



HAL
open science

Chitosan Hydrogels Incorporating Colloids For Sustained Drug Delivery

Soline Peers, Alexandra Montembault, Catherine Ladaviere

► **To cite this version:**

Soline Peers, Alexandra Montembault, Catherine Ladaviere. Chitosan Hydrogels Incorporating Colloids For Sustained Drug Delivery. Carbohydrate Polymers, 2022, 275, pp.118689. 10.1016/j.carbpol.2021.118689 . hal-03362316

HAL Id: hal-03362316

<https://hal.science/hal-03362316v1>

Submitted on 1 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Chitosan Hydrogels Incorporating Colloids For Sustained Drug Delivery

S. Peers, A. Montembault*, C. Ladavière*

University of Lyon, CNRS, Claude Bernard Lyon 1 University, INSA, Ingénierie des
Matériaux Polymères, IMP UMR 5223, 15 bd A. Latarjet, F-69622, Villeurbanne, France

* Corresponding authors: alexandra.clayser-montembault@univ-lyon1.fr (A. Montembault),
catherine.ladaviere@univ-lyon1.fr (C. Ladavière)

Abstract

In today's biomedical research, a huge effort is being made towards the development of efficient drug delivery systems, achieving sustainable and controlled delivery of drugs. Chitosan (CS) hydrogels are high water content materials with very relevant biological properties to that purpose. Their use for a local and delayed delivery has already been demonstrated for a wide variety of therapeutic agents. One relatively recent strategy to improve these CS-based systems consists in the insertion of colloids, embedding drugs, within their three-dimensional matrix. This provides a second barrier to the diffusion of drugs through the system, and allows to better control their release. The main objective of this review is to report the many existing complex systems composed of CS hydrogels embedding different types of colloids used as drug delivery devices to delay the release of drugs. The various biomedical applications of such final systems are also detailed in this review.

Keywords

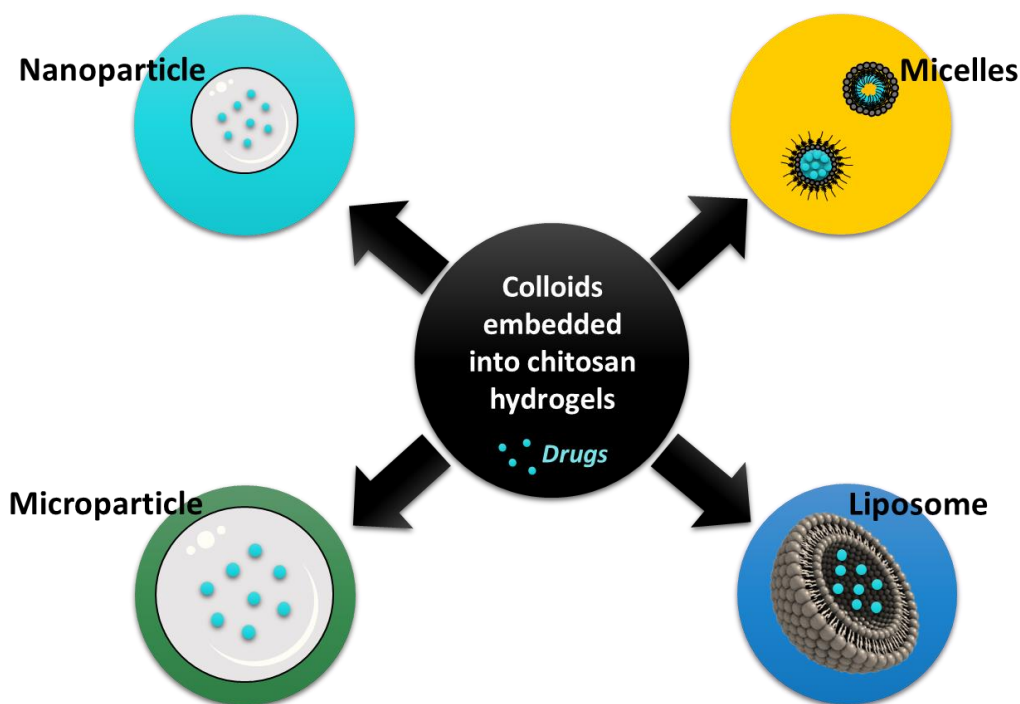
Chitosan; hydrogels; colloids; drug delivery systems

Introduction

32 Chitosan (CS) physical and chemical hydrogels are known to be very efficient and useful
33 systems, not only to delay the release of hydrophilic, hydrophobic molecular or even
34 macromolecular drugs (Bhattarai et al., 2010; Deen & Loh, 2018; Elgadir et al., 2015; Giri et
35 al., 2012; Peers et al., 2020; Ribeiro et al., 2017; Shariatinia & Jalali, 2018), but also to better
36 target the treatment site, with *in situ* injections for example (Ta et al., 2008). They can also be
37 used for specific biomedical applications as wound healing (Hamedi et al., 2018; H. Liu et al.,
38 2018; L. Liu et al., 2016), gastrointestinal (Hejazi & Amiji, 2003), ophthalmic (Wang & Han,
39 2017) treatments or to control angiogenesis (Ishihara et al., 2006). In the literature, three
40 different mechanisms are described to explain the release of drugs from CS hydrogels: i)
41 diffusion-controlled, ii) swelling-controlled, and iii) chemically-controlled release (Azevedo,
42 2015). However, even if CS hydrogels act as diffusion barriers for the entrapped drugs, several
43 teams have already demonstrated that such systems undergo a non-negligible burst-effect. This
44 phenomenon corresponds to the fast and uncontrolled delivery of a high amount of the
45 entrapped drugs immediately upon administration (X. Huang & Brazel, 2001).

46 Even if in some cases the burst release might be desirable (*e.g.*, for encapsulated flavours or
47 local targeted delivery), it is often considered as a detrimental consequence in most of drug
48 delivery applications. Consequently, an intensive research is made to reduce this burst effect
49 by designing more efficient drug delivery systems. One strategy is the incorporation of drugs
50 into colloids that are themselves embedded into the CS hydrogel.

51 The main objective of this review is to report the studies described in the literature about
52 “CS hydrogel–colloids” assemblies, specifically valorized as delivery devices of drugs. The
53 colloids considered in this review are microparticles, nanoparticles, liposomes or even micelles
54 (**Figure 1**). Their biomedical applications are also detailed herein. Note that drug non-
55 proprietary names are employed throughout the review. Hereinafter, the word “drug” refers to
56 active molecules of therapeutic interest, or model molecules (used for proof of concept).
57 Furthermore, it was chosen not to detail cyclodextrin-based systems (Kono & Teshirogi, 2015;
58 Maestrelli et al., 2006; Zhou et al., 2016) and/or nanoemulsions (Moradi et al., 2019; dos Santos
59 et al., 2020; Demisli et al., 2020) in this review.



60

61 **Figure 1.** Schematic representation of different colloids incorporated into CS hydrogels to
 62 control and/or delay the release of drugs, described in the literature.

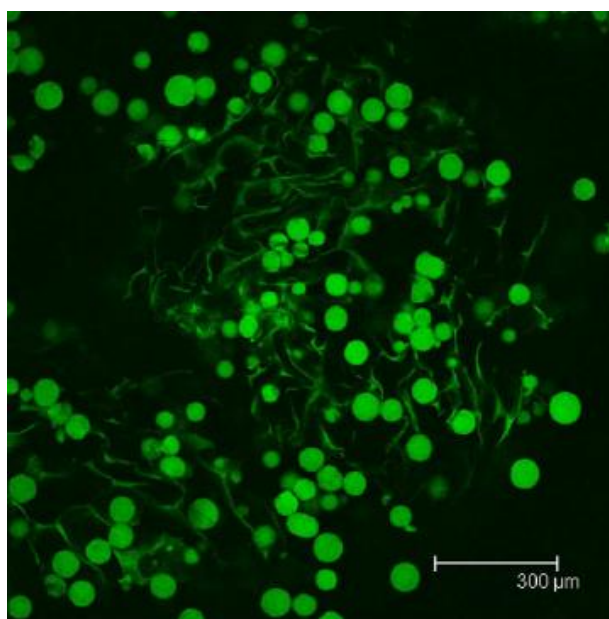
63

64 **1. « Chitosan hydrogel/microparticles » assemblies**

65 The incorporation of drug-loaded microparticles into the hydrogel matrix provides a second
 66 barrier to drugs' diffusion. To this end, drugs have to be firstly incorporated into the
 67 microparticles, and then into the hydrogel network formed by entangled polymer chains. In
 68 most of the cases, the insertion of polymer microparticles in CS hydrogels limits or even
 69 eliminates the burst release phenomenon, that is to say the sudden fast and uncontrolled delivery
 70 of a high amount of drugs entrapped in CS hydrogels in the first minutes/hours after
 71 administration. Burst release is observed with “traditional” hydrogels and is often related to
 72 adsorbed drugs on the hydrogel surface, that are released faster than drug molecules entrapped
 73 within the hydrogel matrix (Pitorre et al., 2017). Several experimental methods can be used for
 74 the elaboration of polymer microparticles (Li et al., 2017). In the literature, one can find phase
 75 separation (*e.g.*, (Zan et al., 2006)), spray-drying (*e.g.*, (Legrand et al., 2007) (Yang et al.,
 76 2017)) or a two-phases nano-emulsion elaboration (mostly « oil-in-water », but also « water-
 77 in-oil », *e.g.*, (Moebus et al., 2009) (Qi et al., 2016) (Dehghan-Baniani et al., 2017)). More than
 78 two phases can also be prepared to create a multi-emulsion (*e.g.*, (Legrand et al., 2007; Zhu et
 79 al., 2018)). Depending on the method employed for the microparticle elaboration, different
 80 sizes, drug incorporation rates, and morphologies of microparticles can be obtained. For

81 example, it has been demonstrated that the incorporation of an antibiotic, the vancomycin
82 (VCM) into (hydroxypropyl)methyl cellulose (HPMC) microparticles lead to more spherical
83 morphologies than microparticles without VCM (Mahmoudian & Ganji, 2017). In most cases,
84 microparticles are embedded into hydrogel before its gelation process by mixing the
85 microparticle suspension and the CS solution. This method is actually mostly used with
86 thermosensitive hydrogels for which the microparticle suspensions are pre-formed with control
87 of the microparticle size and dispersion, and then mixed with CS solution. Some studies thus
88 have revealed that the incorporation of microparticles into thermosensitive hydrogels decreased
89 the gelation time (Dehghan-Baniani et al., 2017; Mahmoudian & Ganji, 2017; Qi et al., 2016;
90 Yang et al., 2017; Zan et al., 2006). In these types of systems, microparticles act as crosslinking
91 nodes and facilitate the gelation process. Such « CS hydrogel/microparticles » assemblies were
92 valorized in different biomedical applications with various administration routes (oral, ocular
93 or direct injection on the injury site).

94 A faster ulcer healing on mice dorsal skin after the application of a CS scaffold in which
95 gelatin microparticles (< 100 μm , elaborated by water-in-oil emulsion) were embedded has also
96 been shown (Park et al., 2009). A growth factor (b-FGF) was firstly incorporated into gelatin
97 microparticles, and the final assembly was observed by confocal microscopy (**Figure 2**). The
98 autofluorescent properties of gelatin and chitosan were used to capture the confocal images. No
99 burst release phenomenon was observed with this assembly, allowing a sustained release of the
100 b-FGF growth factor *in vivo* for 10 days.



