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Note

High-performance liquid chromatography of mercury and phenylmercury as the N-disubstituted dithiocarbamate complexes

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Phenylmercury (PhHg) salts are frequently used as antibacterial preservatives in pharmaceutical products such as eye drops[1] and an assay for PhHg in these products by high-performance liquid chromatography (HPLC) of the morpholinedithiocarbamate (MDTC) complex has been reported from these laboratories[2]. This assay can be succesfully applied to most pharmaceutical products. However, in recent studies on the stability of PhHg salts in the presence of sodium metabisulphite, which is sometimes included in products as an antioxidant, it has been found that the PhHg is degraded to inorganic mercury[3] and that the divalent $Hg(MDTC)_2$ co-elutes with the PhHgMDTC. Simple modification of the mobile phase failed to affect resolution of the two complexes and studies have been undertaken to investigate alternate derivatives to overcome this problem.

The quantitation of heavy metals by HPLC using a diverse range of N-disubstituted dithiocarbamate (DTC) complexing agents has been widely reported in recent years[4,5]. However, little has been published on the relationship between chemical structure of the DTC complexing agents and the chromatographic characteristics of the resultant complexes[6–8]. The separation and quantitation of mercury and organomercury compounds has received considerable attention, the usual complexing agent chosen being diethylaminedithiocarbamate (DEADTC)[9–13]. The resolution of metal ions of differing valency should be particularly amenable to modification of the complexing agent resulting in a differential change in polarity of the complexes. This paper reports the influence of changing the polarity of the DTC on the chromatographic characteristics of Hg^{2+} and $PhHg^{+}$ complexes.

EXPERIMENTAL

General reagents and chemicals

PhHg nitrate was obtained from BDH (Poole, U.K.) and the methanol and acetonitrile were HPLC-grade from Mallinckrodt (Melbourne, Australia). The dimethylamine, diethylamine, morpholine, pyrrolidine, piperidine and carbon disulphide were obtained from BDH or Ajax Chemicals (Sydney, Australia) and redistilled prior to use.

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Complexing reagents

The corresponding amine salts of the N-disubstituted DTC acids were prepared by the slow addition of carbon disulphide to a two-fold excess of the amine by previously reported methods[2] and stored under refrigeration. The complexing reagents were prepared by dissolving 50 mg of the salts in the minimum quantity of water (always less than 5 ml) and making to 100 ml with HPLC-grade methanol or acetonitrile as appropriate. The complexes were prepared by a 1:1 addition of these reagents to a $2 \cdot 10^{-4}$ *M* solution of either PhHg nitrate or Hg nitrate.

Chromatographic equipment and conditions

The liquid chromatograph consisted of a pump and variable-wavelength detector (LC-3, Pye-Unicam, Cambridge, U.K.), 20-μl loop injector (Rheodyne 7125, Cotati, CA, U.S.A.), integrating recorder (Hewlett-Packard 3380 A, Palo Alto, CA, U.S.A.) and a μBondapak C_{18} column (30 cm × 3.9 mm I.D., 10 μm particle size) (Waters Assoc. Sydney, Australia). All HPLC solvents incorporated $1 \cdot 10^{-4}$ *M* disodium EDTA and operated at a flow-rate of 1.5 ml min^{-1}. The monitoring wavelength was 258 nm.

Determination of capacity and separation factors

Capacity factors (k') were determined by duplicate injections of compounds and calculated by the formula: $k' = (t - t_o)/t_o$; and separation factors (α) by the formula: $\alpha = (t_2 - t_o)/(t_1 - t_o)$, where t is the retention time of the substance, t_1 the retention time of PhHg, t_2 the retention rime of Hg, and t_o the retention time of sodium nitrate, all determinations being made at a flow-rate of 1.5 ml min^{-1}.

RESULTS AND DISCUSSION

The capacity factors (k') for $PhHg^+$ and Hg^{2+} complexes derived from a diverse range of DTCs —dimethylamine (DMADTC), morpholine (MDTC), pyrrolidine (PYDTC), piperidine (PIDTC) and diethylamine (DEADTC)— have been determined for practical concentrations of 65–85% methanol in water and 55–80% acetonitrile in water as mobile phases. In some instances dilution of the sample with methanol based or acetonitrile based derivatising reagent resulted in the precipitation of the complexes from solution (consisting of approximately 50% acetonitrile or methanol in water) (Table I). For the sparingly soluble complexes the retention characteristics were determined using $2 \cdot 10^{-5}$ *M* $PhHg^+$ or Hg^{2+}. At the relatively high concentration of $PhHg^+$ used in eye drop formulations only PYDTC presented problems of solubility. Only DEADTC afforded soluble derivatives with both $PhHg^+$ and Hg^{2+} in both acetonitrile–water and methanol–water mobile phases. Numerous studies have been performed demonstrating the relationship between log k' derived from reversed-phase HPLC and calculated or experimentally derived log P values, where P is the octanol–water partition coefficient[14–16]. A similar relationship should exist for the complexes. Table IIA lists the correlation coefficients obtained from the linear plots of log k' and the log P of the amines from which the DTCs are derived, which serve as relative measures of the hydrophobicity of the resultant complexes. Correlation coefficients > 0.94 were obtained for all combinations of ion and solvent composition.

