

1 **4-Sulfonatocalixarene-induced nanoparticle formation of**
2 **methylimidazolium-conjugated dextrans: Utilization for drug encapsulation**

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20 **Abstract**

21 Methylimidazolium side groups were grafted via ether linkage to dextran and the self-
22 assembly of these polymers with 4-sulfonato-calix[n]arenes (SCXn) was studied in aqueous
23 solutions. Dynamic light scattering and zeta potential measurements revealed the mixing ratio
24 ranges of the constituents where stable nanoparticles could be created. The macrocycle size of
25 SCXn and the molecular mass of the polymer barely affected the nanoparticle diameter, but
26 the lowering of the imidazolium degree of substitution substantially diminished the stability
27 of the associates. The pH change from neutral to acidic also unfavourably influenced the self-
28 organization owing mainly to the decrease of the SCXn charge. Cryogenic transmission
29 electron microscopy images proved the spherical morphology of the nanoproducts in which
30 the stoichiometry of the constituents was always close to the one corresponding to charge
31 compensation. The flexible and positively charged dextran-chains are compacted by the
32 polyanionic SCXn. Coralyne, a pharmacologically important alkaloid was efficiently
33 embedded by self-assembly in the produced nanoparticles reaching 99 % association
34 efficiency.

35

36 **Key words**

37 cationic polymer, macrocycle, self-assembly, alkaloid, drug delivery

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39 **1. Introduction**

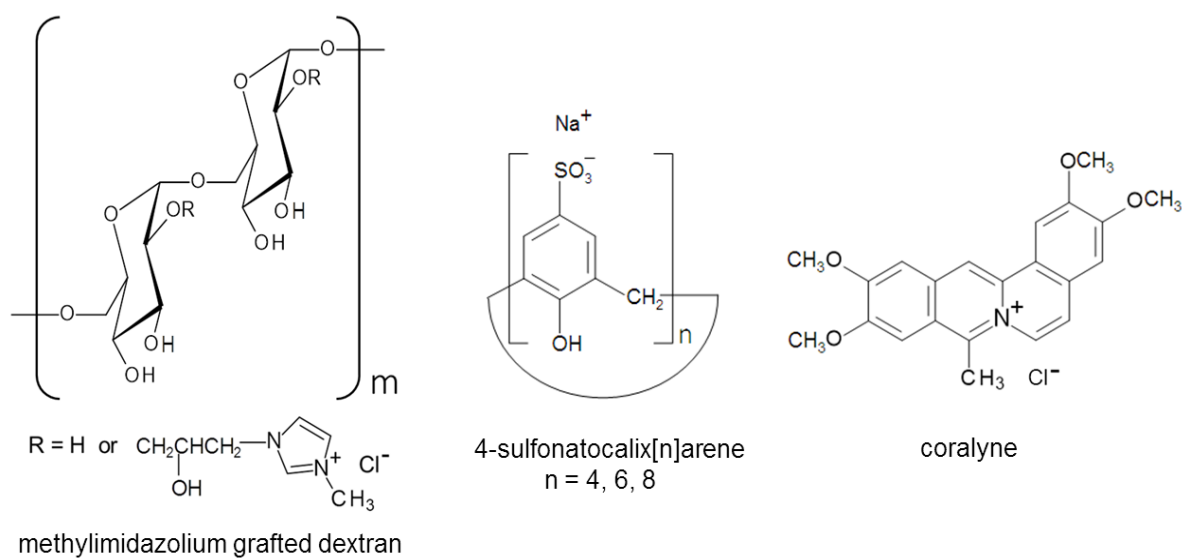
40 The self-assembly of naturally occurring biocompatible and biodegradable polysaccharides
41 and their derivatives have attracted rapidly growing interest (Giri & Ghosh, 2018; Myrick
42 James, Vendra Venkat & Krishnan, 2014; Zheng, Monty & Linhardt, 2015). Dextrans, the
43 glucose polymers of microbial origin, represent a particularly important family of these
44 substances because of their diverse biomedical applications (Heinze, Liebert, Heublein &
45 Hornig, 2006; Maia, Evangelista, Gil & Ferreira, 2014). Their favorable properties offer
46 great potential for the delivery and controlled release of drugs (Huang & Huang, 2018;
47 Mehvar, 2000). Chemical modification of the polymer backbone created conjugates capable
48 of the encapsulation of various compounds via self-organization into stimuli-responsive
49 nanoparticles (Jafarzadeh-Holagh, Hashemi-Najafabadi, Shaki & Vasheghani-Farahani,
50 2018; Son et al., 2018; Tang et al., 2018). The introduction of spermin, histidine, or
51 diethylaminoethyl units led to polycations usable for gene delivery (Hosseinkhani, Azzam,
52 Tabata & Domb, 2004; Pawar et al., 2008; Thomas, Rekha & Sharma, 2012). Improved DNA
53 carrier ability was achieved by grafting of dextran onto branched polyethylenimine (Tseng &
54 Jong, 2003). The association with dextran sulfate was exploited to enhance the bioavailability
55 of poorly-soluble cationic drugs (Cheow & Hadinoto, 2012; Megyesi, Biczók & Görner,
56 2009; Persson, Hugerth, Caram-Lelham & Sundelöf, 2000). A dextran-based prodrug
57 produced micelles from which the pharmaceutically active component was liberated by the
58 hydrolysis at the acidic endosomal pH (Jin, Guo, Dong, Xie & Cao, 2017). The
59 monofunctionalized derivative labelled with a fluorogenic probe exhibited improved cellular
60 imaging capabilities and was efficiently transported into the cytosol (Chyan, Kilgore &
61 Raines, 2018). Dextrans with pendant side chains bearing quaternary ammonium group were
62 synthesized and their aggregation was extensively studied (Bai, Nichifor, Lopes & Bastos,
63 2005; Nichifor, Lopes, Bastos & Lopes, 2004; Nichifor, Stanciu & Simionescu, 2010; (Prado

64 & Matulewicz, 2014). However, much less information is available on the derivatives
65 carrying methylimidazolium substituents. These biodegradable polymers can serve as
66 flocculants in the separation of contaminants of wastewater (Ghimici & Nichifor, 2018).

