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RESEARCH ARTICLE

Albendazole solution formulation via vesicle-to-micelle transition of phospholipid-surfactant aggregates

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ABSTRACT

Objective: To reveal the physicochemical mechanisms governing the solubilization of albendazole in surfactant and phospholipid-surfactant solutions and, on this basis, to formulate clinically relevant dose of albendazole in solution suitable for parenteral delivery.

Significance: (1) A new drug delivery system for parenteral delivery of albendazole is proposed, offering high drug solubility and low toxicity of the materials used; (2) New insights on the role of surface curvature on albendazole solubilization in surfactant and surfactant-phospholipid aggregates are provided.

Methods: The effect of 17 surfactants and 6 surfactant-phospholipid mixtures on albendazole solubility was studied. The size of the colloidal aggregates was determined by light-scattering. The dilution stability of the proposed formulation was assessed by experiments with model human serum.

Results: Anionic surfactants increased very strongly drug solubility at pH = 3 (up to 4 mg/mL) due to strong electrostatic attraction between the oppositely charged (at this pH) drug and surfactant molecules. This effect was observed with all anionic surfactants studied, including sodium dodecyl sulfate, double chain sodium dioctylsulfosuccinate (AOT), and the bile salt sodium taurodeoxycholate. The phospholipid-surfactant mixture of 40% sodium dipalmitoyl-phosphatidylglycerol +60% AOT provided highest albendazole solubilization (4.4 mg/mL), smallest colloidal aggregate size (11 nm) and was stable to dilution with model human serum at (and above) 1:12 ratio.

Conclusions: A new albendazole delivery system with high drug load and low toxicity of the materials used was developed. The high solubility of albendazole was explained with vesicle-to-micelle transition due to the larger interfacial curvature preferred for albendazole solubilization locus.

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Introduction

Albendazole is a benzimidazole anthelmintic drug with recently discovered anticancer properties [1,2]. However, efficient albendazole delivery is hindered by its extremely low aqueous solubility [3], which leads to very poor oral absorption (viz. oral bioavailability) and difficult parenteral application.

Several approaches have been used to enhance albendazole delivery, such as use of cosolvents [4,5], lipid-based drug delivery vehicles [6–8], nanosuspension [9], liposome formulation [10–12], entrapment in nanoparticles [13–17], incorporation in molecular containers [18–22], and surfactant solubilization [23–26].

Thus, solution of albendazole was developed by Torrado et al. by using Transcutol as a co-solvent and its oral absorption was studied in mice [4,5]. At pH ≥4, high albendazole solubility (>1 mg/mL) was obtained only at high Transcutol co-solvent concentrations >60%. Low co-solvent concentration of ca. 10% was effective in increasing albendazole solubility only at very low pH = 1.

Albendazole was formulated in different lipid-based systems like arachis oil suspension [6] and self-microemulsifying solutions based on medium-chain triglycerides, surfactants and PEG 400 [7,8]. All studies reported an increase of oral bioavailability, which was attributed to the increased albendazole solubility in the vehicle and to supersaturation effects.

Wen et al. developed lecithin-based liposomes with size around 100 nm which showed high albendazole entrapment efficiency (75–87%) at an albendazole concentration of 10 mg/mL [10]. These authors demonstrated good efficiency in the treatment of patients with complex alveolar echinococcosis after oral administration [11]. The downside of the proposed formulation is that a multi-step procedure was used for liposome preparation, which consisted of eight steps, including dialysis. Other authors prepared native and PEGylated liposomes with good encapsulation efficiency (>70%) from egg phosphatidylcholine (PC) or its mixture with cholesterol and characterized the *in vitro* drug release rate [12].

Nanoparticles of albendazole were prepared with poly-(D,L-lactide) [13], albumin [14], chitosan [15,16], and chitosan + tripolyphosphate [17]. The poly-(D,L-lactide) nanoparticles were loaded with 0.2 mg/mL albendazole and were administered intravenously to *Echinococcus multilocularis*-infected mice [13]. The injection of 6 mg/kg albendazole in the form of nanoparticles had an equivalent antiparasitic effect as treatment with 1500 mg/kg orally administered free drug, demonstrating the effectiveness of the proposed drug delivery vehicle. The albumin-based albendazole nanoparticles were shown to reduce tumor burden in ovarian cancer xenograft model in mice after *i.p.* administration [14], while the chitosan-based oral formulation displayed targeted delivery to the liver in rats [15] and increased oral bioavailability in rodents [15,16].

The use of molecular containers for albendazole solubility enhancement was explored also in experiments with cyclodextrins

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Supplemental data for this article can be accessed here.

(CD) [18,19], cucurbit[n]urils [20], and acyclic cucurbit[n]urils [21,22]. It was shown that albendazole solubility can be increased above 1 mg/mL when using very high hydroxypropyl-β-CD concentrations (40%) at acidic conditions (pH = 1 or 3), which increased drug oral bioavailability in sheep [18]. Similarly high solubility enhancement was achieved also by the normal and acyclic cucurbit[n]urils, however, at lower molecular container concentration, due to their higher solubilization capacity, compared to CD [20–22]. The acyclic cucurbit[n]urils formulation of albendazole decreased significantly the SK-OV-3 xenograft tumor growth in mice after *i.p.* administration [22].

