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Physicochemical properties of chitosan-lipid emulsions and their stability during the autoclaving process

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Abstract

A new positively charged, submicronized fat emulsion with appropriate stability during the autoclaving process was developed. Only the emulsions prepared with a combination of ABA block co-polymer (F68) and chitosan were stable enough to resist the thermic shock induced by autoclaving sterilization. The results indicate that a mixed film consisting of the ABA block co-polymer and chitosan molecules was formed at the o/w interface with an overall positive surface charge. Conversely, a combination between chitosan with phospholipids and/or with a mixture of phospholipids with ABA block co-polymer showed a phase separation during autoclaving. A chitosan type with a low viscosity was used which was intended for a possible use in the ocular and parenteral application. An experimental factorial design 3^2 was used to investigate the effect of chitosan and F68 concentrations on the physicochemical properties of the system and consequently their influence on the stability of emulsions during autoclaving. Both size and surface charge of emulsions were significantly affected as a function of the chitosan concentration. Formulation with a mean particle size ranging from 125 to 130 nm and with a positive surface charge of 20–23 mV was achieved. Moreover, the chitosan emulsions were autoclaved without a significant change in their particle size. However, increasing the concentration of chitosan needs a higher amount of F68 in order to achieve stable emulsions during autoclaving. This may be due to the interaction between the positively-charged chitosan and the negatively-charged free fatty acids, which are contained in the oil phase (castor oil).

Keywords: Chitosan; Positively-charged emulsions; ABA block co-polymer; Autoclaving process; Experimental factorial design

1. Introduction

Positively-charged systems are receiving more attention as novel colloidal drug carriers for various potential therapeutic applications (Steger and

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Desnick, 1977). For example Groth et al. (1998) and Cortesi et al. (1996) reported a novel use of cationic liposomes to target DNA into the cell nucleus and this allows the replacement of the defective gene product and thus restores the normal cell function. Furthermore, it is well known from emulsion and liposomal studies that the surface charge and the size of the colloidal carrier may affect the biofate of a drug in various organs of the body following i.v. administration (Juliano and Stamp, 1975; Laval-Jeantet et al., 1982).

Moreover, in many papers Benita and his workers (Elbaz et al., 1993; Klang et al., 1994) showed the possibility of producing stable positively-charged submicron emulsions, which are assumed to display several advantages. Davis et al. (1992) suggested that positively charged emulsion droplets can behave differently, when introduced into the bloodstream, to normal (negatively charged) fat emulsion droplets with respect to the uptake of plasma blood components and opsonic factors. Therefore positively-charged systems may alter the pharmacokinetic profile of the incorporated drugs, resulting in a possible drug targeting with enhanced local drug concentration in the organs (Davis et al., 1992).

It would also be interesting to explore the intrinsic effects of the positively charged emulsions in accessible organs such as skin or cornea, which is known to carry a net negative charge (Klang et al., 1996; Zeevi et al., 1994).

Up to now, all positively-charged submicronized emulsions were based on a mixture of phospholipids with Poloxamer and stearylamine, as a cationic emulsifier. Unfortunately stearylamine showed a high toxicity against the tested cell systems in vitro (Adams et al., 1977; Layton et al., 1980; Cambell, 1983). This cytolitic and cytotoxic activity limits the utilization of the advantages of these systems as novel drug delivery carriers (Filion and Phillips, 1998).

The intent of this investigation was, therefore, to formulate a stable positively-charged emulsion with a non-toxic cationic polymer (chitosan) resulting in improved emulsion systems for drug delivery. The experiments were carried out and optimized using an experimental design in order to estimate the appropriate concentrations of chitosan and the non ionic surfactant (ABA block co-polymer). Chitosan has been used in many pharmaceutical formulations but not in the form of lipid emulsions. This could be due to the fact that chitosan is only soluble in an acidic medium (pH below 6) and to interaction with the negatively-charged components, which usually lead to great changes in the physicochemical properties of lipid emulsions, especially during the autoclaving process (Chaturvedi et al., 1992; Bock, 1994; Henriksen et al., 1994).

Chitosan is a natural polysaccharide (*N*-deacetylated-2-acetamido-2-deoxy-β-D-glucan). It is a biodegradable, biocompatible positively-charged polymer, which shows many interesting properties (Muzzarelli et al., 1988; Patel and Amiji, 1996). Moreover, chitosan is a virtually non-toxic polymer with a wide safety margin and it can be used in drug delivery systems as a novel carrier for both oral and intravenous administration (Hirano et al., 1988; Jameela et al., 1996).