67 We have previously shown that 1-alkyl-3-methylimidazolium cations readily bind to
68 4-sulfonatocalixarenes (SCXn) (Wintgens, Biczók & Miskolczy, 2011) and the formed
69 inclusion complexes self-assemble into nanoparticles (NP) or supramolecular micelles
70 (Wintgens et al., 2013) if the number of carbon atoms in the aliphatic substituent of the
71 heterocycle is at least 12. More stable and less toxic NPs may form with SCXn when
72 methylimidazolium groups are connected to polymer chain by covalent bonds. The interior of
73 such species can provide protective matrix for pharmaceutical agents ensuring longer
74 duration of action by sustained release. The confinement in NPs may enhance the
75 bioavailability of **poorly** soluble therapeutics. The utilization of dextrans is expected to be
76 beneficial because of their good water solubility and biocompatibility. The strengthening of
77 the interaction with SCXn polyanionic macrocycles can be achieved by grafting of cationic
78 substituents to the dextran main chain. Despite the importance of the polymer-based NPs,
79 few information has been gathered so far on the SCXn-induced noncovalent crosslinking of
80 macromolecules and on the properties of the nanostructures created thereby. It is unknown
81 how the competitive binding of the pendant moieties to SCXn affects the incorporation of
82 drugs.

83 In the present study, we focus on the association of methylimidazolium-conjugated
84 dextrans with SCXn to reveal how the degree of substitution (DS) of the polymer affects the
85 characteristics of the produced aggregates. Our other goal is to compare the drug loading
86 capability of the created nanoparticles with those previously synthesized from protonated
87 chitosan and SCXn (Harangozó, Wintgens, Miskolczy, Amiel & Biczók, 2016). We linked
88 methylimidazolium cationic groups to dextran because these are very weak acids and cannot

89 be protonated. Hence, the produced polymer can be used in a wide pH range in contrast with
 90 protonated chitosan-SCXn associates, which were applicable only in acidic medium.
 91 Coralyne, a synthetic analogue of natural protoberberine alkaloids, **was** used as a model
 92 compound because it shows high affinity and multiple binding to SCXn. (Megyesi & Biczók,
 93 2010) Coralyne exhibited antitumor activity (Gatto et al., 1996; Kumari, Badana, Mohan,
 94 Shailender Naik & Malla, 2017) and was successfully employed in the phototherapy of
 95 cancer (Bhattacharyya, Gupta, Bandyopadhyay, Patro & Chattopadhyay, 2018;
 96 Bhattacharyya, Saha, Tyagi, Bandyopadhyay, Patro & Chattopadhyay, 2017). The chemical
 97 structures of the investigated compounds are displayed in Scheme 1.



98

99

Scheme 1. Formulas of the studied compounds

100

101 2. Materials and method

102 2.1. Materials.

103 4-Sulfonatocalix[n]arenes (SCX4, SCX6 and SCX8) were purchased from Acros
 104 Organics and used after being dried under vacuum at 343 K overnight. Double distilled water
 105 served as solvent. Dextrans with molar mass of 40 (D40), 70 (D70), 110 (D110) and 500
 106 (D500) kg mol^{-1} were purchased from Amersham Pharmacia (Sweden) and used as received.

107 These samples had a branching ratio below 5% according to the specifications of the
108 manufacturer. 1-Methylimidazole (Acros) and epichlorohydrin (Aldrich) were also employed
109 without further purification. The modified polymers were studied after being dried under
110 vacuum at 343 K overnight. Coralyne chloride (Acros Organics) was recrystallized from
111 ethanol.

112 2.2. *Synthesis of the chemically modified dextrans.*

113 The various modified dextrans were synthesized as reported by Nichifor and
114 coworkers (Nichifor, Stanciu & Simionescu, 2010). In this one-step procedure, typically 1g
115 dextran (6.17 mM anhydroglucose units, AGU) was dissolved in 10 mL water. Then, 2.7 mL
116 1-methylimidazole (33.9 mM) and 2.2 mL epichlorohydrin (27.8 mM) were added, which
117 corresponded to a molar excess (versus AGU) of 5.5 and 4.5 for the former and latter
118 compounds, respectively. The mixture was stirred for 24 hours at 313 K. After cooling, the
119 mixture was set in a dialysis bag (6000-8000 Da molecular weight cut off). Two dialyses
120 against 0.1 M HCl and eight dialyses against water were performed. The final product (~0.8
121 g) was obtained after lyophilization. Following this procedure, three dextran derivatives were
122 prepared with slightly different degrees of substitution: D40Im46, D70Im54, D110Im43,
123 where x and y in $D_x\text{Im}_y$ stand for the starting dextran molar mass (kg mol^{-1}) and the % of
124 imidazolium groups, respectively. The latter quantity, expressed as the number of
125 imidazolium substituents per 100 AGU, was obtained by ^1H NMR spectroscopic
126 measurements in D_2O in the presence of deuterated trifluoroacetic acid as reported in the
127 Supplementary data. In the case of dextrans possessing the highest molecular weight (D500),
128 the synthesis led to gelation. To avoid this, the synthesis was carried out in 25 mL water and a
129 modified dextran with a lower substitution degree was isolated (D500Im25). To enhance the
130 substitution degree, five times higher amounts of each reactants were used in 60 mL water
131 and D500Im44 was obtained. A dextran with the lowest molecular weight and with an

132 imidazolium DS around 0.22 (D40Im22) was also prepared in 10 mL water using twice less
133 amount of 1-methylimidazole and epichlorohydrin than described above.

