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**Physicochemical characterization and toxicity evaluation of steroid-based surfactants  
designed for solubilization of poorly soluble drugs**

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## 1. Introduction

More than 40 % of new drug candidates are water-insoluble and require a large body of formulation when intended for intravenous injection. To overcome their water-insolubility, surfactants are often used (Francis, Lavoie et al., 2003; Narang, Delmarre et al., 2007). Indeed, surfactants are amphiphilic molecules able to self-assemble into water, forming nanostructures like micelles. These micelles are composed of a hydrophilic shell surrounding a hydrophobic core where lipophilic drugs can be solubilized (Francis, Piredda et al., 2003). Unfortunately, a large number of surfactants are cytotoxic due to their ability to disrupt cell membranes. To circumvent this issue, it is important to develop innovative surfactants with low toxicity, biocompatibility and a high ability to solubilize poorly water-soluble drugs (Brito, Marques et al., 2009).

In this study, surfactants were designed by linking naturally occurring hydrophobic and hydrophilic moieties. Cholesterol was first chosen as hydrophobic moiety since it can be obtained from natural sources and several studies report its self-assembling capacity when linked to hydrophilic moieties such as polyethylene glycol (PEG) (Xu, Wang et al., 2010), an anti-HIV nucleoside analogue (Jin, Xing et al., 2009) or oligopeptides (Guo, Tandiono et al., 2008). Bile acids were also used as hydrophobic moieties. They are naturally synthesized in the liver and they are able to form micelles (Matsuoka, Maeda et al., 2003; Moroi and Okabe, 2000; Mukerjee, Moroi et al., 1984). Among the different bile acids, we have chosen ursodeoxycholic acid (UDCA) which has been already used in humans to efficiently dissolve cholesterol gallstones without exhibiting hepato-toxicity (Makino and Nakagawa, 1978; Nakagawa, Makino et al., 1977). Hydrophilic moieties were linked to cholesterol and UDCA using a chemical reaction analogous to the conversion of bile acids into more hydrophilic conjugates that takes place in the liver. These conjugates are excreted into the intestine via the bile duct. This chemical reaction converts hydrophobic bile acids, into water-soluble forms by conjugation to taurine or glycine (Kaler, Morgan et al., 1998; Lee, Kim et al., 2010; Mano, Nishimura et al., 2002). The aim of the conjugation is to prevent bile acids precipitation in presence of acids or divalent cations and to increase their reabsorption in the ileon (Lee, Kim et al., 2010). Our aim is to mimick natural bile acids conversion and to design potentially non-toxic surfactants where conventional headgroups are replaced by natural molecules. In this context, we decided to use amino acid-based surfactants since they have attracted great interest, being more biocompatible than surfactants with traditional headgroups, such as

quaternary ammonium (Sanchez, Mitjans et al., 2007; Vives, Macián et al., 1997). In this study, four surfactants were synthesized. Cholesterol was conjugated to glutamic acid, or to a polypeptide: cholesteryl-poly[*N*-2-hydroxyethyl-L-glutamine] (PHEG). Alternatively, ursodeoxycholic acid (UDCA) was covalently linked to polypeptides such as poly-L-glutamic acid (PGA) or PHEG. Surfactants self-assembly, cytotoxicity and solubilization properties were evaluated and compared to those of polysorbate 80, a reference solubilizer known to induce toxic effects above a certain concentration (Engels, Mathot et al., 2007).

## **2. Materials and methods**

### *2.1 Materials*

Cholesteryl-glutamic acid was synthesized by Technologie Servier. Cholesteryl-PHEG, UDCA-PGA and UDCA-PHEG were synthesized and purchased from Ghent university, Polymer chemistry and biomaterials group. Polysorbate 80 was obtained from Seppic. All surfactants chemical formulas and molecular weights are reported in Table 1. Pyrene, triton X-100 and phosphate buffer saline (PBS) were provided by Sigma-Aldrich. DMSO was purchased from Carlo Erba. Water was purified using a RIOS/synergy system from Millipore. Acetonitrile and methanol gradient grade for liquid chromatography and ammonia solution 25 % for analysis were obtained from VWR. Ammonium formate was provided by Fluka analytical.

