Quantitation of Sodium in Human Albumin Solutions

PAUL K. NG ˣ and JOHN L. LUNDBLAD

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Abstract □ An osmolality method is described for quantitating sodium in isotonic solutions of diafiltered albumin and diafiltered plasma protein fractions. The proposed method shows comparable results with a reference procedure based on atomic absorption spectrometry.

Keyphrases □ Sodium—osmolality method for analysis in isotonic solutions of human albumin and plasma protein fractions □ Osmolality method—analysis of sodium in isotonic solutions of human albumin and plasma protein fractions □ Albumin, isotonic solutions—osmolality method for analysis of sodium □ Plasma protein fractions—osmolality method for analysis of sodium

Preparations of normal serum albumin and plasma protein fraction are clinically useful in plasma volume expansion under similar conditions. The sodium ion in these preparations does not cross vascular membranes instantaneously and, therefore, exerts a transient fluid-drawing or fluid-retaining effect (1). To avoid such a noncolloid osmotic pressure, the sodium content is adjusted to the isotonic range of 130–160 × 10^{-3} *M*.

Accurate measurement of the total sodium and tonicity thus become important criteria for consideration before intravenous administration. This paper reports the sodium quantitation by an osmolality procedure and compares the results with those obtained by atomic absorption spectrometry.

EXPERIMENTAL

Preparations of albumin and plasma protein fraction were obtained by alcohol fractionation according to Cohn *et al.* (2). Removal of alcohol and salt was accomplished by procedures described previously (3, 4). Each solution was adjusted to 5.0 ± 0.3% protein in 0.004 *M* sodium caprylate and 0.004 *M* acetyldetryptophan. The final pH of the protein solution was 6.9 ± 0.5.

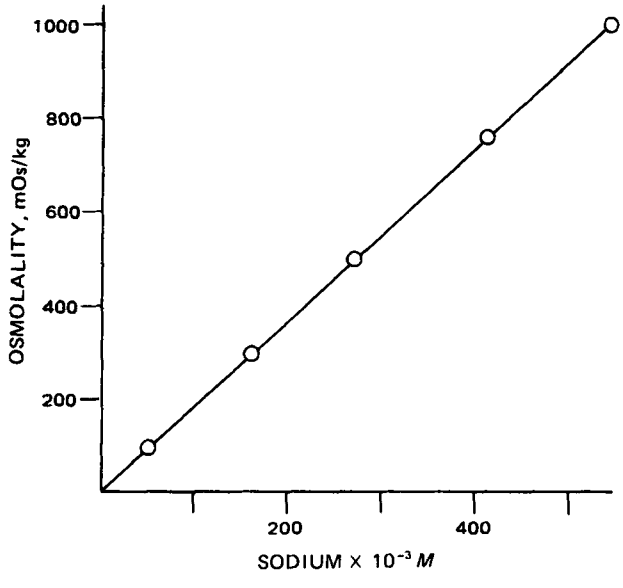

Figure 1—*Correlation of sodium concentration to osmolality.*

Table I—Sodium as Measured by Osmolality Correlation and Atomic Absorption Spectrometry

Sample	Protein, 5.0 ± 0.3%	Osmolality, ±4 mOs/kg	From Correlation, 10^{-3} *M*	From Atomic Absorption Spectrometry, 10^{-3} *M*
1658-64	Albumin	259	140	145
1658-99	Plasma protein fraction	272	147	147
PR2558	Albumin	258	139	142
PR2568	Albumin	248	134	136
PR2589	Plasma protein fraction	280	152	153
1715-13	Plasma protein fraction	269	145	146
1715-16	Plasma protein fraction	266	144	145
1715-41	Plasma protein fraction	282	153	145
1715-48	Plasma protein fraction	269	145	147

The sodium content was assayed by atomic absorption spectrometry[1]. A vapor pressure osmometer[2] measured the solution osmolality. Sodium stock standards were prepared by dissolving reagent grade sodium chloride in water for injection. Osmolality standards of 100, 290, 500, 750, and 1000 mOs/kg were obtained commercially[3].

RESULTS AND DISCUSSION

For the five standard solutions, osmolality *versus* molarity was plotted (Fig. 1). A correlation was obtained by linear regression:

$$\text{sodium} \times 10^{-3}\,M = 0.554 \times \text{osmolality (mOs/kg)} - 3.58 \quad \text{(Eq. 1)}$$

Nine individual samples were tested. Comparison with a reference method based on atomic absorption spectrometry is shown in Table I. The osmolality results were within 10% of the results by atomic absorption spectrometry.

As shown in Table I, interference due to the presence of proteins did not seem to affect the correlation significantly. This result was expected since the albumin solution (at least 96% albumin) or the plasma protein fraction solution (at least 83% albumin) accounted for less than 1 mmole/liter of the total molecules or an osmotic pressure equivalent to less than 25 mm Hg by physical meausrements (5, 6). For an isotonic solution, this value is only 0.5% of the calculated osmotic pressure (~5200 mm Hg).

The electrolytes are the main contributors to the total osmolality. Other ions such as potassium, if present at high enough levels, are interferences and will give erroneous correlation of sodium. In all studied samples, the potassium content did not exceed 2 mmoles/liter and, therefore, had an insignificant influence on the results.

Theoretically, atomic absorption spectrometry measures the total concentration of sodium in a solution and the osmolality procedure measures the total concentration of dissolved particles in a solution without regard for the homogeneity or nonhomogeneity of the molecular species, the molecular weights, the particle sizes, or the densities. By a linear correlation relationship, the osmolality gives a direct reading of sodium content. Since contribution of protein to the total osmolality is

[1] Model 306A, Perkin-Elmer, Norwalk, Conn.
[2] Model 5100B, Wescor, Logan, Utah.
[3] Wescor, Logan, Utah.