134 2.3. Nanoparticle preparation

135 Stock solutions of the dextrans were prepared at least one day before the start of the
136 experiments. The pH of SCXn in water was adjusted to 7 by addition of concentrated NaOH
137 solution. Nanoparticles (NPs) were prepared by mixing appropriate amounts of dextran
138 derivative (5 g L^{-1}) and SCXn (2 mM) solutions in various molar ratios at 276 K under stirring
139 at 150 rpm. Coralyne (Cor) loaded NPs were created using a Cor–SCX8 mixture instead of
140 SCX8.

141 2.4. Instrumentation

142 ^1H NMR spectra were recorded in D_2O in presence of in presence of few drops (~50
143 μL in 1mL solution) of deuterated trifluoroacetic acid on a Bruker Avance II 400 MHz NMR
144 spectrometer. The absorption spectra were taken on an Agilent Technologies Cary60
145 spectrophotometer. Size exclusion chromatography was performed on a chromatograph
146 equipped with a pump P 100 (Spectra-Physics, Fremont, CA, USA). A set of two columns
147 PL-aquagel OH-30 and OH-40 (Polymer Laboratories, Shropshire, UK) was used for the
148 analysis of polymers in aqueous 0.1 M LiNO_3 eluent. Two detectors were connected in series
149 at the end of the columns: a Dawn Optilab Rex differential refractometer and a Dawn Heleos
150 8 light scattering detector (Wyatt Technology, Santa Barbara, CA, USA). The
151 chromatographic analysis of polymers was done with polymer solutions at a concentration of
152 10 mg mL^{-1} . Particle size was determined by dynamic light scattering on a Zetasizer Nano-ZS
153 (Malvern Instrument) equipped with a He-Ne laser ($\lambda = 633 \text{ nm}$, scattering angle 173°) after
154 stabilization at 296 K. Each measurement was the average of 14 runs of 10 seconds. The
155 reported mean diameter (Z-average size) and polydispersity index (PDI) are obtained by

156 cumulant analysis (fit of the logarithm of the correlation function by a 3rd order polynomial).
157 Error limit of $\pm 10\%$ was estimated for the Z-average size determination. NPs were separated
158 from the liquid phase by an ultracentrifuge from Beckman Coulter (Optima Max-XP, type
159 TLA 110 rotor). Total carbon analyses were performed on a Shimadzu TOC-L CSN
160 instrument, which was calibrated by a potassium hydrogen phthalate solution in ultrapure
161 water (2.125 g L^{-1} corresponding to 1000 mgC L^{-1}). Cryo-TEM images were taken on an
162 Ultrascan 2 k CCD camera (Gatan, USA), using a LaB₆ JEOL JEM 2100 (JEOL, Japan) cryo-
163 microscope operating at 200 kV with a JEOL low dose system (Minimum Dose System,
164 MDS) to protect the thin ice film from any irradiation before imaging and to reduce the
165 irradiation during the image capture. The images were recorded at 93 K and digitally
166 corrected using the ImageJ software. The samples were prepared as previously described
167 (Wintgens et al., 2013).

168

169 **3. Results and discussion**

170 *3.1. Polymers*

171 Methylimidazolium substituents were grafted via ether bond to dextran. Synthesis was
172 a one-step procedure in water using epichlorohydrin and 1-methylimidazole as reactants
173 (Nichifor, Stanciu & Simionescu, 2010). The modified dextrans were characterized by ¹H
174 NMR (Figure S1) and the degree of substitution (DS) of the imidazolium moieties is given in
175 Table 1. Four of the prepared polymers with an imidazolium DS around 47% (D40Im46,
176 D70Im54, D110Im43, and D500Im44) were selected to monitor the effect of the molar mass
177 variation from 40 kg mol^{-1} to 500 kg mol^{-1} keeping the imidazolium DS almost constant. We
178 intended to compare the behavior of these polymers with those having an imidazolium DS
179 around 24% (D40Im22 and D500Im25).

180

181 **Table 1.** Characteristics of the modified dextrans

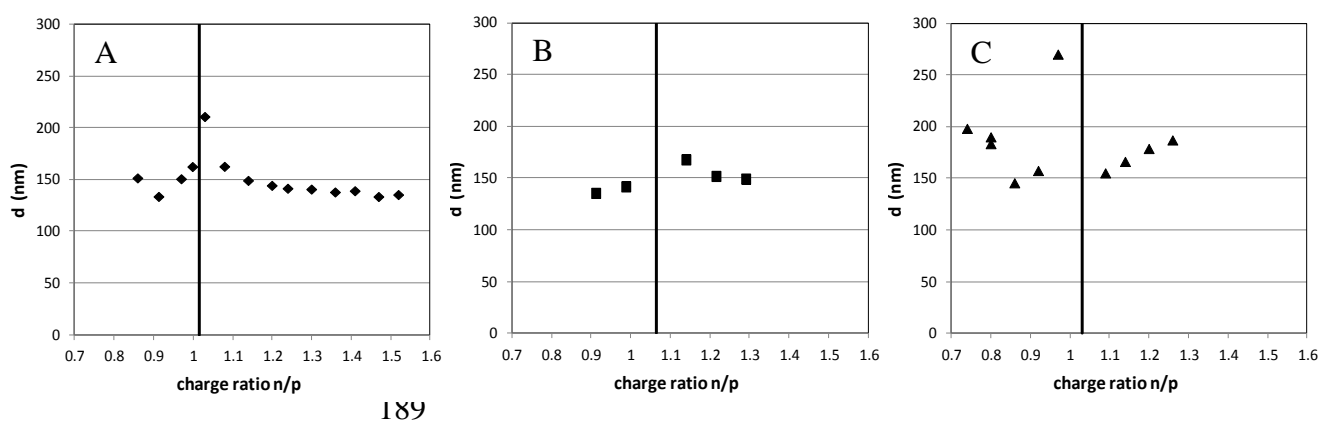
	D40Im22	D40Im46	D70Im54	D110Im43	D500Im25	D500Im44
Dextran molar mass ^a (g mol ⁻¹)	40 000	40 000	70 000	110 000	500 000	500 000
Imidazolium DS	0.22	0.46	0.54	0.43	0.25	0.44

