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Extraction of male steroids and progesterone from water by vegetable oil gels and their determination by partial filling capillary micellar electrokinetic chromatography

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1 **Title**

2 **Isolation and CE determination of male steroids and progesterone from water with**
3 **vegetable oil gels containing cellulose, cotton, or Kraft lignin.**

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11

12 **The running title**

13 **Isolation of steroids with gels and determination with CE**

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16 **Keywords:** gels from microemulsion; vegetable oils; cellulose; Kraft lignin; water samples;
17 capillary electrophoresis.

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Abstract

Microemulsion (ME) gels were synthesized from vegetable oils of macadamia, linseed, olive, walnut, frying oil (mixture of sunflower, palm, and rapeseed oils), rapeseed, sesame, and coconut. The ME were mixed with acrylamide and carbohydrates including celluloses, cotton, or Kraft lignin. The aim was to compare the sorption properties of the sorbents by using them as adsorbents for steroid hormones in water. Out of the sorbents frying, rapeseed, and sesame gels were selected.

The hydrophobicity of the gels was compared by detecting the absorptivity and extraction efficiency of nonpolar androstenedione, testosterone, and progesterone, which are provably detected in wastewater and drinking water. Their quantification was made with micellar electrokinetic capillary chromatography (PF-MEKC).

The results showed that methanol was a perfect eluent for removing androstenedione and testosterone from cellulose acetate and hydroxypropyl cellulose modified gels. On the contrary, progesterone was extracted with high recovery from hydroxypropyl methylcellulose (HPMC) modified gels. However, the recoveries from lignin modified gels were very low. The highest recoveries were obtained with HPMC modified gels which were made of either frying or rapeseed oil. Lignin containing gel materials were hydrophilic and therefore nearly all get moist from water.

61 **1 Introduction**

62 Generally, microemulsions (MEs) are composed of surfactant, water, oil, and alcohol. They have
63 numerous advantages that have gained a lot of attention, especially, being as potential carriers of
64 effective compounds in drug delivery [1-3]. Since MEs are isotropic, thermodynamically stable oil-
65 in-water (o/w) systems, they contain micro sized oil droplets or they have continuous structures [4,5].
66 Microemulsions are frequently transformed into complex semi-solid systems with the use of
67 thickening agents, such as polymers [6-9], low-molecular weighed organic compounds [10], and/or
68 inorganic compounds [11]. An example of synthetizing that kind of gels is to use caprylocaproyl
69 polyoxyl-8-glycerides, diethylene glycol monoethyl ether, and propylene glycol monolaurate. For
70 enhancing its viscosity, carbopol was used, since it formed ME-based gels [12]. Commonly, gels
71 made of surfactant, co-surfactant, water, and oil can be formed to advanced products that are useful
72 in pharmaceutical industry due to their feasibility as drug carriers [13-15]. Especially, the amount and
73 types of gels exploited in biotechnology and food industry have grown greatly [16].

74 In analytics, specific gels are needed to separate biological macromolecules, such as proteins and
75 lipoproteins, by planar or capillary electrophoresis techniques [17]. In gel-aided separations, electric
76 field is used for generation of molecule movement that is depended on molar mass, length,
77 conformation, hydrophobicity, and charge. Those molecular parameters influence in the permeability
78 and compatibility in gel environment [18,19].

79 When gel formation is processed in water, hydroxyethyl cellulose and/or polyvinyl pyrrolidone may
80 be added into solutions to make the material more hydrophobic [20]. Evidence is published about
81 active bio-based nanomaterial films from fish gelatin and chitosan nanoparticles have shown to
82 incorporate with oregano essential oil (*Origanum vulgare L.*). Then, chitosan particles were obtained
83 by ionic gelation with sodium tripolyphosphate to have a spherical morphology (size 40-80 nm) [21].

84 In addition, tensile and water vapor barrier properties of that kind of nanomaterial films showed to
85 vary depending on concentration of essential oil used. Moreover, gelatin from partial hydrolysis of

86 collagen is well-known for its good film-forming characteristics that leads to the formation of
87 transparent and flexible films [22]. However, due to their hydrophilic nature, the films have poor
88 water-vapor barrier [23] and cannot be used for extraction for environmental purposes, since the gel
89 materials need to have hydrophobic properties to be suitable for cleaning of organic contaminants in
90 water.

91 Until now, vegetable oil gels have not been used as solid materials in analytics. However, in literature
92 there are examples of using vegetable oils as part of microemulsions. Mint oil ME has been used in
93 dynamic light scattering (DLS) and electron paramagnetic resonance (EPR) studies, and citrus oil and
94 eucalyptus oil ME in general analytics [24-26]. Modifiers like the surfactant of Brij-30 was needed
95 to enable steroids to absorb through skin [27]. In addition, oil isolated from soya beans has been
96 promising in studies with lecithin as the analysis medium [27].

97 Our group has earlier published results made with capillary electrophoresis using ME electrolytes
98 made of vegetable oils for separation of isoflavonoids, benzodiazepines, steroids, and proteins
99 [28,29]. Since the vegetable oils are heterogeneous, being non-polar and/or polar and containing
100 triglycerides, they are mixtures of fatty acids, esters of glycerol, and fatty acids [30].

