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Physicochemical characterisation of liposomes with encapsulated local anaesthetics

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Abstract

Local anaesthetics may be added to intravenous o/w emulsions of propofol to reduce the initial pain of injection. Due to incompatibility problems of the emulsion on the addition of a local anaesthetic solution, prior encapsulation of the drug within liposomes is suggested. The liposomes were prepared with the sonication method and loaded with either lidocaine hydrochloride (LiHCl) or prilocaine hydrochloride (PrHCl).

The liposomal systems were characterised with photon correlation spectroscopy (PCS) in terms of mean particle size and polydispersity. Lamellarity and lamellar thickness were determined by small angle X-ray scattering (SAXS). Visualisation of drug-loaded liposomes was performed with transmission electron microscopy (TEM) after freeze fracture replication of the samples.

Depending on the manufacturing procedure mean particle sizes of the drug-free liposomes varied from 150±9 nm to 102±2 nm. Drug-loaded liposomes manufactured the same way showed reduced particle sizes of 125 ± 1 nm and 85 ± 7 nm, respectively. Determination of bilayer thickness by SAXS yielded 6.2 ± 0.2 nm in the case of full hydration whereas the bilayer thickness without the hydration shell was just 4.4 ± 0.2 nm. The small particle sizes achieved are appropriate for intravenous administration.

Keywords: Liposomes; Phospholipids; Sonication; SAXS; Lidocaine-HCl; Prilocaine-HCl

1. Introduction

The administration of parenteral o/w emulsions of the narcotic propofol is connected with an initial pain of injection (Eriksson, 1995; Parmar and Koay, 1998; Sadler et al., 1999). The most common method to avoid this pain is the addition of local anaesthetics, because best results were obtained with these drugs (Eriksson, 1995; Nathanson et al., 1996; O'Hara et al., 1997).

However, the addition of solutions of local anaesthetics may affect the stability of propofol emulsions due to various incompatibilities of different local anaesthetics with the emulsions, as has been demonstrated with the substitution of lidocaine HCl (LiHCl) by prilocaine HCl (PrHCl) in propofol emulsions (Kaufke and Krauel, 2000). Although both drugs belong to the amide type and differ only slightly in molecular geometry, the substitution of LiHCl by PrHCl, surprisingly, resulted in an interaction with the emulsifier of the propofol-o/w-emulsion, which

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lead to a breakdown of the emulsion structure and a subsequent phase separation. To avoid this phenomenon local anaesthetic and emulsifier should be kept separated from each other. For that purpose an encapsulation of the local anaesthetic within liposomes is suggested prior to combination with the emulsion. This seems to be reasonable especially because the existence of small unilamellar vesicles actually seems to be likely in phospholipid-stabilized emulsions (Westesen and Wehler, 1992). However, in the present study, the vesicles are considered a possible drug carrier instead of a by-product.

Liposomes as drug carriers are extensively described in literature (Crommelin and Sindelar, 2002). Due to biodegradability and non-toxicity of lecithin as the main constituent of liposomes, liposomes especially for intravenous administration have been widely studied (Weiner et al., 1989), yielding marketed drug formulations (e.g. Ambisome®).

For intravenous injection liposomal size is a crucial parameter. Manufacturing of small unilamellar vesicles (SUVs) is possible with a variety of methods (Rubas and Schreier, 1991), of which many are not appropriate for liposomal dispersions intended for intravenous application, because many methods make use of organic solvents, i.e. chloroform, which can hardly be removed completely. An appropriate method seems to be sonication, which is up to now the most popular method due to its speed and simplicity. Therefore, the preparation of liposomes by sonication was selected for the present study, although some disadvantages exist: (i) high energy input during sonication and thus a high risk of oxidation (Schreier, 1982), and (ii) a direct contact between sample and sonication probe possibly causing metal impurities in the sample.

Since liposomal dispersions often exhibit a broad size distribution, particle size determinations are difficult in many cases. This is especially true for dispersions of multilamellar vesicles. With their broad size distribution it is impossible to measure all particle sizes present with a single technique (Hope et al., 1986). For liposomal dispersions with a mean diameter $d < 1\,\mu m$ a variety of techniques are applicable, among these the photon correlation spectroscopy (PCS) is one of the most common methods and was used in this study.

2. Materials and methods

2.1. Materials

The lecithin used was soybean lecithin Phospholipon® 90 G, kindly provided by Phospholipid GmbH (Köln, Germany). Lidocaine hydrochloride was purchased from Caesar & Loretz GmbH (Hilden, Germany). Prilocaine hydrochloride was donated by AstraZeneca GmbH (Wedel, Germany). All substances were used without further purification. Water was used in bidistilled quality. All concentrations are given in % (w/w).

2.2. Methods

2.2.1. Preparation of liposomes

Samples of 5 ml were prepared. An exactly weighed amount of lecithin was suspended in water. This suspension was mixed for 3 h at room temperature with a magnetic stirrer.

Afterwards the sample was sonicated with various energy input times, idle times and numbers of cycles, respectively. The sonication was performed with a Soniprep 150 MSE (MSE Scientific Instruments, Crawley, UK) at an amplitude of 16 μm. To obtain drug-loaded liposomes the water-soluble drug was dissolved in the aqueous phase prior to sonication, because incorporation of water-soluble drugs after the formation of the liposomes proved to be impossible.

