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Influence of polyethylene glycol-8-lauryl ester in the structural lipid of the elastic liposomes

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This study compared the physicochemical characteristics of conventional and elastic liposomes in terms of surfactant incorporation, flow rate, elasticity, mean diameter, phospholipid content and permeation capability. Surfactants were incorporated into conventional liposomes in order to enhance the transdermal transport of drugs through the *stratum corneum*. The unilamellar liposomes produced composed of egg phosphatidylcholine (PCegg) as the structural component and polyethylene glycol-8-lauryl ester (PEG8L) as the elastic component. Results showed fluidity increased of the lipid bilayer with surfactant incorporation, and particle integrity was preserved. These factors determined the ability of elastic liposomes to permeate nanoporous membranes. The elastic liposomes presented a size of ~100 nm and a polydispersity index of 0.38. The flow rate of the liposomes through membranes obeyed Darcy's law, with characteristics similar to those of water. The findings demonstrate the potential of elastic liposomes for transdermal drug administration.

Key words: Liposomes, surfactants, transdermal transport.

INTRODUCTION

The use of liposomes is one of the most promising techniques developed in recent years (Samad et al., 2007; Allen and Cullis, 2013). This nanocarrier is a spherical vesicle with one or more concentric lipid bilayers that isolate the internal aqueous compartments from the external environment (Gregoriades et al., 1993). The hydrophobic tails of the lipids are orientated towards the interior of the vesicles, and the polar heads are directed to the outside of the bilayer, in contact with the aqueous phase (Torchilin and Weissig, 2003; Ranade, 1989). These systems are able of encapsulating drugs and promoting their controlled release following administration by various means, including oral (Jain et al., 2012; Yang et al., 2013), intravenous (Curic et al.,

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2013), ocular (Abdelbary, 2011; Takashima et al., 2012), pulmonary (Chen et al., 2012) and transdermal (Allen and Cullis, 2013).

Transdermal administration is one of the most innovative and promising techniques in the field of controlled drug release (Prausnitz and Langer, 2008; Pierre and Costa, 2011; Nounou et al., 2008). According to Brown et al. (2006), the main advantages over other administration routes include avoidance of first-pass metabolism, sustained and controlled delivery over a prolonged period of time, reduced side effects associated with systemic toxicity, improved patient compliance, direct access to target or disease sites, and convenient and painless application.

One of the major difficulties encountered in the transdermal route is the nature of human skin, which is a selective barrier that hinders the penetration or elimination of various types of substance (Cevc, 2012). In order to address this problem, Cevc and Blume (1992) introduced the deformable liposomes called Transfersomes® (IDEA AG,Munich, Germany).

Cevc and Blume (2004) and Cevc et al. (2002) prepared Transfersomes® by conventional film method using 89 wt.% soybean phosphatidylcholine as lipid and 11 wt.% sodium cholate as surfactant agent to obtain lipid bilayer flexibility (Cevc, 1996, 1997; Cevc and Gebauer, 2003). Non ionic surfactants with single chain, such as sucrose esters derived of stearic and palmitic acids, mixture of mono-, di- and tri-esters derived from lauric acid or octaethylene glycol laurate (PEG8L) has been introduced in the conventional liposomes. These kind of vesicle were more useful than rigid vesicles in enhancing skin penetration (Bouwstra et al., 1999; Van den Bergh et al., 1999, 2001, Honeywell-Nguyen et al., 2002, 2003a, 2003b; Honeywell-Nguyen and Bouwstra, 2003). Several studies have reported Span 80, Tween 80, sodium deoxycholate and dipotassium glycyrrhizinate as edge activators (El Maghraby et al., 1999, 2000; Trotta et al., 2002, 2004; Boinpally et al., 2003; Lee et al., 2005). These vesicles act to modify the elasticity of the bilayer and increase deformability (El Zaafarany et al., 2010; Uchino et al., 2011; van Smeden et al., 2013a, 2013b; Elsayed et al., 2007).

The studies of active membranes consider theoretical models, such as hydrodynamic models, with regard to the effects of hydrodynamics of the fluid phase. Darcy's law establishes that the total pressure difference across the membrane produces a relative velocity of the fluid with respect to the membrane (Lacoste and Bassereau, 2014). The relationship between the flow rate and the pressure drop is used to determine whether the flow follows Darcy's law, which assumes linearity between these parameters. This law has been extended to represent the flows of many fluids in porous media including membranes (Slattery, 1972). The elasticity of liposomes can be evaluated by measuring their size before and after filtration through a nanoporous filter, according to the flow rate (Cevc and Blume, 2001).

In this paper, the effects of incorporating octaethylene glycol laurate in conventional liposomes composed of egg phosphatidylcholine were described. Characterization of the vesicular systems included measurements of particle size and phospholipid content. Conventional and elastic liposomes were compared in terms of their permeation and elasticity properties using polycarbonate membranes with a nominal pore diameter of 30 nm. The aim of the present study was to investigate the kinetics of surfactant incorporation of PEG 8L into conventional liposomes and evaluated the vesicle flexibility. In addition, it was characterized by particle size and phospholipid content. Conventional and elastic liposomes were compared in terms of their permeation and elasticity properties using polycarbonate membranes with a nominal pore diameter of 30 nm.