2. Materials and methods

2.1. Materials

Purified castor oil and soybean oil were purchased from Henry Lamotte (Bremen, Germany) and medium chain triglyceride (Miglyol 812) was obtained from Hüls (Witten/Ruhr, Germany). Non-ionic ABA copolymer surfactant (Synperonic F68) was provided by ICI (Atlas Chemie, Germany) and chitosan (Chitopure with molecular weight 4.1×10^5, a 92% degree of deacetylation and a viscosity of 14 mPa·s) was furnished by Fish Contract Bremerhaven (Bremerhaven, Germany). Soylecithin (S75) was purchased by Lipoid (Ludwigshafen, Germany). Sorbitol and lactic acid were supplied by Merck (Darmstadt, Germany). For all preparations, doubled distilled water was used. All other chemicals were of reagent grade.

2.2. Preparation of emulsions

The emulsions were prepared as follows: the chitosan was dispersed in 5% aqueous solution of

sorbitol to enable adjustment to isotonicity and an equal amount of a 2% solution of lactic acid (2 g in 100 ml of water) was added, because chitosan is only soluble in an acidic medium. The resulting mixture was stirred vigorously without heating for 60 min until complete solubility was reached. The pH of the resulting solution was adjusted at 5 to avoid any flocculation of chitosan and after this the solution was filtered (0.45 μm filter) to separate the non soluble accompanying fibres. The other emulsifiers (Phospholipids and F68) were dissolved in the oil phase by heating. The oil phase and the aqueous solution were heated separately to about 50–55°C. The oil phase was added to the aqueous solution and this mixture was pre-emulsified using an Ultra-Turrax T25 (Janke and Kunkel, Staufen, Germany) at 8000 rpm for 3 min. Final emulsification was carried out by passing 40 ml of the coarse emulsion through a high pressure homogenizer (Micron Lab 40, APV Gaulin, Lübeck, Germany) eight times at a pressure of 20 MPa. The homogenization was performed at a temperature of 40°C. The pH of the final emulsions was measured directly in the emulsion using a Microprocessor pH/ion Meter pMX 2000 (WTW, Weilhein, Germany) (pH value about 5–5.1). The emulsions were filled into 15 ml vials and sterilized using a steam autoclave (KI5T, Keller, Weinhein, Germany) at 121°C for 20 min.

2.3. Measurements

The mean diameter of the bulk population was determined by photon correlation spectroscopy (PCS) covering the size range 5 nm to approximately 3 μm (Malvern spectrometer RR 102, Malvern, UK, with Helium–Neon laser $\lambda = 632.8$ nm, Siemens, Germany). For size analysis approximately 1 μl fat emulsion was added to 1 ml distilled water in order to obtain the optimum scattering intensity.

Larger particles were detected by laser diffraction analyser LDA (Helos, Sympatec, Clausthal-Zellerfeld, Germany) at a focal length of 20 mm, corresponding to a measurement range of 0.18–35 μm. The emulsions were characterized by Dmax and the D50 and D99 quantiles of the volumetric distribution (that means 50%, 99% or all of the particles were below the given size).

The surface charge (ζ potential) was measured using a ZetaSizer 3 (Malvern Instruments, Malvern, UK). The electrolyte solution used for dilution consisted of double distilled water with a conductivity of 50 μS/cm adjusted by NaCl (0.5 mmol/l). Five hundred μl of each emulsion formulation was diluted with 20 ml electrolyte solution.

2.4. Data analysis

The data were statistically analyzed and the following model equation was used to calculate the contour plots

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2.$$

y is the response parameter of interest [e.g. PCS radius, D (50%) and D (99%) of the volume distribution], x_1 and x_2 are the process parameters (F68 and Chitosan concentrations), and β_0 to β_{22} are fit parameters resulting from the multiple linear regression. The model chosen here takes into account linear and quadratic dependencies as well as interactions of process parameters ($\beta_{12}\ x_1\ x_2$). The exact concentration (coded and real values) of the 3^2 design as well as the results can be found in Table 1.

