Mendeleev Commun., 1997, 7(3), 119–120

Enzymatic determination of organomercury compounds and mercury(II) after their separation by thin-layer chromatography on 'Silufol'

Tatyana N. Shekhovtsova,* Svetlana V. Muginova and Nailya A. Bagirova

Department of Chemistry, M. V. Lomonosov Moscow State University, 119899 Moscow, Russian Federation. Fax: +7 095 939 4675; e-mail: shekhov@chromat.chem.msu.su

A new, selective test technique for the determination of organomercury compounds and mercury(II) combines their preliminary separation by thin-layer chromatography on silica with further enzymatic determination employing *o*-dianisidine oxidation catalysed by horseradish peroxidase.

It is well known that mercury and its derivatives are very dangerous toxicants.[1] Methods available for the differential determination of inorganic mercury and organomercury compounds (OMCs) in water and other environmental samples discussed in the literature of the last 10 years are based as a rule on the different behaviour of mercury compounds towards reduction[2] or sorbtion[3–9] and involve two steps: separation and determination. The separation of OMC mixtures was carried out chromatographically using gas,[3] HPLC,[4–6] paper[7] and thin-layer chromatography (TLC) on silica[8] or Al_2O_3.[9] Various spectroscopic methods were used for the determination of separated forms of organic mercury. However, highly sensitive, simple and rapid enzymatic methods have not yet been applied for these purposes.

Recently[10,11] highly sensitive visual chromogenic spot test procedures for the determination of mercury(II) and methyl-, ethyl- and phenyl-mercury have been developed using paper-immobilised horseradish peroxidase (HRP) with lower limits of analytical concentrations of 50 pmol dm^{-3}, (RSD[†] = 13%, n[‡] = 6), 1 (RSD = 23%, n = 3), 11 (RSD = 18%, n = 3), 12 (RSD = 19%, n = 3) mol dm^{-3}, respectively. Oxidation of *o*-dianisidine, *o*-phenylenediamine and 3,3′,5,5′-tetramethyl-benzidine catalysed by HRP were used as indicators. The determination of mercury(II) was based on its inhibitory effect on enzyme.[10] OMCs were determined using their liberating action[12] on HRP inhibited previously by phenylthiourea. This action may be explained by the interaction of OMCs with phenythiourea, which leads to a decrease in the inhibitory action of the latter and an increase in the HRP activity proportional to the concentration of OMC. The shortcomings of those procedures lie in the impossibility of determining selectively one particular OMC in the presence of others.

The aim of this work was to develop an enzymatic visual test method for the determination of individual OMCs (methyl-, ethyl-, phenyl-mercury) after their separation by TLC on 'Silufol'. The liberating action of OMCs on HRP inhibited by phenylthiourea described above and the inhibitory effect of mercury(II) on the same enzyme provided the basis of this method.

Optimisation of separation conditions on a 'Silufol' plate. – Conditions for the chromatographic separation of CH_3HgI, C_2H_5HgBr and C_6H_5HgCl were studied. A mixture of hexane and ethanol was chosen as the mobile phase according to the data in the publication.[8] It was found that the most effective separation of the OMCs investigated ($R_f > 0.20$) – by one-dimensional TLC on a plate of 'Silufol' (stationary phase) – was observed when the volume ratio of hexane to ethanol in the mobile phase was equal to 5 : 1. Under these conditions their R_f values were 0, 0.63 and 0.42, respectively. Two-dimensional TLC was used to separate OMCs and mercury(II). It was found that the mobile phase should contain a mixture of hexane and ethanol in a volume ratio 5 : 1 for the first step and 9 : 1 for the second step. Under these conditions mercury(II) stayed on the start-line, and R_f of CH_3HgI, C_2H_5HgBr and C_6H_5HgCl (mixed in their volume ratio 1 : 1 : 1) equalled 0.23, 0.85 and 0.41, respectively.

Determination of OMCs and mercury(II) on 'Silufol'. – The reaction of *o*-dianisidine oxidation by H_2O_2 catalysed by native HRP was chosen as an indicator for studying the possibility of determining of OMCs on 'Silufol'. In the course of this reaction the intermediate and final products of *o*-dianisidine oxidation (green and red colours, respectively) were observed visually. The rate of enzymatic reaction was characterised by the time of appearance of the red colour of the final product. The indicator reaction was carried out under optimum conditions stated previously.[§]

These conditions are as follows. Using a micropipette, 5 l of 0.1 M phenylthiourea, 1 l of 12 M peroxidase solution and 2 l of 5 mM *o*-dianisidine solution were applied sequentially to a point on the surface of a 'Silufol' plate according to the R_f value of the individual OMC. In order to minimize spot broadening each spot was dried in the air after dropping each component of the reaction onto the plate. At the moment when 1 l of 5 mM H_2O_2 was added, a stopwatch was started, and the time taken for the spot to assume a red colour was measured. The participation of methyl-, ethyl- and phenyl-mercury, separated preliminary on 'Silufol', in the indicator reaction resulted in a decrease in the time taken for red colour appearance in a spot and in a consequent decrease in the inhibitory effect of phenylthiourea on HRP. It was shown that anion nature has no influence on the rate of the indicator process. In order to determine OMCs after their separation on 'Silufol' it was necessary to conduct a control experiment: to introduce all the components of the reaction (following the procedure described) onto the same plate of 'Silufol' to the point where there was no OMC. The existence of a proportional dependence of the time taken for red colour appearance on the concentration of methyl-, ethyl- and phenyl-mercury cations (for example, for concentrations of methylmercury of 5.7, 28.5, 57 M this time was equal to 32±4, 24±2, 16±3 s, respectively; in the absence of OMCs this time equalled 40±2 s) allowed us to develop chromagenic spot tests for their visual determination on 'Silufol' after their chromatographic separation by TLC with the metrological characteristics presented in Table 1.

