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1 **Applications of membrane techniques for purification of natural products**

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5
6
7 **Abstract**

8
9 The recent development of various membrane-based techniques for the purification of
10 valuable natural products is reviewed. The review covers the important research that
11 has been conducted in the last 5 years on the utilization of microfiltration,
12 ultrafiltration and nanofiltration techniques, either carried out on their own or coupled
13 with other separation techniques, in order to achieve concentration and purification of
14 natural products from their biological source. There is also a special focus on the
15 research that has been undertaken to overcome the membrane fouling encountered in
16 their usage.

17
18 *Keywords: Membrane fouling; Membrane techniques; Microfiltration; Nanofiltration;*
19 *Natural products; Ultrafiltration;*

23 **Introduction**

24

25 The term “natural products” usually refers to chemical substances found in nature that
26 possess distinctive pharmacological or biological activities. They are either directly
27 recovered from their natural sources or are chemically synthesized. In recent years,
28 natural products have been the source for the discovery of new drugs (Newman and
29 Cragg 2003; 2007). It is reported that as many as one quarter of today’s anticancer
30 drugs are natural products, with another quarter being synthetic derivatives of natural
31 products (Davidson 1995). The main categories of natural products include
32 carbohydrates, lipids, proteins and nucleic acids. Taking into consideration that not all
33 natural products can be obtained economically via total chemical synthesis, many of
34 them have to be separated and isolated from higher plants and microorganisms by
35 often tedious and time-consuming processes. Methods to concentrate and purify
36 natural products have become a subject of dramatically increased interest in the last
37 several decades. Advances in the recognition of the value of their various biological
38 properties, and the expansion of food and healthcare products markets have led to the
39 development of state-of-art strategies for the separation and isolation of natural
40 products from plants, microorganisms and marine sources. Generally, modern
41 strategies for the purification of natural products have been based around
42 chromatographic techniques (Andersen and Markham 2006). An earlier review of the
43 application of chromatographic techniques for this purpose has been conducted by
44 Marston and Hostettmann (1991). Recent development of traditional chromatographic

45 separations, and applications for newly developed techniques such as superficial fluid
46 extraction, pressurized liquid extraction, microwave-assisted extraction, vacuum
47 liquid chromatography, preparative pressure liquid chromatography, have been
48 reviewed by Sticher (2008).

49

50 Advances in material science and membrane manufacturing technology have led to
51 membrane-based filtration techniques becoming a mainstream technology since the
52 early 1990s. With a number of advantages such as high efficiency, simple equipment,
53 convenient operation and low energy consumption, membrane technology has become
54 one of the most important industrial separation techniques and has been applied
55 extensively to various fields, *e.g.* concentration of juices (Cheryan 1998), water
56 purification and desalination, dye and sugar separation, and the recovery of valuable
57 products (Brüschke 1995).

58

59 Though the term “membrane technology” refers to a number of separation processes
60 with different characteristics, the general principle is based on the selective
61 permeability of the membrane to allow the target substances to penetrate through the
62 membrane, whilst unwanted substances are normally rejected by the membrane.
63 Various methods, *e.g.* high pressures, concentration gradients and chemical or
64 electrical potential differences, may be adopted as the driving force for membrane
65 separations. Based on the size of the substances to be separated, and the resultant
66 characteristics needed for the membrane, membrane techniques are generally

67 classified into microfiltration, ultrafiltration, nanofiltration, reverse osmosis, dialysis,
68 electro dialysis, pervaporation, gas permeation and membrane distillation. The latter
69 six techniques can usually retain molecules with diameters less than 1 nm and
70 therefore they are not suitable for natural products as these are generally larger
71 molecules. For instance reverse osmosis is based on a solution diffusion mechanism
72 and very high operating pressures are needed for the process. This technique is most
73 appropriate for the molecules in the range of 0.1 ~ 1.0 nm and almost no utilization of
74 reverse osmosis has been reported for natural products. Nanofiltration, ultrafiltration
75 and microfiltration are based on size exclusion mechanisms and found to be suitable
76 to separate various molecules and macromolecules with relative molecular masses
77 ranging from several hundred to several hundred thousand, corresponding to a
78 molecular size in the range from several nm to 1 μm . A key feature of a membrane
79 separation process is the selection of a membrane with appropriate rejection
80 characteristics. It is noticeable that polyvinylidene fluoride (PVDF) membranes have
81 received by far the most attention for adoption in both food and medical fields,
82 because of their chemical stability, durability and biocompatibility (Laroche et al.
83 1995).

