

# DLS Characterization of Non-Ionic Surfactant Vesicles for Potential Nose to Brain Application

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**Abstract:** The aim of this paper is the preparation and characterization of drug delivery systems for a potential brain delivery by intranasal administration. It is possible to reach the central nervous system with alternative routes through which therapeutic agents can bypass the blood brain barrier: that is the nasal administration. Intranasal drug administration is non-invasive and it could be a promising drug delivery method for patients who suffer from chronic and crippling Central Nervous System diseases. Among the formulation strategies for enhanced nose to brain drug delivery, the use of colloidal carriers has become a revolutionary approach. The success of a therapeutic strategy by using nanocarriers depends on their ability to entrap drugs, to penetrate through anatomical barriers, to efficiently release the incorporated drugs, to show a good stability in nanometric size range and good biocompatibility. The use of vesicular systems (niosomes), in nose to brain delivery is here presented. One of the major problems associated with nasal administration is the rapid removal of drugs or drug delivery systems, from the deposition site through mucociliary clearance. This effect is responsible of reduction of contact time between drug or drug delivery systems and nasal epithelium. This problem could be solved by coating nanocarriers with a mucoadhesive agent: chitosan. In this paper the preparation and characterization of hybrid niosomes by Tween 20 and Tween 21 together with dicetyl phosphate or Span 20 and the cationic polyelectrolyte chitosan are described in order to obtain intranasal drug delivery systems. In particular through dynamic light scattering, laser Doppler electrophoresis and fluorescence measurements the aggregation behavior between vesicles and polyelectrolyte can be monitored. Overall phenomenology is well described in terms of the re-entrant condensation and charge inversion behavior, observed in different colloidal systems. The physical stability of hybrid niosomes obtained by the three different surfactants was also evaluated.

**Keywords:** Niosomes, Chitosan, Dynamic Light Scattering, Laser Doppler Electrophoresis, Fluorescence

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## 1. Introduction

The blood brain barrier (BBB) represents the major obstacle to a direct access to the Central Nervous System (CNS) if a conventional pharmaceutical approach is used. The tight junctions between the BBB cells are responsible of the inability to enter into the brain tissue for most compounds, in effect the penetration is possible only for the 2% of small polar molecules. [1]

Actually the difficult BBB crossing has limited the use of a lot of therapeutic agents in Alzheimer's disease, stroke, brain tumor, head injury, depression, anxiety and other CNS disorders treating. It's possible to reach brain with alternative routes through which therapeutic agents can bypass the BBB: intracerebroventricular, intraparenchymal, or intrathecal

administrations, but these routes of administration are invasive, risky, and expensive techniques requiring surgical expertise especially for multiple dosage regimens. [2]

Up to a short time ago, the nasal route has been employed for therapeutic agents delivery in the treatment of local diseases: nasal allergy, sinusitis, nasal infections and nasal congestion; actually the interest of many researchers is now focused on the nasal administration route in the therapy of neurologic diseases in mice, rats, primates and humans. By this administration route drugs in fact is possible to reach the brain through the olfactory region and respiratory epithelium because the olfactory nerve cells and trigeminal nerves are in direct contact with both the environment and the CNS. Moreover intranasal (IN) route is non invasive, resulting in better patient compliance, it doesn't require sterility

requirements or any drug modifications and it has the advantage to achieve liver degradation escape (avoidance of the first passage effect). Through nasal administration the drug come into contact with nasal epithelium where the enzymatic degradation of drugs is lower if compared to that of gastrointestinal tract and liver. Anyway, the drug degradation is possible, so to enhance the stability of the IN administered drugs, one of the possible strategies is the entrapment (especially in case of peptide or protein) in drug delivery systems able to protect the therapeutic agent administration. The use of conventional and new drug delivery systems (DDS) allows to face the major issues in drug release: (1) unfavorable pharmacokinetics and biodistribution which lead to unwanted side effects (e.g. chemotherapy), (2) early drug degradation in the bloodstream by reticulo-endothelial system (RES), and (3) inefficient uptake at target sites that leads to low drug efficacy. In this sense, nanocarriers offer an innovative approach to drug delivery, providing a range of features including cargo protection and increased dose delivery to target sites. Actually, the complexity of human diseases may lead to ineffective in vivo drug treatments, consequently the development of biologically functional nanocarriers that incorporate drugs to selectively bind and target specific cells becomes crucial. [3]

