Evidence by Gel Filtration for Solubilization of NAA by Nonionic Surfactant Micelles

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Abstract. Micelles of two nonionic surfactants (Triton X-114 and Neodol 91) were shown by gel filtration chromatography to solubilize nondissociated NAA molecules in aqueous solutions. Micelle solubilization of nonpolar active ingredients in aqueous spray systems alters the distribution of the chemical in the spray solution and may influence chemical deposit formation and penetration characteristics. Chemical names used: 2-(1-naphthyl)acetic acid (NAA), octylphenoxy polyethoxylate-7.5 POE (Triton X-114), linear alcohol (C₉₋₁₁) polyethoxylate-6 POE (Neodol 91).

Surfactants are frequently used in formulation and spray application of plant growth regulators to improve efficacy (Behrens, 1964; Parr and Norman, 1965). Surfactants may improve spray performance by stabilizing emulsions and suspensions, by increasing wetting and retention of the spray solution by plant surfaces, and by enhancing penetration of the active ingredient into plant tissues. However, surfactants do not always enhance foliar absorption of an applied compound (Jansen et al., 1961; Temple and Hilton, 1963).

Direct measurement of sorption of NAA by isolated cuticles, an early event in the penetration process, has shown that surfactant concentrations above the critical micelle concentration (CMC) depress sorption (Shafer and Bukovac, 1988, 1989). One explanation for this effect is that the surfactant micelles solubilize (entrap) the NAA molecules present in the bulk solution, thereby reducing the effective NAA concentration. This effect occurs only at surfactant concentrations above the CMC and increases with an increase in the micelle population of the solution (Florence and Gillan, 1975; Shafer and Bukovac, 1989). The objective of this study was to investigate surfactant micelle affinity for NAA in aqueous solutions (e.g., a spray solution) using gel filtration chromatography.

In fact, if NAA is solubilized by surfactant micelles, it should be eluted from the gel (along with micelles) at a lower elution

volume when the concentration of the surfactant solution is above the CMC (surfactant in micellar form plus constant level of monomers) than when the concentration is below the CMC. At surfactant concentrations below the CMC (surfactant molecules in monomer form), NAA should elute at a volume similar as without surfactant, unless there is interaction between the NAA and the surfactant monomer.

We adopted the following procedures for detection of the surfactant and NAA. The fractions containing the surfactants were identified using a colorimetric assay (Weber et al., 1964) employing an ammonium thiocyanate/cobalt reaction. The absorption of the reaction product was measured at 620 nm. This reaction proceeds only at surfactant concentrations above the CMC and thus establishes the presence of micelles; NAA at 10⁻⁴ M does not interfere with the assay.

NAA was detected in the fractions by fluorometry after extraction with ethyl acetate. Two milliliters of 5% sodium bicarbonate solution was added to each fraction (≈1.5 ml), and the fraction was shaken for 2 min with 3 ml of ethyl acetate. The organic phase contained the surfactant molecules and the basic aqueous phase the NAA. The presence of NAA was determined by measuring the fluorescence intensity of the aqueous solution (excitation wavelength 280 nm, emission wavelength 327 nm). The accuracy of the analytical procedure is documented in Table 1.

Gel filtration was carried out at 23 ± 1°C on a Sephadex G-25 (Pharmacia Fine Chemicals, Piscataway, N.J.) column (1.6 cm in diameter, bed height 26 cm). A void volume of 40.5 ml was determined using blue dextran. NAA (0.5 ml 1.0 × 10⁻⁴ M in citrate buffer at pH 3.2) was loaded onto the column then eluted with citrate buffer (0.02 M, pH 3.2) or citrate buffer containing Triton X-114 (TX-114) surfactant (Rohm and Haas, Philadelphia) at a concentration below [0.008% (w/v)] or above [1.0% (w/v)] the CMC. The surfactant molecules were present in the eluant below the CMC as monomers and above the CMC as a mixture of monomers and micelles (hereafter referred to as micelles or micellar form). Related studies (Shafer and Bukovac, 1989) established that interaction occurred only between micelles and the nondissociated NAA molecules. The CMC values for octylphenoxy polyethoxylate-9.5 POE (Triton X-100, TX-100), TX-114, and Neodol 91 are 0.019%, 0.009%, and 0.025%, respectively.

