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# Stabilization of unilamellar cationic vesicles induced by $\beta$ -cyclodextrins: A strategy for a tunable drug delivery depot

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## ABSTRACT

The limited stability of cationic vesicles has discouraged their wide use for encapsulation and controlled release of active substances. Their structure can easily break down to form lamellar phases, micelles or rearrange into multilamellar vesicles, as a consequence of small changes in their composition. However, despite the limited stability, cationic vesicles possess an attractive architecture, which is able to efficiently encapsulate both hydrophobic and hydrophilic molecules. Therefore, improving the stability of the vesicles, as well as the control on unilamellar structures, are prerequisites for their wider application range. This study focuses on the impact of  $\beta$ -cyclodextrins for the stabilization of SDS/CTAB cationic vesicles. Molar ratio and sample preparation procedures have been investigated to evaluate the temperature stability of cationic vesicles. Diffusion and spectroscopic techniques evidenced that when  $\beta$ -cyclodextrins are added, unilamellar structures are stabilized above the multilamellar-unilamellar vesicles critical temperature. The results evidence encouraging perspectives for the use of vesicular nanoreservoirs for drug depot applications.

## 1. Introduction

Cationic vesicles are self-assembled nanostructures composed of dilute mixtures of oppositely charged surfactants. They have emerged in the last twenty years as a relevant and exciting new type of vesicle systems (Antunes et al., 2004, 2009; Kaler et al., 1992; Marques et al., 1993; Medronho et al., 2006; Milcovich et al., 2016, 2017; dos Santos et al., 2013). They are usually spherical, their polydispersity can vary remarkably and they can experience either monolayer or multilayer architectures. The advantage of using such nanoreservoirs in drug delivery relies on their elegant ability to compartmentalize both hydrophobic and hydrophilic molecules.

Cyclodextrins (CD) are cyclic oligosaccharides composed by glucose units connected through (1,4)- $\alpha$ -glucoside bonds (Szejtli, 1998). Their 3D molecular structure resembles a truncated cone, with a polar exterior and a less polar inner core (Connors, 1997). Cyclodextrins are able to generate inclusion complexes in aqueous solution by replacing water molecules in their cavity with small hydrophobic molecules or hydrophobic moieties of bigger molecules, and thus changing their solubility. They are mainly produced by starch enzymatic degradation, employing cyclodextrin glycosyl transferases (Biber et al., 2002). So far, these cyclic oligosaccharides exhibit several industrial applications

for pharmaceutical formulations, food products and cosmetics (Davis and Brewster, 2004; Hegdes, 1998). The constant and extensive interest on  $\beta$ -cyclodextrin for pharmaceutical and biomedical applications relies on their approval for human use (Li and Loh, 2008).

They also demonstrated to be able to modulate the rheology properties of hydrophobically modified polymers (Karlson et al., 2002). Depending on their number of glucose units, cyclodextrins can be classified according to Table 1.

An interesting feature of cyclodextrins concerns their aqueous solubility, which is influenced by the number of glucose units. The extremely low water solubility of  $\beta$ -cyclodextrin, compared to  $\alpha$  and  $\gamma$ -cyclodextrin, is due to the high energy H-bond network between the C2 and C3 hydroxyl groups (Szejtli, 1998). Nevertheless, this low solubility is adequate for improving the solubility of the poorly soluble included guests (Tewes et al., 2008; Thi et al., 2009).

Several techniques are described in the literature to study the inclusion of the hydrophobic tail of a surfactant into a cyclodextrin cavity, like NMR (Cabaleiro-Lago et al., 2005; Sehgal et al., 2006), calorimetry (De Lisi et al., 2003), neutron scattering (Alami et al., 2002), sound measurements (Junquera et al., 1993), capillary electrophoresis (Bendazzoli et al., 2010), surface tension (Bai et al., 2008) and conductivity (Junquera et al., 1997; Mehta et al., 2008; Sehgal et al.,

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**Table 1**

Characteristics of cyclodextrins (Connors, 1997; Del Valle, 2004).

Specie	Number of glucose units	Outer diameter	Inner diameter	Height	Solubility
$\alpha$ -cyclodextrin	6	1.46 nm	0.52 nm	0.8 nm	0.1211 M
$\beta$ -cyclodextrin	7	1.54 nm	0.66 nm	0.8 nm	0.0163 M
$\gamma$ -cyclodextrin	8	1.75 nm	0.84 nm	0.8 nm	0.1680 M

2006).  $\beta$ -Cyclodextrins are characterized by a high binding constant toward surfactants (host-guest complexes) (Valente and Söderman, 2014) and the formation of the mentioned complexes competes with surfactant micellization.  $\beta$ -Cyclodextrin was found to form inclusion complexes with the surfactants studied herein for the preparation of catanionic vesicles. Their binding constants are listed below:

- cetyl-trimethyl ammonium bromide (CTAB) with  $\beta$ -cyclodextrin:  $5 \cdot 10^4 \text{ M}^{-1}$  (Cabaleiro-Lago et al., 2005);
- sodium dodecyl-sulfate (SDS) with  $\beta$ -cyclodextrin:  $500 \text{ M}^{-1}$  (Junquera et al., 1993).