2.2.2. Measurement of the liposomal size

Particle size determination of the vesicles was performed with a ZetaSizer III (Malvern Instruments, Malvern, UK) at a temperature of $25 \pm 0.1\,^{\circ}C$. The ZetaSizer was modified with a He/Ne laser ($\lambda = 632.8\,nm$, Spectra Physics, Mt. View, USA). The intensity of the laser light scattered by the samples was detected at an angle of 90° with a photomultiplier. At least three independent samples were taken, each of which was measured at least twice up to four times. For each specimen 10 autocorrelation functions were analysed using a cumulant analysis (Ostrowsky, 1988). From this analysis, the *z*-average was obtained, which is an approximation of the diameter of the liposomes. The particle size distribution was characterised using the polydispersity index, which is a measure for the width of the size distribution.

2.2.3. Determination of the bilayer structure by SAXS

Electron distance distributions of the vesicles were determined with diffuse small angle X-ray scattering (SAXS) measurements. SAXS measurements were carried out in an evacuated Kratky compact camera (Anton Paar, Graz, Austria) at a temperature of 25 °C. The camera was mounted on a PW 1830 generator including a PW 2253/11 broad focus X-ray tube with a copper anode (Philips, Almelo, The Netherlands). Cu Kα X-rays with a wavelength of $\lambda = 0.1542$ nm were emitted whereas the Kβ emission line was filtered out by a nickel foil. The generator was operated at 40 kV and 25 mA. The scattered intensity was detected with a position sensitive detector PSD-50M (MBraun, Garching, Germany). Samples were filled into a refillable quartz capillary (Anton Paar) inserted in a thermostated sample holder with a temperature stability of 0.1 K. Measurement duration was 5000 s with a detector deadtime of less than 2%. The entrance slit width was set to 250 μm, resulting in a range of scattering vectors of $0.11\,\text{nm}^{-1} < h < 5.0\,\text{nm}^{-1}$ in which the scattering vector depends on the scattering angle 2ϑ:

$$h = \frac{4\pi}{\lambda}\sin\vartheta \tag{1}$$

According to the minimum scattering vector, the maximum particle size detectable was $r = 18$ nm. Primary data handling of the scattering curves included subtraction of the solvent scattering, data reduction by means of adjacent averaging and subtraction of the scattering background according to Porod's law (Müller, 1982).

2.2.3.1. Scattering data analysis. Data were evaluated using the Indirect Fourier Transformation program ITP-92 developed by O. Glatter. The algorithm of this method has been described adequately in the literature (Glatter, 1977a,b). The program, which performs a simultaneous slit desmearing and Fourier transformation, finally provides the electron distance distribution $p(r)$, which can be calculated from the scattering function $I(h)$ as follows (Guinier and Fournet, 1955):

$$I(h) = 4\pi\int_0^\infty p(r)\frac{\sin(hr)}{hr}\,\mathrm{d}r \tag{2}$$

The electron distance distribution $p(r)$ gives the probability to find two electrons with distance r in the measured sample. It will be zero for distances r larger than the maximum particle dimension. As the size of the vesicles is much too large to be detected in full extent with the Kratky device, the calculation of the electron distance distribution function in a model-free way according to Eq. (2) will not reveal the complete structure of the liposomes. It is thus reasonable to restrict the calculation of $p(r)$ to a lamellar symmetry in order to obtain the cross-section profile of the multilayer (Glatter, 1980). In analogy to Eq. (2), the lamellar cross-sectional distance distribution function $p_t(r)$ can be calculated via:

$$I(h)h^2 = 4\pi\int_0^\infty p_t(r)\cos(hr)\,\mathrm{d}r \tag{3}$$

The validity of Eq. (3) according to model restriction to lamellar symmetry implies in its ideal case infinitely extended flat particles. In fact, liposomes show a curvature, which is however negligible compared to the maximum thickness of the liposomal shell (Bouwstra et al., 1993).

All scattering curves were reproduced twice with subsequent calculation of the electron distance distribution and yielded identical results. For the figures, a representative curve was selected.

2.2.4. Transmission electron microscopy

To confirm the results by an imaging method, freeze-fracture technique with subsequent transmission electron microscopy was used. For this purpose, the samples were frozen in melting nitrogen (about 63 K), freeze fractured at 173 K and 5×10^{-6} bar in a BAF 400 (Balzers GmbH, Wiesbaden, Germany) and shadowed with platinum carbon at an angle of 45°. Replica were cleaned, first in a chloroform/methanol mixture, and afterwards in water. After cleaning, the obtained replica were observed with a transmission electron microscope (EM 300, Philips, Kassel, Germany) at an accelerating voltage of 80 kV.

