

Alginate-based sorbents in miniaturized solid phase extraction techniques - step towards greenness sample preparation

Natalia Jatkowska

Department of Analytical Chemistry, Faculty of Chemistry, Gdansk University of Technology, 11/12 Narutowicza Str., Gdańsk 80-233, Poland

* natalia.jatkowska@pg.edu.pl

Abstract:

In response to growing concerns about environmental degradation, one of the main areas of research activity in recent years has been to make sample preparation methods more sustainable and eco-friendly. The increasing greenness of this step can be achieved by minimizing the usage of reagents, automating individual stages, saving energy and time, and using non-toxic, biodegradable substances. Therefore, the use of natural materials as sorbents in miniaturized extraction techniques is becoming a main trend. One of the natural material that is increasingly being used, not only due to eco-friendly nature but also because of their easy applicability to various sample preparation techniques, is alginate hydrogel. Following this trend, this review discusses the recent application of alginate-based sorbents in various microextraction techniques, focusing on functionalization approaches that enhance extraction performance. Additionally, the green profile of alginate-based sorbent microextraction approaches, along with the sorbent synthesis, were investigated.

1. Keywords: biosorbents, biopolymers, microextraction, sample preparation, green sorbent, green analytical chemistry

Introducing the ideology of Green Analytical Chemistry (GAC) into analytical laboratories was a turning point in the approach to developing analytical procedures. Since then, the main goal of analysts has been to find a balance between high sensitivity, precision, accuracy, and the sustainability of the developing analytical methodology [1]. Sample preparation is usually considered as the step with the largest environmental impact of the entire analytical procedure due to the extensive use of organic solvents in time-consuming procedures [2]. Therefore, special attention has been paid to improving the greenness of this step. The main strategies include: (i) miniaturization and automation; (ii) reducing or completely eliminating harmful and toxic chemicals; (iii) minimizing energy consumption; and (iv) minimizing waste generation. Another strategy to improve the sustainability of analytical sample preparation is to use biodegradable, reusable reagents easily obtained from renewable sources [3]. Therefore, in recent years, most published papers have focused on the replacement of toxic solvents and petroleum-based materials with more eco-friendly substances. One of the main directions in the area of solid phase extraction techniques is the application of materials of natural origin as sorbents. So far, various natural products such as cork, cotton, bamboo, pollen, sponge, seeds, and biowastes [3,4] have been used directly in different modes of solid-phase techniques. Some of these natural products have also served as sources of biopolymers. Depending on the monomer unit, biopolymers can be classified into three categories, such as polysaccharides, polynucleotides, and polypeptides. According to the literature, polysaccharides, are particularly widely used as sorption materials for sample preparation. Due to their high porosity, good mechanical properties, the possibility of easy chemical or physical modification of their surface, high availability, and low price [5,6]. Indeed, polysaccharides, such as cellulose, starch, chitosan, and agarose have been applied in different miniaturized extraction techniques such as solid-phase extraction (SPE), solid phase microextraction (SPME), dispersive micro solid-phase extraction (D- μ SPE), thin film

44 microextraction (TFME) and many others. A detailed description of these and other applications can
45 be found in the latest published papers [6–11]. However, in recent years, growing interest in the
46 application of another class of polysaccharides, alginates, for sorbent preparation intended
47 formicroextraction has been observed (Fig.1).

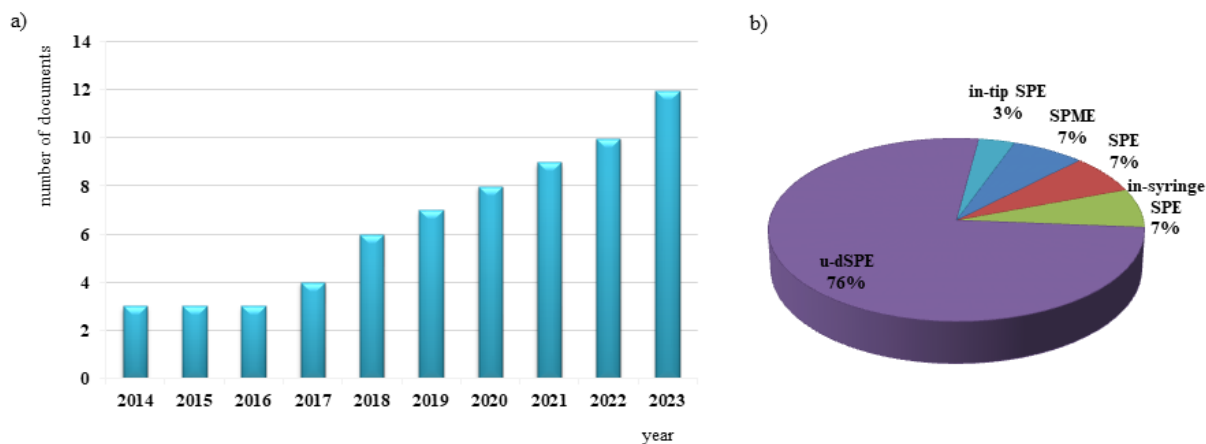


Fig.1.a) Number of publications focused on alginate-based sorbents application in microextraction approaches based on Web of science database (searched keywords: alginate-based sorbent; microextraction, accessed on June 2024);
b) the distribution of articles among various sorbent-based microextraction techniques.

48

49 In the light of the growing interest in the use of alginate-based materials in analytical chemistry, it
50 seems important to collect detailed information about their performance and applicability. Therefore,
51 this literature review presents a comprehensive overview of recent strategies for alginate gel
52 functionalization and their application in microextraction techniques such as SPE, in-syringe SPE,
53 SPME, and D- μ SPE. Additionally, environmental and sustainability issues related to preparation and
54 using alginate-based sorbents in the sample preparation step were considered by application of
55 Analytical Eco-scale tool. Although, few review articles focused on application of bio-sorbents in
56 microextraction processes are published, there is no publication strictly focused on the alginate-based
57 sorbents application in miniaturized extraction techniques, therefore, this review gives the
58 comprehensive knowledge in this area. It is believed that this article will motivate researcher to
59 conduct experiments in this area as considering published data, alginate-based sorbents applied in
60 microextraction processes seems to be a very promising solution.

61

62 2. Chemical structure and properties of alginates

63 Alginates (ALG) are composed of L-guluronic (G) and D-mannuronic (M) units combined with 1,4-
64 glycosidic linkages. The basic structure consists of linear, unbranched units made up of monomers
65 arranged in blocks of M and G in different configurations as homopolymeric blocks of G units (GG),
66 M units (MM), and alternating M and G units (GM), as shown in the Fig. 2 [12].

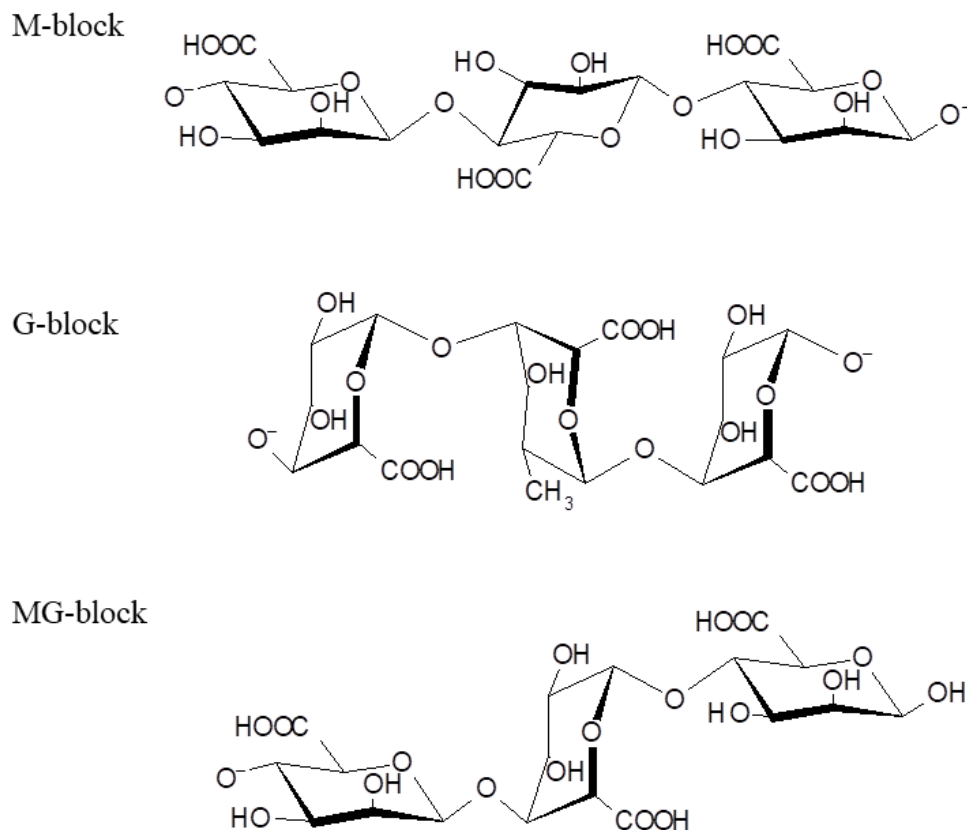


Fig.2. The molecular structures of possible sequences of L-guluronic and D-mannuronic units in alginate polymer.

67

68 The main sources of this biopolymer are marine plants and microorganisms. Seaweeds and brown
 69 algae such as *Ascophyllum nodosum*, *Laminaria digitata*, *Laminaria hyperborea*, and
 70 *Macrocystis pyrifera*, are particularly rich in alginate. The most common form of commercial alginate
 71 is sodium salt ($C_6H_9NaO_7$) [12,13]. One of the special features of alginates is their capability of
 72 forming gel networks. It can be achieved by two methods: ionic crosslinking with divalent cations or
 73 acid precipitation. Furthermore, they are biodegradable, biocompatible, and have not been shown to
 74 accumulate in biological systems. Thus, this material is particularly attractive for medical,
 75 pharmaceutical, cosmetic and food applications as well as for environmental remediation. The other
 76 good feature of alginates is their capability of forming gel independent of temperature, which is unique
 77 compared to other polysaccharides such as gelatin or agar [12,13]. Nonetheless, compared to
 78 conventional synthetic polymers, this biopolymer exhibits some limitations, such as a strong
 79 hydrophilic character, low thermal stability, and weak mechanical properties [14]. Therefore, much
 80 effort has been made to improve its physico-chemical properties. It was found that compounding
 81 alginates gel with other popular sorbents like metal nanoparticles, graphene oxide (GO), molecularly
 82 imprinted polymers, and others contributes to a significant improvement in physico-chemical
 83 properties. What is more, by selecting the appropriate material, both sorption capacity and selectivity
 84 towards selected groups of analytes can be significantly enhanced. In Figure 3 groups of materials
 85 usually combined with alginate gel to improve their sorption properties are presented. The adsorption
 86 properties of different alginate composite materials as well as their application in different
 87 microextraction modes are detailed discussed in the next subsection.

