European Journal of Pharmaceutics and Biopharmaceutics 114 (2017) 305–316

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

A glucose-targeted mixed micellar formulation outperforms Genexol in breast cancer cells

Marcela A. Moretton [a,d], Ezequiel Bernabeu [a,d], Estefanía Grotz [a,d], Lorena Gonzalez [b,d], Marcela Zubillaga [c,d], Diego A. Chiappetta [a,d,*]

[a] Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Tecnología Farmacéutica I, Buenos Aires, Argentina
[b] Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Química Biológica, Buenos Aires, Argentina
[c] Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Física, Buenos Aires, Argentina
[d] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

ARTICLE INFO

Article history:
Received 15 December 2016
Revised 3 February 2017
Accepted in revised form 8 February 2017
Available online 10 February 2017

Keywords:
Paclitaxel
Polymeric mixed micelles
Active targeting
Glycosylated nanocarriers
Breast cancer chemotherapy

ABSTRACT

Breast cancer represents the top cancer among women, accounting 521.000 deaths per year. Development of targeted nanomedicines to breast cancer tissues represents a milestone to reduce chemotherapy side effects. Taking advantage of the over-expression of glucose (Glu) membrane transporters in breast cancer cells, we aim to expand the potential of a paclitaxel (PTX)-loaded mixed micellar formulation based on polyvinyl caprolactam-polyvinylacetate-polyethylene glycol graft copolymer (Soluplus®) and D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) by its surface decoration with Glu moieties. The glycopolymer (Soluplus(Glu)) was obtained by microwave-assisted ring opening reaction of δ-gluconolactone initiated by Soluplus®. The glycosylation was confirmed by ^{1}H NMR and by agglutination assays employing Concanavalin A. The hydrodynamic diameter of Soluplus(Glu) micelles was characterized by dynamic light scattering (100.3 ± 3.8 nm) as well as the critical micellar concentration value (0.0151% w/v). Then, a mixed micelle formulation employing Soluplus®, Soluplus(Glu) and TPGS (3:1:1 wt ratio) loaded with PTX (4 mg/mL) was developed as a multifunctional nanocarrier. Its *in vitro* anticancer performance in MCF-7 (1.6-fold) and MDA-MB-231 (14.1-fold) was significantly enhanced ($p < 0.05$) *versus* the unique commercially available micellar-based PTX-nanoformulation (Genexol®). Furthermore, the *in vitro* PTX cellular uptake assays revealed that the drug intracellular/cell content was significantly ($p < 0.05$) higher for the Glu-containing mixed micelles *versus* Genexol® after 6 h of incubation with MCF-7 (30.5-fold) and MDA-MB-231 (5-fold). Overall, results confirmed the potential of our Glu-decorated mixed colloidal formulation as an intelligent nanocarrier for PTX-targeted breast cancer chemotherapy.

1. Introduction

Worldwide breast cancer is the top cancer among women. Actually, there are 1.67 million new cases and 521,000 deaths from breast cancer each year [1–3]. Nowadays breast cancer therapy involves a "multimodal approach" where chemotherapy remains of vital importance to achieve control or cure of this disease [2,4].

Paclitaxel (PTX) represents one of the most effective antineoplastic drugs with proven activity against a wide variety of malignancies (e.g. breast and ovarian) [5,6]. Due to its poor aqueous solubility (0.3–0.5 μg/mL), PTX is commercially available as Taxol® which has become "a non-patient friendly formulation". It is associated with severe side effects attributable to a surfactant additive (Cremophor EL®) [6,7].

In this context, nanotechnology offers a wide variety of nanotechnological platforms to overcome Taxol® clinical limitations. Among them, an albumin nanoparticle-based formulation (Abraxane®) for recurrent metastatic breast cancer, has been on the spot in the last decade [8]. Also a liposome-based formulation has been approved for its clinical use in China (Lipusu®) against different malignancies [9]. Recently, a polymeric PTX nanoformulation based on polymeric micelles has been approved for its clinical use (Hungria, Bulgaria and South Korea) employing monomethoxy-poly(ethylene glycol)-b-poly(d,l-lactide) as micelle former biomaterial (Genexol®) [10].

These nanotechnological strategies were focused on Cremophor EL® replacement for a safer PTX intravenous administration.

* Corresponding author at: Departmento de Tecnología Farmaceútica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, 956 Junín St., 6th Floor, Buenos Aires CP1113, Argentina
E-mail address: diegochiappetta@yahoo.com.ar (D.A. Chiappetta).

http://dx.doi.org/10.1016/j.ejpb.2017.02.005
0939-6411/

However, other drawbacks are still involved in chemotherapy outcome. Among them, acquired PTX resistance represents one of the main clinical challenges to be coped.

Polymeric micelles represent an attractive nanotechnological platform to enhance aqueous solubility and bioavailability of poorly water-soluble/unstable drugs. These nano-sized carriers result from the self-aggregation of amphiphilic polymers in water upon their critical micellar concentration value [11–14]. Also, the development of mixed micelles combining different types of micelle-former biomaterials was explored. Indeed, parameters as solubility and stability of drug encapsulated within mixed micelles can be enhanced in comparison with single micelles [15–20].

Since cancer chemotherapy is associated with a wide variety of adverse effects, efforts have been directed to the possibility of "target" a certain cancer tissue or solid tumor. In this context, micellar nanocarriers could be used for passive drug targeting into solid tumors, mainly associated with the enhanced permeability and retention (EPR) effect [21,22]. However, the possibility to specifically target (due to ligand–receptor interactions) genes and antineoplastic drugs to certain cancer tissues/cells represents a milestone in cancer chemotherapy.

One of the main characteristics of cancer cells is their increased glucose (Glu) uptake mediated by the over-expression of Glu transporter membrane proteins (GLUTs1-14). Particularly, breast cancer tissues have demonstrated high expression of GLUT1 in association with metastasis [23,24]. This phenomenon suggests that GLUT might be an efficient target for drug delivery to breast tumor tissues. Hence, an active PTX targeting to breast cancer cells could be approached by the surface decoration of polymeric micelles with Glu residues. Surprisingly, to the best of our knowledge, there is no active targeted PTX nanoformulation on current clinical trials [25].

Previously, we developed PTX-loaded mixed micelles employing commercially available biomaterials: polyvinyl caprolactam–polyvinylacetate–polyethylene glycol graft copolymer (Soluplus®) and D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) (4:1 wt ratio). Our nanoformulation demonstrated an improved *in vitro* anticancer activity in breast and ovarian cancer cell lines [26]. In this work, we further expand their potential by incorporating Glu moieties (targeting unit) into the hydrophilic micellar corona. First, we synthesized a glycopolymer (Soluplus(Glu)) by means of ring opening reaction of δ-gluconolactone initiated by Soluplus®. Then, a mixed micellar formulation employing Soluplus®, Soluplus (Glu) and TPGS (3:1:1 wt ratio) loaded with PTX (4 mg/mL) was developed as a multifunctional nanocarrier. Its *in vitro* citotoxicity performance as well as the PTX cellular uptake was investigated in two human breast cancer cell lines (MCF-7 and MDA-MB-231) and the results were compared with those observed for the Glu-free mixed micelles and Genexol®.

2. Materials and methods

2.1. Materials

Paclitaxel (PTX, 99.9%) was purchased from RhenochemAG (Basel, Switzerland), D-α-tocopheryl polyethylene-glycol (PEG) 1000 succinate (TPGS, MW ~1513 g/mol) was from Eastman Chemical Company (Kingsport, TN, USA) and polyvinyl caprolac tam–polyvinylacetate–PEG (Soluplus®, MW ~ 120.000 g/mol) was from BASF (CABA, Argentina). δ-Gluconolactone (Glu; 1,2,3,4,5-pentahydroxycaproic acid δ-lactone, MW = 178.14 g/mol), tin(II) 2-ethylhexanoate ($Sn(Oct)_2$, 95%), bovine serum albumin (BSA), concanavalin A (Con A, from *Canavalia ensiformis*, Jack Bean Type VI) were purchased from Sigma–Aldrich (CABA, Argentina). Tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyme thoxyphenyl)-2-(4-sulfophenyl)-2Htetrazolium], inner salt (MTS) and phenazinemethosulfate (PMS) were purchased from Promega Corporation (Madison, Wisconsin, USA). All solvents were of analytical or high performance liquid chromatography (HPLC) grade and were used following the manufacturer's instructions.

2.2. Glycosylation of Soluplus® copolymer

Copolymer conjugation with Glu was assessed by means of a microwave-assisted ring opening reaction of δ-gluconolactone in presence of Soluplus® (–OH terminal), as previously reported for PEG-based copolymers employing epsilon-caprolactone [4,27–29]. Briefly, Soluplus® (5 g) and Glu (42.7 mg, 15% molar excess) were dissolved in dimethylformamide (10 mL) under magnetic stirring (100 rpm). The mixture was poured into a 250 mL round-bottom flask and dried under vacuum (100–110 °C, glycerol bath, 3 h) before used. Then, $Sn(Oct)_2$ (catalyst, 13.5 μL 1:1 molar ratio to Soluplus®) was added to the mixture and the round-bottom flask was poured in the center of a household microwave oven (Whirlpool®, WMD20SB, microwave frequency 2450 MHz, potency 800 W, Argentina) with ten power levels, adapted in the laboratory to enable the connection of a condenser. The reaction mixture was exposed to microwave radiation for 1 min (power level 2) and 14 min (power level 1) accounting a total reaction time of 15 min. Then the crude was diluted with distilled water (10 mL) and dialyzed (Spectra/Por® Dialysis Membrane, molecular weight cut off = 3500, nominal flat width 45 mm, USA) against distilled water for 3 d to remove unreacted Glu. Finally, the polymer dispersion was frozen (−20 °C, 24 h) and lyophilized (48 h; condenser temperature of −40 °C and 30 μbar pressure; FIC-L05, FIC, Scientific Instrumental Manufacturing, Argentina). The glycosylated Soluplus® copolymer was denoted as Soluplus(Glu).