3. Results

3.1. Optimization of the manufacturing process

Dispersions for intravenous application must not show particles with sizes greater than 5 μm. Although SUVs do not exceed this size in general, the formation

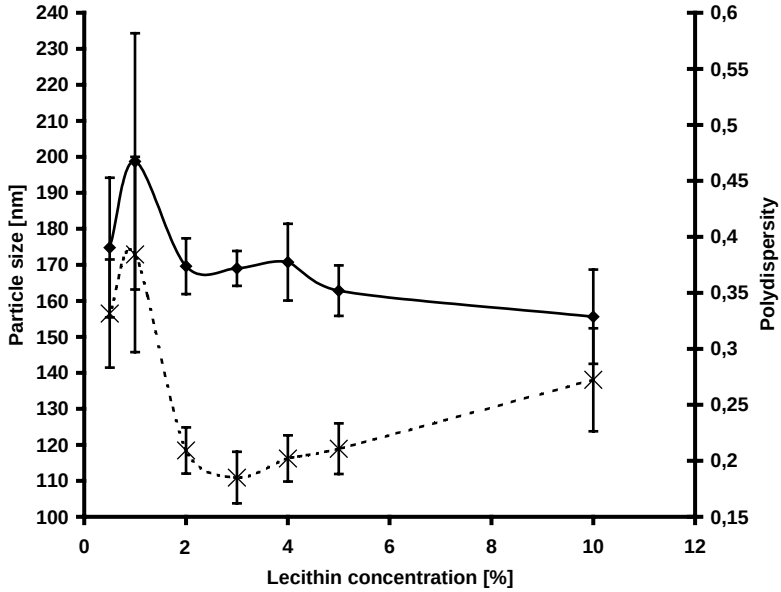


Fig. 1. Particle size (—) and polydispersity index (- - -) depending on the lecithin concentration (60 s energy input, 60 s idle period, 15 cycles with cooling), mean ± S.D. ($n = 40$–60).

of large vesicles during the manufacturing process or due to fusion effects (Mosharraf et al., 1995) has to be carefully excluded. Hence, the aim was to obtain liposomes as small as possible.

In a first step, the optimal lecithin concentration had to be determined to obtain smallest vesicles of highest homogeneity possible. Fig. 1 shows that the mean liposomal size decreases only slightly within the lecithin concentration range of 2–10%. The lowest polydispersity was found at a concentration of 3% lecithin; therefore, a lecithin concentration of 3% was selected for further studies.

In a second step, the energy input of the sonication process had to be evaluated (Fig. 2). Previous studies pointed out that an energy input in cycles yielded the smallest particles (Schubert and Müller-Goymann, 2001). Best results were obtained in cycles with an energy input of 60 s followed by an idle period of 60 s. Cycle numbers exceeding 15 appeared to further reduce the mean particle size only slightly. To limit the energy input as low as possible, a number of 15 cycle reiterations was chosen for further experiments. Furthermore, liposomes manufactured this way revealed a sufficiently low polydispersity.

During sonication the temperature of the sample rises. Therefore, an ice-bath was used for cooling the samples during the manufacturing process. To obtain reproducible particle size distributions, the cooling conditions had to be standardized concerning the ratio of water and ice as well as the total volume in the cooling container (Müller and Müller-Goymann, 2001).

By increasing the idle time in comparison with the energy input time, sample temperature did not exceed 50 °C during the manufacturing process, which proved to be sufficiently low to avoid decomposition of the phospholipids. As a consequence, sample cooling may be omitted if the sonication process is modified to 30 s energy input followed by an idle time of 80 s even up to a cycle number of 30 reiterations. Thus, two different processes will be discussed in the next sections concerning drug incorporation. They are abbreviated as to 60/60/15 and 30/80/30, where the first number represents the energy input time (s), the second number stands for the idle time (s) and the last one gives the number of cycle iterations. Only the 60/60/15 process includes sample cooling.

3.2. Drug loading of liposomes

After evaluation of the manufacturing process, the drug loading had to be optimized. With LiHCl as model drug, the best concentration of the drug (con-

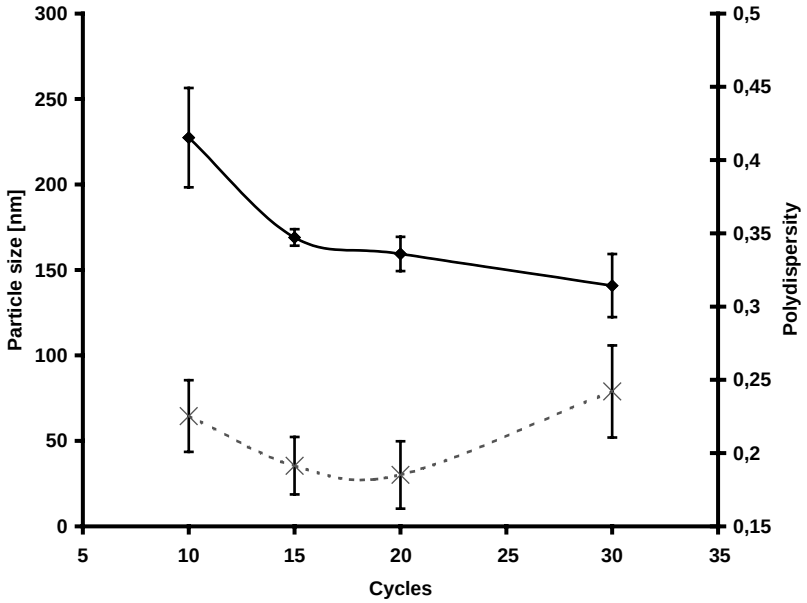


Fig. 2. Particle size (—) and polydispersity index (- - -) depending on the number of cycles (60 s energy input and 60 s idle period), mean ± S.D. (n = 20–30). The samples were cooled during sonication.

