SHORT COMMUNICATIONS

SPECTROPHOTOMETRIC DETERMINATION OF Pt(IV) AFTER EXTRACTION OF ITS 2-FURANTHIOCARBOXHYDRAZIDE-COMPLEX WITH MOLTEN NAPHTHALENE

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SEVERAL hydrazides have been reported for the spectrophotometric determination of Pt(IV)[1-3] But these methods require multiple extraction for quantitative recovery as the distribution equilibrium is slow. In order to eliminate this drawback, a new technique of analysis of metals by solid-liquid separation after liquid-liquid extraction at elevated temperatures using naphthalene is developed. In the proposed method of determining Pt(IV) with 2-Furanthiocarboxhydrazide (FTH), distribution equilibrium was attained rapidly. The sensitivity and selectivity was also enhanced. The method is simple, rapid and selective and also suitable for the determination of Pt(IV) in Pt-Rh alloy.

A solution of platinum chloride was prepared by dissolving 100 mg of pure metal (99.9%) in aqua regia and diluted to 100 ml (0.005 M). It was standardised gravimetrically[4]. 0.01 M solution of FTH was prepared[3] in ethanol and protected from light.

Standard solutions were prepared from A. R. grade reagents. The organic solvents were used after double distillation. The absorbance measurements were carried out with a spectrophotometer (Beckman DU-2) with 1cm quartz cells. The pH measurements were made with a Philips PR 9405 L.

An aliquot of the test solution containing 10 to 80 μg of Pt(IV) and 1 ml of 5% solution of sodium potassium tartrate was taken in an Erlenmeyer flask and to this was added 2ml of 0.01 M FTH solution. The pH of the solution was adjusted to 5.5 with dil HCL and NaOH solutions. The solution was then heated on a steam bath for 15 min followed by the addition of 2g of solid naphthalene. The heating was continued to melt the naphthalene completely. The flask was stoppered, shaken vigorously and cooled. The solidified naphthalene containing the blue complex was filtered washed with water, dried at room temperature by blotting the surplus water in the folds of dry filter and then dissolved in methyl isobutyl ketone (MIBK). The solution was diluted to 25ml with MIBK. The absorbance of the extract was measured at 710nm against MIBK blank.

The FTH in ethanol reacts with platinum(IV) on heating for 15 min on steam bath over a pH range of 5-6.5 to form the complex which is extractable readily into molten naphthalene. The complex in naphthalene MIBK solution exhibits an absorption maximum at 710nm. A twenty fold amount of the complexing reagent is required for full colour development of 2×10^{-6}g of Pt(IV) using 2g of naphthalene. The molar absorptivity of the complex is 33860 L mol^{-1} cm^{-1} and the Sandell sensitivity of the reaction is 0.0057 μg cm^{-2}. Beer's law is obeyed for the range 0.4 to 3.2 μg Pt(IV) ml^{-1}. The composition of the complex was determined by Job's method of continuous variation and the mole ratio is 1:2 with respect to metal and ligand.

The interference of diverse ions was studied by using 2 μg Pt(IV) ml^{-1}, following the recommended procedure. It was found that the tolerance limit for U(VI), Re(VI), W(VI), Mo(VI) and Mn(II) was high as compared to that obtained for FTH method of Shome.[3] The interference due to Co(II) and Au(III) can be removed by masking with thiocyanate and fluoride respectively. EDTA was used as a masking agent for the removal of interfering ions Fe(III), Ni(II), Ru(III) and Cu(II). The interference of Os(VIII) was eliminated by taking advantage of the fact that the Os(VIII) readily forms a complex with FTH in a cold solution at pH 2 which is extractable with CCl_4, while Pt(IV) remains in aqueous phase Upto 2000 fold amounts of tartrate, acetate, phosphate and EDTA, and a 100 fold of thiosulphate, thiourea, thiocynate are without effect in the determination of Pt(IV). Zinc(II), Cd(II), Hg(II), V(V), Ir(III), Ti(IV) and Cr(VI) are tolerated in 50 times that of Pt(IV). The tolerance limit for Pd(II) and Tl(I) is 1:3 while for Se(IV) and Te(IV) it is 1:1.

Five determinations of the synthetic mixtures corresponding to Pt-Rh thermocouple wire containing 22.5 μg of platinum yielded the value 22.75 μg with a relative error 1.1%. The values obtained by this method show excellent agreement with the diethyldithio-carbamate method[5]

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1. Shome, S. C. and Gangopadhyay, P. K. *Z. Anal. Chem.*, 1976, **28**, 143.
2. Gangopadhyay, P. K., Das, H. R. and Shome, S. C. *Anal. Chem. Acta.*, 1973, **66**, 460.
3. Shome, S. C., Nandy, S., Guhathakurta, A., Ghosh, N. C., Das. H. R. and Gangopadhyay, P. K., *Microchim. Acta (Wein).*, 1978(II), 343.
4. Hillbrand, W. E., Lundell, G. E. F., Bright, H. A. and Hofman, J. I. *Applied inorganic analysis.*, 2nd Ed., Wiley, New York, 1953, p 368.
5. Sandell, E. B. *Colorimetric determination of traces of metals.*, 3rd Ed., Interscience, New York, 1965, p 731.

MODIFICATION OF A SINGLE-STEP SEPARATION PROCEDURE FOR SEVERAL PROTEIN CONSTITUENTS OF VENOM OF THE INDIAN COBRA (*NAJA NAJA*)

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SNAKE venoms are the complex mixtures of cardiotoxins, neurotoxins, cytotoxins, enzymes and other protein constituents with several or many pharmacologic activities. The single-step procedures for the fractionation of venom proteins are not uncommon[1-3].