The success of a therapeutic strategy by using nanocarriers depends on their ability to entrap drugs, penetrate through anatomical barriers, release the incorporated drugs, and to show a good stability in nanometric size range. Many of the conventional DDS (e.g. liposomes, niosomes, micelles, and polymer based nanodevices) have reached the late stages of development, and some of them were approved. [3, 4]

In particular, vesicular systems may increase nose-to-brain drug delivery enhancing drug chemical and biological stability. In fact the presence of a hydrophilic core is responsible of poorly water-soluble drug entrapment while the hydrophobic shell protect the system and, entrap lipophilic drugs enhancing brain uptake after intranasal administration. Numerous amphiphilic components together with different preparation techniques, lead to the formation of many vesicular systems: liposomes, niosomes, transfersomes, ethosomes, vesosomes, colloidosomes, and pharmacosomes. [5] Vesicles have spherical structure with a hydrophobic bilayer, and an aqueous core, they are prepared by phospholipids or surfactants that must be biodegradable, biocompatible and with a possible application in neuromedicine. Different studies have also demonstrated that surfactants can be used to prepare stable colloidal supramolecular devices showing a certain degree of selectivity for brain delivery. Surfactant nanocarriers, in particular niosomes, have the capability of entrap both lipophilic and hydrophilic drugs, in the apolar membrane and in the aqueous core, respectively.

They can be functionalized on their surface to obtain different type of targeting or to enhance their biological and/or physical stability. These systems have been widely used in IN delivery but one of the major problems associated with nasal administration, is the rapid removal of drugs or DDS, from the deposition site through mucociliary clearance. This effect is responsible of reduction of contact time between drug

or DDS and nasal epithelium. This problem could be solved by coating nanocarriers with a mucoadhesive agent. In particular the DDS can be coated by Chitosan, a cationic, mucoadhesive polysaccharide, able to form electrostatic interactions with the negatively charged epithelial cells reducing the mucociliary clearance. Chitosan is also able to open reversibly tight junctions enhancing the extracellular pathways through olfactory and trigeminal nerve. [6]

Chitosan originates from chitin and in particular is produced by its deacetylation (Figure 1).

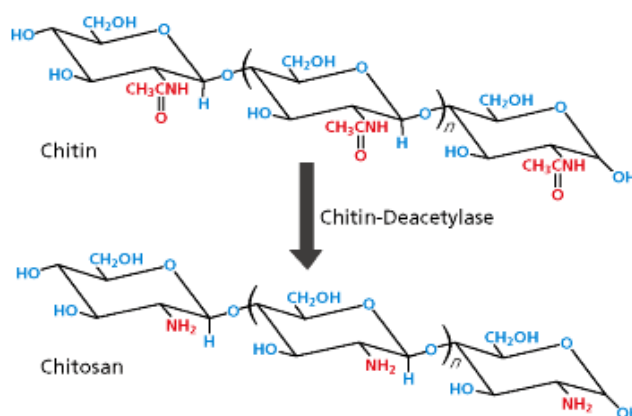


Figure 1. Chitin to chitosan conversion.

The aim of the present study is the evaluation of vesicular systems and chitosan interaction in order to obtain chitosan coated vesicles able to reach the brain by a IN administration avoiding the effect of the mucociliary clearance. Among vesicular systems, non-ionic surfactant ones or niosomes (NSVs) were chosen. Niosomes are similar, in terms of structure and physical properties, to liposomes. [7] They are prepared following the same procedures and under the same variety of conditions to produce unilamellar or multilamellar vesicles. The research interest in niosomal formulations is recently widening because niosomes are able to overcome some disadvantages associated with liposomes: surfactants are easily derivatized, more stable and give a higher versatility to the vesicular structure and moreover they have lower costs than phospholipids. [3]

To this aim, the complexation between negatively charged niosomal vesicles and a cationic, mucoadhesive polysaccharide: the chitosan has been investigated. In this work, three different formulation of unilamellar niosomal vesicles composed by a mixture of polysorbate 20 (Tween 20) or polysorbate 21 (Tween 21) or sorbitan monolaurate (Span 20) with cholesterol have been prepared and characterized.