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minor, application of the present approach could be extended to other protein species.

The method has been used in conjunction with an existing analytical method. Some advantages are that the reagents are stable and that the method requires no special equipment other than the osmometer and it is efficient. Above all, its accuracy is comparable to that achieved by atomic absorption spectrometry.

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Quantitative Determination of Hexylresorcinol in Commercial Antiseptic Solution by High-Pressure Liquid Chromatography

JOSEPH D. MUSTO, JAYANT N. SANE, and VICTOR D. WARNER ˣ

Received July 5, 1977, from the *Department of Medicinal Chemistry and Pharmacology, College of Pharmacy and Allied Health Professions, Northeastern University, Boston, MA 02115.* Accepted for publication June 21, 1978.

Abstract □ High-pressure liquid chromatography was used with a 5-μm silica gel column to quantitate hexylresorcinol in a commercial antiseptic solution following extraction with methylene chloride. This method shows linearity to at least 0.025% hexylresorcinol. A mobile phase consisting of 63% heptane, 34% chloroform, and 5% methanol was used with a UV detector (254 nm) and a flow of 3 ml/min. No interfering substances were observed.

Keyphrases □ Hexylresorcinol—high-pressure liquid chromatographic analysis in commercial preparations □ High-pressure liquid chromatography—analysis, hexylresorcinol in commercial preparations

Methods for the quantitative analysis of phenolic derivatives utilize bromination (1, 2), GLC (3, 4), NMR spectroscopy (5), and high-pressure liquid chromatography (HPLC) (6). This report describes a rapid, quantitative HPLC method for the analysis of hexylresorcinol in a commercial antiseptic solution[1].

EXPERIMENTAL

Chemicals and Reagents—All chemicals and reagents were USP, NF, ACS, or chromatographic grade.

Chromatography—The high-pressure liquid chromatograph[2] was equipped with a UV detector (254 nm) and a recorder. Septum injection was utilized. The column[3] (15 cm × 6.3 mm o.d.) was packed with 5-μm silica.

The chromatographic solvent consisted of 63% heptane, 34% chloroform, and 3% methanol. The temperature was ambient, and the solvent flow rate was 3.0 ml/min (at an inlet pressure of ~70.3 kg/cm²). The detector was set at a sensitivity of 0.08 aufs, and the chart speed was 5.08 cm/min (30.48 cm/hr). The elution order was phenol (2.7 min) and hexylresorcinol (4.7 min); total analysis time was 6 min.

Preparation of Solutions—All solutions of hexylresorcinol and phenol (internal standard) were prepared (weight per volume) in methylene chloride by a simple solution method. An aqueous solution of hexylresorcinol was prepared and used as a primary standard. This aqueous standard was run with each group of samples analyzed. The commercial antiseptic solution was purchased from a local pharmacy.

[1] ST 37 (Calgon).
[2] Waters ALC 202 equipped with a separation injector.
[3] Hi Eff Micropart column (0.5-μm silica), Applied Science Laboratories.

Preparation of Linearity Curve—A 1% hexylresorcinol solution was prepared using methylene chloride containing phenol (0.025%) as the internal standard. Aliquots of this solution were diluted with the methylene chloride containing phenol (0.025%) to yield five standard solutions consisting of 0.025, 0.05, 0.07, 0.10, and 0.125% hexylresorcinol with phenol (0.025%) as the internal standard. A volume of 5–10 μl of each standard was injected, and the peak height ratio of hexylresorcinol to phenol was calculated and plotted *versus* the hexylresorcinol concentration.

The plotted data (Table I) indicated that the hexylresorcinol concentration *versus* the peak height ratio (y) was linear from 0.025 to 0.125% hexylresorcinol (x) ($y = 8.960x + 0.011$; $r = 0.999$), and the straight line intersected zero. Three data points were determined for each concentration. The standard deviations are given in parentheses.

Assay—A 5-ml aliquot of the commercial antiseptic solution was extracted by adding 1 ml of 1 *N* HCl, 0.5 ml of internal standard (0.5% phenol in methylene chloride), and 4.5 ml of methylene chloride. The mixture was hand shaken for 1–2 min and centrifuged; the aqueous supernate was discarded by aspiration with a Pasteur pipet. A 10-μl sample of the extract was injected into the chromatograph. The aqueous standard containing 0.05% hexylresorcinol was carried through the same extraction procedure at the same time as the sample. A 0.1% hexylresorcinol solution in methylene chloride containing phenol (0.025%) was regularly injected during analysis to monitor instrument stability.

Calculation—The following formula was used for calculating concentrations:

$$\% \text{ sample} = \frac{R_s}{R_{std}} \times \% \text{ std} \qquad \text{(Eq. 1)}$$

where % sample is the percent hexylresorcinol in the sample, % std is the percent hexylresorcinol in the standard, R_{std} is the ratio of the peak heights of hexylresorcinol to phenol in the standard, and R_s is the ratio of the peak heights of hexylresorcinol to phenol in the commercial antiseptic.

Table I—Data for Linearity Curve

Hexylresorcinol Standard, %	Peak Height Ratios
0.025	0.232 (0.009)
0.050	0.463 (0.015)
0.070	0.637 (0.014)
0.100	0.905 (0.019)
0.125	1.130 (0.010)

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