84

85 This review is essentially a follow up to the previous reviews completed by Marston
86 and Hostettmann (1991) and Sticher (2008) on strategies for the purification of natural
87 products as a survey of the use of membrane techniques has mainly been neglected by
88 previous authors. Applications of microfiltration, ultrafiltration and nanofiltration

89 techniques in the purification of natural products are described. In addition, special
90 emphasis has been given to the occurrence of membrane fouling, together with
91 strategies that have been adopted to improve the deteriorating permeate flux.

92

93 **Primary purification**

94

95 Although purification by microfiltration and ultrafiltration used in the industrial
96 production of fruit and vegetable juices has been extensively investigated in the past
97 three decades (Vladislavljević et al. 2003), very few studies of the use of these
98 techniques for the purification of natural products from plants and microorganisms
99 have appeared in the literature. Membranes used as simple pretreatment procedures
100 for natural products have been the most frequently reported applications. Cho et al.
101 (2003) studied the clarification and concentration of water soluble pectin, a
102 biopolymer with reported pharmacological properties such as cholesterol-decreasing
103 (Kay and Truswell 1977), immuno-stimulating (Yamada et al. 1989) and anti-ulcer
104 activities (Kiyohara et al. 1994). Pectin was isolated from an extract prepared from
105 mature citrus peel using a 0.20 μm pore size cellulose membrane and a batch mode of
106 operation. The use of a crossflow microfiltration step, with a 75 % saving in the
107 consumption of ethanol, resulted in the recovery yield of pectin being decreased from
108 10.5 % to 9.9 % whilst the galacturonic acid content in the pectin increased from
109 68.0 % to 72.2 %. Further purification was carried out by diafiltration in a fed-batch
110 mode in order to remove the polyphenol impurities. This process was evaluated to be

111 effective and efficient with potential for industrial application. Prodanova et al. (2008)
112 employed ultrafiltration as a sample preparation procedure for polyphenols from
113 almond skins before subsequent high-resolution analysis. A 30 kDa MWCO
114 (molecular weight cut off) polyethersulfone (PES) ultrafiltration membrane has been
115 used for the concentration and pre-purification of R-phycoerythrin from a crude
116 extract of the macro-algae *Grateloupia turuturu* (Denis et al. 2009).

117

118 Membrane-based techniques have also increased in importance as techniques suited to
119 the refining of oligosaccharides present in carbohydrates. Microfiltration and high
120 MWCO ultrafiltration membranes have usually been used to obtain a mixture of
121 oligosaccharides with low molecular weight impurities that have been separated from
122 compounds of higher molecular weight (Playne and Crittenden 1996; Cheryan 1998).
123 The use of low MWCO ultrafiltration and nanofiltration membranes provides an
124 alternative to chromatography to concentrate and separate the target oligosaccharides
125 from these impurities.

126

127 The earliest reports on the application of membranes for the recovery of
128 oligosaccharides can be traced back to the 1990s (Mok et al. 1995; Matsubara et al.
129 1996; Urano et al. 1997; Sarney et al. 2000). More recently, Goulas et al. (2002)
130 reported the nanofiltration on a commercial scale of a galacto-oligosaccharide mixture
131 and investigated the significance of operating parameters, including pressure, feed
132 concentration and filtration temperature, on the purification process. Subsequently, a

133 study of nanofiltration for purifying fructo-oligosaccharides was reported by Li et al.
134 (2004). Two operational modes, variable volume diafiltration and constant volume
135 diafiltration, were applied successfully and 90 % purity was achieved. In addition, the
136 purification of xylo-oligosaccharides for use as low-calorie sweeteners and the
137 removal of monosaccharides and other low molecular weight impurities from rice
138 husk xylan have proved to be feasible using either a 4 kDa MWCO polymeric tubular
139 ultrafiltration membrane or a 1 kDa MWCO ceramic monolithic nanofiltration
140 membrane (Vegas et al. 2008). Nabarlatz et al. (2007) made successful use of
141 commercial thin-film polymeric ultrafiltration membranes with different MWCOs to
142 purify xylo-oligosaccharides from an autohydrolysis solution of almond shells. They
143 were able to separate the xylo-oligosaccharides into fractions containing narrow and
144 reproducible distributions of molar mass. Sjöman et al. (2008) reported the use of
145 nanofiltration to recover xylose from various hemicellulose hydrolysates. The
146 membranes used to recover this pentose sugar in their study had MWCOs between
147 150 and 300 Da. Hyaluronic acid is a natural glycosaminoglycan that can be used for
148 various biomedical purposes such as treatment of arthritis, wound healing and tissue
149 regeneration. Zhou *et al.* (2006) investigated the separation of hyaluronic acid from
150 the fermentation broth of *Streptococcus zooepidemicus* by means of a two-stage
151 tangential flow microfiltration and ultrafiltration process using pure water as
152 diafiltrate. PVDF membranes with 0.45 and 0.20 μm pore size were used for
153 microfiltration and PVDF300 (MWCO 300 kDa) and PVDF100 (MWCO 100 kDa)
154 were used for subsequent ultrafiltration. The process was optimized to achieve an

155 overall yield of hyaluronic acid of 77 % with a purification factor of 1000. Examples
156 of the use of membranes for primary purification purposes are summarized in Table 1.

