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Letter to the Editor

Gel chromatography of heparin

Sir,

In a recent paper Losito et al. [1] describe the separation of a commercial heparin sample into active and inactive fractions and claim that the active material constitutes only about half the total sample. Their method of quantitation appears however to be invalid, since it assumes that the absorptivity (absorbance per unit mass) of all the material in the sample is uniform at 218, 255 and 275 nm. This is inherently extremely unlikely. There is increasing recognition of the variability and complexity of the polymer chains which together constitute "heparin", but no chromophores have so far been observed which could contribute perceptible absorption at 255 or 275 nm, and indeed the absorption of highly purified heparin at these wavelengths is exceedingly low. It is also very variable, since almost all of it results from trace amounts of non-heparin material (usually "bleached" by an oxidising agent such as permanganate) which may or may not be covalently bound to the heparin. Electrostatically bound material may be dissociated from the heparin by the gel chromatographic solvent, in this case 1.0 *M* sodium chloride.

At 218 nm heparin shows some inherent absorption, but a maximum observed at this wavelength is almost certainly a consequence of stray light (however low the inherent stray light of an instrument may be, in a system employing compensation for solvent absorption a spurious peak of characteristic shape is always generated when the solvent transmittance drops towards zero with decreasing wavelength; see, e.g., ref. 2, pp. 65—71 and ref. 3, pp. 99—101). Quantitation then cannot be carried out in any event; in the text of Losito et al. there is no indication which of the three wavelengths was in fact used to give the quoted results.

Although some purification of heparin is achieved by the procedure of Losito et al. more evidence is needed to justify the mass balance described. They quote previous work of ours [4,5], but make no attempt to account for discrepancies between their results and ours, which were obtained using a refractometer as a detector. As a mass-sensitive detector, a refractometer is

very much more satisfactory than an absorptiometer, since variations in specific refraction with molecular structure are negligible when compared with variations in absorptivity.

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1 R. Losito, H. Gattiker and G. Bilodeau, J. Chromatogr., 226 (1981) 61.
2 G.H. Beaven, E.A. Johnson, H.A. Willis and R.G.J. Miller, Molecular Spectroscopy, Methods and Applications in Chemistry, Heywood, London, 1961.
3 C. Burgess and A. Knowles (Editors), Standards in Absorption Spectrometry (UV Spectrometry Group), Chapman and Hall, London, New York, 1981.
4 E.A. Johnson and B. Mulloy, Carbohyd. Res., 51 (1976) 119.
5 B. Mulloy and E.A. Johnson, Thromb. Haemostas., 43 (1980) 192.

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