Separation of Semi-Xylenol Orange from Xylenol Orange by Thin-Layer Chromatography with Solvents Giving Stepwise pH Change on the Plate

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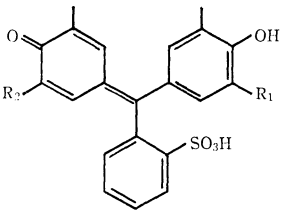
Semi-Xylenol Orange (SXO) is a very suitable reagent as compared with Xylenol Orange (XO) for interpreting the kinetic behavior for complex formation. The *n*-butanol - water - acetic acid solvents reported for the separation of XO and SXO with a cellulose column[1,2] often cause serious tailing and cross-contamination. In the present communication we propose a new procedure with solvents giving stepwise pH change on the plate, whereby the clear-cut separation of SXO from XO is effectively performed by thin-layer chromatography with sandwich technique.



XO · $R_1 = R_2 = CH_2N(CH_2COOH)_2$
SXO · $R_1 = CH_2N(CH_2COOH)_2$, $R_2 = H$

A slurry for one plate (10×20 cm) was made by mixing 6 g of pretreated cellulose powder (Toyo, 300 mesh) with 18 m*l* of water. Developing solvents were prepared shortly before use from various amounts of 28% ammonia and 12 m*l* of *n*-butanol saturated with 10% acetic acid. The solvents formed regions I and II divided by a distinct boundary directly visible (Fig. 1). Region II gave an intense and uniform positive test with the Nessler reagent and a basic color (bluish green) with Bromothymol Blue (BTB), whereas region I showed an almost negative Nessler test and an acid color (orange) with BTB. Uniformity of the sensitive coloration indicates that the development with the given solvent automatically forms two distinct zones with different pH values, region I (*n*-butanol - acetic acid - water) and region II (*n*-butanol - ammonia - ammonium acetate - water).

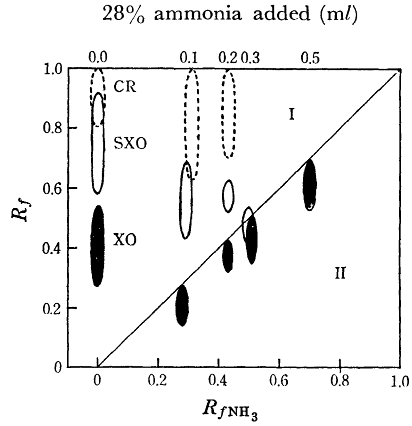


Fig. 1. Chromatograms for SXO and XO as a function of the R_{fNH_3}.
Spot solutions: 0.5 μ*l* of 1×10^{-3} mol/*l* CR, SXO, or XO in *n*-BuOH satd. with 10% HOAc.
The straight line gives the ammonia front.

In Fig. 1 the chromatograms for SXO and XO after 10 cm's development are plotted against R_{fNH_3}, the R_f values of the so-called ammonia front. XO lies always in region II beneath the boundary, whereas SXO is in region I for the R_{fNH_3} values lower than 0.5. When R_{fNH_3} is about 0.4, the cross-contamination of SXO and Cresol Red (CR), one of the raw materials, also disappears. In any case ammonia is very effective to prevent tailing. It is thus concluded that the solvent, 12 m*l* of *n*-butanol saturated with 10% acetic acid and 0.2 m*l* of 28% ammonia ($R_{fNH_3}=0.43$), gives the best separation of SXO from XO within only 1 hr. Preparative thin-layer chromatography for SXO of high purity using the present procedure is now in progress.

The solvent systems as mentioned above would be useful for the chromatographic separation of other series of substances of similar structure, where the subtle differences in charge type and its pH-dependence may be the primary factors for the sharp separation.[3] Further details for such systems will be reported elsewhere.

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1) D. C. Olson and D. W. Margerum, *Anal. Chem.*, **34**, 1299 (1962).

2) M. Murakami, T. Yoshino, and S. Harasawa, *Talanta*, **14**, 1293 (1967).

3) With the given solvents the disubstituted commercial specimen such as GCR, GTB, MTB, and MXB gave at least three distinct spots corresponding to the raw material, mono- and di-substituted derivatives.