cerning particle size and polydispersity index) was determined. Drug concentrations between 1 and 15% show no distinct differences in vesicle size and polydispersity (Fig. 3). For further characterisation of the vesicles, a drug load of 5% (w/w) of LiHCl and of PrHCl was chosen, respectively. The incorporation of the drug went along with a decrease in particle size. Mean hydrodynamic diameter and polydispersity cal-

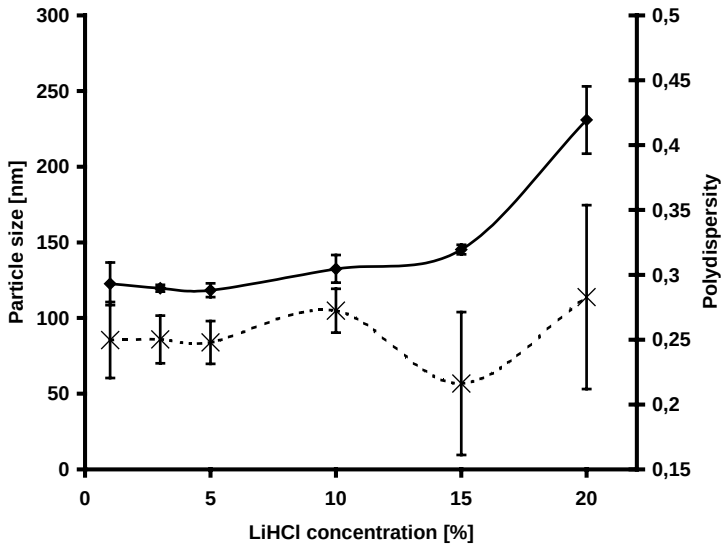


Fig. 3. Particle size (—) and polydispersity index (- - -) depending on the lidocaine-HCl concentration (60 s energy input, 60 s idle period, 15 cycles), mean ± S.D. (n = 30–45).

Table 1
z-Average and polydispersity index of drug-free and LiHCl-loaded liposomes

Manufacturing conditions	System	*z*-Average (nm)	Polydispersity index
(30/80/30) without cooling	Drug-free liposomes	102.1 ± 1.9	0.280 ± 0.017
(30/80/30) without cooling	LiHCl-loaded liposomes	84.5 ± 7.0	0.314 ± 0.027
(60/60/15) with cooling	Drug-free liposomes	149.7 ± 9.2	0.269 ± 0.031
(60/60/15) with cooling	LiHCl-loaded liposomes	124.6 ± 0.5	0.291 ± 0.013

culated by the cumulant method from PCS measurements are shown in Table 1.

By application of liposomes into the body, the contents of the liposomes will be released with and without disintegration of the carrier. A part of the contents will be released, because of a per se permeability of the liposomes. The most significant part of the contents will be released by disintegration of the liposomes. As shown in many studies disintegration from liposomes of phosphatidylcholine in plasma is caused by an interaction with HDL. HDL takes up phospholipids from the liposomes, resulting in a disintegration of the liposome accompanied by a release of the content.

The fate of liposomes in vivo is well reviewed by Senior (1987).

3.3. SAXS measurements

Fig. 4 presents the original scattering curves of the liposomal systems without any beam geometry correction. The small-angle scattering of the drug-free liposomes shows a weak but distinctive peak at $h \approx 0.9\,\mathrm{nm}^{-1}$ that corresponds to a repeat distance of $d \approx 7.0\,\mathrm{nm}$ according to Bragg's law (Eq. (4)), in which n is the order of the diffraction peak. For geometrical reasons, the diffraction peak is found at higher scattering angles after the desmearing process of the scattering curve. Thus, the repeat distance d is shifted to lower values. In the case of the drug-loaded vesicles, a diffuse scattering maximum is visible, both for LiHCl and PrHCl loading.

Obviously, neither the sonication process nor the local anaesthetic itself (LiHCl in comparison with PrHCl) seem to influence the vesicle structure strongly.