Tween 20 and Tween 21 vesicles show a quite negative  $\zeta$ -potential value, not sufficient to perform a stable complexation with chitosan, so a more negative  $\zeta$ -potential value is obtained adding to the formulation a ionic surfactant, dicetyl phosphate (DCP), at an appropriate concentration.

On the other hand vesicles made of Span 20 do not need addition of DCP because they are characterized by a highly negative  $\zeta$ -potential value that guarantees the right interaction with the chitosan.

The study of the aggregation behavior between charged vesicles with the oppositely charged macromolecule has been carried out in terms of evaluation of complexes physical properties such as size, size stability and  $\zeta$ -potential in view of a possible application of such aggregates in brain delivery through nasal administration. These experiments have been performed by dynamic light scattering (DLS) and laser Doppler electrophoretic measurements.

The effect of chitosan/ DCP charge ratio is investigated and discussed within the framework of the re-entrant condensation and charge inversion behavior [8]

## 2. Materials and Methods

### 2.1. Materials

Polysorbate 20 (Tween 20), polysorbate 21 (Tween 21), sorbitanmonolaurate (Span 20), Dicapryl phosphate (DCP), Cholesterol (Chol), Hepes salt {N-(2-idroxyethyl) piperazine-N'-(2-ethanesulfonic acid)}, Sephadex G75, Calcein and Chitosan (low molecular weight) were purchased from Sigma-Aldrich. All other products and reagents were of analytical grade.

### 2.2. Vesicles Preparation and Purification

The thin layer evaporation method was used to prepare non-ionic surfactant vesicles from Tween 20, Tween 21 or Span 20. In each vesicle formulation, Chol and DCP were added at different molar ratios (Table 1). [9, 10]

Table 1. Sample composition.

Sample	Tween20	Tween21	Span20	DCP	Chol
NioTw20	15mM				15mM
NioTw20+DCP	7,5mM			7,5mM	15mM
NioTw21	15mM				15mM
NioTw21+DCP		17,6mM		7,5mM	15mM
NioSpan20			15mM		15mM

All the vesicle constituents were firstly dissolved in an organic mixture (CH<sub>3</sub>OH/CHCl<sub>3</sub> 3/1 v/v), then solvent were removed under vacuum at different temperatures depending on the surfactant used, as reported in the Table 2. The obtained "film" was hydrated with 5 mL of Hepes buffer (0.01M pH 7.4). The suspension was vortexed for about 5 minutes and then sonicated (5 minutes and different amplitudes and temperatures) (Table 2) using a microprobe operating at 20 kHz (VibraCell-VCX 400-Sonics, Taunton, MA, USA).

Table 2. Sonication conditions.

Sample	Temperature (°C)	Amplitude
Nio Tw20+DCP	25	16
NioTw21+DCP	25	16
NioSpan20	60	18

The vesicle suspension was purified by gel permeation chromatography using SephadexG75 (glass column of 50 x 1.2 cm) using Hepes buffer as the eluent. The obtained

purified vesicle suspension was then extruded using cellulose filters with appropriate pore diameters.

### 2.3. Chitosan Solution Preparation

The low molecular weight chitosan solution is obtained in acetate buffer (0.2 M, pH 4.4) after stirring overnight.

### 2.4. Preparation of Hybrid Niosomes

The chitosan-coated niosomes (hybrid niosomes) formation was promoted by adding 1ml of chitosan solution (at an appropriate concentration) to an equal volume of uncoated niosomes. The obtained suspension was stirred for 3 h in a thermostated water bath at 10°C.

