

TLC Resolution Studies of Two-Phase Separation of Metal-2-Mercapto-4-Methyl-5-Phenylazopyrimidine Complexes on Various Surfactant-Impregnated Silica Gel-G Plates

Ashok Kumar

Institute of Chemical Sciences, D.A. University, Indore 452 001, India

Abstract

Mercapto-4-methyl-5-phenylazopyrimidine (MMPAP) complexes of cobalt(II), nickel(II), lead(II), cadmium(II), and copper(II) are resolved using various pure and binary solvent mixtures on silica gel-G plates and on different surfactant-impregnated silica gel-G plates. Thin-layer chromatography of complex mixtures with the binary solvent, acetonitrile-xylene (70:30), provides an effective separation of the complexes, especially when tetrabutyl ammonium hydroxide is used as the surfactant. Slope analysis applied to metal ion distribution experiments from 3M HNO₃ shows a predominant formation of the solvated organic phase complex $M(NO_3)_2 \cdot 2MMPAP$. The developed method is tested for the determination of cobalt(II), nickel(II), lead(II), cadmium(II), and copper(II) in standard alloys.

Introduction

The pyrimidine ring system is one of the most widely distributed systems of heterocyclic compounds, as revealed by various reviews (1-3). Recently, pyrimidines have also been reported as anti-HIV (4,5), antiamebic (6), antimalarial (7), and antiviral (8) agents. In addition, TLC has been applied to drug monitoring studies (9). In the present work, 2-mercapto-4-methyl-5-phenylazopyrimidine (MMPAP) complexes of cobalt(II), nickel(II), lead(II), cadmium(II), and copper(II) have been resolved with various pure solvents and binary solvent mixtures on different surfactant-impregnated silica gel-G plates. The developed method has been tested for the determination of Co(II), Ni(II), Pb(II), Cd(II), and Cu(II) in NKK No. 1021 alloy, JSS 505 high speed steel, and JSS stainless steel.

Experimental

All chemicals were of analytical reagent and guaranteed reagent grades and were obtained from BDH (UK) or Merck

(Germany) unless otherwise specified. 2-Mercapto-4-methyl-5-phenylazopyrimidine (MMPAP) was synthesized and characterized by elemental analysis and infrared and ultraviolet spectroscopy. A 0.1% (w/v) solution of MMPAP in dimethylformamide (DMF) was used for extraction. Stock solutions of Co(II), Ni(II), Pb(II), Cd(II), and Cu(II) (1000 ppm) were prepared in double distilled water. An alloy solution was prepared by dissolving 0.2 g of the standard alloy (supplied by Iron and Steel Institute of Japan; Tokyo, Japan) in 15 mL HCl (1+1) by heating in a water bath and then adding 1-2 mL concentrated HNO₃. The excess of the acid was evaporated by gently heating the mixture in a boiling water bath, and then the volume was diluted to 100 mL in a standard volumetric flask.

Apparatus

A Shimadzu UV 160-A spectrophotometer (Shimadzu Kyoto; Japan) equipped with 10-mm matched quartz cells was used for absorption spectra of organic extractants. IR spectra in Nujol mull between CsI discs were recorded using a Pye Unicam Model 1912 spectrophotometer in the range of 4000-200 cm⁻¹.

TLC plates

Commercially available silica gel and chromatoplates (20 × 10 cm²) were used with an ascending irrigation technique. The glass plates were coated with silica gel-G (thickness, 0.75 mm) with a Stahl type applicator. The plates were air dried and activated by heating at 60°C for 24 h before use.

Metal-MMPAP complexes

Equal volumes (2 mL) of an aliquot of each metal (1000 ppm in 2M nitric acid-30% acetonitrile, v/v) and MMPAP were dissolved in an organic solvent, dimethylformamide. The solution was pipetted into a 60-mL separatory funnel, and the pH was adjusted to 3.0 with Britton-Robinson buffers. Two milliliters of 20% naphthalene solution in acetone was added to the solution. The solid mass (10), which contained metal, ligand, and naphthalene, was filtered, washed several times with distilled water, dried, and dissolved in 10 mL chloroform.

