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Novel *O*-palmitoylscleroglucan-coated liposomes as drug carriers: Development, characterization and interaction with leuprolide

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Abstract

Polysaccharide-coated liposomes have been studied for their potential use for peptide drug delivery by the oral route because they are able to minimize the disruptive influences on peptide drugs of gastrointestinal fluids. The aim of this work was to synthesize and characterize a modified polysaccharide, *O*-palmitoylscleroglucan (PSCG), and to coate unilamellar liposomes for oral delivery of peptide drugs.

To better evaluate the coating efficiency of PSCG, also scleroglucan (SCG)-coated liposomes were prepared.

We studied the surface modification of liposomes and the SCG- and PSCG-coated liposomes were characterized in terms of size, shape, ζ potential, influence of polymer coating on bilayer fluidity, stability in serum, in simulated gastric and intestinal fluids and against sodium cholate and pancreatin.

Leuprolide, a synthetic superpotent agonist of luteinizing hormone releasing hormone (LHRH) receptor, was chosen as a model peptide drug.

After polymer coating the vesicle dimensions increased and the ζ potential shifted to less negative values. These results indicate that both SCG- and PSCG-coated liposomes surface and DSC results showed that PSCG was anchored on the liposomal surface.

The stability of coated-liposomes in SGF, sodium cholate solution and pancreatin solution was increased.

From this preliminary in vitro studies, it seems that PSCG-coated liposomes could be considered as a potential carrier for oral administration.

Keywords: Oral; Sleroglucan; *O*-Palmitoylscleroglucan; Coated-liposomes; Leuprolide

1. Introduction

Liposomes have been extensively studied for their potential use as drug carriers and development of stable liposomes is fundamental for this purpose. Many attempts have been made to enhance the stability of liposomes (New, 1990; Gregoriadis, 1991; Park et al., 1992; Sivakumar and Panduranga Rao, 2001). Among them, surface modification of liposomes is an attractive method to enhance vesicle in vitro and in vivo stability (Jones, 1995; Sagristá et al., 2000; Kato et al., 2004; Lukyanov et al., 2004; Han et al., 2006).

Liposomes have been studied for intraperitoneal and intravenous administration for the delivery of therapeutic or diagnostic agents to specific target tissues. However, there has been increased interest in their potential use for peptide drug delivery by the oral route because they are composed of physiological materials (Fukunaga et al., 1991).

The main problem associated with orally-administered liposomes is their poor stability in the gastrointestinal tract, due to pH, bile salts and pancreatic lipase presence in the GI tract (Kato et al., 1993).

To enhance liposome stability in order to decrease the leakage of entrapped solute and to improve the cellular uptake of liposomes, natural polysaccharides (i.e. mannan, pullulan, amylopectin, dextran, chitosan) were used to coat the outermost surface of liposomal vesicle (Vyas et al., 2005, 2004; Venkatesan and Vyas, 2000; Cansell et al., 1999; Guo et al., 2003).

Coating liposomes with polypeptides or ligands is also an important biomimetic strategy to realize molecular recognition

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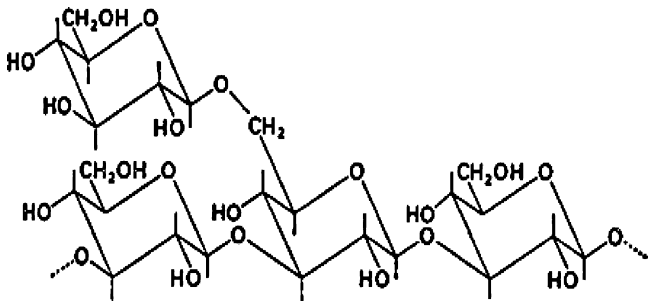

Fig. 1. Polymer structure.

on liposome surface and also to strengthen the mechanical properties of liposomes.

On the other hand, polysaccharide anchoring by adsorption was found to be thermodynamically unstable and pharmaceutically unacceptable (Sihorkar and Vyas, 2001). In order to eliminate these limitations, chemically modified polysaccharide were used to coat liposomes (Sunamoto and Iwamoto, 1986; Lee et al., 2005). In these partially hydrophobized polysaccharides acyl chains were allowed to react covalently with natural polysaccharides and subsequently integrate with the lipid constituents of liposome bilayer.

Scleroglucan is a biocompatible and biodegradable polymer of low toxicity, thus it has been extensively used in the pharmaceutical field (Maggi et al., 1996; Coviello et al., 1998, 2005).

Scleroglucan (SCG) (Fig. 1), a microbial polysaccharide, is a linear chain of 1,3-β linked D-glucopyranose units with single D-glucopyranose residues 1,6-β linked to every third unit of the chain.

