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SOLUBILISATION OF (+)-USNIC ACID IN AQUEOUS MICELLAR SOLUTIONS OF GEMINI AND HETEROGEMINI SURFACTANTS AND THEIR EQUIMOLAR MIXTURE

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The solubilisation of natural compound, (+)-usnic acid, in micellar solutions of gemini (*N*,*N'*-didecyl-*N*,*N*,*N'*,*N'*-tetramethylethane-1,2-diyldiammonium dibromide) and heterogemini (decyl 2-[decyl(dimethyl)ammonio]ethylphosphate) surfactants and their equimolar mixture has been studied. The highest solubility was found for gemini surfactant. The relationship between synergism in surface properties of mixture of surfactants and their solubilisation properties is also discussed.

Keywords: *gemini surfactant – heterogemini surfactant – usnic acid – solubilisation –solubility in aqueous solutions*

INTRODUCTION

Usnic acid (Figure 1) is a phenolic compound. It is an optically active derivative of dibenzofurane and both enantiomers can be found in lichens. It is the constituent of many genera, e.g., *Usnea* (Cansaran et al., 2006, Lukáč, 2010), *Cladonia* (Ingólfsdóttir et al., 1998), *Ramalina* (Tay et al., 2004), or *Alectoria* (Einarsdóttir et al., 2010).

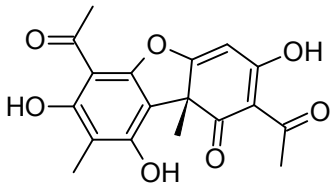


Figure 1 (+)-Usnic acid

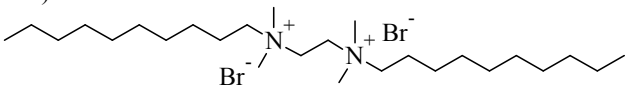
This lichen acid has a wide spectrum of biological activities. It possesses antibacterial, antifungal, antimycobacterial (Ingólfsdóttir, 2002, Melgarejo et al., 2008, Ranković et al., 2008), antiparasitic (Cetin et al., 2008), and antineoplastic activities (Bačkorová et al., 2011, Bazin et al., 2008, Einarsdóttir et al., 2010).

The disadvantage of usnic acid is in its low solubility in aqueous solutions and it complicates its investigations in these media, e.g., microbiological testing. The micellar solubilisation with surfactants, cosolvents or complexation between usnic acid and cyclodextrines was used to increase lichen acid solubility in water solutions (Kristmundsdóttir et al., 2002, 2005; Lira et al., 2009; Segure-Sanchez et al, 2009).

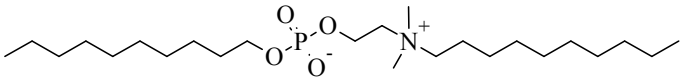
The second disadvantage of usnic acid is its hepatotoxicity. Encapsulation of usnic acid can resolve the problems of hepatotoxicity and also of low solubility in water. Encapsulation of usnic acid into nanocapsules prepared from lactic co-glycolic acid polymer improved antitumour activity of this lichen acid and considerably reduced its hepatotoxicity. Moreover, the prepared suspensions of encapsulated usnic acid in aqueous environments are stable for several months (da Silva Santos et al., 2006).

Surfactants are very often used for solubilisation of sparingly soluble compounds. They can form micelles in aqueous solution and compounds can be solubilised in different parts of the aggregate. Hydrophilic compounds can be adsorbed on the surface of the micelle; compounds with intermediate hydrophilic properties are located in the palisade layer between the hydrophilic groups and the first few carbon atoms of the non-polar groups; and hydrophobic compounds are situated in the inner core of the micelle (Rangel-Yagui et al., 2005). Different types of surfactants can be used in solubilisation of sparingly soluble compounds. They can be represented by anionic, cationic, zwitterionic or nonionic surfactants (Ullah et al., 2012).

The aim of this study was the investigation of the influence of a bisammonium salt, dialkylphosphocholine, and their equimolar mixture on the solubilisation of usnic acid in aqueous solutions. The bisammonium salt and dialkylphosphocholine (Figure 2) were chosen as representative dimeric surfactants. The bisammonium salt represents a cationic surfactant and dialkylphosphocholine represents a zwitterionic surfactant. This study is an extension of our previous work where we investigated the influence of these two surfactants on solubilisation of griseofulvin and rutin in aqueous solutions (Lukáč et al., 2011).