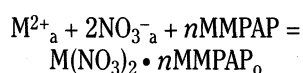
TLC resolution studies

Various surfactants were used in an effort to obtain better resolution for the quantitative separation of metals in complex materials (i.e., alloys): silica gel-G + tetrabutyl ammonium hydroxide (TBAH); silica gel-G + triton X-1003; silica gel-G + tetraethyl ammonium perchlorate (TEAP); silica gel-G + sodium laurylsulfate (SLS). The solutions (0.1 mL) were spotted separately at 2.0 cm from the edge of the plates. The spots were air dried at room temperature (23–25°C) and then placed in a chamber that was saturated with either pure solvent vapors (Table I) or binary solvent vapors (Table II). The plates were developed until the solvent front had travelled a distance of about 16.5 cm; about 45–70 min was usually required. The plates were dried, and the lead and cadmium–MMPAP complexes were made visible by placing the plates in an iodine chamber for 40 s. Co(II), Ni(II), and Cu(II)–MMPAP complexes were detected by their characteristic colors (Table I). For each system, five runs were carried out, and the R_f values were found to be reproducible between runs. For quantitative determination, the spots were carefully scraped off, and the silica gel-G with the respective complex was dissolved in 10 mL chloroform. Because the solutions of lead and cadmium complexes in chloroform were colorless, the lead and cadmium ions were quantitatively determined by the addition of 10^{-3} M solution of cupric nitrate to their respective solutions. The solutions were shaken vigorously in a separating funnel to get a stable yellow color in the chloroform layer. The transmittance and absorbance of the clear chloroform solution were then noted with reference to the calibration graph plotted for the respective metal–MMPAP complexes. The concentration of the metal ion was determined and reported in Table V.

Dependence of the distribution ratio on the concentration of MMPAP

The distribution ratio is defined as the total concentration of metal in the organic phase per milliliter divided by the total concentration of metal in the aqueous phase per milliliter. It is dependent on the concentration of MMPAP. The dependence of the distribution ratio on the MMPAP concentration was studied at a concentration of 3M HNO_3 .

The extraction of metal follows the reaction



where subscripts a and o refer to the species

present in the aqueous and organic phases, respectively. The equilibrium constant (K) is defined by

$$K = [\text{M}(\text{NO}_3)_2 \cdot n\text{MMPAP}]_o / [\text{M}^{2+}]_a [\text{NO}_3^-]_a^2 [\text{MMPAP}]_a^n$$

The equation for the distribution ratio (D) is as follows:

$$D = \log K + 2 \log [\text{NO}_3^-]_a + n \log [\text{MMPAP}]_o$$

The plot of $\log D$ vs $\log [\text{MMPAP}]$ in the organic phase yielded a slope of 2.1, which indicates that the extracted species in the MMPAP–diluent (30% acetonitrile phase) would be predominately of the type $\text{M}(\text{NO}_3)_2 \cdot 2\text{MMPAP}$.

Of the organic solvents used as additives in the study, only

Table I. Thin-Layer Chromatographic Data

Metal complex	pH range	Detection (μg)	Identification*		R_f values in pure solvent†										
			Original	Iodine	A	B	C	D	E	F	G	H	I	J	K
Co	2.5–7.5	1.5	Olive green	Brown	T	T	40	T	40	48	54	58	64	68	72
Ni	3.5–8.0	1.5	Light yellow	Greyish brown	T	T	46	T	46	52	56	63	68	70	76
Pb	2.0–5.5	2.0	Colorless	Bright yellow	T	T	38	T	40	45	48	54	T	64	62
Cd	2.5–7.5	2.0	Colorless	Bright	T	T	36	T	38	42	44	48	T	52	56
Cu	2.5–7.5	1.5	Yellow brown	Greenish brown	T	T	50	T	52	56	60	66	64	75	80

* Both the original color of the spots after development of the plates and the color of spots after development in an iodine chamber are shown.

† T = tailing. Solvent (dielectric constant): A, hexane (1.9); B, cyclohexane (2.1); C, 1,4-dioxane (2.2); D, carbon tetrachloride (2.2); E, benzene (2.3); F, toluene (2.4); G, xylene (2.3); H, chloroform (4.8); I, ethyl acetate (6.0); J, ethyl alcohol (24.6); K, acetonitrile (37.5).