The aim of this work was to synthesize and characterize a modified polysaccharide, *O*-palmitoylscleroglucan (PSCG), to coat unilamellar liposomes for oral delivery of peptide drugs.

To better evaluate the coating efficiency of PSCG, also scleroglucan (SCG)-coated liposomes were prepared.

The SCG- and PSCG-coated liposomes were characterized in terms of size, shape, ζ potential, influence of polymer coating on bilayer fluidity, stability in serum and in simulated gastric and intestinal fluids and against sodium cholate and pancreatin.

Leuprolide, a synthetic superpotent agonist of luteinizing hormone releasing hormone (LHRH) receptor, was chosen as a model peptide drug.

2. Materials and methods

2.1. Materials

Phospholipon 90 (Ph90) was a gift of Nattermann (Germany), cholesterol (CHOL) was purchased from Carlo Erba (Italy), calcein; HEPES salt {*N*-(2-idroxyethyl)-piperazine-*N*′-(2-ethanesulfonicacid)}; Sepharose 4B; Sephadex G-75; pancreatin (from porcine pancreas) and bovine serum were Sigma–Aldrich (Italy) products, sodium 5,6-carboxyfluorescein was purchased by Kodak, scleroglucan (SCG) was provided by Degussa (Germany), Leuprolide was a generous gift of Abbott (USA). All other products and reagents were of analytical grade.

Table 1
Sample composition, expressed as molar fractions

Samples	Phospholipon 90	CHOL
Ph90	1.00	0
P/C1	0.99	0.01
P/C5	0.95	0.05

2.2. Preparation of liposomes

Unilamellar vesicles were obtained by means of the "film" method as previously reported (Carafa et al., 2002), according to the composition reported in Table 1.

For this purpose, Ph90 and, when applicable, CHOL were dissolved in a $CHCl_3/CH_3OH$ (3:1) mixture in a round-bottomed flask.

When the peptide drug (1 mg/ml) was used, the same procedure was carried out adding leuprolide to the constituents before film preparation.

After evaporation of the solvents, the dried film was hydrated by addition of 5 ml of different aqueous phases:

1. HEPES buffer (0.01 M, pH 7.4) for vesicle characterization.
2. Sodium calcein 10^{-2} M in HEPES (0.01 M, pH 7.4) to determine the best sample composition, the entrapment efficiency and for stability studies.
3. Sodium 5,6-carboxyfluorescein 10^{-2} M in HEPES (0.01 M, pH 7.4) to make stability studies in calf serum.

The dispersion was then vortexed for about 5 min and then sonicated for 30 min at 25 °C using a tapered microtip operating at 20 kHz at an amplitude of 18% (Vibracell-VCX 400-Sonics, USA).

2.3. Vesicle purification

In order to separate formed vesicles from not structured materials, the vesicle dispersion was purified by gel-filtration on Sephadex G75 columns (50 cm × 1.2 cm), using HEPES buffer as eluent.

According to the quantitative evaluation of phospholipids proposed by Stewart (1980), carried out on the purified suspension of liposomes, the percentage of phospholipid actually structured in all samples to form the vesicles was determined.

2.4. Derivatization of scleroglucan

Palmitoylscleroglucan (PSCG) was prepared as described by Sunamoto and Iwamoto (1986) for the preparation of *O*-palmitoylpullulan. Briefly, 10 g of scleroglucan were dissolved in 100 ml of dry dimethylformamide at 60 °C. To the resultant solution 16 ml of dry pyridine and 6.1 g of palmitoyl chloride, dissolved in 20 ml of dimethylformamide were added. The mixture was stirred at 60 °C for 2 h followed by 1 h at room temperature. Then, it was slowly poured into 350 ml of absolute ethanol. The precipitate obtained was collected and washed with 200 ml of ethanol and 180 ml of dry diethyl ether. The solid

material obtained was dried in vacuum at 50 °C for 2 h. The polymer obtained has a melting temperature between 242 and 244 °C with decomposition. The polymer was characterized by IR and ^{1}H NMR spectra analyses. The IR spectrum of PSCG (3.5%) incorporated in a KBr tablet, was run on a Perkin-Elmer FTIR 1600 spectrometer.

For ^{1}H NMR spectra analysis a Varian VXR 300-MHz spectrometer was used. The ^{1}H NMR spectrum was obtained in deuterated dimethylsulfoxide solution (DMSO-d_6) using tetramethylsilane (TMS) as internal standard.

2.5. Preparation of polysaccharide-coated liposomes

Before the preparation of coated vesicles, the resistance of liposomes to polymer suspension was tested.

