CHROMSYMP. 1871

Formazan derivatives as the precolumn derivatization reagents in a coupled high-performance liquid chromatographic–spectrophotometric system for trace metal determination

HITOSHI HOSHINO*, KOUJI NAKANO[a] and TAKAO YOTSUYANAGI

Department of Molecular Chemistry and Engineering, Tohoku University, Aoba, Aramaki, Aoba-ku, Sendai 980 (Japan)

ABSTRACT

As precolumn chelating reagents for use in high-performance liquid chromatographic (HPLC) determinations of ultratrace metal ions, several kinds of 3-phenyl- and 3-cyanoformazans were examined and the reagent structure is discussed in connection with the HPLC selectivity for metal ions. These zincon-type formazans, functioning as quadridentate ligands, provide excellent ability in the precolumn derivatization scheme for spectrophotometric detection. The coplanarity and the chelate-cage effect of the reagent skeleton are probably responsible for the unique selectivity for Cu^{II} and Zn^{II} ions, respectively. The formazans possessing oxygen donors give a good resolution of trivalent metal ions, which seems to be ascribable to the hard-base character produced by the oxygen atoms. Detectability for the Ni^{II} ion is only shown with a reagent that provides an α-diimine-like coordination environment consisting of a four-N donor assembly. The limits of detection for most of the metal ions tested are down to the nanomoles per litre level (sub-picomole amounts). Highly sensitive HPLC methods for metal ions at sub-ppb to ppb (10^{-9} g/g) levels are suggested.
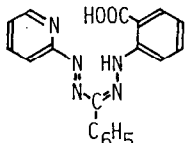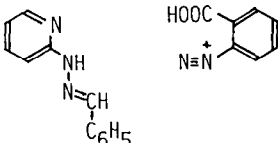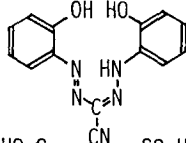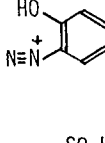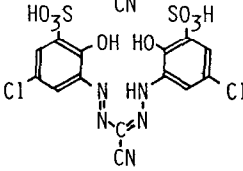
INTRODUCTION
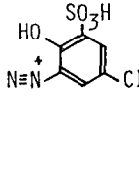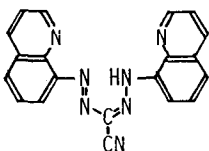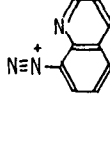
Although many formazan compounds have been investigated as photometric reagents for metal ions[1–5], in fact only three, diphenylcarbazone, diphenylthiocarbazone (dithizone) and zincon, have so far been used in practical applications[6]. Dithizone, which has N,S donor functions, was one of the reagents employed in the high-performance liquid chromatography (HPLC) of metal chelates in its earlier developmental stage[7–9]. In contrast to dithizone, zincon behaves as a potentially

---
[a] Present address: Department of Synthetic Chemistry, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812, Japan.

planar ligand with quadridentate O,O,N,N donor sites[5]. The coordination environ-
ment provided by zincon-type analogues was thus thought to be fairly suitable for
HPLC applications by analogy with quadridentate Schiff base ligands derived from
1,2-phenylenediamine[10]. This idea led us to the present detailed examination of a wide
variety of 3-phenyl- and 3-cyanoformazans as precolumn derivatization reagents for
metal ions in a reversed-phase (RP) HPLC system.

On the basis of the results of our systematic studies, we have concluded that the
unique selectivity as a result of on-column kinetic differentiation of inert from labile
chelates is the key feature of the precolumn derivatization system in conjunction with
an RP-HPLC separation[10–15]. Additionally, the precolumn technique allows the
utilization of the great sensitivity of modern absorbance detectors (the 0.001 a.u.f.s.
range is now available), with freedom from the increased baseline noise caused by the
added reagent in an eluent stream. The molar absorption coefficients of the formazan
chelates, $> 10^4 \, l \, mol^{-1} \, cm^{-1}$, are sufficient to ensure the nanomolar detection of metal
ions. Formazans designed to possess two five-membered and one six-membered
chelate rings are a highly promising choice for HPLC work because they are the most
capable of forming the kinetically inert chelates with many metal ions owing to their
strong "chelate effect"[1]. Indeed, formazan chelates of typically labile cations such as
Cu^{II} and Zn^{II} ions sometimes exhibit well resolved peaks even when working with an
eluent with no added reagent.

