Refractometric Determination of Cryoprotective Agent Concentration

by Michael G. Darwin

One of the most difficult problems confronting the perfusion technician is the accurate determination of cryoprotective agent concentration in body fluids. In the past, two techniques have been employed to determine the concentration of DMSO and glycerol (the two most commonly used protective drugs): specific gravity (SG) as measured hydrometrically and freezing point determination.[1,2] Both techniques have serious drawbacks in accuracy and rapidity, and both are cumbersome if not automated, requiring elaborate control over sample temperature.

Specific gravity, the most commonly used technique, has the most practical disadvantages and the lowest accuracy. Perhaps the principal disadvantage to SG determination is the need for comparatively large sample volumes in order to float the hydrometer (250 to 500 ml., depending on the type of hydrometer and jar used). These large sample volume requirements preclude determination of changes in drug concentrations in cerebrospinal fluid or in small samples collected from peripheral veins or finger sticks.

The second major disadvantage to hydrometery is the effect of blood solids on accuracy. In clinical cryostasis operations it has been found that removal of blood components is extremely difficult, even when large volumes of perfusate are used.[3,4,5] This is particularly true of cases where there has been no cardiopulmonary assist following deanimation. Manipulation of the extremities during perfusion under these conditions has caused large increases in effluent SG, rendering accurate drug concentration impossible to determine. An operation as simple as shutting down the perfusion pump momentarily while reloading the reservoir also causes large fluctuations in effluent SG.[6,7]

The disadvantage common to both hydrometery and freezing point determination is the need for careful control of sample temperature. Warming or chilling large volume samples to a uniform temperature is not only inconvenient, it is likely to be prohibitively expensive as well. Freezing point determination undertaken without the aid of an osmometer is at best a tedious, time consuming operation.

What the perfusion technician needs is a system capable of rapidly, simply, and inexpensively determing drug concentration with extremely small sample volumes (.5 ml. or less). The instrument best able to satisfy these requirements is the American Optical Company Goldberg Refractometer.*

The AO unit is a small, hand held, temperature-compensated refractometer weighing only 275 gm. and capable of working with a sample volume as small as a single free-falling drop. Small volumes of sample material may first be centrifuged in capillary tubes (which additionally allows for microhematocrit determination) or vacutainers to remove blood components which could interfere with measurement. Centrifugation is at this time an economic impossibility when large sample volumes are required. The refractometer has other advantages over freezing point determination and hydrometery in that it is essentially instantaneous and the long-term costs comparatively small.

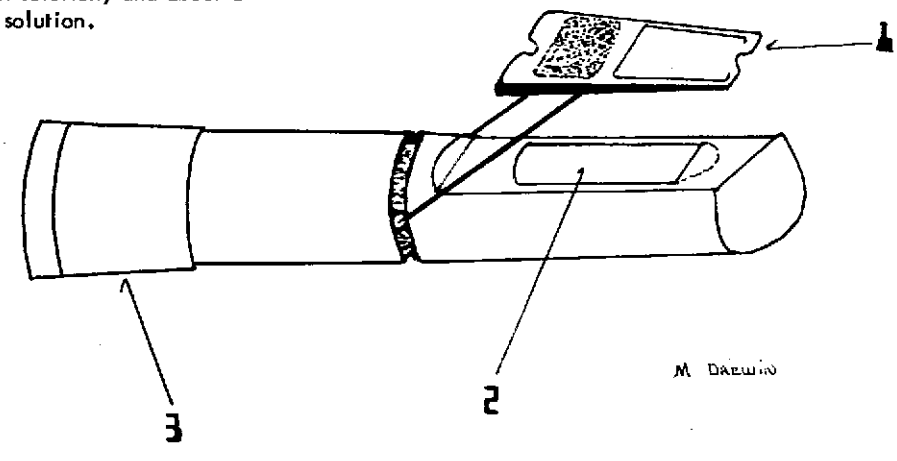
Operation of the refractometer is extraordinarily straightforward. The sample is either dispensed from a small dropper onto the instrument prism, or sucked between the instrument prism and cover plate from the cell column of a broken hematocrit tube by capillary action. Once the sample is in position and the cover plate is in place (see diagram A), the refractometer is held under a bright light and the reading appears -- in refractive index -- where the sharp boundary between the dark and the light fields crosses the scale. The accuracy of the device in determining solute concentrations has been exhaustively documented.[8,9] Because the sample volume is small the sample material comes to rapid thermal equilibrium with the instrument. The instrument is also protected against specious readings from variation in ambient temperature by the presence of a small, optically sequestered gas bubble.

The AO refractometer is a ruggedly built device designed to stand up to years of constant clinical use. The author has worked with AO instruments that have been in continuous duty service for ten years without significant drift in accuracy. Though the initial investment for the refractometer may seem large ($315.00), the long term savings in time and money make it an invaluable purchase.

Standard tables are already available in the literature for glycerol in water solutions.[10] One such table is presented as Table I of this article. These tables do not, however, establish the utility of refractometery in actual working situations. Additionally, tables giving the refractive index for DMSO in water are not to the author's

*Model AO 10402

The upper limits of this refractometer are measuring a 32% (w/w) glycerol solution, and about a 27% DMSO solution.



A

LEGEND

1) Translucent plastic cover plate.

2) Glass prism that sample is dispensed onto.

3) Focusing eyepiece.

knowledge available in the open literature. Therefore, it was decided to conduct a series of experiments to determine a mean Δ in refractive index for each 1% increase in DMSO concentration, and to evaluate the data in the open literature for glycerol under working conditions.