182 ^a molar mass of the starting polymer

183

184 *3.2. Characterization of nanoparticles*

185 **Charge ratio effect.** As shown in Figure S2A, Tyndall effect was observed for different
 186 mixing ratios when SCXn was added to 1 g L⁻¹ D70Im54 solution. This indicated NPs
 187 formation. NPs size was determined by dynamic light scattering (DLS). Representative
 188 examples for correlogram and DLS distribution are given in Figure S3. Figure 1 displays the



189
 190 **Figure 1.** Particle diameter as a function of the mixing charge ratio n/p in the case of SCX4
 191 (A), SCX6 (B) and SCX8 (C) at 1 g L⁻¹ D70Im54 concentration and pH 7. On the left of the
 192 vertical lines NPs were positively charged.

193

194 alteration of NP diameters as a function of the negative/positive charge ratios (n/p) in the
 195 mixed SCXn and D70Im54 components. The data were plotted only for those NPs whose size
 196 remained almost unchanged over two hours. When the relative amount of SCXn was
 197 gradually raised keeping the polymer quantity constant, fast coalescence was found at n/p
 198 close to 1. At ratios below this coalescence point, NPs had a positive zeta potential (around +

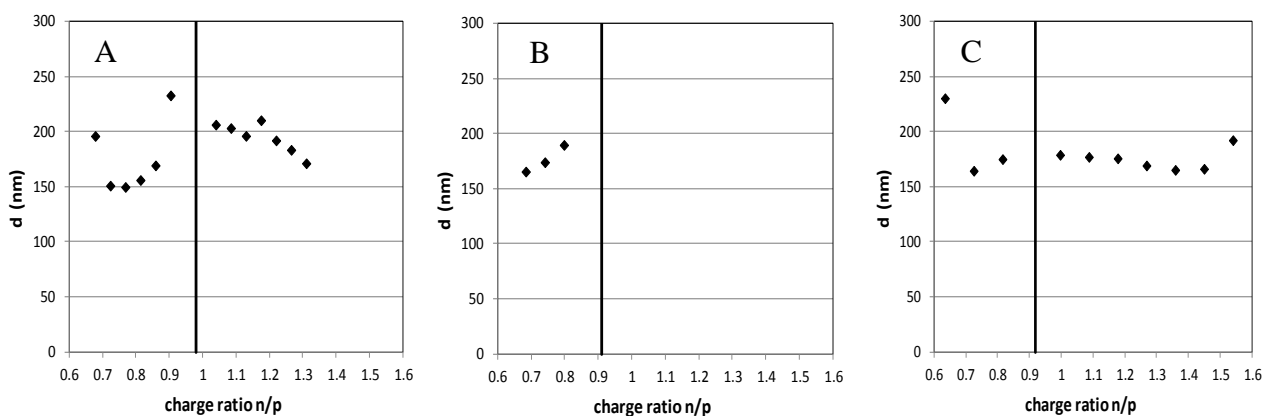
199 22 mV), and reversely, above this point, they had a negative zeta potential (around -23 mV).
200 **Outside the vicinity of the coalescence region, NPs had $\sim 150 \text{ nm} \pm 15 \text{ nm}$ average diameter.**
201 Diameter, PDI and zeta potential values are given for several NPs in Table S2. The
202 macrocycle size barely influenced the NP diameters, but altered the mixing range in which
203 NPs were produced. The largest mixing range was obtained with SCX4 and even a large
204 excess of SCX4 did not destabilize the NPs. The dextran molecular mass exerted a slight
205 effect on the NPs size (Figure S4). The increase of the dextran molecular weight did not
206 improve formation and stability of the NPs. Therefore, further studies were mainly performed
207 with D70Im54 whose highest degree of substitution facilitated the formation of stable NPs.

208 **Imidazolium DS effect.** The SCX_n-induced NP formation was sensitive to the diminution of
209 the imidazolium DS. D40Im22 constituted **negatively charged** NPs stable for at least two
210 hours only with the largest macrocycle SCX8 at a mixing charge ratio n/p of ~ 0.84 . The NPs
211 diameter was $\sim 400 \text{ nm}$, much larger than that obtained with D40Im46 ($\sim 150 \text{ nm}$). D500Im25
212 and SCX4 self-organized at mixing charge ratio 1.04 into stable negatively charged NPs
213 whose diameter was $\sim 200 \text{ nm}$ but unstable NPs emerged with SCX6. On the other hand, both
214 **positively and negatively charged** stable NPs of $\sim 170 \text{ nm}$ diameter were prepared with
215 D500Im25 and the largest SCX8 cavitand in a narrow range of mixing charge ratios.

216 NPs formation and stability depend on the number and density of links that can be
217 established between the modified dextrans and SCX_n. As the lessening of the substitution
218 level diminishes the number and density of links, NPs formation is disfavored. Increasing the
219 dextran molecular weight increases the number of links per chain, but this increase could not
220 compensate the stability loss owing to the too low imidazolium DS in the case of D500Im25.

