November, 1980] © *1980 The Chemical Society of Japan* NOTES *Bull. Chem. Soc. Jpn.*, ***53***, *3365—3366 (1980)* 3365

Purification of Methylthymol Blue and Methylxylenol Blue by High-Performance Liquid Chromatography[†]

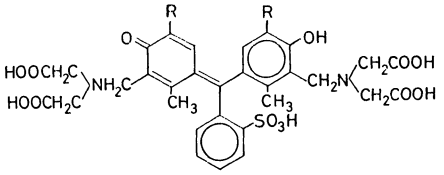
Shoji Nakada, Mutsuo Yamada, Tasuku Ito,[††] and Masatoshi Fujimoto[*]

Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060

(Received April 17, 1980)

Synopsis. Relatively large amounts of 3,3′-bis[bis(carboxymethyl)aminomethyl]thymolsulfonphthalein (Methylthymol Blue, MTB) and 3,3′-bis[bis(carboxymethyl)aminomethyl]-*p*-xylenolsulfonphthalein (Methylxylenol Blue, MXB) are easily purified by high-performance liquid chromatography (HPLC).

Multidentate ligands of triphenylmethane complexon (TPMC) series, MTB and MXB, are metallochromic indicators for the complexometric titration of a number of metal ions in aqueous solutions.1) However, only a few quantitative data on the complex formation of these ligands have been reported. These data show serious discrepancies resulting from the use of specimens of low purity.



MTB : R = CH(CH$_3$)$_2$

MXB : R = CH$_3$

The purity of synthesized or commercial specimens of these ligands is low. MTB usually contains the starting materials, iminodiacetic acid (IDA) and Thymol Blue (TB), and a by-product, Semi-Methylthymol Blue (SMTB). Similarly, MXB contains IDA, *p*-Xylenol Blue (*p*-XB), and Semi-Methylxylenol Blue (SMXB). Since the monosubstituted products, SMTB and SMXB, and IDA also form stable metal complexes, the use of MTB or MXB free of these impurities is essential for quantitative studies of metal complex formation. However, the separation of IDA, SMTB or SMXB, and TB or *p*-XB from MTB or MXB was not easy; the method of purification reported for MTB2) is incomplete, and that for MXB has not been established.

In a previous paper we reported the purification of Xylenol Orange (XO) by preparative HPLC.3) We have applied the method to the purification of MTB and MXB, and succeeded in preparing relatively large amounts of MTB and MXB of high purity.

Ten grams of crude sample of synthesized MTB or MXB was preliminarily purified by cellulose column chromatography (CCC) using a 45×1000 mm column packed with 600 g of Whatman CC-31 cellulose powder and 1-butanol saturated with 1% aqueous acetic acid as an eluent. The last fraction of three colored bands clearly separated on the chromatogram was used as a sample for HPLC. In the case of MXB, the yield of the last fraction by CCC was very low as compared with that of MTB. Paper partition chromatography (PPC) of the pre-treated sample gave no spots of SMTB (or SMXB) and TB (or *p*-XB), but a considerable amount of IDA still remained unremoved.

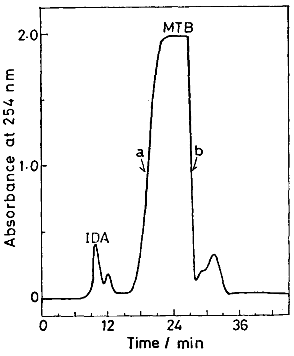


Fig. 1. Elution curve of the pre-purified sample of MTB.

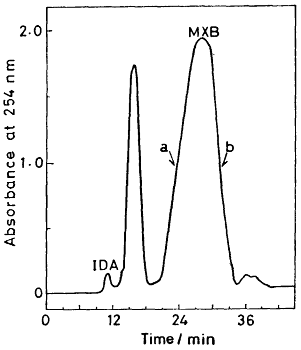


Fig. 2. Elution curve of the pre-purified sample of MXB.

Figure 1 shows a typical elution curve of preparative HPLC for the pre-purified sample of MTB. One gram of the sample pre-purified on CCC was dissolved in 5 cm^3 of water. One cubic centimeter of the sample solution was injected for each run. The separation was accomplished with an eluent, 5% aqueous acetic acid–methanol (60/40, v/v),[**] at a flow rate 6.0 cm^3 min^{-1} and at 1500 psi. MTB was clearly separated from the impurities on a preparative scale, a small peak of an unknown substance being observed in the tail portion of the MTB peak. After elution of MTB the column was regenerated by washing with methanol for 20 min. An unknown reddish yellow material probably containing a trace amount of SMTB was flushed out. The column thus regenerated was used repeatedly.