Table II. $R_f \times 100$ of Metal–MMPAP Complexes in Binary Mixtures

Binary solvents	Ratio	$R_f \times 100$				
		Ni	Co	Pb	Cd	Cu
Chloroform–Benzene	70:30	65	62	59	58	68
Chloroform–Toluene	70:30	63	63	60	58	68
Chloroform–Xylene	70:30	69	65	62	60	70
Ethyl acetate–Benzene	70:30	72	70	T*	T	76
Ethyl acetate–Toluene	70:30	76	74	T	T	78
Ethyl acetate–Xylene	70:30	80	77	T	T	80
Acetonitrile–Benzene	70:30	85	83	80	75	86
Acetonitrile–Toluene	70:30	83	80	77	75	88
1,4-Dioxane–Xylene	70:30	73	70	68	64	75
Ethanol–1,4-Dioxane	60:40	77	75	71	68	80
Xylene–Benzene	60:40	72	69	62	58	76
Chloroform–Toluene	30:70	76	73	68	65	80
Ethyl acetate–Toluene	30:70	80	75	T	T	84
Acetonitrile–Toluene	30:70	82	80	76	72	86

* T = Tailing.

acetonitrile had similar measurements and gave a slope of nearly 2, which shows that the additive does not change the type of solvent species extracted.

Results and Discussion

The MMPAP complexes of Co(II), Ni(II), Pb(II), Cd(II), and Cu(II) absorb strongly at the following wavelengths: Co(II), 430 nm; Ni(II), 380 nm; Pb(II), Cd(II), and Cu(II), 435 nm. All subsequent studies show that only one type of complex is formed in each case. Their TLC resolution data are shown in Tables I–V. All the pure and binary solvent systems used (Tables I and II) gave satisfactory separation of the complexes. When ethylacetate was used, tailing was observed for the complexes of Cd(II) and Pb(II). Tailing persisted even when ethylacetate was

used in combination with benzene, xylene, and toluene. Table III shows the R_f values ($\times 100$) of the metal–MMPAP complexes in the four best combinations of binary solvents using different surfactants. The results show that more satisfactory resolution was obtained in the case of 1% tetra butyl ammonium hydroxide (TBAH) as an adsorbent. The maximum R_f value was obtained for the Cu(II) complex, followed by Ni(II), Co(II), Pb(II) and Cd(II) complexes in all the solvent systems. Pure solvents such as hexane, cyclohexane, and carbon tetrachloride did not help in the development of spots. Another noteworthy observation was that the metal–MMPAP complexes usually gave comparatively high R_f values when solvents of considerably higher dielectric constants or polarizability were used (Table I). It was also observed that high R_f values were obtained in a binary solvent mixture of low and high dielectric constants, in other words, acetonitrile–xylene (dielectric constant, 37.5:02.3) in the presence of 1% tetrabutyl ammonium hydroxide.

Table III. $R_f \times 100$ of Metal–MMPAP Complexes Using Different Surfactants

Adsorbents	Chloroform–Toluene					Ethylacetate–Xylene					Acetonitrile–Xylene					Ethanol–1,4 Dioxan				
	Co	Ni	Pb	Cd	Cu	Co	Ni	Pb	Cd	Cu	Co	Ni	Pb	Cd	Cu	Co	Ni	Pb	Cd	Cu
Silica gel-G	63	65	60	58	68	70	73	68	64	75	80	83	77	75	88	75	77	71	68	80
Silica gel-G + 1% TBAH	72	74	68	67	78	78	81	75	70	83	90	92	87	84	95	84	87	81	78	90
Silica gel-G + 1% triton X-100	65	68	62	60	70	72	75	70	67	78	82	85	80	78	88	78	80	74	70	83
Silica gel-G + 1% TEAP	70	72	66	64	75	75	78	73	67	80	87	89	85	82	92	82	85	78	75	87
Silica gel-G + 1% SLS	68	70	64	62	72	74	77	73	70	80	85	87	83	80	90	80	83	76	72	85

Table IV. Qualitative Analysis of Alloys and Identification of Co, Ni, Pb, Cd, and Cu with Reference to their $R_f (\times 100)$ Values

