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ULTRA TRACE ANALYSIS BY DC POLAROGRAPHY USING CHEMICAL AMPLIFICATION REACTIONS

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THE need to estimate species at ppm and ppb levels and the necessity to analyse small quantities of samples demand analytical techniques with detection limits at sub-ppb level. Analytical signal can be enhanced by electronic amplification. But an electronic amplifier cannot distinguish between signal and background and can only amplify both, whereas to improve sensitivity or detection limit, it is necessary to selectively amplify the signal alone and not both[1]. Such a selective amplification can be realised by chemical amplification reactions.

Chemical amplification reactions can be defined as reactions in which the normal equivalence is altered in some way so that a more favourable measurement can be done. Amplification reactions have been reviewed by Belcher[2]. This communication is concerned with the improvement of detection limit of dc polarography by means of chemical amplification reactions.

For example, under suitable conditions 1 mol of orthophosphate reacts with 12 mol of ammonium molybdate to form the corresponding heteropoly molybdic acid (HPMA) complex. Hence, to estimate phosphorus, if one estimates the molybdenum content of HPMA, the signal is amplified by a factor of 12 atomic times (the mass amplification factor is 37.2). The signal can be enhanced further by coupling this with another cyclic regenerative type of chemical amplification reaction namely estimation of molybdenum by catalytic polarographic wave. The wave due to the reaction of Mo(VI) to Mo(V) in nitrate medium, known as the catalytic wave, exhibits a very much larger height than the diffusion-controlled wave, because of the cyclic regeneration of the Mo(VI) at the electrode surface by the chemical oxidation[3] of Mo(V) by No_3^-. The amplification factor for the second step is dependent on temperature. Under our experimental conditions[4] this factor is 40. By sequentially coupling these two chemical amplification reactions, the overall amplification becomes the product of the individual amplifications (for phosphorus, atomic amplification = 480; mass amplification = 1488). Such a large amplification factor dramatically improves the sensitivity and detection limit of dc polarography by more than two orders of magnitude.

The method has the potentiality of being versatile since at least 35 elements are known to form HPMA. Specificity is ensured by the conditions for HPMA formation and selective extraction of the same. The method involves the following steps.

X ⟶ Heteropolymolybdic acid (HPMA) formation with X as hetero atom ⟶ Selective extraction of HPMA into a suitable organic solvent ⟶ washing the organic solvent to remove the unwanted molybdate reagent which is also simultaneously extracted in the form of isopolymolybdic acid (IPMA) ⟶ Backstripping of HPMA into an aqueous alkali solution to release an equivalent amount of molybdenum corresponding to the original amount of X. ⟶ Estimation of molybdenum by catalytic dc polarographic wave.

Such a chemical amplification procedure has been devised by us for the estimation of trace amounts of phosphorus, arsenic, silicon, boron, cerium, thorium, nitrogen (as ammonium ion), niobium, germanium, vanadium, titanium and thallium. Table 1 summarises the results. From the detection limits reported in the table, it is obvious that these elements could be estimated at ppb and sub-ppb levels by dc polarography. However, in the present study, we find that the actual detection limits for the estimation of these elements are limited by the simultaneous extraction of considerable amounts of isopolymolybdate which is present in large amounts in the system due to the polymerisation of ammonium molybdate reagent. Details of the procedures and methods to decrease the simultaneous extraction of IPMA will be published elsewhere.

Figure 1 shows the dc polarograms for the estim-

Table 1 *Summary of the analytical methods developed for the estimation of trace amounts of phosphorus, arsenic, silicon, germanium, boron cerium, thorium, niobium, vanadium, titanium, thallium and nitrogen (as ammonium ion)*

