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Solubilization of bromhexine hydrochloride in aqueous lecithin dispersions. Physicochemical characterization of interactions between drug and carrier

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In aqueous systems bromhexine hydrochloride (Br−HCl) has a poor solubility (4.54 mg/g) and displays no amphiphilic character e.g. self association. Therefore the drug is molecularly dispersed in water until the solubility product of Br−HCl is exceeded. Solubilization of Br−HCl is linearly increased on addition of lecithin; calculations show that 10 mg Phospholipon® 90G (P 90G) enable solubilization of additional 1.25 mg Br−HCl after the solubility product of Br−HCl has been exceeded. This means that four to five phospholipid molecules are needed for the solubilization of one drug molecule. Ternary systems with P 90G concentrations up to 20% have a lamellar microstructure. The systems are multilamellar vesicle dispersions as polarisation microscopy, transmission electron microscopy and small-angle X-ray diffractometry suggest. Furthermore, Br−HCl solubilization leads to a significant reduction of the interlamellar distance d and increases the elastic properties of the systems. ^{31}P NMR data provide evidence that Br−HCl is solubilized within the lipophilic part of the phospholipid bilayer.

1. Introduction

Bromhexine hydrochloride (Br−HCl) is a mucolytic agent that acts by itself and its metabolites e.g. ambroxol. Due to the low water solubility of Br−HCl and a partition coefficient of 10.60 for n-octanol/buffer of pH 7.4 [1], an investigation of the interactions between the drug and colloidal drug delivery systems (DDS) seems to be appropriate. As colloidal DDS model aqueous lecithin dispersions were chosen. The aim of the present work was to achieve a better understanding of the mode of interactions between the drug and colloidal lecithin associates in aqueous systems and to develop a model that takes into account the structural changes that are caused by the interactions between drug and carrier.

2. Investigations and results

2.1. Characterization of the dissolution behaviour of bromhexine hydrochloride in water

The solubility of Br−HCl in water is poor; from HPLC data (Fig. 1) the maximal amount dissolved at 293 ± 1 K was determined as 4.54 mg/g corresponding to $0.011 \cdot 10^{-3}$ mol/l. The saturated solution of Br−HCl has a clear and transparent appearance and a pH of 3.62 caused by the dissociation of the drug in free base and proton. At concentrations above the solubility product Br−HCl starts to form characteristic crystals that can be detected by means of polarisation microscopy. Table 1 shows that the surface tension already declines at very low concentrations of Br−HCl but stabilizes at a value of about 69 mN/m. The calculated critical micelle concentration (CMC) of Br−HCl in water is 0.06622 mg/g corresponding to $1.5075 \cdot 10^{-5}$ mol/l. For direct micelle detection solutions with up to 4.54 mg/g Br−HCl were investigated with photon correlation spectroscopy (PCS) but no micelles could be detected.

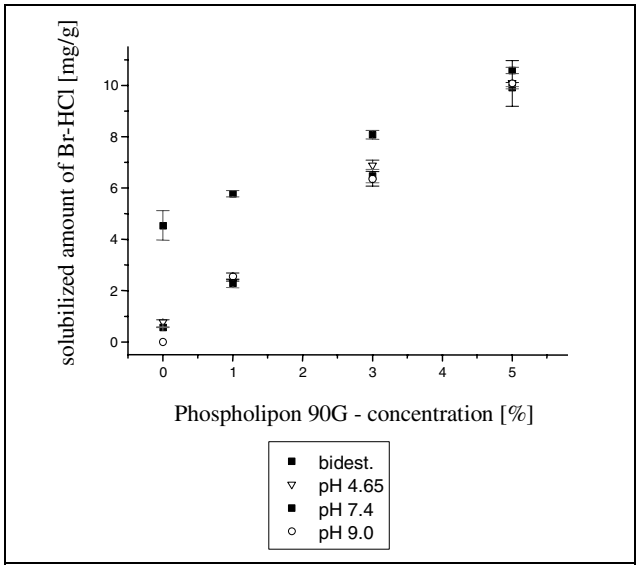

Fig. 1: Influence of lecithin concentration on the maximal solubility of Br−HCl at 293 ± 1 K

Table 1: Surface tension of an aqueous Br–HCl solution

Br−HCl Concentration (mg/g)	Surface tension (mN/m)	sd (mN/m)
0	71.38	0.01
0.036	69.84	0.05
0.071	69.50	0.10
0.142	69.15	0.11
0.214	69.07	0.10
0.355	68.66	0.08
0.498	68.91	0.11
0.711	68.89	0.15
0.853	68.68	0.13
1.066	68.71	0.04