### 2.5. Dynamic Light Scattering Measurements

Vesicles mean diameter (Z-Average) and size distribution (polydispersity index, PDI) were measured at the temperature of 25°C by means of the dynamic light scattering (DLS) technique, using a Malvern ZetaSizerNano 90S, equipped with a 5 mW HeNe laser (wavelength  $\lambda$ = 632.8 nm) and a digital logarithmic correlator. The normalized intensity autocorrelation functions were detected at a 90° angle and analyzed by using the Contin algorithm [11] in order to obtain the decay time of the electric field autocorrelation functions. The decay time is used to obtain the distribution of the diffusion coefficient  $D$  of the particles, which can be converted into an effective hydrodynamic radius  $R_H$  using the Stokes-Einstein relationship  $R_H = K_B T / 6\pi\eta D$ , where  $K_B T$  is the thermal energy and  $\eta$  the solvent viscosity. The values of the radii reported here correspond to the intensity weighted average. [12]

### 2.6. $\zeta$ -Potential Measurements

The electrophoretic mobility measurements were carried out by means of the laser Doppler electrophoresis technique using the Malvern ZetaSizerNano 90S apparatus equipped with a 5mW HeNe laser. The mobility  $u$  was converted into the  $\zeta$ -potential using the Smoluchowski relation  $\zeta = u\eta/\epsilon$ , where  $\eta$  and  $\epsilon$  are the viscosity and the permittivity of the solvent phase, respectively. [8]

### 2.7. Spectrofluorometric Analysis

Niosomal integrity during the chitosan coating process was evaluated by means of fluorescence analyses (Perkin-Elmer LS50B spectrofluorometer).

Calcein loaded vesicles were prepared hydrating the surfactant thin film with a calcein solution (0.01 M of calcein in Hepes buffer 0.01 M pH 7.4). Free calcein was eliminated by extensive dialysis (cellulose dialysis bag membrane 8000 MWCO-Spectrum, The Netherlands). The calcein concentration inside vesicles was approximately 10<sup>-2</sup> M. At this concentration, the dye is self-quenched and is responsible of a low fluorescence signal, while if the probe is released, the dequenching process produce a remarkable increase in fluorescence values due to the dilution of the probe in the

external aqueous medium. [13]

### 3. Results and Discussion

In this study, niosomal vesicles were prepared and characterized in order to obtain a delivery systems for a potential nose to brain application. For this purpose, formulations with different compositions were prepared (Table 1). DLS analyses were carried out to evaluate dimensions and  $\zeta$ -potential characteristics in order to verify their suitability to be coated with chitosan. It can be noticed that, as shown in table 3, Span niosomes were characterized by a sufficient negative charge able to form electrostatic interactions with the positively charged polyelectrolyte.

**Table 3.** Light scattering analysis of vesicular systems in absence and in presence of DCP.

Sample	Z-Average (nm) ±DS	$\zeta$ -Potential (mV) ± DS	PDI ± DS
NioSpan20	202,91 ± 5,31	-48,34 ± 0,23	0,31 ± 0,01
NioTw20	152,53 ± 1,52	-26,42 ± 4,52	0,12 ± 0,02
NioTw20+DCP	157,34 ± 1,24	-44,22 ± 1,61	0,31 ± 0,01
NioTw21	159,32 ± 3,93	-34,23 ± 4,24	0,52 ± 0,01
NioTw21+DCP	136,52 ± 5,21	-51,72 ± 0,62	0,34 ± 0,02

DCP addiction to Tween formulations is needed to achieve a suitable negative  $\zeta$ -potential value useful for the interaction with chitosan. Therefore, the presence of DCP plays a fundamental role in decreasing  $\zeta$ -potential values, and this effect is not associated to a size variation of vesicles. On the other hand, the addiction of DCP leads to opposite effects on PDI values. In particular it produces a PDI increase in the case of Tween 20 vesicles, while it causes a decrease in the case of Tween 21 ones. This phenomenon can be explained looking at the molar ratio between Tweens and DCP (Table 1). The addiction of DCP produces a quite monodisperse vesicular population in both samples, but it can be noticed that it could be better to not use an equimolar amount of DCP.