Calcein loaded liposomes were added to different concentration of polymer solutions (from 0.04 to 0.4%, w/v) and the dequenching of the fluorescent probe was measured.

A suspension of the polymer was prepared: 40 mg of the polymer were added to 25 ml of HEPES buffer, pH 7.4 and the resultant suspension was left under magnetic stirring for 3 days at room temperature and then for 24 h at 50 °C. Polymer suspensions were then filtered on filters of 0.45 μm porosity and then mixed with the liposomes suspension in a 1:2 volumetric ratio.

The contact suspension was maintained under magnetic stirring 12 h at a temperature of 50 °C.

Coated-liposomes were purified by means of gel-filtration on Sepharose 4B columns (50 cm × 1.2 cm), using HEPES buffer as eluent.

2.6. Sample characterization

2.6.1. Freeze-fracture

Vesicles were examined by means of the freeze fracture microscopy technique.

The samples were impregnated in 30% glycerol and then frozen into partially solidified Freon 22, freeze-fractured in a freeze-fracture device (−105 °C and 10^{-6} mmHg) and replicated by evaporation from platinum/carbon gun.

The replicas were extensively washed with distilled water, picked up onto Formvar-coated grids and examined with Philips CM 10 transmission electron microscope.

2.6.2. Size measurements, zeta potential and stability tests

Size measurements, before and after coating were carried out and evaluation of vesicle stability, in terms of vesicle aggregation, was evaluated by means of dynamic light scattering. The vesicle dispersions were diluted about 100 times in the same buffer used for their preparation. Dust particles were eliminated by filtration (0.45 μm) from the buffer solution as well as from the vesicle preparation. Vesicles size distribution was measured on a Malvern Nano ZS90 (Malvern, UK) at 25 °C, with a scattering angle of 90.0°. The used software is DTS Nano, version 4.0, and the mathematical method used for size measurements is the standard data analysis program CONTIN, in terms of a continuous distribution of exponential decay times (Maulucci et al., 2005).

The same apparatus was used for the evaluation of ζ potential using a vesicle preparation appropriately diluted (1:10) in distilled water at 25 °C. The ζ potential determination is based on Smoluchowski relation, that converts the mobility μ of the diffusing aggregates into a ζ potential:

$$\zeta = \frac{\mu\eta}{\varepsilon} \quad (1)$$

where ε is the permittivity of the solution.

The polidispersity index (p.i.) was directly calculated by the software of the apparatus and the values obtained are in agreement with mono disperse vesicular systems. Vesicle stability, in terms of changes in vesicle dimensions after aggregation, was evaluated using of the same technique on samples stored, up to 1 month, at 4, 25 and 37 °C. This type of information was also confirmed by means of ζ potential measurements.

2.6.3. Coating efficiency

The coating amount of polymers was measured as follows: a small amount (0.3 ml) of the polymer-coated liposomal suspensions was centrifuged at 75,000 rpm for 120 min (TL100, Beckman).

Quantitative analysis of polymer in the supernatant was carried out by means of colorimetric assay (Dubois et al., 1956). Briefly, supernatant samples were treated with phenol (80%) and with concentrated sulphuric acid; after 10 min a characteristic yellow-orange colour was developed and the intensity was measured at 490 nm (Perkin-Elmer Lambda 25 UV/VIS spectrometer). The amount of polymer coating the vesicles was calculated from the reduced polymer concentration in the solution after coating.

2.6.4. DSC analyses

DSC measurements were carried out only on samples P/C1, SCG–P/C1 and PSCG–P/C1, containing the lower cholesterol amount, because on samples prepared with CHOL 5% (w/w) is impossible to evidence any variations in obtained thermograms.

The thermal analyses were carried out with a DSC 131 Setaram calorimeter in the temperature range 0–170 °C. The heating and cooling rates were fixed at 1.0 °C/min, under N_2. To calibrate temperature and enthalpy scales in the considered range, caprylic acid was used. Heating and cooling cycles were repeated at least three times to ensure the reproducibility and constancy of the thermal parameters. In all cases, the measured deviations were within the expected experimental error (±0.1 °C for T_m and ±5% for ΔH values).

2.6.5. Drug encapsulation efficiency

The drug entrapment within the vesicles was assesed by HPLC (Perkin-Elmer, LC5 pump, 250 DAD) on purified vesicles, after lyophilization.

The column was a RP18 Lichosphere 5 μm (250 mm × 4.00 mm i.d.); the mobile phase was a 30/70 mixture of CH_3CN/H_2O (CF_3COOH, 0.1%, v/v); the detection was carried out at 220 nm, at a flow rate of 1 ml/min.