MATERIALS AND METHODS

Egg phosphatidylcholine (PCegg) (Ovothin® 160) was supplied by Degussa GmbH (Germany). Octaethylene glycol laurate (PEG8L), used as the elastic component, was purchased from *Lipo* do *Brasil* (Brazil). HEPES buffer was supplied by Sigma (USA), and membranes (30 and 100 nm) were supplied by Osmonics Inc. (USA). All other chemicals were of analytical grade.

Preparation of conventional and elastic liposomes

Liposomes were prepared according to Bangham's method (New, 1990). For the conventional liposomes, 1 and 10 g/L of the lipid (PCegg) was solubilized in an organic solvent mixture (chloroform/methanol, 9:1 v/v) and agitated for 5 min using a rotary evaporator (Fisatom, Brazil) before the evaporation. Organic solvent mixture was removed from the lipid film under vacuum (650 mm Hg, 200 rpm, ~10°C, 2 h, rotary evaporator operation) until a dry film was formed. The film was, then, rehydrated with 10 ml of HEPES buffer (10 mM), at pH 7.4 and 33°C. The liposomal suspension passed through polycarbonate membrane filters (100 nm pore diameter) at 12 atmosphere, using a Model T001 extruder (Lipex Biomembranes, Canada) (Ribas, 1997). Each extrusion performed 15 times. Elastic liposomes were prepared by adding PEG8L (at a molar concentration of 40 mol%) to the conventional liposomes and incubating for 1 h.

Particle size, polydispersity index and zeta potential

Particle size (Z-average) and polydispersity index (PI) were determined by quasi-elastic laser light scattering (QELS) using a Malvern Instruments (UK) Autosizer 4700 instrument. PCS 4700 software was used for data acquisition and analysis. Measurements were made at an angle of 90° relative to the incident light beam from a He-Ne laser. The samples were diluted with HEPES buffer (10 mM, pH 7.4) to reduce the turbidity of the dispersions. Zeta potential values of liposomes measured in purified water adjusting conductivity (50 µS/cm). The zeta potential was calculated from the electrophoretic mobility using the Helmholtz–Smoluchowski equation.

Determination of the total phospholipid content

The liposomes were characterized in terms of the molar concentration

of phospholipids, determined by measuring phosphorus in the lipid using the standard method of Chen et al. (1956). The phospholipid content before and after passage through the membrane was evaluated with the aim of measuring the deformation capability of vesicles after passing through the membrane.

Flow rate and liposome elasticity and deformation

Liposome elasticity was estimated by *in vitro* assays using two polycarbonate membranes with a pore size of 30 nm. The flow rate was determined during passage of 5 ml of the liposome dispersion through the filter, using the Lipex extruder operated at 37°C (above the phase transition temperature of the lipid). The experiments were carried out under a variety of pressures (2.5 to 16 psi) for a maximum time interval of 10 min (Van den Bergh et al., 2001b). The volume of the sample was measured at the end of the procedure. The flow rate was calculated using the expression:

$$J = \frac{V_p\,(mL)}{t\,(min)}, \quad (1)$$

where *J* is the flow rate, *Vp* (ml) is the permeate volume, and *t* (min) is the filtration time. The modulus of elasticity (E) was determined using the expression proposed by Cevc (1995) and Cevc and Gebauer (2003):

$$E \propto J\left(\frac{d_1}{d_p}\right)^2, \quad (2)$$

where d_1 is the mean liposome diameter before extrusion and *dp* is the membrane pore diameter.

The percentage PEG8L incorporation was determined from the change in the surface tension of the medium, relative to the initial value, using a standard curve relating the surface tension to the surfactant concentration. Since the surfactant concentration was above the critical micellar concentration (CMC), the changes in surface tension were determined using samples that had been diluted to obtain values below the CMC. Measurements were performed at 25°C using the Wilhelmy immersion plate method. The surface tension was calculated as:

$$\sigma = \frac{Pw}{Lb.cos\theta}, \quad (3)$$

where σ is the surface tension, *Pw* is the force, *Lb* is the liquid wettability, and θ is the contact angle. The percentage of PEG surface was determined in order to verify the concentration of surfactant in solution and what was the concentration that interacted with liposomes.

Calibration curve for empty liposomes

A plot of surface tension against surfactant molar concentrations in the range 1 to 50 mol% was constructed using empty liposomes at a phosphatidylcholine concentration of 1 mM. The experiments were performed in triplicate. The molar concentration of surfactant in the solution external to the liposomes (*Ce*) was determined using the linear range of the calibration curve (below the CMC).

Determination of the concentration of surfactant incorporated into the lipid bilayer

The surface tension of the elastic liposomes was measured after dilution of the solutions to below the CMC, and the surfactant concentration was determined using the calibration curve constructed previously. The molar concentration of the surfactant incorporated into the lipid bilayer (*Ci*) was then given by:

$$Ci = Ct - Ce, \quad (4)$$

where *Ct* is the total molar concentration of surfactant in the solution. The content of incorporated surfactant *(Ci*)* was expressed as a percentage of the total concentration of surfactant in the solution (Barbosa et al., 2013), according to:

$$Ci^* = \left(1 - \frac{Ci}{Ce}\right) x\ 100. \quad (5)$$

RESULTS

The kinetics of incorporation of 40 mol % PEG8L into the conventional liposomes composed of PCegg (1 g/L) was evaluated, with the objective of studying the alterations in surfactant compositions on the bilayer over time (Figure 1). The Z-average of the vesicles did not vary significantly, with values of around 114 to 133 nm and a standard deviation of 5.5 nm (Figure 1A). The PI also remained stable at around 0.38. These results indicate that there was no major enlargement of the packing of the lipid bilayer when the surfactant was added to the conventional liposomes.