Thus, the presence of cyclodextrin can shift the surfactant CMC (critical micellar concentration) to higher concentrations, in a cyclodextrin-concentration dependent manner (Jiang et al., 2003). Moreover,  $\beta$ -cyclodextrin can interact also with surfactants (see Fig. 1), which composes vesicular aggregates, thus modulating vesicles properties (Yan et al., 2011).

The aim of this study is to understand such interactions, focusing on the influence of molar ratio, sample preparation procedure (i.e. addition of  $\beta$ -cyclodextrin before or after vesicles are generated) and temperature range on the stability of  $\beta$ -cyclodextrin doped catanionic vesicles. We hypothesize that the presence of  $\beta$ -cyclodextrin on catanionic vesicles can lead to an *in situ* stabilizing effect toward multi-to-unilamellar transition of catanionic vesicles, due to their complexation/binding features on the catanionic vesicle components (Fig. 2).

## 2. Materials and methods

### 2.1. Materials and sample preparation

Sodium dodecyl-sulfate (SDS) has been obtained from BDH Chemicals Ltd. Pool. England (purity grade 99,0%), while cetyl-trimethyl ammonium bromide (CTAB) has been purchased from Sigma-Aldrich (purity  $\geq 96\%$ ). Aqueous solutions of CTAB and SDS have been prepared and subsequently mixed in order to obtain vesicular solutions. Different molar ratios ( $R$ , according to equation (1)) have been

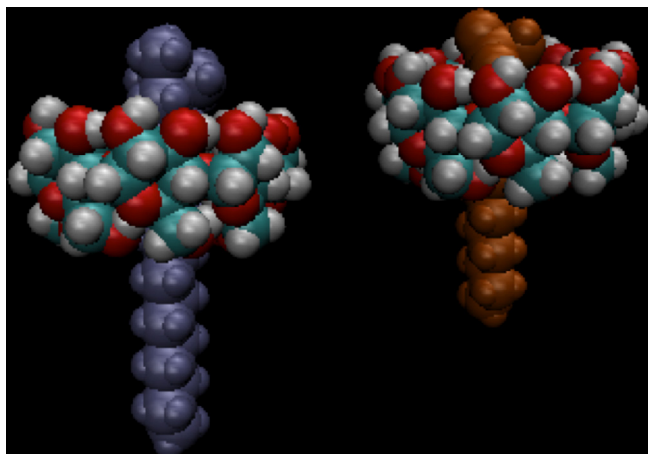


Fig. 1. Schematic representation of surfactant-cyclodextrin binding (Humphrey et al., 1996; Loftsson and Brewster, 1996).

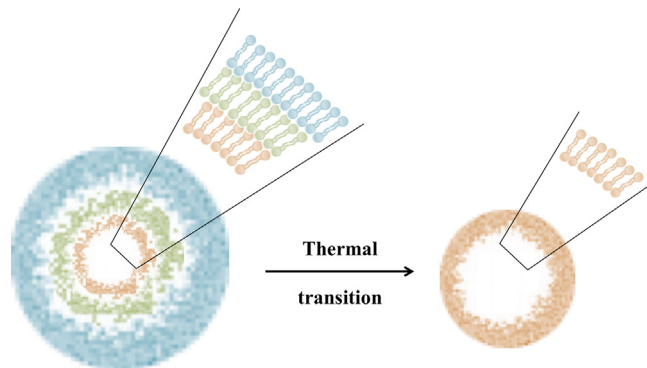


Fig. 2. Schematic model of the catanionic vesicles multi-to-unilamellar transition: the vesicles inner pool is represented as the empty core of the vesicular spherules (Andreozzi et al., 2010; Milcovich and Asaro, 2012).

employed, at a constant concentration of  $C = 6 \text{ mM}$  (i.e. about 0.2% wt).

$$R = \frac{[SDS]}{[CTAB]} \quad (1)$$

$\beta$ -Cyclodextrin (kindly provided from Vectorpharma Spa) was used in a fixed concentration of 1 mM.

The preparation of  $\beta$ -cyclodextrin-vesicles systems by the addition of  $\beta$ -cyclodextrin to the pre-formed vesicles will be referred to “standard preparation”, while the addition of  $\beta$ -cyclodextrin to surfactant monomeric solutions will be referred to “alternative preparation”.

### 2.2. Multinuclear NMR experiments

$^1\text{H}$  NMR measurements were carried out on a Jeol Eclipse 400 NMR spectrometer (9.4 T), equipped with a Jeol NM-EVTS3 variable temperature unit, operating at 400 MHz for  $^1\text{H}$ , with lock on  $\text{CDCl}_3$ , in coaxial tube.  $^{23}\text{Na}$  NMR measurements (Woessner, 2001) were carried out on a Jeol Eclipse 400 NMR spectrometer (9.4 T), equipped with a Jeol NM-EVTS3 variable temperature unit, operating at 105.75 MHz, without field frequency lock. The  $^{23}\text{Na}$ - $R_2$  (transverse relaxation rate  $R_2 = 1/T_2$ ) were measured by Hahn Echo.