Drug encapsulation efficiency (e.e.) was calculated as follows:

$$\text{e.e.} = \frac{\text{mass of incorporated drug}}{\text{mass used for vesicle preparation}} \times 100 \quad (2)$$

2.6.6. Stability in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8–7.4)

To investigate the stability of SCG- and PSCG-coated liposomes in gastric fluid, the suspensions of coated- and uncoated-liposomes were separately added to simulated gastric fluid (SGF) with stirring. SGF was composed of 0.2% sodium chloride, 0.32% pepsin and 0.7% hydrochloric acid. The final solution was adjusted to about pH 1.2 (Guo et al., 2003).

After magnetic stirring for 5 h, leuprolide concentration was determined, in the supernatant by means of HPLC (see Section 2.6.5), after centrifugation (see Section 2.6.3).

The same experiment was also assessed at pH 6.8 in SIF: 0.067 M mixed sodium and potassium phosphate small intestinal fluid ($Na_2HPO_4 \cdot 7H_2O/KH_2PO_4$-Sorensen's buffer).

In the supernatant the lipid concentration were also assed, according to Stewart (1980), as described in Section 2.3.

2.6.7. Stability against sodium cholate solution and pancreatin solution

The study of stability of the SCG- and PSCG-coated liposomes against bile salt and pancreatic lipase is necessary because this carrier is intended to be delivered via the oral route.

The pancreatin solution was prepared by dissolving 10 mg of pancreatin × 100 ml of HEPES buffer, pH 7.4, containing 5 mM of $CaCl_2$ and NaCl for osmolarity adjustment (Kokkona et al., 2000).

Briefly, the suspensions of coated- and uncoated-liposomes were poured into tubes and then the same volume of sodium cholate solution (20 mM HEPES buffer, pH 7.4, containing NaCl in needed amount for osmolarity adjustment) or pancreatin solution were added into the tubes. The stability of coated liposomes was evaluated on the basis of quantitative release of leuprolide after 5 h at 37 °C, determined in the supernatant by means of HPLC (see Section 2.6.5), after centrifugation (see Section 2.6.3).

2.6.8. Stability assay of in the presence of serum

Three hundred microlitres of coated and uncoated purified liposomes, entrapping sodium 5,6-carboxyfluorescein, were added to 50 ml of calf serum (10% in HEPES buffer, v/v) and the samples were incubated at 37 °C, under mild stirring.

To estimate the stability of liposomes, the fluorescence variations were evaluated ($\lambda_{ex} = 486$ nm and $\lambda_{em} = 514$ nm) for 1 h, every 10 min (Allen and Cleland, 1980).

2.7. Statistical treatment

The results were expressed as the mean ± standard deviation (S.D.) of at least three experiments.

Statistical analyses were used to compare the influence of experimental parameters on vesicle stability after polymer coating and were carried out using Student's unpaired *t*-test and one-way analysis of variance (ANOVA). Significance was taken as $P < 0.05$ and $P < 0.01$, respectively.

3. Results and discussion

3.1. Characterization of PSCG

The palmitoylscleroglucan was characterized by IR and ^{1}H NMR.

The IR analysis (Table 2) was used to identify carbonyl groups and to establish that scleroglucan is covalently bound to palmitoyl residues; in particular a characteristic stretching vibration of C=O is observed at about 1735 cm^{-1} (Pavia et al., 1979). However in the PSCG, the stretching vibration was observed at 1655 cm^{-1}. This shift in frequency could be ascribed to a consequence of intramolecular hydrogen bonds between carbonyl and hydroxyl groups that lower the stretching force vibration of C=O band (Sihorkar and Vyas, 2000; Venkatesan and Vyas, 2000). The presence of hydrogen bonds was confirmed from the OH stretching vibration at 3415 cm^{-1} (polymeric band). Furthermore there is a characteristic C–H stretching vibration at 2925 cm^{-1} and C–O stretching at 1100 cm^{-1}. Starting from these data it can be concluded that there exists an ester bond between scleroglucan and palmitoyl residues suggesting palmitoylation of scleroglucan.

Furthermore, the PSCG formation was also confirmed by ^{1}H NMR (Table 3). The proton corresponding to the terminal methyl group of the palmitoyl chain were observed at 0.822 ppm, that of 12-methylene groups were observed at 1.38 ppm, while those at 1.23 and 2.40 ppm were indicative of the presence of β and α methylene groups, respectively, in accordance with those found by Moreira et al. (1997). Altogether the IR and ^{1}H NMR spectrum data strongly suggest that scleroglucan and palmitoyl group were covalently bonded.