### *2.2 Fluorescence spectroscopy: CAC determination*

Pyrene was used as a fluorescent hydrophobic probe to determine the critical aggregation concentration (CAC) of surfactants. Pyrene would preferentially partition into hydrophobic cores of micelles with a concurrent change of the photophysical properties. Experiments were performed using a LS50B spectrofluorimeter (PerkinElmer). A pyrene stock solution ( $2 \times 10^{-4}$  mol/l) was prepared in acetone. A 10  $\mu$ l aliquot of this solution was introduced into empty vials and the solvent was evaporated at 37°C for 1 h. After evaporation, vials were filled with 1 ml of surfactant aqueous solution at pH 7.4 at a fixed concentration and gently stirred overnight at ambient temperature to ensure pyrene incorporation into micelles. Experiments were performed in triplicate. Samples were protected from light throughout the experiment. Samples were excited at  $\lambda = 335$  nm and pyrene emission spectra were recorded from 370 to

400 nm wavelength. Both excitation and emission slit widths were 2.5 nm. Pyrene emission spectra presented vibronic peaks at  $\lambda_1 = 372$  nm (intensity  $I_1$ ) and  $\lambda_3 = 392$  nm (intensity  $I_3$ ). The fluorescence intensity ratio ( $I_1/I_3$ ) was calculated and plotted as a function of surfactant concentration. Experimentally, the CAC was determined from the inflection point of the plot of the fluorescence intensity ratio versus the surfactant concentration (semi-log plot). Using linear regression, the equations describing the two linear parts of the plot were established. The CAC was then obtained from the intersection of these two lines.

### *2.3 Nano-assemblies size distribution*

Size of nano-assemblies formed in solution was measured by dynamic light scattering (DLS) using a Nanophox from Sympatec<sup>®</sup>. Results are presented as a function of intensity. Size measurements were performed in triplicate with surfactant suspensions prepared in purified water.

### *2.4 In vitro toxicity assays*

Human Umbilical Vein Endothelial Cells (HUVEC) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing L-glutamine and a high glucose concentration (4.5 g/l), supplemented with 10 % Fetal Bovine Serum (FBS) and antibiotics (5 IU/ml penicillin and 50 IU/ml streptomycin). They were maintained as an adherent culture and were grown as a monolayer in a humidified incubator, with 5 % CO<sub>2</sub> at 37°C in Petri dishes. DMEM and FBS were purchased from Lonza.

#### *2.4.1 Cell viability assay*

Surfactant toxicity was evaluated using the 3-[4,5-dimethylthiazol-2-yl]-3,5-diphenyl tetrazolium bromide (MTT) assay. This colorimetric test measures the mitochondrial dehydrogenase cell activity, an indicator of cell viability. This assay is based on the reduction, by living cells, of the tetrazolium salt, MTT, which forms a blue formazan product. HUVEC were seeded onto 96 well plates with 200  $\mu$ l medium per well at a density of 40000 cells/well for 24 h. Then, 10  $\mu$ l of surfactant solution at the desired concentrations were added into each well in triplicate and plates were incubated for 72 h in a humidified incubator (5 % CO<sub>2</sub>) at 37°C. After incubation, 20  $\mu$ l of MTT solution (5 mg/ml in PBS) were added into each well,

except for negative control. Cells were further incubated for 2.5 h. After this incubation time, MTT containing media were removed and replaced with 200  $\mu$ l of dimethylsulfoxide in order to dissolve the blue formazan product. MTT absorbance was measured at 570 nm wavelength with a microplate reader (Metertech  $\Sigma$  960, Fisher Bioblock, France). Absorbance value was proportional to the number of living cells.

#### 2.4.2 Membrane damage assay

Lactate dehydrogenase (LDH) is a cytosolic enzyme. During the experiment, a release of LDH in the extracellular medium is relevant of an alteration of cell membrane. LDH catalyzes the oxidation of lactate to pyruvate with the parallel reduction of  $\text{NAD}^+$  into nicotinamide adenine dinucleotide plus hydrogen (NADH). In turn, NADH reacts with an assay reagent (tetrazolium salt) to yield a red formazan product. LDH escaping from the cytoplasm compartment to the extracellular medium was measured through the formation of the red formazan product in medium, measured at 492 nm using a UV/VIS spectrophotometer. LDH was also measured in total cells after removal of medium, washing of cells with medium and solubilization of HUVEC with 1 % (w/v) triton X-100 after an incubation time of 4 h. The fraction of LDH release was then used as an index of cellular membrane integrity and calculated by the equation below, where 'LDH in sample' is LDH released in external medium after treatment with surfactants, 'LDH 0 %' is LDH present in culture medium and 'LDH 100 %' is LDH released after treatment with 1 % (w/v) triton X-100 corresponding to 100 % cell lysis. The activity of LDH was determined using a LDH kit (Promega).