The results of zeta potential and morphology (transmission electron microscopy) of the convetional and elastic liposomes (with 40 mol% PEG8L) were obtained by de Oliveira (2007) and Zanchetta (2009). Zanechatta (2009) showed that the zeta potential of the conventional and elastic liposome showed negative charge close to -10 mv. According zanchetta (2009), conventional liposomes have higher zeta potential (in absolute value) than the elastic liposomes. In this same work, it was also observed that higher concentrations of PEG8L favored smaller electrical charges on the surface of the vesicles. The conventional and elastic liposome morphology was realized by de Oliveira (2007) demonstrated using MET the elastic liposomes (40% PEG-8L) deform, remaining intact after crossing the membranes with pores of 30 nm in diameter.

Declines in the absorbance and the incorporation percentage were observed during the first 20 min of the experiment (Figure 1B). This was due to the re-organization of the liposomes in the presence of 40 mol% PEG8L. The percentage of surfactant incorporated into the liposomes after 5 min of incubation was 66%, and maximum incorporation of 71% was achieved after 120 min. The absorbance values corroborated the Z-average results, with only a small observed variability (0.38 to 0.43).

The relationship between flow rate and pressure during permeation of conventional and elastic liposomes through polycarbonate membranes with a nominal pore diameter of 30 nm in relation water Milli®-Q, conventional and elastic liposomes are shown in Figure 2.

