

Ascorbic Acid: Remission Spectrum of L-Ascorbic Acid Dissolved in Water Admixed Barium Sulfate and Measured on Frosted Quartz

HUGO FREDHOLM

Department of Food Hygiene, Royal Veterinary College, S-104 05 Stockholm, Sweden

Between $\bar{\nu}$ 4.762 μm^{-1} (210 nm) and $\bar{\nu}$ 1.429 μm^{-1} (700 nm) L-ascorbic acid generates a remission spectrum with one strong, sharp minimum of the relative remittance, R'_∞ , at $\bar{\nu}$ 3.774 \pm 0.003 μm^{-1} , 265.0 \pm 0.2 nm, 4.679 \pm 0.003 eV even in amounts of 0.3 ng (1.7×10^{-13} mol) per mm^2 of reflecting area, when measured dissolved in H_2O admixed BaSO_4 on frosted quartz under N_2 (Fig. 1) by the method described earlier.¹ The corresponding maximum of $\log(1/R'_\infty)$ possesses the molar value of

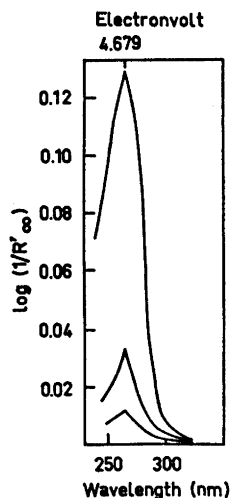


Fig. 1. Remission spectrum of L-ascorbic acid, dissolved in H_2O admixed BaSO_4 , on frosted quartz under N_2 , saturated with H_2O -vapour, at 20°. Lower curve: 0.000 331 μg (1.88×10^{-13} mol). Middle curve: 0.000 912 μg (5.18×10^{-13} mol). Upper curve: 0.00365 μg (2.07×10^{-11} mol) per mm^2 , of L-ascorbic acid dissolved in 0.160 mg H_2O admixed 0.160 mg BaSO_4 . Reference: 0.160 mg H_2O + 0.160 mg BaSO_4 per mm^2 of frosted quartz plate.

6.23×10^9 per mm^2 , when quantities between 1.9×10^{-13} and 2.0×10^{-11} mol of L-ascorbic acid/ mm^2 are measured. The sensitivity, accuracy and precision of the method, applied for the determination of ascorbic acid, is high.

Quantities of 3 ng (0.003 μg = 1.7×10^{-11} mol) are detected and determined with a mean error of about 5% of value, whereas at least 0.5 μg of ascorbic acid is needed for adequate quantitation photometrically by the 2-nitroaniline method² and at least 15 μg of ascorbic acid is needed for determination by the 2,6-dichloroindophenol sodium method.³ For the study of reactions of ascorbic acid *in vivo*, or, e.g., in meat products, a method of high sensitivity, specificity and accuracy is necessary. The magnitude $-\log$ relative remission, ($\log 1/R'_\infty$), applicable to layer thicknesses sufficiently great to prevent transparency, was measured from $\bar{\nu}$ 4.762 μm^{-1} (210 nm) to $\bar{\nu}$ 1.429 μm^{-1} (700 nm). Wavelength values are \pm 0.2 nm, corrected for scanning and sphere errors, controlled with holmium oxide. When substantially greater quantities of L-ascorbic acid are present, the maximum will shift slightly towards higher wavenumbers up to 4.000 μm^{-1} (250 nm). Though ascorbic acid is a rather weak acid, $\text{p}K_1$ 4.17; $\text{p}K_2$ 11.57, dissociation is high at the concentrations used for this report, e.g. 0.331 ng of ascorbic acid in 0.160 mg of H_2O , which means a 1.18×10^{-5} M solution.

Experimental. L-Ascorbic acid, a.r., was used as standard. The measurements shown in Fig. 1 were made with pure solutions of L-ascorbic acid in redist. O_2 -free H_2O . Quantities of L-ascorbic acid, to approximately the same amounts, added to nitrite-free meat products under N_2 , were recovered to 78–92%, depending on kind of meat product, when determined within 2 h.