3. Results and discussion

3.1. The chosen oil phase

As reported in previous studies (Jumaa and Müller, 1998a,b) mixing of castor oil with middle-chain triglycerides leads to a decrease in the viscosity of castor oil and simultaneously to a decrease in the interfacial tension of the oil phase (Fig. 1). This was related to the free fatty acids contained in castor oil, which can act as a co-emulsifier resulting in lower interfacial tension and, simultaneously, in a more stable formulation in comparison with the other oil phases (Washington and Davis, 1987; Yamaguchi et al., 1995).

However, it could be seen from Fig. 1 that a mixture of castor oil with middle-chain triglycerides in ratio 1:1 (w/w) is an appropriate oil mixture with low interfacial tension and relatively low viscosity. This mixture, therefore, was the oil phase of choice.

3.2. Stability of different emulsion formulations containing chitosan during autoclaving

As the aim of this study is to produce a chitosan-lipid emulsion, which can pass the sterilization process by autoclaving without being damaged, a preliminary investigation was carried out using different emulsifier and/or mixtures of emulsifiers. The purpose of this was to choose the appropriate system in order to formulate chitosan in a suitable stable emulsion. The different emulsion formulations were assessed according to their appearance after autoclaving, to find out if any phase separation occurred. The results are summarized in Table 2. From these results it could be clearly seen that only the formulation containing F68 with chitosan displayed good stability during autoclaving, judging by its appearance. These formulations remained stable and showed no phase separation. Conversely, all other formulations showed a great change in their appearance and clearly showed a phase separation. This instability could be attributed to the acidic pH-value (Bock, 1994) and the interaction between the positively-charged chitosan with negatively-charged phospholipids which resulted in a damaged emulsifier film around the oil droplets and, consequently, a coalescence of the droplets (Müller and Heinemann, 1994). Addition of non-ionic surfactant (F68) could not prevent this interaction. Thus, it

Table 1
Experiments of the factorial design (3^2): set up and results[a]

No.	Coded values		Real values		PCS (nm)	D50 (μm)	D99 (μm)	Zeta P (mV)
	Chit (%)	F68 (%)	Chit (%)	F68 (%)				
1	0	0	0.25	2	133.2	0.61	1.48	20.6
2	1	1	0.5	2.5	124.1	0.54	1.4	22.8
3	1	−1	0.5	1.5	131.1	0.59	2.29	22.9
4	−1	1	0.1	2.5	133.9	0.61	1.44	7.6
5	−1	−1	0.1	1.5	149.5	0.72	1.71	6.4
6	0	1	0.25	2.5	130.1	0.57	1.41	19.3
7	1	0	0.5	2	128.3	0.56	1.65	23.2
8	0	−1	0.25	1.5	139.2	0.65	2.4	21.3
9	−1	0	0.1	2	140.5	0.68	1.55	8.5

[a] [$n = 2$].

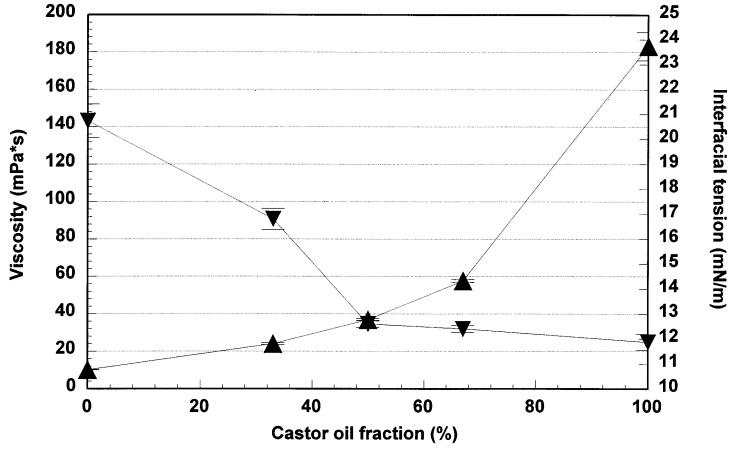

Fig. 1. Viscosity (▲) and interfacial tension (▼) of different oil mixtures (castor oil with MCT) as a function of castor oil fraction.

