Solubility Study of *Azadirachta indica* A. Juss. (Neem) Seed Oil in the Presence of Cosolvent/Nonionic Surfactant at (298.15, 303.15, 308.15, and 313.15) K

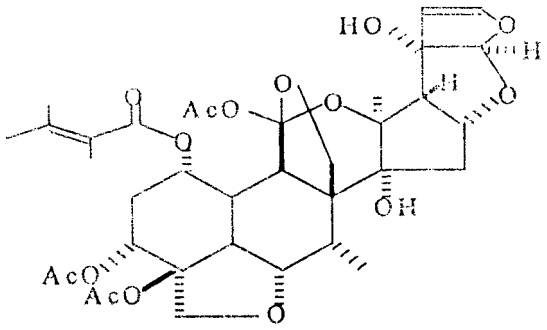
Anandrao R. Kulkarni, Kumaresh S. Soppimath, and Tejraj M. Aminabhavi*

Department of Chemistry, Karnatak University, Dharwad, 580 003 India

Solubility data of *Azadirachta indica* A. Juss. (neem) seed oil (NSO) in aqueous mixtures of methanol or polysorbate-80 obtained from UV spectral measurements are reported at (298.15, 303.15, 308.15, and 313.15) K. Increased solubility of NSO was observed with an increasing amount of methanol in water at all temperatures. However, for the polysorbate-80 + water system, the increase in solubility was significant only at lower concentrations, i.e., up to 0.1 mass %, but at higher concentrations of polysorbate-80, the increase in NSO solubility was not significant.

Introduction

Solubility data of bioactive molecules have wide ranging applications in pharmaceutical and agricultural industries (Dave et al., 1998; Pino-Garcia and Rasmuson, 1998; Dubbs and Gupta, 1998). To crystallize the bioactive molecules in solution, a variety of solvents and mixed aqueous solvents have been employed (Grant and Higuchi, 1990). *Azadirachta indica* A. Juss (commonly known as neem tree) seeds can be extracted to provide neem seed oil (NSO), a somewhat less toxic pesticide that can be handled without producing any hazardous effects. This NSO contains azadirachtin (see structure) as a major component, which is



Azadirachtin

hydrophobic. Commercially, NSO finds applications as a liquid spray to control pests such as aphids, jassids, thrips, mites, and helopelties. Solubility data of NSO in aqueous mixtures containing either organic cosolvent or surfactant are not available in the literature. Such a database has applications in assay procedures and in the development of controlled release (CR) devices (Aminabhavi et al., 1999). In an earlier work (Aminabhavi et al., 1998), we studied the dissolution and the release kinetics of NSO in a variety of solvent mixtures. These results showed an influence on the nature of the medium used. Hence, solubility data of NSO in such media are important in the development of the CR formulations. Therefore, in this paper, the solubility results of NSO in binary aqueous mixtures of methanol + water and polysorbate-80 (i.e., Tween-80) + water were measured from UV spectral studies at (298.15, 303.15, 308.15, and 313.15) K.

* To whom correspondence should be addressed. Fax: 011-91-836-747884. E-mail: rrist@bgl.vsnl.net.in.

Experimental Section

An 80 mass % pure technical grade NSO (calculated by considering the azadirachtin concentration in NSO) was procured from Mangalwadkar Industries, Bijapur, India. Methanol (HPLC grade) and polysorbate-80 (Tween-80) were purchased from s.d. Fine Chemicals, Mumbai, India. Double-distilled water was used and its purity was checked by comparing its density and conductivity at 25 °C with the literature values, which agreed well.

Methanol + water mixtures were prepared by mixing the known volumes of methanol and water in a 100 mL volumetric flask. The amount of methanol transferred was calculated by subtracting the empty mass of the flask from the total mass of volumetric flask + methanol. The polysorbate-80 + water mixtures were prepared by mass, i.e., by adding the calculated mass of polysorbate-80 in 100 mL of water. The mass measurements were taken on a single pan Mettler microbalance (model AE 240, Switzerland) within the accuracy of ±0.01 mg. A total of six compositions were used for mixing with NSO. In each of these flasks, an excess amount (~5 mL) of NSO was added to ensure maximum solubility.The mixtures were shaken thoroughly for 5 min at each temperature and then allowed to stand for 24 h to attain equilibrium as well as to separate the undissolved NSO droplets completely. The flasks were immersed in a stirred circulation constant temperature water bath (Grants, model Y14, UK), the temperature of which was maintained at (298.15, 303.15, 308.15, and 313.15) K within the accuracy of ±0.1 °C at the desired temperature.

