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"Applejack" Technique: New Application of an Old Approach to Solute Concentration

Abstract. *Freezing and slow thawing is a simple and inexpensive means for preparing concentrated solutions from large volumes of water-soluble compounds. Particularly appropriate for colored compounds, it can be adapted easily to colorless solutes for which quantitative tests are available.*

The purification of compounds from plant or animal sources often uses techniques such as dialysis, chromatography, and electrophoresis. These procedures dilute the products in the course of their separation from contaminants in the crude extracts.

The extremely simple technique described here has been used to concentrate the blue-green plant pigment, allagochrome (*1*). Solutions which have been completely frozen in plastic bottles in a deep freeze or dry-ice chest are transferred to a cold room (3° to 4°C) and supported in an inverted position above a collecting container. A several-fold concentration of the solute is obtained in the first effluent. When containers are placed on blocks of dry-ice, freezing is more rapid than in a deep freeze and samples freeze from the bottom up. The solute is thus most concentrated in the last portions of the solution to freeze where it is in a position to drain off first on melting. Maintaining a low constant temperature during thawing permits sufficient equilibration between the outside and the inside of the container so that melting (accompanied by extensive channeling) occurs throughout the sample rather than just in those parts adjacent to the bottle wall.

Table 1 summarizes measurements of the optical density of consecutive 20-ml fractions from three different 500-ml samples of allagochrome of known initial concentrations. Data on the concentration of hemoglobin and flavin mononucleotide are also included. Although the latter compounds exhibited rather unusual patterns of concentration in the effluent fractions, at least a twofold increase in concentration is possible by visually selecting that portion of the melted sample of obviously darker color. Other means would have to be used to estimate the concentrations of colorless solutions.

Between six- and seven-fold concentrations of allagochrome and quantitative recovery of solute have been achieved by this method. Routinely, the melting sample is collected in two fractions: the first 25 percent which contains the major portion of the solute, and the 25 to 80 percent fraction which contains the rest. The second fraction is recycled with a new sample. The ice remaining in the freezing bottle is discarded.

This technique is available to anyone with access to a deep freeze or dry-ice chest and a refrigerator. It provides an economical method for concentrating large volumes of water-soluble materials in laboratories where the facilities for lyophilization are limited.

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Table 1. Relative concentration (optical density) of consecutive 20-ml fractions collected from 500-ml frozen samples. Fractions were collected on an automatic fraction collector with a 20-ml volumetric siphon. Spectra were measured with a Beckman DK-2 recording spectrophotometer. Initial concentrations were as follows: allagochrome, 0.89, 2.18, and 3.01 mg of dried sample per milliliter; flavin mononucleotide (FMN), 0.01 mg/ml; hemoglobin, 0.17 mg/ml.

Allagochrome (673 mμ) at initial concentration			Hemoglobin (406 mμ)	FMN (444 mμ)
0.89	2.18	3.01		
		Initial sample		
0.58	1.42	1.96	1.20	0.24
		Fractions 1 to 7		
2.18	9.50	9.45	0.57	0.39
2.08		6.06	1.20	0.22
	4.56*			
1.99		5.25	1.46	0.26
1.62	2.60	4.85		0.39
			2.62*	
1.35	2.47			0.54
		3.97*		
1.03	2.08		2.66	0.66
0.86	1.77	3.00	0.93	0.61
		Average		
1.59	3.93	5.33	1.72	0.44

* Average for two consecutive fractions.

References and Notes

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Stratospheric Cloud over Northern Arizona

Abstract. *An unusual ring-shaped cloud was widely observed over northern Arizona near sunset on 28 February 1963. From a large number of observers' reports it is known to have appeared overhead near Flagstaff, Arizona. From initial computations based on four photos taken in Tucson, 190 miles south of the cloud, its altitude was approximately 35 kilometers. The most distant observation reported was made 280 miles from the cloud. The cloud remained sunlit for 28 minutes after local sunset. Iridescence was noted by many observers. Tentatively, the cloud may be regarded as similar to a nacreous cloud; but its unusually great height and unusually low altitude, plus its remarkable shape, suggest that it was a cloud of previously unrecorded type.*

Near sunset, on 28 February 1963, a cloud of unusual configuration and coloration was observed in widely scattered localities in Arizona and some surrounding states. The cloud took the form of a large oval ring (clear in the middle) with the long axis running north and south (Fig. 1 and cover photograph, this issue). It remained brightly illuminated well after the sun had set on high cirrus clouds to the west. From Tucson, 190 miles to the south, its angular elevation appeared to be about 6 degrees. A rough computation of its height, based on sunset geometry (*1*), made immediately after the cloud entered the earth's shadow, led me to appeal by press and radio for confirmatory reports in order to establish the approximate location and to secure descriptions from the largest possible number of other observers.

From approximately 150 reports, many communicated by persons well aware that they had seen a type of cloud unprecedented in years of sky-watching, it was quickly established that the cloud lay overhead in the vicinity of Flagstaff, Arizona, that it exhibited iridescence of the sort asso-