N,*N*′-didecyl-*N*,*N*,*N*′,*N*′-tetramethylethane-1,2-diyldiammonium dibromide (***N***)



decyl [2-decyl(dimethyl)ammonio]ethylphosphate (***P***)

Figure 2 Structure of solubilisers

MATERIALS AND METHODS

Surfactants

Decyl 2-[decyl(dimethyl)ammonio]ethylphosphate (***P***) and *N*,*N'*-didecyl-*N*,*N*,*N'*,*N'*-tetramethylethane-1,2-diyldiammonium dibromide (***N***) were prepared according to the described procedure (Lukáč et al., 2009).

Usnic acid

Usnic acid was isolated from *Usnea filipendula* according to the modified procedure described by Ingólfsdóttir et al. (1998). The specimens were collected in Veporské Vrchy Mts., Slovakia (20° 06' 05'' E, 49° 13' 10'' N). The samples were identified according to the Key to European *Usnea* species (Randlane et al., 2009) and were confirmed by other literature (Halonen et al., 1998; Matteucci et al., 2006; Tõrra & Randlane, 2007).

Usnic acid was extracted in a Soxhlet apparatus. About 57 g of thallus were grounded and extracted with 750 ml of diethyl ether within 5 h. After extraction, the solution was evaporated and the crude usnic acid was crystallized from absolute ethanol. Usnic acid obtained was 503 mg.

(+)-Usnic acid, (9b*R*)-2,6-diacetyl-3,8-dihydroxy-7,9,9b-trimethyl-3,9b-dihydro-dibenzo[*b*,*d*]furan-1(2*H*)-one: m.p. = 203 – 205°C, $[\alpha]_{20}^{D}$ = + 491.5° ($CHCl_3$; c = 0.4), ^{1}H NMR ($CDCl_3$) 1.76 (s, 3H), 2.11 (s, 3H), 2.67 (s, 3H), 2.68 (s, 3H), 5.99 (s, 1H), 11.02 (s, 1H), 13.30 (s, 1H), 18.82 (s, 1H)

Solubilisation

Saturated solutions were prepared in 20 ml vials by mixing excess powdered usnic acid (2 mg) with 2.5 ml of deionised water or surfactant solution with a concentration c = 0.01 mol·dm^{-3} and stirring (250 rpm) at a constant temperature t = 25 ± 1 °C for 72 hours before filtering (Millipore, 0.22 m) to remove unsolubilised usnic acid. The extent of dissolution was determined by UV-spectroscopy. The filtered solution (1 ml) was diluted quantitatively with methanol in a 25 ml volumetric flask. Absorbance was measured at the optimum wavelength, 284 nm, which was then compared with the appropriate Beer's law plot for the drug in methanol. Water content in the measured solution was low enough to allow the calibration with methanol solutions to be used without correction. Measurements were performed in triplicate and the results were averaged. Standard deviations were also calculated; considering all sources of error, we estimate a maximum uncertainty in *s* (solubility) of 10%.

RESULTS AND DISCUSSION

Usnic acid was extracted from *Usnea filipendula*. The lichen substance was isolated as an *R*-enantiomer. Usnic acid obtained was 503 mg from 57 g of thallus and it represents 0.88 % of the dry lichen weight. Cansaran et al. (2006) reported similar amount of usnic acid in *U. hirta* (0.68 %) or *U. longissima* (1.12%).

Solubilities of usnic acid measured for deionised water, the surfactants solutions, ***P*** and ***N***, and their mixture, ***P***/***N***, are depicted in Figure 3. The quantity *s* represents the total compound solubility in micrograms of compound solubilised per millilitre of solution. The solubility value of usnic acid in water (s_{water} = 3.0 μg/ml) was similar as that determined previously, 5 μg/ml (Kristmundsdóttir et al., 2005).