The solubilization of albendazole in micellar surfactant solutions has not been systematically studied. Torrado et al. reported albendazole solubility in the range of 0.5–0.7 mg/mL in the presence of 5% Tween 20, sodium lauryl sulfate or Cremophor EL surfactants at pH = 1.2 [4]. Albendazole solubilization by Tween 80 and sodium taurocholate surfactants was much lower, resulting in measured drug solubility of 0.1 and 0.01 mg/mL, for 10% Tween 80 and 4% sodium taurocholate, respectively [23]. These two surfactant systems were studied further in a series of papers, which demonstrated increased oral absorption and oral bioavailability in rats [24,25].

Despite the progress in solubility-enhancing formulations, clinically relevant albendazole solubility in water (*ca.* above 1 mg/mL) can be attained only by using very high solubilizing excipient concentrations (e.g. 40% hydroxypropyl-β-CD [18] or 40% Transcutol [4]) or/and extremely acidic media (pH $\approx$ 1). The only approach that provides sufficiently high albendazole solubility without using high excipient concentrations or very low pH is the liposome solubilization method proposed by Wen et al. [10], which so far has been explored for oral administration only. In fact, the majority of the described techniques for albendazole delivery have been designed for the oral route, with only one nano-particulate formulation being aimed specifically at parenteral delivery [13]. On the other hand, the development of parenterally administered albendazole formulation would be beneficial in view of its potential application in cancer treatment for patients, in the case when oral delivery cannot be used.

Motivated by the identified gaps in the study of albendazole solubility enhancement, in the current study we investigate systematically the albendazole solubilization by classical surfactants and, on this basis, we aim to formulate clinically relevant dose of albendazole in solution suitable for parenteral delivery. To achieve our aims, we studied albendazole solubility enhancement by a series of 17 classical surfactants with different charges (nonionic, anionic, and cationic) and hydrophobic chain-lengths (from 10 to 18 carbon atoms). Proof-of-principle experiments that illustrate the very high solubility of albendazole in solutions of negatively charged amphiphiles were performed with two additional classes of molecules: biosurfactants (sodium taurodeoxycholate) and phospholipids (sodium dipalmitoylphosphatidylglycerol). Mixtures of phospholipids and surfactants were also prepared in order to maximize albendazole solubilization in a biocompatible carrier system. The size of the carrier colloidal aggregates was determined by laser light scattering. The precipitation stability upon dilution in model human serum of a solution containing high concentration of solubilized albendazole was also studied.

Materials and methods

Materials

The relationship between drug solubilization and surfactant molecular structure was investigated by using a series of surfactants with different charges, head-group types, and hydrophobic chain-lengths. Two groups of non-ionic surfactants were studied: polysorbates and alcohol ethoxylates. We have also studied homolog series of anionic surfactants of the alkyl sulfate type, with hydrophobic chain-length of C_{10}, C_{12}, and C_{14}. Other studied anionic surfactants were ethoxylated alkylsulfates with 1 or 3 ethylenoxide groups in the hydrophilic head, and sodium dioctylsulfosuccinate. The cationic surfactants we have used are homologs of the alkyltrimethylammonium bromide type with hydrophobic chain-lengths of C_{12}, C_{14} and C_{16}. Albendazole (CLog*P* = 3.2) was obtained from TCI (purity >98%). The abbreviations and the properties of the surfactants studied, as well as the molecular structure of albendazole are summarized in Table 1, whereas the surfactant critical micellar concentrations (CMC), used for calculation of the solubilization capacity, are presented in the Supplementary information (Supplementary Table S2).

Hydrochloric acid (Sigma, 32%), citric acid (Teokom, > 99%), Na_2HPO_4 (Sigma, 99%), and KH_2PO_4 (Teokom, 99%) were used to prepare buffer solutions for the drug solubilization experiments. The materials used for preparation of the model blood serum were $NaHCO_3$ (Teokom, 99%), albumin, NaCl, KCl, and $CaCl_2$, all products of Sigma with purity >98%. Mobile phase solvents for HPLC analysis include methanol (HPLC grade, Sigma, 99.9%) and phosphoric acid solution (Merck, 85%) in water. All aqueous solutions and phases were prepared using deionized water from water-purification system Elix 3 (Millipore, Billerica, MA).

Experimental methods

Albendazole solubility in buffered surfactant solutions

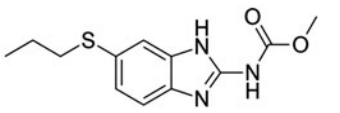
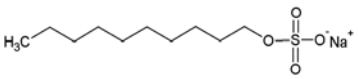
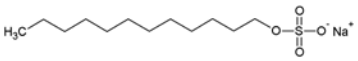
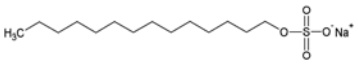
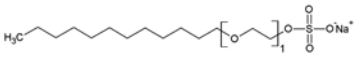
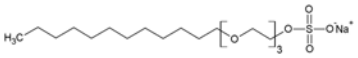
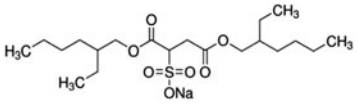
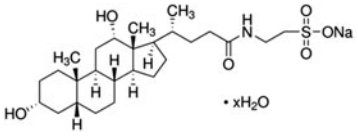
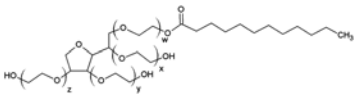
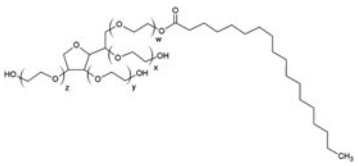
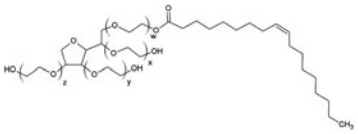
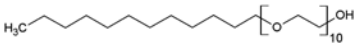
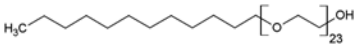
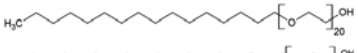
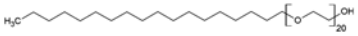
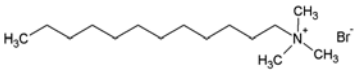
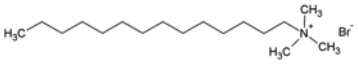
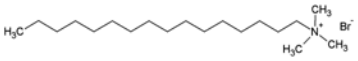
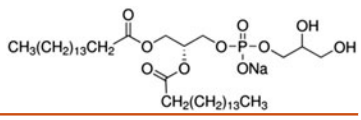
To determine the equilibrium drug solubility in the presence of different surfactants, experiments were performed at pH = 3 (Na_2HPO_4 + citric acid) and 6.5 ($Na_2HPO_4 + KH_2PO_4$). The concentrations of the buffers and the corresponding ionic strength are presented in Supplementary Table S1 in the Supplemental information. Excess amount of drug (100 mg) was weighed in a 20 mL glass bottle in which 10 mL buffered surfactant solution was added. Constant surfactant concentration, $C_S = 40$ mM, much higher than the CMC of all surfactants studied, was used in these experiments. The mixture was stirred with a magnetic stir bar at 400 rpm for 24 h at 37 °C. After this incubation period, the albendazole suspension was filtered at 37 °C through 0.2 μm NYLON syringe filter to eliminate all undissolved particles.