A 10 mL aliquot of the mixtures was removed from the aqueous layer, diluted with the same system, and then absorbance was measured at 214 nm (λ_{max} of azadirachtin in both alcohol and surfactant solution is between 212 and 215 nm depending upon the concentration of NSO) using a UV spectrophotometer (Anthelie, Secomam, France). The λ_{max} of NSO in methanol + water and polysorbate-80 + water mixtures varied according to the concentration of NSO in the solution. The λ_{max} value was approximately 214

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Table 1. Solubility of NSO in Methanol + Water Mixture at Different Temperatures

mass % of methanol	solubility of NSO (μg/mL)			
	298.15 K	303.15 K	308.15 K	313.15 K
20	186.17 ± 0.11	221.38 ± 0.17	225.63 ± 0.11	247.34 ± 0.03
40	279.25 ± 0.13	299.78 ± 0.17	320.95 ± 0.18	388.29 ± 0.10
60	305.85 ± 0.31	338.61 ± 0.23	427.76 ± 0.18	561.17 ± 0.08
80	348.40 ± 0.08	572.02 ± 0.10	733.08 ± 0.71	870.95 ± 0.31
100	398.93 ± 0.11	687.65 ± 0.01	1013.3 ± 0.03	1220.7 ± 0.01

Table 2. Solubility of NSO in Polysorbate-80 + Water Solutions at Different Temperatures

mass % of polysorbate-80	solubility of NSO (μg/mL)			
	298.15 K	303.15 K	308.15 K	313.15 K
0.01	12.76 ± 0.01	35.21 ± 0.17	57.02 ± 0.13	134.04 ± 0.10
0.05	66.80 ± 0.11	96.17 ± 0.13	135.21 ± 0.20	260.63 ± 0.03
0.10	111.7 ± 0.18	192.55 ± 0.08	245.74 ± 0.11	372.34 ± 0.31
0.15	119.14 ± 0.03	202.12 ± 0.30	301.06 ± 0.03	410.63 ± 0.08
0.20	125.53 ± 0.11	223.40 ± 0.07	310.70 ± 0.03	418.08 ± 0.01
0.25	132.23 ± 0.01	233.10 ± 0.17	315.03 ± 0.13	422.31 ± 0.11

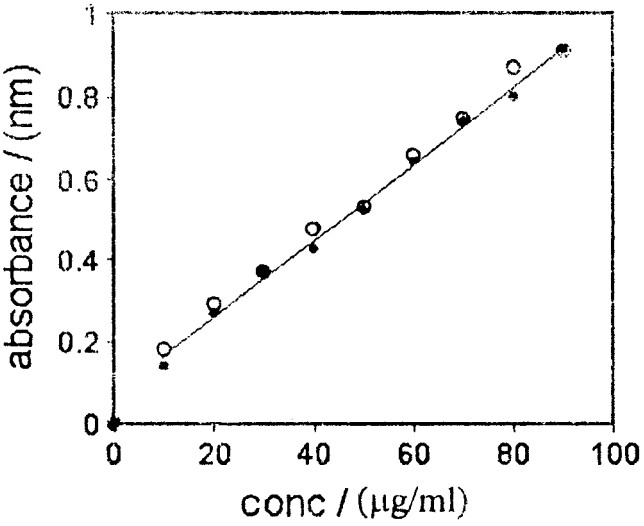

Figure 1. Standard curve of NSO in (●) methanol and (○) 0.01% polysorbate-80 solution.

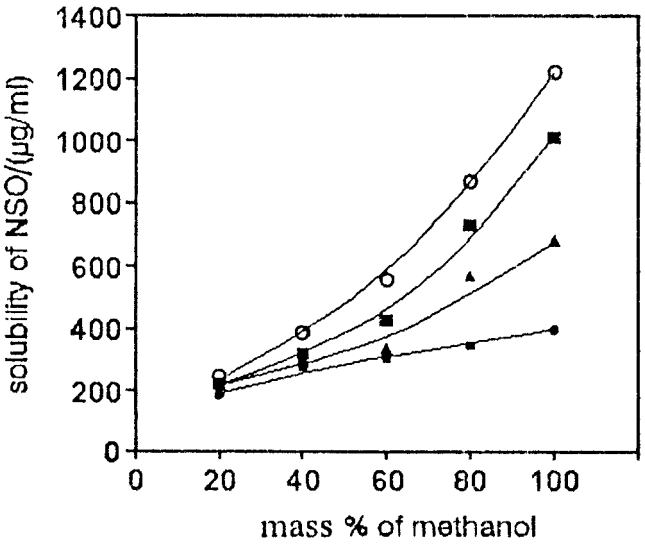

Figure 2. Solubility of NSO in methanol + water mixture at (●) 298.15 K, (▲) 303.15 K, (■) 308.15 K, and (○) 313.15 K.