221
222 **Effect of pH alteration.** Without any pH adjustment, D70Im54–SCX_n mixtures were acidic
223 exhibiting slightly lower pHs than the negative logarithm of the equilibrium constants of the
224 first phenolic OH deprotonation ($\text{pK}_{\text{a}1} = 3.2, 2.7$ and 3.0 for SCX4, SCX6 and SCX8,

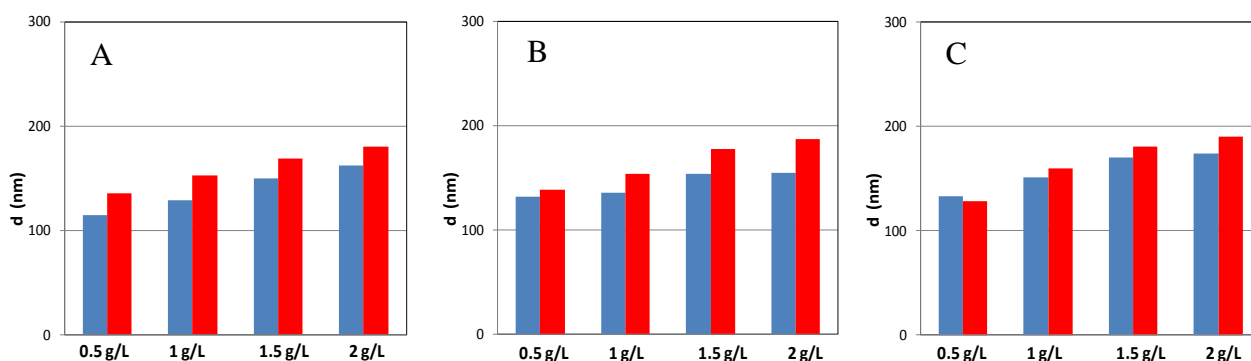
225 respectively) (Suga, Ohzono, Negishi, Deuchi & Morita, 1998). Hence, SCXn macrocycles
 226 had less negative charges than at pH 7 where not only the sulfonic acid moieties but also one
 227 (SCX4 and SCX6) or two (SCX8) phenolic OH group(s) were deprotonated. Figure 2 presents
 228 the NPs diameters as a function of mixing charge ratios n/p. The zeta potential of NPs
 229 changed from positive to negative at mixing n/p close to 1, but this corresponds to higher
 230 SCXn concentrations than those found at pH 7: for example, 0.478 mM and 0.392 mM for



231
 232 **Figure 2.** Particle diameter as a function of the mixing charge ratio n/p in the case of SCX4
 233 (A), SCX6 (B) and SCX8 (C) at 1 g L⁻¹ of D70Im54 without any pH adjustment (2 < pH < 3).
 234 On the left of the vertical line NPs were positively charged.

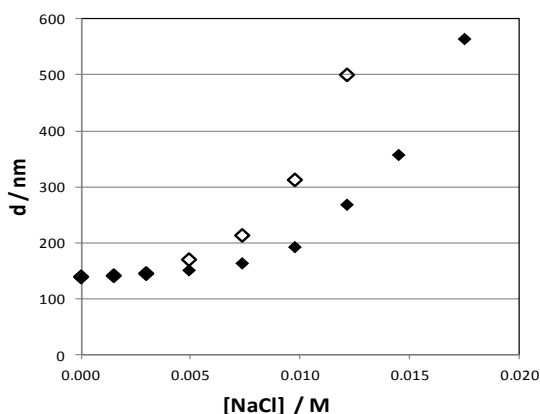
235
 236 SCX4 in water and at pH 7, respectively. This is in accordance with the lower negative charge
 237 of SCX4 in acidic solution. Additionally, the mixing charge ratio range for NP formation and
 238 the stability of the associates were reduced for SCX4 and SCX6 under acidic conditions
 239 compared to those found in neutralized solutions. For instance, negatively charged NPs could
 240 not be created with SCX6 in acidic medium. Because of the more advantageous NP formation
 241 at pH 7, further studies were performed under such conditions.

242 **Concentration effect.** The rise of the modified dextran concentration brought about slight NP
 243 growth (Figure 3) and Tyndall effect enhancement (Figure S2B) in the presence of SCXn. The
 244 diameter increase with D70Im54 concentration depended on the mixing charge ratio. The



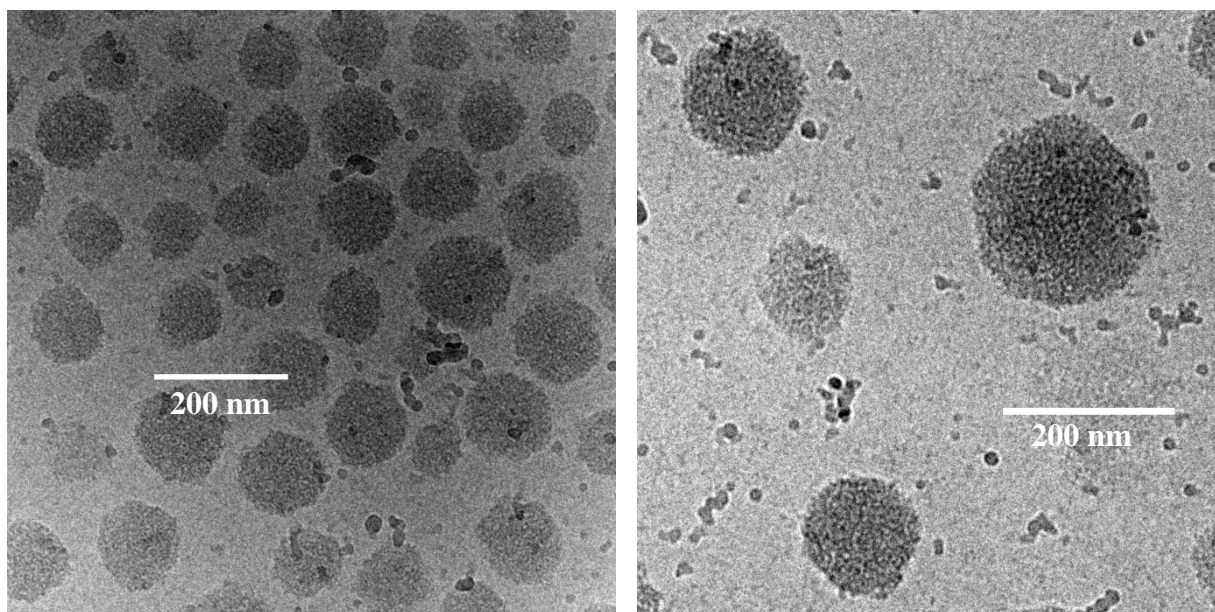
246 **Figure 3.** Particle diameter as a function of D70Im54 concentration (pH 7) with (A) SCX4 at
 247 mixing charge ratio n/p of 0.90 (■) and 1.19 (■); (B) SCX6 at mixing charge ratio n/p of 0.90
 248 (■) and 1.20 (■); (C) SCX8 at mixing charge ratio n/p of 0.91 (■) and 1.13 (■).