After separation of the undissolved particles, the concentration of the dissolved drug in the obtained clear aqueous solution was determined by HPLC (see Supplemental information for experimental details). Every step of the procedure prior to HPLC analysis was carried out at $T = 37$ °C.

Albendazole solubility in phospholipid-surfactant mixtures

Albendazole solubilization in the colloidal aggregates, formed by mixtures of NaDPPG and AOT, was studied by using the following procedure. First, a solution of AOT was prepared, by dissolving an appropriate amount of this surfactant in 3 mL buffer solution (pH = 3), loaded in 5 mL glass bottle. Then, the appropriate amount of NaDPPG was added and the obtained suspension was sonicated for 60 s using a 3 mm diameter sonotrode, set at a power output of 250 W (SKL-650 W sonicator, Syclon). The amounts of the weighed AOT and NaDPPG corresponded to a total amphiphile concentration of 40 mM and were varied to obtain different ratios between these two components. Afterwards, 30 mg of

Table 1. Properties of the drug and amphiphiles studied.

Name	Acronym used in text	Supplier, purity	Molecular mass, g/mol	Structure
Albendazole	–	TCI, 98%	265	
Sodium decyl sulfate	$C_{10}SO_4Na$	Merck, 99%	260	
Sodium lauryl sulfate	$C_{12}SO_4Na$	Arcos, 99%	288	
Sodium tetradecyl sulfate	$C_{14}SO_4Na$	Merck, 95%	316	
Sodium lauryl ethoxy-1 sulfate	$C_{12}E_1SO_4Na$	Stepan Co., 70%	332	
Sodium lauryl ethoxy-3 sulfate	$C_{12}E_3SO_4Na$	Stepan Co., 70%	420	
Sodium dioctyl-sulfosuccinate	AOT	Sigma	445	
Sodium taurodeoxy-cholate	STDC	Sigma, 97%	522	
Tween 20	T20	Sigma–Aldrich	1228	
Tween 60	T60	Sigma–Aldrich	1309	
Tween 80	T80	Sigma–Aldrich	1310	
Polyoxyethylene-10 lauryl ether	$C_{12}E_{10}$	Sigma	627	
Polyoxyethylene-23 lauryl ether	$C_{12}E_{23}$	Sigma–Aldrich	1198	
Polyoxyethylene-20 cetyl ether	$C_{16}E_{20}$	Sigma	1124	
Polyoxyethylene-20 stearyl ether	$C_{18}E_{20}$	Sigma	1152	
Dodecyl trimethyl ammonium bromide	$C_{12}TAB$	Sigma–Aldrich, 98%	308	
Tetradecyl trimethyl ammonium bormide	$C_{14}TAB$	Sigma, 99%	336	
Cetyl trimethyl ammonium bromide	$C_{16}TAB$	Merck, 99%	364	
Sodium dipalmitoyl phosphatidyl glycerol	NaDPPG	NOF, 99%	745	

albendazole was added and the mixture was stirred for 24 h and $T = 37\,^{\circ}C$. After incubation, the albendazole suspension was centrifuged for 30 min at 13,200 × *g* to eliminate all undissolved particles. The concentration of the solubilized drug in the obtained aqueous solutions was determined by HPLC as explained in Supplemental information.

Determination of the aggregate size in the phospholipid-surfactant mixtures

The size of the colloidal molecular aggregates (vesicles or micelles), formed by the mixtures of AOT and NaDPPG in the presence of albendazole, was determined by laser light scattering on a Malvern 4700C apparatus (Malvern Instruments, UK), after sample

centrifugation. The results are averaged over at least two separate measurements and are presented as the mean volume-weighted hydrodynamic diameter of the aggregates.