101 The ratio of various fatty acids varies between vegetable oils [29]. Typically, saturated fatty acids are
102 preferred in gel formation, like palmitoleic and stearic acids in olive, walnut, and linseed oils. The
103 quantity of cis-9-octadecanoic acid (18:1) in rapeseed, olive, walnut, and linseed oils are ca. 32, 84,
104 17, and 19 %. In ME capillary electrophoresis, the electrolyte made of linseed, walnut, olive, and
105 rapeseed oils in steroid separation showed good efficiency [28,29]. In unsaturated vegetable oil the
106 structural and rheological properties have an influence in gel formation and the quantity of the gel
107 product [30]. According to literature, only 0.5 % (w/w) of 12-hydroxy-9-*trans*-octadecenoic acid has
108 been extracted from oils to gels [31]. The small extractability is not a problem, since in case of
109 rapeseed, sesame, and diacylglycerol oils they show to influence the synthesis only, when the acid
110 concentration is low [31-33]. Structure–function studies have shown that carboxyl groups, positions

111 of hydroxyl groups, and lengths of acyl chains play the most important roles in gelation [34].
112 Although, triglyceride-based vegetable oils are important for polymer synthesis, vegetable oils and
113 their modifications are the raw materials for resin preparation. Furthermore, linseed and Tung oil
114 have been used for coating ingredients in oil paints and varnishes [35].

115 The present study was made with vegetable oils of macadamia, linseed, olive, walnut, frying,
116 rapeseed, sesame, and coconut oils synthesized with polyacrylamide and mixed with celluloses,
117 cotton, or Kraft lignin. Consequently, only three selected oil gels have fast gelation and therefore,
118 their performance in purification of steroid in water were studied. The steroid concentrations in the
119 eluates obtained from the extraction procedures were determined with partial filling micellar
120 electrokinetic capillary chromatography (PF-MEKC) with UV detection.

121 **2. Experimental**

122 **2.1 Chemicals**

123 The commonly detected steroids in wastewater were used as the analytes of the study.
124 Androstenedione ($C_{19}H_{26}O_2$, purity $\geq 98\%$), testosterone ($C_{19}H_{28}O_2$, purity $\geq 98\%$), and progesterone
125 ($C_{21}H_{30}O_2$, purity $\geq 98\%$) were purchased from Sigma-Aldrich (Germany). They were used as
126 received, and stored in a dark cold room ($-20\text{ }^{\circ}\text{C}$). Ammonia (min. purity 25%) from VWR
127 International S.A.S (France) and ammonium acetate (purity 98%, AA) from Sigma-Aldrich
128 (Germany) were purchased for the electrolyte solutions in capillary electrophoresis. Methanol
129 (HPLC-MS grade) was from Fisher Scientific (UK). The sodium salt of taurocholic acid monohydrate
130 (BioXtra, purity $\geq 95\%$) and sodium dodecyl sulphate (purity 99%) were from Sigma-Aldrich
131 (Germany). Hydrochloric acid (1.0 M) and sodium hydroxide (1 M) were purchased from Oy FF-
132 Chemicals Ab (Finland). Methanol (HPLC-MS grade) was used as the solvent in standard mixtures
133 and as the eluent in SPE extracts. It was also used the marker of electroosmosis.

134 For gel formation extra pure vegetable oils used were rapeseed, olive, linseed, and walnut oils. The
135 triglyceride fatty acids compositions were alternating the lipid number of C16:0, C17:0, C18:0,

136 C20:0, C16:1, C18:1, C20:1, C22:1, C18:2, and C18:3. The rapeseed oil was manufactured by AOR
 137 N.V. (Belgium) and VitaD'or (Germany), olive oil by Sovena Group (Spain), linseed oil by Elix Oil
 138 Oy (Finland), and walnut oil by Princes Foods B.V. (The Netherlands). Rapeseed oil had approved
 139 the acquisition of *Belgian vegetable oil* distributor *AOR NV* by U.S. grain trader in 2015. The extra
 140 virgin olive oil from Slovenia was classified by UNESCO as Intangible World Heritage since 2013.
 141 Elix Oy is company specialized in production of cold pressed *flaxseed oil* under oxygen-less
 142 environment. Walnut oil was achieved from Lanfranchi, (Italy), macadamia oil from Olivado
 143 (Australia), frying oil (mixture of sunflower, palm, and rapeseed oils), sesame oil from Lanfranchi
 144 (Princes Foods, B.V., The Netherlands), and coconut oil from Coconut Oil (UK).
 145 Sesame oil has linoleic acid, oleic acid, palmitic acid, and stearic acid at 41%, 39%, 8%, and 5%,
 146 respectively (<https://en.wikipedia.org>). Rapeseed oil has the acids at 21%, 61%, 4%, and 2%
 147 concentrations, and frying oil at 3.5-21%, 55-83%, 7.5-20%, 0.5-5%, respectively.
 148 Tris(hydroxymethyl) aminomethane (Tris) and sodium tetraborate decahydrate (Borax) were
 149 purchased from Sigma-Aldrich (Germany). Ammonium persulfate (APS), acrylamide (AA), and
 150 bisacrylamide (N,N'-methylene-bisacrylamide) were from Merck (Darmstadt, Germany).
 151 Tetramethylenediamine (TEMED) was from Thermo Fischer Scientific Oy (Vantaa, Finland). Lignin
 152 (alkali, Kraft, pH 10.5), cotton, cellulose acetate (CA), hydroxypropyl cellulose (HPC), and
 153 hydroxypropyl methylcellulose (HPMC) were from Sigma-Aldrich (Germany).
 154 Ultrapure water (MQ) was purified with a Direct-Q UV Millipore water purification system
 155 (Millipore S.A., Molsheim, France, 18 MΩ, pH 5.80).