Element determined	Nature of HPMA and extraction details	Probable[a] atomic amplification factor	Sensitivity (nA/ppb)	Attainable detection limit (ppb)
P	Molybdophosphoric acid (MPA) Extract with *t*-butyl acetate (*i*-BuOAc)	480	80	0.1
As	Molybdoarsenic acid. Extract with a mixture of *n*-butanol, (*n*-BuOH) ethyl acetate and iso amyl acetate	480	8.5	0.9
Si	Molybdosilicic acid. Extract with methyl isobutyl ketone (MIBK)	480	65	0.1
Ge	Molybdogermanic acid. Extract with a mixture of 1-pentanol and diethyl ether	480	22	0.4
B	Molybdotungstoboric acid. Extract with a mixture of MIBK and *n*-BuOH	240	0.8	10
Ce	Molybdocerophosphoric acid (MCePA) along with MPA. Remove excess MPA by extraction with *n*-BuOH-chloroform mixture. Destroy MCePA to release an equivalent amount of phosphorus. Estimate this phosphorus by once again forming MPA.	240	10	0.8
Th	Molybdothorophosphoric acid (MThPA) along with MPA. Remove Excess MPA with *i*-BuoAC extraction. Then extract MThPA with *n*-BuOH.	440	5	1.6
Nb	Molybdoniobophosphoric acid (MNPA) along with MPA. Extract excess MPA with *i*-BuOAc. Then extract MNPA with *n*-BuOH	440	18	0.4
V	Molybdovanadophosphoric acid (MVPA) along with MPA. Extract excess MPA with *i*-BuoAc. Then extract MVPA with a mixture of 1-pentanol and diethyl ether	440	10	0.8
Ti	Molybdotitanophosphoric acid (MTPA) along with excess MPA. Extract excess MPA with a mixture of chloroform and *n*-BuOH. Then extract MTPA with *n*-BuOH	480	12	0.7
Tl	Thallium molybdophosphate (precipitate) Centrifuge and estimate the molybdenum content of the precipitate	720	5	— (b)
N (as NH_4^+)	Ammonium 12 molybdo phosphate. In the presence of thallium, co-precipitates with thallium molybdophosphate. Centrifuge and estimate the molybdenum content of the precipitate	480	18[c]	— (b)

(a) The amplification factors reported are the most probable ones because some arguments still remain about the exact stoichiometry of some of the HPMAS

(b) Detection limits are decided by the minimum amount of precipitate that can be formed and centrifuged and not reported in this communication

(c) Calibration curve for nitrogen is non linear (but reproducible). The reported value is for the range of 50 to 80 ppb ammonium ion