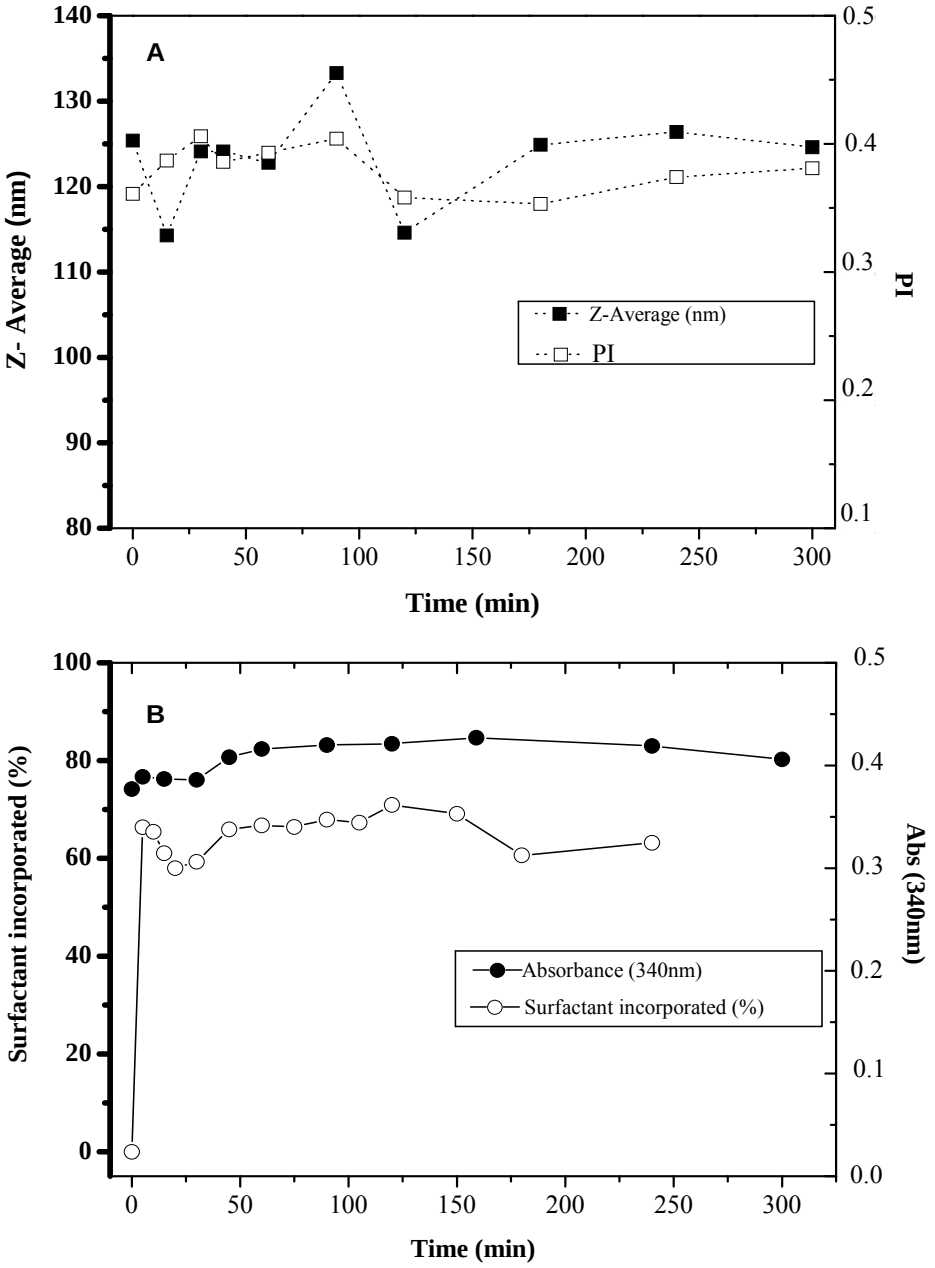

Figure 1. Kinetics of incorporation of PEG8L into conventional liposomes. (A) Z-average and polydispersity index (PI); (B) absorbance and percentage of surfactant incorporated. The conventional liposomes were composed of PCegg (1 g/L), and the surfactant concentration used was 40 mol%.

The elasticity of the liposomes was determined at two lipid concentrations. In the case of conventional liposomes, lower phospholipid concentrations increased elasticity, which ranged between 0.5 and 1.8 ml/min and stabilized above 8 psi (Figure 3A). Liposomes containing surfactant (Figure 3B) showed higher elasticity, compared to the conventional liposomes, with values in the ranges 1.1 to 6.1 and 0.4 to 12.5 ml/min for phospholipid concentrations of 1 and 10 g/L, respectively.

The Z-averages of the conventional and elastic liposomes liposomes as a function of the concentration of PCegg and pressure are shown in Figure 4. For all the samples, the initial diameter was approximately 125 nm. The liposomes containing PEG8L decreased in size as the applied pressure was increased, reaching around 76 nm at 2.5 psi for both lipid concentrations. In the case of the conventional liposomes, there was a gradual decrease in diameter with increasing pressure for the two concentrations tested.

Figure 5 shows the phosphate concentrations for the

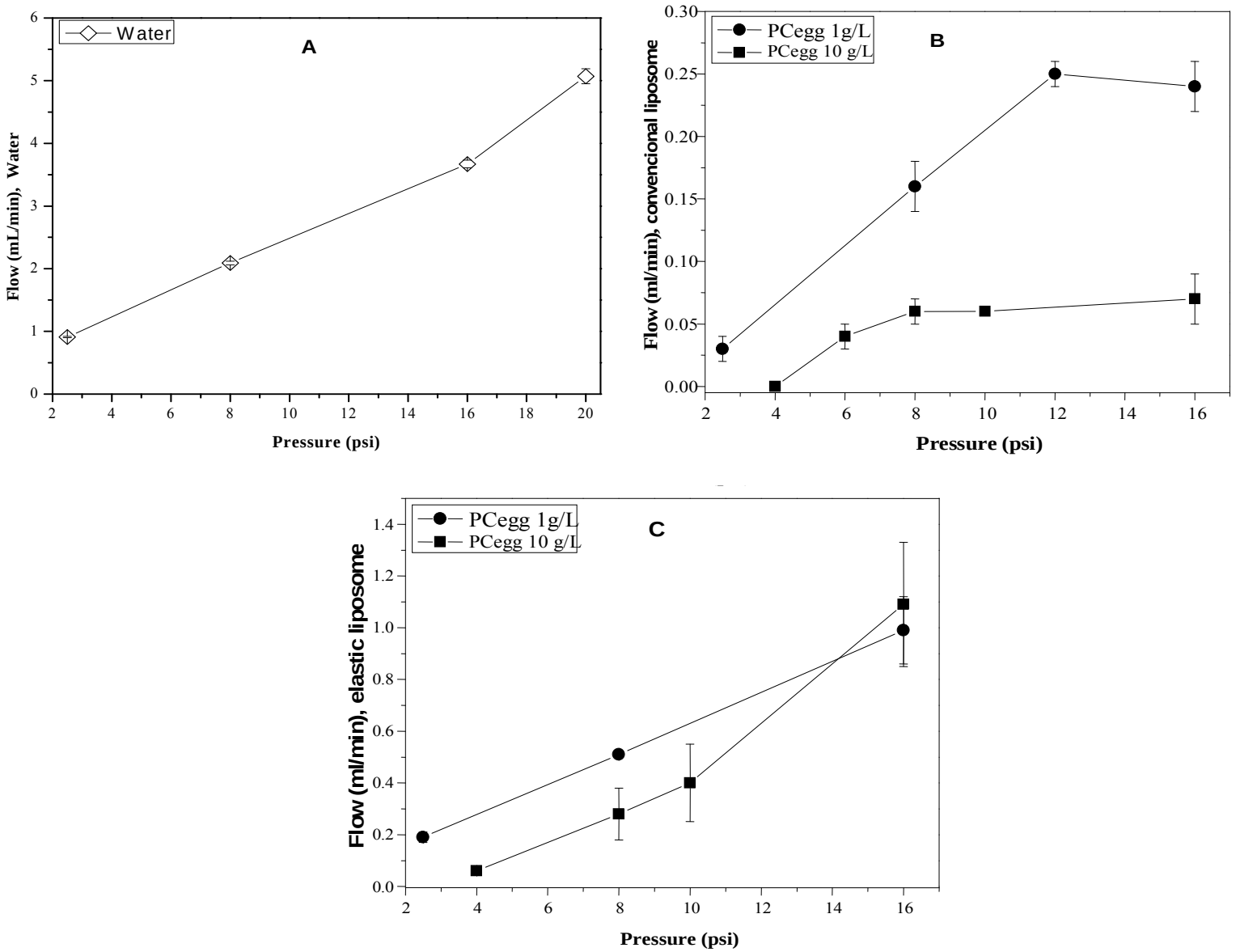

Figure 2. Flow rates using two polycarbonate membranes with a pore size of 30 nm, as a function of PCegg concentration and applied pressure. The surfactant concentration used was 40 mol%. (A) Milli-Q water; (B) conventional liposomes (C) elastic liposomes.