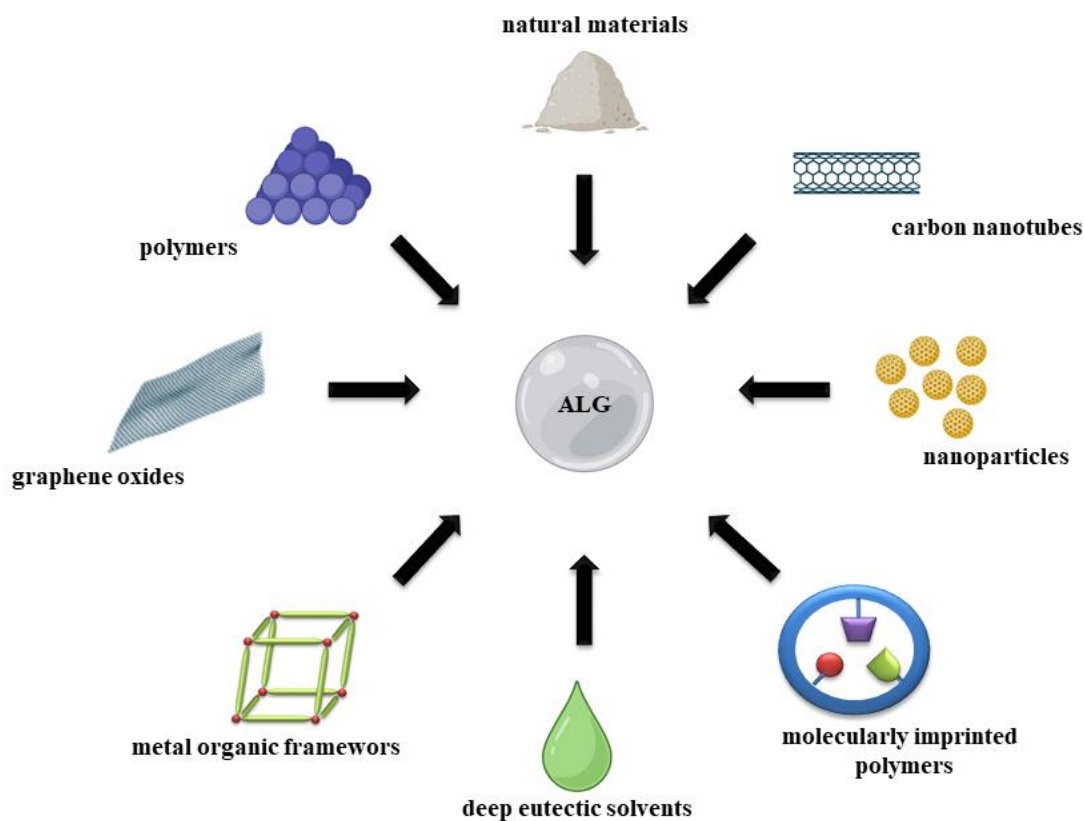


Fig.3. Main groups of sorbents combined with alginate hydrogel and used in solid-phase microextraction techniques.

88

89

90 3. Application of alginate-based sorbents in microextraction techniques

91 So far, alginate-based sorbents have been applied as a sorptive material in some of the miniaturized
 92 extraction techniques, such as SPE, SPME, and D- μ SPE. In general, regardless of the microextraction
 93 mode, the sorbent preparation process is relatively simple and easy to operate. As shown in Fig. 4,
 94 four main stages can be distinguished. In the first step, an alginate solution is prepared by dissolving
 95 alginate in water. The alginate content in an aqueous solution usually ranges from 1 to 2% (w/v). The
 96 second step involves adding specific material to the alginate solution to improve surface and
 97 adsorption properties of the sorbent. In the next step, alginate solution is added to the crosslinker agent
 98 to obtain the alginate-based solid sorbent. Depending on the microextraction approach, the procedure
 99 for obtaining the sorbent shape/format is different. To prepare sorbent beads, the alginate solution is
 100 added dropwise into the crosslinking solution, while for fiber formation, a certain amount of the
 101 alginate solution is added continuously. However, in order to obtain covered fiber, the device is
 102 immersed directly in a crosslinking solution. In almost all of the reported procedures, calcium chloride
 103 (CaCl_2) solution was used as a crosslinker. This stage is the most time-consuming of the entire
 104 procedure. Achieving complete formation of the sorbent requires keeping it in the CaCl_2 solution from
 105 30 minutes to even 2 hours. Then the formed sorbent is separated from the solution, washed by
 106 distilled water and, in most applications, dried. Following these steps, the sorbent becomes ready for
 107 use in the chosen microextraction procedure.

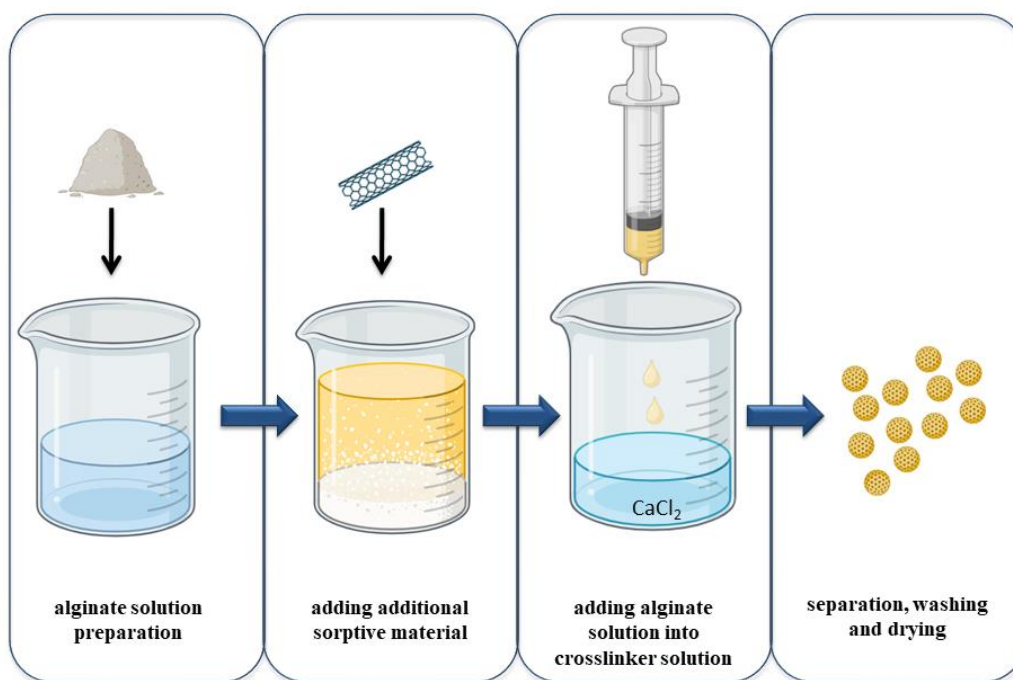


Fig.4. Main steps of alginate-based sorbent preparation.

108

109 Based on a literature review, it can be stated that the application of alginate-based sorbents to extract
 110 analytes in microextraction procedures is not very popular so far. However, the number of published
 111 reports is constantly increasing (as seen in Fig. 1). The vast majority of reports in this field are devoted
 112 to the application of this material in D- μ SPE. Articles focusing on using it in SPE, or SPME format
 113 are much rarer. In this section, the application of alginate-based sorbents in different miniaturized
 114 extraction techniques is described in detail. Moreover, specific parameters of the applied procedures
 115 are summarized in Table 1.

116 3.1. column SPE

117 Column SPE is without any doubt one of the most popular sorbent-based extraction techniques
 118 available today. The main reason for its widespread popularity is the possibility of achieving high
 119 efficiency in the extraction and clean-up processes in a shorter time and at a lower cost. In standard
 120 SPE configuration, the sorbent is placed between two frits in a cartridge or syringe barrel. After the
 121 sorbent conditioning, a sample passes through the sorbent, and the compounds of interest are retained
 122 in the solid phase. Then, the analytes are desorbed by the appropriate solvent [15].

123 Nonetheless, the application of an alginate-based sorbent in this format has not been widely adopted so
 124 far. There are only two papers in the literature describing the application of this approach. In the first
 125 paper, the authors used layered double hydroxides (LDHs) nanosheets-alginate hydrogel to extract and
 126 preconcentrate lead ions from tap and mineral water samples as well as juices and liqueur samples.
 127 The preparation of monolith columns began with the hydrothermal synthesis of NFLS (nickel-iron
 128 layered double hydroxides with laminar structure) nanosheets. Next, the obtained NFLS
 129 nanocomposite was mixed with a sodium alginate solution under ultrasonication. Then, NFLS/alginate
 130 dispersions were dropped into CaCl_2 solution to generate the ionic crosslinked NFLS-gel in the SPE
 131 column. Before loading the sample, the sorbent was conditioned with 2 mL of deionized water. After
 132 the sample passed through, the cartridges were again cleaned with deionized water, and then the
 133 analyte was eluted with 2 mL of HNO_3 and determined by Flame Atomic Absorption Spectrometer

134 (FAAS). The proposed method showed very good extraction efficiency (91.7–99.8%) and good
135 precision (relative standard deviation, 2.65%). Moreover, it has been shown that the incorporation of
136 sulphide intercalated LDHs nanosheets provides more adsorption sites, contributing to the higher
137 selectivity of Pb (II) and almost six times higher adsorption capacity compared to alginate hydrogel
138 [16]. Another approach based on packing alginate microparticles in polypropylene column was
139 proposed by Arantes De Carvalho et al. [17]. This column was prepared for the extraction and
140 preconcentration of 14 rare earth elements from environmental water samples. Briefly, the spherical
141 calcium alginate (CA) beads were synthesized, washed, and freeze-dried. Then, in order to increase
142 the surface area, dried beads were cryogenically ground and sieved. The obtained sorbent (300 mg),
143 with particle sizes ranging from 10 to 180 μm , was dispersed in deionized water and packed as a slurry
144 into a polypropylene column with a porous polyethylene frit at the bottom and at the top. The column
145 was coupled to a flow injection system and conditioned before loading the sample. After loading a
146 sample, the column was dried, the analytes were eluted with 1.0 mL of 0.5 mol/L HNO_3 and analyzed
147 by ICP-MS. However, the obtained recovery values were slightly lower than those obtained using
148 commercially available resins such as iminodiacetate (IDA), C_{18} -polyacrylonitrile (PAN), or alkyl
149 phosphinic acid resin (APAR). The proposed protocol can be a reliable, environmentally friendly
150 alternative due to the possibility of re-utilization of the CA column, even for up to 50 cycles, low cost,
151 and no need to use relatively highly concentrated acids.