Table 2
IR spectral data of PSCG

Observed values (cm^{-1})	Expected values (cm^{-1})	Functional group	Attribution
3415	3600–3200	Hydroxyl groups	Stretching vibration of O–H bond
2925	2960–2950	CH_2CH_3	C–H stretching
1655	1750–1735	C=O	Carbonyl band stretching
1080	1150–1040	C–O	C–O stretching

Table 3
^{1}H NMR spectral data of PSCG

Observed values (ppm)	Expected values (ppm)	Functional group	Attribution
0.82	0.90	$–CH_3$	Terminal methyl group
1.18	1.17	$–(CH_2)_{12}$	Methylene groups
1.23	1.40	$–CH_2\beta$	β-Methylene group
2.40	2.30	$–CH_2\alpha$	α-Methylene group

3.2. Characterization of coated liposomes

It is evident from Fig. 2, that the coating procedure did not modify the vesicular structure, regardless of the cholesterol content in the lipid bilayer.

The formation of a polymer layer on the surface of liposomes was verified by comparing the particle size of the liposomes before and after polymer coating. The increase in particle size of analyzed samples is summarized in Table 4. The obtained results suggested the formation of a coating layer on the surface of liposomes. When the coating amounts of the polymer-coated liposomes were compared, a higher amount of PSCG than SCG was observed both for P/C1 and P/C5 samples (P/C1 + PSCG, 87.0 ± 1.2; P/C1 + SCG, 59.0 ± 2.1; P/C5 + PSCG, 79.1 ± 1.0; P/C5 + SCG, 49.1 ± 0.9) This was explained by the different coating method: a simple physical adsorption of SCG and an anchoring of the hydrophobic chain of PSCG to the liposome bilayer (Takeuchi et al., 1998). Purified coated vesicle dimensions and ζ potential values remained stable during stability tests up to 1 month, this suggesting that there is no significant polymer desorption in the absence of free polymer.

When the polymer concentration of coating was increased, the amount of resultant coating layer on the surface of liposomes was not increased (data not shown).

The ζ potential of the liposomes shifted to less negative values by coating the liposomes with polymers (Table 4) and these changes may be attributed to the increase in thickness of the polymer layer formed on the surface of liposomes that can move the sheared plane into the bulk solution side (Takeuchi et al., 1998).

To evaluate the influence of polymer coating on liposome bilayer characteristics DSC analyses were carried out. The DSC results (Table 5) showed that cholesterol broaden the main transition peak and decreased the transition temperature of Ph90 in