101
102 **Figure 2.** Confocal micrograph of gelatin microparticles in chitosan scaffold (Park et al.,
103 2009)

104

105 Such « CS hydrogel/microparticles » assemblies can thus support tissue regeneration. They
106 can also be used for the sustained and delayed release of drugs incorporated into microparticles.
107 A transforming growth factor (TGF), or a model protein (bovine serum albumin, BSA), were
108 embedded into CS microspheres (0,2-15 μm), then were incorporated into a CS scaffold for the
109 chondrocyte proliferation enhancement and the cartilage regeneration (Kim et al., 2003).
110 Protein release profiles from these CS microspheres (diffusion process) have been presented,
111 but no information was given about the protein release from the final assemblies. This work has
112 interestingly shown a sustained release of TGF with its incorporation into CS microspheres,
113 inducing a high chondrocyte proliferation within the scaffold. This trend has been attributed to
114 the ability of TGF to promote protein synthesis and chondrocyte cellular proliferation.

115 For articular inflammation treatment, other microparticles composed of poly(lactic-co-
116 glycolic acid) (PLGA, microparticles with diameters from 5 to 120 μm) have also been
117 incorporated into CS hydrogels (Joung et al., 2007; Zhu et al., 2018). The release of an anti-
118 inflammatory drug, lornoxicam, from PLGA microspheres, embedded (before the CS gelation
119 process) into an *in situ* gelling CS formulation leading to a chemical hydrogel was studied (Zhu
120 et al., 2018). Only 20% of the initial incorporated drugs have been released in one hour (*versus*
121 30% when the drugs were incorporated into microspheres) with a sustained release of drugs
122 during more than 13 days. In this study, no comparison has been done with the release of
123 lornoxicam directly incorporated into CS hydrogels.

124 *In vivo* drug activity was also studied in mouse after intra-articular injections of particles
125 entrapped in a CS/glycerophosphate (GP) solution (Zhu et al., 2018). Gelation of this CS/GP
126 solution was achieved within 5 minutes after the injection, and that the CS/GP hydrogel formed
127 was maintained at the injection site. With such a « CS/GP hydrogel/microparticles » assembly,
128 the lornoxicam concentration into the blood was lower than in the case of a direct injection of
129 a lornoxicam solution in the delivery site, which was a proof of a sustained delivery of this
130 drug.

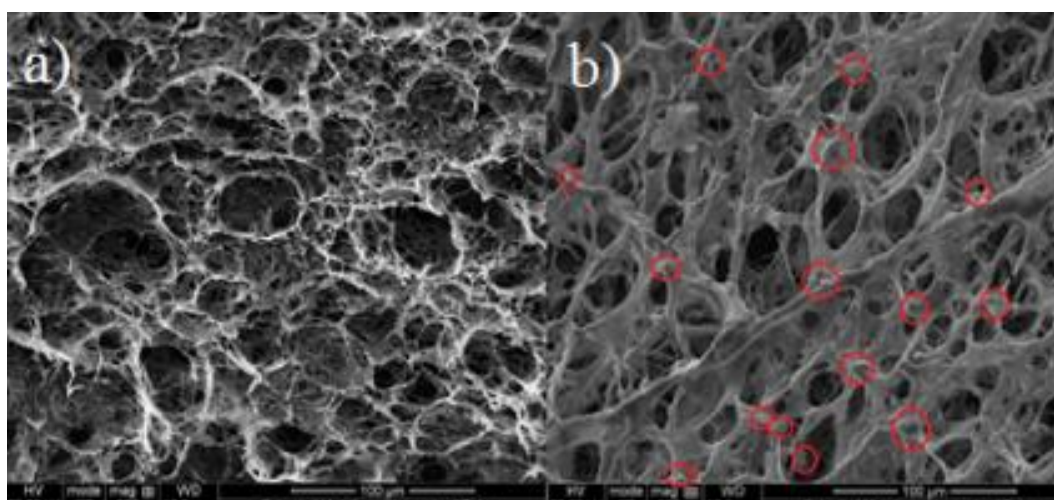
131 Alginate microparticles, with diameters from 1 to 25 μm , loaded with diclofenac have also
132 been elaborated before the CS gelation process (Qi et al., 2016). These microparticles were
133 embedded into thermosensitive CS/GP physical hydrogels for the treatment of articular
134 inflammations (arthrosis). In this study, the drug was progressively released for 5 days *in vitro*.
135 A slowdown in the inflammation propagation was observed *in vivo*, but the cartilage healing

136 was only partial, even after 3 weeks of treatment. However, this treatment is efficient for
137 reducing intra-articular injections, with a minimal immune response.

138 Porous PLGA microparticles, with diameters ranging from 20 to 120 μm and incorporating
139 vascular endothelial growth factor (VEGF), were embedded (before the CS gelation process)
140 into CS/alginate chemical hydrogels associated to an antibiotic, VCM, linked to the CS
141 hydrogel via Schiff's base reaction (J. Huang et al., 2018). This assembly was efficient for the
142 delayed release of VCM, and more particularly for wound healing applications. As a first step,
143 the pores of PLGA microparticles were temporarily "closed" by CS/alginate solution, which
144 drastically slows the release of the VEGF. The release of VCM was here governed by the
145 environmental pH. The VCM and VEGF release profiles could allow not only the treatment of
146 infections *via* the fast release of an antibiotic, but also the healing of wounds by means of the
147 sustained delivery of VEGF, enhancing vascularization and tissue regeneration.

148 The incorporation of PLGA microparticles (before hydrogel gelation) into
149 CS/polyvinylpyrrolidone hydrogels to control the delivery of an anti-inflammatory drug,
150 dexamethasone has also been studied (Saeedi Garakani et al., 2020). The use of a "hybrid"
151 assembly considerably delays the release of the entrapped drug with 80% of the drug released
152 after 30 days, while in the microparticles, the release was completely achieved after 22 days.
153 When the drug was incorporated alone within the hydrogel, it was fully released in few hours,
154 demonstrating the efficiency and interest of this system for wound healing applications.
155 Different parameters of great interest have been studied such as mechanical properties, porosity,
156 swelling ratio, biodegradability or antibacterial activity. For example, they observed an increase
157 in the mechanical properties of the assembly after microparticles addition, attributed to a stiffer
158 structure of the embedded particles compared to the hydrogel. Another interesting point is that
159 an increase in PLGA microparticles content improved not only the mechanical properties, but
160 also the cell viability and antibacterial activity of the systems.

161 Starch microparticles (1.7-2.5 μm) have also been incorporated into thermosensitive
162 CS/monoammonium phosphate physical hydrogel, with the aim of creating a mime of cartilage
163 (Dehghan-Baniani et al., 2017). Starch was selected for its biocompatibility, biodegradability,
164 non-toxicity and low price. These microparticles acted as new crosslinking points (**Figure 3**)
165 by creating interactions between starch and CS chains that enhanced mechanical properties of
166 the assembly (compressive elastic modulus around 28 kPa, *versus* 2 kPa for pure chitosan).
167 Such an assembly could thus be used for the delayed release of drugs, although no study has
168 been done by this team of co-workers.



170
 171 **Figure 3.** SEM images of a) a CS hydrogel with a porous structure, and b) a CS hydrogel
 172 incorporating starch microparticles (0,02g/10 mL CS solution, red circles were added by the
 173 authors to display them), magnification x500 (Dehghan-Baniani et al., 2017)

174
 175 Similarly, VCM was also incorporated into HPMC microparticles (about 5 μm) for the
 176 treatment of osteomyelitis (Mahmoudian & Ganji, 2017). Its release was examined over 12 h.
 177 When VCM was incorporated into a CS chemical hydrogel, the VCM release was delayed over
 178 more than 3 days in comparison with free VCM and VCM-loaded microparticles. Finally, 100%
 179 of incorporated VCM was released (thanks to the double diffusion barrier) over 160 h when it
 180 was firstly incorporated into HPMC microparticles, themselves embedded into CS chemical
 181 hydrogel. Note that the presence of HPMC microparticles inside the CS hydrogel matrix seemed
 182 to decrease the gelation time (8 min for the « CS hydrogel/microparticles » assembly *versus* 13
 183 min for the CS hydrogel without HPMC microparticles).

184 « CS hydrogel/microparticles » assemblies could also be used for the controlled delivery of
 185 anticancer drugs. Indeed, the incorporation of 5-FU into poly-3-hydroxybutyrate (PHB)
 186 microparticles with diameters from 20 to 35 μm , embedded into a thermosensitive CS hydrogel,
 187 has also been studied (Zan et al., 2006). The embedment of PHB microparticles into a CS/GP
 188 hydrogel decreased the rapid initial release from 85% to 29% for the optimized PHB content in
 189 the first 48h. Consequently, the 5-FU release was very interestingly sustained over 10 months
 190 from this « CS hydrogel/PHB microparticles » assembly.

191 The same drug 5-FU has been also embedded into gelatin microsphere, then incorporated
 192 (before gelation process) in chitosan-alginate hydrogels (Chen et al., 2019). The novelty of this
 193 works resides in the incorporation of magnetic nanoparticles inside the microspheres, allowing

194 a tunable release of the entrapped drug under magnetic field. In the early hours, no significant
195 difference was observed between assembly with or without the magnetic field application. After
196 five days, cumulative release rate of 5-Fu showed a significant increase under the external
197 magnetic field, and the final release reached 75% under magnetic fields, against only 55% for
198 the assembly without magnetic field application. A shorter gelation time has been observed,
199 lower swelling ratio and slower degradation process *in vitro* for “hybrid” assemblies compared
200 to the controls.

201 Microparticle insertion into a CS hydrogel could also enhance the bioadhesive properties of
202 CS hydrogel (Yang et al., 2017). They elaborated a thermosensitive CS/GP physical hydrogel
203 incorporating microparticles with diameters between 1.5 and 4.3 μm (made of CS and a
204 polysaccharide extracted from *Bletilla Striata* plant) loading Tenofivir (TFV). This drug
205 partially prevents HIV transmission to women. These TFV-loaded microparticles were
206 incorporated before the CS gelation process. According to their results, the mucoadhesion on
207 vaginal mucosa followed this order: TFV incorporated in microparticles themselves inserted in
208 the CS hydrogel > TFV incorporated in the CS hydrogel » > TFV in the microspheres > only
209 TFV in solution. Nevertheless, note that no information about mucosa/CS hydrogel or
210 mucosa/microparticle interactions was provided. Interestingly, they demonstrated that the burst
211 release phenomenon was strongly limited with this « CS hydrogel/microparticles » assembly:
212 only 13%, 21%, and 32% of TFV released over 15, 30 et 60 min respectively from this
213 assembly, when more than 30% were released over only 30 min from TFV-loaded
214 microparticles (without their incorporation in the CS hydrogel).

215 To conclude this part and in a more exhaustive way, the goal of Table 1 is to report works
216 about the controlled delivery of drugs from these « CS hydrogel/microparticles » assemblies
217 that can be found in the literature, to the best of our knowledge.

