SPEED OF SOUND MEASUREMENTS IN POTENTIAL CONTRAST AGENTS FOR USE IN DIAGNOSTIC ULTRASOUND

by

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R.E.M.

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I. INTRODUCTION.

Current state-of-the-art diagnostic ultrasound equipment enables the physician to visually detect differences between and within body tissues. It allows for the detection of some benign and malignant tumors in such organs as the breast, thyroid, pancreas, liver, and kidney without exposure to the ionizing radiation of xray and radionuclide procedures.

Diagnosis with ultrasound essentially involves the interpretation of observable interactions between the ultrasound beam and tissue interfaces. These interactions include reflection and, to a lesser degree, scattering and depend upon differences between the acoustic impedances of the two tissues forming each interface. Large numbers of tissue interfaces exist; however, the differences among the acoustic impedances of most tissues (normal or abnormal) are not very large.

The usefulness of diagnostic ultrasound in the detection of small tumors or subtle differences between tissues is restricted by ultrasound equipment limitations such as spatial resolution, detector sensitivity, signal to noise ratio, and display capabilities. With current equipment, the ultrasonic differentiation of tumors from normal tissue is at times difficult and, in the case of small tumors, often impossible. The identification and development of ultrasonic contrast materials, which are expected to enhance the acous-

tic impedence mismatch between tissues and hence increase the magnitude of observable interactions, may greatly reduce the limitations of present ultrasound equipment.

Work on ultrasonic contrast in the past has been primarily limited to the use of microscopic air bubbles dissolved in liquids to identify certain vessels in the body (1, 2, 3) No work has been reported on the use of ultrasonic contrast agents to alter the ultrasonic appearance of tissue directly.

The ability of potential ultrasonic contrast agents to produce clinically observable changes in tissue will depend upon the differential uptake of the contrast media by two types of tissue. In this respect, ultrasonic contrast agents will work in an analogous manner to contrast media employed in radiology. Since the amount of observable change will depend upon the difference between the concentration of the contrast agent present in each tissue, it will be necessary for the acoustic properties of the contrast agent to exhibit a marked dependence upon concentration at normal body temperature.

The acoustic impedence of a medium is directly related to the product of its speed of sound and density. For weak aqueous solutions that can be achieved in the body, the speed of sound exhibits the dominant dependence upon concentration. In this present work, precision speed of sound measurements were made for twenty-two compounds in aqueous

solutions as part of an effort to identify and develop potential contrast agents for use in diagnostic ultrasound. Speeds of sound were measured relative to pure water for concentrations up to 1.0 molar (higher concentrations are not likely to be achieved in the body)

Speeds of sound were measured by a comparison method using pulse-echo. The apparatus used represented a modification of available existing equipment. Modification was undertaken in order to increase the precision of the measurements and to eliminate problems associated with the existing equipment's general use.

All solutions were prepared from reagent grade chemicals and distilled water with molar concentration being determined at 20°C. Speeds of sound were then found by placing each solution in a velocimeter, heating to 37°C and measuring the time-of-flight of a sound pulse through the solution. The ratio of the value found for distilled water to that found for a given solution, multiplied by the known speed of sound in pure water yielded the speed of sound for the solution.

The compounds considered were chosen from a list of materials, most of which can be administered intravenously in large (gram) amounts to humans and, in some cases, are known to concentrate in specific organs. These compounds were:

Ammonium Citrate	Lysine Monohydrochloride
Alanine	Mannitol
Arginine Hydrochloride	Methionine
Calcium Gluconate	Potassium Citrate
Cysteine Hydrochloride	Sodium Acetate
Fructose	Sodium Bicarbonate
Glutamic Acid	Sodium Citrate
Glycine	Sodium Lactate
Histidine Hydrochloride	Sorbitol
Lactose	Sucrose
Lithium Citrate	Urea.

The results of these measurements showed that the dependence of the speed of sound on molar concentration was near linear for all compounds with the exception of Lactose and Sucrose. A relatively wide spectrum of speed of sound versus concentration slopes was found. The highest slopes were exhibited by the Group I salts of Citric Acid while the lowest were found for the lower molecular weight organic compounds.