Table 2
Stability of different emulsion formulations with chitosan during the autoclaving

Formulation	Macroscopic aspect	
	Before	After autoclaving
1.5% S75+0.5% Chit[a]	Large oil droplets	Phase separation
1.5% 75+1% F68+0.5% Chit	Some oil droplets	Phase separation
1.5% 75+1.5% F68+0.5% Chit	Some oil droplets	Phase separation
2% F68+0.5% Chit	Homogeneous emulsion	No phase separation
2.5% F68+0.5% Chit	Homogeneous emulsion	No phase separation

[a] Chitosan.

could be deduced that a combination between F68 and chitosan was potentially able to formulate a stable lipid emulsion with chitosan.

This combination, therefore, was suggested for further investigation in order to study the physicochemical properties of these lipid emulsions as a function of chitosan with the aim to optimize the production of this emulsion.

3.3. The effect of chitosan on the physicochemical properties of lipid emulsions

The effect of adding chitosan to lipid emulsions was investigated. Fig. 2 showed the surface plot of the mean particle size obtained from PCS and larger oil droplets (D99) obtained from LDA. The values for D50 are not presented, because they showed the same pattern as the PCS-values and D99 (P-value < 0.03). The results clearly show that there was a parallel decrease in emulsion particle sizes (PCS-values, LDA-values), as the concentration of chitosan was increased from 0.1 up to 0.5% (w/w). Because the decrease in mean particle size was accompanied by a decrease in large particles, this means that the emulsions display a narrow particle size distribution (Bock, 1994).

The addition of the positively-charged chitosan to lipid emulsions led to a change of the surface charge of the oil droplets from the positive to the negative value (from -11 to $+23$ mV). As shown in Fig. 3, the zeta potential was first reversed from negative to positive value (from -10 to 10 mV) (P-value < 0.05) and subsequently increasing the chitosan concentration resulted in a consequent increase of the positive value of the surface charge (zeta potential).

By comparing the zeta potential with the particle size it could be deduced that a decrease in particle sizes of emulsions was accompanied by an increase in the positive surface-charge value. This indicates that chitosan molecules are localized at the interface and intercalated between the nonionic surfactants. Hence, a mixed interfacial film comprising the pluronic F68 and chitosan molecules was formed at the o/w interface which resulted in an overall positive surface charge.

Moreover, as might be expected, increasing the chitosan concentration led to a higher emulsion viscosity (Fig. 4) (P-value < 0.02). This increase of emulsion viscosity, however, did not affect the particle size of chitosan emulsions. In contrast to this it is well established from the literature that increasing the viscosity of emulsion phases led to an increase in the particle size (Jumaa et al., 1998). This behavior may be attributed to the interfacial tension properties of chitosan, because from the particle size and zeta potential results it became clear that chitosan shows interfacial properties (Ropert and Taylor, 1992). Consequently, no change in the particle sizes of chitosan emulsions were observed.

3.4. The effect of chitosan on emulsion stability during autoclaving

Autoclaving is generally a necessary process to sterilize lipid emulsions, as sterile filtration cannot be used due to the large particle size of lipid emulsions (Lucks, 1993). Lipid emulsions, therefore, should display sufficient stability against this stress process if a suitable formulation is to be achieved (Hansrani et al., 1983). Therefore, the change in oil droplet sizes of chitosan emulsions before and after autoclaving was recorded.

Fig. 5 shows the change in mean particle sizes (D50 obtained by LDA and mean particle sizes obtained from PCS). D50 of all systems did not