152 3.1.1. in-syringe SPE

153 Although SPE is a very effective extraction method, it suffers from a few limitations, including using a
154 larger sample volume, solvents for conditioning and desorption, and a larger sorbent amount. So,
155 following GAC principles, a miniaturized version of traditional cartridge-based SPE in the form of a
156 syringe filter holder was proposed [18]. So far, in the area of alginate-based sorbents application in
157 this technique, only one study has been reported. The researchers prepared the sorbent by dispersing
158 biochar (obtained from herb residue) in the sodium alginate matrix and crosslinking it by immersing in
159 a calcium chloride solution. Then the obtained membrane was cut into a round piece (25 mm) and
160 packed in the syringe filter. To extract the nitroimidazoles from tap water, the sample was passed
161 through the filter six times by pulling and pushing the syringe plunger. Subsequently, the analytes
162 were eluted with 1.5 mL of acetonitrile and analysed by ultrahigh performance liquid chromatography
163 coupled to tandem mass spectrometry (UHPLC-MS/MS). Under optimal conditions (pH, extraction
164 time, elution solvent, volume, and extraction cycles), the application of the developed material
165 allowed recoveries of metronidazole (80.2 - 92.5%), secnidazole (86.4 - 99.1%), dimetridazole (79.3 -
166 93.9%), and ornidazole (79.6 - 92.9%) in the analysed samples. The results revealed that
167 immobilization of biochar in alginate gel not only increased analyte adsorption efficiency but also
168 enhanced mechanical properties, allowing it to be reused up to five times. Moreover, the mass of the
169 sorbent used and the limit of detection (LOD) value were significantly lower compared to other solid
170 phase microextraction methods applied for the analysis of nitroimidazoles in water samples. An
171 additional advantage of the proposed methodology was that no additional steps such as centrifugation,
172 filtration, or magnet separation were required, which significantly simplified the entire procedure and
173 reduced the time compared to others [19].

174 3.1.2. In-tip SPE

175 In another format of miniaturized SPE that uses the same steps—conditioning, sample loading,
176 washing, and elution—a small amount of sorbent is packed inside a plastic pipette tip[20]. An
177 alginate-based sorbent has also been applied in this approach. Nurerk et al. introduced a micro-pipette
178 tip device consisting of cotton fiber coated with sorbent, which was prepared by integrating metal-



179 organic frameworks (MIL-101) and cetyltrimethylammonium bromide (CTAB) into alginate hydrogel
180 [21]. In the proposed method, the authors first synthesized MIL-101 via the hydrothermal method and
181 mixed it with CTAB and alginate hydrogel. Subsequently, they used epichlorohydrin as a cross-linker
182 in the reaction between sodium alginate and cotton fibers. Then, cotton fibers were immersed in a
183 CaCl_2 solution to obtain MIL-101/CTAB/ALG/CF. The 500 mg of synthesized sorbent was packed
184 into a 1000 μL pipette tip and connected to a syringe. The proposed lab-made microextraction device
185 was utilized for the extraction of five phthalates from beverage samples. A 10 mL sample was
186 manually passed through the sorbent. The adsorbed analytes were then eluted from the fibers using 2
187 mL of acetonitrile and evaporated to dryness. Finally, the dry residue was dissolved in 1 mL of
188 methanol, and 20 μL were injected into the high performance liquid chromatography coupled to diode
189 array detection (HPLC-DAD) system. The proposed approach yielded a sorbent with a large specific
190 surface area (710.02 m^2/g), resulting in a high recovery rate (85.2–97.8%). Furthermore, the method
191 exhibited a low limit of detection (0.15 $\mu\text{g}/\text{L}$) and high precision (RSD less than 4.9%). The obtained
192 parameters were comparable to another HPLC-DAD method using graphene nanoplates as a sorbent in
193 SPE mode. However, it should be highlighted, that the volume of the sample as well as solvent used
194 for sorbent conditioning in the in-tip SPE approach were significantly reduced (approximately 20
195 times). Moreover, the proposed procedure was much simpler and did not require the use of additional
196 equipment, such as a vacuum pump. Furthermore, the sorbent showed potential to be used multiple
197 times. Even after 15 cycles of sorption-desorption, the analyte recovery remained at 92–93.4%.

198 3.2. SPME

199 Among different miniaturized solid extraction techniques, solid-phase microextraction (SPME),
200 introduced by Pawliszyn and Arthur [22] was found to be one of the most popular and powerful tool to
201 separate and enrich analytes from different complex samples. In this green extraction technique, a
202 small quantity of sorbent is coated on a solid support, typically fiber of a specially constructed
203 syringe-like device. There are two ways to perform the extraction: directly immersing the fiber into the
204 sample (DI-SPME) or placing the fiber above the sample (HS-SPME). Currently, numerous
205 commercial SPME fibers are available with different fiber coatings, such as polydimethylsiloxane
206 (PDMS), divinylbenzene (DVB), polyacrylate (PA), polyethylene glycol (PEG), and composites such
207 as polydimethylsiloxane/carboxen (PDMS/CAR), polydimethylsiloxane/divinylbenzene
208 (PDMS/DVB) and others. However, some of them exhibited limitations in terms of extraction
209 selectivity, pH tolerance, and thermal durability. Therefore, many novel functional materials (e.g.,
210 carbon materials, metal-organic frameworks (MOFs), molecularly-imprinted polymers (MIPs), ionic
211 liquids (ILs), metal nanoparticles (NPs), etc. have been applied as coatings [15].

212 Alginate-based hydrogels have also been applied as efficient coatings in SPME. The first paper
213 describing the usage of alginate sorbent supported on a fiber was published in 2017 by Castilhos et. al.
214 [23]. The researchers prepared an alginate-based hydrogel modified by the incorporation of corn
215 protein-zein for coating a polypropylene hollow fiber. The fiber preparation procedure consisted of the
216 following steps: (i) cutting the fiber into 1.5 cm long pieces; (ii) conditioning in acetone (5 min); (iii)
217 immersing in the alginate/zein dispersion at 60 °C under magnetic stirring (2 h); (iv) inserting, stainless
218 steel rods into the inner lumen of the hollow fiber; (v) immersing the fiber in calcium chloride solution
219 (2 h); and (vi) washing with ultra-pure water. Then the fiber was immersed in a water sample (30 min)
220 to adsorb thirteen polar compounds including hormones, anti-inflammatory agents, antiseptics and
221 analgesics. After the extraction, analytes were desorbed in methanol using ultrasonic energy for 60
222 min, then methanolic extract was dried and derivatization reagent was added. Finally, the sample was
223 injected into gas chromatography coupled to mass spectrometry (GC-MS) system. The authors of this
224 work attributed the superior extraction efficiency especially towards compounds with a low n-octanol-

225 water partition coefficient (K_{ow}), such as estriol, estrone, 17- α -ethinylestradiol, progesterone, and 4-
226 nonylphenol. The increase in the extraction efficiency compared to coating prepared without zein
227 addition could be due to reduced initial amount of water absorbed by the hydrogel, which resulted in
228 better interaction between analytes and the sorbent. It should also be highlighted that covering the
229 fiber with alginate-based gel significantly improved the extraction efficiency for some analytes. The
230 peak areas of paracetamol, atrazine, ibuprofen, and 4-octylphenol were significantly higher when fiber
231 supported by hydrogel was used on a sample preparation step than when using polypropylene (PP)
232 fiber with no modification. Which confirmed that the fiber modification by alginate sorbents
233 significantly improved the affinity and selectivity of the sorptive device. However, the approach
234 proposed by researchers is efficient and sensitive, the fiber modification and extraction steps are quite
235 time- and energy-consuming [23]. Another research group suggested using unmodified alginate gel as
236 a covering material for the extraction of fluoxetine and its metabolite, norfluoxetine, from human
237 plasma samples. A similar methodology, as described above, was used for the preparation of hydrogel
238 fiber device. However, the time of fiber immersing in the hydrogel and calcium chloride solution was
239 significantly shortened (240 min versus 4 min). Moreover, the fiber was coated with a second layer of
240 hydrogel. Obtained SPME fiber was used in DI mode, extracted analytes were eluted in methanol and
241 then injected onto high performance liquid chromatography coupled with fluorescence detector
242 (HPLC-FD). Again, the authors obtained much higher peak areas when using a hydrogel-coated fiber
243 than when only PP fiber was applied, which confirms superior sorption capacity of alginate gel.
244 Moreover, it was also proven that alginate hydrogel acted as a restricted access material (RAM),
245 effectively blocking nearly all (96%) plasma proteins presented in the samples. This feature of the
246 hydrogel is particularly advantageous in the context of its use for biological matrices preparation
247 because it allows simplification of the procedure and shortens the sample preparation time by
248 eliminating steps such as protein precipitation or incorporation of additional RAMs into the extraction
249 phase [24]. Despite the analytical figures of merit not being evaluated (making comparisons of
250 selectivity, sensitivity, and efficiency of the extraction process impossible), the proposed methodology
251 appears intriguing and warrants further development, especially in the context of the application of
252 this format in bioanalysis. Reducing sample preparation steps and costs allows for an increase in the
253 greenness in comparison to commercial SPME devices.