$$\text{LDH release (\%)} = \frac{(\text{LDH in sample} - \text{LDH 0 \%})}{(\text{LDH 100 \%} - \text{LDH 0 \%})} \times 100$$

#### 2.4.3 Haemolytic assay

Blood samples (4.5 ml) were collected in citrated tubes (0.105 M) (BD Vacutainer<sup>®</sup>) from Wistar rats (250–350 g, Charles River Laboratories) by cardiac puncture, under anesthesia with sodium pentobarbital (50 mg/kg). Blood was then centrifuged (2000 rpm, 10 min) with a JOUAN MR1812 centrifuge to isolate red blood cells (RBCs). The erythrocyte suspension was washed twice and dispersed in isotonic PBS buffer (123.3 mM NaCl, 22.2 mM  $\text{Na}_2\text{HPO}_4$  and 5.5 mM  $\text{KH}_2\text{PO}_4$ , 300 mOsmol/l) at pH 7.4. All surfactant solutions were prepared in

PBS buffer in order to obtain a pH close to normal blood pH (pH 7.4). Surfactant solutions (V = 100 µl) were incubated and stirred with RBCs suspension (V = 50 µl) at 37°C for 1 h. Instead of surfactants, minimum (0 %) and maximum (100 %) haemolysis were determined in PBS (V = 100 µl) and in triton X-100, 1 % (w/v) (V = 100 µl), respectively. Each sample was prepared in triplicate. Then, 2 ml of cold PBS were added to each sample to stop haemolysis, except for the 100 % haemolysis standard. For this specific standard, 2 ml of purified water were added. The unlysed RBCs were removed by centrifugation (2000 rpm for 10 min), and the supernatant was analyzed for haemoglobin release by UV/VIS spectroscopy at 450 nm wavelength (Shimadzu). Haemolysis percentage was plotted as a function of surfactant concentration. HC<sub>50</sub>, the concentration where 50 % of RBCs are lysed, was determined for each surfactant. The haemolysis percentage was calculated by the equation below, where 'Abs', 'Abs<sub>0</sub>', and 'Abs<sub>100</sub>' were sample absorbance, control with PBS and control in the presence of haemolytic dose of triton X-100, respectively.

$$\text{Haemolysis (\%)} = \frac{(\text{Abs} - \text{Abs}_0)}{(\text{Abs}_{100} - \text{Abs}_0)} \times 100$$

### *2.5 Drug solubilization*

A number of surfactant concentrations were prepared in purified water (C > CAC). An insoluble model drug (Solubility < 0.15 µg/ml), synthesized by Technologie SERVIER, was added at 20 mg/ml. Magnetic stirring was applied during 24 h. Filtration of the excess drug crystals was then performed through a membrane filter of 0.45 µm pore size (polysulfone). The filtrate was diluted in acetonitrile/purified water (50/50, V/V). The amount of solubilized drug was quantified by HPLC system with UV detection at 250 nm.

## **3. Results and discussion**

### *3.1 Surfactant water solubility*

Water solubility of surfactants was evaluated macroscopically at pH 7.4 (Table 2). All surfactants tested were water-soluble except for cholesteryl-glutamic acid which was poorly soluble in water. Various techniques were used to increase its water solubility without any success: pH adjustments, magnetic stirring and sonication. The water solubility remained in

any case lower than 0.36 mg/ml which was not sufficient to further investigate the physico-chemical properties, cytotoxicity and drug solubilization capacity of the surfactant. The low water solubility of cholesteryl-glutamic acid is probably due to the small volume of its polar head. The chemical structure of this surfactant is composed of a steroidal ring system linked to a negatively charged amino acid, glutamic acid. From this structure, it leads to a hydrophilic/lipophilic ratio too small to obtain a high water solubility. As shown in Table 2, water solubilities of cholesteryl-PHEG, UDCA-PHEG and UDCA-PGA were higher than 100 mg/ml. Polar heads of these three surfactants are polymeric, leading to polar head volumes larger than the one of a single glutamic acid. UDCA is described as a weak acid poorly soluble in water (Igimi and Carey, 1980). Results show that grafting large polymeric polar heads to UDCA is efficient in increasing its water solubility.