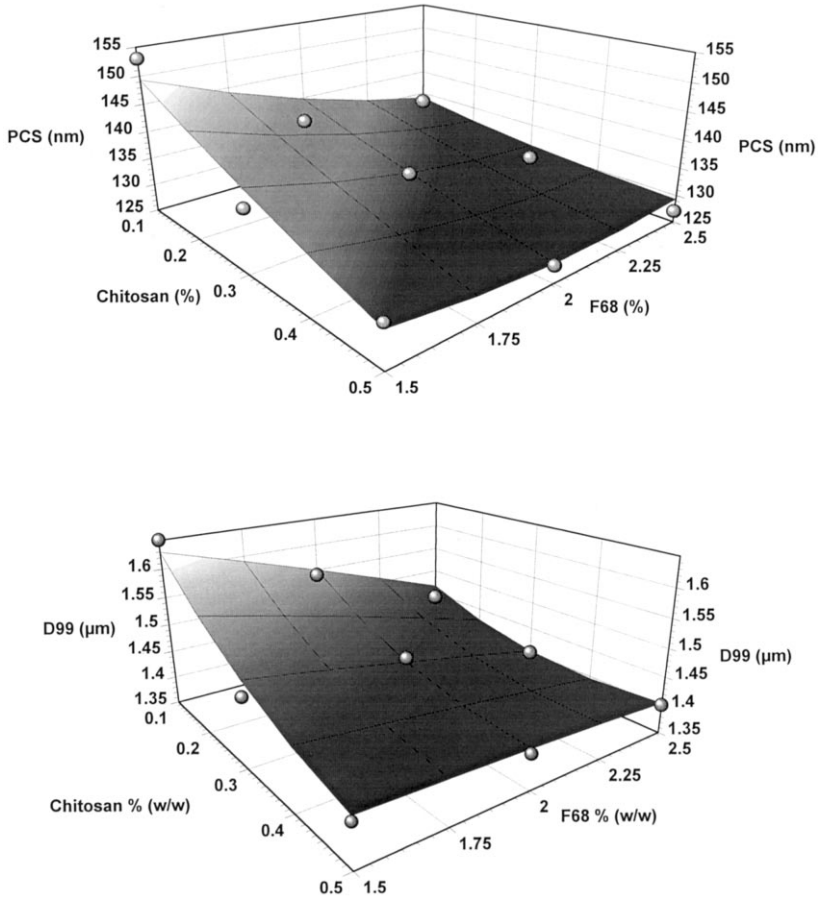

Fig. 2. Surface plot for photon correlation spectroscopy (PCS) radius and D99.

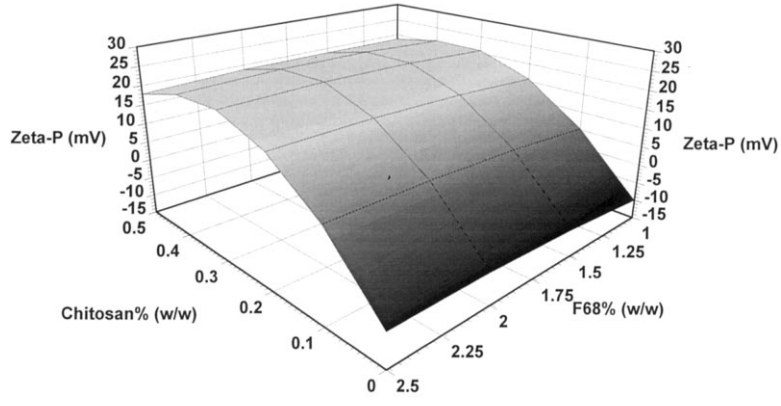

Fig. 3. Surface plot for zeta potential of lipid emulsions.

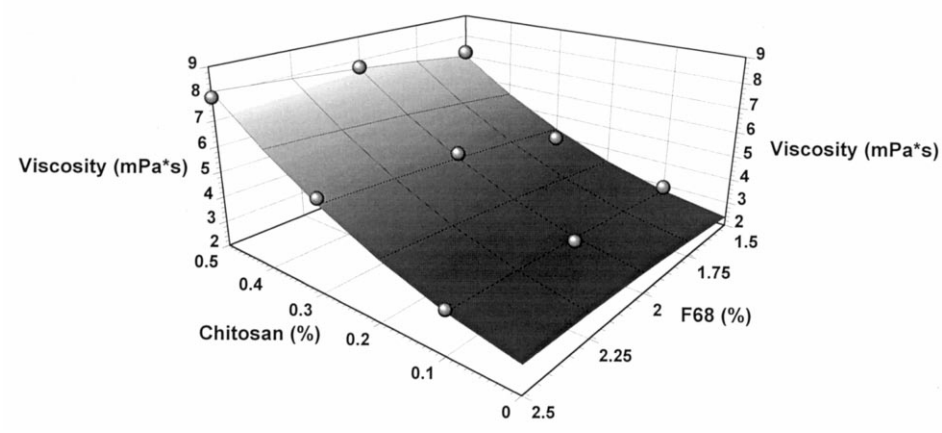

Fig. 4. Surface plot for emulsion viscosity.