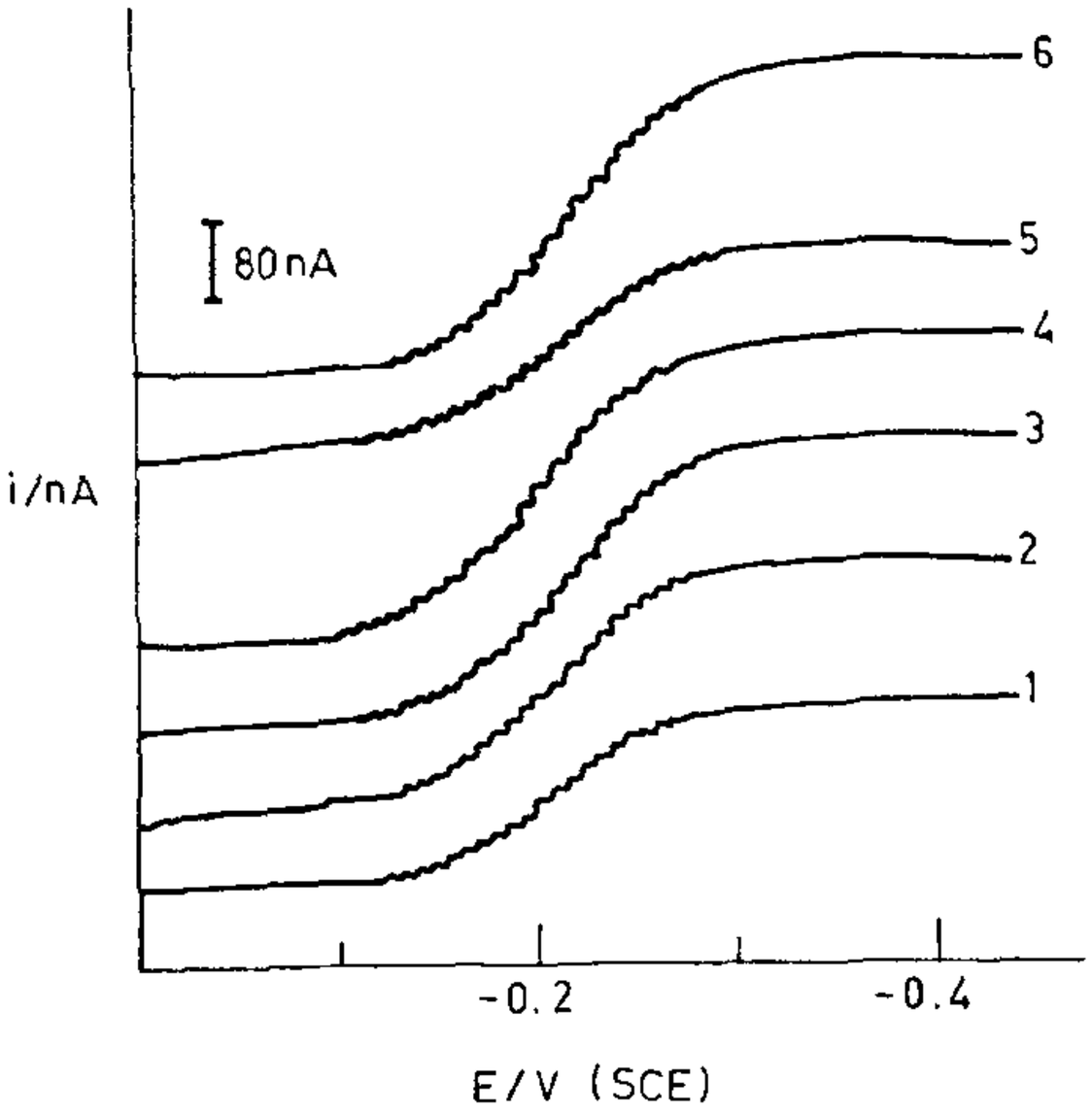

Figure 1. Polarograms of backstripped molybdenum equivalent to (1) 1.9 μg/l silicon; (2) 2.2 μg/l phosphorus; (3) 19.8 μg/l cerium; (4) 100 μg/l boron; (5) 14 μg/l thorium; (6) 60 μg/l nitrogen (as ammonium ion)

ation of trace amounts of silicon, phosphorus, cerium, boron, thorium and nitrogen obtained by the method outlined above. Incidentally, this method is all the more significant for the estimation of these six elements because in these cases not only their sensitivities are amplified by several folds but also this happens to be the only satisfactory polarographic method available for their estimation. The use of the charging current compensated dc (ccc.dc) polarograph[5] further improves the detection limit by effectively removing the dc charging current.

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8-HYDROXYCOUMARIN: AN INTERMEDIATE IN THE MICROBIAL TRANSFORMATION OF QUINOLINE

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QUINOLINE and related heterocyclic compounds occur widely in nature[1] and are extensively used as solvents, intermediates, reagents and drugs[2, 3]; they display toxic[4], carcinogenic and mutagenic activity[5–7]. Rapid degradation of quinoline in sewage has been reported[8], and soil enrichments for quinoline-degrading bacteria have yielded a *Moraxella sp*[9] and a *Pseudomonas sp*[10]. The cleavage of quinoline ring in kynurenic acid, an intermediate of tryptophan catabolism, occurs *via* 7,8-dihydroxykynurenic acid and fission of the benzene ring[11, 12]; a similar sequence is likely for kynurine[13]. However, the microbial metabolism of quinoline molecule itself is not understood. 2-hydroxyquinoline was reported as an intermediate in *Moraxella sp*[9] but further metabolites were not detected. Microbial transformation of quinoline by naphthalene-adapted *Pseudomonas putida* yielded o-aminophenyl-β-hydroxyphenylpropionic acid[14]. The present paper reports the isolation and characterization of 8-hydroxycoumarin as an important metabolite of quinoline in a *Pseudomonas sp* isolated from sewage.

The quinoline-degrading bacterium was isolated from sewage by enrichment in phosphate salts medium[15] containing 0.03% quinoline. The organism was purified by streaking on nutrient-agar plates and phosphate agar-plates exposed to quinoline. The bacterium is gram-negative rod (1 μm × 2–3 μm), motile by a single polar flagellum; aerobic, catalase and oxidase positive; starch and gelatin not hydrolysed; sugars are not utilised as growth substrates; fluorescent pigments not produced and naphthalene not catabolised. The organism has been designated as *Pseudomonas sp*[16] but differs from *P. putida*[14], and the strain isolated earlier in this laboratory[10].

The organism was grown in 1 litre Erlenmeyer flasks containing 300 ml of phosphate salts medium and 0.03% quinoline, with shaking at 30° C. The formation of metabolites was monitored by recording the UV spectrum of cell-free fermentation broths, as well as using phenol reagent to detect phenolic intermediates. Transformation products were isolated from broth by saturation with sodium chloride and extraction with ethyl acetate. After concentration of the extract *in vacuo*, the products were analysed by TLC on silica gel