Figure 2 shows an elution curve for the pre-purified sample of MXB under the same conditions as those for MTB except for the use of 5% aqueous acetic acid–methanol (80/20, v/v)[**] as an eluent. The peak

---

[†] Presented in part at the 1980 Winter Meeting of the Chemical Society of Japan and the Japan Society for Analytical Chemistry, Sapporo, Hokkaido, February 1, 1980, Abstract, p. 36.

[††] Present address: Institute for Molecular Science, Okazaki 444.

[**] Preliminary experiments showed that the retention time for the parent triphenylmethane dyes is much longer than that of the corresponding TPMC and IDA as in the case of XO,3) and that the retention time for TB-series is much longer than that for *o*-CR- and *p*-XB-series. With increase in methanol content in the given eluent, the retention times of *o*-CR, TB, and *p*-XB become shorter. Thus in the case of MTB we applied the eluent of higher methanol content (40%, v/v). The difference in retention time among the three series can be ascribed to the difference in the alkyl substituents in the triphenylmethane skeleton.

TABLE 1. ELEMENTAL ANALYSES AND DSC MEASUREMENTS OF MTB AND MXB

Compound	Formula		Elemental analyses					Mp/°C
			C, %	H, %	N, %	S, %	S/N	
MTB	$C_{37}H_{44}O_{13}N_2S\cdot 3H_2O$	Found	54.80	6.02	3.37	3.93	1.17	201
		Calcd	54.80	6.21	3.46	3.95	1.14	
MXB	$C_{33}H_{36}O_{13}N_2S\cdot 3H_2O$	Found	53.72	5.52	3.59	4.20	1.17	270
		Calcd	52.52	5.61	3.71	4.24	1.14	(dec)

of MXB is much smaller than that of MTB. A colorless component giving a large frontal peak was assigned to an unreactive form of formaldehyde, which was revealed by ultraviolet spectra and chromatographic behavior. As in the case of MTB, a small peak was observed in the tail portion of the MXB peak. This was not assigned to that of the corresponding monosubstituted product, SMXB. The results indicate that the purification of MTB and MXB is not complete by means of a single HPLC even under the best conditions; repeated HPLC is necessary for complete purification.

The effluent between **a** and **b** for each run in Figs. 1 and 2 was collected as the portions of MTB and MXB, respectively. Each effluent was combined, concentrated, and injected again for repeated HPLC. For the effluent after the second HPLC treatment, almost complete purification of MTB or MXB was confirmed by analytical HPLC with a column of Microbondapak C_{18}, 4×300 mm, and a solvent, 5% aqueous acetic acid–methanol (60/40, v/v). The purified MTB or MXB was converted into acid form, concentrated, and dried. For MTB about 300 mg of the acid form of pure specimen was obtained from 1 g of a pre-purified sample. For MXB, however, only 160 mg was obtained from 1 g of a pre-purified sample. The reason for the low yield of MXB can be attributed to structural requirements in MXB.

Experimental

Materials. The solvents used as eluents were purified beforehand. Acetic acid of analytical grade was filtered through a glass filter G4, methanol was distilled, and water was deionized and redistilled. The crude sample of MTB or MXB was synthesized by Mannich condensation from TB or *p*-XB, IDA, and formaldehyde.[4] The sample solutions for HPLC were filtered through a Toyo membrane filter TM-2.

Instruments. A Waters liquid chromatograph Model ALG/GPC 204 equipped with three 7×610 mm columns of Bondapak C_{18}/Porasil B in series and an ultraviolet detector (λ=254 nm).

Free-Acid Form of Purified MTB and MXB. Aqueous solutions of the sodium salts of purified MTB and MXB were passed through a column of Dowex 50W-X8 in the hydrogen form. The effluents were concentrated at 40 °C in a rotary evaporator and dried in a vacuum desiccator. The purity of the specimens of MTB and MXB was confirmed by elemental analyses and DSC measurements (Table 1). The values of pK_a, λ_{max}, and the molar absorptivity of the aqueous solutions of the pure ligands, MTB and MXB, will be reported.[5]

The authors wish to thank Prof. Yoshio Matsunaga for the kindness in the DSC measurements. The present work was supported by a Grant-in-Aid for Scientific Research from Ministry of Education, Science, and Culture (No. 347031).

References

1) B. Budĕšínský, "Chelates in Analytical Chemistry," ed by H. A. Flaschka and A. J. Barnard, Jr., Marcel Dekker, New York, N. Y. (1967), Vol. 1, pp. 15—47.

2) T. Yoshino, H. Imada, T. Kuwano, and K. Iwasa, *Talanta*, **16**, 151 (1969).

3) S. Nakada, M. Yamada, T. Ito, and M. Fujimoto, *Bull. Chem. Soc. Jpn.*, **50**, 1887 (1977).

4) J. Körbl and R. Přibil, *Chem. Ind. (London)*, **1957**, 233.

5) S. Nakada, M. Yamada, T. Ito, and M. Fujimoto, *Bull. Chem. Soc. Jpn.*, **53**, 2252 (1980).