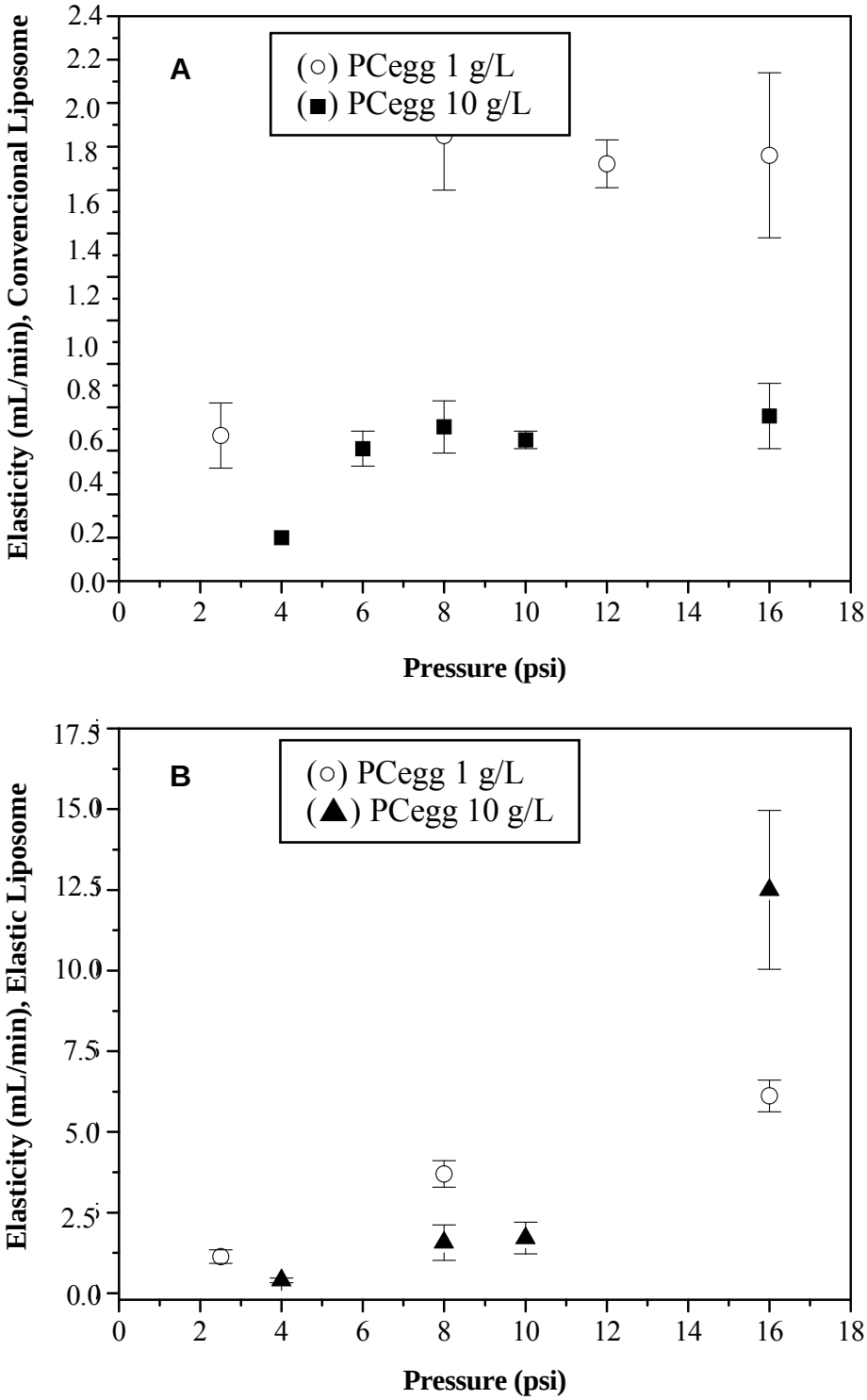

Figure 3. Elasticity results for (A) conventional liposomes and (B) elastic liposomes, using two polycarbonate membranes with a pore size of 30 nm, as a function of PCegg concentration and applied pressure. The surfactant concentration used was 40 mol%.

conventional and elastic liposomes after the elasticity assays, as a function of the PCegg concentration and the applied pressure. Phosphate concentrations were higher for the elastic liposomes produced using 10 g/L of PCegg, and remained stable with increasing pressure. At the same phospholipid concentration, the conventional liposome phosphate concentration decreased drastically when the pressure was increased to 4 psi, after which the