156 **2.2 Instruments and methods**

157 **2.2.1 Capillary electrophoresis and methods**

158 A Hewlett-Packard 3D CE instrument (Agilent, Waldbronn, Germany) equipped with a photodiode
 159 array detector (190-600 nm) was used for the determination of the steroids in the extracts and eluates.
 160 The CE instrument was applied with ChemStation programs (Agilent) for instrument running and

161 data handling. Bare fused silica capillaries (i.d. 50 μm , o.d. 375 μm) were purchased from Polymicro
 162 Technologies (Phoenix, AZ, USA). They were cut to the total length (L_{tot}) of 80 cm with the efficient
 163 length (L_{eff}) of 71.5 cm. Before use, they were conditioned by sequentially flushing with 0.1 M NaOH,
 164 MQ water, and the electrolyte solution for 20 min each at 13.634 p.s.i. (940 mbar). Additionally,
 165 before each analysis, the capillary was flushed with 0.1 M NaOH and the electrolyte solution for 2
 166 min.

167 The analysis method [35] was based on partial filling micellar electrokinetic chromatography (PF-
 168 MEKC) with direct UV detection. The temperature during the analyses was +25 °C. Positive polarity
 169 at constant voltage 25 kV was used in the measurements. The electrolyte solution in PF-MEKC gave
 170 17 μA current. The analysis time was 20 min, but the steroids migrated within 18 min. The samples
 171 were introduced into electrical separation at 0.50 p.s.i. (34.5 mbar) pressure for 6 s. The pilot signals
 172 in detection were 214 (for electroosmosis), 220 (for matrix profiling), and 247 nm (for steroids).

173 **2.2.2 Electrolytes in capillary electrophoresis**

174 The steroids in the extracts from gel filled tubes in SPE treatment were studied with partially filled
 175 micelle mixture in basic 20 mM ammonium acetate solution [36]. Its pH was adjusted to pH 9.68
 176 with 25% ammonia. The micelle solution used in partial pseudo stationary phase was made by mixing
 177 1000 μL of 20 mM ammonium acetate (AA), 440 μL of 100 mM sodium dodecyl sulphate (SDS) in
 178 20 mM AA (pH 9.68), and 50 μL of 100 mM sodium taurocholate, in this specific order. The micelle
 179 and the electrolyte solutions were sequentially introduced into the capillary in order to have the
 180 micelle plug between the BGE solution and the standard or the SPE extracts. After the injection step
 181 the capillary was dipped into BGE for 0.5 min before starting the analysis.

182 **2.2.3 Preparation of ME gels**

183 The ME gels were prepared from macadamia, linseed, olive, walnut, frying oil (used for cooking and
 184 analogues pure unused oil), rapeseed, sesame, and coconut oils (*Supplementary data: 2S*
 185 *Experimental*) according to the schemes informed in Figure 1. Hydrolysis of the vegetable oils were

made at aqueous basic solution in both cases at ambient temperature (recipes 1 and 2) at ambient temperature before adding other solutions. When used in solid phase extraction, the oil gels (1.00 g, *Supplementary data: Figure 2S*) were mixed with lignin, cellulose acetate (CA), cotton, hydroxypropyl cellulose (HPC), or hydroxypropyl methylcellulose (HPMC) (100 mg, 10% of the weight of the oil gel) to stand mechanical stress in packing the material into tubes and against the vacuum during the SPE treatment. The gel formation process was similar to that making PA-type gels, but with the exception that sodium dodecyl sulphate (SDS) was replaced by the vegetable oils. The solutions used were 50 mM sodium tetraborate (STB) buffer (pH 8.5), acrylamide solution (AA, $\geq 99\%$, HPLC grade) at 30% aqueous solution (w/v), 3.9 M ammonium persulphate (APS) solution, and tetramethylethylenediamine (TEMED). The 1.5 M tris(hydroxymethyl) aminomethane (Tris, pH 8.8) solution was prepared into MQ-water for gel synthesis.

2.2.4 Solid-phase extraction

The SPE device Vac Master (Biotage VacMaster 20 Sample Processing Station) was used for solid phase extraction of the water samples. The ME gels (1.00 g or when liquid 3 x 200 μL) added by 100 mg of CA, cotton, HPMC, HPC, or lignin) were placed onto deactivate cotton membrane. In the final studies only frying oil, rapeseed oil and sesame oil based gels were used, since they had the fastest gelation process and thickening performance.

Totally 250 oil gel material including the carbohydrate modified materials were used in the SPE extraction. The steroid sample mixtures in the studies were prepared to 5 $\mu\text{g/mL}$ concentration (15.5 μM androstenedione, 17.3 μM testosterone, and 15.9 μM progesterone) in water.