249
 250 closer this ratio was to that of charge compensation, the more significant diameter increase
 251 was found with polymer concentration. For example, at $[D70Im54] = 2 \text{ g L}^{-1}$, the diameter of
 252 the negatively charged SCX4-containing NPs was 230 nm and 180 nm for mixing charge ratio
 253 of 1.07 and 1.19, respectively. Moreover, close to the charge compensation, the particles were
 254 less stable at larger polymer concentration. But even in more concentrated polymer solutions,
 255 wide mixing ratio ranges were found where both **positively and negatively charged** NPs did
 256 not change within more than one day. Figure S5 shows examples for the temporal stability of
 257 NPs.



258
 259 **Figure 4.** Variation of NP diameters as a function of NaCl concentration at pH 7. NPs were
 260 prepared from 1 g L^{-1} D70Im54 with SCX4 at mixing charge ratio n/p of 0.91 (◆) and 1.20 (◇)

261 **Salt effect.** Addition of NaCl to the NPs suspensions led to NPs destabilization for NaCl
262 concentration larger than 8-10 mM (Figure 4). The charged NPs were mainly stabilized by
263 electrostatic repulsions which were screened by salt addition. Same behavior was observed for
264 other self-assembled nanoparticles made of adamantyl modified dextrans (Wintgens, Layre,
265 Hourdet & Amiel, 2012).



266
267 **Figure 5.** Cryo-TEM images of the NPs prepared from D70Im54 (2 g L⁻¹) with SCX4 at
268 mixing charge ratio n/p of 0.91 at pH 7.

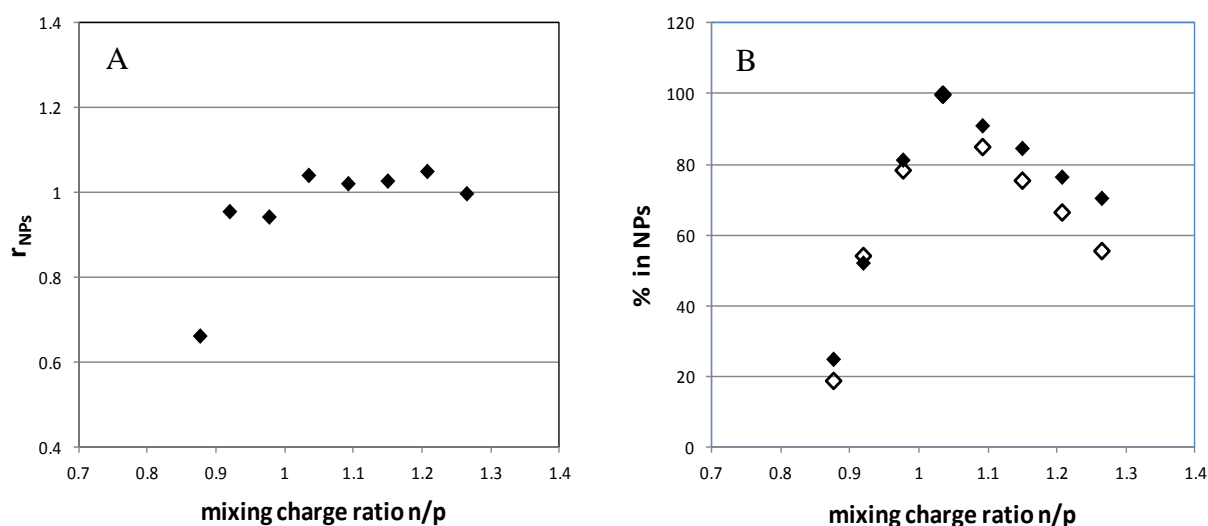
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270 3.3. Morphology and composition of the nanoparticles.

271 **Morphology.** To get information about the shape and the internal structure of NPs, cryogenic
272 transmission electron microscopy (cryo-TEM) images were recorded after mixing the
273 components at 298 K. Figure 5 shows images for positively charged NPs composed of
274 D70Im54 and SCX4 at pH 7. NPs were well-separated, spherical with a grainy appearance, or
275 with small worm-like structures. In a few areas, NPs coalesced at the edge of the amorphous
276 carbon film (Figure S6A), showing their affinity to the surface. The analysis of the cryo-TEM
277 images of 190 NPs provided an average diameter of 112 ± 25 nm in good agreement with the
278 results of DLS measurements. The negatively charged NPs formed from D70Im54 and SCX4

279 were less numerous on the grid, but had the same shape and appearance (Figure S6B). An
280 average diameter of 151 ± 55 nm was determined, also in accord with the DLS data. The NPs
281 formed from D70Im54 and SCX8 led to very similar images (Figure S7).

282
283 **Composition.** At the coalescence mixing ratio, the D70Im54–SCXn particles could be easily
284 filtered and spectrophotometric measurements of the supernatant showed that on average
285 more than 92% of SCXn was incorporated in NPs. Detailed studies were performed using
286 SCX4 to get more information about the composition and formation efficiency of NPs. The
287 suspensions obtained at various mixing ratios were ultra-centrifuged. The amount of SCX4
288 was monitored by spectrophotometry measuring the absorbance of both the SCX4 mother
289 solution and the supernatant at 284 nm. From these absorbances, SCX4 concentrations were
290 obtained in the supernatant and in the NPs (Wintgens et al., 2013). The total organic carbon
291 analysis of the supernatant provided the sum of its SCX4 and D70Im54 concentrations. On
292 the basis of this quantity and the spectrophotometrically determined SCX4 concentrations, the
293 D70Im54 concentrations in the supernatant and in the NPs could be deduced.