As previously described, the hybrid niosomes were obtained by adding a proper amount of chitosan to niosomal suspension. In particular, the aggregation behavior of negatively charged niosomal vesicles in the presence of different amount of chitosan was investigated. In figure 2 the behavior of the Z-Average (panels A, C, E) and the corresponding  $\zeta$ -potential values (panels B, D, F) of different niosomal suspensions in the presence of different concentrations of chitosan solution are reported.

For all the samples, the addition of chitosan promotes the progressive increase of the diameter of the complexes, starting from a value close to the Z-Average of uncoated niosomes when only few polycation molecules adsorb on the vesicle surface, and growing to larger and larger sizes due to the subsequent aggregation of chitosan coated vesicles. Also the value of  $\zeta$ -potential of the hybrid niosomes increases adding

the polyelectrolyte approaching less negative values until the point of charge inversion (isoelectric point) is reached. This behavior is connected to the progressive charge neutralization of the complexes, promoted by the adsorption of positive charges of the chitosan onto the negative charges at the vesicle surface.

Further addition of chitosan promotes the reversal of  $\zeta$ -potential toward positive values and the decrease of the Z-Average of the hybrid niosomes toward lower values. In particular at this higher polyelectrolyte concentration, the hybrid niosomes reach a size similar to the one measured at low chitosan concentrations. This peculiar behavior is known in the colloid literature [14] as re-entrant condensation and is associated to the charge inversion of the chitosan coated vesicles.

The aggregation mechanism and the formation of stable structures are the results of the balance between electrostatic repulsion and short-range effective attraction interactions due to the non uniform charge distribution at the particle surface (charge patch attraction). [15]

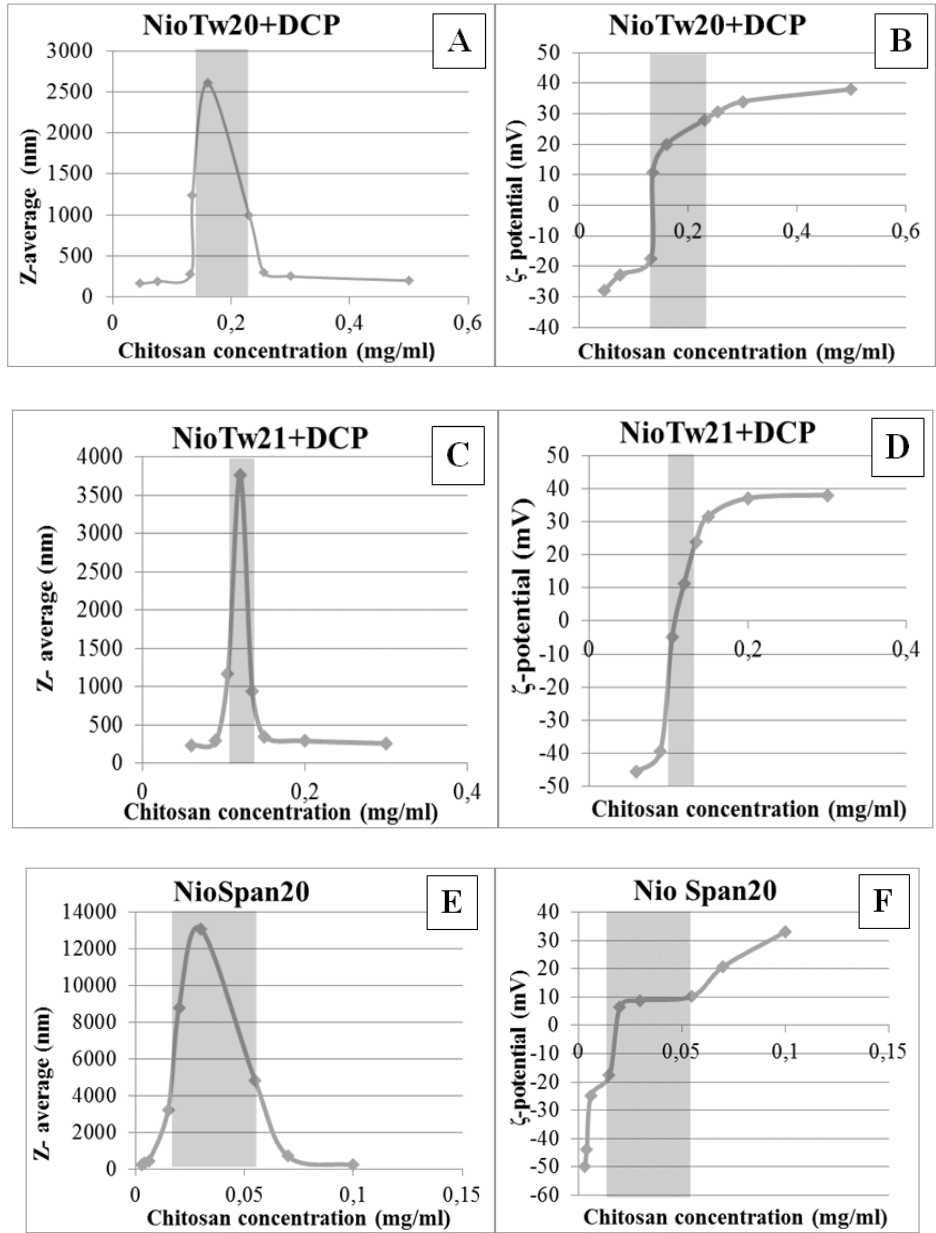
This behavior was observed for all samples studied. The aggregation mechanism and the formation of stable structures were justified in light of a balance between electrostatic repulsion and short-range effective attraction interactions due to the non uniform charge distribution at the particle surface (charge patch attraction). [16]