A series of stock solutions of DMSO in Water for Injection U.S.P. was made up in increments of 1% DMSO concentration from 1% to 15% DMSO (v/v). These stock solutions were then tested on the refractometer and a calibration table was established as shown in Table II. Though there were slight variations, it was found that a 1% increase in DMSO concentration usually resulted in a .0014 or .0015 increase in refractive index.

For the working evaluation of refractometery, rabbit kidneys were serially perfused with 5%, 10%, and 15% (w/w) glycerol in a disodium glycerophosphate based saline. Samples of 1.0 ml. were collected in small glass insuline syringes at two minute intervals from the renal vein.

The samples were divided into two parts: one .1 ml. sample was centrifuged in a heparinized microhematocrit tube manufactured by Dade Company and the liquid fraction of the sample was tested refractometrically. The remaining material was placed in a siliconized blood collection tube and frozen under ultrasonic stimulation (to reduce supercooling) with careful monitoring for the eutectic with a standard freezing point determination thermometer. The freezing point determination was used to verify glycerol concentration as estimated by refractometery. Table III shows the results of refractometery as contrasted with freezing point determination in a single, representative glycerol perfused kidney.

The clinical refractometer has been demonstrated to be a powerful tool for the rapid and accurate determination of dissolved solids concentrations in serology and urology. In our working evaluation it was found to err consistently in amounts we consider insignificant when contrasted with the advantages gained. The error was approximately .5% over what the freezing point determination indicated glycerol concentration was. Application of this method to clinical cryostasis operations, while not straightforward, shows great promise in simplifying and improving the accuracy of stat determination of cryoprotective drug concentrations in diverse body fluids.

REFERENCES

1. Fahy, G. and Federowicz, M., Manrise Technical Review, 3:7-32, 1973.
2. Unpublished suspension records of Bioenergetics, Augusta, Georgia.
3. Quaife, A., Personal Communication.
4. Horn, F., Personal Communication.
5. Unpublished suspension records of Bioenergetics, Augusta, Georgia.
6. Ibid.
7. Unpublished suspension records of Trans Time, Inc., Berkeley, California.
8. Adolph, E.F., Physiological Regulations, Lancaster, p. 219, 1943.
9. Barry, K.G. et al, Journal of Laboratory and Clinical Medicine, 55:803, 1960.
10. Handbook of Chemistry and Physics, 56th ed., Chemical Rubber Publishing Company, p. D-139, 1975.

TABLE I

% Glycerol (w/w)	Molarity	Δ °C	n	Δ
.00	.000	.00	1.3330	.0011
1.00	.109	.20	1.3341	.0012
2.00	.218	.41	1.3353	.0012
3.00	.327	.63	1.3365	.0011
4.00	.438	.85	1.3376	.0012
5.00	.548	1.08	1.3388	.0012
6.00	.660	1.31	1.3400	.0012
7.00	.771	1.56	1.3412	.0012
8.00	.884	1.81	1.3424	.0012
9.00	.997	2.06	1.3436	.0012
10.00	1.110	2.33	1.3448	.0012
11.00	1.224	2.60	1.3460	.0012
12.00	1.338	2.88	1.3472	.0013
13.00	1.453	3.17	1.3485	.0012
14.00	1.569	3.47	1.3497	.0012
15.00	1.685	3.77	1.3509	

(Hoyt, J. Eng. Chem. 26:329)

Δ °C is freezing point depression

TABLE II

% DMSO (v/v)	n	Δ
0	1.3330	.0014
1	1.3344	.0014
2	1.3358	.0013
3	1.3371	.0016
4	1.3387	.0015
5	1.3402	.0014
6	1.3416	.0013
7	1.3429	.0015
8	1.3444	.0016
9	1.3460	.0014
10	1.3474	.0015
11	1.3489	.0015
12	1.3504	.0013
13	1.3517	.0013
14	1.3530	.0017
15	1.3547	

The dimethylsulfoxide used in this study was reagent grade material purchased from the Fisher Scientific Company, Inc., Cleveland, Ohio. The dimethylsulfoxide was measured in a graduated cylinder at 25°C as was the Water for Injection, U.S.P.

TABLE III

% GLYCEROL(w/w) IN PERFUSATE	SAMPLE NUMBER	n	ESTIMATED EFFLUENT GLYCEROL % (w/w)	EFFLUENT GLYCEROL % (w/w) BY FREEZING POINT
0	1	1.3469	0.0	0.0
5	2	1.3491	1.8	1.5
5	3	1.3500	2.5	2.0
5	4	1.3514	3.8	3.2
5	5	1.3523	4.5	3.8
5	6	1.3520	4.4	4.0
10	7	1.3544	6.4	6.0
10	8	1.3561	7.7	7.2
10	9	1.3573	8.7	8.0
10	10	1.3579	9.2	8.5
10	11	1.3580	9.2	9.0
10	12	1.3580	9.2	9.0
15	13	1.3602	11.1	10.6
15	14	1.3614	12.1	11.5
15	15	1.3625	13.0	12.5
15	16	1.3630	13.4	13.0
15	17	1.3632	13.5	13.0
15	18	1.3630	13.4	13.2

Effluent samples were collected at 2 minute intervals during glycerol perfusion. The refractive index of the perfusate was as follows:

5% (w/w) glycerol 1.3529

10% (w/w) glycerol 1.3591

15% (w/w) glycerol 1.3645

This table represents the results of a single perfusion experiment and is considered representative of the other six kidneys perfused in a similar fashion.