2.2. Characterization of the association behaviour of systems with lecithin

Figure 1 shows that the maximal solubility of Br−HCl is higher in ternary systems than in the aqueous drug solution. The maximal solubility increases linearly with growing Phospholipon® 90G (P 90G) concentration. However, the maximal solubility of Br−HCl remains low. For example in bidestilled water addition of approximately 10 mg P 90G is necessary in order to solubilize 1.25 mg Br−HCl i.e. 4 to 5 molecules of P 90G are needed to solubilize one Br−HCl molecule. Despite different P 90G concentration and different drug amount the pH-value of the examined systems in bidestilled water is about 3.6 in all sys-

tems. Buffered systems show a lower maximal solubility in comparison with non buffered systems when no lecithin is employed. Yet, with rising P 90G concentration the maximal solubility of the buffered systems converges with that of the non buffered systems. However, the differences of the solubility product of Br−HCl between the buffered systems are not significant.

Binary systems of P 90G and water and ternary systems display optical anisometry. As characteristic texture a lamellar mesophase was identified in all systems by means of polarisation microscopy. Typical textures for a lamellar mesophase are maltese crosses, either separate or in chains as "oily streaks" [2, 3]. With increasing drug content in ternary systems a decrease of separate maltese crosses and an increase of oily streaks was observed.

Particle size analysis with laser diffraction reveals that incorporation of Br−HCl leads to a concentration independent shift of the particle size to bigger particles. Binary systems of water and P 90G have particles between 1 µm to 100 µm in contrast to ternary system with particles from 5 µm to 120 µm (Fig. 2). Sonicated binary systems of P 90G and water as well as sonicated ternary systems analysed with PCS have a monomodal particle size distribution with a z-average between 60 nm and 120 nm (data not shown). Sonicated drug loaded systems show no significant shift in particle size distribution.

After shock-freezing and replication large multilamellar vesicles (MLV) were observed as typical colloidal structures of the lamellar mesophase for all investigated systems. There is no change in the fundamental structure of the mesophase after solubilization of Br−HCl in systems with lecithin and water. In Fig. 3 a typical micrograph of a lamellar vesicle dispersion is shown.

Interactions between Br−HCl and colloidal lecithin associates cause a concentration dependent change in the rheology of the systems. The elastic modulus G increases for a fixed lecithin concentration constantly with rising Br−HCl concentrations (Fig. 4). Vice versa the phase angle δ decreases with rising Br−HCl concentrations (Fig. 4) until a drug concentration of 0.3% is reached and passes over to a plateau.

The effect of Br−HCl on the colloidal microstructure of lecithin was evaluated by SAXD. The measurements were performed with binary and ternary systems containing water and P 90G in different concentrations. Ternary systems were loaded with the maximal amount of Br−HCl (see Fig. 1 for solubilized Br−HCl amount). Both binary and ternary systems show interference patterns corresponding to a lamellar bilayer microstructure [4]. Furthermore, the incorporation of Br−HCl leads to a significant decrease of the interlamellar distance d from 6.4 nm to 4.2 nm that is independent from the P 90G concentration (Table 2).

^{31}P NMR was used to locate and characterize the site and mode of drug incorporation within the lecithin bilayer. Both binary and ternary systems display one relatively narrow and symmetrical peak. Br−HCl loaded systems show in comparison to drug free systems a minor chemical shift of about 0.05 ppm to more negative ppm values and a smaller half-line width $\Delta\nu_{1/2}$ (Table 3).

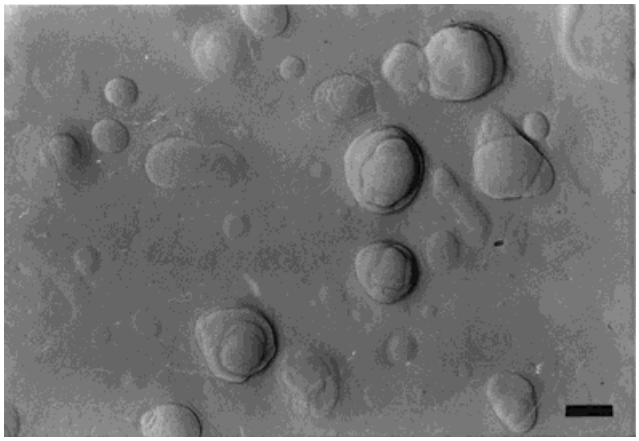

Fig. 3: TEM micrograph of a lamellar vesicle dispersion containing 10% lecithin, 1.5% Br−HCl and 88.5% water (bar = 500 nm)

