

CHROM. 11,724

DISSOCIATION OF METAL DITHIZONATES DURING GEL CHROMATOGRAPHY

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(First received October 13th, 1978; revised manuscript received January 8th, 1979)

SUMMARY

Metal dithizonates in carbon tetrachloride were dissociated through gel chromatographic columns packed with Sephadex LH-20 or Merckogel OR-PVA 2000. The liberated metal ions were adsorbed on the surface of the gels, and the dithizone in its neutral form was eluted from the column. Detailed investigation of this phenomenon was carried out by batch methods. The proposed mechanism is the generation of hydrogen chloride caused by the interfacial reaction of the gels with carbon tetrachloride.

INTRODUCTION

In recent years, gel chromatography has been applied to the investigation of metal complexes¹⁻¹⁰. We have previously^{5,6} suggested that the gel chromatographic behaviour of EDTA complexes in aqueous media can be explained mainly by sieving effect. We have also investigated the behaviour of metal dithizonates on gels in non-aqueous media. When dithizonates of some heavy metals were chromatographed on a Sephadex LH-20 column using carbon tetrachloride as eluent, copper dithizonate was immediately eluted from the column as a dithizonate, whereas the ions Cd^{2+} , Hg^{2+} , Pb^{2+} and Zn^{2+} were adsorbed at the top of the column and the liberated dithizone was easily eluted from the column⁷. This stripping-like phenomenon is obviously due to the dissociation of metal dithizonate and, in this respect, is very similar to the back-extraction process of dithizonate from organic phase to aqueous phase. This suggests that effects more complicated than the sieving effect may participate in the gel chromatographic behaviour of dithizonate in media such as carbon tetrachloride, and we have undertaken further studies to elucidate them.

In this paper, the behaviour of some heavy-metal dithizonates on gel phases is reported. Model experiments were designed in connection with gel matrixes, solvents and solutes, and were carried out by batch operations. Moreover, this phenomenon was applied to simplified purification of dithizone reagent, the concentration of trace metal ions and the fractionation of metal ions.

EXPERIMENTAL

Reagents and sample solutions

Dithizone, carbon tetrachloride and chloroform were purified by the JIS method¹¹, from which dithizone solutions in carbon tetrachloride (0.01%, w/v) and chloroform (0.1%, w/v) were prepared. Metal dithizonate solutions were prepared according to the JIS method: aqueous metal solutions were adjusted to the correct pH (see Table I) with buffer solutions and then extracted successively with the dithizone solutions until they showed no colour changes. These extracted solutions of the metal dithizonates were used as the sample solutions.

TABLE I
EXTRACTION pH OF SOME METAL DITHIZONATES

<i>Metal</i>	<i>Extraction pH</i>	<i>Buffer solution</i>
Silver(I)	7.0	barbital sodium + hydrochloric acid (primary dithizonate)
Cadmium(II)	12.0	sodium phosphate + sodium hydroxide (primary dithizonate)
Copper(II)	3.7	acetic acid + sodium acetate (primary dithizonate)
	12.0	sodium phosphate + sodium hydroxide (secondary dithizonate)
Mercury(II)	3.7	acetic acid + sodium acetate (primary dithizonate)
	12.0	sodium phosphate + sodium hydroxide (secondary dithizonate)
Lead(II)	12.0	sodium phosphate + sodium hydroxide (primary dithizonate)
Zinc(II)	8.9	barbital sodium + hydrochloric acid (primary dithizonate)

The other reagents used were, unless otherwise stated, commercially available analytical or chemical pure reagent grade.

Procedure

Column methods. Sephadex LH-20 (Pharmacia, Uppsala, Sweden), previously swollen with carbon tetrachloride or chloroform for 2 days, was packed in a glass tube (100 × 5.5 mm) to a height of *ca.* 5 cm. At the top of the column, a 1-ml portion of the sample was injected and then eluted with carbon tetrachloride or chloroform.

Batch method 1. This method was used to investigate the dissociation mechanism of the dithizonates. Sephadex LH-20 was swollen in a solvent for 2 days, extracted and air-dried. The dried gel (0.002–0.5 g) was weighed and shaken with a 10-ml portion of the sample solution for a few minutes. The mixture was filtered, and the filtrate was subjected to absorption spectrophotometry.