157

158 **Coupling membrane technologies with other techniques**

159

160 The coupling of membrane techniques together in a series has been employed where a
161 single membrane filtration step is not satisfactory. A classical example is an early
162 application of a three-stage hybrid membrane separation process for the purification
163 of active components from *Echinacea* plants (Johnson et al. 2002). The
164 immunostimulatory activity of *Echinacea* is attributed to its high content of
165 polysaccharides, caffeic acid derivatives and alkyl amides (Bauer 1991).
166 Conventional extraction of the active components from *Echinacea*, e.g. the use of
167 ethanol-water extraction, usually consumes large quantities of solvents and energy.
168 The first stage of the membrane process employed a 200 μm pore size
169 polydimethylsiloxane (PDMS) hollow fibre membrane to remove the ethanol and
170 some water from the crude extract in order to produce a high quality tincture by
171 pervaporation. The second stage involved microfiltration to remove the alkyl amides,
172 which may precipitate during tincture production, from the ethanol-free aqueous
173 product using a 0.04 μm polysulfone (PSU) membrane. In the last stage, the
174 microfiltration permeate was concentrated several-fold by osmotic distillation on a
175 polypropylene (PP) membrane with a nominal pore size of 0.05 μm \times 0.19 μm . This
176 complex hybrid membrane separation process was adopted due to the fact that single

177 osmotic distillation of the ethanol-water extract would result in severe wet through of
178 the hydrophobic membrane. Finally, the microfiltration retentate containing the
179 precipitated alkyl amides was added back to the osmotic distillation retentate to form
180 a highly concentrated product that was suitable for use in capsule production. Similar
181 work has been reported by Tessier et al. (2005) who coupled ultrafiltration (30 and
182 100 kDa MWCO) and nanofiltration (300 Da MWCO) techniques to purify
183 benzylpenicillin from the fermentation broth of *Penicillium chrysogenum* with a
184 recovery yield of nearly 90 %. More recently, Isa et al. (2007) reported the
185 purification of surfactin, a cyclic lipopeptide with antiviral, antibacterial and
186 antitumor properties (Heerklotz and Seelig 2001), from the fermentation broth of
187 *Bacillus subtilis* by a two-step ultrafiltration process.

188

189 There have been frequent reports of the use of membrane separations in conjunction
190 with other purification techniques in order to isolate natural products. Ultrafiltration
191 has been used to purify a plant peroxidase from the leaves of *Ipomoea palmetto*
192 following an aqueous two-phase extraction step (Srinivas et al. 2002). Another case is
193 the use of ultrafiltration as the first clarification step to eliminate large impurities in a
194 lactic acid production process based on conventional and bipolar electro dialysis
195 operations (Bouchoux et al. 2005).

196

197 **Challenging the fouling of membranes**

198

199 The greatest problem that has emerged in the use of membrane-based techniques is
200 the decay of permeate flux caused by concentration polarization and fouling, which in
201 turn increase the operating cost and shorten membrane life. During the membrane
202 separation process, the rejected molecules arrive at the membrane surface by
203 convective transport and thus a concentrated layer of the particles is built up at the
204 membrane surface, forming a concentration gradient termed concentration
205 polarization (Cheryan 1998). Fouling refers to the accumulation of unwanted material
206 on the membrane surface leading to pore constriction and pore blockage that impedes
207 the separation process. The decay of permeate flux caused by concentration
208 polarization can be reversed by modifying the operating conditions, such as flow
209 velocity, feed concentration and transmembrane pressure. However the decay of flux
210 caused by fouling is irreversible and the membrane can usually only be cleaned using
211 chemicals or by back-flushing with water or permeate. Fouling is an unfavourable but
212 inevitable problem in membrane-based techniques and a key issue in the success of
213 membrane separation is the control of fouling. Fouling of membranes is especially
214 prevalent when they are employed in the isolation of natural products from plant
215 sources due to the fact that more than 90 % of the components present after crude
216 extraction are non-pharmacological macromolecules with molecular weights larger
217 than 50 kDa that can easily block the membrane pores.