Dilution stability test by drop-wise addition

The stability to dilution of albendazole solutions, based on the phospholipid-surfactant mixtures, was studied by a procedure adapted from Li et al. [27]. Briefly, an albendazole solution in AOT:NaDPPG was prepared by the procedure described in 'Albendazole solubility in phospholipid-surfactant mixtures' section. After the separation of all undissolved material by centrifugation, 15 μL aliquots of the albendazole solution were subsequently added to a 2 mL model blood serum solution, placed in a quartz cuvette, by using a micropipette. The cuvette was gently shaken to homogenize the solution after each addition. Blood serum was modeled by a solution containing 45 g/L albumin, 140 mM NaCl, 4 mM KCl, 2.4 mM $CaCl_2$ and 25 mM $NaHCO_3$, with a pH of 7.4, in accordance with the normal laboratory values of these components in human serum [28]. Drug precipitation was monitored by determining the light absorption (scattering) at $\lambda = 600$ nm with UNICAM spectrophotometer, equipped with a thermostated cell. The experiment was carried out at $T = 37\,^{\circ}C$.

Experimental results

Effect of surfactant type on albendazole solubility

The effect of 17 surfactants on albendazole solubility at pH = 3 and 6.5 is compared in Figure 1. Drug solubility in anionic surfactant solutions at pH = 3 is very high (150–4033 μg/mL), whereas the solubility in the presence of non-ionic and cationic surfactants at the same pH is much lower (45–100 μg/mL). At pH = 6.5, albendazole solubility is low (10–70 μg/mL) for all surfactants studied.

More detailed analysis of the results presented in Figure 1 shows considerable differences in the albendazole solubility enhancement, for surfactants with the same head-group charge. For example, the solubility of albendazole in solution of $C_{10}SO_4Na$ at pH = 3 is 1078 μg/mL, compared to 4033 μg/mL for $C_{14}SO_4Na$ at the same pH. Both surfactants are anionic and are part of the alkylsulfate homolog series. Similar differences are observed also in the groups of non-ionic and cationic surfactants. Therefore, surfactant molecular structure has significant impact on the albendazole solubilization. To compare the albendazole solubilization efficiency of the different surfactants, we used the molar solubilization capacity, defined as follows [29]:

$$\chi = \left(\frac{S_{tot} - S_W}{C_S - CMC}\right) \times 1000 \quad (1)$$

where S_{tot} is the measured molar drug solubility in the presence of surfactants, S_W is the solubility of the drug in water at the respective pH, C_S is the molar surfactant concentration, and CMC is the critical micelle concentration of the respective surfactant.

The effect of the surfactant head-group for surfactants with C_{12} hydrophobic chain is presented in Figure 2. The solubilization capacity of all studied surfactants at pH = 6.5 is low, $\chi = 2$ to 6 mM/M, regardless of the type of hydrophilic head-group. In contrast, at pH = 3, the negatively charged sulfate groups have very high solubilization capacity. The addition of one ethylene oxide unit in between the sulfate head-group and the C_{12} alkane chain has no significant effect on drug solubilization, whereas the addition of three ethylene oxide units decreases substantially the

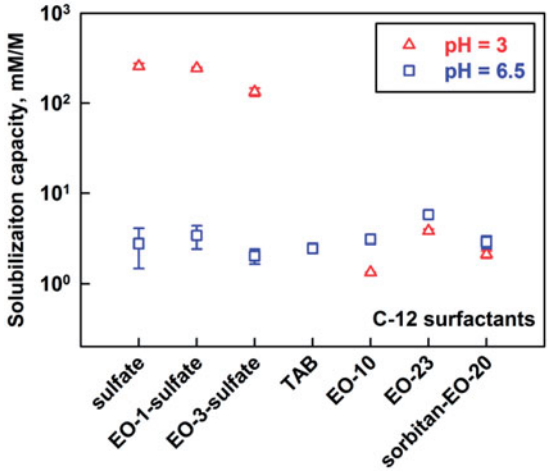


Figure 2. Albendazole solubilization capacity of the micelles of C_{12} chain-length surfactants, as a function of the type of hydrophilic head-group at pH = 3 (red triangles) and 6.5 (blue squares). The error bars can be smaller than the symbols.

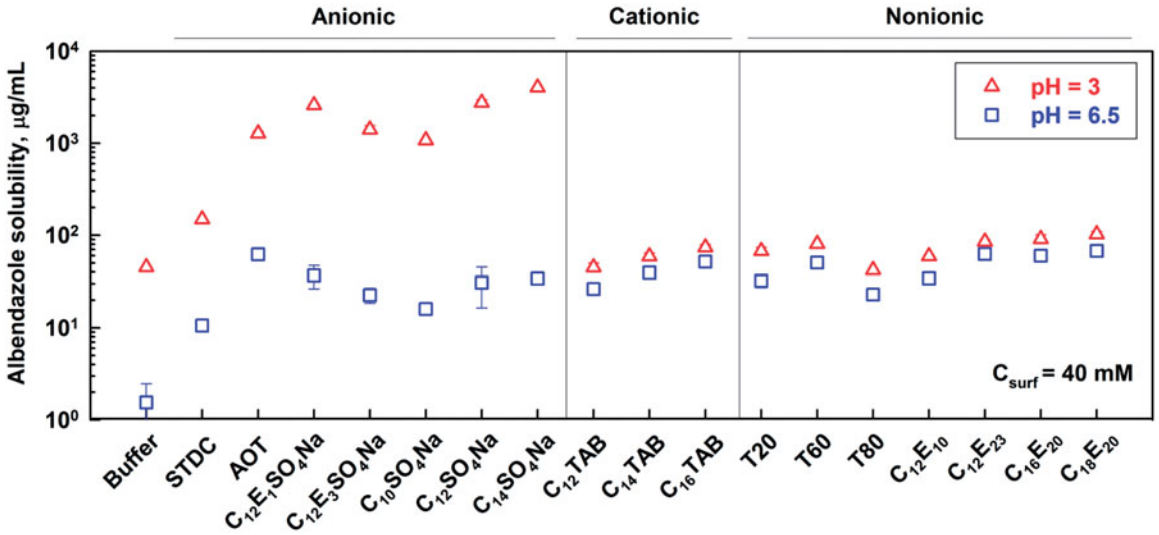


Figure 1. Albendazole solubility as a function of surfactant type at pH = 3 (red triangles) and 6.5 (blue squares). Surfactant concentration is 40 mM. The error bars can be smaller than the symbols.

solubilization capacity from 245 to 133 mM/M. Similarly to the results obtained at pH = 6.5, the positively charged TAB head-group and the non-ionic polyoxyethylene head-groups have low solubilization capacity at pH = 3, viz. $\chi \leq 4$ mM/M.