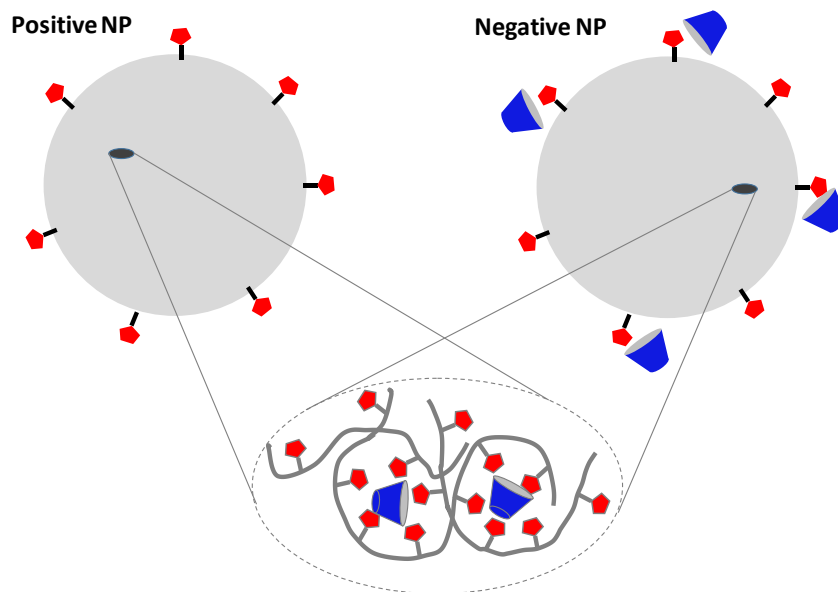
294 **Figure 6.** (A) Charge ratio within NPs (r_{NP}) and (B) percentage of SCX4 (◇) and D70Im54
295 (◆) included in NPs as a function of the mixing charge ratio n/p . $[D70Im54] = 1 \text{ g L}^{-1}$ and pH
296 = 7

297 Figure 6A displays the variation of charge ratio n/p within the NPs (r_{NP}) upon increase
298 of mixing charge ratio. The r_{NP} values were almost constant except in the case of the lowest
299 amount of SCX4. An average value of 1.005 was obtained that indicates a stoichiometry in
300 NPs close to charge compensation like in the case of NPs composed of SCXn and cationic
301 surfactants (Wintgens, Harangozó, Miskolczy, Guigner, Amiel & Biczók, 2017; Wintgens et
302 al., 2013). The fraction of the constituents incorporated in NPs substantially varied with the
303 mixing ratio reaching a maximum around charge neutralization, when almost 99% of both
304 SCX4 and dextran derivative molecules were incorporated in NPs (Figure 6B). The
305 macromolecule–SCX4 associates were able to aggregate into NPs over a large mixing range.
306 Their stoichiometry was close to the charge ratio, but their efficiency of formation attained
307 maximum when the mixing charge ratio was close to 1.

308

309 *3.4. Scheme of NPs formation*

310 Because of its polyanionic character, SCXn can compact the chains of modified
311 dextran by electrostatic interactions via the cationic moieties resulting in NP formation.
312 Hydrogen bonding among polymer chains and also with SCXn probably contributes to the
313 stability of the NPs. Dextran with their flexible backbone may reach the optimal folding to
314 accommodate around the calixarenes. Their persistence length (Rief, Fernandez & Gaub,
315 1998) of ~0.4 nm is lower than the calixarene diameters (1-2 nm). Scheme 2 rationalizes the
316 association mechanism suggesting internal structure with microdomains corresponding to
317 charge neutralization around the calixarene units. Depending on the mixing molar ratio, the
318 **positively charged** spherical nanoparticles are stabilized by methylimidazolium groups on the
319 surface, whereas the **negatively charged** ones are stabilized by an excess of SCXn. The
320 spherical nanoassemblies with a diameter around 150 nm results from the association of
321 numerous dextran chains and the worm-like appearance (as shown on cryo- TEM images)



322
 323 **Scheme 2.** Illustration of **positively and negatively charged** NPs formed between DxImy and
 324 SCX4

325
 326 could be attributed to entanglement of polymer/SCXn microdomains. The experiments have
 327 established that NP sizes mainly depend on the total concentration and the imidazolium DS,
 328 but neither the cavity size nor the polymer molecular weight influences it.

329 Other cationic macromolecules such as protamine or chitosan have been reported to
 330 self-assemble with SCXn (Harangozó, Wintgens, Miskolczy, Amiel & Biczók, 2016; Peng,
 331 Wang, Guo & Liu, 2015; Wang, Guo, Zhao & Liu, 2016). It is worth comparing the
 332 properties of these NPs with those previously found for the product of SCXn self-organization
 333 with protonated chitosan (Harangozó, Wintgens, Miskolczy, Amiel & Biczók, 2016). The
 334 morphology of the NPs differed in the case of the two polysaccharides. DxImy assembled
 335 with SCXn to spherical nano-objects, whereas chitosan produced loose structures with
 336 irregular shape and somewhat larger size. The unlike appearance of the two types of
 337 associates may originate from the less flexible backbone of chitosan compared to dextran.
 338 Persistence length values of 9-12 nm were reported depending on the deacetylation degree of
 339 chitosan (Rinaudo, 2006). The semi-rigid character of chitosan chains may impede the

340 accommodation around SCXn. Therefore, the chitosan nanoparticles were less stable and
341 were formed at much lower chitosan concentration (0.05 g L⁻¹ compared to 1-2 g L⁻¹ in this
342 work).

