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Note

Separation of NTA and EDTA chelates by thin-layer chromatography

Ethylene diaminetetraacetic acid (H_4EDTA) is a widely used complexing agent¹. Nitrilotriacetic acid (H_3NTA), in the form of its trisodium salt, has been actively considered as a technically suitable phosphate substitute in detergents during the past decade² and is even used as such in some countries. Its presence has also been detected in the environment³⁻⁵. In view of the increasing importance of metal chelates of EDTA and NTA, it is desirable to develop effective methods for their identification and separation. The separation of some metal-EDTA complexes by thin-layer chromatography (TLC) on microcrystalline cellulose⁶ and silica gel⁷ has been achieved recently. However, the TLC of metal-NTA chelates has not yet been reported.

We describe here their chromatographic behavior on silica gel as well as their separation from the corresponding EDTA chelates.

Experimental

TLC was performed on silica gel free from gypsum, using precoated plastic sheets (20 × 20 cm, 0.25 mm thick) of Polygram SIL G from Macherey, Nagel & Co., Düren, G.F.R.

The metal-EDTA chelates were synthesized according to the literature^{8,9}. The acid NTA chelates were obtained by the reaction of H_3NTA with aqueous

TABLE I

SOLVENT SYSTEMS FOR SEPARATION OF METAL-NTA, METAL-EDTA CHELATES, AND METAL IONS

<i>Solvent system</i>	<i>Composition (v/v)</i>
A	Water-glycol monomethyl ether- <i>n</i> -butanol-acetone-15 N NH_4OH (40:25:25:10:0.25)
B	(35:30:25:10:0.25)
C	Water-glycol monomethyl ether-methyl ethyl ketone- <i>n</i> -butanol-acetone-15 N NH_4OH (45:25:10:15:10:0.15)
D	Water-glycol monomethyl ether-methyl ethyl ketone-acetone-15 N NH_4OH (40:20:20:20:1.5)
E	Water-methyl ethyl ketone- <i>n</i> -butanol-acetone-15 N NH_4OH (40:25:10:25:1.0)
F	(40:10:10:40:1.5)
G	(30:35:15:20:1.5)
H	(30:20:20:20:1.5)
I	(40:35:10:15:1.5)
J	(40:20:10:30:1.5)
K	Benzene-ethanol-water (30:120:50)
L	(30:110:40)
M	(30:120:30)

solutions of the metallic chloride, hydroxide or sulfate at 100°. The sodium salts of the NTA chelates were prepared under similar conditions by reaction of $\text{Na}_3\text{NTA} \cdot \text{H}_2\text{O}$ with the chloride, nitrate or sulfate of the appropriate metal. All chelates were recrystallized from water or water-ethanol, and gave acceptable microanalytical data for C, H, N and metal(s). Full details of their synthesis, isolation and characterization will be reported elsewhere¹⁰.

TABLE II

COMPOSITION OF SPRAY REAGENTS

No.	Reagent	Composition
1	PAN indicator	(a) 0.25% ethanolic solution of 1-(2-pyridylazo)-2-naphthol (b) 15 N NH_4OH
2	Rubeanic acid	(a) 0.5% ethanolic solution (b) 15 N NH_4OH
3	Sodium sulfide	0.5% aqueous solution, freshly prepared
4	Diphenyl carbazide	(a) 1% ethanolic solution (b) 15 N NH_4OH
5	8-Hydroxyquinoline	1% solution in ethanol saturated with ammonia

TABLE III

CHROMOGENIC BEHAVIOUR OF METAL-NTA, METAL-EDTA CHELATES AND METAL IONS

Compound	Spray reagents				
	1	2	3	4	5
H_3NTA	pale yellow	— ^b	—	—	—
$\text{Na}_3\text{NTA} \cdot \text{H}_2\text{O}$	pale yellow	—	—	—	—
$\text{NaCdNTA} \cdot 2 \frac{1}{2} \text{H}_2\text{O}$	pink	—	yellow	—	pale yellow
CdCl_2	pink	—	yellow	—	pale yellow
$\text{Na}_2\text{CdEDTA} \cdot 2\text{H}_2\text{O}$	pink ^a	—	yellow	—	pale yellow
$\text{NaCuNTA} \cdot \text{H}_2\text{O}$	purple	grey	brown	mauve	—
CuSO_4	purple	grey	brown	mauve	—
$\text{Na}_2\text{CuEDTA} \cdot 1 \frac{1}{2} \text{H}_2\text{O}$	purple	grey	brown	mauve	—
$\text{NaNINTA} \cdot 2\text{H}_2\text{O}$	pale red	purple	—	—	—
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	pale red	purple	greyish brown	—	—
$\text{Na}_2\text{NiEDTA} \cdot 4\text{H}_2\text{O}$	pale red ^a	purple ^a	—	—	—
$\text{NaZnNTA} \cdot \text{H}_2\text{O}$	pink	—	—	—	—
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	pink	—	—	—	—
$\text{Na}_2\text{ZnEDTA} \cdot 3 \frac{1}{2} \text{H}_2\text{O}$	pink	—	—	—	—
$\text{NaCoNTA} \cdot \text{H}_2\text{O}$	dark blue	brown	greyish brown	—	pale yellow
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	dark blue	brown	brown	—	pale yellow
$\text{Na}_2\text{CoEDTA} \cdot 1 \frac{1}{2} \text{H}_2\text{O}$	dark blue	brown	—	—	pale yellow
NaPbNTA	pale red	pale brown	brown	—	pale brown
$\text{Pb}(\text{NO}_3)_2$	pale red	pale brown	brown	—	pale brown
$\text{Na}_2\text{PbEDTA} \cdot \text{H}_2\text{O}$	pale red	pale brown	brown	—	pale brown
Background	deep yellow	pale brown	white	—	pale yellow