II. LITERATURE REVIEW.

A variety of methods have been developed for measuring the speed of sound in liquids (4). Generally, these methods are based on one of three general techniques: the measurement of the wavelength of the sound using an acoustic interferometer (5); measurement of the wavelength using optical diffraction patterns (6); or the measurement of the time-of-flight for a sound pulse over a known distance (7). Each approach can yield precision speed of sound measurements. Accuracies of a few parts in ten thousand are quite common. Values obtained with an acoustic interferometer have even been reported to an accuracy of a few parts per million (4, 8).

Precision speed of sound measurements have been made for many aqueous solutions as a function of temperature, pressure, and concentration. The bulk of these measurements deal with inorganic salts (9, 10) specifically those salts present in sea water (11). These measurements show that the speed of sound, as well as other acoustic parameters, are dependent on concentration. Normally, the speed of sound increases with increasing concentration although exceptions exist (12, 13). Furthermore, the dependence is nearly linear for concentrations lower than 1.0 molar in most cases.

Barthel (12) derived an expression for the concentration dependence of sound speeds in dilute solutions

of nondissipative media which predicted a near linear variation for aqueous solutions at low molar concentrations. He then investigated the behavior of Sodium Chloride, Lead Nitrate, and Sucrose at low concentrations and found agreement with the predicted behavior. Although Barthel used molal concentrations, he pointed out that similar behavior could be obtained using molarity.

A similar behavior at low concentrations can be seen in the work of Freyer (9) for aqueous solutions of certain alkali halides. Freyer's discussion of his results gives and proposes many of the explanations for the behavior of the speed of sound versus concentration for electrolytic solutions mentioned by later authors. One such author, Marks (10), in a well-conceived investigation, provides another illustration and further analysis of the variation of sound speeds with concentrations for electrolytic aqueous solutions. Both authors indicate that the rate of change of sound speed with concentration is dependent upon valance states of the ions in solution, ionic radii, molecular symmetry, and the degree of assocation.

Theoretical expressions have been sought that would allow the prediction of sound speed behavior as a function of concentration. The most promising approaches involve the use of free-volumes and the kinetic theory of liquids (14, 15, 16) Most of these theories deal with binary liquid mixtures and are rather limited in application

at present. More empirical information is needed for the testing and development of these models.

The speed of sound has been determined for a large number of materials in aqueous solution as a function of temperature at various concentrations. Although data is available for some common salts and sugars, NaCl and Sucrose for example, there exists no comprehensive data on injectible solutions.

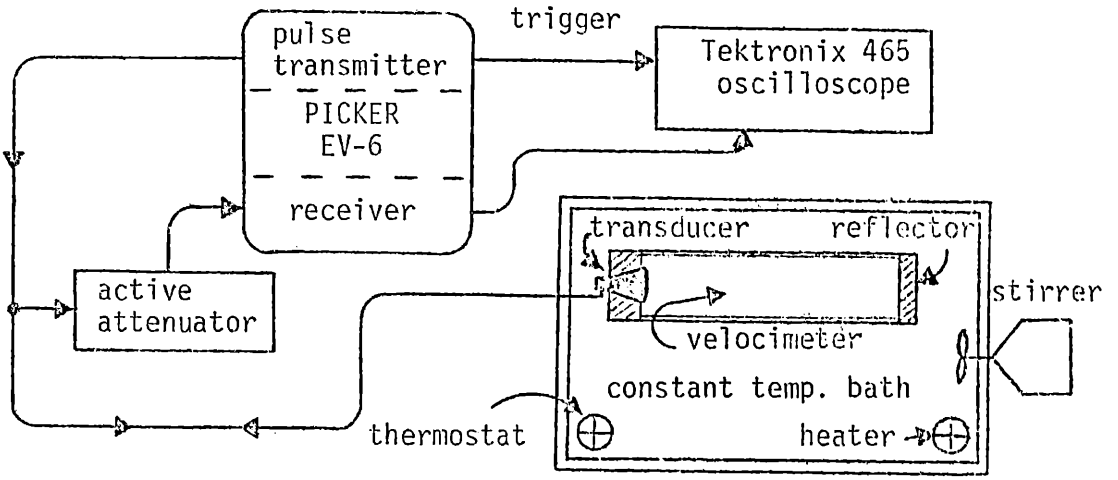
III. MATERIALS AND METHODS.