218
219
220
221
222
223
224
225

226 **Table 1.** Nature and diameters of microparticles, nature of drugs inserted in microparticles of
 227 « CS hydrogel/microparticles » assemblies for the controlled delivery of drugs agents used in
 228 different biomedical applications

Microparticle nature	Microparticle diameter range (µm)	Drugs	Biomedical application	Reference
Chitosan	0,2-1,5	TGF ¹ (growth factor) BSA ² (model protein)	Tissue engineering	(Kim et al., 2003)
	29-40	BSA		(Fan et al., 2017)
Alginate	1-25	Diclofenac (anti-inflammatory drug)	Articular treatment	(Qi et al., 2016)
	2-10	BSA	Tissue engineering	(Xing et al., 2019)
Starch	1,7-4,5	Unspecified	Tissue engineering	(Dehghan-Baniani et al., 2017)
Poly(lactic-co-glycolic acid)	5-75	Indometacin (anti-inflammatory drug)	Unspecified	(Joung et al., 2007)
	6-7	Lornoxicam (anti-inflammatory drug)	Articular treatment	(Zhu et al., 2018)
	20-120	VEGF ³ (growth factor)	Wound healing	(J. Huang et al., 2018)

Hydroxypropyl methylcellulose	1,5-6,4	VCM ⁴ (antibiotic)	Osteomyelitis treatment	(Mahmoudian & Ganji, 2017)
poly hydroxybutarate	20-35	5-FU ⁵ (anticancer drug)	Adenocarcinoma	(Zan et al., 2006)
CS-bletilla striata polysaccharide	1,5-4,3	Tenofovir (antiretroviral)	HIV transmission prevention	(Yang et al., 2017)
Gelatin	40-90	b-FGF ⁶ (growth factor)	Ulcer treatment	(Park et al., 2009)
	0.6	5-FU	Cancer treatment and tissue regeneration	(Chen et al., 2019)
PLGA	1,9-2,9	Dexamethasone	Wound healing	(Saeedi Garakani et al., 2020)

229 ¹ TGF = transforming growth factor
230 ² BSA = bovine serum albumin
231 ³ VEGF = vascular endothelial growth factor
232 ⁴ VCM = vancomycin
233 ⁵ 5-FU = 5-fluorouracil
234 ⁶ b-FGF = basic fibroblast growth factor

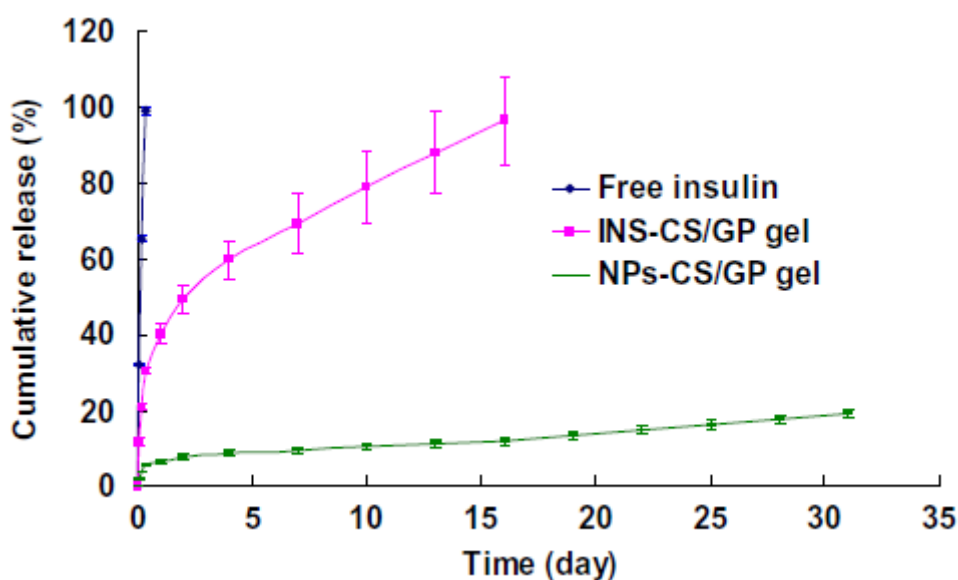
235

236 2. « Chitosan hydrogel/nanoparticles » assemblies

237 Drugs of different nature can also be incorporated in nanoparticles. Nanoparticles could also
238 be incorporated in CS hydrogels, providing a second barrier to the diffusion of drugs, and
239 delaying their delivery (Morantes et al., 2017). As seen before for polymer microparticles,
240 different methods can be employed for the elaboration of polymer nanoparticles (Li et al.,
241 2017). For example, emulsion (mostly water-in-oil) (Peng et al., 2013; Dehghan-Baniani et al.,
242 2017), ionic gelation (Bugnicourt et al., 2014; Li Hui et al., 2016), nanoprecipitation (Legrand
243 et al., 2007) or emulsion/gelation (Qi et al., 2016) processes could be used for such a
244 preparation.

245

246 Another biomedical application of these « CS hydrogel/nanoparticles » assemblies is the
247 regulation of glycaemia with insulin incorporation (which commonly implies daily injections
248 for diabetic patients). A thermosensitive CS/GP physical hydrogel for the controlled delivery
249 of insulin, incorporating poly-3-hydroxybutarate-co-hydroxyhexanoate (PBHBHH)
250 nanoparticles (incorporated before the CS gelation process) has also been elaborated (Peng et
251 al., 2013). *In vitro*, 95% of initially incorporated insulin were released over 16 days when they
252 were incorporated into CS/GP hydrogel. On the contrary, only less than 20% was released when
253 the nanoparticles are firstly incorporated into CS hydrogels (**Figure 4**). Hypoglycaemic activity
254 was also measured *in vivo*, showing an important decrease of blood glucose levels, and thus a
255 better insulin bioavailability.



256
257 **Figure 4.** Cumulative release profiles (% of initially incorporated drug) of free insulin (blue
258 diamonds), insulin into « CS/GP hydrogels » (pink squares), or insulin in « CS/GP
259 hydrogel/insulin loaded-PBHBHH nanoparticles » assemblies (green hyphens) (Peng et al.,
260 2013)

261
262 Another way to delay the release of hydrophobic drugs consists in the elaboration of a colloid
263 suspension surrounded by one or more polymer layer(s) with a final diameter of nanocolloids
264 around 500 nm. This method was for example used for the controlled release of an anticancer
265 drug, camptothecin inserted in nanocolloids, themselves covered by a trimethyl CS layer (Li
266 XingYi et al., 2010). This colloid suspension was then incorporated in a thermosensitive
267 CS/dibasic sodium phosphate physical hydrogel (before the CS gelation process). The drug
268 release and cytotoxicity related to the antitumor activity against specific breast cancer cells

269 were studied *in vitro*. After proving the *in vivo* biodegradability of these assemblies, the *in vitro*
 270 delayed release of camptothecin in PBS at pH 7.4 (diffusion throughout the assembly) has been
 271 studied. 30% of the initially incorporated drug were released over 3 days, 70% over 18 days.
 272 This assembly seemed appropriate not only for protection of drug (as nearly 90% of the
 273 camptothecin were preserved in the lactone form), but also to delay its release *in situ*, while
 274 maintaining its antitumor activity. To conclude this part and in a more exhaustive way, the goal
 275 of Table 2 is to report studies about the controlled delivery of drugs from « CS
 276 hydrogel/nanoparticles » assemblies that can be found in the literature, to the best of our
 277 knowledge.

278

279 **Table 2.** Nature and diameters of nanoparticles, nature of drugs inserted in nanoparticles of «
 280 CS hydrogel/nanoparticles » assemblies for the controlled delivery of drugs used in different
 281 biomedical applications

Nanoparticle nature	Nanoparticle diameter range (nm)	Drugs	Biomedical application	Ref.
Poly-3-hydroxybutyrate-co-hydroxyhexanoate	UNS ¹	Insulin (Peptide hormone)	Glycaemia regulation	(Peng et al., 2013)
Chitosan	500-900	DNA-BMP (DNA plasmid)	Tissue engineering	(Li Hui et al., 2016)
Chitosan	266	FGF ² (growth factor)	Tissue engineering	(Azizian et al., 2018)
Trimethyl CS	500	Camptothecin (anticancer drug)	Ovary cancer treatment	(Li XingYi et al., 2010)

282 ¹ UNS = unspecified

283 ² FGF = fibroblast growth factor

284

285 3. « Chitosan hydrogel/liposomes » assemblies

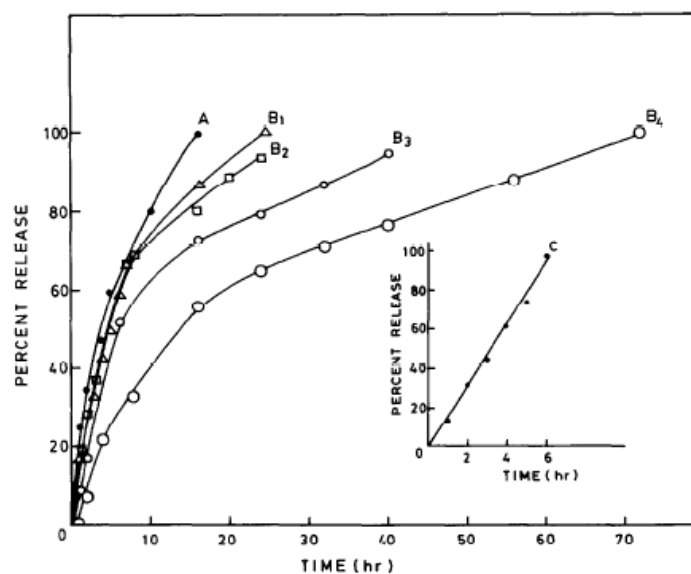
286 Since the early 1990's, several research teams worked on liposomes, used as drug
287 « reservoirs », embedded in CS hydrogels. Indeed, the lipid membrane acts as a supplementary
288 barrier against the drug diffusion throughout the CS hydrogel, while limiting the toxicity of
289 incorporated drugs. This membrane also ensures a better drug stability against surrounding
290 environment. Nevertheless, a review in 2019 entitled “Liposomes-in-Chitosan Hydrogels:
291 Challenges and Opportunities for Biomedical Applications” only mentions about twenty
292 international articles on such assemblies. The combination of two systems (liposomes and CS
293 hydrogels), both of which have the ability to control the delivery of incorporated drugs, was
294 studied with different drugs depending on the targeted biomedical application (Grijalvo et al.,
295 2019).

296

297 *Incorporation of hydrophilic drugs in liposomes*

298 Preliminary studies on « CS hydrogel/liposomes » assemblies were done for the controlled
299 delivery of a water-soluble model molecule, the bromothymol blue (BTB), incorporated into
300 the aqueous cavity of zwitterionic liposomes (phosphatidylcholine, PC). CS hydrogel was
301 chemically crosslinked with the use of ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC)
302 (Rao & Alamelu, 1992). It has been shown that the release of BTB was delayed, with 100% of
303 the initially incorporated BTB into liposomes released over 6 h, *versus* 15-20 h in the case of
304 liposomes incorporated into CS hydrogels. The higher the EDAC crosslinking agent
305 concentration, the slower the release (25h for 5 mg of added EDAC against more than 70h for
306 30 mg, **Figure 5**).