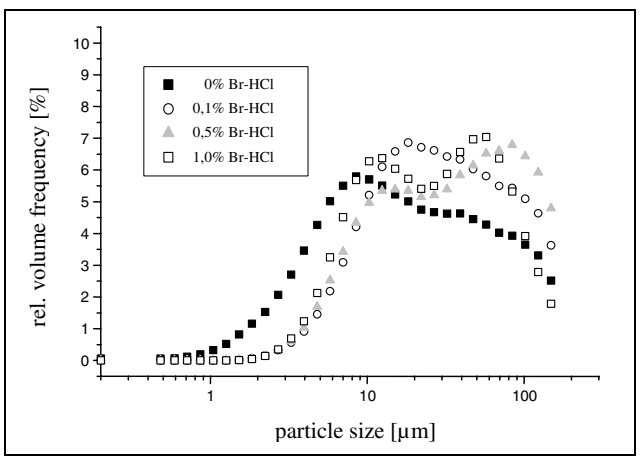

Fig. 2: Comparison of the particle size distribution for a fixed lecithin concentration of 7% (m/m) and varying Br−HCl concentrations (m/m)

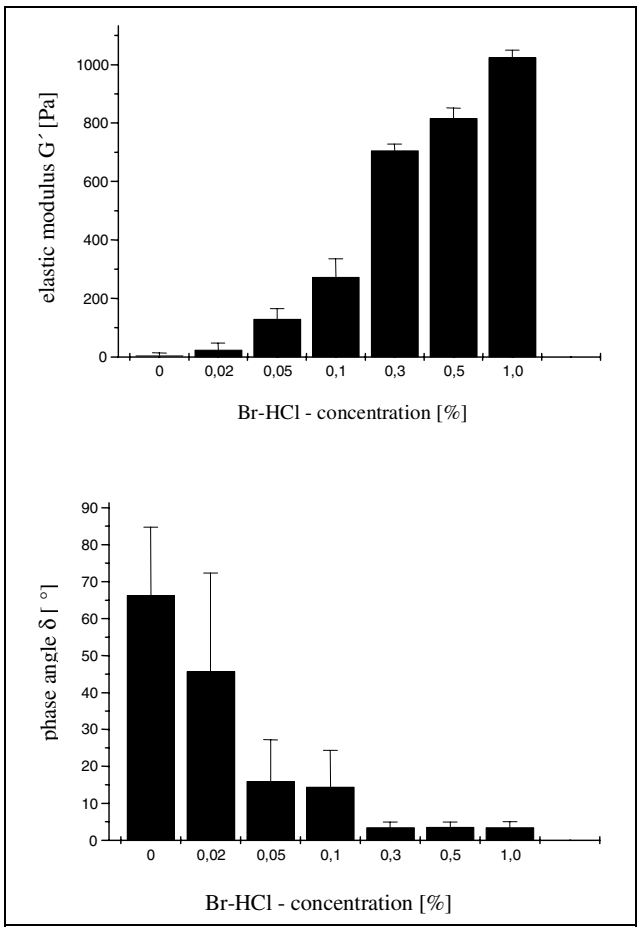

Fig. 4: Influence of the Br−HCl concentration for a fixed lecithin concentration of 20% (m/m) on the rheological properties, n = 3

Table 2: SAXD measurement of the interlayer spacing d in binary and ternary systems

P 90G Concentration (%)	Binary systems: d (Å)	Ternary systems: d (Å)
5	66.6	43.2
10	66.3	43.4
15	66.6	46.2
20	64.9	46.9

Table 3: ^{31}P NMR results of binary and ternary systems

Composition (%)			Peak position (ppm)	$\Delta\nu_{1/2}$ (ppm)
P 90G	D_2O	Br−HCl		
5	95	–	−0.184	0.58
5	94	1	−0.234	0.33
10	90	–	−0.148	0.40
10	89	1	−0.193	0.26

3. Discussion

The partition coefficient of 10.60 as a parameter for the lipophilic respectively hydrophilic behaviour of a substance corresponds to the poor solubility of Br−HCl in water and may already indicate that interactions between Br−HCl and P 90G take place in lipophilic molecule regions of the colloidal carrier. Despite its more lipophilic behaviour Br−HCl possesses also polar molecule regions and therefore possibly amphiphilic properties. Whether the amphiphilic character of Br−HCl is strong enough to display any form of self-association e.g. formation of micelles etc. was examined by means of surface tension measurements and photon correlation spectroscopy (PCS). The observed decline of the surface tension from about 71.5 mN/m to about 69 mN/m is not very distinct and may not be due to a micellization phenomenon of Br−HCl but may be rather induced by the pH decrease and adsorption of the free base at the surface. For that reason, PCS was used for direct micelle detection. Aqueous solutions with up to 4.54 mg/g Br−HCl corresponding to the solubility product of Br−HCl in water were investigated and no micelles could be detected. Thus, it is concluded that Br−HCl has no self association behaviour in water and forms a true solution i.e. molecularly dispersed molecules in water.