Achyuthan *et al* have developed a single-step procedure for the separation of protein constituents of *Naja naja* venom[4]. The advantage of changing the pH of second and third buffers (both from 7.5 to 7.0) in the purification of ATPase is shown elsewhere[5]. Now, we report the effect of the increased column length and decreased flow-rate on the separation of protein constituents of *N. naja* venom.

N. naja venom (Batch No. 127) from Haffkine Institute, Bombay, India, and CM-Sephadex C-25 (4.5 meq/g) from Sigma Chemical Company, Missouri, USA were used. *N. naja* venom was fractionated on CM-Sephadex C-25 column (1.2 × 125 cm) by stepwise elution with phosphate buffers of various molarities and pH, as given in figure 1. Protein was estimated by Miller's modification of Lowry's method[6]. The various enzyme activities were screened using standardized methods. Neurotoxin activity was checked by animal experiments. The identification of the protein fractions are also shown (figure 1). The colorimetric measurements were made in Bausch and Lomb, Spectronic-20.

The increased column length (from 2.5 × 74 cm to 1.2 × 125 cm) and the decreased flow-rate (from 120 ml/ hr to 40 ml/ hr) gives a better separation (more than 20 components instead of 11 components). For instance, Fraction A containing acetylcholinesterase, L-amino acid oxidase and phospholipases has been sub-fractionated into five protein(s) components.

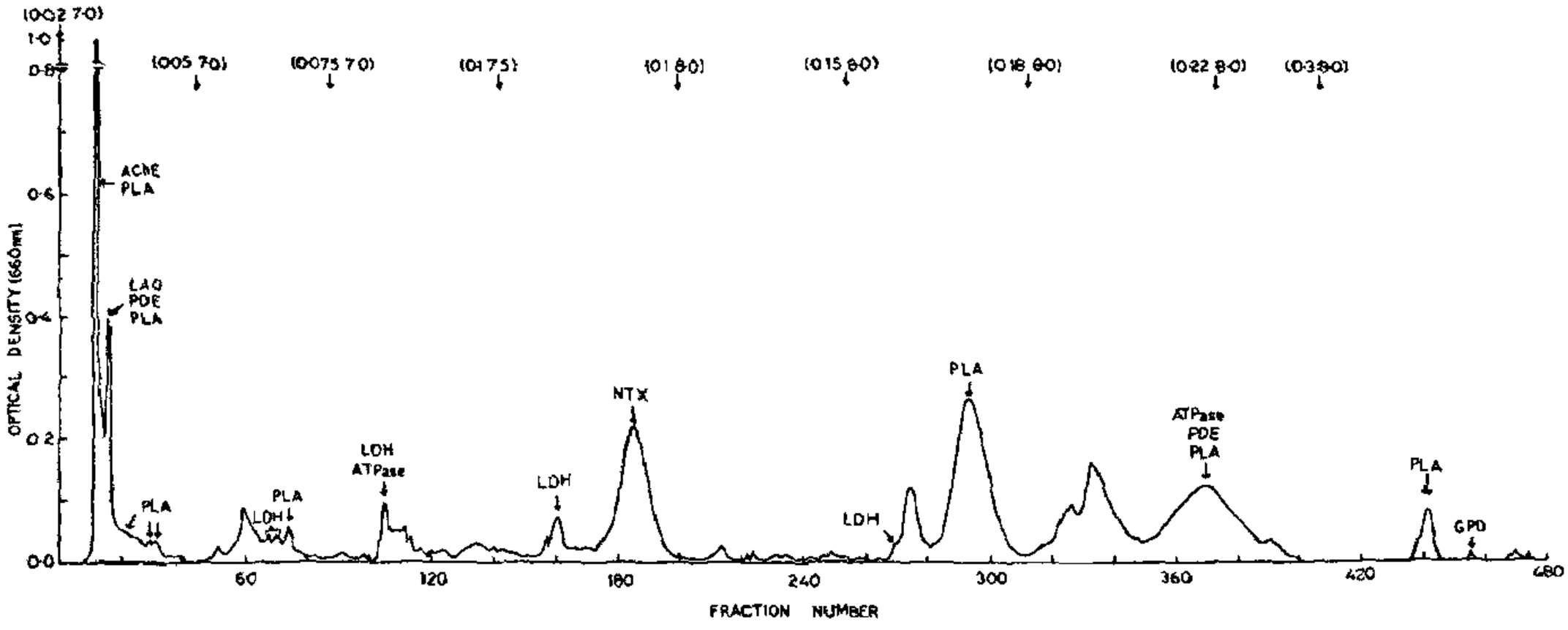

Figure 1. CM-Sephadex column chromatography of *N. naja* venom. Load; 400 mg in 2 ml of 0.02 M phosphate buffer (pH 7); dimensions of column packing, 1.2×125 cm; flow-rate, 40 ml/ hr; fraction volume, 5 ml; temperature, room temperature (~ 26° C). Elution was carried out stepwise with phosphate buffers of the molarities and pH as indicated. Recovery, 83%. AchE—Acetylcholinesterase, LAO—L-Aminoacid oxidase, ATPase—Adenosine triphosphatase, GPD—α-Glycerophosphate dehydrogenase, LDH—Lactate dehydrogenase, NTX—Neurotoxin, PDE—Phosphodiesterase, PLA—Phospholipase.