### 2.3. PGSTE (Pulsed-gradient stimulated-echo) measurements

The  $^1\text{H}$  NMR measurements were carried out on a Varian 500 MHz NMR spectrometer (11.74 T) operating at 500 MHz for  $^1\text{H}$ , equipped with a model L650 Highland Technology pulsed field gradient (PFA) amplifier (10 A) and a standard 5 mm indirect detection, PFG probe. The lock was made on  $\text{CDCl}_3$  in coaxial tube, containing tetramethylsilane (TMS) as  $^1\text{H}$  chemical shift reference. A oneshot sequence has been employed for diffusion measurements (Johnson, 1999; Pelta et al., 2002), with 20 different z-gradient strengths,  $G_z$ , between 0.02 and 0.54 T/m, a pulsed gradient duration,  $\delta$ , of 2 ms, and at different diffusion interval ( $\Delta$ ). At each gradient strength, 64 transients have been accumulated employing a spectral width of 11 ppm over 16 k data points. Solvent suppression was accomplished by presaturation. Gradients were calibrated on the value of  $D = 1.90 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$  for  $^1\text{H}$  in  $\text{D}_2\text{O}$  (99.9%) at 25 °C (Antalek, 2002). The values of the self-diffusion coefficient,  $D$ , were obtained by Tanner equation fitting to experimental data (Tanner, 1970) (Eq. (2)),

$$\frac{E}{E_0} = \exp(-bD) \quad b = (\gamma\delta G)^2 \cdot \left( \Delta - \frac{\delta}{3} \right) \quad (2)$$

where:

- $E$  and  $E_0$  are the signal intensities in the presence and absence of  $G_z$ , respectively,

- $D$  is the diffusion coefficient,
- $\gamma$  is the nuclear gyromagnetic ratio  $26.75 \cdot 10^7 \text{ rad s}^{-1} \text{ T}^{-1}$  for  $^1\text{H}$  nucleus,
- $\delta$  is the gradient pulse width,
- $G$  is the gradient amplitude,
- $(\Delta\delta/3)$  correspond to the diffusion time corrected for the effects of finite gradient pulse.

PGSTE NMR spectra were processed using MestRenova and self-diffusion coefficients were determined by linear regression with Excel.

#### 2.4. UV-Vis (Turbidity) measurements

Spectral and absorbance measurements were carried out by using Shimadzu UV/Vis spectrophotometer model UV-2450, equipped with a Peltier temperature control unit; 1.0 cm thickness matched quartz cells were used for the entire experimental work.

#### 2.5. Polarized light microscopy

A Leitz Pol-Orthoplan microscope, equipped with differential interference contrast (DIC) lenses, was used. The main goal was to check the presence of crystals or anisotropic liquid crystals, such as lamellar phases, under polarized light. Samples were observed at room temperature, both immediately after preparation and several days later.

#### 2.6. Dynamic light scattering (DLS)

The mean particle diameter of the aggregates and polydispersion index were determined by dynamic light scattering (photon correlation spectroscopy, PCS) using an N5 Particle Analyzer (Beckman Coulter Inc., USA), equipped with a Peltier temperature control unit. Data were collected at  $90^\circ$  scattering angle. The time-averaged autocorrelation functions were transformed into intensity-weighted distributions of the apparent hydrodynamic diameter using the available Beckman PCS software. The average values of size were calculated with the data obtained from three measurements  $\pm$  SD.

### 3. Results and discussion

#### 3.1. System characterization

A minimum SDS/CTAB molar ratio of  $R = 1.6$  was found to be required in order to avoid precipitation/flocculates. High molar ratio values ( $R > 2.6$ ) evidenced the co-existence of micelles that can disturb vesicle investigation and observation (Andreozzi et al., 2010). A SDS/CTAB molar ratio of  $R = 1.85$  was investigated, as it ensures the appropriate stability (see Supplementary, Fig. S1).

Supplementary Figs. S1–S4 associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ijpharm.2018.07.026>.

$^1\text{H}$  NMR was performed and  $\beta$ -cyclodextrin peaks have been assigned (see Supplementary, Fig. S2).  $^1\text{H}$  NMR cannot directly detect vesicle signals, due to their large dimension and high relaxation range. On the other hand, the exchanging surfactants and cyclodextrins can be detected, as per Fig. 3. CTAB is completely embedded in the vesicular aggregates, according to the  $^1\text{H}$  NMR, where no CTAB signal is detected (see Supplementary, Fig. S3).

Despite the addition of  $\beta$ -cyclodextrin, solutions remain isotropic, as no liquid crystals were present in any sample, according to polarized light microscopy (data not shown). PGSTE results confirmed those findings, with no shifts in the diffusion coefficient and only a single diffusion coefficient  $D$  detected for  $\beta$ -cyclodextrin signals.

The present system shows the SDS excess throughout vesicle composition. SDS molecules are in exchange between vesicles and bulk solution, as demonstrated by PGSTE NMR diffusion measurements, with a quite lower exchange rate than a micellar system (Fig. 4).

The diffusion coefficients  $D$  were measured and it was possible to assess that  $\beta$ -cyclodextrin is not entrapped in the aqueous inner pool of vesicles, as otherwise  $\beta$ -cyclodextrin  $D$  was expected to be extremely low, compared to a simple  $\beta$ -cyclodextrin water solution (Morris and Johnson, 1993) (see Supplementary, Fig. S4).