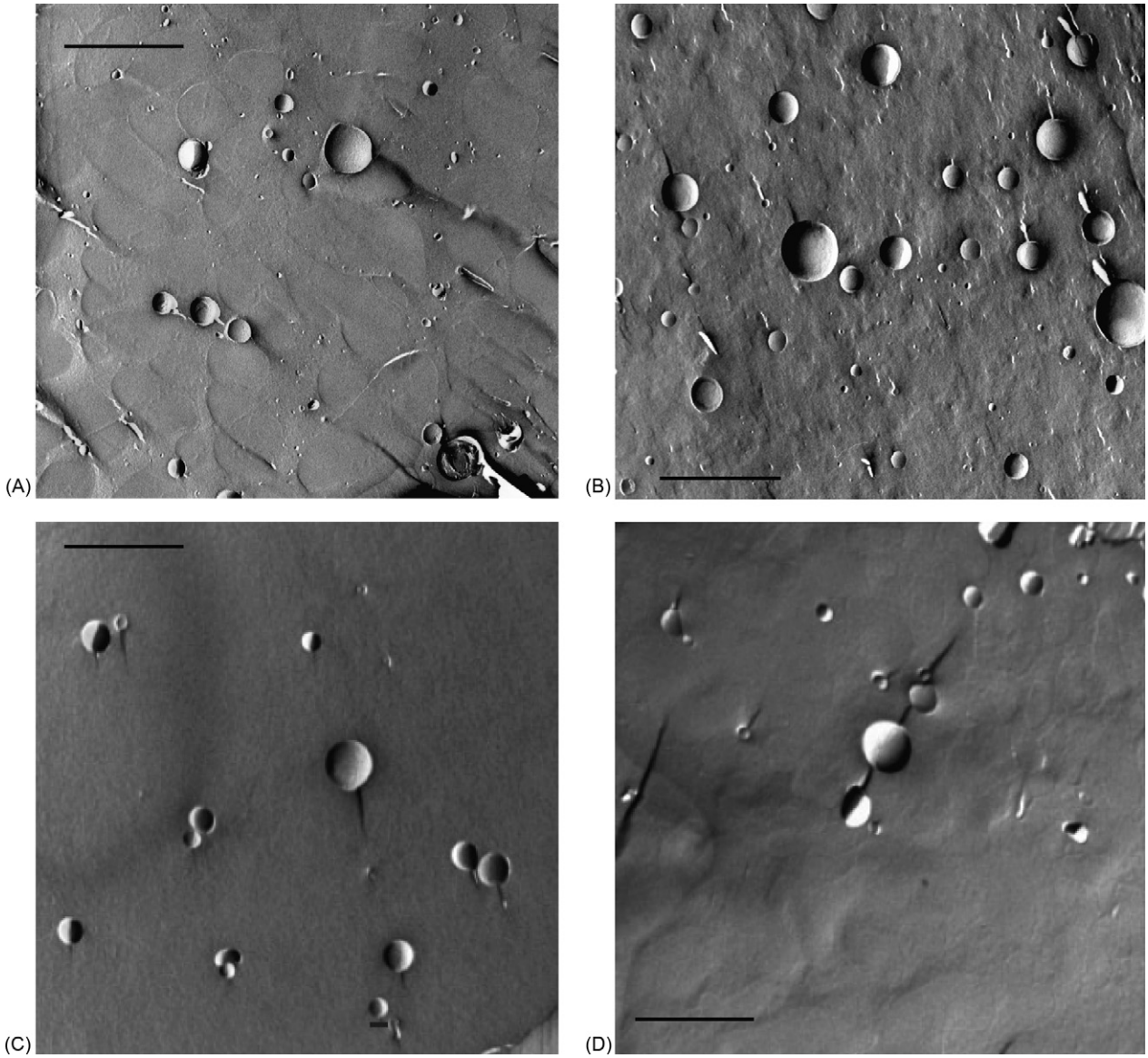

Fig. 2. Transmission electron micrographs of coated-liposomes after freeze-fracture: (A) sample P/C1 + SCG; (B) sample P/C5 + SCG; (C) sample P/C1 + PSCG; (D) sample P/C5 + PSCG (scale bar = 250 nm).

Table 4
Percentage of phospholipid actually structured (%), vesicle dimension (nm) with p.i., ζ potential (mV) of analysed samples ($n = 3$, ±S.D.)

Samples	%	Size (nm)	p.i.	ζ potential (mV)
Ph90	70.2 ± 0.6	105.31 ± 2.45	0.272 ± 0.008	−22.12 ± 0.26
P/C1	69.7 ± 0.3	109.76 ± 4.44	0.293 ± 0.009	−28.50 ± 0.24
P/C5	68.5 ± 0.2	120.46 ± 2.03	0.283 ± 0.009	−38.48 ± 0.28
SCG				−22.84 ± 0.20
PSCG				−11.49 ± 0.51
P/C1 + SCG		116.44 ± 2.29	0.278 ± 0.010	−22.30 ± 0.43
P/C1 + PSCG		112.21 ± 3.36	0.205 ± 0.007	−20.11 ± 0.39
P/C5 + SCG		123.70 ± 2.03	0.284 ± 0.009	−32.15 ± 0.38
P/C5 + PSCG		120.63 ± 3.83	0.240 ± 0.018	−29.98 ± 0.22

Table 5
Effect of polymer coating on Ph90 transition temperature

Samples	T_m (°C)	T_{onset} (°C)
Ph90	22.31 ± 0.21	19.43 ± 0.35
CHOL	149.01 ± 0.09	147.11 ± 0.11
SCG	88.77 ± 0.35	59.99 ± 0.31
PSCG	81.23 ± 0.43	50.47 ± 0.38
P/C1	15.20 ± 0.32	6.12 ± 0.40
P/C1 + SCG	27.13 ± 0.35	20.24 ± 0.30
P/C1 + PSCG	10.09 ± 0.40	4.21 ± 0.41

liposomes, in agreement with data reported by Hirsch-Lerner and Barenholz (1999).

SCG-coated liposomes showed an increase of phospholipid T_m, this probably related to the hydration of liposome surface due to the presence of primary –OH of SCG, in agreement with Fujiwara et al. (1997). On the other hand, PSCG-coated liposomes showed a decrease of phospholipid T_m, probably related to the interdigitation of the hydrophobic chain of PSCG in the liposome bilayer, according to Savva et al. (1999).

These data are in agreement with data reported by Auner et al. (2005) and evidenced that the polymer layer formed on liposome surface modified the sharpness of the phospholipid transition, this probably related to an increased number of molecules forced to cooperate in the transition.