TABLE I

FORMULAE OF THE FORMAZAN REAGENTS EMPLOYED

No.	Reagent	Source of preparation
1		
2		CNCH₂COOH 2
3		CNCH₂COOH 2
4		CNCH₂COOH 2

In this paper the excellent suitability of formazan compounds for metal ions is described in relation to information concerning the HPLC selectivity principle.

EXPERIMENTAL

Apparatus

Absorption spectra and absorbances were recorded on a Shimadzu Model UV-365 recording spectrophotometer. A Hitachi-Horiba F-5 pH meter was used. The HPLC set-up used consisted of a TWINCLE pump unit, a UVIDEC 100-IV UV–VIS spectrophotometric detector and a VL 611 injector with a 100-μl loop from JASCO (Hachioji, Japan). Analytical columns used were a LiChroCart RP-18 (150 mm × 4 mm I.D.) from Cica-Merck Japan and a Zorbax CN (150 mm × 4.6 mm I.D.) from Shimadzu.

Preparation of the formazan reagents

The formazans employed, the structures of which are given in Table I, were (1) 1-(2-pyridyl)-3-phenyl-5-(2-carboxyphenyl)formazan, (2) 1,5-bis(2-hydroxyphenyl)-3-cyanoformazan, (3) 1,5-bis(3-chloro-5-sulpho-6-hydroxyphenyl)-3-cyanoformazan and (4) 1,5-bis(8-quinolyl)-3-cyanoformazan. They were prepared by ordinary coupling reactions of the corresponding diazonium ions to benzaldehyde 2-pyridylhydrazone or cyanoacetic acid[1–5]. The recrystallized products (from ethanol) were HPLC pure and their absorption spectra were identical with those found in the literature[1–5].

Reagents and solutions

Metal ion standard solutions (each 0.01 M) were prepared from the chlorides or the nitrates. Solutions of V^V and Mo^{VI} ions were prepared from ammonium metavanadate and sodium molybdate, respectively. All the metal salts used were of the highest purity available.

The concentration of each formazan solution was 0.01 M. Reagents 1 and 3 were dissolved in 0.01 M sodium hydroxide solution. For reagent 2, a non-ionic surfactant, polyoxyethylene 4-nonylphenoxy ether with 20 oxyethylene units (PONPE-20), was added (2%, w/w) to enhance dissolution. Reagent 4 was dissolved in 2% PONPE-20 solution containing a few drops of concentrated nitric acid.

The mobile phase solutions were acetonitrile–doubly distilled water mixtures containing a pH buffer, disodium EDTA for masking metal interferences and tetrabutylammonium bromide (TBABr) for ion pairing as well as for surface modifying of the RP packings. The detailed eluent conditions are specified for each reagent in the figure captions.

All other reagents and solvents were of analytical-reagent grade.

Procedures

A mixed metal ion solution containing Al^{III}, Be^{II}, Cd^{II}, Co^{II}, Cu^{II}, Fe^{III}, Ga^{III}, Mn^{II}, Mo^{VI}, Ni^{II}, Pb^{II}, V^V and Zn^{II} ions was used for a screening test for the optimization of the complexation conditions and the HPLC parameters.

The complexation reactions were in general carried out in an acetate buffer solution (0.04 M, pH 4.5–5.0) or in the presence of a Tris–HCl buffer (0.01 M,

pH 7.0–8.5). The solution was heated at 90°C for 10 min on a water-bath before diluting to volume. An aliquot of the final solution was loaded on a 100-μl loop. The detection wavelengths and sensitivity settings used are given in the captions of the figures.