According to Table 2,  $\beta$ -cyclodextrin detectable signals correspond to a constant  $D$  coefficient: thus,  $\beta$ -cyclodextrin diffusion is not influenced by the presence of vesicular aggregates.

Further analysis demonstrate that monomeric dodecylsulfate in water possesses a coefficient  $D = 6.2 \cdot 10^{-10} \text{ m}^2/\text{s}$ . Thus, dodecylsulfate

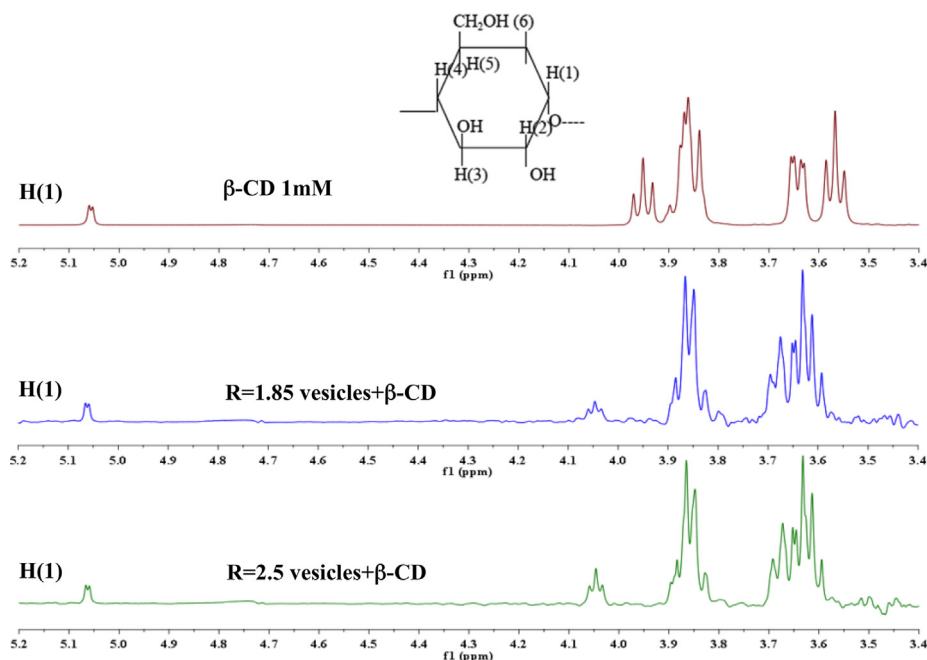


Fig. 3. Comparison of  $^1\text{H}$  NMR spectra in water, all referred to TMS peak.

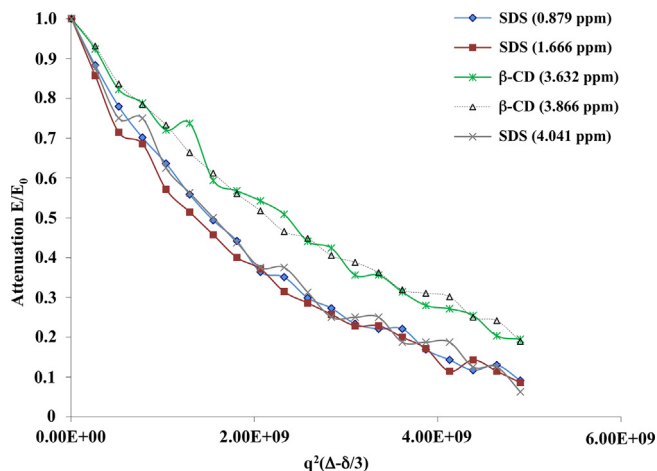


Fig. 4. Plot of the echo decays (only detectable signals) at 30 °C ( $\Delta = 60$  ms) for  $R = 1.85$  vesicles with  $\beta$ -cyclodextrin.

Table 2

Diffusion coefficients of  $\beta$ -cyclodextrin doped vesicles (excess surfactant SDS and  $\beta$ -cyclodextrin diffusion coefficients are reported).

Sample	SDS	$\beta$ -cyclodextrin
SDS + $\beta$ CD	$5.5 \cdot 10^{-10} \text{ m}^2/\text{s}$	$3.1 \cdot 10^{-10} \text{ m}^2/\text{s}$
Vesicles + $\beta$ CD, $R = 1.85$ ("alternative")	$4.2 \cdot 10^{-10} \text{ m}^2/\text{s}$	$3.1 \cdot 10^{-10} \text{ m}^2/\text{s}$
Vesicles + $\beta$ CD, $R = 1.85$	$4.7 \cdot 10^{-10} \text{ m}^2/\text{s}$	$3.1 \cdot 10^{-10} \text{ m}^2/\text{s}$

exhibits a remarkable decrease in terms of diffusion, which might be related to a dynamic complexation with  $\beta$ -cyclodextrin.