2.3. Characterization of Soluplus(Glu) copolymer

In order to characterize the Soluplus® conjugation with Glu, the proton nuclear magnetic resonance (^{1}H NMR) spectra of Soluplus (Glu) was obtained from deuterated chloroform (Sigma–Aldrich, CABA, Argentina) solution at room temperature on a Bruker MSL300 spectrometer (Karlsruhe, Germany), at 300 MHz. Then, Soluplus® was dialyzed against distilled water for 3 d (as previously described for Soluplus-Glu) and its ^{1}H NMR spectra in deuterated chloroform was obtained for comparison.

Further, the critical micellar concentration (CMC) values of Soluplus® and Soluplus(Glu) were determined by dynamic light scattering (DLS, Zetasizer Nano-ZSP, ZEN5600, Malvern Instruments, Worcestershire, United Kingdom). Measurements were conducted at a scattering angle of θ = 173° to the incident beam. Twelve polymer dispersions (between 0.3×10^{-6} and 3% w/v) were prepared in distilled water, equilibrated at 25 °C overnight and analyzed by DLS. The derived count rate was plotted as function of the polymer concentration (% w/v) where the CMC value corresponded to the polymer concentration at which a sharp increase in the scattering intensity was observed [16].

Also the average micellar hydrodynamic diameter (D_h), size distribution (polydispersity index, PDI) and zeta potential of Soluplus® and Soluplus(Glu) dispersions were characterized by DLS as previously described. Colloidal systems were obtained by polymer (3% w/v) dispersion in distilled water under magnetic stirring at 25 °C over 2 h. Then samples were equilibrated for 24 h before the analysis. Before each measurement, samples were equilibrated for 5 min at 25 °C and results were expressed as mean ± standard deviation (S.D.), n = 5.

2.4. Agglutination assays

To further characterize the Soluplus® conjugation with Glu, an agglutination assay was performed employing a water-

soluble carbohydrate-binding lectin (Con A) [30]. Briefly, Soluplus(Glu) micelles (5% w/v) were prepared by polymer dispersion in phosphate buffer (USP 30, pH 7.4) and they were stabilized for 24 h at 25 °C before use. Then BSA (60 mg) was added to the micellar dispersions (2 mL) and samples were vortexed (1 min) and incubated at 25 °C for 30 min. Next, samples were diluted (1/2) with Con A phosphate buffer solution (pH 7.4, 10 μM) and magnetically stirred for 2 h at 25 °C. Micelle agglutination was visualized by transmission electron microscopy (TEM, Philips CM-12 TEM apparatus, FEI Company, The Netherlands). Aliquots (5 μL) were placed onto a clean grid and covered with a Fomvar film. Then, sample was negatively stained with 5 μL of phosphotungstic acid solution (1% w/v), washed with distilled water (5 μL) and dried into a silicagel container before the analysis. Soluplus®-based micelles were used as controls.

Additionally, micelle agglutination was confirmed by a complementary technique as DLS. Briefly, Soluplus® and Soluplus (Glu) micelles were prepared in phosphate buffer pH 7.4 and BSA (60 mg) was added to the colloidal dispersions (2 mL). Samples were incubated at 25 °C for 30 min, diluted (1/2) with a Con A phosphate buffer solution (pH 7.4 10 μM) and magnetically stirred for 6 h at 25 °C. Finally, samples were equilibrated for 5 min at 25 °C and the micellar size and size distribution results were expressed as mean ± standard deviation (S.D.), n = 5.

2.5. Preparation and characterization of PTX-free and PTX-loaded glycosylated mixed micelles

The CMC values of the mixed micelles with and without Glu were determined by DLS as previously described on Section 2.3. In this case, twelve polymer dispersions (between 0.5×10^{-6} and 5% w/v) were prepared in distilled water and equilibrated at 25 °C overnight before the assay.

Glycosylated mixed micelles (5% w/v) were prepared by dispersion of the appropriate amount of each polymer (Soluplus®, Soluplus(Glu), and TPGS; 3:1:1 wt ratio) in distilled water under magnetic stirring at 25 °C for 2 h. Then samples were equilibrated for 24 h at 25 °C before use. Glycosylated mixed micelles were denoted as Glu-mixed micelles. For comparison, Glu-free mixed systems were prepared as previously described employing only Soluplus® and TPGS (4:1 wt ratio). These mixed micelles were denoted as Glu-free mixed micelles.

Encapsulation of PTX within Glu-mixed micelles was obtained as reported elsewhere by an acetone diffusion technique [26]. Briefly, a PTX solution in acetone (30 mg/mL, 1.33 mL) was added drop wise to the mixed micellar dispersion (10 mL) under magnetic stirring (500 rpm, 10 h) at 25 °C. Then, micellar dispersions were frozen (−20 °C) and lyophilized (48 h, condenser temperature of −40 °C and 30 μbar pressure; FIC-L05, FIC, Scientific Instrumental Manufacturing, Argentina).

PTX concentration was determined by a validated RP-HPLC method [31]. The analytical method consisted in a Fluophase PFP reverse phase C18 column (4.6 mm × 250 mm, 5 μm, Thermo, USA) with a mobile phase composed of a mixture of acetonitrile: water (50:50, v/v) eluted at a flow rate of 1 mL/min. The detection was performed at 227 nm (UV-Detector, Shimadzu SPD-10A, Japan), employing an injection volume of 20 μL at room temperature. The same procedure was followed to obtain PTX-loaded Glu-free mixed micelles.

Finally, the morphology of PTX-loaded (4 mg/mL) Glu-mixed micelles was investigated by means of TEM as described on Section 2.4. Samples (5% w/v, 5 μL) were negatively stained with 5 μL of phosphotungstic acid solution (1% w/v).

2.6. Micellar size and size distribution

The D_h values, size distribution and zeta potential of PTX-free and PTX-loaded (4 mg/mL) Glu-mixed micelles in aqueous media were measured by DLS. Samples were equilibrated for 24 h before the analysis at 25 °C and they were equilibrated for 5 min at 25 °C before each measurement. Results were expressed as mean ± standard deviation (S.D.), n = 5. Glu-free mixed micelles were used as controls.

2.7. Micellar physical stability under administration conditions

Micellar size and size distribution (25 °C) of PTX-loaded (4 mg/mL) nanoformulations (Glu-mixed and Glu-free mixed micelles) diluted (1/10) in normal saline solution (ClNa 0.9% w/v) and in dextrose 5% w/v was investigated over 30 min as an attempt to characterize its physical stability after commonly preparation conditions for clinical PTX intravenous administration [32]. Results are expressed as mean ± standard deviation (S.D.), n = 3.

2.8. In vitro PTX release

The PTX *in vitro* release profiles from Glu-mixed micelles, Glu-free mixed micelles and Genexol-PM® were investigated by using a dialysis method. Briefly, freeze-dried systems were re-dispersed in distilled water (PTX concentration 4 mg/mL), diluted (1/10) with distilled water and aliquots (100 μg) were placed into dialysis membranes (Spectra/Por®3 Dialysis Membrane, molecular weight cut off = 3500, nominal flat width 18 mm, USA) which were sealed and placed in Falcon® conical tubes (50 mL) containing the release medium (phosphate buffer USP 30 pH 7.4 with Tween 80 0.5% v/v, 50 mL). The tubes were maintained at 37 ± 1 °C in an orbital water bath shaking at 40 rpm for 96 h. The release medium (50 mL) was sampled at predetermined time intervals (2, 4, 8, 24, 48, 72 and 96 h) and replaced with equal volume of fresh medium preheated at 37 °C. Then, PTX content was determined by RP-HPLC as previously described above in Section 2.5. Results are expressed as mean ± standard deviation (S.D.), n = 3.

2.9. Cell culture

MCF-7 and MDA-MB 231 human cancer cell lines were obtained from the American Type Culture Collection (ATCC) (Rockville, MD, USA). Cells were maintained in Dulbecco's minimum essential medium (DMEM®, supplemented with 50 μg/mL gentamycine, 2 mM l-glutamine (Invitrogen, Argentina) and 10% fetal bovine serum (FBS)) in an incubator (5% CO_2, MCO-19AIC(UV), SANYO Electric Co., Biomedical Division, SANYO, Japan) at 37 °C.

2.10. In vitro citotoxicity assays

Cells were seeded in clear 96-well plates (Corning Costar, Fisher Scientific, USA) at a density of 5000 cells/well and incubated for 24 h. Then, cells were incubated with free-loaded mixed micelles (without and with Soluplus(Glu)), PTX-loaded Glu-free mixed micelles, PTX-loaded Glu-mixed micelles and Genexol® for 72 h. After incubation, the medium was removed, the wells were washed with PBS and fresh medium was added. Finally, the water soluble tetrazolium salts (WST) solution (CellTiter 96®aqueous non-radioactive cell proliferation assay, Promega) was added and cells were incubated for 2 h. The absorbance at 490 nm was measured using a microplate reader (ELx800™ Absorbance Microplate Reader, BioTek® Instruments, USA). PTX concentrations leading to 50% cell-killing (IC_{50}) were determined from the concentration-dependent cell survival curves. Values were expressed in terms of percent of untreated control cells set as 100%. Blank TPGS

micelles (1% w/v) were assayed for comparison. Results are expressed as mean ± standard deviation (S.D.), n = 3.

2.11. In vitro cellular uptake assays

Quantitative cellular uptake of PTX encapsulated within Glu-mixed micelles, Glu-free mixed micelles and Genexol® was investigated in two different breast cancer cell lines: MCF-7 and MDA-MB 231. Briefly, MCF-7, MDA-MB231 cells suspensions were seeded in 6-well plates to yield 4×10^5 cells/well. Then each plate was incubated (37 °C, 5% CO_2, MCO-19AIC (UV), SANYO Electric Co., Biomedical Division, SANYO, Japan) for 24 h to allow cell attachment. The cells were then incubated with PTX, PTX-loaded mixed micelles (with and without Glu) and Genexol-PM® at a concentration of 25 μg/mL PTX for 0.5, 2, 4 and 6 h. At every time-point, cells were rinsed with PBS (1 mL/well, pH 7.4) and 0.25 mL Trypsin-EDTA (0.25% w/v) was incorporated. Supernatants were collected, incubated at 8 °C for 10 min and then centrifuged (13,000 rpm, 10 min, MiniSpin®plusTM, Eppendorf, Germany). The protein content in the cell lysate (50 μL) was determined using Bradford assay. After centrifugation (13,000 rpm, 10 min, MiniSpin®plusTM, Eppendorf, Germany), the drug content in the supernatants (150 μL) were measured by HPLC (as described above) and values were normalized by protein content in each sample. Results are expressed as mean ± standard deviation (S.D.), n = 3.