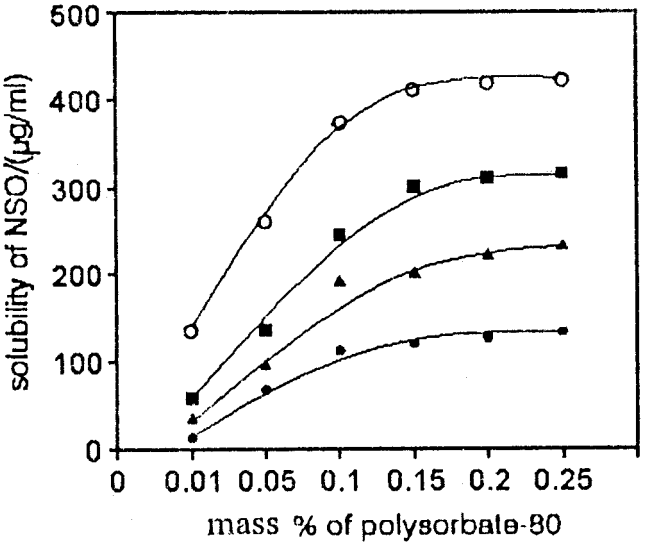

Figure 3. Solubility of NSO in polysorbate-80 + water mixture at (●) 298.15 K, (▲) 303.15 K, (■) 308.15 K, and (○) 313.15 K.

nm at the NSO concentrations of 10−100 μg/mL. The standard curve for NSO was taken in both solutions (see Figure 1). From the slope of the line, the solubility of NSO present in the binary mixture was calculated.

Results and Discussion

The standard curve for NSO was obtained in both pure methanol and in 0.01 mass % of polysorbate-80 solution, and the absorbance data of NSO with respect to its concentration remained similar to that shown in Figure 1. The selection of polysorbate-80 concentration is made according to its capacity to dissolve NSO, i.e., 10−100 μg of NSO could be easily dissolved in the selected polysorbate-80 concentration range. However, even if the higher concentration of polysorbate-80 was used, it did not affect the calibration data because the same solution was used as a blank without NSO. The Beer−Lambert law was obeyed for the range of NSO concentrations studied and slope of the line was 0.0094. The solubility results of NSO in water + methanol and polysorbate-80 + water solutions at different temperatures are presented in Figures 2 and 3, respectively. Experiments were conducted in triplicate, but only the average values are presented along with the standard deviations in Tables 1 and 2.

As shown in Figure 2, the solubility of NSO increased with an increasing amount of methanol in methanol + water mixtures at all temperatures. However, the increase in NSO solubility was greater at higher temperatures (i.e., at (308.15 and 313.15) K) than at lower temperatures. On the other hand, as shown in Figure 3, the increase in solubility of NSO with the concentration of polysorbate-80

in water as well as with temperature is lower when compared to the methanol + water mixture. The solubility of NSO reached an optimum value at about 0.1 mass % of polysorbate-80, beyond which no considerable increase in solubility was observed.

NSO is a hydrophobic molecule which results in its poor solubility in water. With an increment of 20 mass % of methanol in the mixture, its solubility increases nearly by 2-fold. For polysorbate-80, with an addition of 0.05 mass % of polysorbate-80, the solubility of NSO increases nearly by 5-fold. However, with higher concentrations of polysorbate-80, i.e., from 0.15 to 0.25 mass %, the NSO solubility did not increase further, probably because of the crossover of the critical micellar concentration of the polysorbate-80 surfactant.

Conclusions

The focus of the present research was to study the improvement in NSO solubility by the addition of either methanol or polysorbate-80 in water. Solubility data are presented for NSO in two binary aqueous mixtures containing methanol or polysorbate-80 at four different temperatures and under varying amounts of methanol or polysorbate-80. The solubility of NSO in aqueous polysorbate-80 solution is much lower than that observed for the methanol + water mixture. Solubility enhancement with temperature is also much higher in the latter mixture.

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