Batch method 2. This method was used to recover the metal ion adsorbed on the gel. Sephadex LH-20 was swollen in a solvent for 2 days, extracted and air-dried. The dried gel (0.2–0.4 g) was weighed and shaken with a 10-ml portion of the sample solution for a few minutes. The suspension was filtered, and the filtered gel was air-dried again. The dried gel was washed with redistilled water on the glass filter. The filtrate was subjected to atomic absorption spectrophotometry.

Apparatus

A Hitachi Model EPS 20 UV spectrophotometer was used to identify metal complexes. To determine the amount of metal ion adsorbed on the gel, a Hitachi Model 508 atomic absorption spectrophotometer was used. To determine the concentration of hydrogen ions and chloride ions liberated from the gel, a pH meter (Hitachi-Horiba F-5) and a chloride ion meter (Toa Ion Meter 1M1B) were used. The absorption spectrum of the gas released from the swollen gel was measured with a Nippon Bunko DS-403G IR absorption spectrophotometer.

RESULTS AND DISCUSSION

Column chromatographic behaviour of some metal dithizonates

At the top of the column, *ca.* 1 ml of the sample solution (metal dithizonate of $2-4 \cdot 10^{-5}$ M in carbon tetrachloride) was injected and then eluted with carbon tetrachloride at $20 \pm 1^\circ$. When a sample solution of a zinc(II), cadmium(II), lead(II) or mercury(II) complex was chromatographed, the colour of the solution immediately turned greenish at the top of the column bed. This green band was easily eluted by successive elution with carbon tetrachloride. The visible absorption spectrum of the eluate was identical with that of dithizone in carbon tetrachloride solution. On the other hand, the dithizone complex of copper(II) was not affected by this treatment.

According to the selective adsorption on the gel column, metal ions can be easily separated into two groups: one is retained tightly in the column as a result of the dissociation of the dithizonates (zinc(II), cadmium(II), lead(II) and mercury(II)) and the other is eluted as the dithizonate (copper(II)). The metal ions retained in the column were eluted almost completely with prewashing (*ca.* 20 ml of acetone) and *ca.* 5 ml of aqueous EDTA solution. Experimental results of the percentage recoveries of the metal ions adsorbed on the gel are given in Table II.

TABLE II
RECOVERIES OF ADSORBED METAL IONS IN THE COLUMN METHOD

Metal ion	Taken (μg) *	Found (μg) **	Recovery (%)
Zinc(II)	9.50	8.50	89.5
	7.50	7.38	98.4
Cadmium(II)	5.03	4.74	94.2
	8.31	8.46	101.8
Lead(II)	28.8	22.8	78.2
	8.49	8.24	97.1

* Atomic absorption spectrophotometry after back-extracting sample solution with HCl (1 + 10).

** Atomic absorption spectrophotometry.

By this procedure, zinc(II), cadmium(II) and lead(II) present as impurities in commercial dithizone of analytical reagent grade were eliminated and were analysed by the atomic absorption method (zinc(II), 0.016%; cadmium(II), not detectable; lead(II), 0.004%). The method is applicable to the simple and rapid purification of the dithizone reagent by the removal of these contaminants.

Dissociation of metal dithizonates by batch method I

Some characteristically coloured metal dithizonates were turned greenish during the gel chromatographic procedure, suggesting that dissociation occurs. Batch procedure I was used to study this phenomenon in more detail. A typical absorption spectra change for the dithizonate of lead (II) is shown in Fig. 1. The dithizonates of silver(I), cadmium(II) and zinc(II) showed the same pattern of dissociation. The dithizonates of copper(II) and mercury(II) showed different patterns of dissociation (Figs. 2 and 3).

