow}^a	Sorption of HAs	Coagulation		Hybrid process: Coagulation (PAX-18) + nanofiltration	
					PIX-113	PAX-18	SF10	DS5DK
					Removal, %			
Estrone	270.4	13.0	3.43	61.9	73.6	79.1	91.8	97.2
Estradiol	272.4	13.0	3.94	54.3	70.6	70.2	91.9	97.2
Estriol	288.4	13.0	2.81	38.5	59.5	68.8	81.5	95.4
Ethinylestradiol	296.4	4.80	4.15	63.4	67.7	84.3	94.3	93.5
Mestranol	310.4	0.30	4.67	50.0	59.9	84.2	91.9	99.9
Diethylstilbestrol	268.3	12.5	5.07	51.6	78.2	91.5	97.9	99.9

^a K_{ow} partition coefficient between n-octanol phase and water

of retention coefficient are within the range from 95.4% (E3) to 99.9% (DES), with better results being observed for the membrane made from polyamide (NF-DS5DK). The dependence between retention coefficient of estrogens and the value of $\log K_{ow}$ was of linear character.

Conclusions

The work involved the evaluation of the retention of organic micropollutants such as natural estrogens and synthetic compounds from the EDs group in the coagulation process and in the pressure membrane process such as nanofiltration.

Coagulation applied in the first step removed the investigated compounds partially, with better results being obtained with the application of polyaluminum chloride (PAX-18). A considerable part of estrogens was absorbed on the particles of humic acids.

The application of nanofiltration in the second step of water treatment made it possible to remove the investigat-

ed estrogens to a high degree (>90%), with slightly better results being observed for the membrane made from polyamide (NF-DS5DK).

Acknowledgements

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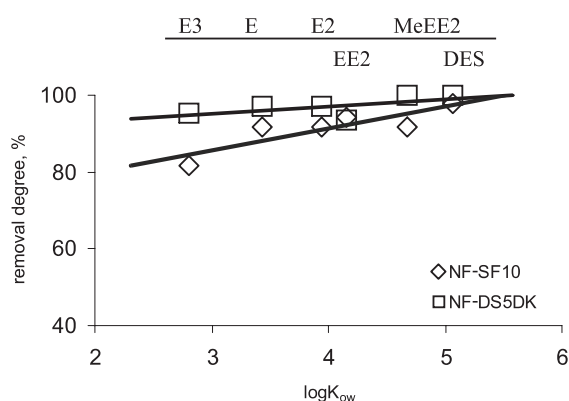


Fig. 5. Dependence of $\log K_{ow}$ on the obtained retention coefficient of estrogens.

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