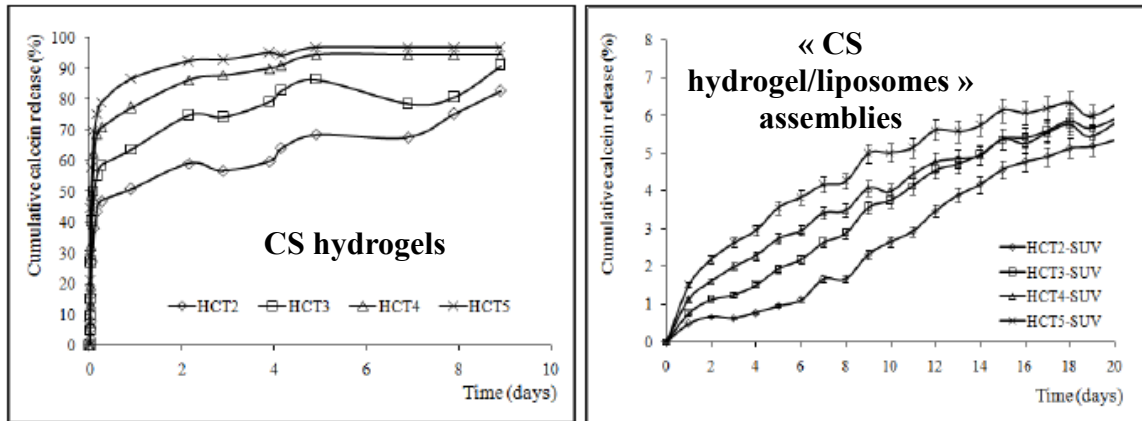
307



308
 309 **Figure 5.** Influence of the EDAC crosslinking agent concentration on the cumulated release
 310 (% of total initially incorporated drug) of BTB incorporated into PC liposomes embedded in a
 311 CS hydrogel (A) without EDAC, (B1) with 5 mg EDAC, (B2) 10 mg EDAC, (B3) 20 mg
 312 EDAC, (B4) 30 mg EDAC. These release profiles were performed in a phosphate buffer at
 313 pH = 7.4 The C curve is representative of the release of BTB from free liposomes (Rao &
 314 Alamelu, 1992)

315
 316 More recently, a sustained release of calcein over more than 20 days when this water-soluble
 317 molecule was incorporated into Phospholipon®-90G liposomes, themselves embedded in
 318 CS/gelatin chemical hydrogels. Hydrogels are crosslinked with two different crosslinking
 319 agents: glutaraldehyde and sodium sulphate or sodium triphosphate (Ciobanu et al., 2014). In
 320 this work, a significant burst effect when calcein was directly incorporated into the hydrogel
 321 has been observed. On the contrary, the incorporation of calcein into liposomes (calcein-loaded
 322 liposomes incorporated before the CS gelation process) creates a second diffusion barrier and
 323 delayed the release of calcein throughout the final assembly. Similar results have been obtained
 324 with chemical CS/gelatin hydrogels, crosslinked by glutaraldehyde or sodium tripolyphosphate,
 325 incorporating Phospholipon®-90G liposomes (also incorporating calcein) (Paun et al., 2016).
 326 An assembly composed of CS hydrogel and Phospholipon®-90G liposomes incorporating
 327 calcein has also been elaborated (Popa et al., 2017). These hydrogels were physically
 328 crosslinked with the addition of tannic acid. The ability of this assembly to remove the burst
 329 release phenomenon, with a sustained release of calcein over few days (less than 10% of
 330 initially incorporated calcein were released after 10 days, **Figure 6** right *versus* **Figure 6** left

331 displaying data obtained for CS hydrogels without liposome) has been demonstrated. This trend
332 can be attributed to the diffusion of liposomes outside the hydrogel matrix. The liposome lipid
333 bilayer would be then destabilized, slowly releasing the entrapped calcein.
334



335
336 **Figure 6.** *In vitro* cumulative release of calcein under magnetic stirring (in PBS, at 37°C, 60
337 rpm) directly incorporated in CS/tannic acid hydrogels (left), or incorporated into
338 Phospholipon®-90G liposomes embedded into CS/tannic acid hydrogels (right) with different
339 molar ratios in CS and tannic acid (Popa et al., 2017)
340

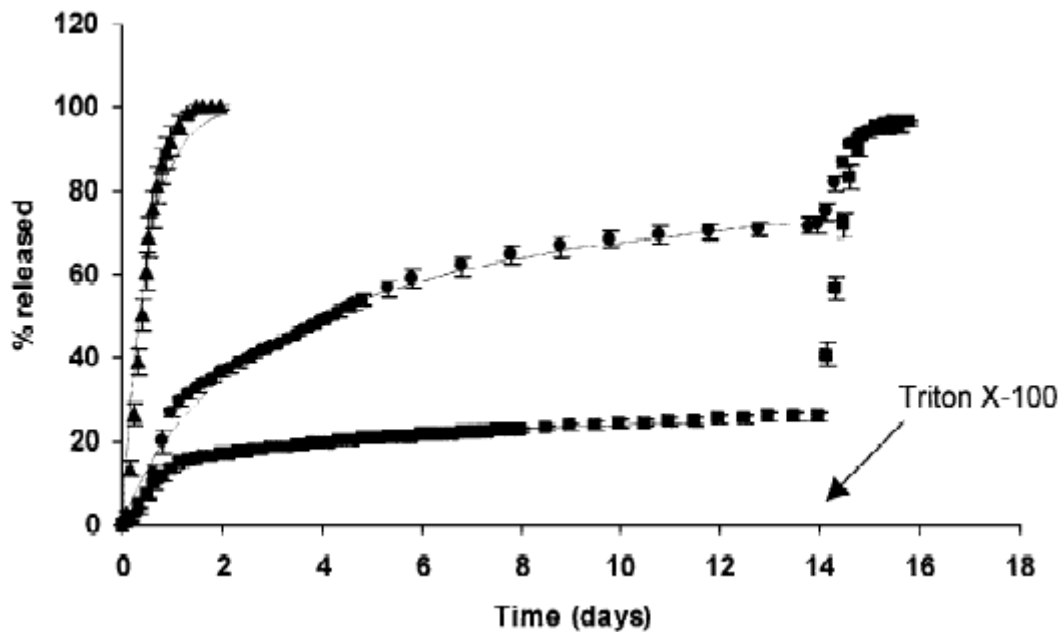
341 Similar assemblies have been studied for wound healing after burn injuries (Hurler et al.,
342 2012; Hurler & Škalko-Basnet, 2012). Mupirocin, an antimicrobial agent, was incorporated into
343 Phospholipon®-90G liposomes (mupirocin encapsulation rate between 50 and 75%),
344 embedded (before the CS gelation process) in CS/glycerol physical hydrogels. The double
345 diffusion barrier of « CS hydrogel and liposomes » assemblies limits the release of mupirocin
346 with less than a half of the initially incorporated drug released within 24 h, whereas the total
347 amount was released from the hydrogel without liposome over the same time. These results
348 were confirmed by skin penetration studies, with release profiles similar to the ones obtained
349 *in vitro*, which is very promising for burn injuries treatments.

350 Very recently, the incorporation of desferrioxamine (DFO), a chelating agent of iron and
351 trivalent aluminium, in the aqueous cavity of dipalmitoyl-sn-glycero-3-phosphocholine
352 (DPPC), 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethyleneglycol)-
353 2000] (DSPE-PEG), and monostearoylphosphatidylcholine (MSPC) liposomes with a molar
354 ratio of 85.3:9.7:5.0, respectively has been examined (O'Neill et al., 2017). Note that the DFO
355 incorporation rate into liposomes was not mentioned in this work. These liposomes were then
356 embedded into a thermosensitive CS/GP physical hydrogel.

357

358 Another assembly composed of doxorubicin incorporated into PC liposomes (with a
359 diameter around 95 nm) embedded (before the CS gelation process) into thermosensitive
360 CS/GP physical hydrogels has been elaborated (Ren et al., 2016). Two major advantages of
361 these assemblies are: i) they are maintained on the damaged site (herein, the liver), and ii) the
362 drug toxicity on surrounding tissues and organs is decreased. According to their results, such
363 assemblies could delay the release of doxorubicin and decrease the burst release in the 4th first
364 hours after injection. Indeed, only 12% of doxorubicin were released during this time for the
365 « CS hydrogel/liposomes » assembly (drug incorporation rate in liposomes around 98%)
366 against around 25% when free drug was directly incorporated in CS hydrogel. After that, the
367 doxorubicin was slowly released from the two systems. This trend was attributed to: i) the
368 release of doxorubicin from the cavity of the liposomes towards the hydrogel, followed by a
369 diffusion process from the hydrogel, ii) the diffusion of the liposomes from hydrogel to the
370 outside if their diameter is below to 100 nm. A mathematical model of this second step from
371 « CS hydrogel/liposomes » assemblies has been established, and have demonstrated that only
372 the smaller liposomes could go through the hydrogel whereas the bigger ones stayed entrapped
373 into de hydrogel matrix (Ruel-Gariépy et al., 2002). These bigger liposomes thus acted as drug
374 reservoirs that delayed the release of doxorubicin through the system. In this latter study (Ruel-
375 Gariépy et al., 2002), a water-soluble model molecule, carboxyfluorescein (CF), was
376 incorporated into PC, PC/cholesterol, or distearoylphosphatidylcholine (DSPC)/cholesterol
377 liposomes (with a CF encapsulation rate in liposomes < 8%). These liposomes were then
378 embedded into CS/GP physical hydrogels. This study has shown that the CF diffusion was
379 strongly influenced by size and composition of liposomes embedded into hydrogels (**Figure 7**).
380 Furthermore, Triton-X100 was introduced to induce breakage of the lipid membrane, proving
381 the liposomes integrity before this addition. Furthermore, the bigger the liposomes, the slower
382 the release (with a CF release during several weeks for assemblies with liposomes with a
383 diameter of 280 nm, **Figure 7**).

384



385
 386 **Figure 7.** *In vitro* cumulative release (% of total initially incorporated drug, in PBS at 37°C)
 387 of free CF (triangles), or CF incorporated into 100 nm (circles) or 280 nm (squares)
 388 PC/cholesterol liposomes (Ruel-Gariépy et al., 2002)
 389

390 In ophthalmic applications, a too small amount of drugs reaching the injury site (such as
 391 cornea) is often deplored, partially due to the tear production. Patients thus need repeated drug
 392 administrations for an efficient treatment. Moreover, the cornea epithelial membrane represents
 393 a barrier to the drug diffusion across the eye (Kaur & Kanwar, 2002). To solve this issue,
 394 ofloxacin antibiotic has been incorporated into multilamellar PC liposomes (obtained by the
 395 thin film hydration method or inverse phase evaporation) embedded into thermosensitive
 396 CS/GP physical hydrogels (Hosny, 2009). Ofloxacin incorporation rates into liposomes were
 397 found to be highly variable (between 13 and 65%) depending on the preparation method of
 398 liposomes, and their lipid composition (*e.g.*, the lipid charge nature). For example,
 399 incorporation rates were higher for liposomes obtained by thin film evaporation than those
 400 obtained by inverse phase evaporation (with same lipids and composition). With the liposome
 401 suspension, ofloxacin diffusion across the cornea was multiplied by 5 (*versus* an aqueous
 402 solution of ofloxacin). This could be explained by attractive interactions between anionic
 403 cornea and PC liposomes. Moreover, a significant increase in ofloxacin diffusion was even
 404 more observed when liposomes were embedded in CS/GP hydrogels (multiplied by 7 *versus*
 405 the free ofloxacin solution). Finally, it was demonstrated that the mucoadhesive properties of
 406 CS makes the interactions between liposomes and transcorneal membrane easier.

407 A similar study was carried out for the controlled release of an anticancer drug, the
408 cytarabine (Mulik et al., 2009). This drug presents a low molar mass (*ca* 240 g.mol⁻¹), a high
409 water-solubility, and a very short half-life in human plasma. For all these reasons, it is arduous
410 to formulate it in a stable way, as well as to control its delivery. That is why cytarabine has been
411 incorporated in PC liposomes (named CLLS, for cytarabine-loaded liposomal suspension, with
412 an encapsulation rate of *ca* 85%), then embedded before gelation process in thermosensitive
413 CS/GP physical hydrogels (named CGPCLL, for chitosan-glycerophosphate containing
414 cytarabine-loaded liposomes).