TABLE I

SOLUBILITY OF DITHIOCARBAMATE COMPLEXES FOLLOWING ADDITION OF REAGENT

Dithiocarbamate substituent	*Solvent*			
	Methanol		*Acetonitrile*	
	$PhHg^+$	*Hg^{2+}*	*$PhHg^+$*	*Hg^{2+}*
Dimethylamine	Soluble	Insoluble	Soluble	Insoluble
Morpholine	Soluble	Insoluble	Soluble	Insoluble
Pyrrolidine	Insoluble	Insoluble	Soluble	Insoluble
Diethylamine	Soluble	Soluble	Soluble	Soluble
Piperidine	Soluble	Insoluble	Soluble	Soluble

As the Hg^{2+} forms divalent complexes with DTCs, whereas $PhHg^+$ forms only monovalent complexes, the log k' for Hg^{2+} would be influenced to twice the extent of $PhHg^+$ by change in hydrophobicity of the side-chain groups. This is demonstrated in Table IIB where linear relationships are demonstrated between the two sets of data of slope close to 2.00 and with high correlation coefficients (> 0.99). As expected, the separation factors (α) are far more influenced by relative hydrophobicities of the DTC complexes than modification of solvent composition, due to the differing valency of

TABLE II

RELATIONSHIPS BETWEEN LOG CAPACITY FACTORS (k') AND LOG P FOR $PhHg^+$ AND Hg^{2+} COMPLEXES

Log P values used in calculations: morpholine, -1.08[17]; diethylamine, $+0.55$[18]; piperidine, $+0.85$[18]; dimethylamine, -0.49; pyrrolidine, $+0.32$. Dimethylamine and pyrrolidine values were calculated from the values of diethylamine and piperidine respectively, using the method of Rekker[18].

Percent modifier	*(A) Correlation coefficients of lines through log k' versus log P of corresponding amines*		*(B) Correlation coefficients and slopes of lines through log k' Hg^{2+} versus log k' $PhHg^+$*	
	$PhHg^+$	*Hg^{2+}*	*Slope*	*Correl. coeff.*
Methanol				
65	0.955	0.948	1.944	0.9969
70	0.966	0.967	1.945	0.9977
75	0.960	0.967	1.965	0.9970
80	0.965	0.971	1.972	0.9980
85	0.994	0.998	1.886	0.9975
Acetonitrile				
55	0.978	0.972	1.978	0.9992
60	0.969	0.971	1.975	0.9996
65	0.966	0.974	1.977	0.9983
70	0.961	0.973	2.046	0.9979
75	0.967	0.969	1.984	0.9989
80	0.967	0.974	1.896	0.9919

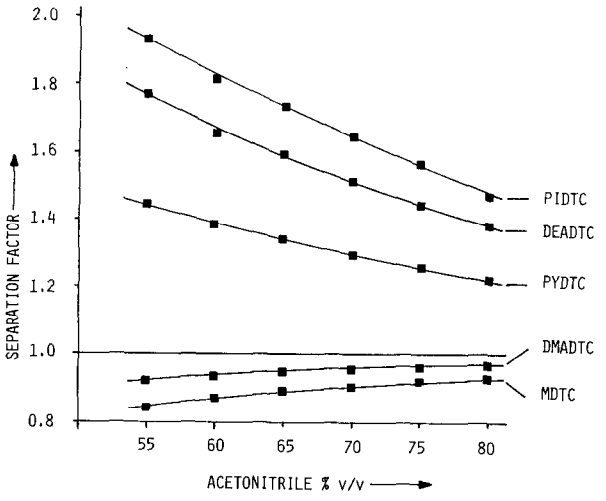

Fig. 1. Relationship between separation factor (α) and the acetonitrile content of the mobile phase and the nature of the dithiocarbamate complex. MDTC = morpholinedithiocarbamate; DMADTC = dimethylaminedithiocarbamate; PYDTC = pyrrolidinedithiocarbamate; DEADTC = diethylaminedithiocarbamate, and PIDTC = piperidinedithiocarbamate.

the ions (Figs. 1 and 2), the separation being improved by the use of more hydrophobic DTC complexing agents. For the quantitation of $PhHg^+$ in eye drops, any of the investigated DTC complexing agents would serve, provided no Hg^{2+} is present, with the exception of PYDTC, in which the complex is sparingly soluble in the derivatising solvent. For samples containing both $PhHg^+$ and Hg^{2+} the DEADTC would appear to be the best DTC agent due to its excellent separation and solubility of both com-

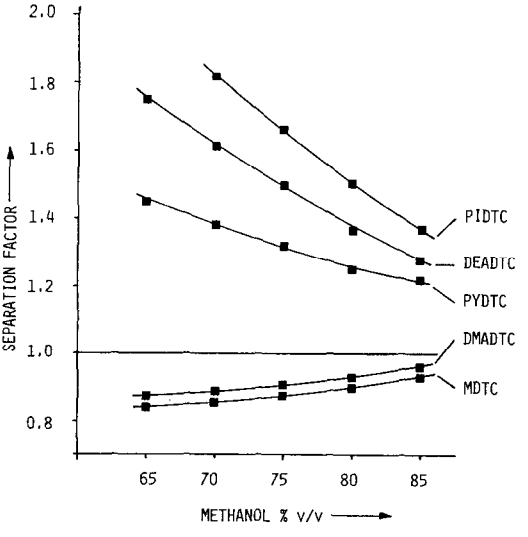

Fig. 2. Relationship between separation factor (α) and the methanol content of the mobile phase and the nature of the dithiocarbamate complex.

plexes in the mobile phases. The use of alternative DTC agents should enable separations of $PhHg^+$ and eye drop components to be achieved where the previously reported MDTC agent fails to resolve the $PhHg^+$ complex from other components in the formulation.

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