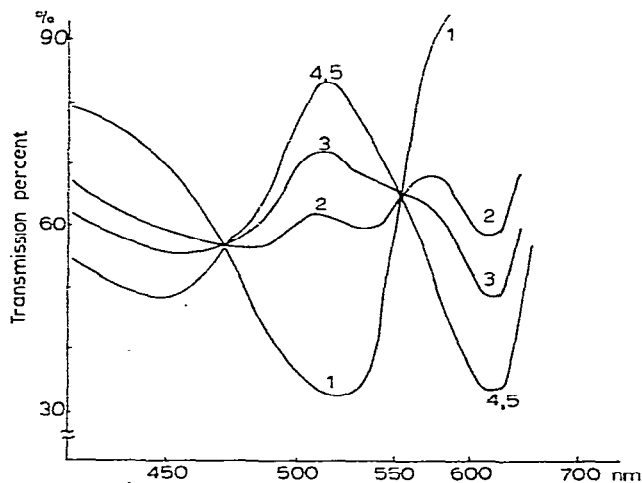


Fig. 1. Spectral change of lead(II) dithizonate on addition of swollen gel. Amounts (g) of swollen gel added in the dithizonate-carbon tetrachloride solution: 1, none; 2, 0.005; 3, 0.008; 4, 0.052; 5, 0.063.

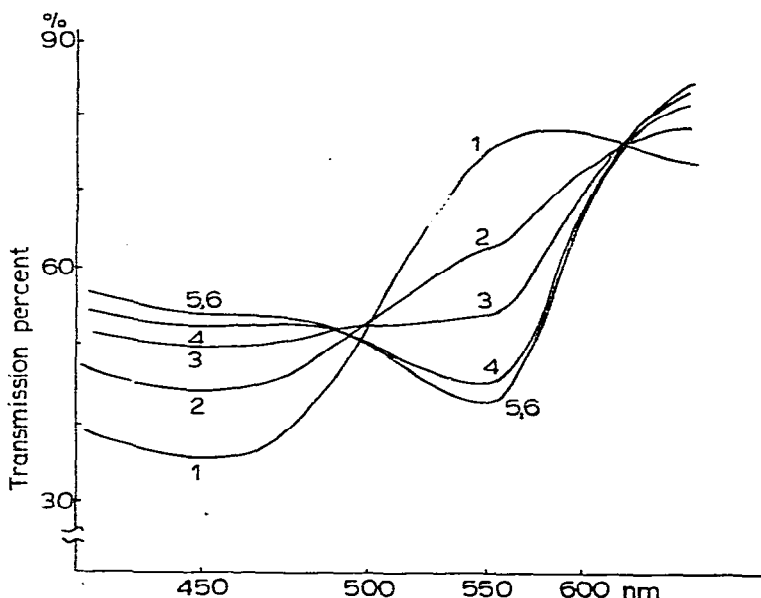


Fig. 2. Spectral change of copper(II) dithizonate on addition of swollen gel. Amounts (g) of swollen gel added in the dithizonate-carbon tetrachloride solution: 1, none; 2, 0.021; 3, 0.085; 4, 0.232; 5, 0.314; 6, 0.512.

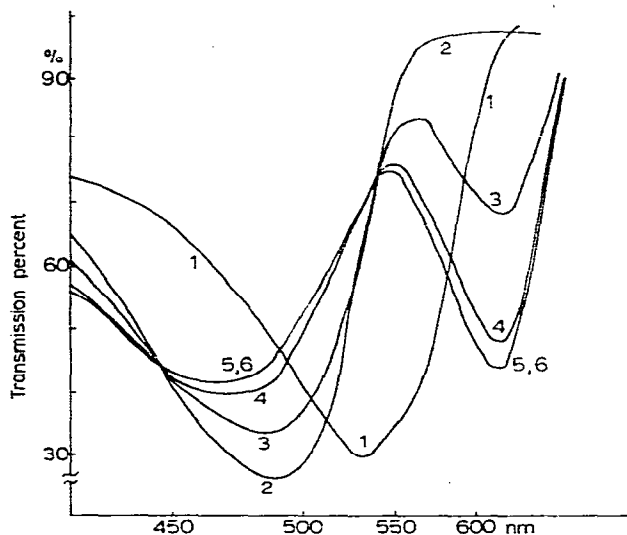


Fig. 3. Spectral change of mercury(II) dithizonate on addition of swollen gel. Amounts (g) of swollen gel added in the dithizonate-carbon tetrachloride solution: 1, none; 2, 0.045; 3, 0.081; 4, 0.091; 5, 0.118; 6, 0.130.