218

219 Various approaches to the theoretical analysis and prediction of membrane fouling
220 phenomena have been reported (Boerlage et al. 2002; Tung et al. 2001; Hwang et al.

221 2007; Kawakatsu et al. 1995; Vrouwenvelder et al. 2006). Much effort has been paid
222 to the prevention of membrane fouling, *e.g.* dosing with oxidant or anti-fouling agents
223 (Durham and Walton 1999; Sakol and Konieczny 2004). Some recent studies have
224 developed strategies to recover the decreasing flux. Ceramic membranes have been
225 extensively employed in the purification of active components from many Chinese
226 herbs. Li et al. (2006) observed the effect of pH on the adsorption behaviour to
227 zirconia particles of macromolecular impurities in an aqueous extract of *Radix*
228 *Rehmanniae*. These authors have also studied the fouling phenomena that occur on
229 the surface of a zirconia membrane when used in a microfiltration process with the
230 aqueous extract. The work indicated that adsorption reached its maximum rate and
231 extent when the pH was near the isoelectric point of the zirconia particles. This was in
232 agreement with the pH value at which the minimum membrane flux and the highest
233 rejection of total solids occurred with the zirconia membrane.

234

235 Modification of the properties of a membrane has also been investigated by
236 introducing various functional groups into the existing membrane chemistry. A
237 generally accepted hypothesis is that hydrophilic membranes are usually less
238 susceptible to fouling than hydrophobic ones (Hester and Mayes 2002). Consequently
239 the hydrophilicity of the membranes has been increased by introducing polymeric
240 hydrophilic groups to the membrane. Xu et al. (2005) reported the modification of a
241 PVDF ultrafiltration membrane with PVP (polyvinylpyrrolidone) for use in the
242 purification of pharmacological flavonoids (Kubota et al. 2001) from extracts of

243 *Ginkgo biloba*. The modified membrane contains a polyamide structure that may
244 show a high and selective affinity for flavonoids due to hydrogen-bond effects
245 between flavonoids and the PVP groups. With the modified PVDF-PFP membrane,
246 the permeate flux was observed to increase linearly at low transmembrane pressures
247 and gradually reached a steady value at high pressure; the flavonoid content in the
248 product was increased from 21.3 % to 34.8 %. For polyacrylonitrile (PAN)
249 membranes, the simplest way to increase the hydrophilicity of the membrane is to
250 hydrolyze the membrane by immersion into 2M NaOH (Qiao et al. 2007). The
251 hydrophilic PAN membranes so obtained were then used to purify danshensu and
252 protocatechuic aldehyde from an aqueous extract from *Salvia miltiorrhiza*. The study
253 suggested that increased permeate flux could be obtained by increasing the
254 hydrophilicity of the membrane. In addition, permeates with different contents and
255 ratios of the two desired components could be achieved by using PAN membranes
256 with different hydrophilicities. Another approach is the use of inorganic acids as
257 hydrophilization agents. González Muñoz et al. (2006) immersed a nanofiltration
258 membrane into a 1 % w/v hydrofluoric acid (HF) solution for 14 days in order to
259 improve the membrane performance. Although no investigation was carried out on the
260 nature of the chemical modification that had occurred, improved permeate flux was
261 demonstrated for the membrane after HF treatment. Although various synthetic
262 polymer membranes have gained increased popularity due to the stability of their
263 chemical structure and the availability of abundant functional groups, novel
264 membranes containing regenerated cellulose composites may be an attractive

265 alternative because of the occurrence of less fouling, improved mechanical strength
266 and easy regeneration (van Reis and Zydney 1999).

267

268 A method used by You et al. (2007) to study the fouling of membranes by wastewater
269 influent may be illuminative for similar fouling problems when membranes are used
270 to purify natural products. They investigated the use of ozone to oxidize the fouling
271 matter on PVDF ultrafiltration membranes. Ozonation was performed by direct
272 continuous dosing of ozone into the wastewater influent at a flow rate of 8.79 mg/min
273 and the residual concentration of ozone in the influent flow was steadily maintained at
274 4.02 mg/L throughout the test. It was observed that after 1 h, the permeate flux
275 dropped by 40 % for a membrane not subjected to ozonation, whilst the flux with the
276 treated membrane fell by only 10 %. On the basis of infra-red and energy dispersive
277 x-ray spectroscopy analyses, the authors concluded that the ozone treatment destroyed
278 aromatic rings within the PVDF membrane to “produce functional oxygenated groups
279 (aldehydic, ketonic, and especially carboxylic acid), which may be coupling with
280 calcium ions to form soluble calcium complexes that reduce the formation of calcium
281 carbonates”.