254 3.3. Dispersive micro-solid phase extraction

255 Without a doubt, dispersive micro-solid-phase extraction (D- μ SPE) is technique where alginate-based
256 sorbents are most frequently used. In this sub-mode of traditional SPE method, low sorbent amount (in
257 the low milligram range) is directly introduced into the sample solution and dispersed by vortexing or
258 external energy source such as ultrasounds. Due to the sorbent dispersion in the sample, the contact
259 area between the extractant and analytes increases significantly thus allowing to improve the
260 extraction efficiency and reduced the extraction time. Subsequently, the sorbent containing adsorbed
261 analytes is separated from the sample matrix, typically through centrifugation or filtration.
262 Advantageous features of the D- μ SPE, such as simplicity, short sample preparation time, minimized
263 solvent usage, as well as simplicity of alginate-based sorbent preparation have caused so wide interest
264 among analysts. Silva et. al. prepared ALG-MWCNT microbeads by mixing sodium alginate solution
265 with multi-walled carbon nanotubes that were used for the extraction of polycyclic aromatic
266 hydrocarbons (PAHs) from environmental water samples [25]. The procedure entailed adding ALG-
267 MWCNT microbeads to an aqueous sample and thoroughly mixing the entire solution on a platform
268 shaker for 30 minutes. After this time, the microbeads were separated by decantation and dried. The
269 authors tested several solvents and their volumes for analytes desorption. Finally, 0.1 mL of ethyl
270 acetate was used. In order to improve desorption, ultrasonication was applied. Fluorene, phenanthrene,



271 and fluoranthene determination was carried out using an gas chromatography coupled to flame
272 ionization detector (GC-FID) system, resulting in LODs ranging from 0.22 to 0.42 ng/mL. In another
273 work, also aimed at extracting PAHs from real water samples, alginate beads based on granular
274 activated carbon were applied. The authors obtained carbon material from waste polyethylene
275 terephthalate (PET) bottles, which is a very interesting solution in terms of reducing the amount of
276 plastic waste and thus environmental protection. The plastic bottles were first cut into small pieces,
277 washed, and dried. Then a small portion of the material was subjected to carbonization in a cylindrical
278 furnace at 800 °C for 1 h under a nitrogen atmosphere. Cooled and powdered activated carbon was
279 mixed with sodium alginate solution, and sorbent beads were formed. The obtained sorbent exhibited a
280 sphere shape with a rough surface and porous structure, which ensured sufficient inner area for PAHs
281 sorption, resulting in recovery rates ranging from 93.5% to 102.22%. 50 mg of sorbent was found to
282 be sufficient for obtaining satisfactory extraction efficiency, and equilibrium was achieved under
283 shaking in just 8 minutes. Moreover, the developed method exhibited very good linearity, and
284 precision (RSD <6.7%), low LOD of 0.01 ng/mL (much lower than other procedures reported in the
285 literature for PAHs extraction) and low LOQ of 0.03 ng/mL [26]. In 2022, Asghari and co-workers
286 introduced an innovative variant of gel-based sorbent composed of an eutectogel derived from the
287 combination of calcium alginate and deep eutectic solvent (DES). In this procedure, a hydrophobic
288 DES consisting of choline chloride (ChCl) and polyethylene glycol (PEG) in molar ratio 1:1 was
289 encapsulated in alginate beads (beads diameter in the range of 414–746 µm). After drying at room
290 temperature, the small portion (30 mg) of sorbent was used for the rapid extraction (completed in 10
291 min) of 5-hydroxymethylfurfural (HMF) from coffee samples. The analyte desorbed from the sorbent
292 with 200 µL of acetonitrile was determined by HPLC-UV. The introduction of green solvent to the
293 alginate beads provided high recovery (79-102%) due to the formation of dipolar interactions and
294 hydrogen bonding between PEG chains and HMF molecules. The validation results demonstrate broad
295 linear range (7.49–1000 µg/kg), low LOD (2.25 µg/kg), and good precision (RSD < 4.7%) of proposed
296 method. Moreover, the authors assessed the environmental impact of the proposed method using two
297 green analytical matrix tools: the Complex Green Analytical Procedure Index (Complex GAPI) and
298 the Analytical Eco-Scale. The obtained pictogram as well as the final Eco-Scale score of 63 out of 100
299 indicate that the method can be classified as an acceptable green [27]. A similar approach, involving
300 encapsulation of DES in alginate beads, was proposed by Karami and Seresthi [28]. The researchers
301 combined thymol and methacrylic acid in a 1:1 molar ratio and subjected it to the polymerization
302 process using ammonium persulfate (APS) as a radical initiator and ethylene glycol dimethacrylate
303 (EGDMA) as a crosslinker to obtain a polymer named ([poly(MAA-co EGDMA)]:[Thy] DES). The
304 sorbent was then utilized to extract twenty four pesticides from non-alcoholic malt-based beverages. A
305 comparison between the extraction efficiency of unmodified alginate hydrogel and the eutectogel
306 showed a considerable role of the DES in enhancing extraction capabilities, resulting in 1.5–3.4 times
307 higher chromatographic responses than unmodified ones. As can be seen, the main shape of the
308 alginate-based sorbent used in the D-µSPE is a bead; however, in one report, scientists proposed the
309 use of other structural configuration, such as fiber. They prepared sorbent named zirconia
310 nanoparticles-decorated calcium alginate hydrogel fibers (ZNCAHF) by constant injection with
311 syringe zirconia/sodium alginate suspension into CaCl₂ solution. After drying, the sorbent (0.1 g) was
312 immersed in water and juice samples to extract three compounds from organophosphorus pesticides
313 group under stirring for 5 min. Afterward, the sorbent was withdrawn from the solution, washed,
314 dried, and transferred to a small tube with toluene/dichloromethane mixture to desorb analytes. The
315 method exhibited very high recovery rate (90-105%) and very low LOD (0.001-0.004 µg/L).
316 According to the authors, these excellent results was achieved due to high specific area resulting with
317 sorbent thin fibrous shape and also zirconia nanoparticles content. However, the influence of the
318 sorbent shape on the extraction efficiency has not been investigated [29]. In another study, researchers



319 prepared sorbent by combining activated carbon (AC) and polyvinylpyrrolidone (PVP) and
320 encapsulating it in alginate beads (AC@PVP@ALG) [30]. The synthesized material was employed for
321 ultrasound-assisted dispersive micro-solid phase extraction (UA-D μ -SPE) of antiretroviral drugs in
322 environmental water samples, and then followed up with HPLC-DAD determination. Due to its high
323 porosity and large specific surface area (372 m²/g), the developed sorbent exhibited a favourable
324 extraction capability, ranging from 91.6 to 99.1%. Moreover, the application of ultrasounds as an
325 enhancing extraction factor could potentially affect this high recovery factor, but the authors have not
326 conducted any comparison studies. In comparison to SPE-Oasis HLB cartridges, mostly used for
327 nevirapine and zidovudine extraction, the elaborated procedure exhibited a notable higher recovery
328 with a much lower quantity of the sorbent used.

329 3.4. Magnetic dispersive micro-solid phase extraction (MD- μ SPE)

330 If the sorbent has magnetic properties, it can be separated from the sample matrix using an external
331 magnetic field. Based on the data gathered in Table 1 it can be concluded that this sub-version of D-
332 μ SPE is the preferred choice of researchers. The iron oxide, namely magnetite (Fe₃O₄) is one of the
333 most frequently used due to its superparamagnetism and facile synthesis. However, unmodified iron
334 oxide suffers from some drawbacks such as poor selectivity, sensitivity to acids and tendency to
335 aggregation. To overcome these limitations, hybrid materials composed of Fe₃O₄ and other materials
336 of different sizes and physicochemical properties are synthesized [31]. So far, GO, carbon nanotubes
337 (CNTs), silica-based materials, MOFs, and MIPs have been the most frequently used in the
338 preparation of magnetic alginate-based sorbents.

339 Nurerk et. al. proposed a magnetic composite sorbent named PANI/ALG/Fe₃O₄ consisting of
340 polyaniline (PANI) coated Fe₃O₄ nanoparticles entrapped in alginate beads [32]. These sorbent beads
341 were combined with the HPLC-FLD method for the extraction and determination of three PAHs in
342 reservoir, river and wastewater samples. The proposed method showed very good extraction efficiency
343 (86–98%) and good precision (RSD <6%). Moreover, it has been shown that the addition of PANI has
344 a beneficial effect on PAHs adsorption efficiency. Compared to Fe₃O₄, Fe₃O₄/alginate sorbents,
345 PANI/alginate/Fe₃O₄ exhibited much higher adsorption capability (more than 4 times) of the target
346 analytes via π - π interactions. The adsorption capacity for benzo[a]anthracene, benzo[b]fluoranthene,
347 and benzo[a]pyrene was determined as 1.25, 1.19, and 1.15 μ g/g, respectively and remained constant
348 even after six cycles of sorption-desorption. Similar sorbent, have also been used to extract
349 fluoroquinolones from honey, milk, and egg samples. However, in the case of this study, the core of
350 the sorbent consists, apart from magnetite, also GO and octadecyl-bonded silica. The combination of
351 these high-affinity materials and the formation of a double-pore network in the hydrogel provided a
352 high sorption capacity of 27.84 m²/g and a good extraction efficiency ranging from 80 to 98% [33]. In
353 the next publication by the same authors, another material, polypyrrole, was used to cover the alginate
354 beads. The sorbent exhibited high adsorption capacity towards estriol, β -estradiol, and bisphenol A,
355 without significant loss even after 16 regeneration cycles [34]. A polypyrrole-based sorbent named
356 porous nanostructured polypyrrole, reduced GO, magnetite nanoparticle alginate hydrogel
357 microspheres (PPy-rGOx-Fe₃O₄) was also applied for phthalate extraction from bottled water and
358 beverage samples. PPy-rGOx-Fe₃O₄ was prepared by adding magnetite nanoparticles, GO, and
359 calcium carbonate to an alginate solution. To reduce GO, hydrogel microspheres were immersed in
360 0.2% w/v ascorbic acid and heated at 85°C for 6 h. While, to obtain the hierarchical porous
361 nanostructure, the composite was added to 20% w/v hydrochloric acid. With the reaction of CaCO₃
362 in the hydrogel microspheres with the hydrochloric acid, bubbles of CO₂ were released, which created a
363 porous double network. The obtained composite were then coated with polypyrrole. The analytes
364 desorbed from the sorbent with ethyl acetate were determined using GC-MS/MS. Under optimized