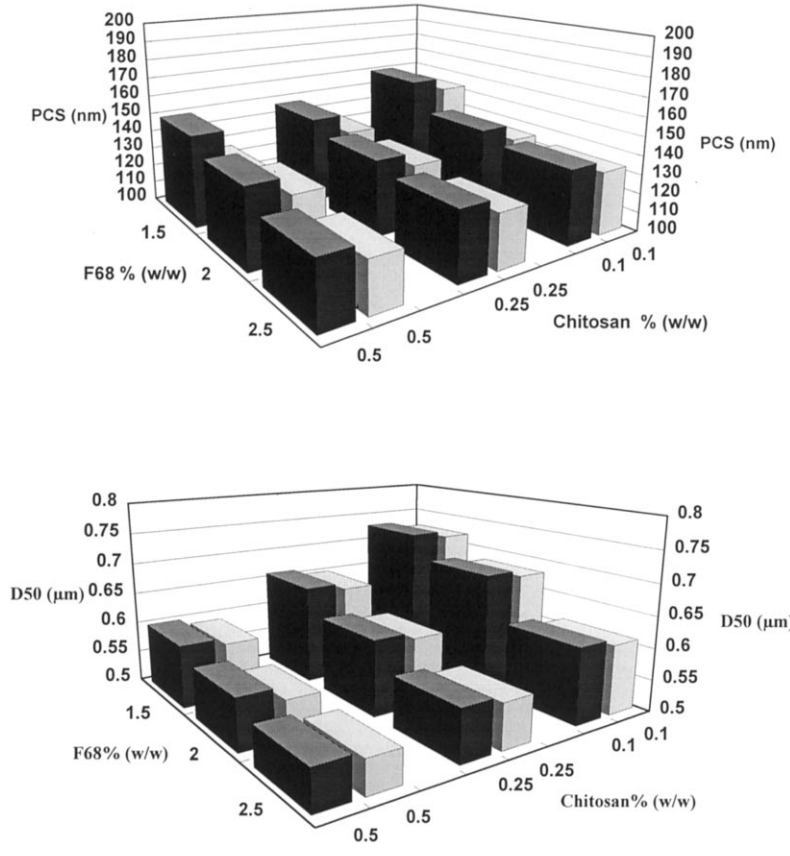

Fig. 5. Influence of chitosan concentration on the emulsion mean particle sizes during autoclaving (photon correlation spectroscopy, PCS-value and D50) [before (open) and after autoclaving (closed)].

show a significant change in the emulsion particle size before and after autoclaving, whereas PCS-values of formulations containing 1.5% F68 as well as formulations with 0.5% chitosan and 2% F68 showed a slight increase after the autoclaving process. Furthermore, the larger particles of the studied systems (D99 and Dmax) displayed the same behavior. As could be seen from Fig. 6, at least a 2% concentration of ABA block co-polymer was necessary in order to obtain a chitosan emulsion with sufficient stability during autoclaving. Hence, the physicochemical properties of the mixed surfactant interfacial film were strong enough to prevent droplet coalescence upon the thermic process. Moreover, by increasing the chitosan concentration above 0.25% a higher concentration of ABA block co-polymer was required to achieve a stable formulation during the thermic process. This may be due to the interaction between the positively-charged chitosan and the negatively-charged free fatty acids, which are contained in the castor oil. Thus, by increasing chitosan concentration this interaction will be more effective and, therefore, a greater amount of the ABA block co-polymer is required in order to overcome this unfavorable interaction and to produce a stable emulsion formulation.