2.2.5 Preparation of samples

The stock solutions of the steroids were 1000 mg/L. The water samples in the SPE contained 5 mg/L of the steroids. The compounds removed from the gel material during the extraction into methanol. The water parts were also analyzed and therefore they were concentrated by removal of the solvent.

First, the eluates were evaporated to dryness at 40 °C temperature under nitrogen. Then, the precipitates were dissolved into 300 µL of methanol. The whole solution was put into a 250 µL vial.

3. Results

Vegetable oils used in preparation of gels have noticed to improve properties of the new preparations and have shown to exploit them in novelty valued applications [31,32]. In the present research, vegetable oil ME gels were prepared for removing hydrophobic steroids from water. All layers in gel formation (Figure 1), which were the upper fraction from the vegetable oil, the middle layer from oil-in-water (o/w), and the lower fraction from water, were used for the studies. The gels as such could be spread to film format (*Supplementary, Figure 3S*) and used in gel electrophoresis for protein separation (*Supplementary, Figure 4S*). When they were used for the targeted water cleaning, the gel durability against mechanical pressure needed to be increased. Therefore, they were mixed with bio materials such as Kraft lignin, cotton, cellulose acetate (CA), hydroxypropyl cellulose (HPC), and hydroxypropyl methylcellulose (HPMC). Then, the gels could be used to remove of nonpolar steroids from water. The steroids were selected as the analytes, since they are found in wastewater and drinking water [36,37]. Furthermore, the steroids were markers to follow the behaviour of the gel materials and compare them with each other.

3.1 Behaviour of the gel materials for extraction

Eight different gels from macadamia, linseed, olive, walnut, rapeseed, sesame, coconut, and frying oils (Figure 1; *Supplementary data: Table 1S, Figure 1S*) were used. Differences in their chemical behaviour was observed due to the saturation state of the fatty acids. Frying oil was included in the study, since it has been scientifically pointed that frying oil that is made of olive oil, has equal or superior properties compared with the refined vegetable oil [38]. In the present study, the frying oil contained sunflower, palm, and rapeseed oils. Its behavior was not previously known. After modification with lignin, cotton, or celluloses hydrophobicity of all synthesized gels was changed compared to the basic materials. The present study showed that the most decrease in hydrophobicity

235 was obtained with lignin, because it was from alkali Kraft process (pH 10.5). On the other hand, the
236 most increase in the hydrophobicity was observed after HPMC modification of any bio gel. Recent
237 literature supports the observations, since oregano oil has noticed to increase the hydrophobicity of
238 bioactive materials that were made of fish gelatine and chitosan [21].

239 The strong hydrophobic character of the gels was important since using them for cleaning of
240 contaminants from water. In the present work, hydrophobicity, wettability, and floating properties of
241 the selected gel mixtures were studied by observations with water repulsion of the gel materials. The
242 differences between the ME gels were studied using experimentally measured total steroid
243 concentrations from methanol extract by comparing them with the concentrations in water
244 concentrate. The gels from macadamia, linseed, walnut, and coconut were also used in the
245 examination, but they were excluded from the final studies (Supplementary data: Figures 5S, 6S, 7S,
246 and 8S). The reasons were their low recoveries in extraction, quite high matrix effect in
247 electrophoretic separation, and especially, the very slow gel formation at ambient temperature (Figure
248 1).

249 **3.2 Determination of steroids with capillary electrophoresis**

250 For the gel studies, the PF-MEKC method was optimized at 247 nm UV-wavelength in determination
251 of the steroids. It was recently used for analyses of eight steroids in wastewater and drinking water
252 samples [36,37,39,40]. In the earlier studies, the steroids were isolated from authentic water with
253 commercial nonpolar polymer-based SPE materials. In the present study the water samples were
254 artificially prepared, since the behaviour of the gel materials were studied. Usually, the steroids are
255 at very low concentrations (pg/L-ng/L) in environmental water, although in emission sources the
256 concentrations may be 1000-100000 times higher [39]. Using the PF-MEKC method in CE, UV
257 detecting of the steroids needs concentrations above 0.1 mg/L. According to literature it means that
258 the sample volumes should be 2 L – 5 L to enrich the steroids for the PF-MEKC analyses [36]. Thus,

259 standard samples were used to evaluate the advantages and differences of the gels without unknown
260 effects from other biological matrices except the gel components.

261 Figures 2 and 3 show the ordinary steroid profiles from the water eluates from the o/w phase gels
262 made of sesame oil modified with CA, frying oil with Kraft lignin, and sesame oil gel containing
263 HPMC. It was noticed that in case of lignin modification the eluates gave always a baseline that was
264 10 times higher than that obtained in presence of HPMC. The reason was the matrix that reached the
265 UV detector in six minutes and showed a high absorption thereafter. Since all the oils used contained
266 linoleic acid, oleic acid, palmitic acid, and stearic acid, it could be supposed that the alkyl chains of
267 the oil structures would give rather specific than similar gel properties that could be detected from
268 the individual electropherogram.