365 conditions, the elaborated method showed favourable recovery rates ranging from 87.5 to 99.1% and a
366 low LOD of 0.005-0.01 $\mu\text{g/L}$ [35]. Another sorbent applied to magnetic D- μSPE was proposed by
367 Sereshti et. al. [36], who synthesized a three-component eutectogel, composed of a crosslinked
368 copolymeric DES and polyvinylpyrrolidone-coated Fe_3O_4 nanopowder (PVP- Fe_3O_4). The copolymeric
369 DES, consisting of 2-hydroxyethyl methacrylate (HEMA)/thymol (Thy) in a 1:1 M ratio, was prepared
370 through thermally-induced free-radical polymerization with azobisisobutyronitrile, as an initiator, and
371 EGDMA as a crosslinker. The resulting material exhibited excellent and rapid (extraction occurred in
372 10 min) sorption capability of melamine from milk and dairy products. Additional advantages of the
373 proposed approach include low sorbent mass (10 mg) and low volume of desorption solvent (300 μL).
374 Once again, a significantly greater recovery of analyte was obtained using DES-modified gel beads
375 (66.9%) than that of unmodified calcium alginate beads (33.3%). Furthermore, it was found that the
376 extraction efficiency of the sorbent was significantly influenced by the pH of the sample solution. The
377 highest recovery (87.6%) was obtained at pH adjusted at 6 due to electrostatic interactions between $-\text{O}^-$
378 and $-\text{COO}^-$ groups in sorbent and $-\text{NH}_3^+$ groups in melamine. One more magnetic eutectogel beads
379 composed of a polymeric DES, carboxylated multiwall carbon nanotubes and polyvinylpyrrolidone
380 coated- Fe_3O_4 nanocrystals ($\text{Fe}_3\text{O}_4@/\text{CA}/[\text{PNVP}]:[\text{BA}]/\text{MWCNT-COOH}$) was applied for
381 determination of pesticide residues in honey samples [37]. Another interesting sorbent was proposed
382 by Khongkla et. al. [38]. The authors integrated ZnO nanoparticles with magnetic molecularly
383 imprinted polymer and incorporated them into alginate hydrogel beads. This innovative sorbent,
384 known as $\text{ZnO}/\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-NH}_2/\text{MIP}/\text{alginate}$ was utilized for determination of two compounds from
385 sulphonamides group in milk samples. Through the application of molecular imprinting technique it
386 was possible to obtain not only efficient, with recovery ranging from 80.7-98.5% but also highly
387 selective material. Only the target sulfonamides have been extracted from such complex sample as
388 milk without interference compounds presented in the matrix. Moreover, the sorbent demonstrated
389 satisfactory reusability for up to ten sorption-desorption cycles. The process of sorbent preparation
390 was also investigated in terms of repeatability. Despite the demonstrated good reproducibility, with
391 RSDs $< 1.3\%$, the unquestionable disadvantage is the long duration of the entire process (almost 52
392 hours). Sorbent beads containing magnetic graphene oxide (MGO) as a magnetic core were used to
393 extract endocrine disrupting compounds (EDCs) such as bisphenol A (BPA) and epichlorohydrin
394 (ECH) from river water and industrial wastewater [39]. Shah and Jan first synthesized GO, and then,
395 by adding a solution of Fe (II) and Fe (III) in molar ratio 1:2, in alkali conditions, they synthesized
396 MGO. Subsequently, they added to the sodium alginate solution and stirred for 2 h. The procedure of
397 alginate beads preparation was the same as described elsewhere. However, instead of CaCl_2 solution,
398 the commonly used crosslinking agent, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ solution, was used. The combination of sodium
399 hydrogel and MGO (MGO-ALG) yielded a sorbent with a large specific surface of 303.68 m^2/g .
400 Significantly higher than these calculated for GO and MGO, equal to 43.64 m^2/g and 274.67 m^2/g ,
401 respectively. The optimized conditions of the sample preparation step resulted in a very high recovery
402 rate of 97.17% for ECH and 99.46% for BPA. Using an HPLC-UV at the final determination stage,
403 the authors achieved low LODs of 8.25 and 13.99 ng/L , which was lower than in similar reported
404 methods. Nonetheless, regeneration study results showed that five times the use of the sorbent
405 contributed to reducing the recovery to 87% and 83% for BPA and ECH, respectively. In another
406 study, the authors investigated the applicability of a hydrogel sorbent consisting of MGO, MIL-101,
407 and silica-coated magnetite nanoparticles for the selective extraction and quantification of phthalates
408 in water, juice, and tea samples [40]. GOx/MIL-101/ $\text{Fe}_3\text{O}_4\text{-SiO}_2$ sorbent in the form of fiber showed a
409 satisfactory extraction efficiency toward four phthalate esters, ranging from 80.7-89.9%. According to
410 the test results, the synergistic effect of GOx and MIL-101 and significantly boosted the adsorption
411 capacity of the sorbent, almost 2 times compared to MIL-101/ $\text{Fe}_3\text{O}_4\text{-SiO}_2$, and 1.2 times compared to



412 GOx/Fe₃O₄-SiO₂. Moreover, the sorbent demonstrated thermal stability and allowed for up to 16
413 reuses without significantly decreasing its extraction performance.

414

415

416

417

418

419

420

421

422

423

424

425

426

427

428

429

430

431

432

433

434

435

436

437

438

439

Table 1. Summary of application of alginate-based sorbents in microextraction techniques.

Sorbent (mass [mg])	Adsorption capacity [mg/g]	analyte	Sample (volume/mass)	desorptive solvent (volume [mL])	Final determination	LOD	LOQ	Recovery [%]	RSD [%]	reusability	Ref
SPE											
NFL/ALG (n.d.)	18.86	Pb ²⁺	water, beverages (2 mL)	3 M HNO ₃ (2)	FAAS	0.39 [ug/L]	1.31 [ug/L]	91.7-99.8	2.65	n.d.	[16]
Calcium ALG microparticles (10)	n.d.	rare earth elements	water (100 mL)	0.5 M HNO ₃ (1)	ICP-MS	0,01-0,03 [ng/L]	n.d.	81-101	n.d.	50 cycles	[25]
in-syringe SPE											
P – ZrO ₂ CeO ₂ ZnO nanoparticles/ALG (150)	62.89 292.93	Ca, Li	environmental water (100 mL)	1 M HNO ₃ (10)	ICP-MS	0.03 0.102 [ng/L]	0.099 0.34 [ng/L]	57.0– 147.7 33.12– 116.5	n.d.	n.d.	[41]
biochar/SA MMM (n.d.)	n.d.	nitroimidazoles	environmental and drinking water (10 mL)	ACN (1.5)	HPLC-MS/MS	0.006- 0.014 [ng/mL]	0.021- 0.048 [ng/mL]	79-99	7.1- 8.2	5 cycles	[19]
in-tip SPE											
MIL-101/CTAB/ALG/CF (500)	3.48, 4.99	phthalates	beverages (10 mL)	ACN (2)	HPLC-DAD	0.15 [ug/L]	n.d.	85.2- 97.8	0.1 – 4.9	15 cycles	[21]
SPME											
ALG hydrogel (n.d.)	n.d.	luoxetine, norfluoxetine	plasma (0.32 mL)	MeOH (0.2)	HPLC-FD	n.d.	n.d.	n.d.	n.d.	5 cycles	[24]
ALG/zein hydrogel (n.d.)	n.d.	drugs	water (10 mL)	MeOH (0.19)	GC-MS	1-50 [ng/L]	n.d.	n.d.	n.d.	n.d.	[23]
μ-dSPE											
ALG/GAC hydrogel (n.d.)	n.d.	PAHs	environmental water (100 mL)	toluene (2)	HPLC-UV	0.01-0.07 [ng/mL]	0.03-0.23 [ng/mL]	93.5- 102.2	4.2– 6.7	n.d.	[26]
CA/[poly(MAA-co-EGDMA)]:[Thy] (n.d.)	n.d.	pesticides	beverages (3 mL)	EtOH (0.15)	GC-MS	0.004- 0.069 [ug/L]	0.013- 0.23 [ug/L]	68.2- 115.6	1.4– 10.2	4 cycles	[28]
ALG-g-	n.d.	Pb ²⁺	corn cereal	1 M HNO ₃	MIP OES	n.d.	n.d.	100	n.d.	10 cycles	[42]



PAAM/MWCNT-f (10)		Cd ²⁺		(10)							
SFE/ALG beads (200)	n.d.	Pb ²⁺	environmental water (200 mL)	0.8 M HCl (2)	FAAS	2 [ug/L]	7 [ug/L]	94.8-100.2	< 5	6 cycles	[43]
ZNCAHF (n.d.)	n.d.	pesticides	beverages (5 mL)	toluene/DC M (0.25)	GC-MS	0.001-0.004 [ng/mL]	0.003-0.012 [ng/mL]	90-105	3.2-7.8	n.d.	[29]
ALG-MWCNT (50)	n.d.	PAHs	environmental water (50 mL)	EtOAc (0.1)	GC-FID	0.22-0.42 [ng/mL]	0.732-1.38 [ng/mL]	71,2-104	0.4-12.4	n.d.	[25]
CA/[ChCl]:[PEG] (30)	n.d.	5-hydroxymethylfurfural	coffee (0.5 g)	ACN (0.2)	HPLC-UV	2.25 [ug/kg]	7.49 [ug/kg]	79-102	<4.7	n.d.	[27]
AC@PVP@alginate (100)	42.2, 44.4	nevirapine, zidovudine	environmental water (20 mL)	ACN (5)	HPLC-DAD	0.20-0.23 [ug/L]	0.67-0.75 [ug/L]	91.3-99,1	≤6.8	6 cycles	[30]
Mμ-dSPE											
Fe-ALG- MGO (5)	6.73, 7.01	BPA, ECH	environmental water (20 mL)	MeOH (n.d.)	HPLC-UV	13.99 [ug/L]	8.25 [ug/L]	97.17-99.46	1.20-6.87	5 cycles	[39]
GOx/MIL-101/Fe ₃ O ₄ -SiO ₂ (n.d.)	n.d.	phthalates	beverages (10 mL)	ACN (2)	HPLC-DAD	3-5 [ug/L]	10-15 [ug/L]	80.7-89.9	0.1-7.3	16 cycles	[40]
Fe ₃ O ₄ @CA/[(HEMA)m-co-(EGDMA)n]:[Thy] (10)	n.d.	melamine	milk and diary products (0.5 g)	MeOH (0.3)	HPLC-UV	0.5 [ug/kg]	2 [ug/kg]	77.4-105	<6.9	3 cycles	[36]
HP-PEDOT@Fe ₃ O ₄ @ALG (250)	n.d.	PAHs	meat (5 g)	IPA (1)	GC-MS/MS	0.2-0.4 [ug/kg]	0.5-1 [ug/kg]	81.5-99.4	<7	9 cycles	[44]
Fe ₃ O ₄ @C ₁₈ @Ba ²⁺ -ALG (100)	n.d.	PAHs and PAEs	environmental water (20 mL)	ACN (0.5)	HPLC-FLD	2-53 [ng/L]	(n.d.)	72-108	1-9	8 cycles	[45]
Fe ₃ O ₄ /MWCNTs/ALG (200)	n.d.	PAHs	environmental water (10 mL)	ACN/toluene (4)	HPLC-FLD	0.05-0.01 [ng/L]	0.02-0.04 [ng/L]	96-98	<10	10 cycles	[46]