As HPLC measurements show, the maximal solubility of Br−HCl in ternary systems increases linearly with growing P 90G content. In addition, the pH-value is only of minor influence on the solubility product of Br−HCl when lecithin is employed. Thus can be concluded, that the solubilization of the drug by the colloidal carrier results from interactions between drug and carrier. The changing rheological properties i.e. an overall increase in the elastic properties of the examined systems with rising Br−HCl concentration and the shift of particle size distribution caused by incorporation of Br−HCl are further signs of interactions that take place between Br−HCl and P 90G even though the proper site and mode of interaction is not yet understood. But as the increase of elastic properties and the shift of the particle size suggest, Br−HCl incorporation causes a transformation of the vesicle dispersion into a gel that was also macroscopically observed. As polarisation microscopy shows a decrease of maltese crosses and an increase of oily streaks with the incorporation of Br−HCl, defect structures within the bilayers of the multilamellar vesicles become less probable whereas planar arrangement of infinitely extended multilayers becomes more probable. This is in accordance with the gelation effect.

Polarisation microscopy, TEM and SAXD demonstrate that the bilayer structure of lecithin in water remains unchanged in principle by the incorporation of Br−HCl although the distance d in the lamellar microstructure resulting from the phospholipid bilayer and interlamellarly bound water is reduced significantly. Therefore, the decrease of d after the incorporation of Br−HCl results from either a reduction of interlamellarly bound water or a new arrangement of the fatty acid chains in the bilayer. However, the extrusion of interlamellarly bound water is unlikely due to the fact that water as the main component ($\geq$ 80%) in the investigated systems is available in excess and that the solubilized amount of Br−HCl in ternary systems is above the solubility product of Br−HCl in water. In addition, the more lipophilic character of Br−HCl seems to favour a drug incorporation within the lipophilic part of the phospholipid bilayer. It is likely that the drug incorporation causes increasing flexibility of the lipophilic chains within the phospholipid bilayer and thus a new arrangement of the fatty acid chains resulting in a decrease of the interlamellar distance d.

^{31}P NMR measurements display for both binary and ternary systems one relatively narrow and symmetrical peak, reflecting isotropic motional averaging and arising mostly from lateral diffusion and Brownian tumble [5]. This finding corresponds well to the PCS data for the sonicated systems where all investigated systems have a monomodal particle size distribution with a z-average value between 60 nm and 120 nm. As no shift split is observed a modification of possibly existing interparticular interactions can be excluded and the incorporation within the bilayer structure appears to be homogeneous. The small chemical shift of 0.05 ppm to more negative ppm values of Br−HCl loaded systems in comparison to drug free systems shows that the incorporation of Br−HCl has only a minor influence on the phosphate group of P 90G. Therefore it can be concluded that incorporation of Br−HCl takes place in the lipophilic part of the phospholipid bilayer. Furthermore, Br−HCl reduces the half-line width ($\Delta\nu_{1/2}$) of the systems from which a more homogeneous and narrower particle size distribution can be deduced.

4. Experimental

4.1. Materials

Bromhexine hydrochloride (Br−HCl) was supplied by Thomae (D-Biberach/Riß). Phospholipon® 90G (P 90G), a highly purified sojabean lecithin containing at least 90% phosphatidylcholine was supplied by Rhône-Poulenc-Rorer (D-Köln). Water was used in bidistilled quality. Deuterium oxide (D_2O) was purchased from Aldrich (D-Steinheim).

4.2. Methods

4.2.1. Preparation of binary and ternary systems

All components depending on the composition of the systems were weighed in sealed containers up to a lecithin concentration of 20% (m/m). The mixtures were stirred with a Teflon coated magnet for 20 min at 333 K. Stirring was continued until room temperature was reached. pH-values of 4.65, 7.4 and 9.0 were obtained by use of acetate puffer (0.1 mol/l), phosphate puffer (0.1 mol/l), borate puffer (0.1 mol/l) and HCl (0.1 mol/l) and NaOH (0.1 mol/l) for pH adjustment.

4.2.2. Preparation of small unilamellar vesicles (SUVs)

The various systems were prepared as described 4.2.1. and afterwards sonicated with a Soniprep 150 (MSE Scientific Instruments, GB-Crawley) for 10 cycles with 60 s on and 60 s off each.