269 Based on the electrophoresis profiles of water (Figures 2 and 3) the migration order of the target
270 compounds was androstenedione, testosterone, and progesterone. The figures also show that the water
271 eluates contain less matrix compounds than the correspondingly obtained methanol extract (Figure
272 4). Even though, the sensitivities of the steroids were higher in methanol than in water extracts.

273 The concentrations of androstenedione, testosterone, and progesterone were used to calculate the lipid
274 absorptivity of frying, rapeseed, and sesame oil gels (Figures 2, 3, and 4). In all cases, the steroid
275 profiles were identified by spiking with a mixture of 2 µg/mL (6.98 µM androstenedione, 6.93 µM
276 testosterone, and 6.36 µM progesterone) concentration to justify correctness of their migration.
277 Concentration calibration was made with 0.5-10.0 µg/mL solution mixtures by resulting in the ranges
278 of 1.75-35.0 µM for androstenedione, 1.73-34.6 µM for testosterone, and 1.59-31.8 µM for
279 progesterone. The corresponding calibration lines were $y=0.632x-0.029$ (r^2 0.940), $y=0.779x-0.213$
280 (r^2 0.962), and $y=4.315x-4.077$ (r^2 0.968). Therefore, the limits of detection (LOD) were 1.75 µM,
281 1.30 µM, and 0.35 µM and the quantification limits (LOQ) 5.25 µM, 4.00 µM, and 1.02 µM,
282 respectively.

283 Steroids adsorbed onto frying oil-based ME gels were better removed to methanol than to water. In
284 presence of Kraft lignin methanol was as good eluent as water (MQ water, pH 5.80) for steroid
285 release. All of the most hydrophobic HPMC modified vegetable oil-based gels were very selective
286 for methanol elution. Figure 5 shows the results that were more centralized in steroid extracts from
287 frying oil gel than from sesame oil gels. It can also be concluded that Kraft lignin modification was
288 not selective enough to clean the water samples from steroids, since those gels absorb water.
289 Especially, when Kraft lignin was mixed to rapeseed oil gel that was originated from the upper phase
290 of the synthesis (Figure 1), PF-MEKC analyses gave identically small recoveries from extraction of
291 steroids with both methanol and water. Similarly, the similar type small peaks with low
292 concentrations could be obtained in both CA and HPC modified rapeseed oil from the middle (o/w)
293 phases due to the low yields of steroids from those materials.

294 Figure 6 shows the results of steroids extracted from rapeseed, sesame, and frying oil gel materials.
295 The Figures 6A and B show the compilation of the results in CA, cotton, and HPC modified gels with
296 adsorbed steroids. It can be seen that methanol and water in elution had nearly equal selectivity jointly
297 with the w/o emulsion fractions (Figure 1, middle). On the contrary, HPMC or Kraft lignin
298 modification was more suitable for removing steroids from water combined with all the oil layers
299 (upper) in methanol elution. The more nonpolar the gel, the longer the steroids retained. Thus, in
300 those cases methanol was particularly selective for the steroid extraction. The analyte recoveries from
301 nonpolar phases were 70-85% for all the steroids. This was in accordance with our earlier studies
302 [36,37]. The obtained recoveries were also comparable with the study on extraction of diclofenac
303 formulation with ME gel made of caprylocaproyl polyoxyl-8 glycerides, diethylene glycol monoethyl
304 ether, and propylene glycol monolaurate allowing the recoveries of 77% and 70% [12].

305 In the presence of Kraft lignin, CA, and cotton as the gel modifiers the results of the extracts were
306 more centralized than in using nonpolar HPC and HPMC modification. The hydrophobicity and the
307 strength of interaction increased with increase in the alkyl chain length, but the absorptivity decrease

308 [41], as is visualised also in Figure 6. It shows that the analytes were merely detected from the upper
 309 (oil) and middle (o/w) layers in nonpolar HPMC modified oil gels (*Supplementary data: Figure 10S*).

310 **3.3 Water resistance of the gels**

311 Polar groups and ionization of the gel mixtures decreased their hydrophobic character. The decrease
 312 of hydrophobicity of gels modified with Kraft lignin, cotton, and celluloses gave the order of lignin
 313 > cotton > cellulose acetate > HPC > HPMC. When the additives had a role in the wettability of the
 314 gels, they could be detected by sinking to the bottom of the water container. Those materials that
 315 could be floating spread slightly out in presence of water. Then, the best material combinations with
 316 similar floating properties as the two best water resistant were CA and HPMC modified rapeseed,
 317 frying, and sesame oil based gels, but also lignin modified rapeseed and sesame oil gels. All CA and
 318 HPMC based gels and a few Kraft lignin based, some of cotton modified, and a few HPC modified
 319 gels were floating more than 5 days, 120 h, 120 h, and 2 h, respectively (Figure 7).

320 On the contrary, gels made from rapeseed and sesame oils, and finalized with lignin and cellulose
 321 acetate, became hydrophobic and thus they could not absorb water (Figure 7). The similar effect was
 322 generated, when the ingredients were rapeseed, sesame, and frying oils modified with HPMC or CA.
 323 Frying oil and alkali Kraft lignin ended up to hydrophilic gels, and sank immediately below the water
 324 surface (*Supplementary data: Figure 9S*). It was recognized that hydrophilic lignin had a significant
 325 role in wettability. Similarly, frying oil gels modified by cotton resisted water only less than a minute
 326 before absorption and gone down into water. However, CA and HPMC in rapeseed, sesame, and
 327 frying oil gels enhanced the material hydrophobicity accomplishing lightweight materials.