RESULTS AND DISCUSSION

Absorption spectra and complexation reactions
The absorption spectra of some metal chelates of reagents 3 and 4 are shown in Figs. 1 and 2. As the formazan chelates were assumed to have a 1:1 stoichiometry (metal-to-ligand ratio), the absorption spectra were recorded for equimolar solutions of metal ion and the reagent. The molar absorptivity (ε) values are $3.2 \cdot 10^4$ l mol^{-1} cm^{-1} at 680 nm and $2.6 \cdot 10^4$ l mol^{-1} cm^{-1} at 645 nm for the reagent 3 chelates of Al^{III} and Zn^{II} ions, respectively. The ε value of the reagent 4 chelate of Ni^{II} is small, $5.9 \cdot 10^3$ l mol^{-1} cm^{-1} at 650 nm, compared with that of the Zn^{II} chelate of $1.5 \cdot 10^4$ l mol^{-1} cm^{-1} at 545 nm. The spectral properties of the chelates seem to be unattractive for the usual spectrophotometric applications, but the data in Figs. 1 and 2 show promise for the excellent ability for the sensitive detection of metal ions at sub-ppb to ppb (10^{-9} g/g) levels using HPLC–spectrophotometry. Spectral overlapping no longer causes a serious problem in the precolumn derivatization technique.

As the complexation reactions of the formazans are often slow at room temperature, the reaction mixture was heated at 90°C for 10 min. Two pH ranges for the precolumn derivatization studies were employed, 4.5–5.0 with acetate buffer for reagents 1 and 3 and 7.0–8.5 with Tris–HCl buffer for reagents 2 and 4. The criteria for the choice of the pH conditions were the stability and height of the peak signals. A sufficient excess of the reagent, finally established as 10^{-4} M, was added in the precolumn derivatization protocol.
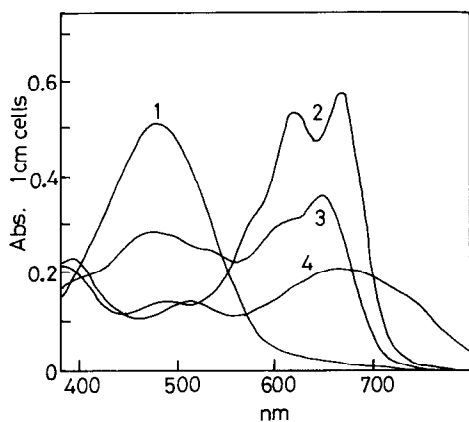

Fig. 1. Absorption spectra of reagent 3 and the chelates in slightly acidic aqueous solution. 1 = Reagent 3, $1.84 \cdot 10^{-5}$ M; 2 = Al^{III}, $2.00 \cdot 10^{-5}$ M; 3 = Zn^{II}, $2.05 \cdot 10^{-5}$ M; 4 = Co^{II}, $1.96 \cdot 10^{-5}$ M. pH 5.0 acetate buffer solution (0.04 M).
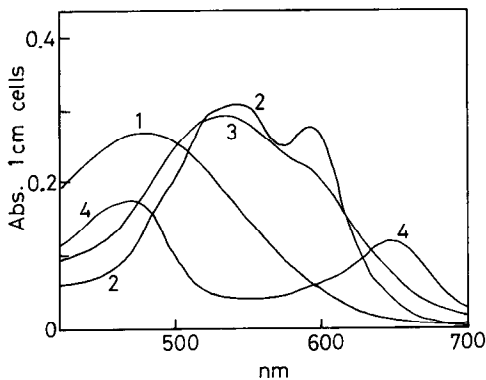
Fig. 2. Absorption spectra of reagent 4 and the metal chelates in aqueous 0.16 wt.-% PONPE-20 solution at pH 7.0. 1 = Reagent 4, $2.0 \cdot 10^{-5}$ M; 2 = Zn^{II}, $2.05 \cdot 10^{-5}$ M; 3 = Cd^{II}, $2.40 \cdot 10^{-5}$ M; 4 = Ni^{II}, $2.03 \cdot 10^{-5}$ M. pH 7.0 phosphate buffer solution (0.01 M).