### 3.2. In situ stabilization of cationic vesicles by $\beta$ -cyclodextrins

When prepared, vesicle dispersion appears milky, but the turbidity dramatically decreases by heating. This is related to a multi-to-unilamellar transition together with a decrease of the vesicle size, as reported in literature (Andreozzi et al., 2010). This phenomenon is anticipated by sodium dissociation, as per  $^{23}\text{Na}$  NMR transverse relaxation rates ( $R_2$ ), and it involves an uptake of free dodecylsulfate into aggregates, as revealed by  $^1\text{H}$  NMR. Accordingly,  $^{23}\text{Na}$  transverse relaxation rates show that  $\text{Na}^+$  dissociation anticipates the thermal transition (Milcovich and Asaro, 2012).

The addition of  $\beta$ -cyclodextrins leads to an increase of the critical transition temperature,  $T_c$ , and to lower  $^{23}\text{Na}$ - $R_2$  trends. This is supported by the reduction of free dodecylsulfate in the bulk, due to its complexation with  $\beta$ -cyclodextrin.

Moreover, vesicular aggregates do not allow  $\beta$ -cyclodextrin to permeate the bilayer, as shown by PGSTE NMR measurements. The decrease in turbidity is anticipated by a sodium dissociation; this trend is evident in  $\beta$ -cyclodextrin doped samples as well.

Specifically, for what concerns the shift in the critical transition temperature,  $T_c$ , normal vesicles usually hold a  $T_c = 47$  °C, while for  $\beta$ -cyclodextrin doped vesicles values around 49–50 °C have been detected. Therefore  $\beta$ -cyclodextrins addition is able to expand the temperature range for multilamellar vesicle, reporting a higher  $T_c$ . Viceversa, above the  $T_c$ ,  $\beta$ -cyclodextrins interaction leads to unilamellar vesicles with higher stability.

The critical temperature ( $T_c$ ) depends on molar ratio  $R$ : lower  $T_c$  values correspond to higher molar ratios,  $R$ . Thus, it is proposed that  $^{23}\text{Na}$ - $R_2$  trends are related to vesicular dimension and dodecylsulfate amount inside the aggregates (Fig. 5).

Lower  $R$  values show higher amounts of free  $\text{Na}^+$ , probably due to a complexation of dodecylsulfate with  $\beta$ -cyclodextrin, which seems not to participate in aggregates, as inferred by previous  $^1\text{H}$  NMR integral

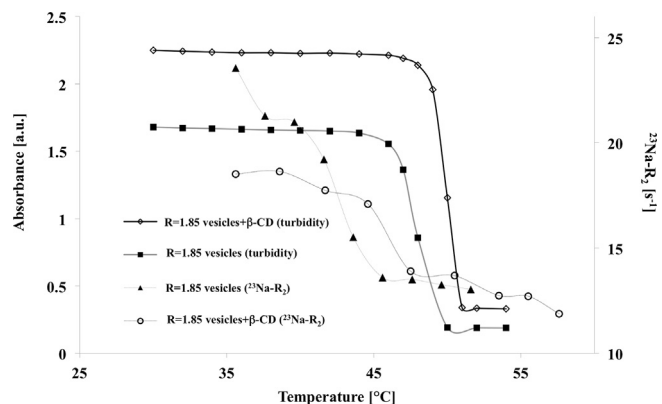


Fig. 5. Effect of  $\beta$ -cyclodextrin addition on turbidity and  $^{23}\text{Na}$ - $R_2$  trends vs. temperature.

analyses.

$^{23}\text{Na}$  transverse relaxation rates evidence that the preparation procedure can influence very much the  $\text{Na}^+$  dissociation, as evidenced in Fig. 6. Thus, even though the thermal transition leads to a single final

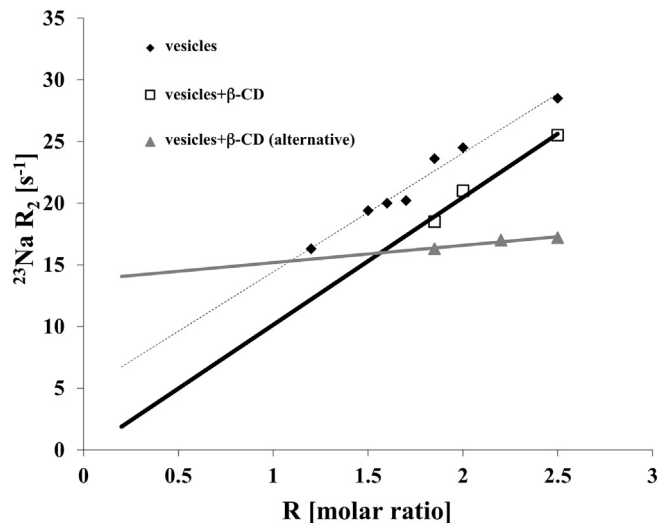


Fig. 6.  $^{23}\text{Na}$ - $R_2$  trends vs. molar ratio before thermal transition, with  $\beta$ -cyclodextrin (both preparation procedures) and without.