These dissociation patterns were classified in the following three categories.

(1) Primary dithizonate \rightarrow metal ion + dithizone molecule

Silver(I), cadmium(II), lead(II) and zinc(II) belong to this group.

(2) Secondary dithizonate \rightarrow primary dithizonate \rightarrow metal ion + dithizone molecule

Mercury(II) belongs to this group.

(3) Secondary dithizonate \rightarrow primary dithizonate

This dissociation does not proceed as far as the free ion and dithizone molecule. Copper(II) belongs to this group.

These three patterns show a good correlation with the back-extraction of metal dithizonates from organic solvents to aqueous media: the changes of the absorption spectra of these dithizonates suggest that addition of the gel swollen in carbon tetrachloride to metal dithizonate solutions corresponds to the acidification of the aqueous phase in the back-extraction procedure. The third category classified on the basis of absorption spectrometry seems to be in good agreement with the conventional experimental fact that the quantitative back-extraction of copper(II) dithizonate is difficult and incomplete even in highly acidic conditions^{12,13}.

Adsorbed metal ion and the recovery by batch method 2

Free metal ions were not detected either in the green effluent from the Sephadex LH-20 column or in the filtrate in the batch procedure. These facts suggest that the free metal ions liberated from their dithizonates are retained in the gel. In order to recover completely the metal ions adsorbed on the gel, batch method 2 was used. In the column method, the recoveries listed in Table II fluctuated too much and the procedure was somewhat difficult, perhaps owing to the different swelling properties of the gel in the presence of acetone and carbon tetrachloride. These difficulties are

avoided in the batch procedure. As mentioned in the experimental section, the gel adsorbing the metal ions was first dried and then washed on a sintered glass filter plate with various solutions, such as 0.1 *M* HCl, 0.01 *M* EDTA and redistilled water. It turned out that the redistilled water was just as effective as the other two. The data listed in Table III show that satisfactory recoveries were obtained by washing with redistilled water, except for mercury(II) dithizonate.

TABLE III
RECOVERIES OF ADSORBED METAL IONS IN THE BATCH METHOD

Metal ion	Taken (μg) *	Found (μg) **	Recovery (%)	Gel amount added (g)
Zinc(II)	8.76	8.63	98.5	0.2
Cadmium(II)	13.83	14.05	101.6	0.2
Lead(II)	10.6	10.5	99.1	0.2
Mercury(II)***	17.45	13.44	77.0	0.2
Mercury(II)***	11.23	10.03	89.3	0.4

* Atomic absorption spectrophotometry after back-extracting sample solution with HCl (1 + 10).

** Atomic absorption spectrophotometry.

*** Back-extraction with HCl (1 + 1).

Behaviour of metal dithizonates in carbon tetrachloride with Merckogel OR-PVA 2000

Merckogel OR-PVA 2000 (Merck, Darmstadt, G.F.R.), a cross-linked poly(vinyl acetate) gel, showed the same dissociation phenomena with the metal complexes. Zinc(II) dithizonate in carbon tetrachloride, for example, was dissociated with the gel swollen in carbon tetrachloride (batch method 1). Fig. 4 shows the absorption spectra of the dithizonate from the two gels. The results suggest that the dissociative effect of Merckogel is somewhat less than that of Sephadex LH-20.