Equilibrium stability data for the chelate formation reaction of 3-cyano-formazans were reported by Budesinsky and Svec[1]. They pointed out that the coplanarity and the chelate cage effect of the ligands are responsible for the unusual stability of the chelates with Zn^{II}, Cu^{II} and Pd^{II} ions. However, little quantitative information is available on the chelate formation reactions of hard metal ions such as Al^{III}, Ga^{III} and V^{V}. Additionally, no kinetic data have yet been given for the complexation reactions of the formazans.

HPLC separation studies

A preliminary experiment using commercially available zincon showed that it was almost useless because a number of unknown peaks due to impurities appeared. Therefore, even though zincon gave peaks for Co^{II}, Cu^{II} and V^{V} ions, a more detailed investigation was not attempted.

Acetonitrile–water as the eluent was more suitable for the resolution of the formazan chelates than methanol–water. The (apparent) pH of the mobile phase was maintained at 7–7.5 with sodium acetate. Better resolution was often achieved using a relatively polar Zorbax CN packing. The addition of TBABr was found to be a very important factor influencing the retention and the resolution of the chelates. When anionic chelates are predominant in the RP-HPLC process (all the chelates of reagent 3 and the reagent 2–V^{V} chelate), the TBA^{+} cation undoubtedly enhances their retention by ion-pair formation. In contrast, even in the separation of neutral or cationic chelates (most of the chelates of reagents 1, 2 and 4), this onium ion probably controls their retention and resolution, adsorbing both on the bonded phase and on the residual ionized silanol groups present in the surface. The further addition of sodium bromide was very effective in resolving the Cu^{II} and Ni^{II} chelates of reagent 4. The optimum concentrations of TBABr dissolved in the mobile phase are given in captions of the figures.

Typical chromatograms for the reagent systems are shown in Figs. 3–5. Most of the metal chelates which did not give peaks possibly decomposed on the HPLC column, because the free concentration of the reagent in the vicinity of the chelate bands was extremely low. This phenomenon is probably based on the difference in the kinetics of the chelate dissociation processes in an HPLC column (typically, the aquation reactions). In our continuing studies, emphasis has been placed on this "kinetic differentiation" of metal chelates which results in the unique selectivity acquired in the HPLC approach[10–15].

It is thus noteworthy that the Zn^{II} ion appears in the reagent systems 2 and 3. The formazan ligand structure may contribute to the stabilization of this "labile cation" chelate, seemingly in accordance with the considerations of Budesinsky and Svec[1]. Additionally, the Cu^{II} gives the peaks in all the systems examined. The coplanarity of the formazan ligands again seems to be favourable for this ion in the RP-HPLC separation. Co^{II} ion gives peaks in reagent systems 1 and 2, in which the oxidation state of cobalt is presumed to be the trivalent judging from its inert nature.

Advantages were found with reagent 3. In addition to Cu^{II} and Zn^{II} ions, this reagent is compatible with the detectability of several trivalent cations such as Al^{III}, Ga^{III} and Mn^{III}. The most likely reason is the hard-base nature of the reagent resulting from the donor assembly with two oxygen atoms. The added form of manganese was the Mn^{II}, but oxidation readily occurred to give the Mn^{III} chelate. As shown in Fig. 4A,

the peaks for Al^{III} and Mn^{III} ions are seriously overlapped, but this was effectively circumvented by adding sodium fluoride $(1 \cdot 10^{-3} M)$ to masking Al^{III} at the derivatization stage. As can be seen in Fig. 4B, the peak due to Al^{III} ion disappears in the presence of fluoride.

On the other hand, only with reagent system 4 was Ni^{II} ion detected. This can be explained in terms of its α-diimine-like structure; bi-, tri- and quadridentate ligands which have five-membered conjugated N,N-coordination (2,2'-bipyridyl-like moiety) probably show selectivity for Ni^{II} ion[10,16,17]. The discussion of this aspect will be presented elsewhere[15].