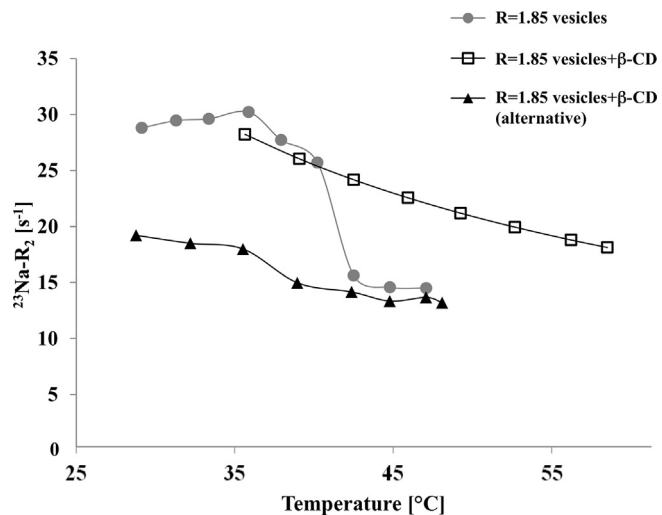


Fig. 7.  $^{23}\text{Na}$ - $R_2$  rates, for samples of vesicles  $R = 1.85$  with  $\beta$ -cyclodextrin ("standard" and "alternative" preparation), compared with simple vesicles.



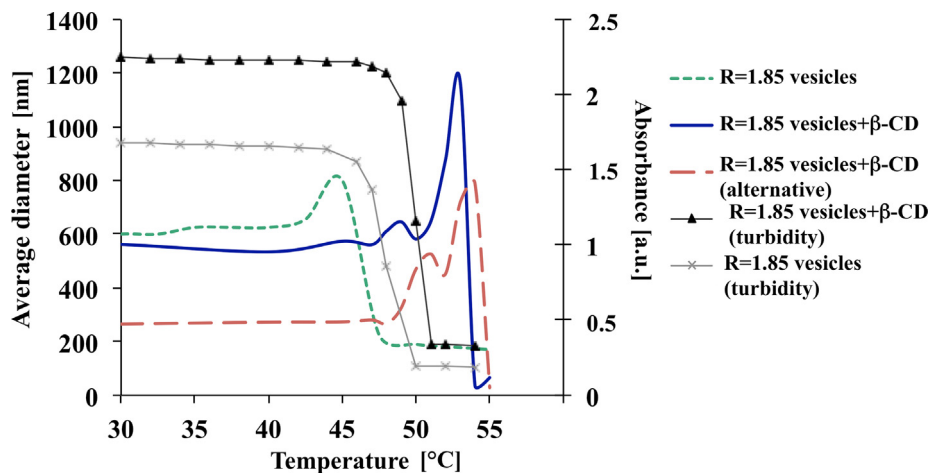


Fig. 8. DLS and turbidity ( $\lambda = 550$  nm) vs. temperature for vesicles and  $\beta$ -cyclodextrin doped vesicles (both preparations).

$R_2$ , the addition of  $\beta$ -cyclodextrin to monomeric starting solutions enhances the  $\text{Na}^+$  dissociation at the early stage.

The “alternative preparation” is supposed to influence remarkably the structural features of the system:  $^{23}\text{Na}$ - $R_2$  values exhibit a linear dependence on  $R$ , with the same slope for both simple vesicles and  $\beta$ -cyclodextrin doped vesicles (“standard preparation”). Moreover, the preparation protocol plays a key role in terms of stability of the overall system. Indeed, the  $^{23}\text{Na}$ - $R_2$  trends after the thermal transition reveal that the “standard” fabrication protocol has a stabilization effect on the free  $\text{Na}^+$  exchange between the vesicles and the bulk (see Fig. 7), leading to a more easily tunable control toward the vesicle architecture.

These findings allow to determine that the production method referred as “standard” followed by a first thermal transition cycle, leads to stable unilamellar vesicles.

In terms of DLS assessment, when a particle is relatively small and can undergo random thermal (Brownian) motion, time-dependent fluctuations occur and the distance among particles is therefore constantly changing. Constructive and destructive interference of the scattered light by neighboring particles gives rise to the intensity fluctuation at the detector plane. When the particles are extremely small, compared with the light wavelength, the intensity of the scattered light is uniform in all directions (Rayleigh scattering); whereas for larger particles (diameter  $\geq 250$  nm), the intensity is angle-dependent (Mie scattering).

The size, shape and molecular interactions in the vesicular samples determine frequency shifts, polarization and intensity of the scattered light. Thus, matching DLS characteristics with the other results, allows to gain information about the structure and molecular dynamics of the scattering sample.

DLS analysis (Fig. 8) evidences the presence of a sort of pre-transition, which anticipates the real thermal transition. The results show remarkable differences between the thermal behavior of normal vesicles and  $\beta$ -cyclodextrin doped vesicles.  $\beta$ -Cyclodextrin doped aggregates are also susceptible to a further transition, leading, after the thermal ramp, to quite small aggregates.

As a consequence, the preparation conditions have a major influence on both the size and the thermal behavior of the aggregates, as confirmed by spectroscopic techniques.

#### 4. Conclusions

The results confirm the stabilization of vesicles induced by  $\beta$ -cyclodextrin. PGSTE experiments show an increase of dodecylsulfate in the bulk, mainly in a complexed form together with  $\beta$ -cyclodextrin, as confirmed by  $^{23}\text{Na}$ - $R_2$  trends (decrease of sodium dissociation).