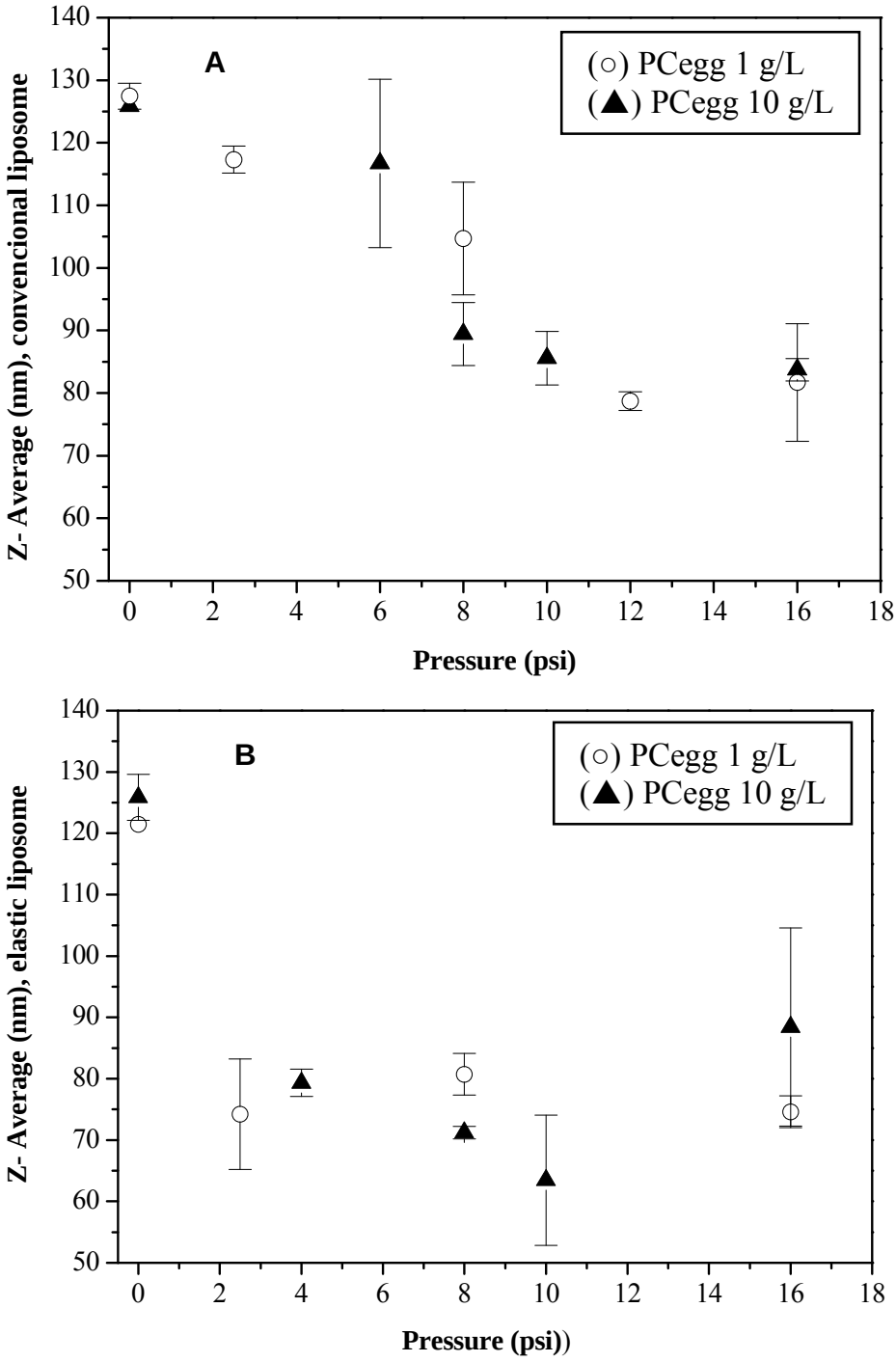

Figure 4. Z-averages for (A) conventional liposomes and (B) elastic liposomes, using two polycarbonate membranes with a pore size of 30 nm, as a function of PCegg concentration and applied pressure. The surfactant concentration used was 40 mol%.

phosphate content increased.

DISCUSSION

Enhanced bioavailability of topical actives is desired for many researches for treatment of diseases or cosmetic application. Mezei and Gulasekharam (1980) were the first to suggest liposome as drug delivery in topical administration. These researches loaded triamcinolone acetonide into liposome and evaluated it in rabbit skin. Results showed increase of drug in the skin and low levels