282

283 In addition to the efforts devoted to modifying the structure and chemistry of
284 membranes, considerable work has been focused on developing new membrane
285 systems with reduced fouling characteristics and enhanced permeate flux. Earlier
286 work towards this objective includes the adoption of conical-shaped rotors (Vogel and

287 Kroner 1999), cylindrical Taylor vortex systems (Parnham et al. 1995), rotating disk
288 filters (Lee et al. 1995) and helical coiled Dean vortex devices (Luque et al. 1999;
289 Gehlert et al. 1998). Recently, Auddy et al. (2004) developed a stainless steel
290 rectangular cross-flow cell that worked as a turbulence promoter to enhance the
291 permeate flux in a nanofiltration system. Significant transmembrane flux
292 enhancement in the range of 40-100 % was achieved and was even more pronounced
293 at higher flow velocity. The researchers concluded that turbulence was promoted by
294 the wires placed in the cross-flow cell and helped to reduce concentration polarization
295 and resulted in less solute deposition on the membrane surface. Although the work
296 was carried out in a nanofiltration process for dye solutions, the feasibility of flux
297 enhancement in may result in the design of novel devices that work in similar ways to
298 challenge the fouling that occurs during membrane purification processes for natural
299 products.

300

301 **Conclusions and outlook**

302

303 This mini review covers the most recent developments in the purification of natural
304 products in which one or more membrane-based techniques are utilised as either a
305 primary treatment or as a complete recovery process. From the work carried out so far,
306 the general observation is that single membrane techniques, such as microfiltration,
307 ultrafiltration and nanofiltration, have been able to perform a primary purification of
308 natural products from plant and microorganism sources due to their inherent

309 advantages, *e.g.* high efficiency, simple equipment, convenient operation and low
310 energy consumption. In addition, these techniques can be used in conjunction with
311 other separation processes, to achieve various demands for higher degrees of
312 purification of natural products. Much effort has been devoted to improve the
313 selectivity of membrane separations while maintaining their inherent high-throughput
314 characteristics (van Reis and Zydney 2001).

315

316 The outlook for membrane-based techniques in this field will be the development of
317 better membrane filtration system with high selectivity for target substances and
318 better anti-fouling properties. An important trend is the improvement of the
319 membrane structure and the emergence of functional membranes, which with
320 appropriate function groups grafted onto their surface, are highly selective for target
321 substances, and in addition are able to resist fouling more effectively. In conclusion,
322 the abundant qualities of membrane-based techniques will ensure that they will
323 remain productive tools in the purification of natural products.

324

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464 **Table 1** Applications of membrane techniques for primary purification of natural products.

Solute	Raw material	Membrane technique	Membrane material	Module	Surface area (m ²)	Pore size (μm)	Flux ^c (L/m ² h)	Reference
phenolics	almond skins	centrifugal ultrafiltration	n/a	n/a	n/a	10 ~ 50k ^a	n/a	Prodanova et al.
cycoerythrin	macro-algae <i>Grateloupia turuturu</i>	ultrafiltration	PES ^b	tubular	0.033	25 ~ 30k ^a	35.1	Denis et al.
pectin	mature citrus peel	crossflow microfiltration	cellulose	flat sheet	0.1	0.2	25.9	Cho et al.
oligosaccharide	commercial mixture	nanofiltration	cellulose	flat sheet	n/a	n/a	n/a	Goulas et al.
oligosaccharides	commercial mixture	nanofiltration	n/a	n/a	1.77	n/a	n/a	Li et al.
oligosaccharides	rice husk xylan	ultrafiltration	PES ^b	tubular	n/a	4k ^a	21	Vegas et al.
		nanofiltration	ceramic	tubular	n/a	1k ^a	34	
oligosaccharides	almond shells	ultrafiltration	polymeric	flat sheet	1.26×10^{-3}	8k ^a	227 ~ 579	Nabarlatz et al.
xylan	hemicellulose hydrolyzate feeds	nanofiltration	PS ^b	flat sheet	0.18	150 ~ 300 ^a	18.2 ~ 66.2	Sjöman et al.
lactic acid	<i>S. zooepidemicus</i> fermentation broth	tangential flow microfiltration and ultrafiltration	PVDF ^b	flat sheet	10.4×10^{-4}	100 ~ 300k ^a	23.0 ~ 24.9	Zhou et al.

465 ^a molecular weight cut off, MWCO.

466 ^b PES: polyethersulfone; PS: polysulfone; PVDF: polyvinylidene fluoride

467 ^c dependent on specific pressure in each work

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