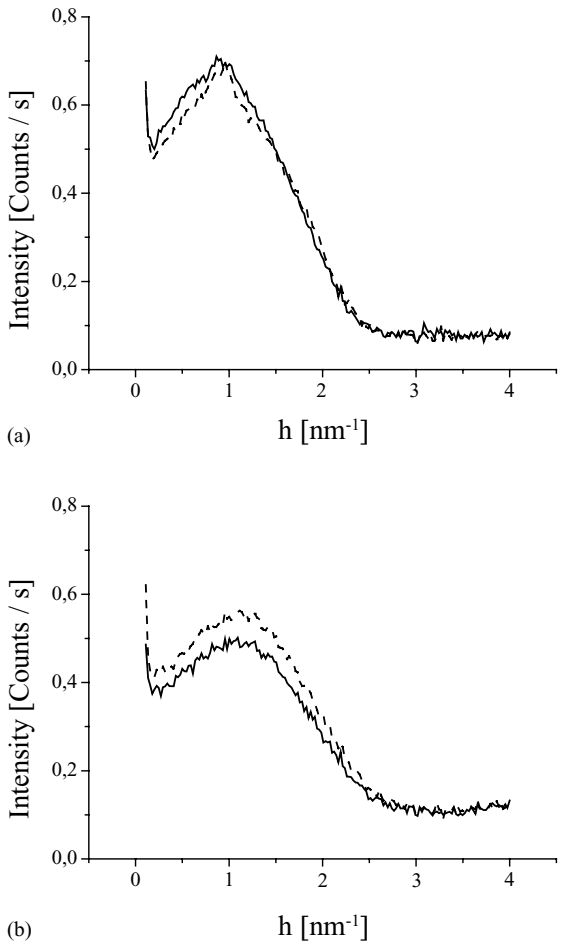
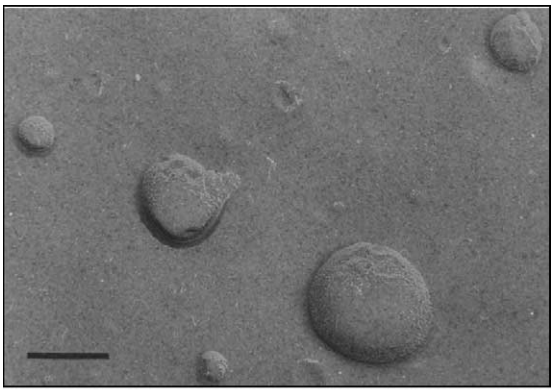
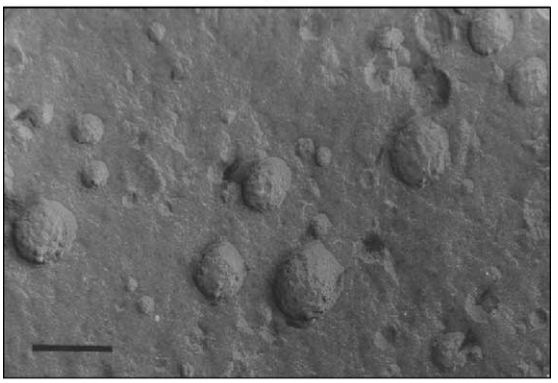


Fig. 4. (a) Original scattering curves of aqueous dispersions of 3% lecithin, i.e. drug-free liposomes, prior to the desmearing process: (—) sonication 30 s energy input, 80 s idle period, 30 cycles; (- - -) sonication 60 s energy input, 60 s idle period, 15 cycles. (b) Original scattering curves of liposomes, which consist of 3% lecithin in water, prior to the desmearing process: (—) loaded with 5% lidocaine-HCl; (- - -) loaded with 5% prilocaine-HCl. Sonication 30 s energy input, 80 s idle period, 30 cycles.



(a)



(b)

Fig. 5. Transmission electron micrographs of freeze-fractured samples. (a) Drug-free vesicles in an aqueous dispersion of 3% lecithin, sonicated with 60 s energy input, 60 s idle period, 15 cycles (bar: 150 nm). (b) Drug-loaded liposomes containing 5% LiHCl; 3% lecithin in water; sonicated with the same method (bar: 150 nm).

$$n\lambda = 2d \times \sin\vartheta = \frac{hd\lambda}{2\pi} \Rightarrow d = \frac{2\pi n}{h}. \quad (4)$$

3.4. Transmission electron microscopy (TEM)

TEM micrographs of the sample replica were taken to visualize the vesicles (Fig. 5a and b). The sonication process for each sample was 60/60/15. Thus, the drug-loaded vesicles can be compared to the drug-free ones. Fig. 5a shows unloaded liposomes. Besides some smaller vesicles, a large oligolamellar vesicle is visible in the micrograph. Fig. 5b represents liposomes loaded with LiHCl. In this micrograph, only small unilamellar vesicles can be observed.

4. Discussion

Two major conclusions may be drawn from the PCS measurements. First, the drug-loaded liposomes are distinctly smaller than the drug-free vesicles. Second, the liposomal size depends on the sonication process. Liposomes manufactured with the 60/60/15 method are clearly larger than those sonicated with the 30/80/30 process (Table 1). Since the latter variation did not involve cooling, it may be concluded, that the sonication process without cooling reduces the particle size to a greater extent than the process where cooling was applied. That means that higher temperature is more effective in particle size reduction.

As the light scattering intensity I is proportional to d^6, in which d means the particle diameter, even a small population of oligolamellar large vesicles as they presumably exist in the drug-free systems, leads to a strong increase in I. Thus, the z-average increases, because it is weighted by the intensity. This effect is assumed to be the reason for the decrease in particle size of the drug-loaded liposomal systems, in which only small unilamellar vesicles in a comparably narrow distribution exist.

The difference in particle size of liposomes manufactured with the two different sonication methods is affected by the cooling. Since both methods apply the same amount of energy to the samples, no differences in liposomal size should be expected. Part of the energy is used to rise the temperature of a cooled sample, whereas a higher amount of energy is used for size reduction of the liposomes unless the sample is cooled. Thus, using the 30/80/30 method without cooling results in a more efficient decrease in particle size.