Binary solvents	Alloys*	Identification	$R_f \times 100$ (using different surfactants)			
			Silica gel-G + 1% TEAP	Silica gel-G + 1% TBAH	Silica gel-G + 1% triton X-100	Silica gel-G + 1% SLS
Chloroform–Toluene	A	Co, Ni, Cu	70, 72, 75,	72, 74, 78,	65, 68, 74,	68, 70, 72,
	B	Co, Ni, Cu, Pb	70, 75, 66, 75	71, 75, 68, 78	64, 66, 62, 70	68, 74, 64, 73
Ethylacetate–Xylene	A	Co, Ni, Cu	75, 78, 80,	78, 81, 83,	72, 75, 78,	74, 77, 80,
	B	Co, Ni, Cu, Pb	75, 79, 73, 80	76, 80, 75, 82	72, 75, 70, 79	73, 77, 73, 81
Acetonitrile–Xylene	A	Co, Ni, Cu	87, 89, 92,	90, 92, 95,	82, 84, 88,	85, 87, 90,
	B	Co, Ni, Cu, Pb	86, 88, 85, 91	89, 91, 87, 95	81, 84, 80, 87	85, 86, 83, 91
Ethanol–1,4-Dioxane	A	Co, Ni, Cu	82, 85, 87,	84, 87, 90,	78, 80, 83,	80, 83, 85,
	B	Co, Ni, Cu, Pb	81, 84, 78, 88	83, 86, 85, 89	78, 80, 74, 83	80, 84, 76, 85

* A = JSS, 607, high speed steel: C, 0.750; Co, 4.720; Mo, 0.030; Cr, 4.140; Al, 0.002; V, 0.860; S, 0.006; P, 0.012; Ni, 0.015; Cu, 0.028; Mn, 0.300; W, 16.960. B = MKK NO. 916 high speed steel: C, 0.03; Co, 0.003; Mg, 0.100; Cr, 0.050; Ti, 0.100; V, 0.020; Pb, 0.040; Si, 0.410; Ca, 0.030; B, 0.0066; Zr, 0.050; Ni, 0.060; Cu, 0.270; Mn, 0.110; Bi, 0.030; Zn, 0.050; Sn, 0.050; Fe, 0.540.

Table V. Determination of Cobalt, Nickel, and Copper in Alloys*

Certified alloy	Composition (%)		Amt. taken	Amt. found	RSD (%)	$R_f \times 100$
NKK No. 1021 (Al-Si-Cu-Zn alloys)	S: 15.560	Fe: 0.990	Cu: 2.72	2.69	0.65	95
	Sb: 0.010	Zr: 0.010	Ni: 5.70	5.67	1.10	92
	Hg: 0.290	Ni: 0.140				
	Cr: 0.030	Cu: 2.720				
	Ti: 0.040	Mn: 0.200				
	V: 0.007	Bi: 0.0100				
	Pb: 0.180	Zn: 1.760				
	Sn: 0.100	Ca: 0.004				
JSS, 505 High speed steel	C: 0.200	S: 0.0086	Cu: 1.00	0.98	1.02	95
	P: 0.020	Si: 0.300	Ni: 3.64	3.60	0.95	92
	Mo: 0.220	Ni: 11.825				
	Cr: 0.640	Cu: 0.100				
	Al: 0.026	Mn: 0.300				
JSS, 655 Stainless steel	Te: 0.030	Nb: 0.060	Co: 1.12	1.10	1.45	90
	Cr: 18.540	Mn: 1.580	Ni: 2.28	2.30	1.20	92
	Mo: 0.051	Si: 0.060	Cu: 4.40	4.36	1.40	95
	C+S+P: 0.094					

* Absorbents: silica gel-G + 1% tetrabutyl ammonium hydroxide (TBAH). Solvent: acetonitrile + xylene (70:30). Alloy sample was supplied by Iron and Steel Institute of Japan, Tokyo, Japan.

