upon excess tellurium was filtered off. Weight of unreacted tellurium, 0.2770 g, calc. 0.2642 g.

To 11.5 g tetraphenylarsonium cyanide, dissolved in 200 ml acetonitrile, was added 3.58 g tellurium powder. The reaction mixture was stirred for 6 h, and the slightly yellow product precipitated slowly. The reaction mixture was finally slowly heated to 40°C to dissolve the product, the solution was set aside for some minutes, and the clear solution was decanted from traces of unreacted tellurium. The residue was washed with 20 ml acetonitrile, making the total volume 220 ml. After 2 h at −7°C, the clear solution was decanted from the precipitated product, and the crystals were washed with cold acetonitrile and finally with ether, yielding 5.5 g pure tetraphenylarsonium tellurocyanate. Removal of the mother liquor from the product by filtration was found to cause severe decomposition, as the crystals, when wet by acetonitrile, were rapidly attacked by moisture.

The volume of the mother liquor was reduced in vacuum to 50 ml, and an additional amount of 4.5 g pure tetraphenylarsonium tellurocyanate thereby precipitated, making the total yield 10 g, or 66 % based on consumed tellurium. By addition of diethyl ether to the mother liquor, unreacted tetraphenylarsonium cyanide and the rest of the tellurocyanate could be precipitated.

In a similar experiment, but where the reaction mixture was evaporated to dryness after completed reaction and the product recrystallized from acetone after filtration, the yield of pure tetraphenylarsonium tellurocyanate, from 7.95 g tetraphenylarsonium cyanide and excess tellurium, was 5.5 g, or 53 %. (Found: C 56.62; H 3.98; N 2.57. Calc. for $C_{25}H_{20}AsNTe$: C 55.92; H 3.75; N 2.61.).

Tetramethylammonium tellurocyanate. To tetramethylammonium cyanide in 250 ml acetonitrile, made *in situ* from 26.4 g tetramethylammonium chloride and 31.0 g potassium cyanide,[6] and filtered, was added 12.76 g tellurium powder, and the mixture was stirred for 3 h at room temperature. Traces of unreacted reactants were filtered off, and the solvent was removed in vacuum, yielding 21.6 g raw product, 95 % based on added tellurium, of rather high purity. Pure samples could be obtained in more than 50 % yield through one crystallization from acetone. (Found: C 26.43; H 5.45; N 11.32: Calc. for $C_5H_{12}N_2Te$: C 26.33; H 5.30; N 12.29.).

Acknowledgement. The authors are indebted to Dr. P. Klæboe, University of Oslo, for making the Perkin-Elmer 225 Grating Infrared Spectrophotometer available to them, and to Dr. O. Foss for valuable suggestions.

1. Downs, A. W. *Chem. Commun.* **1968** 1290.
2. Greenwood, N. N., Little, R. and Sprague, M. J. *J. Chem. Soc.* **1964** 1292.
3. Songstad, J. and Stangeland, L. J. *Acta Chem. Scand.* **24** (1970) 804.
4. Coetzee, J. F. *Pure Appl. Chem.* **1967** 429.
5. Gutman, V. *Coordination Chemistry in Non-aqueous Solvents*, Springer, Vienna 1968.
6. Andreades, S. and Zahnow, E. W. *J. Am. Soc.* **91** (1969) 4181.

Received December 2, 1970.

Fluorimetric Determination of Homovanillic Acid in Tissues, Using Anion Exchange Separation and Mixed Solvent Elution

J. KORF,[a] B. E. ROOS[b] and B. WERDINIUS[b]

[a] *Department of Biological Psychiatry, State University, Groningen, the Netherlands, and* [b] *Department of Pharmacology, University of Göteborg, Göteborg, Sweden*

Several methods have been employed for the quantitative determination of homovanillic acid (HVA, 3-methoxy-4-hydroxyphenylacetic acid) in biological material such as brain tissue, cerebrospinal fluid and urine. The final assay procedures for this important metabolite of dopamine include, *e.g.*, gas-liquid chromatography, spectrophotometry and spectrofluorimetry, after suitable chemical rearrangement of the HVA.