2.12. Statistical analysis

Statistical analysis was performed was performed by one-way ANOVA test and Newman-Keuls multiple comparisons post-hoc test using GraphPadPrism version 6.01 for Windows (GraphPad Software, USA). Results were considered statistically different if $p < 0.05$. Assays were done by triplicate and results expressed as mean ± S.D.

3. Results and discussion

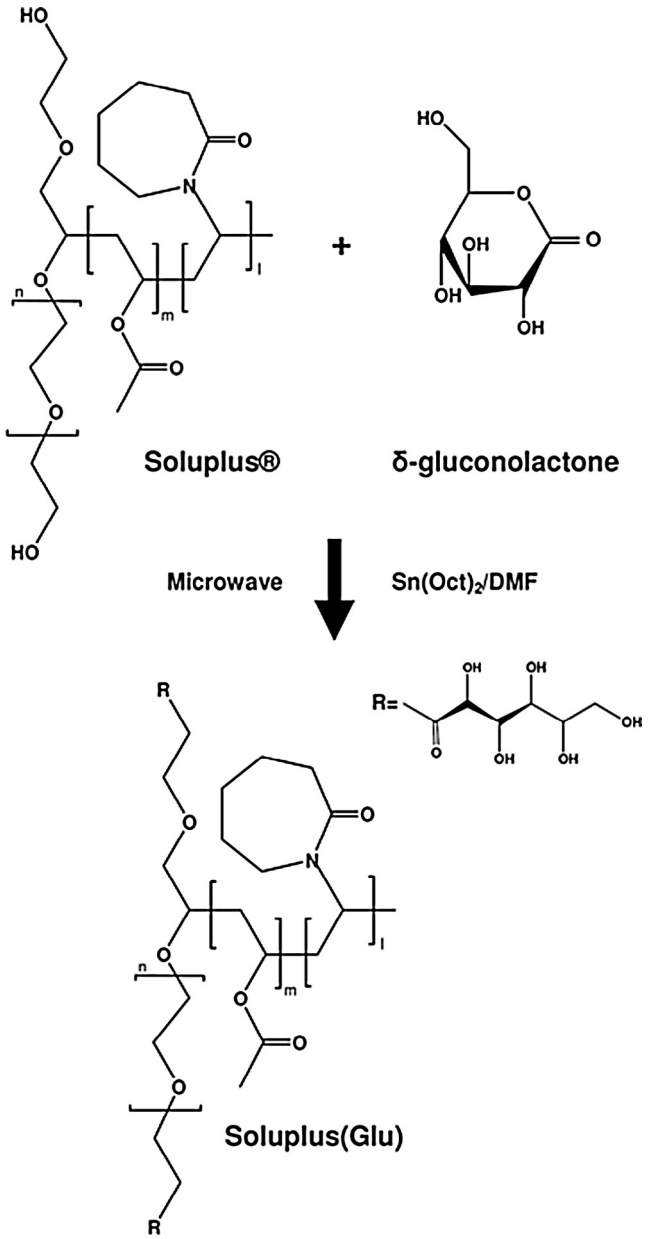
3.1. Glycopolymer preparation and characterization

Glycopolymers have been on the spot for the last decade due to the possibility of an active targeting of drugs and genes to a certain cell/tissue/organ mediated by specific ligand-receptor interactions [33–35]. Particularly, in cancer chemotherapy, efforts have been directed to develop drug delivery nanosystems surface decorated with ligands that could interact with a receptor over-expressed in a certain tumor tissue [36–38]. Among these receptors, the family of GLUT1-14 integral membrane proteins, which mediates the transport of carbon compounds as monosacharides in almost every human cell type [39], has been founded to be over-expressed in different cancer tissues due to their requirement of continuous Glu supply [40]. This fact makes GLUT membrane transporters an excellent potential target to actively direct antineoplastic drugs. Particularly, breast cancer cells over-express GLUT1 which exhibits high affinity for Glu as its main physiological substrate [23,24,41]. Hence the conjugation of a biopolymer with Glu residues becomes an attractive strategy for the development of a potential targetable nanocarrier to optimize breast cancer chemotheraphy.

As we have already mentioned, our group developed a PTX-loaded nanoformulation based on mixed micelles for breast and ovarian cancer therapy [26]. Hereby, we aimed to synthesize a glycopolymer employing Soluplus® and Glu to develop a glycosylated PTX-loaded micellar mixed multifunctional formulation for the potential *in vitro* active targeting to breast cancer cells, independently of their hormonal dependence.

In this context, aldonolactones (especially aldono-1,4- and -1,5-lactones) has been well investigated in recent years for the generation of novel glycopolymers [42]. Previously, we have successfully synthesized di- and tri-block copolymers by the ring opening polymerization of a lactone (epsilon-caprolactone) initiated by poly (ethylene glycol) (PEG) (–OH terminal) and assisted by microwave radiation to improve the reaction performance [4,27–29]. Hence, we synthesized a glycopolymer (Soluplus(Glu)) by the ring opening reaction of the sugar lactone (δ-gluconolactone) in presence of Soluplus® (–OH terminal), employing SnOct as catalyst. The total reaction time was only 15 min due to employment of microwave radiation and the yield was greater than 90% (Scheme 1).

In order to characterize the glycosylated derivative, ^{1}H NMR spectroscopy was employed. Typical Soluplus® peaks were observed at (i) 3.65 ppm (polyethylene glycol) and (ii) between 1.29 and 3.25 ppm for polyvinyl caprolactam and polyvinyl acetate. For instance, proton signals corresponding to –OCOCH3 (from



Scheme 1. Synthesis of Soluplus(Glu) by ring opening reaction of δ-gluconolactone in presence Soluplus® (-OH terminal) assisted by microwave.

polyvinyl acetate) and –CH2– (from caprolactam) were observed at 2.04 ppm and 1.78 ppm, respectively (Fig. 1a). Similar results were observed for this copolymer in deutered cloroform (Fig. 1a) [43]. Interestingly, these signals were also observed in the Soluplus (Glu) ^{1}H NMR spectrum with the addition of the sugar moiety peak at 4.08 ppm (Fig. 1b). These results were in concordance with previous studies of glycosylated polymers where Glu moieties signals were commonly assigned between 3.0 and 4.5 ppm [44,45].

Since we aimed to develop a Glu surface decorated micellar nanocarrier, we investigated its aggregation behaviour before and after Glu conjugation. It has been fully described that polymeric micelles are dynamic colloidal systems and their aggregation behaviour may be influence by either, chemical modifications to the amphiphilic polymer and environment factors as temperature, salt content and pH [46]. Thereafter, micellar aqueous physical stability could be affected after polymer glycosylation. Then, the CMC values of Soluplus(Glu) and its un-modified counterpart (Soluplus®) were compared. As it was expected, there was an increase (6.9-fold) of the glycopolymer CMC value (0.0151%w/v) in comparison with the sugar-free copolymer (0.0022%w/v) at 25 °C. It has been demonstrated that the micellar thermodynamic stability depends on (i) copolymer hydrophobic block length and (ii) the hydrophilic block interactions inside the micellar corona and with the aqueous environment. An increase on hydrophilic polymer content might lead to an increase of the polymer molecules steric hindrance [46]. In this context, the differences observed for the CMC values, before and after polymer glycosylation, could be explained due to the incorporation of hydrophilic moieties (Glu) to the polymer backbone which increases the copolymer hydrophilic/hydrophobic balance, resulting in an enhanced micellar corona hydrophilic character and a decrease of the Soluplus(Glu) tendency to self-aggregate into polymeric micelles.