The effect of surfactant hydrophobic chain-length on albendazole solubilization capacity is determined by using homolog series of surfactants with the same head-group, see Figure 3. The increase of chain-length increases the solubilization capacity of charged (anionic and cationic) surfactants at both pH = 3 and 6.5. For the non-ionic surfactants, at both studied pH values, small increase in drug solubilization is observed when the chain-length is increased from 16 to 18 carbon atoms.

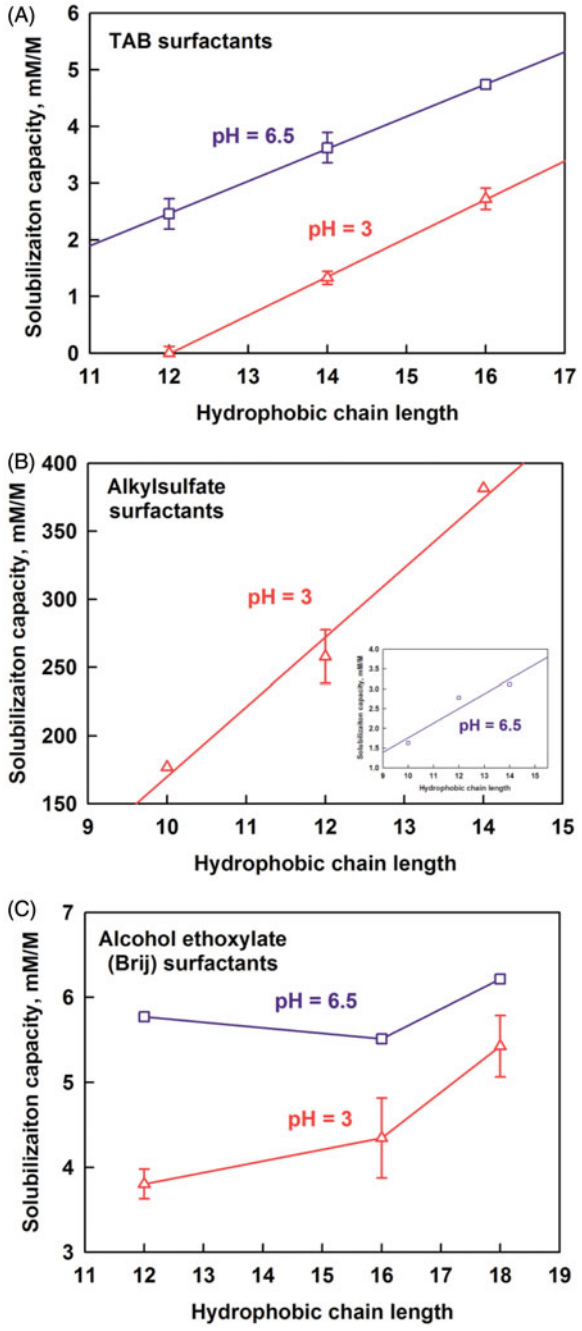


Figure 3. Albendazole solubilization capacity of the micelles of (A) TAB surfactants, (B) alkylsulfates, and (C) alcohol ethoxylates, as a function of surfactant chain-length at pH = 3 (red triangles) and 6.5 (blue squares). The error bars can be smaller than the symbols.

Phospholipid-surfactant mixtures: albendazole solubility, aggregate size, and dilution stability

In the previous section, we showed a dramatic increase of albendazole solubility in solutions of negatively charged surfactants at pH = 3. However, the surfactants with best effect (the alkylsulfates) have relatively high toxicity and are not appropriate for use as excipients in parenteral solutions. Thus, negatively charged amphiphiles with higher biocompatibility were identified and studied further with the aim to prepare albendazole solutions with low toxicity. The chosen materials were sodium dioctyl sulfosuccinate (AOT, sodium docusate) and a negatively charged phospholipid – sodium dipalmitoylphosphatidylglycerol (NaDPPG), both of which are approved for use in parenteral preparations [30]. Citrate buffer was used to keep pH = 3, which is required for sufficient albendazole ionization and solubilization, and is still in the range of pH values that are acceptable for parenteral administration [31,32].

The solubility of albendazole as a function of NaDPPG fraction, f_{DPPG}, in phospholipid-surfactant mixtures at constant molar concentration of 40 mM and pH = 3 is presented in Figure 4(A). Albendazole solubility passes through a maximum ($\approx$4400 μg/mL) at $f_{DPPG} = 0.4$, and decreases with further increase of NaDPPG in the mixture. Relatively high albendazole solubility (>1325 μg/mL) can be obtained at a NaDPPG fraction as high as 0.8, whereas NaDPPG alone dissolves $\approx$460 μg/mL albendazole.