It is interesting to highlight that the chitosan concentration responsible of charge inversion is similar for Tween vesicles and is higher in comparison with the one necessary to obtain charge inversion of Span 20 vesicular suspension. Probably this is due to the presence of PEG chains on Tween vesicle surfaces. PEG can act as a kind of shield to an initial chitosan surface deposition and interaction.

In order to check the integrity of the hybrid niosomes during the whole aggregation process, the leakage of calcein entrapped in the core of the hybrid niosomal vesicles was monitored. The release of calcein induced by the coatings with the polyelectrolyte was studied as a function of chitosan concentration at a temperature of 25°C. The percent of dye leakage from the aggregates ( $I_F$ ) was calculated as:

$I_F = (I - I_N) / (I_E - I_N)$ , where  $I$  is the fluorescence intensity of the hybrid niosomal suspension,  $I_N$  is the one of the uncoated niosomes, and  $I_E$  the value corresponding to the complete rupture of the vesicles caused by adding, for example, ethanol to the suspension.

In the hybrid niosomes aggregates, calcein is present at a self-quenched concentration inside of the single vesicle aqueous core. To eliminate the calcein from the external medium, an exhaustive dialysis was carried out, but a residual fluorescence was observed. This fluorescence is probably due to some amount of calcein that remain entrapped in the external polyethylene hydrophilic layer on the surface of niosomes alone and of hybrid niosome aggregates.



**Figure 2.** Z-Average (panel A, C, E) and the corresponding  $\zeta$ -potential values (panel B, D, F) of different niosomal suspensions in the presence of different concentrations of chitosan solution are reported. The shaded area represents the region of instability.

The evaluation of increase of calcein fluorescence in the hybrid niosomal suspension, due to probe dequenching and dilution following complete rupture of the vesicles is useful to follow the resistance of the vesicles during polymer coating.

In tables 4, 5 and 6 are reported the fluorescence of the samples formed by the three different surfactants, prepared with different amount of chitosan.