4.2.3. Surface tension measurements

The surface tension of binary drug/water systems was determined by using a Lecomte de Noüy ring tensiometer (Lauda, D-Königshofen) at 293 K in equilibrium.

4.2.4. High performance liquid chromatography (HPLC)

Br−HCl analyzis was performed by reversed phase chromatography using a column of Hypersil® ODS, 125 × 4 mm (Grom, D-Herrenberg). The mobile phase consisted of acetonitrile and phosphate buffer of pH 7.0 (80 : 20) with a flow rate 1.5 ml/min using a spectroflow 400 pump (Kratos, D-Weiterstadt). Peaks were detected with a Beckman System Gold Detector Module 166 (Beckman, D-München) at a wave length of 240 nm, peak identification and integration was carried out by Beckman System Gold Software Version 6.01 (Beckman, D-München). Calibration was performed within a range of 10 ng/ml to 50 ng/ml with a correlation coefficient greater than 0.999.

4.2.5. Polarisation microscopy

Binary and ternary systems were observed with a photo microscope type III (Zeiss, D-Oberkochen) using crossed polarizers and a λ-plate to generate a path difference of 550 nm.

4.2.6. Laser diffraction

Laser diffraction was used to determine the particle size and particle size distribution of binary and ternary systems above 500 nm. The measurements were performed with a Mastersizer MS 20 (Malvern, D-Herrenberg). Immediately after diluting 50 mg of sample with 100 ml bidestilled water the measurement was started. Results were calculated with Malvern SB O9 software using the Fraunhofer approximation.

4.2.7. Photon correlation spectroscopy (PCS)

For particles smaller than 500 nm. i.e. sonicated systems (see 4.2.2) and micelle detection PCS was applied. PCS measurements were performed with a Zetasizer 3 (Malvern, D-Herrenberg) modified with a He/Ne laser model 127 (Spectra Physics, USA-Mt. View, Cal). The different systems were investigated under an angle of 90° in a measuring cell AZ 10 tempered at 293 K. Each sample was diluted with filtrated bidistilled water until the appropriate concentration of particles was achieved to avoid multiscattering events and measured with a sample time of 30 ms for 10 min in serial mode. Each measurement was performed in triplicate and the particle z-average diameter was determined.

4.2.8. Rheological measurements

Rheological measurements were performed at 293 K on a rheometer CVO/CS (Bohlin Instruments, GB-Cirencester) with a cone and plate measuring geometry CP 4/40. Oscillation measurements were performed in the linear viscoelastic region applying a shear stress sweep from 0.060 Pa to 10 Pa and a constant frequency of 1 Hz. As the most relevant rheological parameters phase angle δ and the elastic modulus G′ were assessed.

4.2.9. Transmission electron microscopy (TEM) of freeze-fractured specimen

Binary and ternary systems with a lecithin content up to 20% (m/m) were shock-frozen in melting nitrogen at 63 K between two flat gold holders. The frozen samples were fractured at 173 K in a BAF 400 instrument (Balzers, D-Wiesbaden). Samples were shadowed with platinum/carbon (2 nm) at 45° and with pure carbon at 90° for replica preparation. A chloroform-methanol mixture (1 : 1 (v/v)) was used for cleaning the replicas. The replicas on uncoated grids were viewed using a transmission electron microscope EM 300 (Philips, D-Kassel).

4.2.10. Small-angle X-ray diffractometry (SAXD)

SAXD studies were performed in a compact small-angle system connected to a PW 1710 generator including a PW 2213-20 X-ray tube with a copper anode (Philips, D-Kassel). A nickel foil served as K_β-filter. The tube voltage was 40 kV and the anode current 25 mA. An OED PSD-50M position sensitive detector (Braun, D-München) was linked to a Canberra MCA 8100 multichannel analyzer (Canberra Electronics, D-Frankfurt) for interference analysis. Binary and ternary systems with a different P 90G content were investigated for 800 s between kapton foils in vacuum to reduce scattering effects.

4.2.11. ^{31}P nuclear magnetic resonance (^{31}P NMR)

^{31}P NMR spectra were obtained on an AC Bruker 200 MHz spectrometer (Bruker, D-Rheinstetten) at 293 K with orthophosphoric acid as external reference. The various systems were prepared as described above and afterwards sonicated with a Soniprep 150 (MSE Scientific Instruments, GB-Crawley) for 10 cycles with 60 s on and 60 s off each.

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Received June 21, 2000
Accepted January 31, 2001

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