Fe ₃ O ₄ @CA/[PNVP]:[BA]/MWCNT (10)	n.d.	pesticides	honey	ACN (0.1)	GC-MS	0.007-0.31 [µg/kg]	0.023-1.02 [µg/kg]	73.2-110.8	2.3-8.1	n.d.	[37]
PANI/ALG/Fe ₃ O ₄ (400)	1.15-1.25	PAHs	environmental water (10 mL)	ACN (1 mL)	HPLC-FLD	0.01 [µg/L]	0.04 [µg/L]	86-98	<6	6 cycles	[32]
PPy-rGOx-Fe ₃ O ₄ ALG hydrogels (750)	n.d.	phthalates	beverages (20 mL)	EtOAc (3)	GC-MS/MS	0.005-0.01 [µg/L]	0.02-0.04 [µg/L]	87.5-99.1	0.6-7.7	18 cycles	[35]
ZnO/Fe ₃ O ₄ @SiO ₂ -NH ₂ /MIP/ALG (500)	n.d.	sulfonamides	milk (10 mL)	MeOH/acetic acid (2)	HPLC-DAD	0.03-0.06 [µg/L]	0.1-0.2 [µg/L]	87-98.5	<6	10 cycles	[38]
PANI/GOx/ C ₁₈ -SiO ₂ -Fe ₃ O ₄ (500)	n.d.	fluoroquinolones	honey, milk, eggs (10 mL)	ACN/DCM/acetic acid (3)	HPLC-FLD	0.001-0.01 [µg/L]	0.003-0.04 [µg/L]	80-98	<9	7 cycles	[33]
polypyrrole/Fe ₃ O ₄ /ALG (n.d.)	n.d.	estriol, β-estradiol, BPA	environmental water (10 mL)	ACN (2)	HPLC-FLD	0.5 [µg/L]	n.d.	90-98	<5	16 cycles	[34]
MIL-101(Cr)-NH ₂ (300)	n.d.	herbicides	environmental water (10 mL)	ACN (1)	HPLC-MS/MS	0.43-16 [ng/L]	n.d.	75.3-155.3	1.51-9.5	10 cycles	[47]

AC- activated carbon; ALG- alginate; BPA-bisphenol A; CTAB- cetyl trimethyl ammonium bromide; ECH-epichlorohydrin; EGDMA- ethylene glycol dimethacrylate; EtOH-ethanol; FAAS- Flame Atomic Absorption Spectrometer; FID- Flame ionization detector; GAC- granular activated carbon; GC-Gas Chromatography; HEMA-2-Hydroxyethyl methacrylate; HP- Hierarchically Porous; HPLC-High Performance Liquid Chromatography; ICP- Inductively Coupled Plasma; IPA-2-propanol; MeOH-methanol; MIP OES - Microwave-Induced Plasma Optical Emission Spectrometer; MIL-amino-functionalized metal-organic framework; MIP-molecularly imprinted polymers; MGO-magnetic graphene oxide; MMM- Mixed matrix membrane; MS-Mass Spectrometer; MWCNTs – multiwalled carbon nanotubes; PAAM-poly(acrylamide); PAHs-polyaromatic hydrocarbons; PAEs-phthalate esters; PANI- polyaniline; PEDOT- poly(3,4-ethylenedioxythiophene); PEG-polyethylene glycol; PVP- polyvinylpyrrolidone; Thy-thymol; UV-ultraviolet; ZNCAHF-zirconia nanoparticles-decorated calcium alginate hydrogel fibers.

441 4. Green profile

442 Because of their eco-friendly nature, the application of alginate-based sorbents in microextraction
443 techniques is undoubtedly a significant step toward improving the greenness of analytical procedures.
444 Alginates are renewable, non-toxic, and easily biodegradable materials, therefore using them as a
445 sorbent is in line with some of the GAC principles. Based on the data presented in the Table 1, it can
446 be stated that the amount of the sorbent, volume of the sample, and desorption solvent used in various
447 miniaturized alginate-based sorbent approaches are relatively small. Additionally, the sample
448 preparation procedures are usually simple and do not consist of many steps. From a GAC point of
449 view, the possibility of regeneration and multiple reuses of the sorbent is also an undeniable
450 advantage. However, it should be highlighted that in the vast majority of procedures, modified alginate
451 hydrogels were used. The surface modification procedures or combination with other materials mostly
452 require the use of large quantities of additional reagents, solvents, and washing solutions. Whereby the
453 overall amount/volume of chemicals and generated wastes is increased. Moreover, some stages of
454 sorbent modification require the utilization of ultrasound energy or long-term heating, which
455 significantly reduces environmental friendliness of the entire analytical procedure.

456 To study whether the green profile of the analytical procedure is consistent with that of the alginate-
457 based sorbent synthesis, selected approaches proposed by different researchers were evaluated using
458 one of the tools intended to assess how green the analytical methods are, named Analytical Eco-scale
459 [48]. This green chemistry metric proposed by Gałuszka et al. [49] is based on the penalty points,
460 which are assigned in four main aspects: (i) the amount and hazards of reagents used; (ii) the energy
461 consumption; (iii) occupational hazards; and (iv) waste generation. The Eco-scale score is calculated
462 by subtracting all penalty points from 100. In this evaluation, the higher the score, the greener and
463 more sustainable the analytical procedure is. This metric tool was selected over others such as GAPI,
464 AGEEPrep [50,51], and others because it primarily concentrates on the sample preparation step,
465 disregarding issues related to sample collection, transportation, preservation, or sample throughput that
466 are not relevant to the sorbent synthesis.

467 Ten representative studies have been selected from those collected in the Table 1. At least one study
468 from each type of microextraction technique was selected. Due to the larger number of publications in
469 the dispersive micro-solid phase extraction techniques section, two publications from the D- μ SPE
470 group and four from the MD- μ SPE group were selected. Additionally, the diversity of the sorbent
471 composition was taken into account at the stage of reports selection. Table 2 includes the penalty
472 points assigned to a given category and the eco-scale score of the selected studies, considering the
473 analytical procedure (without sorbent preparation process) and the alginate-based sorbent synthesis.

474

475

476

477

478

479



Table 2. Penalty Points and Analytical Eco-scale scores of the selected reports, with consideration of the analytical procedure and the sorbent synthesis.

Microextraction technique	sorbent	Analytical procedure					Sorbent synthesis					Ref.
		Reagents PPts	Energy PPts	Occupational haz.	Waste PPts	Eco-scale score	Reagents PPts	Energy PPts	Occupational hazard	Waste PPts	Eco-scale score	
SPE	NFL/ALG	2	2	0	6	90	11	3	0	6	80	[16]
in-syringe SPE	biochar/ALG	8	3	3	8	78	5	4	3	6	82	[19]
in-tip SPE	MIL-101/CTAB/ALG/CF	10	2	3	8	77	52	6	0	8	34	[21]
SPME	ALG/zein	11	2	3	8	76	5	1	0	6	88	[51]
μ -dSPE	CA/[poly(MAA-co-EGDMA)]:[Thy]	4	3	0	6	87	18	1	0	6	75	[28]
	ALG-g-PAAc/MWCNT-f	4	3	3	6	84	34	3	0	8	55	[47]
M μ -dSPE	MIL-101(Cr)-NH ₂	4	3	3	8	82	43	7	0	8	42	[47]
	Fe-ALG-MGO	11	3	0	8	78	41	3	3	8	45	[39]
	ZnO/Fe ₃ O ₄ @SiO ₂ -NH ₂ /MIP/ALG	16	5	3	8	68	51	4	3	8	34	[38]
	PANI/GOx/C ₁₈ -SiO ₂ -Fe ₃ O ₄ ALG	12	1	3	8	76	70	4	3	8	15	[33]

480

481

482

483

484

485

486 As it can be seen in Table 2, a significant majority of the selected analytical procedures obtained total
487 Eco-scale scores above 75, indicating an excellent green analysis. Only one procedure, with a rating of
488 68, qualifies as an acceptable green analysis. However, considering the sorbent synthesis approach, it
489 becomes evident that not all these steps are as environmentally friendly as their analytical application.
490 Just four of the ten investigated synthesis protocols achieved scores ≥ 75 . In these protocols, only a
491 slight modification of alginate hydrogel was conducted, consisting of compounding with natural
492 sorbents and DES (references [4, 7, 9, 19]). The other procedures necessitated the use of significantly
493 more reagents, primarily those hazardous and energy-consuming processes like long-term high-
494 temperature processes or sonication, thereby increasing the PPs associated with reagents and energy
495 consumption. The PANI/GOx/C₁₈-SiO₂-Fe₃O₄ alginate sorbent synthesis yielded the lowest eco-scale
496 score rating of 15. This result is mainly influenced by the PPs associated with usage of a significant
497 amount of reagents (classified as “danger”) at the stage of tedious and involves several steps in the in
498 the synthesis of individual sorbent components. Similarly, in the case of the other two protocols with
499 an eco-scale score lower than 40, the main factor affecting the decrease in green profile are PPs
500 assigned to the reagents used (PPs for reagents greater than 50). This suggests that the main factor
501 reducing the environmental sustainability of the synthesis is the excessive use of hazardous reagents.

502 5. Summary and future trends

503 Alginates gels seem to be a very attractive material capable of being employed in various miniaturized
504 extraction techniques. In comparison to synthetic polymers, they offer many favourable features, such
505 as biodegradability, low cost, abundant availability, and easy preparation. These characteristics make
506 alginates ideal green sorptive materials from the GAC point of view. Unfortunately, alginates gels in
507 their pure form show low extraction capacity, which limits their use as a neat extraction material.
508 Nevertheless, their adsorption performance can be easily enhanced by compounding with other
509 sorptive materials, including carbon and magnetic nanoparticles, GO, MIPs, MOFs, DESs, and others.
510 So far, alginate composites sorbents have been successfully applied as efficient adsorbents in SPE, in-
511 syringe SPE, in-tip SPE, SPME, and D μ -SPE, with the last being the most preferred approach. In all
512 presented methods, the tested sorbents showed effective binding capabilities for specific analytes.
513 Despite the fact that the idea of using biodegradable material as a sorbent contributes to improving the
514 green profile of analytical methodology, some of the sorbent preparation processes are not as green as
515 desirable. As discussed above, in some research, the alginate gel modification step required the use of
516 a large amount of hazardous reagents and energy- and time-consuming processes. Therefore, future
517 efforts should be made to find a balance between sorbent formulation process sustainability and
518 alginate-based sorbent efficiency. This goal could be achieved by using materials coming from natural
519 sources and/or natural deep eutectic solvents as modifiers; replacing organic solvents used during the
520 sorbent synthesis by greener alternatives, and using modifiers that will contribute to increasing the
521 reusability of the sorbent. What is more, it is also important to take sorbent synthesis into
522 consideration when the environmental sustainability of the procedure is considered.