### *3.2 Surfactant self-assembling capacity*

#### *3.2.1 Pyrene fluorescence measurements*

Self-assembling capacity of surfactants was investigated using pyrene fluorescence measurements to determine their CAC values at pH 7.4 (Table 2). Data were compared to those obtained for polysorbate 80 (Fig. 1). Fig. 1 presents the  $I_1/I_3$  ratio as a function of surfactant concentration (semi-log plot). The  $I_1/I_3$  ratio decreases when hydrophobic domains are present in solution as surfactant self-assemble into nano-objects. Surprisingly, as UDCA-PGA concentration increases the  $I_1/I_3$  ratio remains constant even at high concentrations (10 mg/ml) indicating that pyrene was always surrounded by a hydrophilic environment in these conditions (Fig. 1). UDCA-PGA therefore didn't self-assemble in the concentration range tested. On the contrary, UDCA-PHEG surfactant self-assembled as witnessed by the decrease of  $I_1/I_3$  ratio, leading to the determination of a CAC of 120  $\mu\text{g/ml}$ . The difference between UDCA-PHEG and UDCA-PGA probably arises from the difference in the molecular weight of hydrophilic moieties and thus to their hydrophilic lipophilic balance (HLB). The HLB of UDCA-PGA is probably too high leading to a high water solubility, therefore preventing its self-assembly. As shown in Fig. 1, cholesteryl-PHEG was able to self-assemble and its CAC value was 17  $\mu\text{g/ml}$ , close to the value obtained for polysorbate 80 (14  $\mu\text{g/ml}$ ). Low CAC values mean that the surfactant remains self-assembled under highly diluted conditions after an intravenous injection (Hu, Liu et al., 2009). Our results show that PHEG is more efficient than PGA to form self-assembled nanostructures when it is linked to UDCA. In addition, the

existence of groups where intermolecular hydrogen bonding can occur, namely the N-H groups in UDCA-PHEG, can further enhance the solubility of the hydrophilic moiety and the self-assembly of the surfactant in comparison with UDCA-PGA (Boettcher, Schade et al., 2001; Brito, Marques et al., 2006).

### 3.2.2 *Size measurements with DLS*

DLS measurements show that polysorbate 80 forms micelles with a mean diameter of 8 nm in agreement with previous DLS and scanning electron microscopy results (Heydenreich, Westmeier et al., 2003). UDCA-PGA does not form nano-objets consistently with pyrene fluorescence measurements. On the contrary, UDCA-PHEG spontaneously forms nano-objets with a mean diameter of 250 nm leading to a turbid solution, whereas cholesteryl-PHEG self-assembles into nano-objets whose mean diameter is 13 nm. The size difference may arise from the presence of two hydroxyl groups in UDCA chemical structure. Indeed, hydroxyl groups have been shown to play an important role in the aggregation process of bile salts (Seret and Bahri, 2009). Cholesterol moiety is more hydrophobic than UDCA which is a dihydroxylated bile acid. As a consequence, its high hydrophobicity leads to the formation of smaller nano-objets with cholesteryl-PHEG which probably possess more condensed hydrophobic cores. In addition, the cholesterol was linked to PHEG by the cyclohexyl alcohol, ring A, which allows the freedom of the flexible chain located at the extremity of the surfactant. This flexible chain probably plays an important role in surfactant self-assembly. Indeed, Wang *et al.* have shown that the main driving force to form the compact aggregates stems primarily from van der Waals interactions of the hydrophobic cholesteryl tails of cholesteryl-DIMEB surfactant. These interactions lead to the formation of a compact hydrophobic inner core consisting of cholesteryl moieties (Wang et al., 2011). By contrast UDCA is covalently linked to PHEG by the alkane chain, yielding a less flexible and more voluminous hydrophobic core.