**Table 4.** Fluorescence of NioTw20+DCP+Calcein in presence of different concentrations of chitosan solution.

Chitosan concentration (mg/ml)	Fluorescence (AU)
Uncoated	88,49
0,045	70,69
0,060	67,74
0,131	52,41
0,135	35,60

0,150	32,16
0,255	38,50
0,300	38,26
0,500	37,56

**Table 5.** Fluorescence of NioTw21+DCP+Calcein in presence of different concentrations of chitosan solution.

Chitosan concentration (mg/ml)	Fluorescence (AU)
Uncoated	74,10
0,060	68,26
0,090	61,41
0,105	60,29
0,120	56,30
0,135	47,84
0,150	46,33
0,200	40,633
0,300	38,937

**Table 6.** Fluorescence of NioSpan20+Calcein in presence of different concentrations of chitosan solution.

Chitosan concentration (mg/ml)	Fluorescence (AU)
Uncoated	78,92
0,003	75,26
0,004	72,94
0,006	70,81
0,015	69,36
0,020	58,14
0,030	57,27
0,055	47,86
0,070	40,71
0,100	37,11

Obtained data show that hybrid niosomal fluorescence never exceeds the one of uncoated niosomes (at the same dilution) confirming that no calcein release following vesicular degradation occurs. These results indicate that vesicular membranes of all analyzed samples are undamaged while chitosan coating occurs. For all the formulation, a fluorescence decrease with the increase of the chitosan concentration can be observed. Probably chitosan coating on vesicular surface, act as a fluorescence-screen for calcein molecules that are present on the hybrid niosome surface. The

vesicles integrity was also confirmed by DLS measurements (data not shown).

Vesicle integrity after chitosan coating, was evaluated during a time interval of 24 hours. In particular no variations of fluorescence and size were detected (data not shown), so it can be concluded that samples are not damaged by the chitosan coating.

Vesicular formulations were stored at 4°C and 25°C for a period of 90 days to evaluate their physical stability. Samples from each batch were withdrawn at definite time intervals (1 day, 30 days, 60 days and 90 days) to measure  $\zeta$ -potential and the mean hydrodynamic diameter. These kind of measurements were carried out on a limited number of samples in particular those characterized by similar size to uncoated sample and by a positive  $\zeta$ -potential. Apart from providing the hydrodynamic diameter and  $\zeta$ -potential value, DLS provides also valuable information on the homogeneity of the suspension. A single sharp peak in the DLS profile implies the existence of a single population of scattering particles that correspond to low PDI value. In particular all samples are characterized by PDI value that is always lower than 0.4, and vesicular suspensions can be considered as monodisperse systems.

**Table 7.** Mean hydrodynamic diameter (Z-Average) and  $\zeta$ -Potential values of uncoated vesicles and of selected chitosan coated vesicles.

Sample	Z-Average (nm) $\pm$ DS	$\zeta$ -Potential (mV) $\pm$ DS	PDI $\pm$ DS
NioTw20+DCP	157,34 $\pm$ 1,24	-44,22 $\pm$ 1,61	0,31 $\pm$ 0,01
NioTw20+DCP+Chitosan (0,3mg/ml)	252,91 $\pm$ 4,42	37,73 $\pm$ 0,72	0,31 $\pm$ 0,02
NioTw21+DCP	136,52 $\pm$ 5,21	-51,72 $\pm$ 0,62	0,34 $\pm$ 0,02
NioTw21+DCP+Chitosan (0,2mg/ml)	262,12 $\pm$ 2,41	33,92 $\pm$ 0,71	0,21 $\pm$ 0,01
NioSpan20	202,91 $\pm$ 5,31	-48,34 $\pm$ 0,23	0,31 $\pm$ 0,01
NioSpan20+Chitosan (0,1mg/ml)	292,73 $\pm$ 6,81	43,43 $\pm$ 0,52	0,21 $\pm$ 0,01

In table 8 physical stability data of the three different uncoated and coated formulations are reported. These studies were carried out to investigate if significant changes of the size and  $\zeta$ -potential of surfactant vesicle dispersion occur during storage. The measurements carried out by DLS indicate that all selected surfactants form vesicular structures with no significantly different sizes and  $\zeta$ -potential. The uncoated and coated systems are characterized by no

significant variations of dimension and  $\zeta$ -potential during the storage period, especially if they are maintained at 4°C. The high negative or positive  $\zeta$ -potential values are important in preventing aggregation phenomena. In fact, it has been reported that to prevent aggregation due to electrostatic interactions, a nanovesicle should have a  $\zeta$ -potential value of at least -30 mV or greater + 30 mV. [17]

**Table 8.** Physical stability in terms of dimensions and  $\zeta$ -potential variations of uncoated (panel A) and coated (panel B) vesicles. Data were collected at days 1 and 90 and are the mean of three experiments  $\pm$  SD.