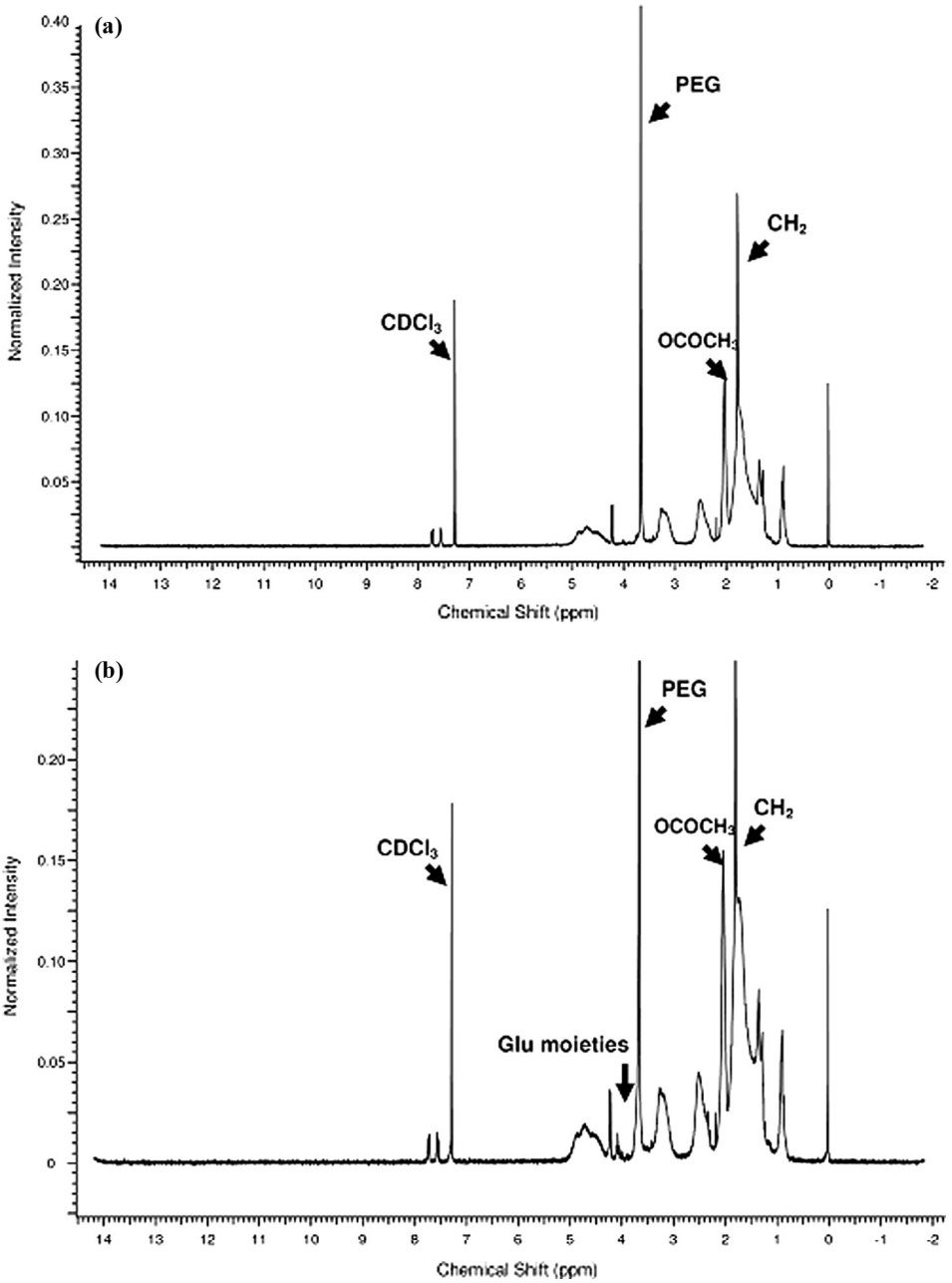


Fig. 1. ^{1}H NMR spectra of (a) Soluplus® and (b) Soluplus(Glu) in $CDCl_3$ with the assignment of the main proton signals.

Similar results were observed after the incorporation of sugar residues in a poly(D,L-lactide)-based amphiphilic copolymer [47].

It is well known that Soluplus® can self-assemble in water into polymeric micelles upon its CMC value [26,48]. Then, taking into account the CMC value obtained for Soluplus® and Soluplus(Glu), we prepared micelles with a copolymer concentration of 3% w/v (>>CMC) by simple copolymer dispersion in distilled water. In this case, Soluplus® micelles demonstrated a monomodal size distribution with a D_h value of 73.3 ± 1.8 nm at 25 °C. Then, Soluplus(Glu) micelles showed a D_h value of 100.3 ± 3.8 nm maintaining an unimodal size distribution. In this case an increment on micellar size was observed after polymer glycosylation. These results also suggest the presence of hydrophilic moieties in the micellar corona, improving its hydrophilic character and increasing micellar mean size, as previously described [47]. Moreover an increase on sample polydispersion was observed since PDI values for Soluplus® and Soluplus(Glu) micelles increased from 0.046 ± 0.022 to 0.224 ± 0.002, respectively. Zeta potential values for the sugar-free micelles (−0.012 ± 0.045 mV) and the glycosylated micelles (−0.247 ± 0.026 mV) remained almost neutral at 25 °C.

3.2. Agglutination assays of Soluplus® and Soluplus(Glu)

To further confirm Soluplus® glycosylation and the surface decoration with Glu moieties, agglutination assays employing a water-soluble lectin (Con A) were performed. Con A is a globular protein which specifically binds Glu and mannose residues [49,50] and it has been well investigated for the study of novel glycopolymers and sugar surface decorated nanocarriers [30,50,51]. It exhibits a tetrameric form above pH 7.0 which results useful to interact with several sugar residues present on nanocarrier surface and form large aggregates [49]. Further, this lectin demands the presence of Ca^{2+} ions to improve the sugar binding. Then, we added BSA to the colloidal systems as a Ca^{2+} supplement [30]. We investigated the Con A interactions with Soluplus® and Soluplus(Glu) micelles (5% w/v) employing two complementary techniques: TEM and DLS [30,49,50]. As is shown in Fig. 2a, individual rod-shape Soluplus® micelles could be observed without the presence of large micellar aggregates after Con A incubation. On the other hand, a different behaviour was observed after lectin incubation with the Glu decorated micelles. In this case, large non-spherical micellar aggregates were observed, confirming the sugar residues location on the micellar corona of Soluplus(Glu) micelles (Fig. 2b).

These results were confirmed by DLS where micellar size and size distribution of Soluplus® and Soluplus(Glu) dispersions, before and after Con A incubation, are summarized in Table 1. First, BSA exhibited a unimodal size distribution with an only peak at 9.0 nm.

On the other hand, Soluplus® and Soluplus(Glu) dispersions, demonstrated a monomodal size distribution after BSA incorporation. In case of Soluplus® micelles a D_h value of 104.2 ± 4.2 nm (PDI: 0.168 ± 0.011) was observed and a similar size pattern with only one size peak (121.1 ± 4.9 nm, PDI: 0.384 ± 0.005) was obtained after Con A incubation for 6 h, being this behaviour stemming from the absence of Glu on the micellar corona. Conversely, a different behaviour was observed for the glycosylated system. In this case, only one peak was observed after the BSA incorporation (112.5 ± 0.1 nm, PDI: 0.136 ± 0.007). However, a bimodal size distribution was observed after the incubation of the Soluplus(Glu) micelles with the sugar-binding lectin. For instance, a major peak of 123.5 ± 4.9 nm and a second peak of 4698.0 ± 60.7 nm (PDI: 0.393 ± 0.004) were observed after incubation for 6 h (Table 1). These results suggest the Con A-mediated micellar agglutination due to the presence of sugar moieties on the surface of the glycosylated copolymer leading to the generation of large micellar aggregates (>4 μm). Moreover, no aggregates were observed for those micelles without the incorporation of sugar moieties were the size distribution was not affected by the lectin addition. Similarly large micellar aggregates were obtained after Con A

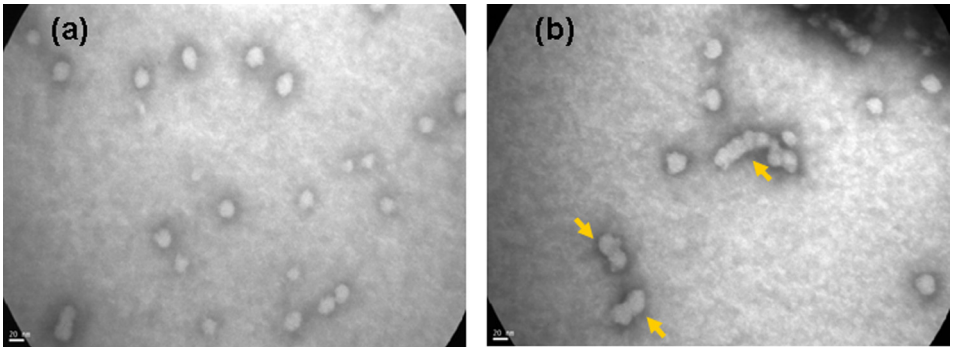


Fig. 2. TEM micrographs of 5%w/v micelles incubated with Con A 10 μM. (a) Soluplus and (b) Soluplus(Glu). Arrows point out the formation of non-spherical micellar aggregates in (b). Scale bar: 20 nm.

Table 1
Micellar size and size distribution (PDI) of Soluplus® and Soluplus(Glu) dispersions after BSA addition, before and after incubation with Con A at 25 °C over 6 h, as measured by DLS.

Sample	Con A	Size (nm) (±S.D.)				
		Peak 1[b]	% Intensity	Peak 2[c]	% Intensity	PDI (±S.D.)
BSA[a]	–	9.0 (0.3)	100.0	–	–	0.151 (0.029)
Soluplus®	–	104.2 (4.2)	100.0	–	–	0.168 (0.011)
	√	121.1 (4.9)	100.0	–	–	0.384 (0.005)
Soluplus(Glu)	–	112.5 (0.1)	100.0	–		0.136 (0.007)
	√	123.5 (4.9)	91.3	4698.0 (60.7)	8.7	0.393 (0.004)

[a] BSA 1.5%w/v solution in phosphate buffer pH 7.4.
[b] Smaller size population.
[c] Larger size population.

incubation with Glu-surface decorated thermoresponsive polymeric micelles [51].

Overall, the agglutination assays confirm the Soluplus® conjugation with Glu and the presence of sugar moieties on the micellar corona, resulting in a potential novel drug delivery nanocarrier for an active targeting to breast cancer cells.

3.3. Characterization of free and PTX-loaded glycosylated mixed micelles

Polymeric micelles represent one of the most attractive nanotechnological platforms for the development of drug delivery systems. The preparation of polymeric micelles combining a mixture of amphiphilic polymers, known as "mixed micelles", has been on the spot due to the possibility of combine the properties of different biomaterials, being a well investigated strategy in cancer chemotherapy [26,52]. In our study, after the obtention of a glycopolymer with Glu pendant moieties, we developed a mixed polymeric formulation employing not only Soluplus® and Soluplus(Glu) but also TPGS. The last is commonly known as the "water-soluble" form of Vitamin E, a versatile biomaterial and pharmaceutical additive, which has been approved by the FDA as a "pharmaceutically safe adjuvant" [53] Particularly, for cancer chemotherapy, it has been studied due to its unique properties of (i) P-glycoprotein (P-gp) inhibition as an efflux pump which mediates multidrug resistance in tumor cells [26] and (ii) its specific cytotoxic activity on breast cancer cells [54].

Previously, we could successfully encapsulate PTX within a mixed micelles nanoformulation of Soluplus® and TPGS [26], then we developed a mixed system employing Soluplus:Soluplus(Glu): TPGS at a weight ratio of 3:1:1, denoted as Glu-mixed micelles. The self-aggregation behaviour of the glycosylated mixed micelles was found to be lower than its counterpart without Glu moieties. For instance, the CMC value for the glycosylated system was 0.0032% w/v, being 4-fold higher than the CMC value of the Glu-free system (0.0008% w/v) (Table 2). The presence of Glu moieties in the micellar corona of the mixed system, due to the incorporation of Soluplus(Glu), increases its hydrophilic character leading to a decrease on the system self-aggregation tendency in water at 25 °C. In a similar manner, Soluplus(Glu) micelles demonstrated higher CMC value than Soluplus® micelles, as previously described.