415

416 *In vitro* (in PBS, at 37°C) or *in vivo* (intramuscular injections of 5.4 mg of cytarabine/kg in
417 albino mice) tests have revealed that the combination of CS/GP hydrogel and liposomal
418 suspension (CGPCLL) delayed the cytarabine release. Indeed, 85% of initially incorporated
419 cytarabine were released over 60 h for assemblies (Erreur ! Source du renvoi
420 introuvable.“CGPCLL”), whereas 90% of cytarabine incorporated in liposomes were released
421 in less than 10 h (“CLLS”).

422 Very recently, a successful elaboration of biocompatible “liposomes in hydrogels”
423 assemblies without any crosslinking agent or additives, for the delayed delivery of various
424 water-soluble molecules has been described (Peers et al., 2019). Cumulative final releases of
425 carboxyfluorescein (fluorescent dye), rifampicin (antibiotic), and lidocaine (anaesthetic) have
426 revealed a delayed release of 16.9%, 11.6%, and 7.5% between “drug-in-liposomes-in-
427 hydrogel” and “drug-in-hydrogel” assemblies, respectively. Besides the data, we have shown
428 that the embedment of liposomes in the CS three-dimensional matrix had no impact on the
429 rheological properties of the hydrogel, regardless of the entrapped molecule. Finally, the
430 presence of liposomes inside the CS hydrogel network was interestingly confirmed, for the first
431 time, by environmental scanning electron microscopy.

432

433 *Incorporation of hydrophobic drugs in the lipid membrane of liposomes*

434 The controlled delivery of hydrophobic drugs from such assemblies is also mentioned in the
435 literature. The first study dealt with the incorporation of an antimicrobial agent, the dapson, in
436 the lipid membrane of PC liposomes (encapsulation rate of *ca* 16%), themselves embedded
437 (before the CS gelation process) in CS chemical hydrogels, crosslinked with the addition of
438 EDAC (Alamelu & Panduranga Rao, 1994). This not only led to the liposome stabilization
439 (Weiner et al., 1985), but also to a controlled release of dapson. In a phosphate buffer at pH

440 7.6, the release of dapsone was found to be 3 times slower for assemblies (86% of dapsone
441 released over 24 h). The chemical crosslinking between liposomes and the hydrogel has doubled
442 the time necessary to release the same amount of dapsone (in comparison with hydrogel without
443 EDAC) supported by a decrease of the burst effect phenomenon.

444 In 2014, a « CS hydrogel/liposomes » assembly has been elaborated for the controlled
445 delivery of an anticancer drug, the doxorubicin (López-Noriega Adolfo et al., 2014). This latter
446 was incorporated in thermosensitive liposomes (composed of DPPC, MSPC, and DSPE-
447 PEG2000 with an encapsulation rate > 90%). This thermosensitive property was brought by the
448 addition of 10% molar of MSPC lysolipid in the formulation, which has changed the phase
449 transition temperature of the liposome membrane, and formed pores inside the membrane with
450 the increase of the temperature (Grüll & Langereis, 2012; Negussie et al., 2010, 2011)). These
451 thermosensitive liposomes were then embedded (before the CS gelation process) in a
452 thermosensitive CS/GP physical hydrogel. Such assemblies have shown two major advantages:
453 i) the hydrogel formation was operated at physiological temperature, and ii) the « on-demand »
454 release of doxorubicin with the thermosensitive liposomes was possible. It has been also
455 demonstrated that the temperature increase (42°C) created pores in the lipid membrane
456 (supported by a high release of incorporated doxorubicin). As a conclusion, these assemblies
457 have revealed a better *in vitro* antitumor activity than free doxorubicin. Note that when no
458 temperature increase was applied, only 15% of doxorubicin were released over 7 days.

459 Very recently, the controlled delivery of curcumin (molecule with antioxidant, anti-
460 inflammatory, and anti-bacterial properties) from liposomes with various surface charges (with
461 an encapsulation rate between 43% and 76%, depending on the liposome formulation),
462 themselves embedded in a CS hydrogel for wound dressing applications has been investigated
463 (Ternullo et al., 2020). Neutral liposomes were made of PC and polysorbate (weight ratio
464 85:15). Stearylamine (weight ratio to PC 1:9) was added for cationic liposome elaboration.
465 Anionic liposomes were elaborated from PC and sodium deoxycholate. A sustained penetration
466 of curcumin from resulting assemblies through the *ex-vivo* full human skin, which is really
467 promising for advanced dermal delivery and wound healing applications has been evidenced.

468

469 *Incorporation of macromolecular drugs in liposomes*

470 « CS hydrogel/liposomes » assemblies were also used for the controlled delivery of
471 macromolecular drugs, such as peptides or proteins. Aiming at a wound healing application
472 after burn injuries, the incorporation of an epidermal growth hormone (EGF, *ca* 134 000 g.mol⁻¹

473 ¹) into DPPC liposomes, themselves embedded (before the gelation process) in CS physical
474 hydrogels has also been studied (Değim et al., 2011). EGF promotes cell proliferation, as well
475 as an extracellular matrix formation during the healing process. Hormone incorporation in
476 liposomes (with an encapsulation rate close to 60%) increased the stability of the hormone
477 (Alemdaroğlu et al., 2008; Yerushalmi et al., 1994). Daily administrations during 14 days of
478 different formulations (free EGF, liposomal EGF, and « CS hydrogel/-EGF-loaded liposomes »
479 assemblies) on rats' second-degree burn had been done to study the EGF release. According to
480 measurements of fibroblast diameters and epidermis thickness, the healing was faster for these
481 two formulations. This could thus stabilize the incorporated macromolecular drugs, and control
482 their release, promoting wound healing after burn injuries.

483 Glutathione (GSH) has also been incorporated in extruded DSPC and
484 dioleoylphosphatidylethanolamine (DOPE) liposomes (with a not mentioned incorporation rate
485 of GSH in liposomes) (Alinaghi et al., 2013). Liposomal GSH was then embedded in a
486 thermosensitive CS/GP physical hydrogel, which increased the retention time of GSH into the
487 peritoneal cavity in mouse.

488 In 2012, the incorporation of ovalbumin (*ca* 45,000 g.mol⁻¹), a model protein acting as an
489 antigen, into PC and stearylamine liposomes with an encapsulation rate of around 23% has been
490 studied (Gordon et al., 2012). These liposomes were then embedded in a thermosensitive CS/GP
491 physical hydrogel. This work has shown a wider immune response for these assemblies,
492 revealing their potential use as vaccines. However, this study did not result in an overall
493 improvement in immunogenicity.

494

495 To conclude this part about liposomes incorporated into CS hydrogels, the latter embedded
496 in CS hydrogels seem to be very efficient for the controlled release of different drugs
497 incorporated into liposomes. The lipid membrane and the hydrogel create a double barrier to
498 drug diffusion across the assembly. Note that no study in the literature deals with the
499 incorporation of drugs into liposomes, themselves embedded in “pure” CS physical hydrogels,
500 that is to say, with no additive of crosslinking agent in the final composition of the hydrogel.

501

502

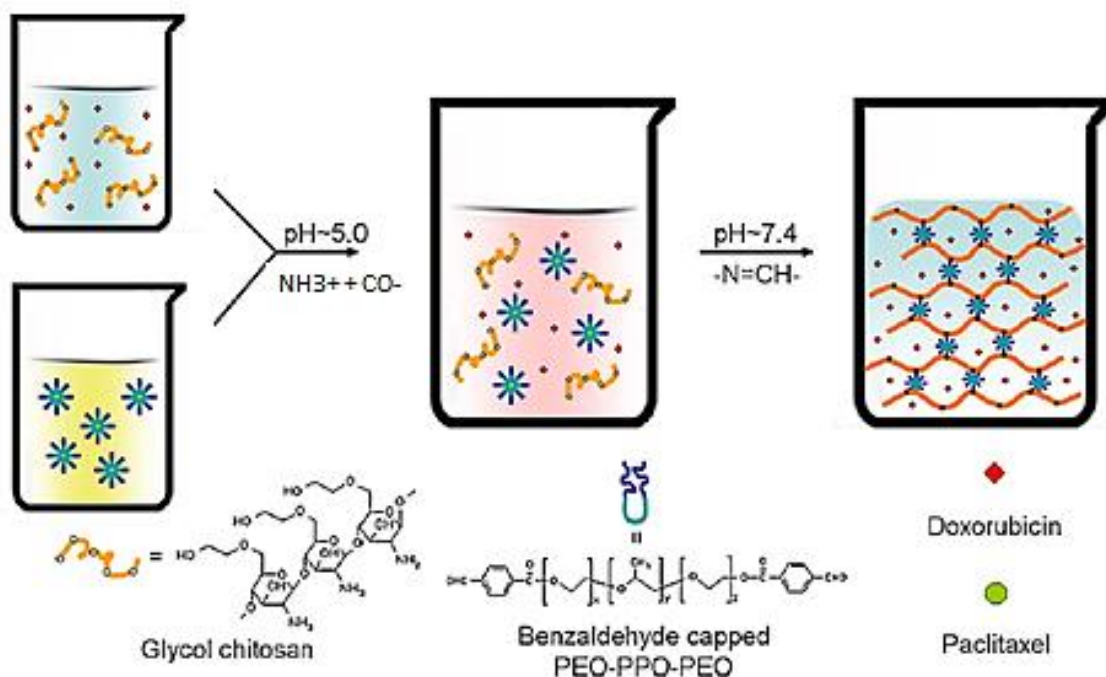
503 **4. « Chitosan hydrogel/micelles » assemblies**

504 As well as microparticles, nanoparticles and liposomes, micelles composed of molecular
505 surfactants or amphiphilic polymers can also be incorporated into CS hydrogels to delay the

506 release of drugs incorporated in them. Hydrophobic drugs can thus be entrapped in the inner
 507 core of direct micelles, and hydrophilic drugs, in reversed micelles. Both types of micelles act
 508 as a secondary barrier to the drug diffusion across the assembly, improving drug bioavailability.
 509 This implies the use of non-toxic additives, but also reduces the dose and associated side effects
 510 (Lundberg et al., 2003). The following part deals with such assemblies for the sustained delivery
 511 of drugs.

512 Glycol-CS chemical hydrogels associated to poly(ethylene glycol)-b-poly(propylene
 513 glycol)-b-poly(ethylene glycol) (PEO-PPO-PEO) for the controlled delivery of two anticancer
 514 drugs (doxorubicin and paclitaxel) has been elaborated (Zhao et al., 2011). Benzoic-imine
 515 bonds were thus created with a pH increase. Hydrophilic doxorubicin was dissolved in the CS
 516 solution, whereas hydrophobic paclitaxel was incorporated in the inner core of the (PEO-PPO-
 517 PEO) micelles (**Figure 8**). This micellar colloidal suspension was added before the CS gelation
 518 process.

519



521 **Figure 8.** Schematic representation of the preparation of « doxorubicin-loaded CS chemical
 522 hydrogel/paclitaxel-loaded-PEO-PPO-PEO micelles » by Zhao *et al.* (Zhao et al., 2011)

523 It has been shown that this « doxorubicin-loaded CS chemical hydrogel/paclitaxel-loaded-
 524 PEO-PPO-PEO micelles » assembly can delay the *in vitro* release of these two drugs over 2
 525 weeks. The release of doxorubicin seemed to be more influenced than paclitaxel by its
 526 incorporation rate and the crosslinker content. A value of pH close to 6.8 increases the release

527 of both drugs. This was attributed to the benzoic-imine bond within the hydrogel that is labile
528 at this pH (but stable at a physiological pH). This is very interesting for anticancer applications
529 because of slightly acid tumour environment. *In vivo*, anti-tumour activity of both drugs was
530 sustained which limited the tumour growth. Consequently, the survival of treated animals was
531 enhanced (23 days for mice treated with assemblies, *versus* 10 days for free paclitaxel, 14 days
532 for free doxorubicin, and 12 days for untreated mice). Such a « doxorubicin-loaded CS chemical
533 hydrogel/paclitaxel-loaded-PEO-PPO-PEO micelles » assembly can thus be valorized for the
534 simultaneous delivery of drugs with different natures.