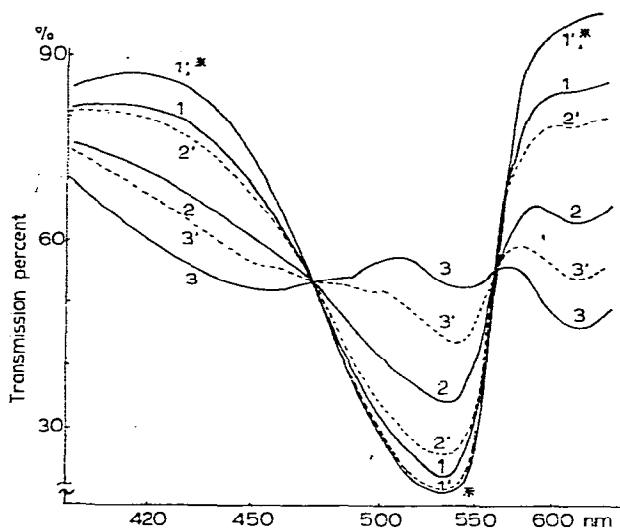


Fig. 4. Spectral change of zinc(II) dithizonate on addition of swollen gel. Dashed curves: Merckogel OR-PVA 2000; continuous curves: Sephadex LH-20. Amounts (g) of swollen gels added in the dithizonate-carbon tetrachloride solution: 1 and 1', 0.02; 2 and 2', 0.05; 3 and 3', 0.10. The asterisk denotes the dithizonate-carbon tetrachloride solution.

The effect of solvent

The dissociation effects were studied in solvents other than carbon tetrachloride: chloroform, dichloromethane, acetone and cyclohexane. Sephadex LH-20 or Merckogel OR-PVA 2000 were swollen in these solvents for about 2 days, filtered and air-dried. In these solvents, the metal dithizonates used in this study showed no change with the air-dried gel (batch method 1), except that in chloroform a slight change occurred after *ca.* 5 days.

Investigation of the dissociation of metal dithizonates

Formation of hydrogen ions on the gel by carbon tetrachloride. As the dissociation of metal dithizonate in the carbon tetrachloride-gel system is very similar to the back-extraction procedure, it is reasonable to consider that hydrogen ions may play an important role in the dissociation. It could be assumed that hydrogen ions originate from the interaction between the solvent and the gel. To verify this assumption, qualitative tests of pH in the solvent-gel mixed system were undertaken. Each 10 ml of solvents was mixed with 0.3 g of gel (Sephadex LH-20), and the suspended solutions were left for about 2 days. Pieces of pH test-papers were directly immersed in the solution and in the solvent itself, and the colour change was compared with the reference. A distinct colour change, indicating the existence of hydrogen ions, occurred in the carbon tetrachloride solvent. No colour changes were observed for solvents such as dichloromethane, acetone, cyclohexane, toluene and dioxane. A slight colour change occurred in chloroform, indicating a very low hydrogen ion activity.

For the carbon tetrachloride solvent, more detailed tests using pH paper were carried out. The suspension was filtered through a sintered glass disk, and the filtrate and the swollen gel were checked with pH paper. The former did not contain any hydrogen ions, but the latter showed strong hydrogen ion activity. This fact clearly indicates that the hydrogen ions are located on the surface of the gel.

The hydrogen ions should be accompanied by some negative counter-ions. When the suspension of the gel in carbon tetrachloride was titrated with dilute AgNO_3 solution, a white precipitate (AgCl) was observed. The hydrogen ions on the surface of the gel were not detected in the filtrate, but they were eluted when the gel swollen in carbon tetrachloride was air-dried and washed with redistilled water. The quantitative determination of hydrogen ions and chloride ions was carried out by batch procedure. After the gel was swollen in carbon tetrachloride for 4–30 days, the swollen gel was air-dried and washed with 10 ml of redistilled water. The rinsed water was then used to determine both hydrogen ion and chloride ion. These ions were measured by pH meter and chloride ion selective electrode, respectively. The results are listed in Tables IV and V. These data suggest that the concentrations of the hydrogen ions and the chloride ions released from the gel are almost comparable and that their concentrations increase with the increasing amount of gel added to the solvent. The concentrations of these ions also increased, to some extent, with increased swelling time. After the swollen gel was air-dried, a small amount of the gel was sealed in the IR absorption cell (gas cell) and warmed; the characteristic rotation spectrum of hydrogen chloride was observed. The spectrum was positive proof of the existence of hydrogen chloride on the gel.