	NioTw20+DCP		NioTw20+DCP+Chitosan (0,3mg/ml)					
	25°C		4°C		25°C		4°C	
	Z-Average (nm)	$\zeta$ -Potential (mV)	Z-Average (nm)	$\zeta$ -Potential (mV)	Z-Average (nm)	$\zeta$ -Potential (mV)	Z-Average (nm)	$\zeta$ -Potential (mV)
t=0	157,34 $\pm$ 1,24	-44,22 $\pm$ 1,61	157,34 $\pm$ 1,24	-44,22 $\pm$ 1,61	252,91 $\pm$ 4,42	37,73 $\pm$ 0,72	252,91 $\pm$ 4,42	37,73 $\pm$ 0,72
t=90	153,91 $\pm$ 1,43	-41,01 $\pm$ 2,23	166,12 $\pm$ 7,32	-42,84 $\pm$ 1,11	241,94 $\pm$ 4,43	33,82 $\pm$ 1,43	337,01 $\pm$ 4,82	38,22 $\pm$ 0,73

**Table 8.** continue.

	NioTw21+DCP		NioTw21+DCP+Chitosan (0,2mg/ml)					
	25°C		4°C		25°C		4°C	
	Z-Average (nm)	$\zeta$ -Potential (mV)	Z-Average (nm)	$\zeta$ -Potential (mV)	Z-Average (nm)	$\zeta$ -Potential (mV)	Z-Average (nm)	$\zeta$ -Potential (mV)
t=0	136,52 $\pm$ 5,21	-51,72 $\pm$ 0,62	136,52 $\pm$ 5,21	-51,72 $\pm$ 0,62	262,12 $\pm$ 2,41	33,92 $\pm$ 0,71	262,12 $\pm$ 2,41	33,92 $\pm$ 0,71
t=90	121,12 $\pm$ 3,43	-31,11 $\pm$ 1,02	113,01 $\pm$ 3,64	-53,11 $\pm$ 2,02	197,01 $\pm$ 3,92	31,62 $\pm$ 1,01	253,82 $\pm$ 2,31	36,22 $\pm$ 1,72

Table 8. continue.

	NioSpan20		NioSpan20+Chitosan (0,1mg/ml)					
	25°C		4°C		25°C		4°C	
	Z-Average (nm)	ζ-Potential (mV)	Z-Average (nm)	ζ-Potential (mV)	Z-Average (nm)	ζ-Potential (mV)	Z-Average (nm)	ζ-Potential (mV)
t=0	202,91±5,31	-48,34±0,23	202,91±5,31	-48,34±0,23	292,73±6,81	43,43±0,52	292,73±6,81	43,43±0,52
t=90	258,11±7,83	-32,22±1,52	224,82±7,91	-48,51±1,52	275,82±6,72	25,72±1,22	272,92±7,62	36,22±1,71

## 4. Conclusions

In this work, the complexation process between negatively charged vesicles built up by three different surfactants (Tween 20, Tween 21 and Span 20) and chitosan, a cationic polyelectrolyte, with mucoadhesive properties, is described.

The aggregation behavior can be deeply investigated by size, ζ-potential and fluorescence evaluations, in order to select the most appropriate samples to perform intranasal administration. From the obtained results the selected Tw20, Tw21 and Span vesicles coated with a chitosan concentration able to obtain a positively charged hybrid vesicle, are: a) homogenous and monodisperse vesicular population; b) characterized by dimensions compatible with the proposed route of administration; c) stable in terms of dimension and ζ average variation, if stored at 4°C.

These kind of measurements can be useful to perform an initial screening and needs to be confirmed by a complete preclinical evaluation.

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