343

344 3.5. Drug loading in the nanoparticles

345 SCXn can encapsulate alkaloids of pharmaceutical interest (Megyesi & Biczók, 2006, 2010;
346 Yatsimirsky, 2012). Previous results demonstrated that these cavitands are capable of binding
347 as many coralyne (Cor) molecules as the number of their 4-hydroxybenzenesulfonate units
348 and the largest association efficiency is reached with SCX8 (Megyesi & Biczók, 2010).
349 Hence, we used this alkaloid as a model compound to test whether dextran derivative–SCX8
350 NPs are able to entrap drug. D70Im54 or D500Im44 was mixed with Cor–SCX8 complex
351 solutions at pH 7 keeping the polymer amount (1 g L⁻¹) constant. The total SCX8
352 concentration in the initial mixture ($[SCX8]_T$) was varied to change the mixing charge ratio
353 n/p and to produce both **positively and negatively charged** NPs. (The positive charge of Cor
354 was taken into account for n/p determination). The molar ratio of the total concentrations in
355 the initial solutions ($[Cor]_T/[SCX8]_T$) was around 0.5. Although Cor and SCX8 can form
356 complexes with stoichiometry as high as 8 NPs were destabilized by Cor confinement and
357 large aggregates were produced when $[Cor]_T/[SCX8]_T$ exceeded 1. To determine the fraction
358 of Cor embedded in NPs, the suspensions were ultracentrifuged and the concentration of
359 alkaloid in the supernatant ($[Cor]_S$) was determined by spectrophotometric measurements
360 monitoring its absorbance at 423 nm. The association efficiency (AE) and the loading capacity
361 (LC) of NPs were calculated as follows:

$$362 \quad AE = \frac{[Cor]_T - [Cor]_S}{[Cor]_T} \times 100 \quad (1)$$

$$363 \quad LC = \frac{[Cor]_T - [Cor]_S}{[SCX8]_T + [Dext]_T} \times 100 \quad (2)$$

364 where $[Cor]_T$, $[SCX8]_T$, and $[Dext]_T$ represent the total concentrations of Cor, SCX8, and
 365 dextran derivatives, respectively. Table 2 summarizes the variation of the NP characteristics
 366 with the concentrations of the constituents in the starting mixtures at pH 7. Efficient Cor
 367 confinement was achieved, which insignificantly changed the size and stability of the
 368 prepared NPs at $[Cor]_T/[SCX8]_T \approx 0.5$. The AE and LC values were similar for both
 369 **positively and negatively charged** aggregates alike suggesting that alkaloid binding occurs not
 370 on the surface but inside the NPs. The affinity of Cor was slightly higher to the particles
 371 comprising D70Im54 instead of D500Im44. For the former dextran derivative, almost
 372 quantitative Cor encapsulation could be reached.

373

374 **Table 2.** SCX8 concentration, mixing charge ratio n/p and $[Cor]_T/[SCX8]_T$ molar ratio used
 375 for the preparation of NPs at 1 g L⁻¹ modified dextran concentration and pH 7. Association
 376 efficiency (AE), loading capacity (LC), zeta potential (ζ), mean diameter (d), and
 377 polydispersity index (PDI) of the NPs

	$[SCX8]_T$ (mM)	Mixing charge ratio n/p	Molar ratio $[Cor]_T/[SCX8]_T^a$	AE (%)	LC (weight %)	ζ (mV)	d (nm)	PDI
D70Im54	0.178	0.88	0.51	92.6	2.5	^b	174	0.10
	0.188	0.93	0.50	97.2	2.7	+17.0	210	0.11
	0.199	0.98	0.50	99.1	2.9	-16.1	189	0.06
	0.210	1.03	0.49	96.3	2.9	^b	159	0.16
D500Im44	0.144	0.82	0.46	88.4	1.9	^b	180	0.08
	0.155	0.88	0.47	93.1	2.1	+19.1	177	0.11
	0.177	1.01	0.47	95.8	2.4	-21.5	162	0.14
	0.188	1.06	0.48	84.9	2.3	^b	160	0.17

378 ^a molar ratio of the total Cor and SCX8 concentrations in the initial mixture, ^b not determined

379 The presence of SCXn allows the encapsulation of alkaloids such as coralyne in the
380 NPs. Our previous study with chitosan showed that it was possible to load as much as 4.5 Cor
381 per SCX8. In this work, the maximum loading is 0.5 Cor per SCX8 was reached. NPs were
382 destabilized in the presence of more Cor. The association between SCX8 and the modified
383 dextran is less cooperative when the negative charges of SCX8 are partly neutralized by Cor.
384 Despite the lower LC in DxImy–SCX8 NPs than in chitosan–SCX8 NPs, the total amount of
385 encapsulated Cor is at least twice **as high** in the former case because larger NP concentration
386 can be attained due to the more substantial solubility of DxImy. Another advantage of
387 methylimidazolium-conjugated dextrans is that they readily produce NPs with SCX8 even at
388 pH 7 where the application of chitosan is hindered by its low solubility. It is beneficial that
389 narrower NP size distribution can be obtained when DxImy polymer is applied instead of
390 protonated chitosan and both positively and negatively charged NPs can incorporate drugs.

391

392 **4. Conclusions**

393 SCXn polyanionic macrocycles not only induce self-assembly with
394 methylimidazolium-conjugated dextrans into NPs by supramolecular crosslinking of the
395 polymer chains but also can serve as molecular containers within NPs to encapsulate the
396 biomedically important coralyne alkaloid. Despite its cationic character, similar amount of
397 coralyne can be confined in both positively and negatively charged NPs because of the large
398 binding affinity of the alkaloid to the SCXn constituents. The hydrophilic and flexible
399 backbone of dextran polycations is advantageous for the association with SCXn into stable
400 spherical aggregates. The molecular mass of DxImy polymers and the size of the SCXn
401 constituent barely influence the properties of the self-organized NPs, but the low degree of
402 substitution with methylimidazolium moieties on the dextran backbone exerts unfavorable
403 effect.

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407

408 **Appendix A. Supplementary data**

409 Supplementary material related to this article can be found, in the online version, at
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411

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