From the numerous methods available for determining the speed of sound in liquids, a modification of the pulse-echo method introduced by Pellam and Galt (7) which is generally referred to as the comparison method (17) was adopted. The method offers one of the best combinations of convenience (ease-of-use) and high accuracy (4). It involves the measurement of the pulse transit times for a set propagation distance in both the medium of interest and a medium with known propagation speed. Since aqueous solutions were of interest here and since its speed of sound is very well known, pure water was taken as the reference medium. Hence, all speed measurements herein are relative to pure water at 37°C.

The existing apparatus appears in Figure 1. The velocimeter, consisting of a plexiglas tube with a Automation Industries 2.0 megahertz transducer sealed to one end and a plane stainless steel reflector sealed to the other, was submerged in a GCA/Precision Scientific constant temperature bath. A Picker EV-6 with a receiver modification served as both transmitter and receiver, and a Tektronix 465 oscilloscope with calibrated delay sweep was used to determine the time delay between the transmitted pulse and the received echo.

Problems were encountered with this initial equip-

FIGURE 1: Block Diagram of Existing Apparatus



ment. The one microsecond resolution of the oscilloscope's delay sweep limited the determination of the speed of sound to a certainty of 3.5 parts in a thousand. Although this was probably sufficient for the nature of this work, it was much worse than the accuracy reported in the literature for pulse-echo techniques. The use of plexiglas for the body of the velocimeter caused several problems. Due to the themal conductivity of plexiglas, extensive time was required for the sample and bath to reach thermal equilibrium. Thermal expansion of the velocimeter also caused transit time measurements to be inordinately temperature sensitive. The most significant problem with the velocimeter, however, was the low chemical resistance of the plexiglas to certain aque-

ous solutions.

To resolve these problems, modification of the existing apparatus was undertaken. An increase in the temporal resolution was achieved by replacing the existing electronics with the arrangement in Figure 2. The basic improvement resulted from the use of a 40 megahertz crystal clock circuit with digital delay. This increased the temporal resolution to 25 nanoseconds and thus limited the determination of the speed of sound to an optimal certainty of about one part in ten thousand. Next, the difficulties caused by the plexiglas velocimeter were greatly reduced by replacing it with a borosilicate glass velocimeter. Two velocimeters were constructed, having lengths of 20 and 35 centimeters (Figure 3)

All solutions were prepared from reagent grade chemicals without further purification. When possible, concentrations of .125, .25, .50, and 1.00 molar were used with molarity determined at 20°C. A Sartorius 2354 precision balance with an accuracy of ±.01 gram was used to determine mole quantities for each concentration. These were then diluted with distilled water to a volume of one liter using volumetric flasks calibrated to an accuracy of .3 milliliters.

All speed measurements were made at 37°C ± .1°C (normal body temperature) using the new apparatus in Figure 2.

Briefly, operation went as follows. A single cycle pulse (7 volts p-p with load) from a 1.6 megahertz sine wave (1.6 megahertz yielded best transducer response) was applied to the transducer to create a short-duration stress pulse in the liquid. The transducer's response to the echo from the stainless steel reflector (normal to pulse-echo path) was amplified by a wideband amplifier and displayed on the oscilloscope. The pulse and echo were matched in amplitude and transit time accurately measured by recording the number of clock pulses from a 40 megahertz crystal clock that occurred between the pulse and the echo. The speed of sound in the sample was then found from the ratio of clock pulses for distilled water to that of the liquid sample, and the known speed of sound in pure water at 37°C, 1523.62 meters per second (18). Values were checked using the two velocimeters (ratio of lengths 4 : 7).