## 3.3 *In vitro toxicity evaluation of surfactants*

### 3.3.1 *Cell viability assay*

In this study, HUVEC cell model was chosen to investigate the cytotoxicity of new designed surfactants because it allows the determination of surfactant interactions with endothelial

cells, constitutive of blood vessel walls. HUVEC are thus an interesting model to predict surfactant toxicity after intravenous injection. Cell viability percentage is plotted as a function of surfactant concentration (semi-log plot) (Fig. 2). Surfactant concentrations responsible for a decrease in cell viability of 50 % ( $IC_{50}$ ) are shown in Table 3 for easier comparison. For UDCA-PHEG, cell viability decreases down to 65 % for concentrations above 2.4  $\mu\text{g/ml}$  and no  $IC_{50}$  value could be determined. For UDCA-PGA, cell viability decreases down to 35 % for concentrations higher than 20  $\mu\text{g/ml}$ , leading to an  $IC_{50}$  value of 7  $\mu\text{g/ml}$ . For cholesteryl-PHEG, cell viability decreases abruptly from 90 % for 50  $\mu\text{g/ml}$ , down to 0 % for concentrations higher than 200  $\mu\text{g/ml}$ , leading to an  $IC_{50}$  of 75  $\mu\text{g/ml}$ . For polysorbate 80, cell viability decreases from 80 % for 100  $\mu\text{g/ml}$  down to 2 % (Fig. 2). Polysorbate 80  $IC_{50}$  is around 250  $\mu\text{g/ml}$  in agreement with previous studies (Hirama, Tatsuishi et al., 2004), being therefore cytotoxic at concentrations above its CAC value. For UDCA-based surfactants, the viability seems to decrease smoothly as a function of concentration with a more pronounced effect for UDCA-PGA. In addition, HUVEC viability does not seem to depend of the CAC since UDCA-PGA does not self-assemble. The difference between UDCA-PGA and UDCA-PHEG may arise from the difference of interactions of polar heads with HUVEC membrane due to the presence of the side-chain in the PHEG monomer. On the other hand, for polysorbate 80 and cholesteryl-PHEG, HUVEC viability is clearly related to self-assembly since viability decreases abruptly after the CAC is reached. Most probably, in this case, the decrease of viability arises from solubilization of HUVEC membrane. The measurement of LDH release will help confirming this hypothesis. To conclude, MTT assays show that surfactant cytotoxicity follows the following order: UDCA-PHEG < Polysorbate 80 < Cholesteryl-PHEG < UDCA-PGA.

### 3.3.2 Membrane damage assay

Toxicity of surfactants against cell membrane was assessed by a LDH assay. LDH release is presented as a function of surfactant concentration (semi-log plot) in Fig. 3. For both polysorbate 80 and cholesteryl-PHEG, LDH release is not significant below the CAC value, but it increases dramatically above the CAC. These surfactants were cytotoxic around their CAC value. This suggests that their toxic effects are in relation with a destabilization or a disruption of cell membranes (Inacio, Mesquita et al., 2011). UDCA-PGA and UDCA-PHEG have very different behaviors. LDH release occurs for UDCA-PGA concentrations higher

than 100 µg/ml with an IC<sub>50</sub> of 1450 µg/ml, whereas for UDCA-PHEG almost no release can be observed in the concentration range studied, either below or above its CAC. These results suggest that membrane destabilization induced by UDCA-PGA occurs via interaction between the PGA moiety and the HUVEC membrane. The UDCA hydrophobic part could be inserted in cell membranes without leading to a LDH release. Indeed, Güldütuna *et al.* demonstrated that similarly to cholesterol, UDCA is able to maintain membrane structure, its stability and membrane functionalities. In addition, UDCA prevents cell membrane damages induced by mechanical or chemical stress (Güldütuna *et al.*, 1997). UDCA penetration could be partial or complete and could induce a slow toxicity. The decrease in cell viability below the CAC value for the two UDCA derivatives tends to show that this penetration of surfactant monomers actually occurs. This assay emphasizes UDCA-PHEG biocompatibility with HUVEC, an important factor to develop new surfactants intended for intravenous injection. These results corroborate the viability assays: the mechanism of toxicity differs for UDCA-based surfactants on the one hand, and for polysorbate 80 and cholesteryl-PHEG on the other hand. For the first group, toxicity arise from adsorption of surfactant monomers into cell membrane. It induces cell membrane disruption for UDCA-PGA whereas the PHEG moiety does not interact with HUVEC membrane. Cytotoxicity is thus related to the nature of polar head groups as Inacio *et al.* evidenced in their study (Inacio, Mesquita *et al.*, 2011). For the second group of surfactants, toxicity arises from membrane solubilization as attested by LDH release above surfactant CAC.