As this work was basically undertaken to develop the potential utility of formazan compounds in RP-HPLC, the practical applicability to real samples was not been studied in detail. The estimated limits of detection (LOD) of major metal ions are listed in Table II. As the baseline stability of the detector used here is about $2 \cdot 10^{-5}$ absorbance, the LOD is defined as the concentration which gives a signal three times the baseline noise, $1 \cdot 10^{-4}$ absorbance. It is striking that the LODs of most of the metal ions examined are down to the nanomolar range (sub-ppb to ppb level). These data suggest that spectrophotometric methods coupled with RP-HPLC would be complementary to atomic absorption or emission spectrometric techniques. The development
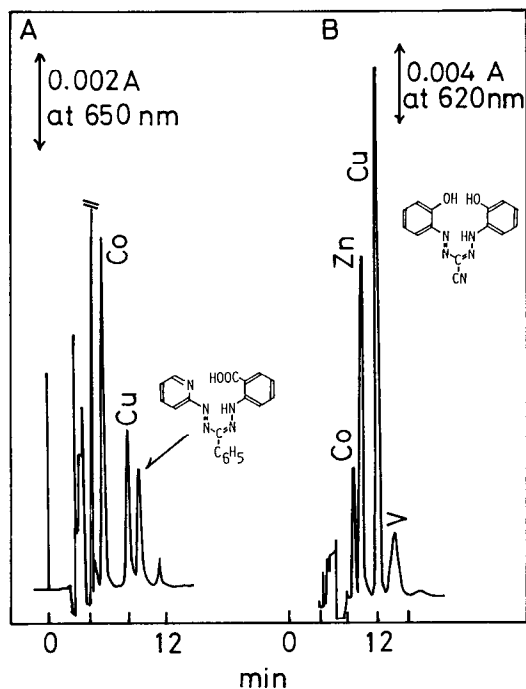

Fig. 3. Typical chromatograms for the formazan reagents 1 (left) and 2 (right). Metal ion concentrations $(10^{-6} M)$: Al 1.00, Be 1.03, Mn 0.990, Ga 0.989, Co 1.09, Cu 1.01, V 1.00, Ni 1.02, Zn 1.02, Cd 1.20, Pb 1.0 and Mo 1.0. Reagent concentrations each $1 \cdot 10^{-4} M$. pH 5.0 (acetate) for 1 and 7.5 (Tris–HCl) for 2. (A) Column, Zorbax CN; mobile phase, 38.1% (w/w) acetonitrile–water, 7.76 mM TBABr, 5 mM sodium acetate, 0.1 mM Na$_2$EDTA; flow-rate, 0.5 ml/min. (B) Column, Zorbax CN; mobile phase, 39.0% (w/w) acetonitrile–water, 3.1 mM TBABr, sodium acetate and Na$_2$EDTA added as in (A); flow-rate, 0.3 ml/min.
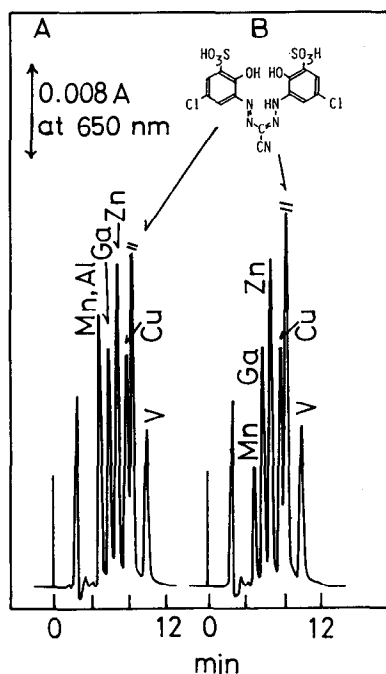
Fig. 4. Chromatograms for the formazan reagent 3 in the absence (left) and presence (right) of sodium fluoride in the sample solution. Concentrations of metal ions and reagent 3 as in Fig. 3; $1 \cdot 10^{-3}$ M NaF added in the final solution; pH 5.0 (acetate). Column, LiChroCart RP-18; mobile phase, 39.0% (w/w) acetonitrile–water, 3.1 mm TBABr, concentrations of sodium acetate and Na_2EDTA as in Fig. 3; flow-rate, 0.5 ml/min.
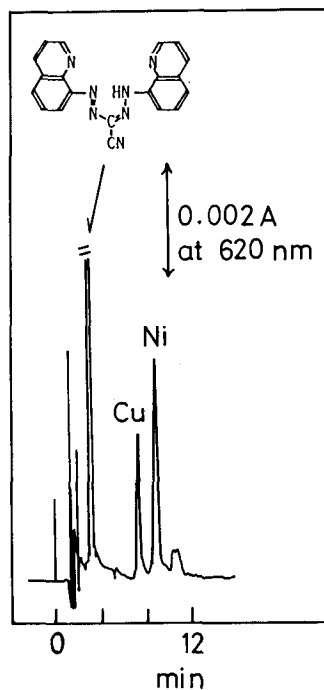
Fig. 5. Typical chromatogram for the formazan reagent 4. Concentrations of metal ions and reagent 4 as in Fig. 3; pH 7.5 (Tris–HCl). Column, Zorbax CN; mobile phase, 60.0% (w/w) acetonitrile–water, 1.86 mm TBABr, 55.8 mm sodium bromide, concentrations of sodium acetate and Na_2EDTA as in Fig. 3; flow-rate, 1.0 ml/min.