The average size by volume of the phospholipid + surfactant aggregates is plotted in Figure 4(B), as a function of the fraction of NaDPPG in the surfactant-phospholipid mixtures. Interestingly, this dependence passes through a deep minimum: the big aggregates formed at $f_{DPPG} = 1$ and 0.8 ($d_H \approx 270$ and 460 nm, respectively) appear to be disrupted upon further increase of the AOT fraction in the mixture which manifests as a strong decrease of the aggregate size. Thus, a minimum value of the aggregate diameter, $d_H = 11$ nm, is reached at a NaDPPG fraction of 0.4. Further decrease of NaDPPG fraction increases the aggregate size: $d_H = 20$ nm is measured at NaDPPG fraction of 0.2, and the aggregates of pure AOT (NaDPPG fraction of 0) have $d_H \approx 70$ nm.

To understand better the mechanism of albendazole solubilization we determined the size of the empty surfactant-phospholipid aggregates at $f_{DPPG} = 0.4$, where a maximum in the solubility and a minimum in the aggregate size (in the presence of albendazole) was observed. We found that the empty AOT + NaDPPG aggregates are much bigger ($\approx$100 nm) than the drug-loaded aggregates (11 nm).

The dilution stability of the surfactant-phospholipid-albendazole solution was tested by its sequential addition in small aliquots to a model human serum solution of larger volume, see 'Experimental methods' section for more details. The investigated solution was composed of AOT + NaDPPG at a ratio of 6:4 and pH = 3. The onset of precipitation was detected by turbidity measurement at $\lambda = 600$ nm, see Figure 5. Significant increase in the solution turbidity was observed only after $\geq$165 μL of the albendazole solution was added to 2 ml of the model serum, which corresponded to $\leq$12 times dilution.

Discussion

Effect of surfactant structure on drug solubilization

Effect of hydrophilic head group

From the data presented in Figure 1, it can be calculated that the anionic surfactant micelles contain up to 21% albendazole molecules at pH = 3, which is a clear indication for formation of mixed surfactant + albendazole micelles. The latter hypothesis is confirmed also by the experimentally determined very high

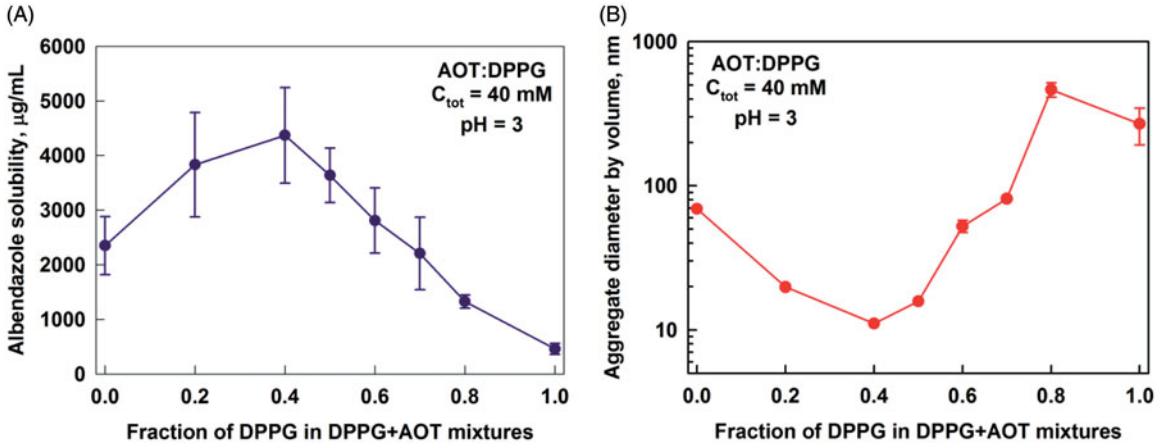


Figure 4. (A) Albendazole solubility and (B) mean hydrodynamic diameter by volume, d_H, as a function of the fraction of phospholipid in NaDPPG + AOT mixtures at total concentration of 40 mM and pH = 3 (50 mM citrate buffer). The error bars can be smaller than the symbols ($n \geq 2$).

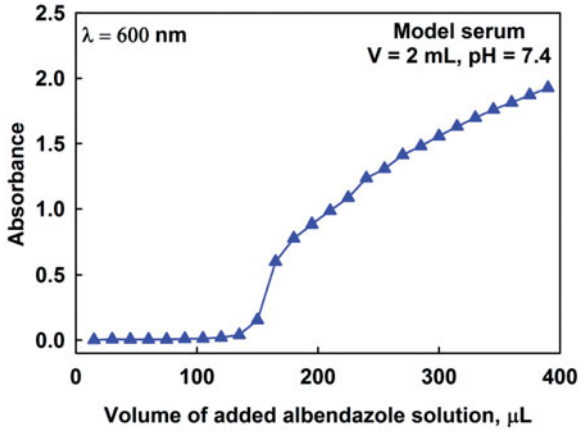


Figure 5. Absorbance at λ = 600 nm of model human serum solution, as a function of the volume of added albendazole solution. Albendazole was dissolved in 40 mM solution of 1:1 NaDPPG + AOT at pH = 3.

dissolution rate of albendazole at these conditions: 50% of the maximum drug solubility (corresponding to ≈ 1.4 mg/mL) in $C_{12}SO_4Na$ solution at pH = 3 is reached for 5 min only. Such very quick dissolution is typical when mixed micelles are formed, and was observed also with ibuprofen in the presence of anionic and non-ionic surfactants [33,34].