^a See Discussion.

^b — indicates absence of visible spots for the levels indicated in the *Experimental* section.

TABLE IV

 $R_F \times 100$ VALUES OF VARIOUS METAL-NTA CHELATES

Compound	$R_F \times 100$ in solvent												
	A	B	C	D	E	F	G	H	I	J	K	L	M
H ₃ NTA	68	62	78	61	48	47	18	30	51	53	61	61	54
Na ₃ NTA·H ₂ O	67	61	78	61	48	47	18	30	51	53	61	61	54
Cd ₃ (NTA) ₂ ·H ₂ O	65 ^b	59 ^b	80	—	—	—	—	—	—	—	56 ^b	53 ^b	—
NaCdNTA·2½H ₂ O	65 ^b	59 ^b	80	—	—	—	—	—	—	—	56 ^b	53 ^b	—
HCdNTA·4H ₂ O	— ^b	59 ^b	80	—	—	—	—	—	—	—	56 ^b	54 ^b	—
NaCuNTA·H ₂ O	66	62	82	—	—	—	—	—	—	—	73	77	69
HCuNTA·2H ₂ O	65	62	82	—	—	—	—	—	—	—	73	77	69
H ₄ Cu(NTA) ₂ ·2H ₂ O	65	61	82	—	—	—	—	—	—	—	73	77	69
Cu ₃ (NTA) ₂ ·6H ₂ O	65	61	82	—	—	—	—	—	—	—	73	77	69
NaNiNTA·2H ₂ O	68	64	86	77	65	62	28	36	55	56	72	71	63
HNiNTA·3H ₂ O	68	64	86	77	64	60	27	35	55	54	72	71	64
H ₄ NiNTA·2H ₂ O	67	64	86	74	64	58	26	33	54	52	72	71	64
NaZnNTA·H ₂ O	—	—	—	—	—	—	—	—	—	—	74	73	68
HZnNTA·2½H ₂ O	—	—	—	—	—	—	—	—	—	—	73	72	67

^a Partially decomposed.^b — indicates complete decomposition. Solvents D to J (inclusive) produce double fronts.

TABLE V

 $R_F \times 100$ VALUES OF VARIOUS METAL IONS, METAL-NTA AND METAL-EDTA CHELATES

Compound	$R_F \times 100$ in solvent												
	A	B	C	D	E	F	G	H	I	J	K	L	M
NaCdNTA·2½H ₂ O	65 ^b	59 ^b	80 ^a	—	—	—	—	—	—	—	56 ^b	53 ^a	—
CdCl ₂	0	0	0	0	0	0	0	0	0	0	60	54	—
Na ₂ CuEDTA·2H ₂ O	57	54 ^b	71 ^b	79 ^b	65 ^b	70 ^b	32 ^b	41 ^b	54 ^b	63 ^b	34	35	24
NaCuNTA·H ₂ O	66	62	82	—	—	—	—	—	—	—	73	77	69
CuSO ₄	0	0	0	0	0	0	0	0	0	0	2	2	2
Na ₂ CuEDTA·1½H ₂ O	59	59	77	83 ^b	69	—	—	—	—	61 ^b	46	37	30
NaNiNTA·2H ₂ O	68	64	86	77	65	62	28	36	55	56	72	71	63
NiCl ₂ ·6H ₂ O	2	2	0	0	0	0	0	0	0	0	60	56	31
Na ₂ NiEDTA·4H ₂ O	60	56	75	86	70	76	37	46	61	69	43	44	30
NaZnNTA·H ₂ O	— ^b	—	—	—	—	—	—	—	—	—	74	73	68
ZnSO ₄ ·7H ₂ O	0	0	2	0	0	0	0	0	0	0	10	20	6
Na ₂ ZnEDTA·3½H ₂ O	62 ^a	59 ^b	80	—	—	—	—	—	—	—	52	48	33
NaCoNTA·H ₂ O	65	65	87	—	65 ^b	—	—	—	—	—	63	76	73
CoCl ₂ ·6H ₂ O	0	0	0	0	0	0	0	0	0	0	64	56	31
Na ₂ CoEDTA·1½H ₂ O	64	59	78	87	67	73	36	44	61	68	63	42	33
NaPbNTA	—	—	—	—	—	—	—	—	—	—	70	66	65
Pb(NO ₃) ₂	0	0	0	0	0	0	0	0	0	0	0	0	0
Na ₂ PbEDTA·H ₂ O	67	60	76	79	68	71	30	39	56	63	38	29	17

^a Partially decomposed.^b — indicates complete decomposition.