TABLE II

LIMITS OF DETECTION (LOD)[a] FOR METAL IONS

Reagent	LOD (nM)[b]						
	V	Mn	Co	Ni	Cu	Zn	Ga
1			15				
			(0.89)				
2					4.2	6.4	
					(0.27)	(0.42)	
3	7.8	11				4.2	6.2
	(0.40)	(0.58)				(0.27)	(0.43)
4				25			
				(1.4)			

[a] LOD is defined as the concentration which gives an absorbance signal of 10^{-4}.

[b] LOD in ppb in parentheses.

of RP-HPLC–spectrophotometric methods using formazan reagents is therefore worthy of investigation in future trace metal analysis.

REFERENCES

1 B. W. Budesinsky and J. Svec, *Inorg. Chem.*, 10 (1971) 313.
2 A. Kawase, *Bunseki Kagaku*, 16 (1967) 1364; 21 (1972) 578.
3 H. Ishii, T. Mizoguchi and T. Odashima, *Bunseki Kagaku*, 26 (1977) 540.
4 M. I. Ermakova, N. L. Vasil'eva and I. Ya. Postovskii, *Zh. Anal. Khim.*, *Engl. Transl.*, 16 (1961) 6.
5 R. M. Rush and J. H. Yoe, *Anal. Chem.*, 26 (1954) 1345.
6 K. L. Cheng, K. Ueno and T. Imamura, *CRC Handbook of Organic Analytical Reagents*, CRC Press, Boca Raton, FL, 1982.
7 M. Lohmuller, P. Heizmann and K. Ballschmiter, *J. Chromatogr.*, 137 (1977) 165.
8 D. E. Henderson, R. Chaffee and F. P. Novak, *J. Chromatogr. Sci.*, 19 (1981) 79.
9 E. B. Edward-Inatimi, *J. Chromatogr.*, 256 (1983) 253.
10 M. Kanbayashi, H. Hoshino and T. Yotsuyanagi, *J. Chromatogr.*, 386 (1987) 191.
11 H. Hoshino and T. Yotsuyanagi, *Chem. Lett.*, (1984) 1445.
12 H. Hoshino and T. Yotsuyanagi, *Anal. Chem.*, 57 (1985) 625.
13 N. Uehara, M. Kanbayashi, H. Hoshino and T. Yotsuyanagi, *Talanta*, 36 (1989) 1031.
14 H. Hoshino, K. Nakano and T. Yotsuyanagi, *Analyst (London)*, 115 (1990) 133.
15 N. Iki, H. Hoshino and T. Yotsuyanagi, *Analyst (London)*, submitted for publication.
16 J. W. O'Laughlin, *Anal. Chem.*, 54 (1982) 178.
17 M. Mangia and M. T. Lugari, *J. Liq. Chromatogr.*, 6 (1983) 1073.