3.3. Leuprolide entrapment efficiency

The interaction of leuprolide with uncoated- and coated-liposomes led to an increase in vesicle dimensions and to a decrease in ζ potential values (Table 6), but did not increase vesicle lamellarity (Fig. 2). Leuprolide is water-soluble and posses positive charge at pH 7.4 (ζ potential = +4.02 mV) and the collected data are in agreement with a "partition" of the drug between the aqueous core of liposomes and the outer surface. It has been reported that the interaction of positively charged peptides with lipid membrane depends both on electrostatic attraction at the polar head group level and the apolar part of membrane (Lo and Rahman, 1995), this leading to a higher drug entrapment efficiency for uncoated-liposomes (Table 6) with more negative surface charge, in agreement with data reported by Guo et al. (2003).

After entrapment efficiency experiments, only samples containing 1% of cholesterol were used for stability studies.

3.4. Stability in SGF and SIF

There was a leakage of leuprolide from P/C1L liposomes upon dilution in SGF and SIF no matter whether the pH is acidic or neutral (Fig. 3). On the other hand, the coating procedure with both natural and derivatized polymers seems to prevent drug leakage in SGF, without significative differences between

Table 6
Effect of the interaction of leuprolide (L) with coated- and uncoated-liposomes; entrapment efficiency (e.e.) is expressed as % of leuprolide loading dose ($n = 3$, ±S.D.)

Samples	Size (nm)	p.i.	ζ potential (mV)	e.e. (%)
P/C1	109.76 ± 4.44	0.293 ± 0.009	−28.50 ± 0.24	
P/C1 + SCG	116.44 ± 2.29	0.278 ± 0.010	−22.30 ± 0.43	
P/C1 + PSCG	112.21 ± 3.36	0.205 ± 0.007	−20.11 ± 0.39	
P/C1L	155.02 ± 1.76	0.238 ± 0.013	−13.64 ± 0.55	76.01 ± 0.10
P/C1L + SCG	155.76 ± 1.09	0.254 ± 0.012	−12.62 ± 0.46	40.10 ± 0.07
P/C1L + PSCG	160.10 ± 3.34	0.205 ± 0.007	−8.84 ± 0.13	37.07 ± 0.04
P/C5	120.46 ± 2.03	0.283 ± 0.009	−38.48 ± 0.28	
P/C5 + SCG	123.70 ± 2.03	0.284 ± 0.009	−32.15 ± 0.38	
P/C5 + PSCG	120.63 ± 3.83	0.240 ± 0.018	−29.98 ± 0.22	
P/C5L	162.10 ± 1.14	0.219 ± 0.022	−7.64 ± 0.39	47.02 ± 0.12
P/C5L + SCG	154.54 ± 4.05	0.165 ± 0.006	−8.85 ± 0.38	17.30 ± 0.34
P/C5L + PSCG	168.92 ± 2.60	0.20 ± 0.011	−12.63 ± 0.30	29.43 ± 0.18

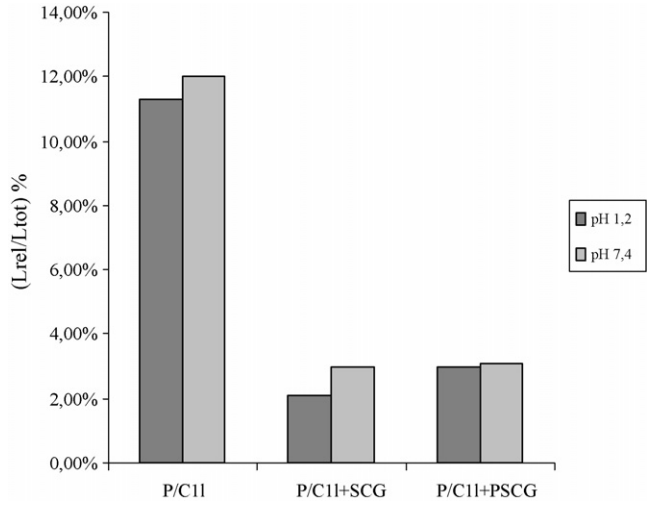

Fig. 3. Stability in SGF and SIF, at 37 °C, expressed as leuprolide leakage, after 5 h. The results are the mean of three experiments.

samples P/C1L + SCG and P/C1L + PSCG (Fig. 3). The drug leakage is probably related to vesicle disruption, in agreement with free phospholipid amount determined in the analyzed samples (Table 7).

3.5. Stability against sodium cholate solution

Leakage of leuprolide from liposomal suspensions was examined with respect to the evaluation of stability in presence of bile acid.

The degree of drug release in sodium cholate solution was dependent on polymer coating. The non-coated liposomes and the SCG-coated liposomes clearly showed a drug leakage. On the other hand, the leakage was slight from PSCG-coated liposomes (Fig. 4).

