A COMPARISON OF THREE CHEMICAL METHODS OF ASSAY OF HEPARIN

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It is frequently necessary to determine the amount of sulphated polysaccharides (SP) such as heparins, chondroitin sulphates or carrageenans in solution. The difficulties associated with biological methods usually results in restriction of their use to situations where information about a particular biological activity is required. However, sulphate ester content is often closely associated with activity and chemical reactions in which that group engages may be used to determine amounts of SP present. Methods based on interaction of SP with cationic dyes have appeared but no comparison of the methods is available, hence choice is usually arbitrary. Using two pure heparins, pig mucous (Leo, 155 $u.mg^{-1}$) and beef lung (Upjohn, 158 $u.mg^{-1}$) three such methods have been investigated, namely those using toluidine blue (McIntosh, 1941), A, azure-A (Lam & others, 1976), B, and alcian blue (Whiteman, 1973), C, respectively. A and B required no special modification; C required a particular $MgCl_2$ concentration, 0.2M, and the volume of heparin solution used was 200µl.

Table 1. Data for three methods of determination of heparin

Method	Heparin	useful range of heparin µg	regression equation y =	standard deviation of scatter about regression	correlation coefficient
A	pig mucous	10 - 110	0.011x-0.020	0.042	0.995
	beef lung	10 - 110	0.013x-0.006	0.045	0.995
B	pig mucous	1 - 5	0.061x+0.026	0.016	0.966
	beef lung	1 - 5	0.065x-0.011	0.024	0.971
C	pig mucous	2 - 10	0.086x+0.012	0.030	0.993
	beef lung	2 - 10	0.088x-0.013	0.008	0.999

In method A the precipitated SP-dye complex is taken to a water-petroleum ether interface and the depletion of dye in the aqueous phase is measured; in B direct spectrophotometric reading of the SP-dye solution is quantitative; in C the precipitated centrifuged SP-dye complex is dissolved in a concentrated surfactant solution before spectrophotometric reading, hence, the complexity of operations and convenience of the methods vary. In method A concentration of NaCl is somewhat critical limiting the nature of solutions in which SP can be measured without modification; toluidine blue is a mixture of dyes which may vary slightly, and its solution should be reasonably fresh, but the amount of heparin which can be measured is relatively high. Method B is the simplest technically; the dye is reasonably stable and relatively pure and the method is easily adapted. In Method C $MgCl_2$ concentration and pH are critical and vary for different SP; manipulations are the most complex of the three methods although some information relative to its use in determination of SP in biological fluids has accumulated (Whiteman, 1973,b). The excellent linearity of the absorbance versus heparin concentration plots and the reproducibility within the useful range, indicated in Table 1 for each method, suggest that the choice of method may safely be made on the basis of such factors as convenience, ease of adaptation to particular circumstances and complexity of fluids containing the heparin to be determined.

Lam, L.H., Silbert, J.E. & Rosenberg, R.D. (1976). Biochem. Biophys. Res. Comm., 69, 570-574.
McIntosh, F.C. (1941). Biochem. J., 36, 776-782.
Whiteman, P. (1973a). Ibid. 131, 343-350.
Whiteman, P. (1973b). Ibid. 131, 351-357.