535 Similarly, the simultaneous delivery of two different drugs from « CS hydrogel/micelles »
536 assemblies has been studied (Wei et al., 2009). The doxorubicin was incorporated into micelles
537 composed of a triblock copolymer of (poly(glutamic acid)-b-poly (propylene oxide)-b-
538 poly(glutamic acid), *ca* 11,000 g.mol⁻¹), themselves embedded (before the CS gelation process)
539 into a thermosensitive CS/poly(vinyl alcohol) (PVA) chemical hydrogel containing
540 acetylsalicylic acid. More than 75% of the initially incorporated acetylsalicylic acid was
541 released over 12 h contrary to the same amount of doxorubicin that was released from micelles
542 over more than a week. A pH-sensitive release of acetylsalicylic acid has been shown: the
543 higher the pH, the faster the release. This behaviour was can be explained by the hydrogel
544 retraction and a water expulsion, which fostered the acetylsalicylic acid release (*in vitro*
545 measurements carried out at pH=4.0; 5.5; 7.4, and 8.4). Finally, an increase in local temperature
546 (from 20 to 37°C) leads to a faster release of drugs. This study interestingly proved that the
547 drug release can be influenced by various stimuli such as pH or temperature, and that the drug
548 release could be delayed after drug incorporation into polymer micelles.

549 Micelles can also incorporate hydrophobic antibiotics. A hydrophobic antibiotic, the
550 tetracycline, has been incorporated into polymer micelles composed of PEG and PLA (di-block,
551 *ca* 7,000 g.mol⁻¹) (Ito et al., 2018). These PEG-PLA micelles were then embedded into PEG
552 modified-CS chemical hydrogels (CS chains were modified by PEG chains to adjust the CS
553 gelation properties). It has been shown that no matter the length of PEG or PLA chains in
554 micelles (with a diameter of micelles \simeq 20 nm), a slower release was obtained (*versus* free drug
555 directly incorporated into the hydrogel matrix). Micelles acted as a diffusion barrier, thus
556 delaying the release of tetracycline. Only 30% of tetracycline were released over 20 h from
557 « PEG-CS hydrogel/tetracycline-loaded PEG-PLA micelles » assemblies, *versus* more than
558 50% when the tetracycline was directly incorporated into the PEG-CS hydrogel matrix.

559 To conclude this part about « CS hydrogel/micelles », as previously for micro- and nano-
560 particles, and liposomes, micelles seem to be efficient for delaying the release of drugs when
561 incorporated in these assemblies. Different types of drugs can also be embedded in these
562 systems, improving drastically the treatment efficiency.

563

564 **Discussion and conclusion**

565 This paper reviews studies described in the literature about the elaboration and the
566 biomedical uses of complex CS-based systems, composed of drugs incorporated in « CS
567 hydrogel/colloids » assemblies, with the aim of reducing the burst effect, and delaying the
568 release of drugs over longer periods. Drugs are usually firstly incorporated in the colloidal
569 objects, themselves embedded in the CS solution before the CS gelation process. Different
570 colloids were considered such as micro- and nanoparticles, liposomes, as well as micelles. One
571 can note that the most of studies that dealt with polymer micro and nanoparticles, whereas the
572 number of researchers working on the incorporation of micelles into CS hydrogels is the most
573 limited. Colloids' elaboration, drug loading, stability, and (bio)compatibility are key parameters
574 to take in account to optimize the final systems even before their incorporation into hydrogels.
575 All these final “hybrid” assemblies demonstrate a very interesting potential for drugs delivery
576 applications, with the colloids acting as a second diffusion barrier of drugs regarding of the type
577 of colloid employed. Their release through the systems is not only delayed, but their side effects
578 are also reduced. Furthermore, the embedment of drugs with different natures (hydrophilic,
579 hydrophobic, and macromolecular) is facilitated. In particular, the incorporation of hydrophobic
580 or macromolecular drugs can be efficient with the use of appropriate colloids, while this
581 incorporation could be challenging directly in CS hydrogels due to the hydrophilic behaviour
582 of CS. Furthermore, as shown by different research teams, drugs can be released in two stages,
583 which could be of interest for specific treatments such as wound healing for example. These
584 “hybrid” assemblies thus open the door to a wider range of biomedical applications.

585 However, there is still plenty of room left for new researches and future improvements. For
586 instance, the structural characteristics of CS used (*i.e.*, its molar mass and acetylation degree)
587 are not systematically given in papers. Only ca 45% of the papers cited in this review provide
588 the molar mass (from 50,000 to 670,000 g.mol⁻¹, the other papers only mention
589 “low/medium/or high molar mass), and 30% of the papers, the viscosity of the resulting chitosan
590 solution. And ca 70% of the papers mention the value of the acetylation degree: rather low

591 acetylation degrees, from 5 to 25%. Consequently, it would be interesting to determine if these
592 characteristics can be linked to release properties, and to targeted biomedical applications.

593 Furthermore, hydrogels composed of CS and another polymer (*e.g.*, interpenetrating
594 polymer network hydrogels) could be useful to give to the final material some interesting
595 properties such as a denser hydrogel that could favour a more sustained drug delivery. Another
596 possible goal for future researches could be to work on the elaboration of such systems with
597 less (if any) chemical or physical crosslinkers in the final state, to create more health-friendly
598 systems. Indeed, chemical and physical crosslinkers could represent a real danger for such
599 biomedical applications. Finally, a better understanding of mechanisms involved after
600 administration of these “hybrid” assemblies could be relevant to even more enhance the
601 sustainable release of the entrapped drugs, and thus improve the treatment efficiency.

602

603 **Acknowledgments**

604 This work was financially supported by a grant from the French Ministry of Higher
605 Education and Research.

606

607 **References**

608

609 Alamelu, S., & Panduranga Rao, K. (1994). Liposomes sequestered in chitosan gel as a delivery
610 device for dapsone. *Carbohydrate Polymers*, 24(3), 215–221.

611 [https://doi.org/10.1016/0144-8617\(94\)90133-3](https://doi.org/10.1016/0144-8617(94)90133-3)

612 Alemdaroğlu, C., Degim, Z., Celebi, N., Şengezer, M., Alömeroglu, M., & Nacar, A. (2008).

613 Investigation of epidermal growth factor containing liposome formulation effects on
614 burn wound healing. *Journal of Biomedical Materials Research Part A*, 85A(1), 271–
615 283. <https://doi.org/10.1002/jbm.a.31588>

616 Alinaghi, A., Rouini, M. R., Daha, F. J., & Moghimi, H. R. (2013). Hydrogel-embedded vesicles,

617 as a novel approach for prolonged release and delivery of liposome, in vitro and in vivo.

618 *Journal of Liposome Research*, 23(3), 235–243.

619 <https://doi.org/10.3109/08982104.2013.799179>

620 Azevedo, E. P. D. (2015). Chitosan hydrogels for drug delivery and tissue engineering

621 applications. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(12),

622 8–14.

623 Azizian, S., Hadjizadeh, A., & Niknejad, H. (2018). Chitosan-gelatin porous scaffold

624 incorporated with Chitosan nanoparticles for growth factor delivery in tissue

625 engineering. *Carbohydrate Polymers*. <https://doi.org/10.1016/j.carbpol.2018.07.023>

626 Bhattarai, N., Gunn, J., & Zhang, M. (2010). Chitosan-based hydrogels for controlled, localized

627 drug delivery. *Advanced Drug Delivery Reviews*, 62(1), 83–99.

628 <https://doi.org/10.1016/j.addr.2009.07.019>

629 Bugnicourt, L., Alcouffe, P., & Ladavière, C. (2014). Elaboration of chitosan nanoparticles:

630 Favorable impact of a mild thermal treatment to obtain finely divided, spherical, and

631 colloiddally stable objects. *Colloids and Surfaces A: Physicochemical and Engineering*

632 *Aspects*, 457, 476–486. <https://doi.org/10.1016/j.colsurfa.2014.06.029>

633 Chen, X., Fan, M., Tan, H., Ren, B., Yuan, G., Jia, Y., Li, J., Xiong, D., Xing, X., Niu, X., &
634 Hu, X. (2019). Magnetic and self-healing chitosan-alginate hydrogel encapsulated
635 gelatin microspheres via covalent cross-linking for drug delivery. *Materials Science and*
636 *Engineering: C*, *101*, 619–629. <https://doi.org/10.1016/j.msec.2019.04.012>

637 Ciobanu, B. C., Cadinoiu, A. N., Popa, M., Desbrières, J., & Peptu, C. A. (2014). Modulated
638 release from liposomes entrapped in chitosan/gelatin hydrogels. *Materials Science and*
639 *Engineering: C*, *43*, 383–391. <https://doi.org/10.1016/j.msec.2014.07.036>

640 Deen, G. R., & Loh, X. J. (2018). Stimuli-Responsive Cationic Hydrogels in Drug Delivery
641 Applications. *Gels*, *4*(1), 13. <https://doi.org/10.3390/gels4010013>

642 Değim, Z., Çelebi, N., Alemdaroğlu, C., Deveci, M., Öztürk, S., & Özoğul, C. (2011).
643 Evaluation of chitosan gel containing liposome-loaded epidermal growth factor on burn
644 wound healing. *International Wound Journal*, *8*(4), 343–354.
645 <https://doi.org/10.1111/j.1742-481X.2011.00795.x>

646 Dehghan-Baniani, D., Bagheri, R., & Solouk, A. (2017). Preparation and characterization of a
647 composite biomaterial including starch micro/nano particles loaded chitosan gel.
648 *Carbohydrate Polymers*, *174*, 633–645. <https://doi.org/10.1016/j.carbpol.2017.06.095>

649 Demisli, S., Mitsou, E., Pletsa, V., Xenakis, A., & Papadimitriou, V. (2020). Development and
650 Study of Nanoemulsions and Nanoemulsion-Based Hydrogels for the Encapsulation of
651 Lipophilic Compounds. *Nanomaterials*, *10*(12), 2464.
652 <https://doi.org/10.3390/nano10122464>

653 dos Santos, M. K., Kreutz, T., Danielli, L. J., De Marchi, J. G. B., Pippi, B., Koester, L. S.,
654 Fuentefria, A. M., & Limberger, R. P. (2020). A chitosan hydrogel-thickened
655 nanoemulsion containing Pelargonium graveolens essential oil for treatment of vaginal
656 candidiasis. *Journal of Drug Delivery Science and Technology*, *56*, 101527.
657 <https://doi.org/10.1016/j.jddst.2020.101527>

658 Elgadir, M. A., Uddin, Md. S., Ferdosh, S., Adam, A., Chowdhury, A. J. K., & Sarker, Md. Z.
659 I. (2015). Impact of chitosan composites and chitosan nanoparticle composites on
660 various drug delivery systems: A review. *Journal of Food and Drug Analysis*, 23(4),
661 619–629. <https://doi.org/10.1016/j.jfda.2014.10.008>

662 Fan, M., Ma, Y., Tan, H., Jia, Y., Zou, S., Guo, S., Zhao, M., Huang, H., Ling, Z., Chen, Y., &
663 Hu, X. (2017). Covalent and injectable chitosan-chondroitin sulfate hydrogels
664 embedded with chitosan microspheres for drug delivery and tissue engineering.
665 *Materials Science and Engineering: C*, 71, 67–74.
666 <https://doi.org/10.1016/j.msec.2016.09.068>