On the other hand, size and size distribution assays of the Glu-mixed micelles demonstrated that there was only a slight micellar size increment after PTX encapsulation since D_h values increase from 110.7 ± 2.3 nm to 117.6 ± 2.0 nm, for free and drug-loaded mixed micelles, respectively. Also, a narrow size distribution was observed before and after PTX encapsulation. Furthermore, a similar size pattern was obtained for Glu-free mixed micelles (Table 2). These results suggest that the incorporation of Soluplus(Glu) did not directly affect micellar size and size distribution of the colloidal system, regardless the presence on PTX.

Zeta potential values of both mixed nanocarriers (in absence and presence of Glu) remained almost neutral, regardless the encapsulation of PTX (Table 2).

Finally, the morphological characterization of the PTX-loaded glycosylated mixed systems demonstrated rod-shape micelles with a unimodal size distribution (Fig. 3a). The non-spherical morphology observed is due to the Soluplus® chemical composition. Similar results were observed previously [48]. Further, it is worth stressing that the PTX-loaded glycosylated mixed micelles could be freeze-dried, without the employment of lyoprotectant additives, presenting an uniform macroscopic aspect and D_h value of 99.8 ± 2.0 nm (PDI: 0.102 ± 0.015). Then, samples could be easily re-dispersed in distilled water by simple hand-shanking obtaining a translucent colloidal dispersion (Fig. 3b). This characteristic is very important for a possible conservation of the formulation under room temperature storage conditions as Abraxane® and Genexol® which are lyophilized products.

Table 2
Micellar size, size distribution (PDI) and CMC values in distilled water for free-loaded and PTX-loaded mixed micelles with and without Soluplus(Glu) at 25 °C.

Samples	PTX (4 mg/mL)	Size (nm) (±S.D.)	PDI (±S.D.)	Zeta potential (mV) (±S.D.)	CMC[c] (% w/v)
Glu-free mixed micelles[a]	–	107.1 (1.4)	0.103 (0.017)	+0.122 (0.018)	0.0008
	√	113.5 (1.3)	0.075 (0.010)	+0.154 (0.091)	–
Glu-mixed micelles[b]	–	110.7 (2.3)	0.120 (0.016)	+0.125 (0.080)	0.0032
	√	117.6 (2.0)	0.122 (0.010)	+0.103 (0.062)	–

[a] Soluplus:TPGS, weight ratio 4:1.
[b] Soluplus:Soluplus(Glu):TPGS, weight ratio 3:1:1.
[c] Determined by DLS at 25 °C.

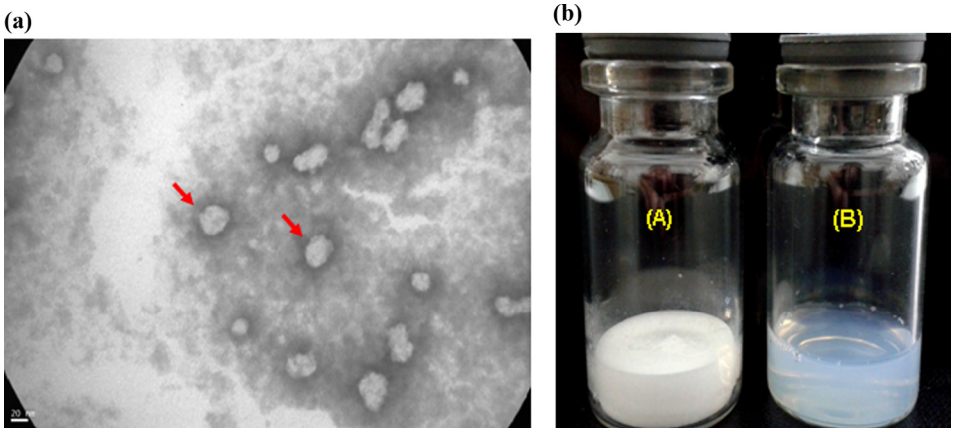


Fig. 3. (a)TEM micrograph of PTX-loaded (4 mg/mL) Glu-mixed micelles (Scale bar: 20 nm). (b) PTX-loaded (4 mg/mL) freeze-dried Glu-mixed micelles before (A) and (B) re-dispersion in distilled water at 25 °C.

The present investigation was focused on the development of a PTX-loaded active targeting nanoformulation for parenteral administration. Typically, Taxol® is intravenous administrated after its dilution at a final concentration of 0.3–1.2 mg/mL with either (i) normal saline solution (NaCl 0.9% w/v) or (ii) dextrose 5% w/v. Further, Genexol® is also diluted in these solutions to get a clinically relevant drug concentration for intravenous administration [32,55]. Since polymeric micelles are dynamic systems, results crucial the evaluation of their physical stability after dilution with different physiological solutions containing salts or dextrose, commonly employed for PTX intravenous administration. Taking this is mind, we investigated the physical stability of our glycosylated mixed micelles after its dilution with NaCl 0.9% w/v and dextrose 5% w/v over 30 min. In this case, samples were diluted 1/10 to obtain a clinically relevant PTX concentration. Results showed a unimodal size distribution with no size increment over time, regardless the dilution medium employed (Fig. 4). For instance, samples diluted in normal saline solution showed D_h values of 75.0 ± 0.7 nm and 75.7 ± 1.3 nm at 0 and 30 min, respectively. Further, Glu-containing micelles diluted with dextrose solution demonstrated a slight size increment in comparison with their counterparts diluted in normal saline solution (Fig. 4). In this case, the D_h values observed were 91.1 ± 1.5 nm and 89.9 ± 0.7 nm at 0 and 30 min, respectively. These are promising results since no secondary size populations or micellar aggregation was observed suggesting an optimal physical stability of the glycosylated mixed micelles after its dilution with conventional mediums for PTX intravenous administration. Further, Glu-free mixed micelles demonstrated a similar behaviour as is shown in Fig. 4. In this case, a monomodal size distribution was also observed where a slight size increment from 85.9 ± 1.1 nm to 88.5 ± 1.4 nm was obtained for micelles diluted with normal saline solution and dextrose solution, respectively.

3.4. In vitro PTX release

As an attempt to characterize the *in vitro* PTX release from the mixed micelles (without and with Soluplus(Glu)) and compare their performance with a commercially available PTX-loaded micellar formulation (Genexol®), we assessed the drug cumulative release profiles from the mixed micelles and Genexol® at pH 7.4 (37 °C) over 96 h. Genexol® demonstrated a sustained PTX release

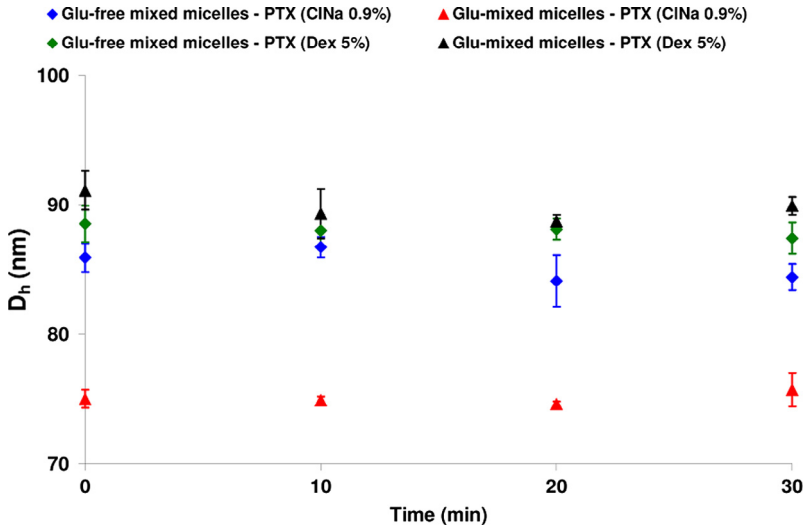


Fig. 4. Micellar size of PTX-loaded (4 mg/mL) mixed micelles diluted (1/10) in ClNa 0.9%w/v and in dextrose 5%w/v over 30 min at 25 °C. Data represents mean ± standard deviation (S.D.), n = 3.

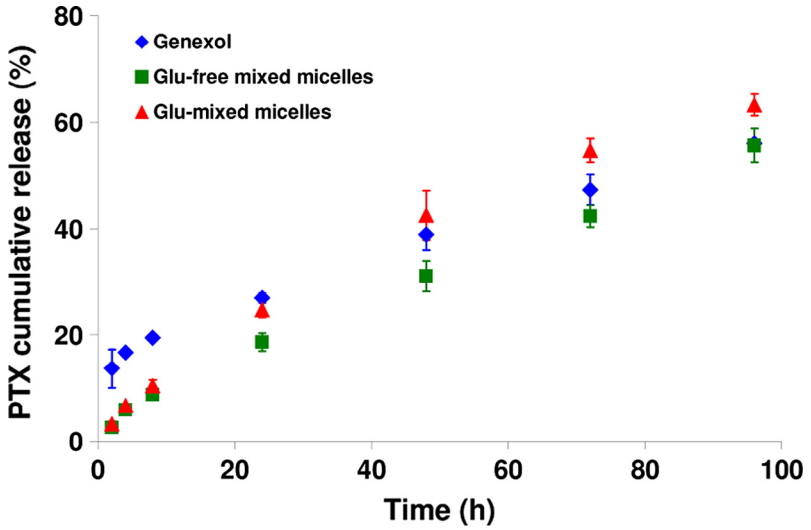


Fig. 5. *In vitro* PTX release profile from Glu-mixed micelles, Glu-free micelles and Genexol® at 37 °C over 96 h. ®. Results are expressed as mean ± standard deviation (S.D.), n = 3.

over time and no burst effect was observed, being the cumulative PTX release of 56% over 96 h (Fig. 5).

On the other hand, both mixed micellar systems demonstrated a similar behaviour as Genexol® were a drug sustained release was observed over 96 h. For the Glu-free mixed micelles, the cumulative amount of PTX release after 8 and 96 h was 8.8% and 55.7%, respectively. Further, for the sugar-containing mixed micelles, the amount of drug release was 10.5% and 63.3% at 8 and 96 h, respectively (Fig. 5). Interestingly, it was observed a faster PTX release from the Glu-mixed micelles *versus* Glu-free mixed micelles between 8 and 96 h. These results could be related with the presence of Glu moieties on the micellar corona. Variations on the length of the hydrophilic/hydrophobic portions of the micelle-former biopolymers, affect the drug release profiles from the polymeric micelles. In general, as the hydrophilic content is increased, the drug is released into a greater extend. Hence, the hydrophilic Glu pendant moieties in our Glu-mixed micelles increase the hydrophilic character of the micellar corona, resulting in a faster PTX release from the polymeric matrix *versus* the Glu-free mixed counterparts. Similar results were observed for polymeric micelles decorated either with Glu and maltose residues [56].