All available CTAB molecules are participating in the vesicle

formation, as no CTAB signals were detected (vesicle  $^1\text{H}$  NMR signals cannot be directly detected, due to very high relaxations). These findings demonstrate that, despite their ‘soft’ nature, cationic vesicles can successfully resist to the addition of saccharide-based molecules, such as  $\beta$ -cyclodextrins, leading to an *in situ* vesicular nanoreservoirs stabilization. Indeed, the increase in multi-to-unilamellar transition temperature  $T_c$  determines the availability of a broader temperature range for multilamellar vesicles handling and application.

Conversely, above the transition temperature  $T_c$ , the addition of  $\beta$ -cyclodextrins elicits the formation of smaller unilamellar vesicles, with higher stability. Overall, depending on the characteristics of the bioactive molecule to be delivered, it is possible to tune the most suitable vesicles size, lamellar composition and temperature range. Thus, such system looks promising for future application in drug delivery, e.g. to decrease bioactive molecules cytotoxicity, enhance small molecules solubility, boosting the absorption of aerosolized bioactive molecules and/or proposes as innovative topical drug delivery solutions.

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#### References

- Andreozzi, P., Funari, S.S., La Mesa, C., Mariani, P., Ortore, M.G., Sinibaldi, R., Spinuzzi, F., 2010. Multi-to unilamellar transitions in cationic vesicles. *J. Phys. Chem. B* 114, 8056–8060.
- Alami, E., Abrahamsen-Alami, S., Eastoe, J., Grillo, I., Heenan, R.K., 2002. Interactions between a nonionic gemini surfactant and cyclodextrins investigated by small-angle neutron scattering. *J. Colloid Interface Sci.* 255, 403–409.
- Antalek, B., 2002. Using pulsed gradient spin echo NMR for chemical mixture analysis: How to obtain optimum results. *Concepts Magn. Reson. Part A* 14, 225–258.
- Antunes, F.E., Marques, E.F., Gomes, R., Thuresson, K., Lindman, B., Miguel, M.G., 2004. Network formation of cationic vesicles and oppositely charged polyelectrolytes. Effect of polymer charge density and hydrophobic modification. *Langmuir* 20, 4647–4656.
- Antunes, F.E., Marques, E.F., Miguel, M.G., Lindman, B., 2009. Polymer-vesicle association. *Adv. Colloid Interface Sci.* 147–148, 18–35.
- Bai, Y., Xu, G.Y., Xin, X., Sun, H.Y., Zhang, H.X., Hao, A.Y., Yang, X.D., Yao, L., 2008. Interaction between cetyltrimethylammonium bromide and cyclodextrin: surface tension and interfacial dilational viscoelasticity studies. *Colloid Polym. Sci.* 286, 1475–1484.
- Bendazzoli, C., Mileo, E., Lucarini, M., Olmo, S., Cavrini, V., Gotti, R., 2010. Capillary electrophoretic study on the interaction between sodium dodecyl sulfate and neutral cyclodextrins. *Microchim. Acta* 171, 23–31.
- Biber, A., Antranikian, G., Heinzle, E., 2002. Enzymatic production of cyclodextrins. *Appl. Microbiol. Biotechnol.* 59, 609–617.
- Cabaleiro-Lago, C., Nilsson, M., Soderman, O., 2005. Self-diffusion NMR studies of the