The incorporation of such high fraction of albendazole molecules in the anionic micelles is explained by the electrostatic attraction between the oppositely charged drug and surfactant molecules, as at pH = 3 the albendazole molecule is positively charged due to protonation of the nitrogen in the benzodiazole ring (see Supplementary information for the effect of pH on albendazole solubility). Strong electrostatic interactions between drug and surfactant pairs have been documented for a wide variety of ionizable drugs, such as ibuprofen [34], procaine [35], mefenamic acid and nimesulide [36], chlorpromazine [37], trifluoperazine [38], β-blockers (atenolol and nadolol), benzodiazepines (midazolam and nitrazepam) [39], and indomethacine [40]. However, much higher drug solubilization capacity of the oppositely charged micelles, compared to micelles of other surfactant, was measured only in the case of mefenamic acid and nimesulide [36]. In contrast, for ibuprofen and procaine the solubilization was in the same order of magnitude [34] or even lower [35]. Clearly, the solubilization capacity is determined not only by the drug–surfactant electrostatic and hydrophobic interactions, but also by the geometric restrains (packing of drug and surfactant molecules) in the mixed micelles.

As can be expected for such an electrostatics-driven effect, substantial increase in albendazole solubility was observed for all anionic surfactants studied, with different molecular structures. Classical surfactants with single chain (C_nSO_4Na, $C_{12}E_nSO_4Na$) and double chain (AOT) were shown to solubilize very efficiently albendazole at low pH (Figure 1). Very strong increase in solubility at low pH, compared to pH = 6.5, was observed also for the bile salt STDC.

The results presented in Figure 2 show that the addition of three ethylene oxide units in between the sulfate head-group and the dodecyl alkane chain decreases significantly the solubilization at both studied pH values. This effect is most likely due to steric hindrance in the $C_{12}E_3SO_4Na$ micelles – the ethylene oxide units are bulkier than the methylene groups, resulting in more difficult packing of the drug and surfactant molecules together in the micelles. The latter explanation is supported by the observation that the aggregation number of ethoxylated dodecyl sulfates decreases with increasing the number of ethylene oxide units [41], which clearly demonstrates that the surfactant packing in these micelles is more difficult.

Effect of hydrophobic chain length

The increased albendazole solubilization with increased surfactant hydrophobic chain-length (Figure 3) is a general effect, observed with a wide variety of drug molecules like fenofibrate and danazol [42], erythromycin [43], timobesone acetate [44], β-areether [45], and mefenamic acid [46]. For purely hydrophobic, simple molecules (e.g. alkanes), this effect is attributed to the increased volume of the micellar hydrophobic core [47], which increases the volume available for solubilization of the hydrophobic solute. However, at pH = 3 albendazole must be located in closer proximity to the micelle surface (*viz.* in the so-called 'palisade' layer), due to its charge and the resulting electrostatic attraction with the anionic surfactant head-group. At higher pH = 6.5, albendazole is unionized and could penetrate deeper into the micelle. However, it is unlikely that this drug molecule resides entirely in the anhydrous hydrophobic core of the micelles, due to the presence of hydrophilic groups in its structure. Thus, the effect of surfactant hydrophobic chain-length is most likely due to the increased volume of the micelle palisade layer which increases the space available for albendazole solubilization, as has been shown for other drugs, such as fenofibrate and danazol [42].

Albendazole solubility in NaDPPG + AOT mixtures

In Figure 4, it was shown that mixing of NaDPPG with AOT in the presence of oppositely charged albendazole decreases the aggregate size until small aggregates with diameter = 11 nm are obtained at $f_{DPPG} = 0.4$, which corresponds to the maximum of albendazole solubilization. Indeed, very good inverse correlation is obtained between albendazole solubility and the mean aggregate diameter in the surfactant-phospholipid mixtures (Supplementary Figure S4). The better incorporation of albendazole in aggregates with smaller size is a puzzling result, which could be explained only if the surface curvature of the aggregates is very important for drug solubilization. To check if this is the case, we plotted albendazole solubility as a function of the surface curvature (Figure 6). One observes a very good positive correlation between these two parameters which confirms the hypothesis that albendazole is solubilized preferentially in aggregates with larger surface curvature.

The obtained experimental results also showed that albendazole is crucial for the formation of small micellar aggregates ($d_H \approx 11$ nm) in the surfactant-phospholipid mixtures. In contrast, the size of the empty surfactant-phospholipid aggregates at $f_{DPPG} = 0.4$ was ≈ 100 nm. For conventional surfactants, the diameter of the spherical micelles can be approximated as twice the length of their extended-chain conformation. If we assume that NaDPPG determines the size of the mixed surfactant-phospholipid aggregates due to its longer chain length, the assumption for micellar shape would yield a micelle diameter of ≈ 6 nm. This value is more than an order of magnitude smaller than the experimentally determined aggregate diameter of ≈ 100 nm, which demonstrates that the aggregates cannot physically have a micellar structure. On the other hand, it has been reported that the aggregates of the individual NaDPPG and AOT are vesicles [48,49]. The latter observation shows that the critical packing parameter (CPP) of both AOT and NaDPPG is ≈ 1 and thus both amphiphiles are prone to formation of bilayer aggregates [50]. We can therefore conclude that the empty AOT + NaDPPG aggregates should be vesicles.