In addition, it was shown that mercury(II) might be also determined on 'Silufol' after its separation from OMCs. The determination of mercury(II) is based on its inhibitory effect,

[†] Relative standard deviation (%) at observed concentration and confidence level (P) 95%.

[‡] The number of replicate procedures.

[§] *Experimental details.* Horseradish peroxidase (1.11.1.7) was obtained from Reanal (Budapest, Hungary) (RZ = A_{403}/A_{287} = 3.28). The enzyme water solutions were obtained by dissolving the commercial enzyme preparation in sodium borate buffer (pH 7.0), containing 20 vol.% of 0.1 M sodium nitrate to maintain constant ionic strength. Solutions of *o*-dianisidine (Sigma), sulfur-containing organic compounds (thiourea and phenylthiourea, analytical grade reagents from 'Soyuz Reactive', Moscow, Russia), OMCs (CH_3HgI, C_2H_5HgBr, C_6H_5HgCl, synthesized and purified specially) were prepared daily by dissolving accurately weighed amounts in ethanol, solutions of H_2O_2 (Merck) and mercury(II) nitrate (Ekoanalitika, Russia) in water. Double-distilled and demineralized water was used. 'Silufol' plates were obtained from 'Kovaler' (Czech Republic).

Table 1 Metrological characteristics of the chromagenic spot tests for the determination of individual OMCs on 'Silufol' using peroxidase oxidation of *o*-dianisidine in the presence of phenylthiourea.

Cation of OMC	C_1^a/ M	t^b/s	RSD^c (%) (n = 3, P = 95%)
CH_3Hg^+	5.7±3.6	32±4	26
$C_2H_5Hg^+$	6.0±3.7	28±1	25
$C_6H_5Hg^+$	6.7±3.8	24±4	23

[a]The lower limit of analytical concentration. [b]Time of appearance of the red colour in a spot. [c]The relative standard deviation for a cation of OMC at its concentration equal to C_1.

which increased in the presence of thiourea, on HRP in the reaction of *o*-dianisidine oxidation (C_{min} = 50 pmol dm^{-3}). The optimum conditions for this reaction were the following: 1 l of 12 M peroxidase solution, 1 l of 25 mM thiourea solution, 2 l of 5 mM *o*-dianisidine solution and 1 l of 5 mM H_2O_2. All the components were added to the corresponding point on the start-line. The analytical signal and the mean of its measurement were the same as in the procedure for OMC determination.

Thus, a combination of preliminary chromatographic separation of CH_3HgI, C_2H_5HgBr, C_6H_5HgCl and mercury(II) by TLC on 'Silufol' with visual enzymatic detection makes it possible to determine these compounds selectively and with good sensitivity.

References

1 X. Zigel and A. Zigel, *Nekotorye voprosy toksichnosti metallov*, Mir, Moscow, p. 366 (in Russian).
2 Yu. V. Zelyukova and M. M. Novoselov, *Rtut' v rekakh i vodoemakh. Tezisy dokladov Vsesoyuznogo simpoziuma* (*Mercury in rivers and water bodies. Abstr. of USSR symposium*), Novosibirsk, 1990, p. 62 (in Russian).
3 C. J. Cappon and T. J. Joribara, *LC and GC*, 1986, **74**, 1010.
4 S. Corrado, *Pittsburg Conference Presents*, New Orleans, 1992, p. 310.
5 W. Langsith, *J. Chromat.*, 1988, **438**, 414.
6 E. Bulska, D. C. Baxter and W. Frech, *Anal. Chim. Acta*, 1991, **249**, 545.
7 M. Hempel, H. Hintelman and R.-D. Wilken, *Analyst*, 1992, **117**, 669.
8 M. P. Volynets, R. F. Gur'eva, T. V. Dubrova, B. F. Myasoedov and S. B. Savvin, *Zh. Anal. Khim.*, 1991, **46**, 1595 [*J. Anal. Chem. USSR* (*Engl. Transl.*), 1991, **46**, 1160].
9 V. V. Ispravnikova, *Trudy instituta prikladnoi geofiziki*, 1990, **76**, 131 (in Russian).
10 T. N. Shekhovtsova and S. V. Chernetskaya, *Anal. Lett.*, 1994, **27**, 2883.
11 T. N. Shekhovtsova, S. V. Chernetskaya and N. A. Bagirova, *International Workshop on Peroxidase Biotechnology and its Applications*, Pushchino, Russia, 1995, p. 59.
12 T. Keleti, *Basic Enzyme Kinetics*, Academia Kiado, Budapest, 1990, p. 234.

Received: Moscow, 20th November 1996
Cambridge, 4th February 1997; Com. 6/08044H