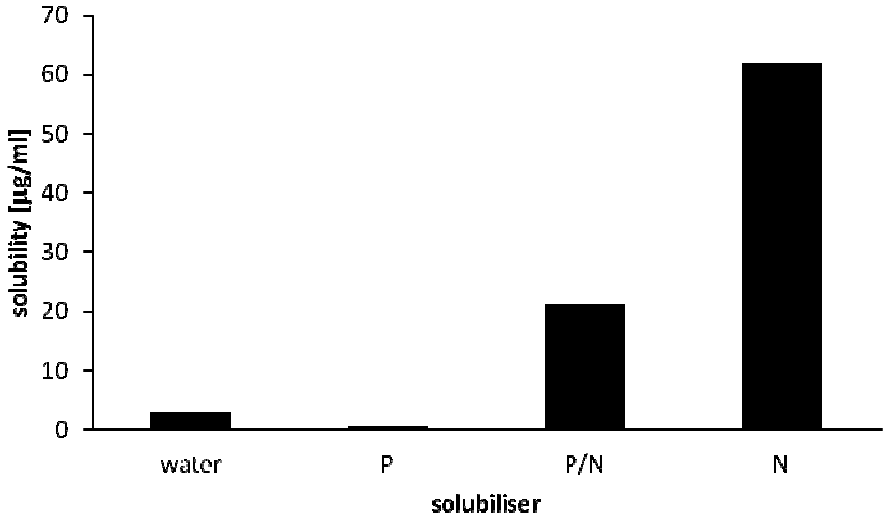


Figure 3 Solubility of usnic acid in water and aqueous micellar solutions

The influence of ***P***, ***N*** and ***P***/***N*** was studied on solubilisation of lichen acid. ***P*** and ***N*** are compounds with similar chemical structure. Both compounds are gemini surfactants with two alkyl chains, which contain 10 carbon atoms, and with a spacer, whose length is 2 methylene groups. ***N*** is a bisammonium salt, a true gemini surfactant because it is symmetrical and contains two cationic polar head groups. ***P*** differs from ***N***. One cationic polar head group is replaced with phosphate, an anionic polar head group. ***P*** is a heterogemini surfactant.

N improves the total solubility of usnic acid in the solution (s_N = 61.7 μg/ml). A different situation was observed in the case of ***P*** (s_P = 0.6 μg/ml). The solubility of usnic acid in ***P*** solution was decreased in comparison to solubility in water. The possible reason of lower solubility of compound in ***P*** than in deionised water consists in the character of the aggregates of ***P*** formed in solution. ***P*** forms coacervates and vesicles besides micelles in aqueous solutions (Lukáč et al., 2011, Menger & Peresypkin, 2001). The interaction between usnic acid and the coacervates/vesicles caused precipitation of ***P*** and the compounds were "glued" together. Solubility of usnic acid in ***P***/***N*** is between the solubility in ***P*** and ***N*** ($s_{P/N}$ = 21.0 μg/ml). The synergisms between equimolar mixture of ***P*** an ***N*** were not observed in surface tension reduction efficiency, surface tension reduction effectiveness, and mixed micelle formation (Lukáč et al., 2011). In addition no synergisms were observed in solubilisation properties

of ***P***/***N*** on solubilisation of usnic acid; *s* of lichen acid in solution of ***P***/***N*** is lower than $s_N+s_P/2$.
The influences of ***P***, ***N*** and their mixtures were also previously studied on solubilisation of rutin and griseofulvin (Lukáč et al., 2011). Some similarities between griseofulvin, rutin and usnic acid were observed. The compounds caused very low solubility in solutions of ***P***. The reason was explained previously. ***N*** increased the solubility of all compounds (usnic acid, griseofulvin and rutin). The difference is in the solubilisation influence of ***P***/***N*** on solubilisation of the studied compounds. The solubility of usnic acid in ***P***/***N*** is between its solubility in ***P*** and ***N***. However, griseofulvin and rutin were more soluble in ***P***/***N*** than in ***P*** or ***N*** alone.
One has to take into account that the concentration of surfactants is well above their critical micelle concentrations ($cmc_N = 4.8 \times 10^{-3}$ mol·dm^{-3}; $cmc_P = 2.2 \times 10^{5}$ mol·dm^{-3} (Lukáč et al., 2011)), so changes in the shape and structure of the micelles could play a distinctive role in the solubilisation of usnic acid.

CONCLUSION

In conclusion we can say that micellar solution of ***N*** and ***P/N*** increased the solubility of usnic acid in its aqueous solution in comparison with its solubility in deionised water. The solubility of lichen acid was more than 20 times higher in ***N*** and 7 times higher in ***P/N*** than the solubility of lichen acid in water. The solubility of usnic acid in an aqueous solution of ***P*** was decreased in comparison with the pure solvent.

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SOLUBILIZÁCIA KYSELINY (+)-USNOVEJ VODNÝMI MICELÁRNYMI ROZTOKMI GEMINI A HETEROGEMINI TENZIDOV A ICH EKVIMOLÁRNEJ ZMESI

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Bola študovaná solubilizácia prírodnej zlúčeniny, kyseliny (+)-usnovej, v micelárnych roztokoch gemini (*N,N'*-didecyl-*N,N,N',N'*-tetrametyletán-1,2-diyldiamóniumdibromid) a heterogemini (decyl 2-[decyl(dimetyl)ammónio]etylfosfát) tenzidov a ich ekvimolárnej zmesi. Najvyššia rozpustnosť bola zaznamenaná v prípade gemini tenzidu. Diskutuje sa aj vzťah medzi synergizmom povrchových vlastností zmesi tenzidov a ich solubilizačnými vlastnosťami.

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