328 The PF-MEKC-UV studies showed that the highest peak areas correlating to the highest
 329 concentrations and recoveries were obtained for androstenedione and testosterone in methanol
 330 extracts from CA and HPC modified gels. In that case, the extraction properties were high, although
 331 HPC modification was not very practical due to the poor floating properties. On the contrary, the
 332 largest recoveries for progesterone were obtained in both methanol and water, when the gels were

333 modified with HPMC. Unfortunately, the peak areas of androstenedione were the smallest, which
334 showed that water was not a good eluent and the steroid retained on the HPMC containing gels. The
335 smallest recoveries were observed in water extracts for both testosterone and progesterone in lignin
336 modified gels (*Supplementary data: Figure 9S*).

337 To compare the gels by extraction properties, the best vegetable oil based gel was rapeseed oil, since
338 in the presence of HPMC the gel gave the best recoveries for all steroids. Furthermore, frying oil from
339 upper phase (Figure 1) had excellent absorptivity against the lipid steroid and also verified its floating
340 capability on water surface. The worst material for extraction of steroids was CA modified from the
341 lower phases of frying, sesame, and rapeseed oils.

342 The study showed that vegetable oil based gels can be used to isolate steroids from water matrix. In
343 addition, the selected eluents in SPE showed that the recoveries were high. Nearly all steroids could
344 be removed from the sorbent to enable their reusability.

345 **4 Conclusions**

346 The study shows that the gels made of vegetable oils and modified with Kraft lignin, cotton, and
347 cellulose related substances are capable to adsorb nonpolar steroids from water. When the oil gel
348 materials are not sank in water, they may be used for water pretreatment to collect nonpolar
349 compounds, like steroid-type compounds and other lipids from surface water. Capillary
350 electrophoresis techniques showed to work excellent for comprehensive profiling of the steroids after
351 solid phase extraction enrichment made by the new oil gels.

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356 **6 References**

357 [1] A. Froelich, T. Osmałek, A. Snela, P. Kunstman, B. Jadach, M. Olejniczak, G. Roszak, W. Białas,
358 Novel microemulsion-based gels for topical delivery of indomethacin: Formulation, physicochemical

- properties and in vitro drug release studies, *Journal of Colloid and Interface Science* 507 (2017) 323–336.
- [2] M.J. Lawrence, G.D. Rees, Microemulsion-based media as novel drug delivery systems, *Adv. Drug Deliv. Rev.* 64 (2012) 175–193.
- [3] S. Talegaonkar, A. Azeem, F. Ahmad, R. Khar, S. Pathan, Z. Khan, Microemulsions: A Novel Approach to Enhanced Drug Delivery, *Recent Pat. Drug Deliv. Formul.* 2 (2008) 238–257.
- [4] D.J. McClements, Nanoemulsions versus microemulsions: terminology, differences, and similarities, *Soft Matter* 8 (2012) 1719–1729.
- [5] Y.S. Rhee, J.G. Choi, E.S. Park, S.C. Chi, Transdermal delivery of ketoprofen using microemulsions. *Int J Pharm* 228 (2001) 161–170.
- [6] A. Froelich, T. Osmalek, P. Kunstman, R. Roszak, W. Białas, Rheological and textural properties of microemulsion-based polymer gels with indomethacin, *Drug Dev. Ind. Pharm.* 42 (2016) 854–861.
- [7] M. El-Badry, G. Fetih, F. Shakeel, Comparative topical delivery of antifungal drug croconazole using liposome and micro-emulsion-based gel formulations, *Drug Deliv.* 21 (2014) 34–43.
- [8] B. Rozman, A. Zvonar, F. Falson, M. Gasperlin, Temperature-sensitive microemulsion gel: an effective topical delivery system for simultaneous delivery of vitamins C and E, *AAPS Pharm Sci Tech* 10 (2009) 54–61.
- [9] S. Miao, P. Wang, Z. Su, S. Zhang, Vegetable-oil-based polymers as future polymeric biomaterials, *Acta Biomaterialia*, 10 (2014) 1692–1704.
- [10] G. Feng, Y. Xiong, H. Wang, Y. Yang, Gelation of microemulsions and release behavior of sodium salicylate from gelled microemulsions, *Eur. J. Pharm. Biopharm.* 71 (2009) 297–302.
- [11] B. Rozman, M. Gosenca, M. Gasperlin, K. Padois, F. Falson, Dual influence of colloidal silica on skin deposition of vitamins C and E simultaneously incorporated in topical microemulsions, *Drug Dev. Ind. Pharm.* 36 (2010) 852–860.
- [12] B. Hajjar, K.-I. Zier, N. Khalid, S. Azarmi, R. Löbenberg, Evaluation of a microemulsion-based gel formulation for topical drug delivery of diclofenac sodium, *Journal of Pharmaceutical Investigation* 48(3) (2018) 351–362.
- [13] Y. Zheng, W.-Q. Ouyang, Y.-P. Wei, S. F. Syed, C.-S. Hao, B.-Z. Wang, Y.-H. Shang, Effects of Carbopol® 934 proportion on nanoemulsion gel for topical and transdermal drug delivery: a skin permeation study, *Int J Nanomedicine* 11 (2016) 5971–5987.
- [14] A. Spornath, A. Aserin, Microemulsions as carriers for drugs and nutraceuticals, *Adv. Colloid Interface Sci.* 128–130 (2006) 47–64.
- [15] I. Kirill, S.C. Roberge, O. Zabeida, M. Robert, J. E. Klemberg-Sapieha, *J Mater Sci: Mater Med* 20 (2009) 681–689.