The changes in liposome stability can be related to the incorporation of cholate molecules into the lipid bilayer (Moreira et al., 1996) and the interdigitation of the hydrophobic chain of PSCG in the liposome bilayer might prevent this fenomenon, in agreement with free phospholipid amount determined in the analyzed samples, after 5 h (Table 7).

Therefore, it can be suggested that the PSCG-coated liposomes can be used to deliver drugs to the intestinal tract via oral administration.

3.6. Stability against pancreatin solution

Leakage of Leuprolide from liposomal suspensions were examined with respect to the evaluation of stability in presence of pancreatin.

Table 7
Lipid amount, expressed as % of structured lipid, after liposome disruption in stability studies ($n = 3$, ±S.D.)

Samples	SGF	SIF	Na cholate	Pancreatin
P/C1L	33.1 ± 1.5	30.9 ± 1.5	45.1 ± 0.9	27.3 ± 0.3
P/C1L + SCG	10.4 ± 2.0	8.3 ± 0.9	30.2 ± 1.5	24.4 ± 1.3
P/C1L + PSCG	13.2 ± 1.7	12.2 ± 2.1	19.3 ± 1.2	20.5 ± 0.8

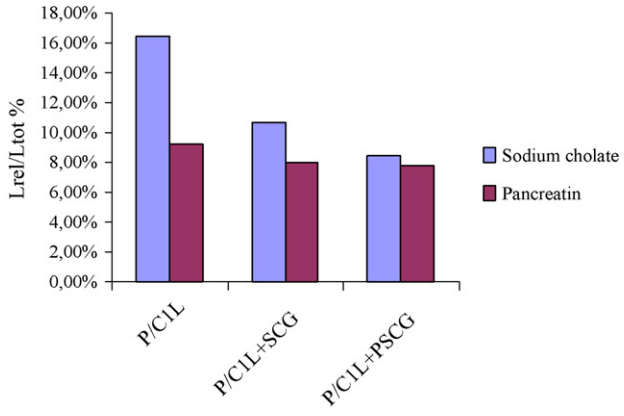

Fig. 4. Stability against sodium cholate solution (20 mM) and pancreatin solution, at 37 °C, expressed as leuprolide leakage, after 5 h. The results are the mean of three experiments.

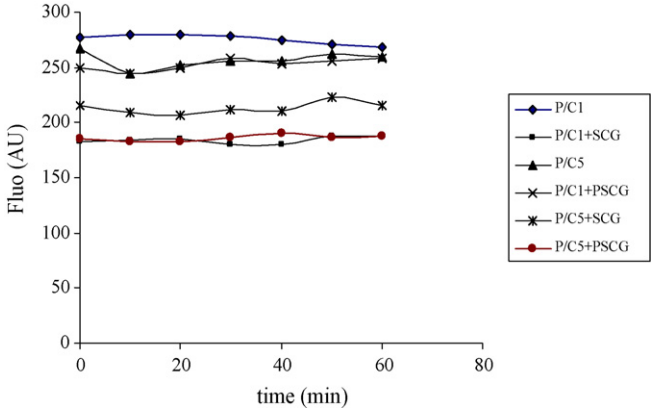

Fig. 5. Stability in calf serum at 37 °C, expressed as 5,6-carboxyfluorescein leakage. The results are the mean of three experiments.

The degree of drug release in pancreatin solution was not dependent on polymer coating (Fig. 4 and Table 7), this related with the good stability of uncoated-liposomes due to the presence of equimolar amount of cholesterol (Kokkona et al., 2000).

3.7. Stability in serum

The effects of polymer coating on the stability of liposomes in calf serum was evaluated by measuring the release of 5,6-carboxyfluorescein, in order to evaluate the potential use of the tested formulations also for parenteral administration. Comparing the release profiles (Fig. 5) from uncoated-liposomes after incubation in calf serum to those from polymer-coated liposomes, it can be evidenced that coating procedure did not modify vesicle stability in serum, at 37 °C.

4. Conclusions

In this study, scleroglucan and *O*-palmitoylscleroglucan-coated liposomes were prepared, characterized and evaluated for their potential use in oral drug delivery. Scleroglucan, a natural polysaccharide, was chemically modified into its palmitoyl derivative and used for the coating of liposomes.

After polymer coating the vesicle dimensions increased and the ζ potential shifted to less negative values. These results indicate that both SCG and PSCG coated liposome surface and DSC results showed that PSCG was anchored on the liposomal surface. The polymer coating improved the stability of liposomes in SGF and bile salts solutions. From this preliminary in vitro studies, it seems that PSCG-coated liposomes could be considered as a potential carrier for oral administration.

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