A spectrofluorimetric method commonly used is that of Andén *et al.*,[1] which with minor modifications has been adopted by other workers.[2–4] The method involves oxidation of the sample, using potassium ferricyanide in ammonia. This results in the formation of a highly fluorescent

dimerization product of HVA, the structure of which has been established.[5] Some other compounds closely related to HVA also give a similar blue fluorescence upon oxidation with potassium ferricyanide.[6]

All methods require preliminary extraction and purification steps in order to remove interfering substances and concentrate the small amounts of HVA present in biological material. Various solvent extraction techniques are commonly used prior to the spectrofluorimetric assay.[1,2,4] Frequent internal standardization is necessary due to somewhat fluctuating recoveries throughout the extraction procedure.

Column chromatographic anion exchange procedures have also been employed for the purification of HVA.[2-4] Thus, Juorio *et al.*[3] employed a column of Dowex 1 × 2 (4 × 25 mm, acetate form) for the sorption of HVA from a brain tissue extract. Elution was performed with about 5 ml of 0.1 N HCl, followed by fluorimetric determination. However, in our experience, larger volumes (10 – 15 ml) were necessary for the complete elution, thus interfering with the concentration of the HVA to a small sample. Possibly, non-ionic interaction forces between the aromatic resin matrix and the HVA might interfere with the elution step. The intensity of the fluorescence was moreover depressed by the presence of even small amounts of HCl in the final sample, and the linearity of the fluorescence seemed to be somewhat impaired. In other methods for urinary assay of HVA [2,4] the substance was eluted from a Dowex 1 × 4 column (chloride form), using a large volume (22 ml) of 1.5 N sodium chloride. This eluate had then to be subjected to further solvent extraction steps prior to fluorimetric assay.

In the course of a study to separate various phenolic acids we observed that HVA, previously adsorbed to a column of Dowex 1 × 4 (chloride form), could be eluted completely in a small volume of HCl containing various organic solvents. Of several organic solvents tested, including methanol, ethanol, propanol, ethylene glycol, methyl and ethyl cellosolve, none was significantly more effective than ethanol. The elution pattern of HVA using 0.05 N ethanolic (50 % v/v) HCl compared to that using 0.05 N aqueous HCl is shown in Fig. 1.

Roughly, a fourfold decrease in the elution volume was noted in favour of the mixed solvent. Moreover, the absolute fluorescence intensity obtained after oxidation of HVA in various mixed solvents was increased in proportion to the decreased polarity of the solvent, without a corresponding increase in the blank fluorescence. Thus, the fluorescence of HVA was almost doubled when the oxidation was performed in the above-mentioned HCl-ethanol (50 %) mixture compared to the corresponding aqueous solution, in agreement with other reports.[4] A corresponding increase in fluorescense could also be noted when the ethanol was added after the termination of the reaction.

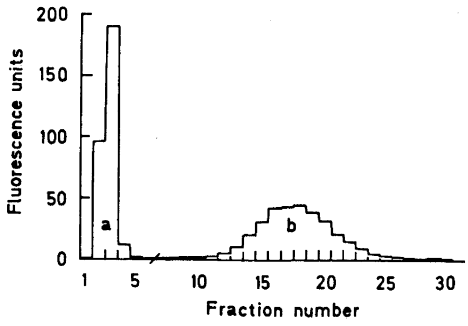

Fig. 1. Elution pattern of 10 μg HVA. Resin bed: Dowex 1 × 4 (chloride form, 200 – 400 mesh) 4 × 50 mm. Flow rate: 0.6 ml/min. Eluent: (a) 0.05 N ethanolic (50 % v/v) HCl; (b) 0.05 N aqueous HCl. Each fraction contains 1 ml. The fluorescence is given in arbitrary fluorescence units, after oxidation of each fraction and reading against an appropriate standard of the same solute composition.

In this communication some further details will be given of the assay procedure for HVA, as applied to brain tissue.

Columns (4 × 50 mm) of the anion exchange resin Dowex 1 × 4 (chloride form, 200 – 400 mesh) are prepared immediately before use. The columns are pretreated with 20 ml of 0.05 N ethanolic (50 %) HCl in order to remove interfering fluorescent material, and then rinsed with water to neutrality.

The brain tissue (generally 0.5 to 1 g) is homogenized in 10 ml of 0.4 N ice-cold perchloric acid. After centrifugation and filtration, the clear supernatant fluid is neutralized to pH 6.5 with 5 N potassium hydroxide added drop-wise. The slightly soluble potassium perchlorate is spun down at 0°C. The neutralized extract is allowed to pass through

the pretreated Dowex column, at a rate of 0.6 ml/min, followed by 10 ml of water. Elution is then performed with 0.05 N ethanolic (50 %) HCl. The first ml is discarded, and the following 5 ml are collected.