- 393 [16] L.S. Nair, C.T. Laurencin, Polymers as biomaterials for tissue engineering and controlled drug
394 delivery. *Adv Biochem Engin/Biotechnol* 102 (2006) 47-90.
- 395 [17] J. Kopecek, Hydrogel biomaterials: a smart future? *Biomaterials* 28 (2007) 5185–5192.
- 396 [18] H. Omidian, K. Park, Hydrogels. In *Fundamentals and Applications of Controlled Release Drug*
397 *Delivery*. Edited J. Siepmann, R Siegel, M Rathbone. 2012. Springer New York, NY.
- 398 [19] J.H. van Esch, B.L. Feringa, New Functional Materials Based on Self-Assembling Organogels:
399 From Serendipity towards Design, *Angew Chem Int Ed Engl* 2000, 39: 2263-2266.
- 400 [20] U.A. Krut, A. Guevara, J. Huang, A.A. Shaposhnikov, A.I. Vezentsev, Method for preparation
401 of bioactive sorbent gel composition. *Russ.* (2017), RU 2616250 C1 Apr 13, 2017.
- 402 [21] S.F. Hosseini, M. Rezaei, M. Zandi, F. Farahmandghavi, Development of bioactive fish
403 gelatin/chitosan nanoparticles composite films with antimicrobial properties *Food Chem.* 194 (2016)
404 1266-1274.
- 405 [22] M.B. Pérez-Gago, Protein-Based Films and Coatings, in *Edible Coatings and Films to Improve*
406 *Food Quality*, 2nd Ed. (Eds. A.E. Baldwin, R. Hagenmaier, J. Bai) 2012, pp.14-58. Taylor and Francis
407 Group, Boca Raton, USA, CRC Press.
- 408 [23] S.F. Hosseini, M. Zandi, M. Rezaei, F. Farahmandghavi, Two-step method for encapsulation of
409 oregano essential oil in chitosan nanoparticles: preparation, characterization and in vitro release
410 study. *Carbohydr Polym.* 95 (2013) 50-56.
- 411 [24] F. Zhong, M. Yu, C. Luo, C.F. Shoemaker, Y. Li, S. Xia, J. Ma, Formation and characterization
412 of mint oil/S and CS/water microemulsions, *Food Chem* 115 (2009) 539-544.
- 413 [25] M. Fanun, Properties of microemulsions with mixed non-ionic surfactants and citrus oil, *Colloids*
414 *Surf. A: Physicochem Eng Aspects* 369 (2010) 246–252.
- 415 [26] B. Biruss, H. Kählig, C. Valenta, Evaluation of a Eucalyptus Oil Containing Topical Drug.
416 *Delivery System for Selected Steroid Hormones*, *Int J Pharm* 328 (2007) 142–151.
- 417 [27] V. Papadimitriou, E.D. Tzika, S. Pispas, T.G. Sotiroudis TG, Xenakis A., *Colloids Surf A:*
418 *Physicochem Eng Aspects* 2011, 382: 232-237.
- 419 [28] H. Sirén, A. Karttunen, Microemulsion electrokinetic chromatography for the separation of
420 carbamazepine, oxcarbazepine, and their metabolites, *J Chrom B* 783 (2003) 113–124.
- 421 [29] H. Sirén, S. Vesanen, J. Suomi, Separation of steroids using vegetable oils in microemulsion
422 electrokinetic capillary chromatography, *J Chrom B* 945/946 (2014) 199– 206.
- 423 [30] A.J. Wright, A.G. Marangoni, Formation, structure and rheological properties of ricinelaidic
424 acid-vegetable oil organogels. *J. Amer. Oil Chem. Soc.* 83 (2006) 497–503.
- 425 [31] N. Karak, *Vegetable Oil-Based Polymers: Properties. Processing and Applications*, 2012.
426 Cambridge, UK, Woodhead Publ. (Elsevier Ltd).

