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Purification of Xylenol Orange by the High-Speed Liquid Chromatography

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Synopsis. A relatively large amount of 3,3′-bis[*N*, *N*-bis(carboxymethyl)aminomethyl]-*o*-cresolsulfonphthalein (Xylenol Orange, XO) is purified easily by the high-speed liquid chromatography (HSLC).

Despite of many interesting problems concerning the solution chemistry of metal complexes of a multidentate ligand XO, serious discrepancies have often been noted among the data reported by different authors. The discrepancies are primarily caused by the use of commercial or incompletely purified XO, which usually contains iminodiacetic acid (IDA), Semi-Xylenol Orange (SXO), and/or *o*-Cresol Red (*o*-CR) as the starting materials or a by-product of the synthesis of XO.[1–4] Since SXO and IDA also form metal complexes, the use of XO free from these impurities is essential for the quantitative studies of metal complex formation. Among the impurities *o*-CR is easy to remove, but the separation of IDA and SXO from XO is quite difficult.[3] Recently, a new method was established for the sharp separation of SXO and XO by the preparative thin-layer chromatography.[3] However, the method for easy separation of XO and IDA on a preparative scale has not been reported so far. In the present study we applied a technique of preparative HSLC and obtained easily a relatively large amount of pure XO.

The sample for HSLC was preliminarily purified by the cellulose column chromatography using an eluent, 1-butanol saturated with 10 or 25% aqueous acetic acid.[2,5] The paper partition chromatography (PPC) of the pretreated sample gave no spots of SXO and *o*-CR, but IDA still remained unremoved.

Figure 1 shows a typical elution curve of the preparative HSLC for the pre-purified sample. One gram of the sample was dissolved in 5 ml of water. Six hundred microliters of the solution were injected for each run. For the elution 5% aqueous acetic acid–methanol (80/20, v/v) was used as a solvent at a flow rate of 6.0 ml min^{-1} (pressure: 2400 psi). XO was clearly separated from the impurities on a preparative scale. The retention time assigned for IDA, XO, and SXO was measured beforehand with corresponding pure substances. Even a trace amount of SXO undetected by PPC was sharply discriminated as a peak of the preparative HSLC. The peak of *o*-CR, if any, is expected to appear at a retention time longer than 2 h under this condition.* The component for a peak at the retention time of 10 min was yellow and unidentified. After the elution of SXO the column was regenerated by washing with methanol for 20 min. This procedure flushed out the unknown yellow material. The column thus regenerated was used repeatedly.

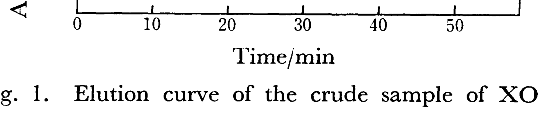
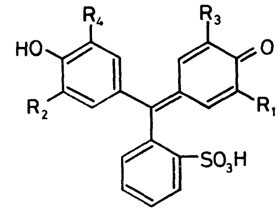


Fig. 1. Elution curve of the crude sample of XO.



XO: $R_1 = R_2 = CH_2N(CH_2COOH)_2$,
$R_3 = R_4 = CH_3$
SXO: $R_1 = CH_2N(CH_2COOH)_2$,
$R_2 = H$, $R_3 = R_4 = CH_3$

The effluent between **a** and **b** as shown in Fig. 1 was collected as a portion of pure XO for each run. Each effluent was combined and concentrated in a rotary evaporator at 60—65 °C and dried in a vacuum desiccator. About 560 mg of the sodium salt of pure XO were obtained on eight runs from 960 mg of the pre-purified sample. From the elution curve and the amount of the isolated XO, the injected sample was found to consist of more than 58% XO, at most 19% IDA, 0.01% SXO, and other unknown materials.

Increasing methanol content in the developing solvent accelerated the elution of XO and SXO, but did not affect the retention time of IDA. The solvent composed of 5% aqueous acetic acid and methanol in the ratio 80/20 (v/v) gave the best separation of XO from IDA and SXO. This solvent is volatile and is more suitable for the subsequent treatment than the 1-butanol–acetic acid–water system used for the ordinary chromatographic method.

The pre-purification is not essential for the purification of XO by HSLC. By lowering the methanol content of the eluent, the present technique of HSLC is applicable to the sample obtained directly from the synthesis. *o*-CR, which is present in the synthetic mixture and is removable in the step of pre-purification, is

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* A preliminary work of HSLC with a small column Microbondapak C_{18}, 4 mm × 300 mm, and a solvent of higher methanol content as 5% aqueous acetic acid–methanol (60/40, v/v) revealed that the retention time of *o*-CR was 17 min and much longer than that of IDA, XO, and SXO, 1.8, 3.5, and 8 min, respectively.

easily eliminated by the HSLC. However, since a crude XO obtained directly from the synthesis contains usually a fairly large amount of SXO, the unavoidable cross-contamination of SXO and XO might occur. In the present study, therefore, the pre-purification was carried out for the sake of the better yield and efficiency.

It is interesting to note that the sodium salt of the XO purified by the HSLC was not retained at all on a column of Dowex 50W-X8, a strongly acidic cation-exchange resin of medium cross-linking and in the hydrogen-form, whereas the sodium salt of the XO purified by the ordinary cellulose column chromatography was always partly and strongly retained on the exchanger.

Experimental

Materials. Acetic acid of analytical grade and an HSLC grade methanol were used for the elution chromatography. Water was deionized. A crude XO was synthesized according to the literature.[2]

Instruments. All separations were carried out with a Waters Model ALC/GPC 244 chromatographic instrument equipped with two 7 mm×610 mm columns in series and a spectrophotometric detector (λ=254 nm). The columns were packed with Bondapak C_{18}/Porasil.

The Free Acid of the XO. An aqueous solution of the sodium salt of the purified XO was passed through a column of Dowex 50W-X8 in the hydrogen-form. The effluent was concentrated at *ca.* 60 °C in a rotary evaporator and dried in a vacuum desiccator. Mp *ca.* 286 °C (dec). Found: C, 53.33; H, 4.94; N, 4.14; S, 4.67%; S/N =1.13. Calcd for $C_{31}H_{32}O_{13}N_2S \cdot H_2O$: C, 53.91; H, 4.96; N, 4.06; S, 4.64%; S/N=1.14.

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