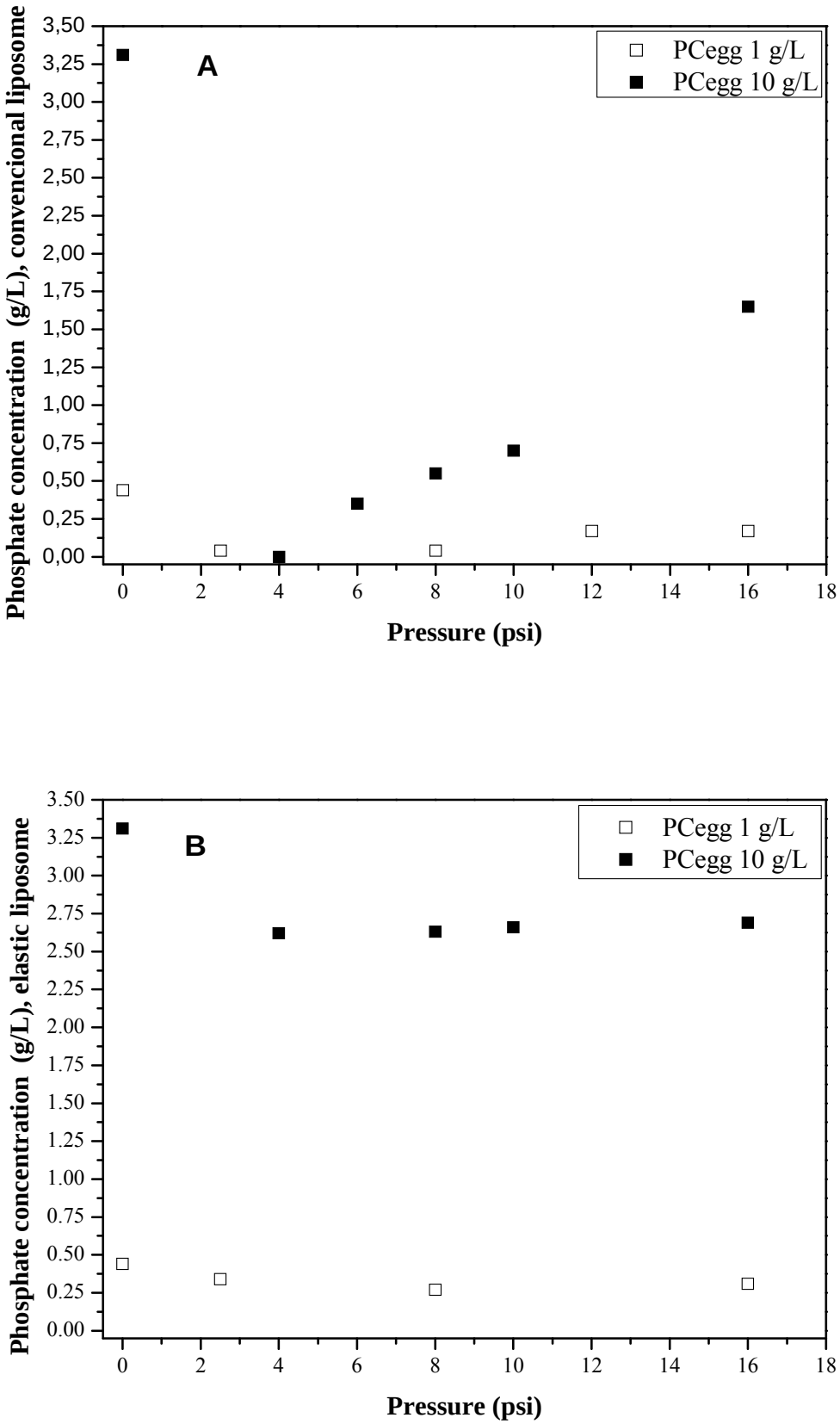

Figure 5. Phosphate concentrations for conventional and elastic liposomes after the elasticity assays, as a function of PCegg concentration and applied pressure. The surfactant concentration used was 40 mol%.

of percutaneous absorption.

Liposome can be produced by several methods, however, Bangham method is a very popular preparation technique that requires extrusion to form homogeneous liposomal formulation. This method is generally accepted due its reproducibility and possibility to scale up. Example is Lipex® Biomembranes Inc., a system invented for extrusion ranging from small to large scale can be used in high temperatures (Wagner and Vorauer-Uhl, 2011). Recent studies have described the incorporation of PEG8L into liposomes in order to improve membrane flexibility and increase skin absorption (Cereda et al., 2013; Chen et al., 2013; Barbosa et al., 2013).

Similar results were obtained by Severino et al. (2012), who produced elastic liposomes using egg phosphatidylcholine with cholesterol as surfactant. This formulation was loaded with benzophenone-3 sunscreen in order to increase the effectiveness of the chemical when applied to the skin. After extrusion, the liposomes loaded with benzophenone-3 presented a size of around 100 nm, a PI of 0.2, and an incorporation percentage of 20.34% (m/m). The *in vitro* results indicated that use of the liposomes should enhance the sun protection factor, compared to free benzophenone-3.

Our group, in a previous study, reported the incorporation of PEG8L into liposomes and demonstrated the capacity of the fluid bilayer to accommodate the surfactant without enlargement. Similar results were observed for liposomes in the presence of 40 mol% PEG8L as shown in Figure 1. However, incorporation of PEG8L into magnetoliposomes caused a reduction in the fluidity of the bilayer due to the inclusion of iron in the lipid structure (Barbosa et al., 2013).

Uchino et al. (2011) loading ketorolac in rigid and elastic vesicles, showed that elastic vesicles were produced with mixture of surfactants sucrose-ester laurate, octaethylene glycol laurate (PEG8L) and sulfosuccinate (TR-70). After incorporation, surfactants vesicles showed little variation of size (90 to 150 nm).

Researchers reported that drug transport across the skin is increase in elastic liposome (Cevc, 1995; Cevc and Gebauer, 2003; Uchino et al., 2011). To verify the fluidity of liposome membranes calculate flow rate, elasticity and deformation.