667 Giri, T. K., Thakur, A., Alexander, A., Ajazuddin, Badwaik, H., & Tripathi, D. K. (2012).
668 Modified chitosan hydrogels as drug delivery and tissue engineering systems: Present
669 status and applications. *Acta Pharmaceutica Sinica B*, 2(5), 439–449.
670 <https://doi.org/10.1016/j.apsb.2012.07.004>

671 Gordon, S., Young, K., Wilson, R., Rizwan, S., Kemp, R., Rades, T., & Hook, S. (2012).
672 Chitosan hydrogels containing liposomes and cubosomes as particulate sustained
673 release vaccine delivery systems. *Journal of Liposome Research*, 22(3), 193–204.
674 <https://doi.org/10.3109/08982104.2011.637502>

675 Grijalvo, S., Eritja, R., & Díaz, D. D. (2019). Liposomes-in-Chitosan Hydrogels: Challenges
676 and Opportunities for Biomedical Applications. In *Materials Science and Technology*
677 (pp. 1–32). American Cancer Society. <https://doi.org/10.1002/9783527603978.mst0484>

678 Grüll, H., & Langereis, S. (2012). Hyperthermia-triggered drug delivery from temperature-
679 sensitive liposomes using MRI-guided high intensity focused ultrasound. *Journal of*
680 *Controlled Release*, 161(2), 317–327. <https://doi.org/10.1016/j.jconrel.2012.04.041>

681 Hamed, H., Moradi, S., Hudson, S. M., & Tonelli, A. E. (2018). Chitosan based hydrogels and
682 their applications for drug delivery in wound dressings: A review. *Carbohydrate*
683 *Polymers*, *199*, 445–460. <https://doi.org/10.1016/j.carbpol.2018.06.114>

684 Hejazi, R., & Amiji, M. (2003). Chitosan-based gastrointestinal delivery systems. *Journal of*
685 *Controlled Release*, *89*(2), 151–165. [https://doi.org/10.1016/S0168-3659\(03\)00126-3](https://doi.org/10.1016/S0168-3659(03)00126-3)

686 Hosny, K. M. (2009). Preparation and Evaluation of Thermosensitive Liposomal Hydrogel for
687 Enhanced Transcorneal Permeation of Ofloxacin. *AAPS PharmSciTech*, *10*(4), 1336–
688 1342. <https://doi.org/10.1208/s12249-009-9335-x>

689 Huang, J., Ren, J., Chen, G., Li, Z., Liu, Y., Wang, G., & Wu, X. (2018). Tunable sequential
690 drug delivery system based on chitosan/hyaluronic acid hydrogels and PLGA
691 microspheres for management of non-healing infected wounds. *Materials Science and*
692 *Engineering: C*, *89*, 213–222. <https://doi.org/10.1016/j.msec.2018.04.009>

693 Huang, X., & Brazel, C. S. (2001). On the importance and mechanisms of burst release in
694 matrix-controlled drug delivery systems. *Journal of Controlled Release*, *73*(2–3), 121–
695 136. [https://doi.org/10.1016/S0168-3659\(01\)00248-6](https://doi.org/10.1016/S0168-3659(01)00248-6)

696 Hurler, J., Berg, O. A., Skar, M., Conradi, A. H., Johnsen, P. J., & Škalko-Basnet, N. (2012).
697 Improved Burns Therapy: Liposomes-in-Hydrogel Delivery System for Mupirocin.
698 *Journal of Pharmaceutical Sciences*, *101*(10), 3906–3915.
699 <https://doi.org/10.1002/jps.23260>

700 Hurler, J., & Škalko-Basnet, N. (2012). Potentials of Chitosan-Based Delivery Systems in
701 Wound Therapy: Bioadhesion Study. *Journal of Functional Biomaterials*, *3*(1), 37–48.
702 <https://doi.org/10.3390/jfb3010037>

703 Ishihara, M., Obara, K., Nakamura, S., Fujita, M., Masuoka, K., Kanatani, Y., Takase, B.,
704 Hattori, H., Morimoto, Y., Ishihara, M., Maehara, T., & Kikuchi, M. (2006). Chitosan

705 hydrogel as a drug delivery carrier to control angiogenesis. *Journal of Artificial Organs*,
706 9(1), 8–16. <https://doi.org/10.1007/s10047-005-0313-0>

707 Ito, T., Takami, T., Uchida, Y., & Murakami, Y. (2018). Chitosan gel sheet containing drug
708 carriers with controllable drug-release properties. *Colloids and Surfaces B:*
709 *Biointerfaces*, 163, 257–265. <https://doi.org/10.1016/j.colsurfb.2017.12.054>

710 Joung, Y. K., Choi, J. H., Park, K. M., & Park, K. D. (2007). PLGA microparticle-embedded
711 thermosensitive hydrogels for sustained release of hydrophobic drugs. *Biomedical*
712 *Materials*, 2(4), 269. <https://doi.org/10.1088/1748-6041/2/4/010>

713 Kaur, I. P., & Kanwar, M. (2002). Ocular Preparations: The Formulation Approach. *Drug*
714 *Development and Industrial Pharmacy*, 28(5), 473–493. [https://doi.org/10.1081/DDC-](https://doi.org/10.1081/DDC-120003445)
715 120003445

716 Kim, S. E., Park, J. H., Cho, Y. W., Chung, H., Jeong, S. Y., Lee, E. B., & Kwon, I. C. (2003).
717 Porous chitosan scaffold containing microspheres loaded with transforming growth
718 factor- β 1: Implications for cartilage tissue engineering. *Journal of Controlled Release*,
719 91(3), 365–374. [https://doi.org/10.1016/S0168-3659\(03\)00274-8](https://doi.org/10.1016/S0168-3659(03)00274-8)

720 Kono, H., & Teshirogi, T. (2015). Cyclodextrin-grafted chitosan hydrogels for controlled drug
721 delivery. *International Journal of Biological Macromolecules*, 72, 299–308.
722 <https://doi.org/10.1016/j.ijbiomac.2014.08.030>

723 Legrand, P., Benoit, J.-P., Briançon, S., Fattal, E., Fessi, H., & Passirani, C. (2007). Sphéroïdes
724 et formes vectorisées. In Maloine (Ed.), *Pharmacie Galénique: Formulation et*
725 *Technologie pharmaceutique* (pp. 221–250). Maloine. [https://hal.archives-](https://hal.archives-ouvertes.fr/hal-00385473)
726 [ouvertes.fr/hal-00385473](https://hal.archives-ouvertes.fr/hal-00385473)

727 Li Hui, Ji Qiuxia, Chen Ximin, Sun Yan, Xu Quanchen, Deng Panpan, Hu Fang, & Yang
728 Jianjun. (2016). Accelerated bony defect healing based on chitosan thermosensitive
729 hydrogel scaffolds embedded with chitosan nanoparticles for the delivery of BMP2

730 plasmid DNA. *Journal of Biomedical Materials Research Part A*, 105(1), 265–273.
731 <https://doi.org/10.1002/jbm.a.35900>

732 Li, Q., Cai, T., Huang, Y., Xia, X., Cole, S. P. C., & Cai, Y. (2017). A Review of the Structure,
733 Preparation, and Application of NLCs, PNPs, and PLNs. *Nanomaterials*, 7(6), 122.
734 <https://doi.org/10.3390/nano7060122>

735 Li XingYi, Kong XiangYe, Zhang Juan, Wang YingJing, Wang YuJun, Shi Shuai, Guo Gang,
736 Luo Feng, Zhao Xia, Wei YuQuan, & Qian ZhiYong. (2010). A novel composite
737 hydrogel based on chitosan and inorganic phosphate for local drug delivery of
738 camptothecin nanocolloids. *Journal of Pharmaceutical Sciences*, 100(1), 232–241.
739 <https://doi.org/10.1002/jps.22256>

740 Liu, H., Wang, C., Li, C., Qin, Y., Wang, Z., Yang, F., Li, Z., & Wang, J. (2018). A functional
741 chitosan-based hydrogel as a wound dressing and drug delivery system in the treatment
742 of wound healing. *RSC Advances*, 8(14), 7533–7549.
743 <https://doi.org/10.1039/C7RA13510F>

744 Liu, L., Gao, Q., Lu, X., & Zhou, H. (2016). In situ forming hydrogels based on chitosan for
745 drug delivery and tissue regeneration. *Asian Journal of Pharmaceutical Sciences*, 11(6),
746 673–683. <https://doi.org/10.1016/j.ajps.2016.07.001>

747 López-Noriega Adolfo, Hastings Conn L., Ozbakir Burcin, O'Donnell Kathleen E., O'Brien
748 Fergal J., Storm Gert, Hennink Wim E., Duffy Garry P., & Ruiz-Hernández Eduardo.
749 (2014). Hyperthermia-Induced Drug Delivery from Thermosensitive Liposomes
750 Encapsulated in an Injectable Hydrogel for Local Chemotherapy. *Advanced Healthcare*
751 *Materials*, 3(6), 854–859. <https://doi.org/10.1002/adhm.201300649>

752 Lundberg, B. B., Risovic, V., Ramaswamy, M., & Wasan, K. M. (2003). A lipophilic paclitaxel
753 derivative incorporated in a lipid emulsion for parenteral administration. *Journal of*
754 *Controlled Release*, 86(1), 93–100. [https://doi.org/10.1016/S0168-3659\(02\)00323-1](https://doi.org/10.1016/S0168-3659(02)00323-1)

755 Maestrelli, F., González-Rodríguez, M. L., Rabasco, A. M., & Mura, P. (2006). Effect of
756 preparation technique on the properties of liposomes encapsulating ketoprofen–
757 cyclodextrin complexes aimed for transdermal delivery. *International Journal of*
758 *Pharmaceutics*, 312(1–2), 53–60. <https://doi.org/10.1016/j.ijpharm.2005.12.047>

759 Mahmoudian, M., & Ganji, F. (2017). Vancomycin-loaded HPMC microparticles embedded
760 within injectable thermosensitive chitosan hydrogels. *Progress in Biomaterials*, 1–8.
761 <https://doi.org/10.1007/s40204-017-0066-x>

762 Moebus, K., Siepmann, J., & Bodmeier, R. (2009). Alginate–poloxamer microparticles for
763 controlled drug delivery to mucosal tissue. *European Journal of Pharmaceutics and*
764 *Biopharmaceutics*, 72(1), 42–53. <https://doi.org/10.1016/j.ejpb.2008.12.004>

765 Moradi, S., Barati, A., Salehi, E., Tonelli, A. E., & Hamed, H. (2019). Preparation and
766 characterization of chitosan based hydrogels containing cyclodextrin inclusion
767 compounds or nanoemulsions of thyme oil. *Polymer International*, 68(11), 1891–1902.
768 <https://doi.org/10.1002/pi.5899>

769 Morantes, S. J., Buitrago, D. M., Ibla, J. F., García, Y. M., Lafaurie, G. I., & Parraga, J. E.
770 (2017). Composites of hydrogels and nanoparticles. In *Biopolymer-Based Composites*
771 (pp. 107–138). Elsevier. <https://doi.org/10.1016/B978-0-08-101914-6.00005-3>

772 Mulik, R., Kulkarni, V., & Murthy, R. S. R. (2009). Chitosan-Based Thermosensitive Hydrogel
773 Containing Liposomes for Sustained Delivery of Cytarabine. *Drug Development and*
774 *Industrial Pharmacy*, 35(1), 49–56. <https://doi.org/10.1080/03639040802178144>