FIGURE 2. Block Diagram of the New Apparatus

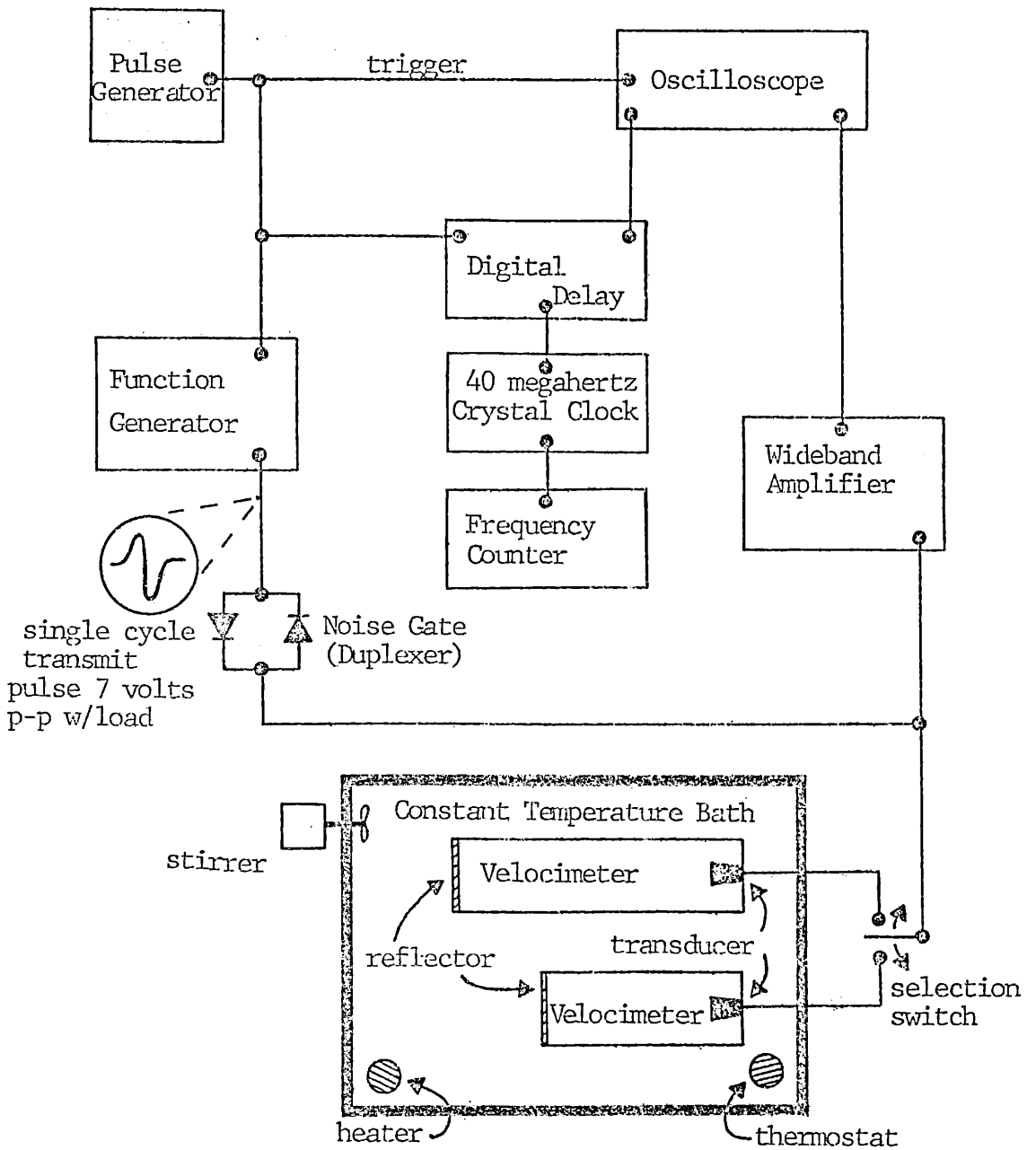
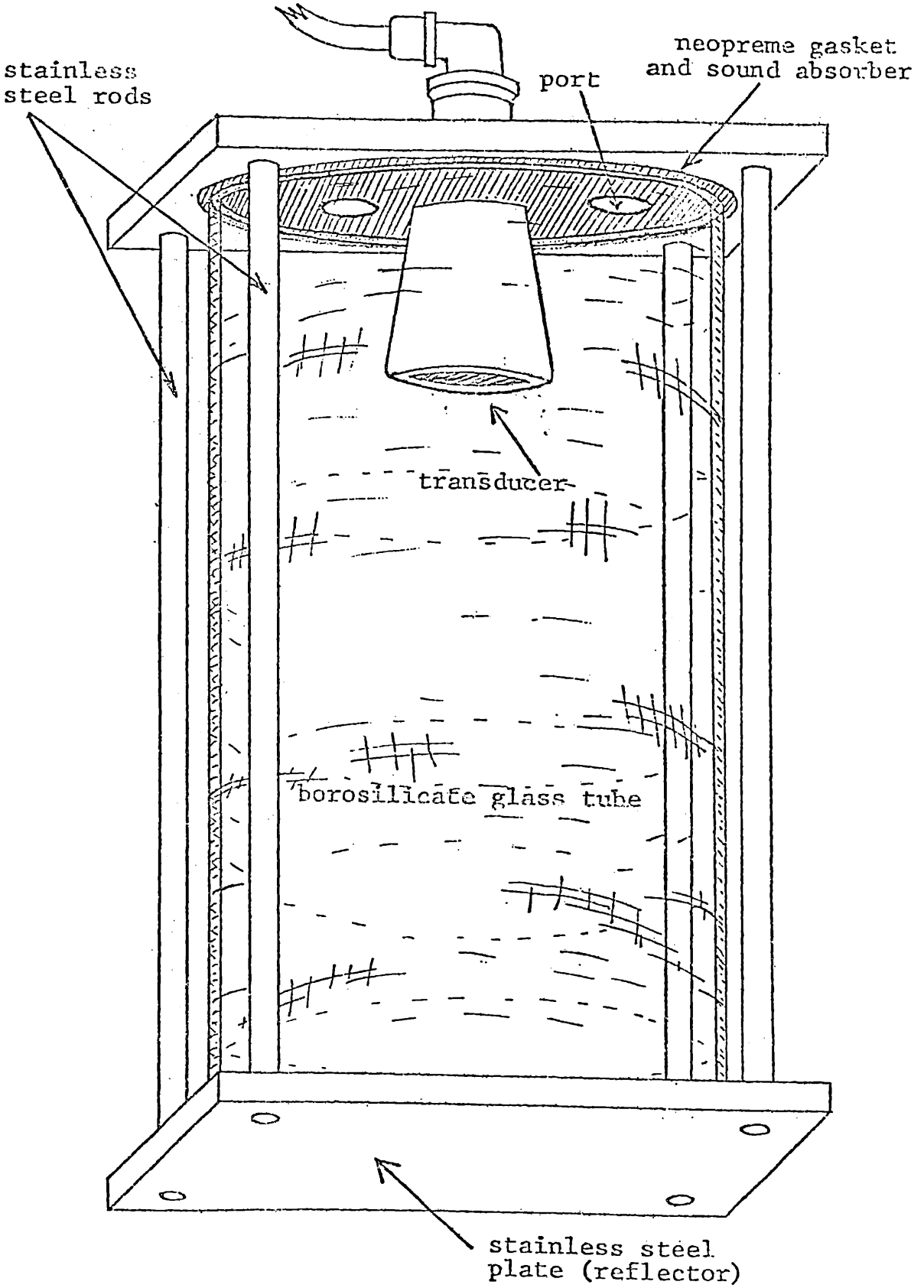