Qualitative evaluation of thin-layer chromatograms

The spectrophotometric determination of nickel, cobalt, lead, cadmium, and copper complexes with binary solvent and surfactants was carried out. Those combinations of binary solvents were used in which the interference by foreign ions is relatively low due to the difference in the $R_f (\times 100)$ values of the various metal complexes. The qualitative presence of nickel, cobalt, cadmium, lead, and copper in the alloys is confirmed by comparing the R_f values obtained during the analysis of alloys with the R_f values obtained for the individual metal complexes in that specific solvent mixture. Cadmium was not usually present in the alloy. In the quantitative analysis of the alloys by TLC, 5.47 μg copper, 3.20 μg cobalt, and 2.10 μg nickel in 10 mL chloroform gave mean absorbance values of 0.25, 0.18, and 0.20, respectively, with a relative standard deviation of 1.10%, 0.92%, and 1.00%. In the determination of lead(II) after replacement with $\text{Cu}(\text{NO}_3)_2$, 5.0 μg lead in 10 mL chloroform gave a mean absorbance value of 0.35 with a relative standard deviation of 1.00%.

Conclusion

A simple method is suggested for enhancing the extraction of cobalt, nickel, lead, cadmium, and copper. The various interfering radicals were effectively eliminated as a result of the difference in the $R_f \times 100$ values by the TLC method of separation and resolution.

The method proved to be convenient and highly selective. In addition, accurate results were achieved. The sensitivity of the method may be improved by using other optical techniques, such as flameless atomic absorption spectrophotometry or high-performance liquid chromatography.

Acknowledgment

The author wishes to thank the Council of Scientific and Industrial Research, New Delhi, for providing financial assistance.

References

1. R.C. Elderfield. *The Chemistry of Heterocyclic Compounds*, vol. 5. Wiley Interscience, New York, NY, 1968.
2. L.A. Peguette. *Principles of Modern Heterocyclic Chemistry*. N.A. Benjamin, New York, NY, 1989.
3. K. Hirota, Y. Kitade, K. Simada, S. Senda, and Y. Maki. Pyrimidine derivatives and related compounds. Part 45. Synthesis of 4-allophanoylpyrazoles via a pyrimidine-to-pyrazole ring transformations. *J. Chem. Soc. Perkin Trans. 6*: 1293-97 (1983).
4. K. Haraguchi, Y. Itoh, H. Tanaka, and T. Miyasaka. Preparation and reactions of 2'- and 3'-vinylbromides of uracil nucleosides: versatile synthesis for anti-HIV agents. *Tetrahedron Lett.* **32**: 3391-94 (1991).
5. S. Czerneck and A. Ezzitouni. Synthesis of Various 3'-branched 2',3'-unsaturated pyrimidine nucleosides as potential anti HIV agents. *J. Org. Chem.* **57**: 7325-28 (1992).
6. N. Kalyanam, P.C. Parthasarthy, L. Ananthan, S.G. Manjunatha, and M.A. Likhate. Study of antiamoebic compounds, part IV. Synthesis of hexahydropyrimidines and tetrahydroimidazoles. *Ind. J. Chem.* **31B**: 243-47 (1992).
7. S.J. Kester, M.J. Degnan, M.J. Hung, and L.M. Werbel. Antimalarial drugs synthesis and antimalarial properties of 1-imino-derivatives of 7-chloro-3-substituted-3,4-dihydro-1,9 (2H, 10H) aeridinediones and related compounds. *J. Heterocycl. Chem.* **21**: 741 (1992).
8. R.P. Tripathi, A. Hasan, R. Pratap, and D.S. Bhakuni. Studies in nucleosides: Part XVII—Synthesis of 4-amino-6-methoxypyrazolo(3,4 a) pyrimidine & 4-amino-6-methylthio pyrazolo (3,4 d) pyrimidine ribosides & their antiviral activity. *Ind. J. Chem.* **26**: 851 (1988).
9. E.H. Taylor and B.H. Ackerman. Free drug monitoring by liquid chromatography and implication for therapeutic drug monitoring. *J. Liq. Chromatogr.* **10**: 323-44 (1987).
10. A. Kumar. Spectrophotometric determination of palladium, tellurium and iridium after extraction with 2-mercapto-4-methyl-5-phenylazopyrimidine. *Anal. Sci.* **11**: 281-84 (1995).

Manuscript accepted September 8, 1995.