The metal ions and chelates were applied in aqueous solutions. The amount spotted was 0.5 μg for the metal ions and NTA chelates, and 2 μg for the EDTA chelates.

Reagent-grade chemicals were used to prepare the solvent systems listed in Table I. The chromatographic tank (Desaga, for 20 × 20 cm plates) containing 100 ml of solvent was allowed to equilibrate for 2 h, with a development time varying from 2 to 6 h and a run of 15 cm. The solvent systems chosen are listed in Table I.

The spots were revealed by the spraying technique and the colors observed in daylight. The composition of the spray reagents used and their chromogenic behavior are summarized in Tables II and III, respectively. The R_F values are recorded in Tables IV and V, and are the average of four determinations.

Discussion

The TLC of EDTA and NTA chelates requires a judicious choice of solvent systems, because the stability of these compounds is highly dependent on pH. Accordingly, the pH of the solvent system has to be kept within narrow limits to prevent decomposition of the chelates during chromatography. Furthermore, the NTA chelates are much less soluble in water — the only solvent in which they dissolve reasonably — than their EDTA counterparts; this could pose certain problems in spotting. A third important consideration is the choice of spray reagent. When the latter is not adequately sensitive, solvent systems that give double fronts cannot be detected; also apparent spots may be obtained when in fact decomposition of the chelates occurs during chromatography. In this study, PAN was found to be the most suitable spray reagent: it revealed all the compounds studied (Table III), giving characteristic colors. Double fronts and chelate decomposition were also readily detected with that reagent. In the case of the EDTA chelates of Cd and Ni, prior hydrolysis with 12 *N* HCl-ethanol (1:1, v/v) was required before revelation with PAN.

The chromatographic behavior of some chelates of NTA with divalent metals is summarized in Table IV. At high pH (solvents containing more than 1% of ammonia) chelate decomposition occurs (solvents D to G) and, in several cases, double fronts are observed. Only the nickel-NTA chelates are stable under these conditions. In solvents A, B, C (weakly basic) and K, L, M (neutral), separation occurs and reliable R_F values are obtained. These are identical for the different NTA chelates of the same metal, irrespective of the number of NTA moieties in the molecule (Table IV). On the other hand, when the sodium salts of the 1:1 NTA chelates are compared, it is possible to distinguish between the chelates of different metals on the basis of their R_F values (Table V). It would thus appear that the influence of the metal ion is more important than that of the NTA moiety in determining the migratory aptitudes of the chelates studied.

The TLC method is particularly effective for separating mixtures of EDTA and NTA chelates. The metal cations, being in most instances non-migrating in the solvents used, can also be distinguished from one another by the characteristic colors observed with the different spray reagents used. This color differentiation is also of special significance in the case of chelates with closely similar R_F values. Thus, the cobalt-EDTA and copper-EDTA chelates appear as overlapping spots in solvent M; however, their presence is unequivocally established on the basis of their distinct colors after spraying with PAN reagent (Table III). Another such instance is that of the copper- and zinc-NTA chelates in solvent L. The EDTA and NTA chelates are otherwise readily separable by TLC (Table V). Furthermore,

as expected, for a given divalent metal, the NTA chelate migrates faster in the same solvent than its EDTA analog, being less polar than the latter.

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- 1 A. WALLACE, *Ann. N.Y. Acad. Sci.*, 88 (1960) 361.
- 2 N. S. THOM, *Water Res.*, 5 (1971) 391.
- 3 Y. K. CHAU AND M. E. FOX, *J. Chromatogr. Sci.*, 9 (1971) 271.
- 4 L. RUDLING, *Water Res.*, 5 (1971) 831.
- 5 B. K. AFGHAN AND P. D. GOULDEN, *Environ. Sci. Technol.*, 5 (1971) 601.
- 6 Z. MASOONI AND D. T. HAWORTH, *J. Chromatogr.*, 48 (1970) 581.
- 7 J. VANDERLEEN, *J. Chromatogr.*, 39 (1969) 521.
- 8 D. T. SAWYER AND J. M. MCKINNIE, *J. Amer. Chem. Soc.*, 82 (1960) 4191.
- 9 D. T. SAWYER AND P. J. PAULSEN, 81 (1959) 816.
- 10 F. J. M. RAJABALEE AND S. LAHAM, in preparation.

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