523 References:

- 524
- 525 [1] S. Armenta, S. Garrigues, M. de la Guardia, *Green Analytical Chemistry*, *TrAC - Trends Anal.*
526 *Chem.* 27 (2008) 497–511. <https://doi.org/10.1016/j.trac.2008.05.003>.
 - 527 [2] S. Armenta, F.A. Esteve-Turrillas, S. Garrigues, M. de la Guardia, *Alternative green solvents*
528 *in sample preparation*, *Green Anal. Chem.* 1 (2022) 100007.
529 <https://doi.org/10.1016/j.greeac.2022.100007>.
 - 530 [3] J. Millán-Santiago, G. Lasarte-Aragonés, M. Laura Soriano, A.I. López-Lorente, R. Lucena, S.

- 531 Cárdenas, Natural Sorbents in Green Sample Preparation, *Green Sample Prep. Tech.* (2023)
532 150–178. <https://doi.org/10.1039/BK9781839166419-00150>.
- 533 [4] J. Werner, R. Frankowski, T. Grześkowiak, A. Zgoła-Grześkowiak, Green sorbents in sample
534 preparation techniques – naturally occurring materials and biowastes, *TrAC Trends Anal.*
535 *Chem.* 176 (2024) 117772. <https://doi.org/10.1016/J.TRAC.2024.117772>.
- 536 [5] N.H. Godage, E. Gionfriddo, Use of natural sorbents as alternative and green extractive
537 materials: A critical review, *Anal. Chim. Acta.* 1125 (2020) 187–200.
538 <https://doi.org/10.1016/J.ACA.2020.05.045>.
- 539 [6] J. Werner, A. Zgoła-Grześkowiak, T. Grześkowiak, R. Frankowski, Biopolymers-based
540 sorbents as a future green direction for solid phase (micro)extraction techniques, *TrAC -*
541 *Trends Anal. Chem.* 173 (2024). <https://doi.org/10.1016/j.trac.2024.117659>.
- 542 [7] H. Tabani, M. Alexovič, J. Sabo, M. Ramos Payán, An overview on the recent applications of
543 agarose as a green biopolymer in micro-extraction-based sample preparation techniques,
544 *Talanta.* 224 (2021) 121892. <https://doi.org/10.1016/J.TALANTA.2020.121892>.
- 545 [8] J. Płotka-Wasyłka, A. Chabowska, S. Pantanit, O. Bunkoed, M.Y. Fares, M. Sajid, D.
546 Lambropoulou, A. Kurowska-Susdorf, N. Jatkowska, Natural/bio-based sorbents as greener
547 extractive materials for endocrine disrupting compounds in samples of different matrix
548 composition, *TrAC - Trends Anal. Chem.* 176 (2024) 117773.
549 <https://doi.org/10.1016/j.trac.2024.117773>.
- 550 [9] A.R. Bagheri, N. Aramesh, H.K. Lee, Chitosan- and/or cellulose-based materials in analytical
551 extraction processes: A review, *TrAC Trends Anal. Chem.* 157 (2022) 116770.
552 <https://doi.org/10.1016/J.TRAC.2022.116770>.
- 553 [10] I. Pacheco-Fernández, D.W. Allgaier-Díaz, G. Mastellone, C. Cagliero, D.D. Díaz, V. Pino,
554 Biopolymers in sorbent-based microextraction methods, *TrAC - Trends Anal. Chem.* 125
555 (2020). <https://doi.org/10.1016/j.trac.2020.115839>.
- 556 [11] V.D. Alampanos, D.A. Lambropoulou, Hydrogel sorbent-based sample preparation processes
557 as green alternatives for the extraction of organic contaminants followed by chromatographic
558 analysis, *TrAC - Trends Anal. Chem.* 174 (2024) 117687.
559 <https://doi.org/10.1016/j.trac.2024.117687>.
- 560 [12] S.H. Ching, N. Bansal, B. Bhandari, Alginate gel particles—A review of production techniques
561 and physical properties, *Crit. Rev. Food Sci. Nutr.* 57 (2017) 1133–1152.
562 <https://doi.org/10.1080/10408398.2014.965773>.
- 563 [13] K. Adamiak, A. Sionkowska, State of Innovation in Alginate-Based Materials, *Mar. Drugs.* 21
564 (2023) 1–26. <https://doi.org/10.3390/md21060353>.
- 565 [14] S. Liu, Y. Li, L. Li, Enhanced stability and mechanical strength of sodium alginate composite
566 films, *Carbohydr. Polym.* 160 (2017) 62–70. <https://doi.org/10.1016/j.carbpol.2016.12.048>.
- 567 [15] J. Płotka-Wasyłka, N. Szczepańska, M. de la Guardia, J. Namieśnik, Miniaturized solid-phase
568 extraction techniques, *TrAC - Trends Anal. Chem.* 73 (2015) 19–38.
569 <https://doi.org/10.1016/j.trac.2015.04.026>.
- 570 [16] J. Wang, Q. Yang, L. Zhang, M. Liu, N. Hu, W. Zhang, W. Zhu, R. Wang, Y. Suo, J. Wang, A
571 hybrid monolithic column based on layered double hydroxide-alginate hydrogel for selective
572 solid phase extraction of lead ions in food and water samples, *Food Chem.* 257 (2018) 155–
573 162. <https://doi.org/10.1016/j.foodchem.2018.02.143>.
- 574 [17] G.G. Arantes De Carvalho, D.F.S. Petri, P. V. Oliveira, Calcium alginate microparticles for rare
575 earth elements preconcentration prior to ICP-MS measurements in fresh water, (2018).
576 <https://doi.org/10.1039/c8ay01626g>.
- 577 [18] X. Sun, X. yue Jiao, J. Li, L. Xu, A miniaturized sorbent phase-based extraction device in the
578 form of syringe filter holder using molecularly imprinted polymer as sorbent and its application
579 to extract benzophenones, *J. Chromatogr. A.* 1543 (2018) 1–13.
580 <https://doi.org/10.1016/J.CHROMA.2018.02.039>.

- 581 [19] H. Zhang, S. Zhang, Y. Li, L. Li, X. Hou, Biochar/sodium alginate mixed matrix membrane as
582 adsorbent for in-syringe solid-phase extraction towards trace nitroimidazoles in water samples
583 prior to ultra-high-performance liquid chromatography-tandem mass spectrometry analysis, *J.*
584 *Sep. Sci.* 46 (2023) 2300316. <https://doi.org/10.1002/jssc.202300316>.
- 585 [20] J. Płotka-Wasyłka, N. Jatkowska, M. Paszkiewicz, M. Caban, M.Y. Fares, A. Dogan, S.
586 Garrigues, N. Manousi, N. Kalogiouri, P.M. Nowak, V.F. Samanidou, M. de la Guardia,
587 Miniaturized solid phase extraction techniques for different kind of pollutants analysis: State of
588 the art and future perspectives – PART 2, *TrAC Trends Anal. Chem.* 165 (2023) 117140.
589 <https://doi.org/10.1016/J.TRAC.2023.117140>.
- 590 [21] P. Nurerk, R. Chaowana, W. Limbut, O. Bunkoed, A hierarchical composite adsorbent of
591 cotton fibers modified with a hydrogel incorporating a metal organic framework and cetyl
592 trimethyl ammonium bromide for the extraction and enrichment of phthalate esters,
593 *Microchem. J.* 158 (2020) 105220. <https://doi.org/10.1016/J.MICROC.2020.105220>.
- 594 [22] C.L. Arthur, J. Pawliszyn, Solid Phase Microextraction with Thermal Desorption Using Fused
595 Silica Optical Fibers, *Anal. Chem.* 62 (1990) 2145–2148.
596 <https://doi.org/https://doi.org/10.1021/ac00218a019>.
- 597 [23] N.D.B. Castilhos, N.M.F.M. Sampaio, B.C. da Silva, I.C. Riegel-Vidotti, M.T. Grassi, B.J.G.
598 Silva, Physical-chemical characteristics and potential use of a novel alginate/zein hydrogel as
599 the sorption phase for polar organic compounds, *Carbohydr. Polym.* 174 (2017) 507–516.
600 <https://doi.org/10.1016/j.carbpol.2017.06.079>.
- 601 [24] C.S. Guzella, D.E.P. Souto, B.J.G. Silva, Alginate-based hydrogel fiber as a restricted access
602 material for microextraction of drugs in biological samples, *Carbohydr. Polym.* 294 (2022)
603 119810. <https://doi.org/10.1016/j.carbpol.2022.119810>.
- 604 [25] A.S. Abboud, M.M. Sanagi, W. Aini, W. Ibrahim, A.S.A. Keyon, H.Y. Aboul-Enein, J. Bahru,
605 Calcium Alginate-Caged Multiwalled Carbon Nanotubes Dispersive Microsolid Phase
606 Extraction Combined With Gas Chromatography-Flame Ionization Detection for the
607 Determination of Polycyclic Aromatic Hydrocarbons in Water Samples, *J. Chromatogr. Sci.* 56
608 (2018) 177–186. <https://doi.org/10.1093/chromsci/bmx095>.
- 609 [26] R. Hamadan, M.M. Al-Tae, A.B. Mahdi, S.K. Hadrawi, N.K.A. Dwijendra, M. Kavitha, Y.F.
610 Mustafa, A.K. Wadday, Entrapment of polyethylene terephthalate derived carbon in Ca-
611 alginate beads for solid phase extraction of polycyclic aromatic hydrocarbons from
612 environmental water samples, *Inorg. Chem. Commun.* 146 (2022) 110147.
613 <https://doi.org/10.1016/j.inoche.2022.110147>.
- 614 [27] Z. Asghari, H. Sereshti, S. Soltani, H. Rashidi Nodeh, M. Hossein Shojaee AliAbadi, Alginate
615 aerogel beads doped with a polymeric deep eutectic solvent for green solid-phase
616 microextraction of 5-hydroxymethylfurfural in coffee samples, *Microchem. J.* 181 (2022)
617 107729. <https://doi.org/10.1016/J.MICROC.2022.107729>.
- 618 [28] F. Karami, H. Sereshti, Copolymerized Natural Deep Eutectic Solvent Encapsulated in
619 Calcium Alginate Beads: Application as a Green Sorbent for Analysis of Multiclass Pesticides
620 in Nonalcoholic Malt Beverages by GC-MS/SIM, *ACS Sustain. Chem. Eng.* 11 (2023) 2455–
621 2467. <https://doi.org/10.1021/acssuschemeng.2c06436>.
- 622 [29] M. Zare, Z. Ramezani, N. Rahbar, Development of zirconia nanoparticles-decorated calcium
623 alginate hydrogel fibers for extraction of organophosphorous pesticides from water and juice
624 samples: Facile synthesis and application with elimination of matrix effects, *J. Chromatogr. A.*
625 1473 (2016) 28–37. <https://doi.org/10.1016/j.chroma.2016.10.071>.
- 626 [30] M. Ndilimeke Akawa, K. Mogolodi Dimpe, P. Nosizo Nomngongo, An adsorbent composed of
627 alginate, polyvinylpyrrolidone and activated carbon (AC@PVP@alginate) for ultrasound-
628 assisted dispersive micro-solid phase extraction of nevirapine and zidovudine in environmental
629 water samples, *Environ. Nanotechnology, Monit. Manag.* 16 (2021) 100559.
630 <https://doi.org/10.1016/j.enmm.2021.100559>.
- 631 [31] R. Chen, X. Qiao, F. Liu, Ionic liquid-based magnetic nanoparticles for magnetic dispersive