When citrate buffer was used as the eluant, NAA was eluted in fractions 90 to 98, representing an elution volume of ≈140 ml (Fig. 1). The large elution volume indicates a strong affinity of the gel for NAA. When the column was eluted with citrate buffer containing the monomeric form of the surfactant (0.008%), a similar elution profile was obtained as with citrate buffer alone (Fig. 1), indicating that no significant interaction occurred between the NAA molecules and the surfactant monomers and that the latter did not increase the NAA-solubilizing capacity of the buffer. However, the elution volume (≈68 ml, fractions 35 to 47) was dramatically reduced when TX-114 molecules were present in the eluate in the micellar form, i.e., at a concentration (1.0%) above the CMC (Fig. 1). Further, the TX-114 surfactant micelles without NAA eluted in these same fractions (35 to 42). These data provide evidence that the NAA was readily desorbed from the column by the micelle-rich surfactant solution and that the micelles have a high affinity (solubilizing) for NAA.

The slight difference in elution volume between the surfactant micelles with (≈68 ml) and without (≈58 ml) NAA can be readily explained by the effect of the solubilized solute (NAA) on micelle size and/or shape (Kano et al., 1985). Working with the surfactant TX-100, Kano et al. (1985) showed that micelle size was reduced by ≈50% on solubilizing aniline derivatives, and a reduc-

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Table 1. Accuracy of the analytical procedure used to determine NAA.

Actual concn (ng/ml)	n ²	Concn found (ng·ml ⁻¹)	SD	CV (%)
<i>In aqueous solution</i>				
43.40	7	44.09	0.50	1.13
<i>In aqueous solution after extraction with ethyl acetate³</i>				
43.40	9	41.55	2.35	5.66

²Number of samples.

³Tests were made using TX-100 [0.1% (w/v)] surfactant solutions containing NAA at the concentration indicated above.

Fig. 1. NAA elution profiles from a Sephadex G-25 column using citrate buffer (0.02 M, pH 3.2) alone or with TX-114 at below [0.008% (w/v), surfactant in monomer form] or above [1.0% (w/v), micelle-rich solution] the CMC.

tion in micelle size would be expected to increase elution volume. A similar shift in elution volume following micelle solubilization of a solute has been observed with other surfactant systems (Herries et al., 1964).

Further evidence was obtained that surfactant micelles solubilize NAA by following the elution pattern of buffered NAA (pH 3.0) from a Sephadex G-25 column with Neodol 91 (Shell Development Co., Houston) surfactant at concentrations below [0.005% (w/v)] and above [1.0% (w/v)] its CMC. The elution profile of NAA was established by measuring UV absorbance (280 nm). Again, the elution volume was dramatically lower for the micelle- than for the monomer-containing surfactant solution, being 67 ml vs. 135 ml, respectively.

Convincing evidence for NAA solubilization by micelles was obtained by observing a UV spectral shift (280 to 282.5 nm) for NAA in the eluate containing the Neodol 91 micelles (data not presented). No such shift was found

in the presence of monomers. Such a red spectral shift is generally interpreted as confirmation of molecular association between micelles and a micelle-soluble molecule (Cardinal and Mukerjee, 1978). Thus, our data show conclusively that there is a strong association between the micelles of these two surfactants and the nondissociated NAA molecule.

Our findings have practical implications. For example, in spray application systems, nonpolar active ingredients should be solubilized by micelles of a variety of commonly used micelle-forming surfactants when added at concentrations above their CMCs. This may affect deposit formation on plant surfaces and/or alter penetration characteristics of the active ingredient. Similarly, in laboratory studies where one is following sorption or penetration from aqueous systems, if micelles solubilize the active ingredient, the concentration will decrease in the intermicellar solution and the driving force for sorption/penetration will decrease accordingly.

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