Overall, the glycosylated mixed micelles represents a feasible platform as PTX delivery system for parenteral administration since a sustained drug release was observed over 96 h. Further, the drug release pattern was similar than the PTX release profile observed from Genexol®.

3.5. In vitro citotoxicity assays

To compare the *in vitro* anticancer performance of the Glu-mixed micelles *versus* Genexol®, MCF-7 (estrogen-dependent) and MDA-MB-231 (estrogen independent) human breast cancer cells were exposed to different PTX concentrations encapsulated within the mixed micelles or Genexol®. Further, free-loaded mixed micelles (with and without Glu) were evaluated for comparison. The cancer cell lines for the cytotoxic assays were chosen taking into account PTX clinical applications.

Results demonstrated that PTX-based mixed micellar formulations showed a significant ($p < 0.05$) improvement on their cytotoxic effect in comparison with Genexol®, regardless the cancer cell line (Table 3, Fig. 6). Further, PTX-loaded mixed systems demonstrated statistically ($p < 0.05$) different IC_{50} values in comparison with free-loaded mixed micelles, both with and without Soluplus(Glu) (Table 3). Interestingly, free-loaded mixed micelles also demonstrated cytotoxic effect, being this result probably related with the presence of TPGS in the mixed system. It has been stated that this biomaterial exhibits specific cytotoxic effect in breast cancer cells along with an inhibitory activity of efflux protein P-gp. Recently, the addition of TPGS to Genexol® has been evaluated as a potential strategy to overcome multi-drug resistance in tumor cells [57]. Further, we assayed TPGS (1% w/v) in both cancer cells lines, where the IC_{50} values for MCF-7 (18.7 ± 2.4 μg/mL) and MDA-MB-231 (21.5 ± 1.8 μg/mL) clearly demonstrated the *in vitro* antitumoral effect of this biomaterial.

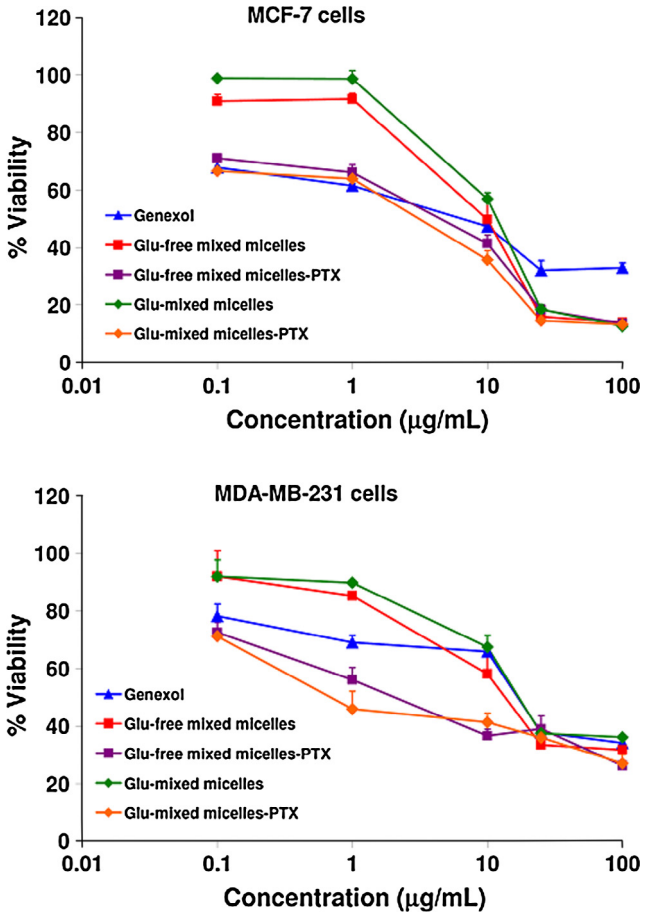


Fig. 6. Cell viability of MCF-7 and MDA-MB-231 cells after 72 h of treatment with free-loaded mixed micelles (without and with Glu), PTX-loaded mixed micelles (without and with Glu) and Genexol®. Results are expressed as mean ± standard deviation (S.D.), n = 3.

Also, it has been reported that TPGS might be part of the novel "mitocans" group since it has been demonstrated that α-tocopherol succinate exhibits a mitochondria-associated apoptotic effect [58]. Then, the cytotoxic effect observed in the different cell lines might result from the combination of both (i) the encapsulated PTX and (ii) the micelle-former TPGS, conforming the mixed micellar system.

Particularly, for the estrogen-dependent cell line assayed (MCF-7), the IC_{50} values for the drug-loaded mixed micellar systems without (7.77 μg/mL) and with (6.06 μg/mL) Glu were 1.2-fold

Table 3
IC_{50} (mean ± S.D.) values in MCF-7 and MDA-MB-231 after 72 h treatment by Genexol®, free-loaded mixed micelles and PTX-loaded mixed micelles with and without Glu.

Cell line	IC_{50} (μg/mL)				
	Genexol®	Free-loaded		PTX-loaded	
		Glu-free mixed micelles	Glu-mixed micelles	Glu-free mixed micelles	Glu-mixed micelles
MCF-7	9.53 (1.04)	11.76 (0.56)[a]	13.20 (0.50)[a]	7.77 (0.64)[a,b,c,d]	6.06 (0.28)[a,b,c]
MDA-MB-231	17.07 (1.00)	15.40 (0.36)[a,c]	18.09 (0.63)[b]	1.72 (0.17)[a,b,c]	1.21 (0.27)[a,b,c]

Note: Multiple comparisons were performed using one-way ANOVA and Newman-Keuls multiple comparisons post hoc test ($n = 3$ experiments).
[a] Significant difference compared to Genexol® ($p < 0.05$).
[b] Significant difference compared to free-loaded Glu-free mixed micelles ($p < 0.05$).
[c] Significant difference compared to free-loaded Glu-mixed micelles ($p < 0.05$).
[d] Significant difference compared to PTX-loaded Glu-mixed micelles ($p < 0.05$).

and 1.6-fold lower than Genexol®, respectively ($p < 0.05$). Furthermore, if we compare both PTX-loaded micellar systems, without and with Glu, there was also a significant ($p < 0.05$) difference on their *in vitro* anticancer activity against MCF-7 cells. Indeed, the IC_{50} value for the glycosylated system was significantly ($p < 0.05$) lower than that observed for its counterpart without the presence of Glu (Table 3 and Fig. 6). The reason for this might be that the ligand of the modified micelles, can target actively the nanocarriers to the tumor cells. Therefore, Glu-mixed micelles could be more efficiently taken up and deliver more PTX into cells, resulting in an increased cytotoxicity.

A similar behaviour was observed for the other breast cancer line assayed, the estrogen-independent MDA-MB-231. In this case, the decrease on the IC_{50} values for the drug-loaded mixed micelles *versus* Genexol® was more pronounced. For instance, PTX-loaded glycosylated mixed micelles showed an IC_{50} value almost 14.1-fold lower than the commercially available micellar formulation (Table 3).

It is worth stressing than breast cancer MDA-MB-231 tumors always demand chemotherapy since they do not respond to conventional hormonal therapy. Hence the improvement of the cytotoxic effect in the estrogen-independent cancer cell line due to PTX encapsulation within the glycosylated mixed micelles is clinically relevant to enhance breast cancer chemotherapy.

3.6. In vitro cellular uptake assays

One of the main goals of the present investigation was the development of a multifunctional PTX delivery micellar nanocarrier for a potential breast cancer cellular-specific targeting (active targeting). Recently, Genexol® has been approved in some countries as the first micellar-based nanoformulation for metastasic breast cancer chemotherapy [59]. Surprisingly, micellar-based commercially available nanomedicines have not been developed for an active drug targeting to tumor cells. Indeed, they are based on a passive drug targeting to cancer tissues mainly associate with the EPR effect [60]. In line with the premise that antineoplastic drugs must be uptaken by tumor cells and accumulated in an optimal concentration to get an efficient cytotoxic effect, we decided to explore the benefits of employing sugar pendant residues on the corona of a mixed multifunctional micellar drug carrier. Hence, we assessed the *in vitro* PTX cellular uptake from the sugar-containing mixed micelles in MCF-7 and MDA-MB-231 cancer cells. As is shown in Fig. 7, there was a significant increase ($p < 0.05$) in PTX intracellular/cell content for the Glu-mixed micelles *versus* Genexol® after 4 h (11.1-fold) and 6 h (30.5-fold) of incubation with MCF-7 cells. Similarly, a significant increase ($p < 0.05$) in the PTX intracellular/cell content was also observed for the glycosylated systems *versus* Glu-free mixed micelles after 4 h (5.2-fold) and 6 h (8.5-fold) of incubation with MCF-7 cells (Fig. 7).

On the other hand, promising results were also observed after the incubation of the nanocarriers with MDA-MB-231 cells. In this case, Glu-modified micelles also demonstrated significantly ($p < 0.05$) higher drug intracellular/cell levels (2.89 μg PTX/mg protein) than Genexol® (0.58 μg PTX/mg protein) at 6 h of incubation, representing an increment of ~5-fold. Besides, it was also observed a significant ($p < 0.05$) increment in the PTX intracellular/cell levels for the glycosylated systems *versus* their un-modified counterparts at 4 (2.9-fold) and 6 h (2.9-fold) of incubation (Fig. 7). These results of a successful PTX cellular uptake from our Glu-containing nanocarriers suggests their potential for a selective targeting to breast cancer cells in comparison with the other micelle-based nanocarriers without the presence of a surface-located ligand (Genexol® and Glu-free mixed micelles).