- host-guest interaction between beta-cyclodextrin and alkyltrimethylammonium bromide surfactants. *Langmuir* 21, 11637–11644.
- Connors, K.A., 1997. The stability of cyclodextrin complexes in solution. *Chem. Rev.* 97, 1325–1357.
- Davis, M.E., Brewster, M.E., 2004. Cyclodextrin-based pharmaceuticals: past, present and future. *Nat. Rev. Drug Discov.* 3, 1023–1035.
- De Lisi, R., Lazzara, G., Milioto, S., Muratore, N., Terekhova, I.V., 2003. Characterization of the Cyclodextrin–Surfactant interactions by volume and enthalpy. *Langmuir* 19, 7188–7195.
- Del Valle, E.M.M., 2004. Cyclodextrins and their uses: a review. *Process Biochem.* 39 (9), 1033–1046.
- Hegdes, V., 1998. Industrial applications of cyclodextrins. *Chem. Rev.* 98, 2035–2044.
- Humphrey, W., Dalke, A., Schulten, K., 1996. VMD – visual molecular dynamics. *J. Mol. Graph.* 14 (1), 33–38.
- Jiang, B.-Y., Du, J., Cheng, S.-Q., Pan, J.-W., Zeng, X.-C., Liu, Y.-J., Shunzo, Y., Yoshimi, S., 2003. Effects of cyclodextrins as additives on surfactant CMC. *J. Dispers. Sci. Technol.* 24 (1), 63–66.
- Johnson Jr, C.S., 1999. Diffusion ordered nuclear magnetic resonance spectroscopy: principles and applications. *Progr. Nucl. Magn. Reson. Spectrosc.* 34, 203–256.
- Junquera, E., Tardajos, G., Aicart, E., 1993. Effect of the presence of beta-cyclodextrin on the micellization process of sodium dodecyl sulfate or sodium perfluorooctanoate in water. *Langmuir* 9, 1213–1219.
- Junquera, E., Pena, L., Aicart, E., 1997. A Conductimetric study of the interaction of beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin with dodecyltrimethylammonium bromide in water solution. *Langmuir* 13, 219–224.
- Kaler, E.W., Herrington, K.L., Murthy, A.K., Zasadzinski, J.A.N., 1992. Phase behavior and structures of mixtures of anionic and cationic surfactants. *J. Phys. Chem.* 96, 6698–6707.
- Karlson, L., Thuresson, K., Lindman, B., 2002. A rheological investigation of the complex formation between hydrophobically modified ethyl (hydroxy ethyl) cellulose and cyclodextrin. *Carbohydr. Polym.* 50, 219–226.
- Li, J., Loh, X.J., 2008. Cyclodextrin-based supramolecular architectures: syntheses, structures, and applications for drug and gene delivery. *Adv. Drug Deliv. Rev.* 60 (9), 1000–1017.
- Loftsson, T., Brewster, M.E., 1996. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. *J. Pharm. Sci.* 85 (10), 1017–1025.
- Marques, E., Khan, A., Miguel, M.G., Lindman, B., 1993. Self-assembly in mixtures of a cationic and an anionic surfactant: the sodium dodecyl sulfate-didodecyltrimethylammonium bromide-water system. *J. Phys. Chem.* 97, 4729–4736.
- Mehta, S.K., Bhasin, K.K., Dham, S., Singla, M.L., 2008. Micellar behavior of aqueous solutions of dodecyltrimethylammonium bromide, dodecyltrimethylammonium chloride and tetradecyltrimethylammonium chloride in the presence of  $\alpha$ -,  $\beta$ - and  $\gamma$ -cyclodextrins. *J. Colloid Interface Sci.* 321, 442–451.
- Milcovich, G., Asaro, F., 2012. Insights into cationic vesicles thermal transition by NMR spectroscopy. *Progr. Coll. Polym. Sci.* 139, 35–38.
- Milcovich, G., Antunes, F., Golob, S., Farra, R., Grassi, M., Voinovich, D., Grassi, G., Asaro, F., 2016. Thermo-responsive hydrogels from cellulose-based polyelectrolytes and cationic vesicles for biomedical application. *J. Biomed. Mater. Res. A* 104 (7), 1668–1679.
- Milcovich, G., Lettieri, S., Antunes, F.E., Medronho, B., Fonseca, A.C., Coelho, J.F.J., Marizza, P., Perrone, F., Farra, R., Dapas, B., Grassi, G., Grassi, M., Giordani, S., 2017. Recent advances in smart biotechnology: hydrogels and nanocarriers for tailored bioactive molecules depot. *Adv. Colloid Interface Sci.* 249, 163–180.
- Morris, K.F., Johnson Jr., C.S., 1993. Resolution of discrete and continuous molecular size distributions by means of diffusion-ordered 2D NMR spectroscopy. *J. Am. Chem. Soc.* 115 (10), 4291–4299.
- Pelta, M.D., Morris, G.A., Stchendorff, M.J., Hammond, S.J., 2002. A one-shot sequence for high-resolution diffusion-ordered spectroscopy. *Magn. Reson. Chem.* 40, S147–S152.
- dos Santos, S., Medronho, B., dos Santos, T., Antunes, F.E., 2013. Amphiphilic molecules in drug delivery systems. *Drug Delivery Systems – Advanced Technologies Potentially Applicable in Personalized Treatment.* Springer.
- Sehgal, P., Sharma, M., Wimmer, R., Larsen, K.L., Otzen, D.E., 2006. Interactions between anionic mixed micelles and  $\alpha$ -cyclodextrin and their inclusion complexes: conductivity, NMR and fluorescence study. *Colloid Polym. Sci.* 284, 916–926.
- Szejtli, J., 1998. Introduction and general overview of cyclodextrin chemistry. *Chem. Rev.* 98, 1743–1754.
- Tanner, J.E., 1970. Use of the stimulated echo in NMR diffusion studies. *J. Chem. Phys.* 52, 2523–2526.
- Tewes, F., Brillault, J., Couet, W., Olivier, J.-C., 2008. Formulation of rifampicin-cyclodextrin complexes for lung nebulization. *J. Control. Release* 129 (2), 93–99.
- Thi, T.H.H., Azaroual, N., Flament, M.-P., 2009. Characterization and in vitro evaluation of the formoterol/cyclodextrin complex for pulmonary administration by nebulization. *Eur. J. Pharm. Biopharm.* 72 (1), 214–218.
- Valente, A.J., Söderman, O., 2014. The formation of host-guest complexes between surfactants and cyclodextrins. *Adv. Colloid Interface Sci.* 205, 156–176.
- Woessner, D.E., 2001. NMR relaxation of spin-3/2 nuclei: Effects of structure, order, and dynamics in aqueous heterogeneous systems. *Concepts Magn. Reson.* 13, 294–325.
- Yan, Y., Jiang, L., Huang, J., 2011. Unveil the potential function of CD in surfactant systems. *PCCP* 13, 9074–9082.