On the other hand, Sephadex LH-20 itself was somewhat decomposed by contact with carbon tetrachloride. The colour of the gel in carbon tetrachloride varied

TABLE IV

VARIATION OF HYDROGEN ION AND CHLORIDE ION CONCENTRATIONS IN THE PRESENCE OF DIFFERENT AMOUNTS OF GEL AFTER 5 DAYS SWELLING

Gel amount (g)	$[H^+]$ value (M)	$[Cl^-]$ value (M)
0.006	$4.90 \cdot 10^{-4}$	$2.41 \cdot 10^{-4}$
0.022	$1.32 \cdot 10^{-3}$	$9.26 \cdot 10^{-4}$
0.028	$1.62 \cdot 10^{-3}$	$1.23 \cdot 10^{-3}$
0.039	$2.04 \cdot 10^{-3}$	$1.69 \cdot 10^{-3}$
0.053	$3.02 \cdot 10^{-3}$	$2.76 \cdot 10^{-3}$

TABLE V

VARIATION OF HYDROGEN ION AND CHLORIDE ION CONCENTRATION AS A FUNCTION OF SWELLING TIME

Day	$[H^+]/g$	$[Cl^-]/g$	Ratio
4	$2.37 \cdot 10^{-2}$	$1.41 \cdot 10^{-2}$	1.68:1
5	$5.68 \cdot 10^{-2}$	$4.54 \cdot 10^{-2}$	1.25:1
30	$6.80 \cdot 10^{-2}$	$6.00 \cdot 10^{-2}$	1.13:1

from the normal white to light yellow after about a week and turned brownish after about a month. This coloration was very similar to the caramelization of the gel caused by warming the suspension of the gel in dilute HCl solution for *ca.* 30 min. Moreover, the gel swollen in carbon tetrachloride showed a positive sugar test with the tryptophan-sulphuric acid reaction or the phenol-sulphuric acid reaction. These results suggest that Sephadex LH-20 gel is gradually hydrolysed by hydrogen chloride formed during the swelling process in carbon tetrachloride.

Mechanism of dissociation of metal complexes in carbon tetrachloride with Sephadex LH-20 gel. The results of this study can be summarized as follows.

- (1) Considerable amounts of hydrogen and chloride ions are formed when a gel such as Sephadex LH-20 is swollen in carbon tetrachloride.
- (2) The hydrogen and chloride ions appear only at the interface of the gel phase and cannot be detected in the filtrate that is separated from the gel.
- (3) Metal dithizonates, except for the copper(II) complex, are completely dissociated into metal ions and free dithizone in contact with the swollen gel, and the metal ions remain in the gel phase. The adsorbed metal ions are recovered almost completely by washing with redistilled water after drying the gel.

The results are very similar to those obtained in the back-extraction of metal complexes in organic phase into aqueous phase. The aqueous phase corresponds to the gel phase in the present carbon tetrachloride system. When viewed from the point of the effectiveness of back-extraction, the hydrogen ion activity arising from the swollen gel under the usual conditions may be insufficient to cause dissociation of copper(II) dithizonate. In practice, the dissociation of copper(II) dithizonate was observed when the gel was allowed to stand for about a month in contact with carbon tetrachloride before the copper(II) dithizonate was added to the mixture.

The formation of hydrogen chloride was not characteristic of gel matrices used, but was also observed in mixtures of high molecular ethers, such as ethylene glycol dimethyl ether, and carbon tetrachloride. This suggests that some of the functional

groups in the gel should play an important role in the generation of hydrogen chloride.

From the above results, the following mechanism for the dissociation of metal dithizonates can be proposed.

(1) The interfacial reaction at the gel between carbon tetrachloride and water sorbed in the gel matrix gives rise to the evolution of hydrogen chloride.

(2) The hydrogen chloride is adsorbed on to the surface of the gel, owing to its strong polarity.

(3) The dissociation of metal dithizonate into metal ion and free dithizone will be caused by the active hydrogen chloride on the surface of the gel in such a fashion that the metal ion bonded to dithizone is substituted by the hydrogen ion.

(4) The liberated dithizone molecules would not be further protonated unless the hydrogen ion concentration is very high, and then they are left in the carbon tetrachloride phase.

(5) The metal ions are adsorbed on to the surface of the gel, probably as chlorides.

If non-aqueous solvents such as carbon tetrachloride or chloroform, which contained chlorine, are used as eluents in chromatography with amphoteric gels such as Sephadex LH-20 or Merckogel OR-PVA 2000, then the generation of hydrogen chloride on the surface of the gels should be considered to be likely to occur.

ACKNOWLEDGEMENT

This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Japan.

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