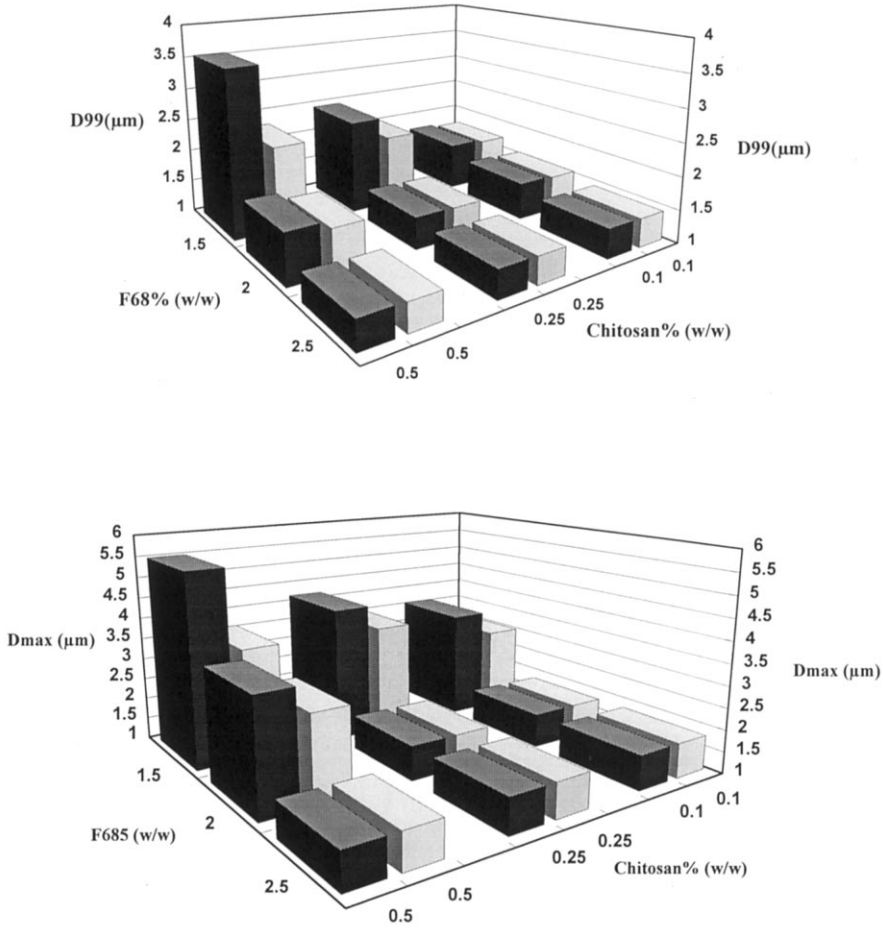

Fig. 6. Influence of chitosan concentration on the emulsion large particles during autoclaving (D99 and Dmax) [before (open) and after autoclaving (closed)].

4. Conclusion

A new positively-charged lipid emulsion was developed. The use of chitosan allowed clarification of its role as a cationic polymer in achieving a positive surface charge. Only emulsions prepared with a combination between chitosan and ABA block co-polymer showed sufficient stability during the autoclaving process. Furthermore, by increasing chitosan concentration the ABA block co-polymer amount should also be increased too in order to produce a stable formulation. Chitosan has a wide safety margin and, therefore, the development of such a system can open pioneering work in the search for positively-charged emulsions without significant toxicity.

References

Adams, D.A., Richardson, G.J., Ryman, B.E., Wisniewski, H.M., 1977. Liposome toxicity in mouse central nervous system. J. Neurol. Sci. 31, 173–179.

Bock, T., 1994. Emulsionen als parenterale Arzneiträgersysteme (Herstellung, Charakterisierung und Optimierung). PhD thesis, Kiel.

Cambell, P.I., 1983. Toxicity of some charged lipids used in liposome preparations. Cytobios 37, 21–26.

Chaturvedi, P.R., Patel, N.M., Lodhi, S.A., 1992. Effect of terminal heat sterilization on the stability of phospholipids-stabilized submicron emulsions. Acta. Pharm. Nord. 4, 51–55.

Cortesi, R., Esposito, E., Menegatti, E., Gambari, R., Nastruzzi, C., 1996. Effect of cationic liposome composition on in vitro cytotoxicity and protective effect on carried DNA. Int. J. Pharm. 139, 69–78.

Davis, S.S., Illum, L., Washinghton, C., Harper, G., 1992. Studies on the interaction of charge reversed emulsions with reticuloendothelial system. Int. J. Pharm. 82, 99–105.

Elbaz, E., Zeevi, A., Klang, S., Benita, S., 1993. Positively-charged submicron emulsions—a new type of colloidal drug carrier. Int. J. Pharm. 96, R1–R6.

Filion, M.C., Phillips, N.C., 1998. Major limitations in the use of cationic liposomes for DNA delivery. Int. J. Pharm. 162, 159–170.

Groth, D., Keil, O., Lehmann, C., Schneider, M., Rudolph, M., Reszka, R., 1998. Preparation and characterization of a new lipospermine for gene delivery into various cell-lines. Int. J. Pharm. 162, 143–157.

Hansrani, P.K., Davis, S.S., Groves, M.J., 1983. The preparation and properties of sterile intravenous emulsions. J. Parent. Sci. Technol. 37, 145–150.

