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Resonance Raman Spectra Produced by the Reaction of 5,5′-Dithiobis(2-nitrobenzoic acid) with Glutathione and with Sulphydril Groups of Biological Fluids

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A standard spectrophotometric method of estimation of sulphydril group concentrations in biological fluids is based on the reaction of the thiol groups with 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's reagent–ESSE) [1].

$$ESSE + RSH \rightarrow ESSR + ESH$$

$$ESH \rightarrow ES^{(-)} + H^{+} \text{ (neutral pH)}$$

The resultant solution is yellow, due to an absorption of $ES^{(-)}$ which has a maximum at 412 nm. The method is satisfactory for use with small molecules containing sulphydril groups but interferences from the band edge of albumin and other proteins or from the visible spectrum of haemoglobin limit the use and sensitivity in biological fluids. For example, in the determination of serum sulphydril concentration in clinical studies, the 412 nm band is present as a shoulder and careful adjustment of concentration and of the composition of the reference solution are required.

A comparison of the Raman spectrum of Ellman's reagent in solid (Fig. 1 spectrum 1) and solution at 10^{-3} m (Fig. 1 spectrum 2) indicates a resonance effect. Bands due to vibrations of the nitro group, ring modes, –C–S and –S–S– groups in the solid spectrum were assigned by comparison with the spectra of related compounds. On addition of glutathione to Ellman's reagent, two strong bands due to the nitro-group vibrations were predominant in the spectrum. An Ellman's reagent concentration of 10^{-5} molar proved suitable for measurement (Fig. 1, spectrum 3) using the 457.9 nm laser line. The nitro-group modes are assigned to a symmetric stretch (1325 cm^{-1}) and an in-plane symmetric bend (850 cm^{-1}), both of which have A_1 symmetry.

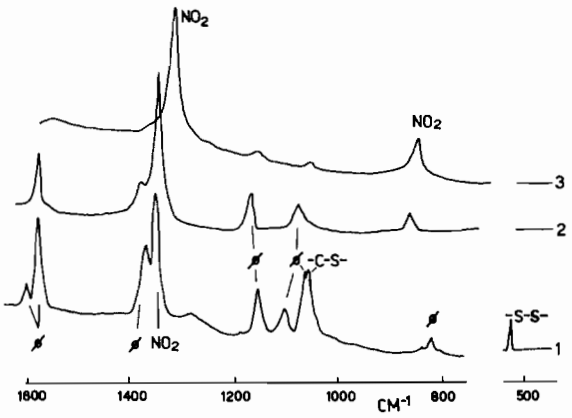

Fig. 1. The Raman spectrum of solid Ellman's reagent (1), a 10^{-3} molar solution of Ellman's reagent (2) and a 10^{-5} molar solution of Ellman's reagent and glutathione (3). The groups giving rise to each peak are assigned as shown, with aromatic modes marked ϕ.

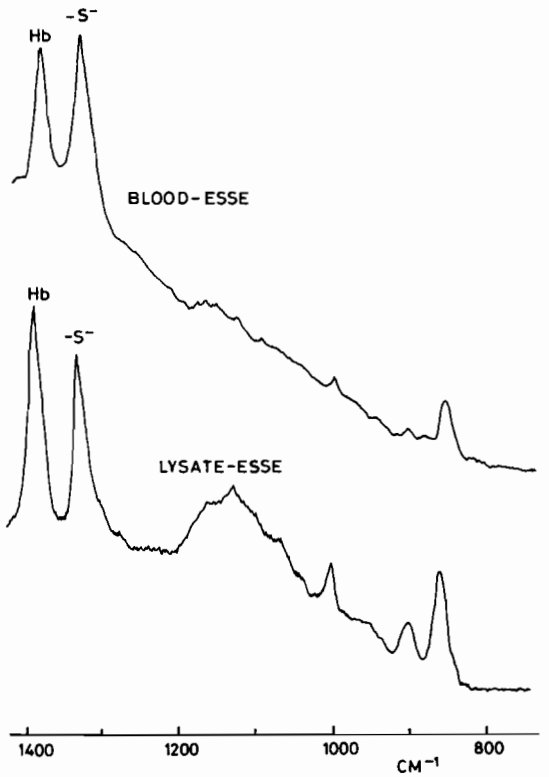

Fig. 2. The spectrum of red-cell lysate containing Ellman's reagent and of blood containing Ellman's reagent. The peak marked Hb is due to haemoglobin, that marked $-S^{-}$ is due to ES^{-} (see text).

These results suggest that the absorption of $ES^{(-)}$ at 412 nm is due almost entirely to an $n \rightarrow \pi^*$ transition of the nitro group and that this transi-

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tion gives rise to a Frank-Condon-type resonance effect. The enhancement of the aromatic modes is less marked, and includes modes with different symmetries. It would appear that this resonance effect is of the Hertzberg-Teller type and is due to a pre-resonance from a $\pi \rightarrow \pi^*$ aromatic transition further into the ultraviolet region.

The spectra were obtained with a modified Cary 81 Spectrometer and a Spectra Physics 171-09 Argon Ion Laser, using 100 m.w. of power. The intensity of the Raman spectrum increased as the wavelength of the exciting line was changed to ultraviolet.

The main advantage of a resonance Raman measurement for thiols in biological systems would be in selectivity and in sensitivity. We have applied the method to the determination of the sulphydril group concentration in red-cell lysate which contains haemoglobin (Fig. 2 spectrum 1). In this case the haemoglobin spectrum will also be resonant and, consequently, it is possible to use the 1375 cm^{-1} band due to haemoglobin as a standard. The sulphydril group concentration was determined at 1.2×10^{-3} *M* and it was confirmed by standard addition of glutathione to the red-cell lysate. A similar measurement can be taken using suspended unlysed red cells (Fig. 2 spectrum 2) but the result is more difficult to interpret, requiring an estimation of homogeneity and the degree of lysis of the cells. However, it is clear that the thiol concentration per haemoglobin molecule is higher than in red-cell lysate. In conclusion, it would seem that this technique has potential in biological investigation in that it can be used in coloured solutions at low concentrations and is quantitative.

Reference

1 G. L. Ellman, *Arch. Biochem. Biophys.*, *82*, 70 (1959).