THE USE OF ELECTRICAL CONDUCTIVITY TO ASSESS THE POTENTIAL ACTIVITY OF CRYOPROTECTIVES

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There is substantial evidence that cryoprotective additives owe their activity in minimising cellular and liposomal freeze-thaw damage to their colligative properties rather than to specific biochemical mechanisms (Meryman et al 1977). These properties may be examined by several techniques including the measurement of electrical conductivity changes with temperature; on cooling of an aqueous cell suspension ionic movement is reduced, and the resistance of the system reaches a maximum on solidification. Although cooling profiles have been used to examine mechanisms of cryoprotection, there are no indications in the literature that they may be useful in predicting the degree of protection afforded against freeze-thaw damage. The purpose of this present work therefore was to assess the feasibility of using electrical conductivity measurements in this way.

Cryoprotection was determined by measurement of streptomycin leakage from lecithin liposomes following a freeze-thaw cycle (Higgins et al 1984). Electrical conductivities of cryoprotectives in 0.02 M phosphate buffer, pH 6.5, were made using 3.0 ml solution in an insulated conductivity cell fitted with stainless steel electrodes connected to the Wayne Kerr Automatic Precision Bridge. This was linked to a BBC microcomputer which also received the amplified temperature recording from a type K thermocouple and digital thermometer; both temperature and conductivity data were stored on disk. The conductivity cell was cooled by immersion in liquid nitrogen, and resistance in the range 200Ω to 20 MΩ was measured between 0 and -150°C. Plots of resistance against temperature always showed a linear region between 1 KΩ and 100 KΩ during cooling and the temperature differential corresponding to this change in resistance is tabulated against streptomycin leakage below.

Additive	3% additive		6% additive	
	% drug loss*	Temperature change °C*	% drug loss	Temperature change °C
Sucrose	29	11		
Mannitol	26	13		
Alanine	16	22	17	22
Glycerol	19	23	14	27
Betaine	15	26	8	34
DMSO	12	28	9	37

* Typical coefficients of variation for replicate determinations of % drug loss and temperature change were 0.07 and 0.05 respectively.

It can be seen that the most effective cryoprotectives (at the bottom of the table) exhibit a much slower change in resistance as the temperature is reduced. If the above results for the 3% and 6% additive are plotted and subjected to linear regression they give correlation coefficients of -0.979 and -0.961 respectively. Thawing profiles were not found to correlate with cryoprotection. These results indicate that the resistance-temperature cooling profile is likely to be of value as a means of rapid, preliminary assessment of potential cryoprotectives.

Higgins, J. et al (1984) J. Pharmac. Pharmacol. 36: Supplement 24P
Meryman, H. et al (1977) Cryobiology 14: 287-302