The eluate is assayed for HVA essentially according to the oxidation procedure described earlier.[1,5] To 1 ml of the eluate is added 1 ml 5 N ammonia and 0.05 ml 0.75 % zinc sulfate ($ZnSO_4 \cdot 7H_2O$). To this mixture is further added 0.2 ml of 0.02 % potassium ferricyanide, and the oxidation is terminated after 4 min with 0.2 ml 0.5 % cysteine hydrochloride. An internal standard is prepared by adding a known amount (0.5 to 2 μg) of authentic HVA to another sample, and the fluorescence developed is compared to that of an external standard, and a reagent blank, with the same solute composition. An unoxidized tissue blank is also included, prepared by neutralizing the oxidant with the cysteine before the eluate sample is added. The fluorescence developed is read in an Aminco-Bowman spectrophotofluorimeter, with the activating and fluorescence wave-lengths set at 310 and 425 nm, respectively (uncorrected instrumental values).

The internal standard is usually in a very good agreement (95 to 105 %) with the external standard, making further corrections unnecessary. The addition of zinc ions to the reaction mixture seems to improve the recovery somewhat. The reason for this is not clear; possibly the zinc ions form complexes with catechol compounds present in tissue, which would otherwise interfere with the oxidation. If the tissue proteins are precipitated with zinc hydroxide instead of perchloric acid, the present method, slightly modified, gives good recoveries of HVA without addition of further zinc ions.[7]

In an earlier communication [8] we reported on the column chromatographic separation and assay of urinary HVA, using a mixture of 0.3 N acetic acid and ethanol (60:40 v/v) for elution. This modification of the eluent was necessary to prevent the elution of interfering substances in the urine.

The use of mixed organic solvents in anion exchange chromatography has been reported earlier, *e.g.* for the separation of certain organic acids and phenols [9,10] (for review, see *e.g.* Samuelson [11]). In a few papers the successful separation of biogenic amines and their basic metabolites from strong cation exchange resins have been reported, using various mixtures of organic solvents and mineral acids for elution.[12,13] Generally, however, these mixed solvents seem to have attracted only a limited interest in microanalytical work on biological material.

The present study, as well as further work in progress, suggest a wider application of mixed organic solvents to the differential anion exchange separation and assay of acid compounds of biological interest.

Acknowledgements. This work was supported by grants from the *Faculty of Medicine*, Göteborg, *Läkemedelsindustriföreningen* (LIF), and the *Swedish Medical Research Council* (B71-21X-165-07 and B71-14×2218-05). For skilful technical assistance we are indebted to Miss Majbritt Hansson and Mrs. Annbritt Forsström.

1. Andén, N.-E., Roos, B.-E. and Werdinius, B. *Life Sci.* **2** (1963) 448.
2. Sato, T. L. *J. Lab. Clin. Med.* **66** (1965) 517.
3. Juorio, A. V., Sharman, D. F. and Trajkov, T. *Brit. J. Pharmacol.* **26** (1966) 385.
4. Geissbühler, F. *Clin. Chim. Acta* **26** (1969) 231.
5. Corrodi, H. and Werdinius, B. *Acta Chem. Scand.* **19** (1965) 1854.
6. Gjessing, L. R., Vellan, E. J., Werdinius, B. and Corrodi, H. *Acta Chem. Scand.* **21** (1967) 820.
7. Lewander, T. *Personal communication.*
8. Korf, J., Werdinius, B. and Roos, B.-E. *4th Intern. Congr. Pharmacol.*, Basel 1969, (*Congress Abstracts*), p. 463.
9. Carrol, K. K. *Nature* **176** (1955) 398.
10. Shelley, R. N. and Umberger, C. J. *Anal. Chem.* **31** (1959) 593.
11. Samuelson, O. *Ion Exchange Separation in Analytical Chemistry*, Almqvist & Wiksell, Stockholm, and Wiley, New York 1963, p. 138 ff.
12. Glowinski, J., Iversen, L. L. and Axelrod, J. *J. Pharmacol. Exptl. Therap.* **151** (1966) 385.
13. Atack, C. V. and Magnusson, T. *J. Pharm. Pharmacol.* **22** (1970) 626.

Received December 8, 1970.