- 632 solid-phase extraction: A review, *Anal. Chim. Acta.* 1201 (2022) 339632.
633 <https://doi.org/10.1016/j.aca.2022.339632>.
- 634 [32] P. Nurerk, P. Kanatharana, O. Bunkoed, Polyaniline-coated magnetite nanoparticles
635 incorporated in alginate beads for the extraction and enrichment of polycyclic aromatic
636 hydrocarbons in water samples, *Int. J. Environ. Anal. Chem.* 97 (2017) 145–158.
637 <https://doi.org/10.1080/03067319.2017.1291808>.
- 638 [33] P. Klongklaew, P. Kanatharana, O. Bunkoed, Development of doubly porous composite
639 adsorbent for the extraction of fluoroquinolones from food samples, *Food Chem.* 309 (2020)
640 125685. <https://doi.org/10.1016/j.foodchem.2019.125685>.
- 641 [34] O. Bunkoed, P. Nurerk, R. Wannapob, P. Kanatharana, Polypyrrole-coated alginate/magnetite
642 nanoparticles composite sorbent for the extraction of endocrine-disrupting compounds, *J. Sep.*
643 *Sci.* 39 (2016) 3602–3609. <https://doi.org/10.1002/jssc.201600647>.
- 644 [35] S. Pinsrithong, O. Bunkoed, Hierarchical porous nanostructured polypyrrole-coated hydrogel
645 beads containing reduced graphene oxide and magnetite nanoparticles for extraction of
646 phthalates in bottled drinks, *J. Chromatogr. A.* 1570 (2018) 19–27.
647 <https://doi.org/10.1016/j.chroma.2018.07.074>.
- 648 [36] H. Sereshti, Z. Mohammadi, S. Soltani, M. Taghizadeh, Synthesis of a magnetic micro-
649 eutectogel based on a deep eutectic solvent gel immobilized in calcium alginate: Application
650 for green analysis of melamine in milk and dairy products, *Talanta.* 265 (2023) 124801.
651 <https://doi.org/10.1016/j.talanta.2023.124801>.
- 652 [37] Z. Asghari, H. Sereshti, S. Soltani, M. Taghizadeh, S. Karami, M. Esmaeili Bidhendi, S.
653 Rezania, An alginate-based eutectogel impregnated with polyvinylpyrrolidone/benzoic acid
654 deep eutectic solvent and magnetic carboxylated multiwalled carbon nanotubes: Evaluated as
655 sorbent in green microextraction of pesticides, *J. Chromatogr. B Anal. Technol. Biomed. Life*
656 *Sci.* 1229 (2023) 123865. <https://doi.org/10.1016/j.jchromb.2023.123865>.
- 657 [38] S. Khongkla, S. Jullakan, S. Saeai, P. Nurerk, A. Kliangsuwan, O. Bunkoed, A composite
658 zinc oxide and magnetic molecularly imprinted polymer hydrogel adsorbent for the extraction
659 of sulfonamides in milk, *Microchem. J.* 191 (2023) 108865.
660 <https://doi.org/10.1016/j.microc.2023.108865>.
- 661 [39] J. Shah, M.R. Jan, Eco-friendly alginate encapsulated magnetic graphene oxide beads for solid
662 phase microextraction of endocrine disrupting compounds from water samples, *Ecotoxicol.*
663 *Environ. Saf.* 190 (2020) 110099. <https://doi.org/10.1016/j.ecoenv.2019.110099>.
- 664 [40] N. Orachorn, P. Klongklaew, O. Bunkoed, A composite of magnetic GOx@MOF incorporated
665 in alginate hydrogel fiber adsorbent for the extraction of phthalate esters, *Microchem. J.* 171
666 (2021) 106827. <https://doi.org/10.1016/j.microc.2021.106827>.
- 667 [41] N. Hokonya, C. Mahamadi, N. Mukaratirwa-Muchanyereyi, T. Gutu, C. Zvinowanda,
668 Enrichment of lead and cadmium from water using P–ZrO₂CeO₂ZnO nanoparticles/alginate
669 beads: Optimization and determination of significant factors and interaction using response
670 surface methodologies, *Sci. African.* 17 (2022) e01340.
671 <https://doi.org/10.1016/j.sciaf.2022.e01340>.
- 672 [42] E.C. Silva, C.G. Gomes, M.A. Vieira, A.R. Fajardo, Composite hydrogel based on alginate-g-
673 poly(acrylamide)/carbon nanotubes for solid phase extraction of metals from corn cereal
674 samples, *Int. J. Biol. Macromol.* 242 (2023) 124586 Contents.
675 <https://doi.org/10.1016/j.ijbiomac.2023.124586>.
- 676 [43] J. Van Beik, K.B. Fontana, D.C.C.S. Medeiros, A.C.N. Sydney, E.S. Chaves, Feasibility of
677 calcium alginate beads to preconcentrate lead in river water samples prior to determination by
678 flame atomic absorption spectrometry, *Environ. Monit. Assess.* 193 (2021) 666.
679 <https://doi.org/10.1007/s10661-021-09453-9>.
- 680 [44] S. Jullakan, S. Pinsrithong, O. Bunkoed, Hierarchically porous adsorbent alginate beads
681 incorporating poly(3, 4-ethylenedioxythiophene) for dispersive liquid-solid phase extraction of
682 five polycyclic aromatic hydrocarbons, *J. Food Compos. Anal.* 111 (2022) 104629.

- 683 <https://doi.org/10.1016/j.jfca.2022.104629>.
- 684 [45] S. Zhang, H. Niu, Y. Cai, Y. Shi, Barium alginate caged Fe₃O₄@C18 magnetic nanoparticles
685 for the pre-concentration of polycyclic aromatic hydrocarbons and phthalate esters from
686 environmental water samples, *Anal. Chim. Acta.* 665 (2010) 167–175.
687 <https://doi.org/10.1016/j.aca.2010.03.026>.
- 688 [46] O. Bunkoed, P. Kanatharana, Extraction of polycyclic aromatic hydrocarbons with a magnetic
689 sorbent composed of alginate, magnetite nanoparticles and multiwalled carbon nanotubes,
690 (n.d.). <https://doi.org/10.1007/s00604-015-1484-x>.
- 691 [47] S.C. Tan, H.K. Lee, A hydrogel composite prepared from alginate, an amino-functionalized
692 metal-organic framework of type MIL-101(Cr), and magnetite nanoparticles for magnetic
693 solid-phase extraction and UHPLC-MS/MS analysis of polar chlorophenoxy acid herbicides,
694 *Microchim. Acta.* 186 (2019). <https://doi.org/10.1007/s00604-019-3679-z>.
- 695 [48] A. Gutiérrez-Serpa, R. González-Martín, M. Sajid, V. Pino, Greenness of magnetic
696 nanomaterials in miniaturized extraction techniques: A review, *Talanta.* 225 (2021) 122053.
697 <https://doi.org/10.1016/j.talanta.2020.122053>.
- 698 [49] A. Gałuszka, Z.M. Migaszewski, P. Konieczka, J. Namieśnik, Analytical Eco-Scale for
699 assessing the greenness of analytical procedures, *TrAC - Trends Anal. Chem.* 37 (2012) 61–72.
700 <https://doi.org/10.1016/j.trac.2012.03.013>.
- 701 [50] J. Płotka-Wasyłka, A new tool for the evaluation of the analytical procedure: Green Analytical
702 Procedure Index, *Talanta.* 181 (2018) 204–209. <https://doi.org/10.1016/j.talanta.2018.01.013>.
- 703 [51] W. Wojnowski, M. Tobiszewski, F. Pena-Pereira, E. Psillakis, AGREeprep – Analytical
704 greenness metric for sample preparation, *TrAC Trends Anal. Chem.* 149 (2022) 116553.
705 <https://doi.org/10.1016/J.TRAC.2022.116553>.

706

707

708 Figure captions:

709

710 Fig.1. Number of publications focused on alginate-based sorbents application in microextraction
711 approaches based on Web of Science database (searched keywords: alginate-based sorbent;
712 microextraction, accessed on June 2024).

713 Fig.2. The molecular structures of possible sequences of L-guluronic and D-mannuronic units in
714 alginate polymer.

715 Fig.3. Main groups of sorbents combined with alginate hydrogel and used in solid-phase
716 microextraction techniques

717 Fig.4. Main steps of alginate-based sorbent preparation.

718 Fig 5. The distribution of articles among various sorbent-based microextraction techniques.

719

720 Table captions:

721 Table 1. Summary of application of alginate-based sorbents in microextraction techniques.

722 Table. 2. Penalty Points and Analytical Eco-scale scores of the selected reports, with consideration of
723 the analytical procedure and the sorbent synthesis.

724

725