In this study, conventional and elastic liposomes in two lipid concentration (1 and 10 g/L) and comparative data were obtained using a flow of Milli®-Q water. In order to mimic human skin, the membrane pore size simulated that of the *stratum corneum* (30 nm), and it was assumed that the membrane pores possessed cylindrical geometry and had identical diameters. As expected, the flow rate of Milli®-Q water was higher than that of the formulations, under the same experimental conditions, and the permeation of elastic liposomes exceeded that of conventional liposomes. For conventional liposomes, higher flow rates were obtained for samples containing lower concentrations of lipids, while no significant difference was observed for elastic liposomes.

According to Figure 2C it is observed that greater pressure was required for more concentrated samples. As the applied pressure was increased, the elasticity values increased up to a limit value that represented the maximum deformation capacity of a spheroidal structure (Bruinsma, 1996), with the effect being more evident for the conventional liposomes.

In the pressure range investigated, nonlinear behavior was observed for the conventional liposomes, while the flow rate of vesicles containing surfactant was proportional to the pressure applied, thus satisfying the Darcy flow law (Hunter and Frisken, 1998). Darcy's law assumes linearity between flow rate and pressure drop, and the proportionality constant is the permeability of the medium, which only depends on its physical properties. The law was subsequently extended to represent the flows of many fluids in porous media including membranes (Slattery, 1972). These results clearly show the difference in deformability between the elastic and the conventional liposomes. For conventional liposomes, permeation was limited by the rigidity of the bilayer, which restricted the flow as the pressure increased. Moreover, a minimum pressure of 4 psi was required to enable these liposomes to flow through the membrane.

Elasticity was also evaluated in two concentration of lipid (1 and 10 g/L) for conventional and elastic liposomes. Lower phospholipid concentration was noted in conventional liposomes, and lower lipid concentration with favorable membrane to exhibit less ordered structure forming a more compact and more fluid in improved elasticity. This is favored by the addition of surfactant that showed the membrane with higher elasticity (Figure 3).

Van den Bergh et al. (1999) studied the influence of elastic and conventional liposomes on the structure and permeability of the skin. It was found that elastic liposomes containing PEG8L surfactant disorganized the lipid bilayers, creating or modifying pathways for possible drug penetration. As reported previously (Huang et al., 2011), the presence of surfactant molecules in liposomes reduces the surface tension of the vesicles, leading to a reduction in particle size. The incorporation of PEG8L into the liposomes demonstrated the capacity of the fluid bilayer to accommodate the surfactant without disruption (Barbosa et al., 2013). Trotta et al. (2002) investigated the elasticity of conventional and elastic liposomes and found that the quantity of surfactant influenced the ability of the particles to change shape and deform under stress. Larger quantities of surfactant promoted highly curved structures and decreased the energy required for particle deformation, favoring liposome penetration in pores.

Figure 4 shows a reduction of the size of liposome added to the surfactant. Huang et al. (2011) observed similar results for liposomes containing soy phosphatidylcholine, cholesterol, and Tween® 80 as the surfactant. An increase on the concentration of Tween®

80 caused a gradual decrease in both liposome size and PI, indicative of a fairly homogeneous vesicle population.

Teixeira et al. (2010) prepared elastic polymeric nanocapsules by the pre-formed polymer interfacial deposition method. According to authors, nanocapsules without an additional surfactant are retained on the membranes, because the capillary repulsion forces between membrane pores and particles. However, 0.2% m/v of PEG8L can favors the wettability of pores and take the particles to cross the barrier. Moreover, the high permeability of the nanocapsules is probably because of the system's deformability characteristics. According to Honeywell-Nguyen et al. (2003), elastic vesicles were able to extrude the formulations through membranes with pore sizes of 30 nm; on the other hand, rigid vesicles could not even be extruded through membranes with pore sizes of 50 nm.

Figure 5 showed the results of phosphate concentrations for the conventional and elastic liposomes after the elasticity assays. Elastic liposomes using 10 g/L of PCegg were superior recovery phosphate concentrations, and remained stable with increasing pressure. However, the conventional liposome phosphate concentration reduced with increased to 4 psi, after which the phosphate content increased. These results suggest that the higher phosphate concentrations after filtration may be associated with a higher deformability of vesicle to passing through membrane (elastic liposome). Further, some particles can undergo coalescence during extrusion promoting clogging of pores with consequent retention of sample (conventional liposome) (Teixeira et al., 2010).

Conclusions

The kinetics of surfactant incorporation revealed that, over time, the free surfactant in the solution became incorporated in the bilayer as was observed. The elasticity was higher for liposomes containing PEG8L, probably due to the high hydrophilicity of the surfactant, which increased flexibility and enabled the vesicles to traverse barriers with diameters four times smaller than the original particle diameter. The flow behavior of the elastic liposomes obeyed Darcy's law, with a linear relationship between flow and pressure drop that was independent of concentration, demonstrating the capacity for deformation during the process of nanopore permeation. Conventional liposomes showed different behavior, with pressure and size reduction limits indicating disruption of vesicles.

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Declaration of Interest

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Conflict of Interest

The author(s) have not declared any conflict of interests.

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