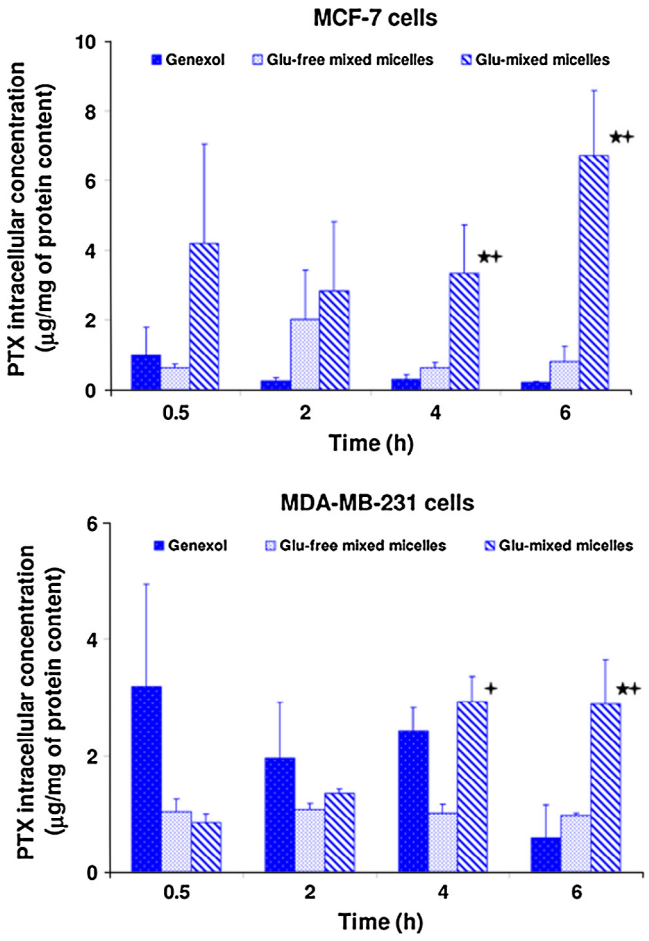


Fig. 7. Time-dependent intracellular/cell PTX levels in MCF-7and MDA-MB-231 cancer cell lines for drug-loaded mixed micelles *versus* Genexol®. Drug amount was normalized by protein concentrations of the cell lysates. Results are expressed as mean ± standard deviation (S.D.), n = 3. ★ Significant difference ($p < 0.05$) compared to Genexol®. + Significant difference ($p < 0.05$) compared to Glu-free mixed micelles.

Although GLUT1 results up-regulated in many cancer cells types, it has also been demonstrated that other GLUT isoforms can be over-expressed in tumor cells where the expression of proteins from other cell types could be observed [61]. Particularly, for MCF-7 it has been found that not only GLUT1 but also GLUT12 result over-expressed [62,63]. Hence, an enhanced PTX intracellular/cell concentration, due to the drug encapsulation within the Glu-containing nanocarrier, was expected for estrogen-dependant breast cancer cell line *versus* MDA-MB-231 cells, as is shown in Fig. 7.

Overall, data confirms that the nanocarrier glycosylation with Glu residues resulted in a successful strategy to design a novel PTX delivery system for a potential active targeting to breast cancer cells. Taking into account that the different commercially available nanoformulations based on PTX are not currently design for a selective drug targeting, our PTX-loaded Glu-decorated mixed micelles are excellent candidates to improve breast cancer chemotherapy.

4. Conclusion

In the present investigation we successfully design a multifunctional mixed micellar system decorated with Glu and loaded with a clinically relevant PTX concentration for an enhanced breast cancer therapy. The *in vitro* antitumoral activity as well as its the PTX

intracellular/cell levels were significantly improved in comparison with Genexol® in MCF-7 and MDA-MB-231 cancer cell lines.

Overall, our novel mixed nanocarrier represents an attractive strategy for the development of an intelligent PTX delivery system to improve breast cancer chemotherapy. Furthermore, to the best of our knowledge, this is the first Glu-containing micellar nanocarrier explored for a potential active PTX targeting to breast cancer cells.

Acknowledgements

Authors thank the Universidad de Buenos Aires (Grant UBACyT 20020130200038BA and UBACyT 20020130200005BA). Estefania Grotz is supported by doctoral scholarship of CONICET. Marcela A. Moretton, Ezequiel Bernabeu, Lorena Gonzalez, Marcela Zubillaga and Diego A. Chiappetta are partially supported by CONICET, Argentina. The authors express their gratitude to BASF Argentina S.A. (Carla Neirone) for providing Soluplus® samples.

References

[1] World Health Organization (WHO) http://www.who.int/cancer/detection/breastcancer/en/ (Accessed November 2016).
[2] World Health Organization (WHO) <http://www.who.int/mediacentre/factsheets/fs297/en/> (accessed November 2016).
[3] GLOBOCAN, Breast Cancer Fact Sheets <http://globocan.iarc.fr/old/FactSheets/cancers/breast-new.asp> (accessed November 2016).
[4] E. Bernabeu, G. Helguera, M.J. Legaspi, L. Gonzalez, C. Hocht, C. Taira, D.A. Chiappetta, Paclitaxel-loaded PCL–TPGS nanoparticles: in vitro and in vivo performance compared with Abraxane®, Colloids Surf. B Biointerfaces 113 (2014) 43–50.
[5] E.K. Rowinsky, The development and clinical utility of the taxane class of antimicrotubule chemotherapy agents, Annu. Rev. Med. 48 (1997) 353–374.
[6] A.K. Singla, A. Garg, D. Aggarwal, Paclitaxel and its formulations, Int. J. Pharm. 235 (2002) 179–192.
[7] H. Gelderblom, J. Verweij, K. Nooter, A. Sparreboom, Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation, Eur. J. Cancer 37 (2001) 1590–1598.
[8] A. Sparreboom, C.D. Scripture, V. Trieu, P.J. Williams, T. De, A. Yang, B. Beals, W. D. Figg, M. Hawkins, N. Desai, Comparative preclinical and clinical pharmacokinetics of a cremophor-free, nanoparticlealbumin-bound paclitaxel (ABI-007) and paclitaxel formulated in cremophor (Taxol), Clin. Cancer Res. 11 (2005) 4136–4143.
[9] Š. Koudelka, J. Turánek, Liposomal paclitaxel formulations, J. Control. Release 163 (2012) 322–334.
[10] T.-Y. Kim, D.-W. Kim, J.-Y. Chung, S.G. Shin, S.-C. Kim, D.S. Heo, N.K. Kim, Y.J. Bang, Phase I and pharmacokinetic study of genexol-PM, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies, Clin. Cancer Res. 10 (2004) 3708–3716.
[11] D.A. Chiappetta, C. Hocht, C. Taira, A. Sosnik, Oral pharmacokinetics of the anti-HIV efavirenz encapsulated within polymeric micelles, Biomaterials 32 (2011) 2379–2387.
[12] M.A. Moretton, L. Cohen, L. Lepera, E. Bernabeu, C. Taira, C. Höcht, D.A. Chiappetta, Enhanced oral bioavailability of nevirapine within micellar nanocarriers compared with Viramune®, Colloids Surf. B Biointerfaces 122 (2014) 56–65.
[13] M.A. Moretton, C. Taira, S. Flor, E. Bernabeu, S. Lucangioli, C. Höcht, D.A. Chiappetta, Novel nelfinavir mesylate loaded D-α-tocopheryl polyethylene glycol 1000 Succinate micelles for enhanced pediatric anti HIV therapy: in vitro characterization and in vivo evaluation, Colloids Surf. B Biointerfaces 123 (2014) 302–310.
[14] D.A. Chiappetta, A. Sosnik, Poly(ethylene oxide)-Poly(propylene oxide) block copolymer micelles as drug delivery agents: Improved hydrosolubility, stability and bioavailability of drugs, Eur. J. Pharm. Biopharm. 66 (2007) 303–317.
[15] A. Ribeiro, A. Sosnik, D.A. Chiappetta, F. Veiga, A. Concheiro, C. Alvarez-Lorenzo, Single and mixed poloxamine micelles as nanocarriers forsolubilization and sustained release of ethoxzolamide for topical glaucomatherapy, J. R. Soc. Interface 9 (2012) 2059–2069.
[16] D.A. Chiappetta, G. Facorro, E. Rubin de Celis, A. Sosnik, Synergistic encapsulation of the anti-HIV agent efavirenz within mixed poloxamine/poloxamer polymeric micelles, Nanomedicine 7 (2011) 624–637.
[17] M. Goutam, V. Kumar, S.K. Shukla, P.K. Singh, R.I. Mahato, EGFR-targeted polymeric mixed micelles carrying gemcitabine for treating pancreatic cancer, Biomacromolecules 17 (2016) 301–313.
[18] Y. Chen, W. Zhang, Y. Huang, F. Gao, X. Sha, X. Fang, Pluronic-based functional polymeric mixed micelles for co-delivery of doxorubicin and paclitaxel to multidrug resistant tumor, Int. J. Pharm. 488 (2015) 44–58.
[19] X. Zhang, H. Wang, T. Zhang, X. Zhou, B. Wu, Exploring the potential of self-assembled mixed micelles in enhancing the stability and oral bioavailability of an acid-labile drug, Eur. J. Pham. Sci. 62 (2014) 301–308.
[20] J. Dou, H. Zhang, X. Liu, M. Zhang, G. Zhai, Preparation and evaluation *in vitro* and *in vivo* of docetaxel loaded mixed micelles for oral administration, Colloids Surf. B Biointerfaces 114 (2014) 20–27.
[21] F. Suriano, R. Pratt, J.P.K. Tan, N. Wiradharma, A. Nelson, Y.-Y. Yang, Synthesis of a family of amphiphilic glycopolymers via controlled ring-opening polymerization of functionalized cyclic carbonates and their application in drug delivery, Biomaterials 31 (2010) 2637–2645.
[22] V. Torchilin, Tumor delivery of macromolecular drugs based on the EPR effect, Adv. Drug Deliv. Rev. 63 (2011) 131–135.
[23] M.L. Macheda, S. Rogers, J.D. Best, Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer, J. Cell. Physiol. 202 (2005) 654–662.
[24] R.A. Medina, G.I. Owen, Glucose transporters: expression, regulation and cancer, Biol. Res. 35 (2002) 9–26.
[25] J. Li, F. Wang, D. Sun, R. Wang, A review of the ligands and related targeting strategies for active targeting of paclitaxel to tumours, J. Drug Target. 24 (2016) 590–602.
[26] E. Bernabeu, L. Gonzalez, M. Cagel, E.P. Gergic, M.A. Moretton, D.A. Chiappetta, Novel Soluplus®-TPGS mixed micelles for encapsulation of paclitaxel with enhanced in vitro cytotoxicity on breast and ovarian cancer cell lines, Colloids Surf. B Biointerfaces 140 (2016) 403–411.
[27] M.A. Moretton, R.J. Glisoni, D.A. Chiappetta, A. Sosnik, Molecular implications in the nanoencapsulation of the anti-tuberculosis drug rifampicin within flower-like polymeric micelles, Colloids Surf. B Biointerfaces 79 (2010) 467–479.
[28] M.A. Moretton, M. Cagel, E. Bernabeu, L. Gonzalez, D.A. Chiappetta, Nanopolymersomes as potential carriers for rifampicin pulmonary delivery, Colloids Surf. B Biointerfaces 136 (2015) 1017–1025.
[29] P. Taich, M.A. Moretton, M.J. Del Sole, U. Winter, E. Bernabeu, J.O. Croxatto, J. Oppezzo, G. Williams, G.L. Chantada, D.A. Chiappetta, P. Schaiquevich, Sustained-release hydrogels of topotecan for retinoblastoma, Colloids Surf. B Biointerfaces 146 (2016) 624–631.
[30] M.A. Moretton, D.A. Chiappetta, F. Andrade, J. das Neves, D. Ferreira, B. Sarmento, A. Sosnik, Hydrolyzed galactomannan-modified nanoparticles and flower-like polymeric micelles for the active targeting of rifampicin to macrophages, J. Biomed. Nanotechnol. 9 (2013) 1076–1087.
[31] E. Bernabeu, S. Flor, C. Hocht, C. Taira, D. Chiappetta, V. Tripodi, S. Lucangioli, Development and validation of a highly sensitive HPLC method fordetermination of paclitaxel in pharmaceutical dosage forms and biological samples, Curr. Pharm. Anal. 8 (2014) 185–192.
[32] R. Panchagnula, Pharmaceutical aspects of paclitaxel, Int. J. Pharm. 172 (1998) 1–15.
[33] E.-H. Song, M.J. Manganiello, Y.-H. Chow, B. Ghosn, A.J. Convertine, P.S. Stayton, L.M. Schnapp, D.M. Ratner, In vivo targeting of alveolar macrophages via RAFT-based glycopolymers, Biomaterials 32 (2012) 6889–6897.
[34] K. Kizjakina, J.M. Bryson, G. Grandinetti, T.M. Reineke, Cationic glycopolymers for the delivery of pDNA to human dermal fibroblasts and rat mesenchymal stem cells, Biomaterials 33 (2012) 1851–1862.
[35] Y. Wang, X. Zhang, P. Yu, C. Li, Glycopolymer micelles with reducible ionic cores for hepatocytes-targeting delivery of DOX, Int. J. Pharm. 441 (2013) 170–180.
[36] X. Ying, H. Wen, W.-L. Lu, J. Dua, J. Guo, W. Tian, Y. Men, Y. Zhang, R.J. Li, T.Y. Yang, D.W. Shang, J.N. Lou, L.R. Zhang, Q. Zhang, Dual-targeting daunorubicin liposomes improve the therapeutic efficacy of brain glioma in animals, J. Control. Release 141 (2010) 183–192.
[37] X. Li, H. Zhou, L. Yang, G. Du, A.S. Pai-Panandiker, X. Huang, B. Yan, Enhancement of cell recognition in vitro by dual-ligand cancer targeting gold nanoparticles, Biomaterials 32 (2011) 2540–2545.
[38] J. Niu, A. Wang, Z. Ke, Z. Zheng, Glucose transporter and folic acid receptor-mediated Pluronic P105 polymeric micelles loaded with doxorubicin for brain tumor treating, J. Drug Target. 22 (2014) 712–723.
[39] M. Mueckler, B. Thorens, The SLC2 (GLUT) family of membrane transporters, Mol. Aspects Med. 34 (2013) 121–138.
[40] L. Szablewski, Expression of glucose transporters in cancers, Biochim. Biophys. Acta 2013 (1835) 164–169.
[41] V. Ganapathy, M. Thangaraju, P.D. Prasad, Nutrient transporters in cancer: relevance to Warburg hypothesis and beyond, Pharmacol. Ther. 121 (2009) 29–40.
[42] N.M. Xavier, A.P. Rauter, Y. Queneau, Carbohydrate-based lactones: synthesis and applications, Top Curr. Chem. 295 (2010) 19–62.
[43] D. Xia, H. Yu, J. Tao, J. Zeng, Q. Zhu, C. Zhu, Y. Gan, Supersaturated polymeric micelles for oral cyclosporine A delivery: the role of Soluplus–sodium dodecyl sulfate complex, Colloids Surf. B Biointerfaces 141 (2016) 301–310.
[44] X.-H. Dai, H.-D. Zhang, C.-M. Dong, Fabrication, biomolecular binding, in vitro drug release behavior of sugar-installed nanoparticles from star poly(3-caprolactone)/glycopolymer biohybrid with a dendrimer core, Polymer 50 (2009) 4626–4634.
[45] Z. Du, L. Wang, G. Wang, S. Wang, Synthesis, surface and aggregation properties of glucosamide-grafted amphiphilic glycopolysiloxanes, Colloids Surf. A Physicochem. Eng. Asp. 381 (2011) 55–60.
[46] S.C. Owen, D.P.Y. Chana, M.S. Shoichet, Polymeric micelle stability, Nano Today 7 (2012) 53–65.