The broad maximum in the small angle X-ray scattering curves of the drug-loaded systems (Fig. 4b) suggests the presence of unilamellar liposomes (Bouwstra et al., 1993). Furthermore, Bouwstra et al. compared calculated model scattering curves from liposomes of various bilayer numbers with measured scattering curves. As a result, an increase in sharpness of the scattering curve goes along with an increasing mean number of lamellae in the liposome walls. Thus, the scattering curves of the unloaded liposomes (Fig. 4a) can be regarded as superpositions of the scattering of unilamellar vesicles with diffraction peaks resulting from multilamellar vesicles. Compared with the results from Bouwstra et al., a population of unilamel-

lar liposomes with a non-negligible fraction of oligolamellar vesicles (2–10 lamellae) is suggested from Fig. 4a. In the same context, the drug-loaded systems give no hint of multilamellar vesicles, hence a reduction of lamellarity due to drug incorporation into the lamellae can be concluded. At this point, it should be emphasized that SAXS proved to be the only method which is able to resolve the lamellarity of vesicles with such accuracy.

To investigate the structure of the liposomal walls, the electron distance distribution of the lamellar cross section according to Eq. (3) was calculated. As neither the drug itself nor the sonication process influence the vesicular structure strongly, a differentiation just between drug-free and drug-loaded liposomes (Fig. 6a) may be done.

The strong oscillations around the abscissa are due to the discrete regions of the phospholipid bilayer having high and low electron densities with regard to the surrounding water (Laggner et al., 1977). The bilayer thickness can be obtained from the point where $p(r)$ finally approaches zero (Laggner et al., 1977), which in the case of unilamellar vesicles is the third abscissa section of the $p(r)$-function. According to this, the bilayer thickness for both drug-free and drug-loaded vesicles is about $d = 4.4 \pm 0.2$ nm, which is in accordance with the results from Laggner, obtained with dimyristoylphosphatidylcholine vesicles (Laggner, 1982). However, the drug alters the bilayer microstructure slightly but distinctly. This fact points at an intercalating incorporation of the drug molecules into the phospholipid lamellae which of course alters the electron density of the vesicular walls. Furthermore, the $p(r)$-function of the drug-free liposomes shows weak fluctuations around the abscissa for $r > 4.4$ nm, which are probably caused by additional bilayers occurring in oligolamellar vesicles.

Additional information about the vesicle structure is given by the model-free evaluation of the scattering curves. Fig. 6b shows the electron distance distribution $p(r)$ averaged over all three dimensions (Eq. (2)), thus avoiding misinterpretation due to model restriction. The $p(r)$-function of the drug-loaded system has only one distinct oscillation at small r due to regions of high and low electron density within the liposomal bilayer. The drug-free system, however, further shows fluctuations that indicate oligolamellar liposomes. The distance between the maxima gives the repeat distance of the lamellae, which in this case is about $d = 6.2 \pm 0.2$ nm. This factor does not agree with the lamellar thickness $d = 4.4 \pm 0.2$ nm from the cross-section evaluation, but roughly fits to the repeat distance of slightly less than 7.0 nm calculated from the faint peak in the non-desmeared scattering curves (Fig. 4a). The reason for this discrepancy has to be seen in the surrounding of the lamellae. First of all, fully hydrated phosphatidylcholine membranes as they exist in liposomes show a bilayer thickness between 6.0 and 6.5 nm. As in oligo-/multilamellar vesicles the surrounding of the hydration water dif-

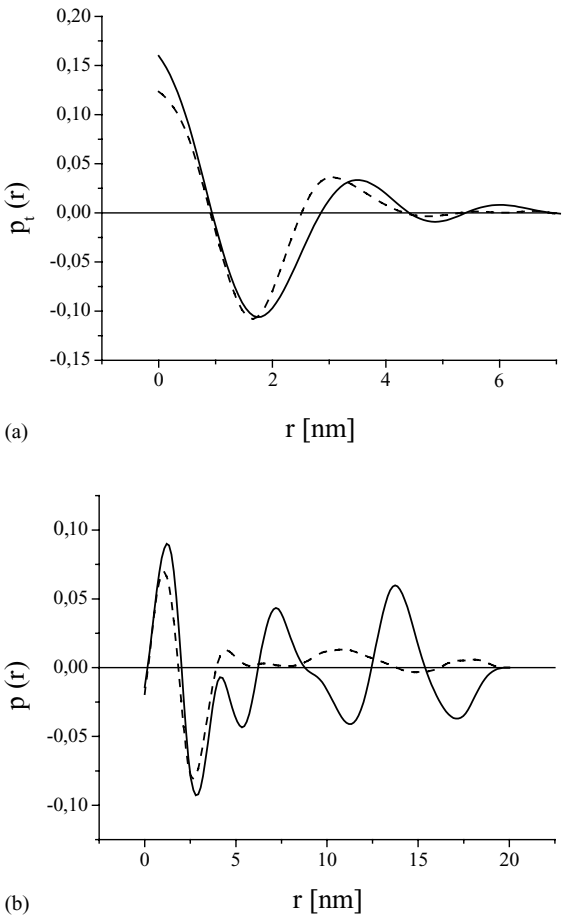


Fig. 6. (a) Electron distance distribution $p_t(r)$, restricted to the lamellar cross section of the liposomal walls: (—) unloaded system; (- - -) system loaded with 5% prilocaine-HCl. (b) Electron distance distribution $p(r)$ calculated without any model assumption: (—) unloaded system; (- - -) system loaded with 5% prilocaine-HCl.