Henriksen, I., Smistad, G., Karlsen, J., 1994. Interaction between liposomes and chitosan. Int. J. Pharm. 101, 227–236.

Hirano, S., Seino, H., Akiyama, Y., Nonaka, I., 1988. Biocompatibility of chitosan by oral and intravenous administration. Polym. Mater. Sci. Eng. 59, 897–901.

Jameela, S.R., Latha, P.G., Subramoniam, A., Jayakrishnan, A., 1996. Antitumour activity of mitoxantrone-loaded chitosan microspheres against ehrlich ascites carcinoma. J. Pharm. Pharmacol. 48, 685–688.

Juliano, R.L., Stamp, D., 1975. The effect of particle size and charge on the clearance rates of liposomes and liposomes encapsulated drugs. Biochim. Biophys. Commun. 63, 651–658.

Jumaa, M., Müller, B.W., 1998a. The effect of oil components and homogenization conditions on the physicochemical properties and stability of parenteral fat emulsions. Int. J. Pharm. 163, 81–89.

Jumaa, M., Müller, B.W., 1998b. The stabilization of parenteral fat emulsion using non-ionic ABA copolymer surfactant. Int. J. Pharm. 174, 29–37.

Jumaa, M., Kleinebudde, P., Müller, B.W., 1998. Mixture experiments with the oil phase of parenteral emulsions. Eur. J. Pharm. Biopharm. 46, 161–167.

Klang, S., Frucht-Pery, J., Hoffman, A., Benita, S., 1994. Physicochemical characterization and acute toxicity evaluation of a positively-charged submicron emulsion vehicle. J. Pharm. Pharmacol. 46, 986–993.

Klang, S.H., Baszkin, A., Benita, S., 1996. The stability of piroxicam incorporated in a positively-charged submicron emulsion for ocular administration. Int. J. Pharm. 132, 33–44.

Laval-Jeantet, A.M., Laval-Jeantet, M., Bergot, C., 1982. Effect of particle size on the tissue distribution of iodized emulsified fat following intravenous administration. Invest. Radiol. 17, 617–620.

Layton, D., Luckenbach, G.A., Andreesen, R., Munder, P.G., 1980. The cell interaction of liposomes with cells: the relation of cell specific toxicity to lipid composition. Eur. J. Cancer 16, 1529–1538.

Lucks, S., 1993. Parenterale Fettemulsionen als Arzneistoffträger (Herstellung, Charakterisierung und Stabilität. PhD thesis, Kiel.

Müller, R.H., Heinemann, S., 1994. Fat emulsions for parenteral nutrition. IV. Lipofundin MCT/LCT regimens for total parenteral nutrition (TPN) with high electrolyte load. Int. J. Pharm. 107, 121–131.

Muzzarelli, R., Baldassarre, V., Conti, F., Ferrara, P., Biagini, G., 1988. Biological activity of chitosan: ultrastructural study. Biomaterials 9, 247–252.

Patel, V.R., Amiji, M.M., 1996. Preparation and characterization of freeze-dried chitosan-poly(ethylene oxide) hydrogels for site-specific antibiotic delivery in the stomach. Pharm. Res. 13, 588–593.

Ropert, G.A.F., Taylor, K.E., 1992. Surface activity and foam-enhancing properties of *N*-(2-hydroxyalkyl) chitosans. Advanced in Chitin and Chitosan. Elsevier, Amsterdam, pp. 179–186.

Steger, D.L., Desnick, J.R., 1977. Enzyme therapy VI: comparative in vivo fates and effects on lysosomal integrity of enzymes entrapped in negatively and positively charged liposomes. Biochim. Biophys. Acta. 464, 530–546.

Washington, C., Davis, S.S., 1987. Ageing effects in parenteral fat emulsions: the role of fatty acid. Int. J. Pharm. 39, 33–37.

Yamaguchi, T., Nishizaki, K., Itai, S., Hayashi, H., Ohshima, H., 1995. Physicochemical characterization of parenteral lipid emulsion: influence of cosurfactants on flocculation and coalescence. Pharm. Res. 12, 1273–1278.

Zeevi, A., Klang, S., Alard, V., Brossard, F., Benita, S., 1994. The design and characterization of a positively-charged submicron emulsion containing a sunscreen agent. Int. J. Pharm. 108, 57–68.