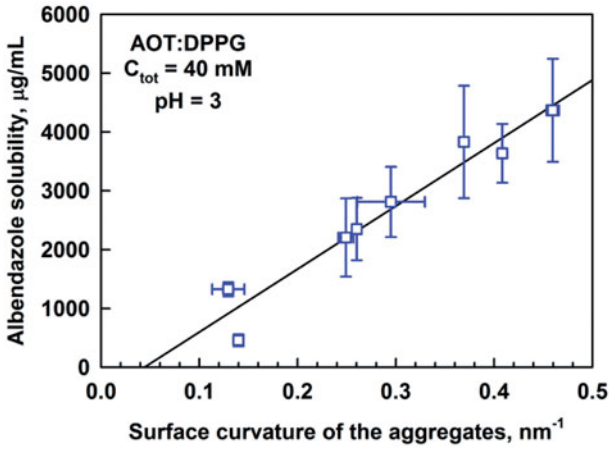


Figure 6. Correlation between albendazole solubility in NaDPPG + AOT mixtures and the surface curvature of the aggregates. The total amphiphile concentration is 40 mM and pH =3 (50 mM citrate buffer). The error bars are in both X- and Y-directions and can be smaller than the symbols ($n \geq 2$).

Most likely, the addition of small, oppositely charged albendazole molecules to the surfactant-phospholipid aggregates induces a transition from vesicles to micelles (see Figure 7 for schematic representation of the process), due to the larger surface curvature preferred by albendazole. Such transitions are commonly observed when small surface active molecules (*viz.* classical surfactants, bile salts) are introduced to dispersions of phospholipid liposomes, which is again explained with the different geometry of the molecules which perturb the lipid bilayer and induce a transition from liposomes to micelles with much larger surface curvature [51].

Dilution stability of the albendazole + NaDPPG + AOT formulation

One of the main challenges in *i.v.* administration of concentrated parenteral solutions is their precipitation stability upon dilution in the blood stream. In particular, the increase of pH from 3 to 7.4 during intravenous application of the formulation could be expected to deprotonate albendazole and thus induce its precipitation, which can lead to pain and phlebitis. The results presented in Figure 5 suggest that if the ratio between the blood flow rate and the *i.v.* infusion flow rate is higher than 12, albendazole should not precipitate in the point of injection. As the blood flow rate in the cephalic vein is 28 mL/min [52], it follows that the infusion rate should be <2.3 mL/min in order to avoid drug precipitation.

Summary

We studied the effect of 17 surfactants on albendazole solubility at pH = 3 and 6.5 which allowed us to reveal the link between surfactant molecular structure and albendazole solubilization. Based on these results, we identified the most promising candidates for formulation of albendazole solution suitable for parenteral application: a mixture of the biocompatible surfactant AOT and phospholipid NaDPPG. The solubility of albendazole, the aggregate size and the dilution stability of these surfactant-phospholipid mixtures were characterized. The main conclusions from the study can be summarized as follows:

- Albendazole solubility increases strongly in micellar solutions of negatively charged surfactants at pH = 3, where albendazole molecules are positively charged.

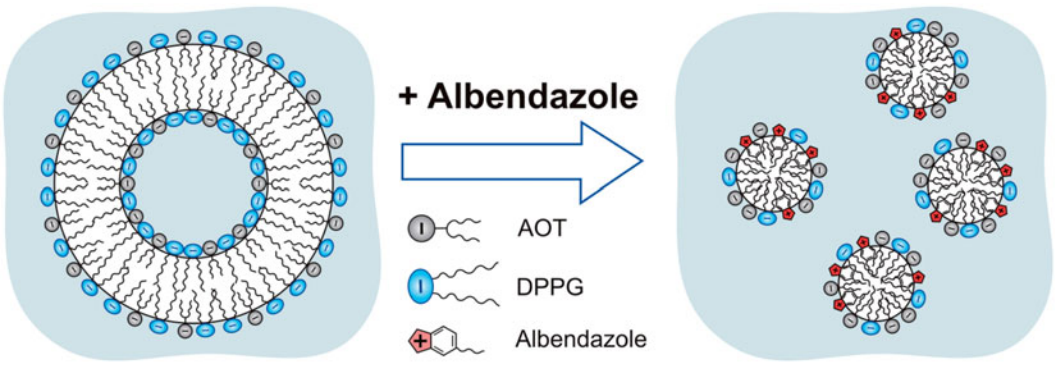


Figure 7. Schematic representation of the vesicle-to-micelle transition induced by albendazole solubilization in AOT + NaDPPG aggregates at pH = 3.

- Enhanced drug solubilization at pH = 3 is driven by electrostatic attraction between the oppositely charged surfactant and drug molecules, which results in the formation of mixed micelles that contain up to 21% albendazole molecules.
- The observed synergy between surfactant and pH is operative for negatively charged classical surfactants (alkylsulfates, ethoxylated alkyl sulfates), biosurfactants (sodium taurodeoxycholate), and surfactant-phospholipid mixtures (AOT + NaDPPG).
- Albendazole induced vesicle-to-micelle transition of the AOT + NaDPPG aggregates at NaDPPG fraction of 0.4 and pH = 3 (citrate buffer). As a result, mixed micelles loaded with clinically relevant drug concentrations of up to 4.4 mg/mL were obtained.
- Dilution tests in model human serum showed no precipitation at dilutions higher than 1:12. Such dilutions can be obtained by using standard *i.v.* infusion equipment at low infusion rate.

The presented study offers important insights on the mechanism of albendazole solubilization which could be used to develop theoretical models for prediction of drug solubility in surfactant solutions, as has been done in the area of microemulsions [53,54]. A new colloidal drug delivery system with high drug load, low toxicity of the materials used, and straightforward preparation procedure was developed. The major limitation of the proposed formulation is the acidic medium (pH = 3), which could induce local irritation upon parenteral administration. Further studies are required to explore the drug loading capacity and the *in vivo* antitumor properties of this promising system at physiological conditions.

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Disclosure statement

The authors report no declarations of interest.

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