775 Negussie, A. H., Miller, J. L., Reddy, G., Drake, S. K., Wood, B. J., & Dreher, M. R. (2010).
776 Synthesis and in vitro evaluation of cyclic NGR peptide targeted thermally sensitive
777 liposome. *Journal of Controlled Release*, 143(2), 265–273.
778 <https://doi.org/10.1016/j.jconrel.2009.12.031>

779 Negussie, A. H., Yarmolenko, P. S., Partanen, A., Ranjan, A., Jacobs, G., Woods, D., Bryant,
780 H., Thomasson, D., Dewhirst, M. W., Wood, B. J., & Dreher, M. R. (2011). Formulation
781 and characterisation of magnetic resonance imageable thermally sensitive liposomes for
782 use with magnetic resonance-guided high intensity focused ultrasound. *International*
783 *Journal of Hyperthermia : The Official Journal of European Society for Hyperthermic*
784 *Oncology, North American Hyperthermia Group*, 27(2), 140–155.
785 <https://doi.org/10.3109/02656736.2010.528140>

786 O'Neill, H. S., Herron, C. C., Hastings, C. L., Deckers, R., Lopez Noriega, A., Kelly, H. M.,
787 Hennink, W. E., McDonnell, C. O., O'Brien, F. J., Ruiz-Hernández, E., & Duffy, G. P.
788 (2017). A stimuli responsive liposome loaded hydrogel provides flexible on-demand
789 release of therapeutic agents. *Acta Biomaterialia*, 48, 110–119.
790 <https://doi.org/10.1016/j.actbio.2016.10.001>

791 Park, C. J., Clark, S. G., Lichtensteiger, C. A., Jamison, R. D., & Johnson, A. J. W. (2009).
792 Accelerated wound closure of pressure ulcers in aged mice by chitosan scaffolds with
793 and without bFGF. *Acta Biomaterialia*, 5(6), 1926–1936.
794 <https://doi.org/10.1016/j.actbio.2009.03.002>

795 Paun, V. A., Popa, M., Desbrieres, J., Peptu, C. A., Dragan, S. V., Zegan, G., & Cioca, G.
796 (2016). Liposome loaded chitosan hydrogels, a promising way to reduce the burst effect
797 in drug release a comparativ analysis. *Mater. Plast.*, 53(4), 590–593.

798 Peers, S., Alcouffe, P., Montembault, A., & Ladavière, C. (2019). Embedment of Liposomes
799 into Chitosan Physical Hydrogel for the Delayed Release of Antibiotics or Anaesthetics,
800 and its First ESEM Characterization. *Carbohydrate Polymers*, 115532.
801 <https://doi.org/10.1016/j.carbpol.2019.115532>

802 Peers, S., Montembault, A., & Ladavière, C. (2020). Chitosan hydrogels for sustained drug
803 delivery. *Journal of Controlled Release*, 326, 150–163.
804 <https://doi.org/10.1016/j.jconrel.2020.06.012>

805 Peng, Q., Sun, X., Gong, T., Wu, C.-Y., Zhang, T., Tan, J., & Zhang, Z.-R. (2013). Injectable
806 and biodegradable thermosensitive hydrogels loaded with PHBHHx nanoparticles for
807 the sustained and controlled release of insulin. *Acta Biomaterialia*, 9(2), 5063–5069.
808 <https://doi.org/10.1016/j.actbio.2012.09.034>

809 Pitorre, M., Gondé, H., Haury, C., Messous, M., Poilane, J., Boudaud, D., Kanber, E.,
810 Rossemond Ndombina, G. A., Benoit, J.-P., & Bastiat, G. (2017). Recent advances in
811 nanocarrier-loaded gels: Which drug delivery technologies against which diseases?
812 *Journal of Controlled Release*, 266(Supplement C), 140–155.
813 <https://doi.org/10.1016/j.jconrel.2017.09.031>

814 Popa, M., Ciobanu, B. C., Ochiuz, L., Desbrieres, J., & Peptu, C. A. (2017). Controlling the
815 release kinetics of calcein loaded liposomes from chitosan/tannic acid and
816 chitosan/poly(vinyl alcohol)/tannic acid hydrogels. *Cellulose Chemistry and*
817 *Technology*, 52(5–6), 353–370.

818 Qi, X., Qin, X., Yang, R., Qin, J., Li, W., Luan, K., Wu, Z., & Song, L. (2016). Intra-articular
819 Administration of Chitosan Thermosensitive In Situ Hydrogels Combined With
820 Diclofenac Sodium–Loaded Alginate Microspheres. *Journal of Pharmaceutical*
821 *Sciences*, 105(1), 122–130. <https://doi.org/10.1016/j.xphs.2015.11.019>

822 Rao, K. P., & Alamelu, S. (1992). Effect of crosslinking agent on the release of an aqueous
823 marker from liposomes sequestered in collagen and chitosan gels. *Journal of Membrane*
824 *Science*, 71(1), 161–167. [https://doi.org/10.1016/0376-7388\(92\)85015-B](https://doi.org/10.1016/0376-7388(92)85015-B)

825 Ren, S., Dai, Y., Li, C., Qiu, Z., Wang, X., Tian, F., Zhou, S., Liu, Q., Xing, H., Lu, Y., Chen,
826 X., & Li, N. (2016). Pharmacokinetics and pharmacodynamics evaluation of a

827 thermosensitive chitosan based hydrogel containing liposomal doxorubicin. *European*
828 *Journal of Pharmaceutical Sciences*, 92, 137–145.
829 <https://doi.org/10.1016/j.ejps.2016.07.002>

830 Ribeiro, L. N. M., Alcântara, A. C. S., Rodrigues da Silva, G. H., Franz-Montan, M., Nista, S.
831 V. G., Castro, S. R., Couto, V. M., Guilherme, V. A., & de Paula, E. (2017). Advances
832 in Hybrid Polymer-Based Materials for Sustained Drug Release. *International Journal*
833 *of Polymer Science*, 2017, 1–16. <https://doi.org/10.1155/2017/1231464>

834 Ruel-Gariépy, E., Leclair, G., Hildgen, P., Gupta, A., & Leroux, J.-C. (2002). Thermosensitive
835 chitosan-based hydrogel containing liposomes for the delivery of hydrophilic
836 molecules. *Journal of Controlled Release*, 82(2–3), 373–383.
837 [https://doi.org/10.1016/S0168-3659\(02\)00146-3](https://doi.org/10.1016/S0168-3659(02)00146-3)

838 Saeedi Garakani, S., Davachi, S. M., Bagher, Z., Heraji Esfahani, A., Jenabi, N., Atoufi, Z.,
839 Khanmohammadi, M., Abbaspourrad, A., Rashedi, H., & Jalessi, M. (2020). Fabrication
840 of chitosan/polyvinylpyrrolidone hydrogel scaffolds containing PLGA microparticles
841 loaded with dexamethasone for biomedical applications. *International Journal of*
842 *Biological Macromolecules*, 164, 356–370.
843 <https://doi.org/10.1016/j.ijbiomac.2020.07.138>

844 Shariatinia, Z., & Jalali, A. M. (2018). Chitosan-based hydrogels: Preparation, properties and
845 applications. *International Journal of Biological Macromolecules*, 115, 194–220.
846 <https://doi.org/10.1016/j.ijbiomac.2018.04.034>

847 Ta, H. T., Dass, C. R., & Dunstan, D. E. (2008). Injectable chitosan hydrogels for localised
848 cancer therapy. *Journal of Controlled Release*, 126(3), 205–216.
849 <https://doi.org/10.1016/j.jconrel.2007.11.018>

850 Ternullo, S., Schulte Werning, L. V., Holsæter, A. M., & Škalko-Basnet, N. (2020). Curcumin-
851 In-Deformable Liposomes-In-Chitosan-Hydrogel as a Novel Wound Dressing.
852 *Pharmaceutics*, 12(1), 8. <https://doi.org/10.3390/pharmaceutics12010008>

853 Wang, K., & Han, Z. (2017). Injectable hydrogels for ophthalmic applications. *Journal of*
854 *Controlled Release*, 268, 212–224. <https://doi.org/10.1016/j.jconrel.2017.10.031>

855 Wei, L., Cai, C., Lin, J., & Chen, T. (2009). Dual-drug delivery system based on
856 hydrogel/micelle composites. *Biomaterials*, 30(13), 2606–2613.
857 <https://doi.org/10.1016/j.biomaterials.2009.01.006>

858 Weiner, A. L., Carpenter-Green, S. S., Soehngen, E. C., Lenk, R. P., & Popescu, M. C. (1985).
859 Liposome–collagen gel matrix: A novel sustained drug delivery system. *Journal of*
860 *Pharmaceutical Sciences*, 74(9), 922–925. <https://doi.org/10.1002/jps.2600740903>

861 Xing, L., Sun, J., Tan, H., Yuan, G., Li, J., Jia, Y., Xiong, D., Chen, G., Lai, J., Ling, Z., Chen,
862 Y., & Niu, X. (2019). Covalently polysaccharide-based alginate/chitosan hydrogel
863 embedded alginate microspheres for BSA encapsulation and soft tissue engineering.
864 *International Journal of Biological Macromolecules*, 127, 340–348.
865 <https://doi.org/10.1016/j.ijbiomac.2019.01.065>

866 Yang, T.-T., Cheng, Y.-Z., Qin, M., Wang, Y.-H., Yu, H.-L., Wang, A.-L., & Zhang, W.-F.
867 (2017). Thermosensitive Chitosan Hydrogels Containing Polymeric Microspheres for
868 Vaginal Drug Delivery. *BioMed Research International*, 2017, 1–12.
869 <https://doi.org/10.1155/2017/3564060>

870 Yerushalmi, N., Arad, A., & Margalit, R. (1994). Molecular and Cellular Studies of Hyaluronic
871 Acid-Modified Liposomes as Bioadhesive Carriers for Topical Drug Delivery in Wound
872 Healing. *Archives of Biochemistry and Biophysics*, 313(2), 267–273.
873 <https://doi.org/10.1006/abbi.1994.1387>

874 Zan, J., Zhu, D., Tan, F., Jiang, G., Lin, Y., & Ding, F. (2006). Preparation of Thermosensitive
875 Chitosan Formulations Containing 5-Fluorouracil/Poly-3-hydroxybutyrate
876 Microparticles Used as Injectable Drug Delivery System. *Chinese Journal of Chemical
877 Engineering*, 14(2), 235–241. [https://doi.org/10.1016/S1004-9541\(06\)60064-5](https://doi.org/10.1016/S1004-9541(06)60064-5)

878 Zhao, L., Zhu, L., Liu, F., Liu, C., Shan-Dan, Wang, Q., Zhang, C., Li, J., Liu, J., Qu, X., &
879 Yang, Z. (2011). PH triggered injectable amphiphilic hydrogel containing doxorubicin
880 and paclitaxel. *International Journal of Pharmaceutics*, 410(1), 83–91.
881 <https://doi.org/10.1016/j.ijpharm.2011.03.034>

882 Zhou, H. Y., Wang, Z. Y., Duan, X. Y., Jiang, L. J., Cao, P. P., Li, J. X., & Li, J. B. (2016).
883 Design and evaluation of chitosan- β -cyclodextrin based thermosensitive hydrogel.
884 *Biochemical Engineering Journal*, 111, 100–107.
885 <https://doi.org/10.1016/j.bej.2016.03.011>

886 Zhu, S., Tian, H., Dou, M., & Huang, G. (2018). Chitosan Temperature-Sensitive Gels: Reduce
887 the Burst Release of Microspheres Containing Lornoxicam and Enhance Drug
888 Targeting. *MOJ Bioequiv Availab*, 5(1), 00076.

889