[47] N. Handke, V. Lahaye, D. Bertin, T. Delair, B. Verrier, D. Gigmes, T. Trimaille, Elaboration of glycopolymer-functionalized micelles from an *N*-vinylpyrrolidone/lactide-based reactive copolymer platform, Macromol. Biosci. 13 (2013) 1213–1220.
[48] M. Wegmann, L. Parola, F.M. Bertera, C.A. Taira, M. Cagel, F. Buontempo, E. Bernabeu, C. Höcht, D.A. Chiappetta, M.A. Moretton, Novel carvedilol paediatric nanomicelle formulation: in-vitro characterization and in-vivo evaluation, J. Pharm. Pharmacol. (2016), http://dx.doi.org/10.1111/jphp.12605.
[49] G. Sánchez-Pomales, T.A. Morris, J.B. Falabella, M.J. Tarlov, R.A. Zangmeister, A lectin-based gold nanoparticle assay for probing glycosylation of glycoproteins, Biotechnol. Bioeng. 109 (2012) 2240–2249.
[50] D. Pei, Y. Li, Q. Huang, Q. Ren, F. Li, T. Shi, Biomimetic glycopolymers tethered gold nanoparticles: preparation, self-assembly and lectin recognition properties, Colloids Surf. B Biointerfaces 126 (2015) 367–373.
[51] K. Sun, S.W.A. Bligh, H.-L. Nie, J. Quan, L.-M. Zhu, Lectin recognizing thermoresponsive double hydrophilic glycopolymer micelles by RAFT polymerization, RSC Adv. 4 (2014) 34912–34921.
[52] D.S. Pellosi, I.R. Calori, L.B. de Paula, N. Hioka, F. Quaglia, A.C. Tedesco, Multifunctional theranostic Pluronic mixed micelles improve targeted photoactivity of Verteporfin in cancer cells, Mater. Sci. Eng. C 71 (2017) 1–9.
[53] Y. Guo, J. Luo, S. Tan, B.O. Otieno, Z. Zhang, The applications of Vitamin E TPGS in drug delivery, Eur. J. Pharm. Sci. 49 (2013) 175–186.
[54] C.M. Neophytou, C. Constantinou, P. Papageorgis, A. Constantinou, D-alpha-tocopheryl polyethylene glycol succinate (TPGS) induces cell cycle arrest and apoptosis selectively in Survivin-overexpressing breast cancer cells, Biochem. Pharmacol. 89 (2014) 31–42.
[55] T.-Y. Kim, D.-W. Kim, J.-Y. Chung, S.G. Shin, S.-C. Kim, D.S. Heo, Phase I and pharmacokinetic study of genexol-PM, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies, Clin. Cancer Res. 10 (2004) 3708–3716.
[56] K.-Y. Peng, M.-Y. Hua, R.-S. Lee, Amphiphilic polyesters bearing pendant sugar moieties: synthesis, characterization, and cellular uptake, Carbohydr. Polym. 99 (2014) 710–719.
[57] Z. Fan, C. Chen, X. Pang, Z. Yu, Y. Qi, X. Chen, Adding Vitamin E-TPGS to the formulation of genexol-PM: specially mixed micelles improve drug-loading ability and cytotoxicity against multidrug-resistant tumors significantly, PLoS One 10 (2015) e0120–e0129.
[58] Y. Bao, M. Yin, X. Hu, X. Zhuang, Y. Sun, Y. Guo, A safe, simple and efficient doxorubicin prodrug hybrid micelle for overcoming tumor multidrug resistance and targeting delivery, J. Control. Release 235 (2016) 182–194.
[59] H. Cabral, K. Kataoka, Progress of drug-loaded polymeric micelles into clinical Studies, J. Control. Release 190 (2014) 465–476.
[60] H. Maeda, G.Y. Bharate, J. Daruwalla, Polymeric drugs for efficient tumor-targeted drug delivery based on EPR-effect, Eur. J. Pharm. Biopharm. 71 (2009) 409–419.
[61] A.N. McCracken, A.L. Edinger, Nutrient transporters: the Achilles' heel of anabolism, Trends Endocrinol. Metab. 24 (2013) 200–208.
[62] A. Godoy, K. Salazar, C. Figueroa, G.J. Smith, M.A. Garcia, F.J. Nualart, Nutritional channels in breast cancer, Mol. Oncol. 13 (2009) 3973–3984.
[63] J. Pujol-Gimenez, F. Pérez de Heredia, M.A. Idoate, R. Airley, M.P. Lostao, A.R. Evans, Could GLUT12 be a potential therapeutic target in cancer treatment? A preliminary report, J. Cancer 6 (2015) 139–143.