- 427 [32] Z.S. Petrovic, Polyurethanes from vegetable oils, *Polym. Rev.* 48 (2008)109–155.
- 428 [33] C Zhang, TF Garrison, SA Madbouly, MR Kessler, Recent Advances in Vegetable Oil-Based
429 Polymers and Their Composites. *Progress in Polymer Science* 2017, 71, 91-143.
- 430 [34] J. Daniel, R. Rajasekharan, Organo gelation of Plant Oils and Hydrocarbons by Long-Chain
431 Saturated FA, Fatty Alcohols, Wax Esters, and Dicarboxylic Acids. *JAOCs* 2003, 80(5): 417-421.
- 432 [35] M. Samadzadeh, S.H. Boura, M. Peikari, A. Ashrafi, M. Kasiriha, Tung oil: an autonomous
433 repairing agent for self-healing epoxy coatings, *Prog. Org. Coat.* 2011 70, 383-387.
- 434 [36] H. Sirén, S. El Fellah, Androgens, oestrogens, and progesterone concentrations in wastewater
435 purification processes measured with capillary electrophoresis, *Environmental Science and Pollution*
436 *Research* (2017), 24(20), 16765-16785.
- 437 [37] S. El Fellah, G. Duporté, H. Sirén, Steroid hormones, inorganic ions and botrydial in drinking
438 water. Determination with capillary electrophoresis and liquid chromatography-orbitrap high
439 resolution mass spectrometry, *Microchemical Journal* 133 (2017) 126-136.
- 440 [38] A. Chiou, N. Kalogeropoulos, Virgin Olive Oil as Frying Oil, *Comprehensive Reviews in Food*
441 *Science and Food Safety* 16 (2017) 632-646.
- 442 [39] H. Sirén, S. El Fellah, Steroids contents in waters of wastewater purification plants:
443 determination with partial-filling micellar electrokinetic capillary chromatography and UV detection,
444 *International Journal of Environmental Analytical Chemistry* 96 (2016) 1003–1021.
- 445 [40] S. El Fellah, H. Sirén, Recent Developments in Capillary Electrophoresis of Steroids and Sterols,
446 *Journal of Biomedical Research and Practice* 2(1) (2018) 1-21, Article ID: 100006.
- 447 [41] J.A. Queiroz, C.T. Tomaz, J.M.S. Cabral, Hydrophobic interaction chromatography of proteins.
448 *J. Biotechnol.* 87 (2001) 143-159

Figure 1. Procedures for synthesis of vegetable oil gels. All the phases (upper, middle, lower) for each vegetable oil were used in the study. The three layers contained mostly oil (upper), mixture of oil and water (middle), and water (lower). TEMED and APS were used for gelation of the water layer. Upper layer was oil phase, middle layer was water-in-oil/oil in water layer, and lower layer was water layer. The concentration and pH values of the buffers were optimized, since those parameters effected gelation.

Figure 2. Electropherograms of water eluates of steroids. (A) Sesame oil-CA, middle phase from recipe 1 (Fig. 1); (B) Frying oil-lignin, middle phase from recipe 2 (Fig. 1). Separation conditions as explained in Experimental part. Peaks (1) androstenedione, (2) testosterone, (3) progesterone. Detection at 247 nm. Method: PF-MEKC with ammonium acetate (pH 9.68) as the electrolyte and mixed micelle solution made of SDS and STC as the pseudo stationary phase. Volume in hydrodynamic injection was 55.67 nL (CE Expert Lite, SCIEX). Capillary dimensions: 80 cm (L_{tot}) and 71.5 cm (L_{det}).

Figure 3. Electropherograms of eluates obtained with HPCM modified ME sorbents. (A) Canola oil gel and (B) Frying oil gel. Elution was made with MQ water. Fraction is the middle phase from recipe 1 (Fig. 1). Volume in hydrodynamic injection was 55.67 nL (CE Expert Lite, SCIEX). Capillary length 80 cm.

Figure 4. Electropherogram of the steroid profile obtained from SPE methanol made on HPCM modified sesame oil based gel, middle phase made by the recipe 1. Methanol extract. Detection at 247 nm. Other details as in Fig.2. Volume in hydrodynamic injection was 55.67 nL (CE Expert Lite, SCIEX). Capillary dimension were 70 cm (L_{tot}) and 61.5 cm (L_{det}).

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540 Figure 5. Statistical data from correlation of total peak areas of steroids in electropherograms measured
541 with PF-MEKC from methanol and water extracts. SPE were made with canola oil, sesame oil, and frying oil
542 gels modified with HPCM, HPC, CA, cotton, and lignin. The total peak areas were calculated from the
543 steroid profile in the electropherogram from sum of peaks in methanol extract subtracted by the sum of
544 those peaks in water extract. Analysis conditions as in Figure 2. Zero-level correspond equal total
545 concentrations in methanol extract and water extract.

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562 Figure 6. Comparison of methanol and water solvents in extraction of steroids from cellulose, cotton, and
563 lignin modified ME gels. (A) Numbers 1 and 2 present the recipes used for the synthesis (Figure 1); (B)
564 upper, middle, and lower present the phases obtained with ME gel formation. Analysis conditions as in
565 Figure 2. The colours in the figure A and B represent the same vegetable oil. The spots in the figures A and
566 B represent the same phases made with the individual recipe. The spots below the horizontal line mean
567 that the steroids are extracted with water from the gels. Those above the line mean that the steroids are
568 extracted with methanol.

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Figure 7. Comparison of floating properties of the ME gels modified with celluloses, cotton, and lignin. The ME gels studied were made with recipes 1 and 2. Upper, middle, and lower phases were studied. Selected data from PF-MEKC –UV analyses were used to clarify the differences. The analysis conditions are informed in Figure 2.