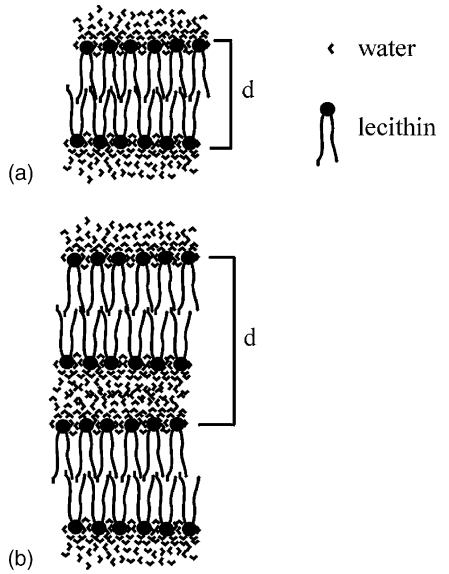


Fig. 7. Bilayer thickness d of different liposomal walls, measured with SAXS: (a) of a fully hydrated unilamellar vesicle in an aqueous surrounding, and (b) of a fully hydrated multilamellar vesicle.

fers in electron density, the hydration water layer is seen as part of the bilayer and therefore has to be added to the bilayer thickness (Fig. 7). This results in repeat distances of the observed $d = 6.2 \pm 0.2$ nm. Unilamellar vesicles are certainly hydrated in the same manner as the oligo-/multilamellar ones. In this case, however, the electron density difference between the hydration shell of the lecithin molecules and the surrounding water is too low to be resolved. For this reason, the hydration shell becomes invisible in SAXS measurements (Kratky, 1982) and the bilayer thickness decreases to the thickness of a pure unhydrated phospholipid bilayer (Fig. 7). Thus, the smaller value of $d = 4.4 \pm 0.2$ nm can be explained.

From the electron micrographs, the particle size of the vesicles could be determined directly. Fig. 5a of a drug-free system shows one oligolamellar vesicle of a particle size of about $d = 200$ nm besides many small particles of sizes from $d = 25$ nm to $d = 75$ nm. For the drug-loaded liposomes, a mean particle size of $d = 60$ nm is obtained (Fig. 5b) with a narrow distribution. Thus, the results from PCS and SAXS measurements are in good agreement. Mean particle sizes of the liposomes decrease by incorporation of a local anaesthetic. In the drug-free systems a small population of oligolamellar vesicles exists.

For the understanding of the results, it is useful to explain the apparent difference between the results of this work and those of Dörfler et al., who observed a complete transition from multilamellar to unilamellar liposomes after 20 min of continuous sonication (Dörfler et al., 1995). To obtain better results concerning particle size and polydispersity, it turned out in the present study to perform the sonication in periodic cycles of energy input and idle time rather than in a continuous way. Summing up the energy input times results in a complete energy input time of 15 min for both applied methods (60/60/15 and 30/80/30), which is less time than Dörfler et al. used in their sonication procedure. This may explain the small population of oligolamellar vesicles besides unilamellar liposomes in the present study.

5. Conclusion

The results of this study suggest the encapsulation of local anaesthetics in liposomes, as described in literature (Hammel et al., 2002; el-Ridy and Khalil, 1999; Yu et al., 2002), to be possible and yielding particle sizes small enough for intravenous application. The loading of liposomes with local anaesthetics causes a decrease in vesicle size and results in a narrower particle size distribution. There are several options to explain this reduction in particle size. First, the packing parameter of lecithin (Israelachvili et al., 1976) will be modified if the lecithin molecules interact with drug molecules (Schütze and Müller-Goymann, 1998). Second, interactions of the chloride anion with the positively charged trimethylammonium group of the lecithin molecule alter the surface charge of the vesicles.

Further possibilities are the decrease in pH occurring with the drug-loading as well as a replacement of lecithin hydration water due to interactions between drug and lecithin molecules. In any case, further spectroscopic studies to substantiate one of these explanations are indispensable and already in progress.

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References

Bouwstra, J.A., Gooris, G.S., Bras, W., Talsma, H., 1993. Small angle X-ray scattering: possibilities and limitations in characterization of vesicles. Chem. Phys. Lipids 64, 83–98.

Crommelin, D.J.A., Sindelar, R.D., 2002. Pharmaceutical Biotechnology: An Introduction for Pharmacists and Pharmaceutical Scientists. Routledge, Taylor & Francis Group, London, pp. 96–99.

Dörfler, H.-D., Görner, G., Bendzko, P., 1995. Kalorimetrische Untersuchungen an peptidmodifizierten Liposomen. Pharmazie 50, 553–557.

el-Ridy, M.S., Khalil, R.M., 1999. Free versus liposome-encapsulated lignocaine hydrochloride topical applications. Pharmazie 54, 682–684.

Eriksson, M., 1995. Prilocaine reduces injection pain caused by propofol. Acta Anaesth. Scand. 39, 210–213.