L-Ascorbic acid was extracted from sample with redist. O_2 -free H_2O under N_2 in the presence of EDTA to avoid catalytical influence of traces of heavy metals, solution centrifuged and purified with small quantities of low crosslinked dextran gel (Sephadex G. 75, 100–200 mesh), all operations under N_2 . Since quantitative separation of ascorbic acid on silica gel is very difficult, paper chromatography under N_2 was used for separation.³ Parallel runs of L-ascorbic acid, a.r., were always carried out. This is necessary to localize spots.

BaSO_4 was prepared by diffusion of 0.1 M Na_2SO_4 , a.r., into 0.1 M BaCl_2 , a.r., precipitate

washed and centrifuged 5 times, dried *in vacuo*.

The measurements reported in this paper were made without cover quartz plates. The work with cover plates is easier, it does not require as much of care in keeping temperature and humidity constant; but the cover plates reduce the relative remittance of the sample.

Acknowledgement. This work was financially supported by the *Magn. Bergvall's Foundation*.

1. Fredholm, H. *Tetrahedron Letters* **31** (1971) 2903.
2. Mohr, H. *Mitt. Geb. Lebensmittelunters. Hyg.* **47** (1956) 20.
3. Strohecker sen., Heimann, W. and Matt, F. *Z. anal. Chem.* **145** (1955) 401.

Received November 19, 1971.

Specific Radioactivities of Protein Discs in a Polyacrylamide Gel

KARI HEMMINKI

Department of Medical Chemistry, University of Helsinki, SF-00170 Helsinki 17, Finland

Turn-over rates of proteins are heterogeneous and, therefore, determination of a turn-over rate from a mixture of proteins yields only little information concerning a single species. Application of polyacrylamide gel electrophoresis has emerged as a method of choice for simultaneous measurement of specific radioactivities of an array of purified proteins.

The procedure introduced in this paper is highly reproducible allowing determination of absolute specific radioactivities of individual discs in a polyacrylamide gel. The stained protein bands are cut from a gel on a device producing cylinders of equal size, followed by solubilization and protein estimation by means of the bound dye. The solutions are then evaporated to dryness and processed for liquid scintillation counting.

Experimental. Samples: Rat brain cortex was used in all experiments. For the incorporation study 20 $\mu\text{Ci}({}^3\text{H})$ -leucine (20 μl) was injected into a lateral ventricle and the animals were killed at various times. The cortices were homogenized and centrifuged at 100 000 *g* for 30 min to yield the soluble proteins in the supernatant and the insoluble proteins in the pellet; both fractions were then treated with "the sample solvent"¹ for electrophoresis. The solvent contained 50 mM K_2CO_3 , 8 M urea, 10 % mercaptoethanol, and 5 % Triton X-100.

Electrophoresis. Polyacrylamide gel electrophoresis was performed according to Lim *et al.*¹ by replacing bis(*N,N'*-methylene bisacrylamide) by 0.4 % ethylene diacrylate (Borden Chemical Co.) in the small pore gel solution to make the gel alkali labile.² 200 μl of the sample was applied onto 30 \times 0.5 cm columns and a current of 3 mA/tube was connected until the methyl green marker had migrated to the cathode end of the gel.

Quantitation of protein and radioactivity. The gels were stained with 0.5 % amido black in 10 % acetic acid for 30 min; the destaining was made by diffusion in a solution containing 10 % acetic acid and 20 % ethanol.

A sketch was drawn to indicate the positions of the major bands. All parallel gels were placed on a slicer (Fig. 1) adjusted to produce 2 mm cylinders and the major bands were cut according to the sketch making sure that each band was contained in a cylinder. To provide background readings for protein and radioactivity four additional slices were taken from such parts of the gel which were apparently devoid of protein. The slices obtained were dissolved in 1 ml 1 M NH_4OH and the absorbance of the solution was measured at 625 nm³ followed by evaporation to dryness at 80°. The residue was mixed with 200 μl of Soluene 100 (Packard), 20 μl of water and 10 ml of toluene scintillation fluid and counted in a Packard scintillation spectrometer to a small counting error. The results were corrected for color quenching by internal standardization. The quenching was at most 10 % of the total counts. The counting efficiency was 15 %.

Standard curve for protein. To relate the amido black absorbance and protein content, protein discs were dissolved in NH_4OH and the absorbance was measured at 625 and 750 nm. The samples were then evaporated and dissolved in NaOH followed by estimation of protein content by Lowry's method⁴ at 750 nm. The reading for amido black at 750 nm was subtracted from that obtained with Lowry's method.