### 3.3.3 Haemolytic assay

To further assess biocompatibility of surfactants after intravenous injection, haemolytic assays were performed with rat RBCs (Fig. 4). Haemolysis assays are of extreme relevance to assess the initial and immediate damage caused by surfactants to RBCs (Brito, Marques *et al.*, 2009). A 50 % release of haemoglobin from RBCs occurs with polysorbate 80 at 760 µg/ml. Cholesteryl-PHEG also destabilizes RBCs membrane and release of haemoglobin starts for concentrations higher than 300 µg/ml. For cholesteryl-PHEG, HC<sub>50</sub> was 1250 µg/ml, higher than polysorbate 80 HC<sub>50</sub> value. With UDCA-PGA and UDCA-PHEG, no release of haemoglobin could be detected within the range of concentrations tested, proving these are indeed compatible for intravenous injection. The discrepancy between LDH and haemolytic assays may arise from a difference in lipid composition of cell membranes. Cholesterol does not possess a haemolytic activity. However our study evidenced the haemolytic activity of

cholesteryl-PHEG whereas cholesteryl-poly (ethylene glycol) studied by Baba *et al.* is reported as a non haemolytic material (Baba *et al.*, 2004). The binding of PHEG to cholesterol thus probably modifies the surfactant interaction with RBCs, as evidenced in this study. Furthermore, cholesterol and UDCA were not linked to PHEG by the same side. In the case of cholesteryl-PHEG, the side chain of cholesterol was free which could favor its incorporation into RBCs membrane due to its higher flexibility (Folmer, 2003).

### 3.4 Drug solubilization capacity

The ability of surfactants in increasing water solubility of an anticancer drug was finally evaluated. The drug tested is insoluble in water ( $< 0.15 \mu\text{g/ml}$ ), according to solubility specifications of the European Pharmacopoeia 7.2 (Insoluble drug: solubility  $< 0,1 \text{ mg/ml}$ ). Fig. 5 shows drug solubilization results obtained with the four surfactants. In addition, Table 4 shows solubilization results for a fixed surfactant concentration of 50 mg/ml and presents corresponding drug loadings. Polysorbate 80 is able to solubilize the drug up to a concentration of 17.3 mg/ml ( $\times 100000$ ) whereas cholesteryl-PHEG increases drug solubility up to 2 mg/ml ( $\times 15000$ ). UDCA-PHEG and UDCA-PGA are far less efficient since drug solubility only reaches concentrations of 204 ( $\times 1500$ ) and 28  $\mu\text{g/ml}$  ( $\times 200$ ) respectively. In addition, after drug solubilization, the size of the nano-objects was close to 200 nm in the case of polysorbate 80, cholesteryl-PHEG and UDCA-PHEG, with no macroscopic increase of solution viscosity (Table 2). The size of these nano-objects, lower than 1  $\mu\text{m}$ , is fully compatible with an intravenous injection. Drug solubility enhancement can be related to the molecular structure of surfactants and to their self-assembling ability. UDCA-PGA that does not self-assemble is unefficient in increasing drug solubility. For PHEG-based surfactants that self-assemble, cholesterol seems more efficient than UDCA in increasing drug solubilization, although their chemical structures are very similar. However UDCA is slightly less hydrophobic than cholesterol due to the presence of two hydroxyls groups bonded to aromatic rings. As stressed out above, although cholesterol and UDCA are both steroids, cholesterol was linked to PHEG by the cyclohexyl alcohol, ring A, whereas PGA is linked to the carboxylic side chain of ring D. Therefore, UDCA-PHEG presents itself with a rigid and bulky tetracyclic end, whereas cholesteryl-PHEG ends with a small and flexible alkyl chain. The high flexibility of this part of the molecule might play an important role in drug solubilization process. Although cholesteryl-PHEG allows to increase solubility, this

surfactant remains less efficient than polysorbate 80. In addition, cholesteryl-PHEG is either as toxic as polysorbate 80 or even more toxic depending on the toxicity assay.

#### 4. Conclusion

Innovative surfactants made of naturally occurring moieties were successfully designed and synthesized. PGA hydrophilic moiety does not seem to be favorable to self assembly whereas PHEG moiety leads to surfactants with low CAC values. From a toxicological point of view, cholesteryl-PHEG possesses a marked cytotoxicity and haemolytic activity whereas UDCA-PHEG is non-toxic and non-haemolytic. However, UDCA-PHEG does not lead to a significant increase of drug solubility (200 µg/ml). Although cholesteryl-PHEG allows to increase solubility up to 2 mg/ml, this surfactant remains less efficient than polysorbate 80. In addition, cholesteryl-PHEG is either as toxic as polysorbate 80 or even more toxic depending on the assay.

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