Glatter, O., 1977a. A new method for the evaluation of small-angle scattering data. J. Appl. Cryst. 10, 415–421.

Glatter, O., 1977b. Data evaluation in small angle scattering: calculation of the radial electron density distribution by means of indirect Fourier transformation. Acta Phys. Austriaca 47, 83–102.

Glatter, O., 1980. Evaluation of small-angle scattering data from lamellar and cylindrical particles by the indirect transformation method. J. Appl. Cryst. 13, 577–584.

Guinier, A., Fournet, G., 1955. Small-Angle Scattering of X-rays. John Wiley & Sons, New York (Chapter 2).

Hammel, M., Uhrikova, D., Balgavy, P., 2002. Partion of local anaesthetic heptacaine homologs between phosphatidylcholine bilayers in unilamellar liposomes and aqueous: UV-Vis spectrophotometry study. Pharmazie 57, 499.

Hope, M.J., Bally, M.B., Mayer, L.D., Janoff, A.S., Cullis, P.R., 1986. Generation of multilamellar and unilamellar phospholipid vesicles. Chem. Phys. Lipids 40, 89–107.

Israelachvili, J.N., Mitchell, D.J., Ninham, B.W., 1976. Theory of self-assembly of hydrocarbon amphiphiles into micelles and bilayers. J. Chem. Soc. Farad. Trans. II 72, 1525–1568.

Kaufke, S., Krauel, K., 2000. Einfluss von Lokalanästhetika auf die Teilchengröße von Propofol-Fettemulsionen. Krankenhauspharmazie 21, 284–286.

Kratky, O., 1982. A survey. In: Glatter, O., Kratky, O. (Eds.), Small Angle X-ray Scattering. Academic Press, London (Chapter 1).

Laggner, P., Glatter, O., Müller, K., Kratky, O., Kostner, G., Holasek, A., 1977. The lipid bilayer structure of the abnormal human plasma lipoprotein X. Eur. J. Biochem. 77, 165–171.

Laggner, P., 1982. Lipoproteins and membranes. In: Glatter, O., Kratky, O. (Eds.), Small Angle X-ray Scattering. Academic Press, London (Chapter 10).

Mosharraf, M., Taylor, K.M.G., Craig, D.Q.M., 1995. Effect of calcium ions on the surface charge and aggregation of phosphatidylcholine liposomes. J. Drug. Target. 2, 541–545.

Müller, K., 1982. Experimental practice. In: Glatter, O., Kratky, O. (Eds.), Small Angle X-ray Scattering. Academic Press, London (Chapter 7).

Müller, M., Müller-Goymann, C.C., 2001. Influence of temperature on the manufacturing of liposomes. Arch. Pharm. Pharm. Med. Chem. 334, 79.

Nathanson, M.H., Gajraj, N.M., Rusell, J.A., 1996. Prevention of pain on injection of propofol: a comparison of lidocaine with alfentanil. Anesth. Analg. 82, 469–471.

O'Hara, J.R., Sprung, J., Laseter, J.T., Maurer, W.G., Carpenter, T., Beven, M., Mascha, E., 1997. Effects of topical nitroglycerin and intravenous lidocaine on propofol-induced pain on injection. Anesth. Analg. 84, 865–869.

Ostrowsky, N., 1988. Particle characterisation by photon correlation spectroscopy. In: Lloyd, P.J. (Ed.), Particle Size Analysis. Wiley & Sons, New York, pp. 19–30.

Parmar, A.K., Koay, C.K., 1998. Pain on injection of propofol. A comparison of cold propofol with propofol premixed with lignocaine. Anesth. Analg. 53, 79–83.

Rubas, W., Schreier, H., 1991. Liposomen: Fortschritte in Herstellungs-Technologie und Therapie. Pharmazie in unserer Zeit 20, 255–270.

Sadler, P.J., Thompson, H.M., Maslowski, P., Liddle, A., Rowbotham, D.J., 1999. Iontophoretically applied lidocaine reduces pain on propofol injection. Br. J. Anaesth. 82, 432–434.

Schreier, H., 1982. Liposomen-ein neuartiger Arzneistoffträger. Pharmazie in unserer Zeit 11, 97–108.

Schubert, M., Müller-Goymann, C.C., 2001. Solubilization of bromhexine hydrochloride in aqueous lecithin dispersions. Physicochemical characterization of interactions between drug and carrier. Pharmazie 56, 734–737.

Schütze, W., Müller-Goymann, C.C., 1998. Phase transformation of a liposomal dispersion into a micellar solution induced by drug-loading. Pharm. Res. 15, 538–543.

Senior, J.H., 1987. Fate and behavior of liposomes in vivo: a review of controlling factors. CRC 3, 123–193.

Weiner, N., Martin, F., Riaz, M., 1989. Liposomes as a drug delivery system. Drug Dev. Ind. Pharm. 15, 1523–1553.

Westesen, K., Wehler, T., 1992. Physicochemical characterization of a model intravenous oil-in-water emulsion. J. Pharm. Sci. 81, 777–786.

Yu, H.Y., Li, S.D., Sun, P., 2002. Kinetic and dynamic studies of liposomal bupivacaine and bupivacaine solution after subcutaneous injection in rats. J. Pharm. Pharmacol. 54, 1221–1227.