FIGURE 3. Velocimeter.



IV. RESULTS.

The twenty-two compounds listed in the introduction were divided into four groups. The speed of sound measurements as function of concentration for each group appear in tabular and graphical form in Tables 1 through 4 and Graphs 1 through 4, respectively. All measurements are for 37°C, normal body temperature. Graph 5 compares the compounds from each group that exhibited the largest dependence of speed of sound on concentration.

From linear least-square fits to the data, the slopes and intercepts were determined and appear in Table 5. All slopes were normalized to the highest slope found and also appear in Table 5.

TABLE 1: SUGARS: Speed of sound (meters per second) versus molar concentration at 37°C.

Compound	.125M	.25M	.5M	1.0M
Fructose (m. w. 180.16)	1531.19	1538.92	1553.85	1586.52
d-Lactose ($1H_2O$) (m. w. 360.32)	1535.87	1548.56	1575.78	1639.19
d-Mannitol (m. w. 182.18)	1531.32	1539.00	1554.97	1588.33
d-Sorbitol (m. w. 182.17)	1531.23	1538.48	1555.01	1588.24
Sucrose (m. w. 342.20)	1534.14	1545.16	1568.83	1625.14

TABLE 2: SALTS OF CITRIC ACID: Speed of sound (meters per second) versus molar concentration at 37°C. **

Compound	.125M	.25M	.5M	1.0M
Ammonium Citrate (m. w. 226.19)	1537.64	1551.45	1579.93	1637.80
Potassium Citrate ($1H_2O$) (m. w. 324.22)	1546.67	1569.31	1614.07	1704.41
Sodium Citrate ($2H_2O$) (m. w. 294.10)	1546.63	1569.28	1614.05	1704.54
	.125M	.25M	.32M	.64M
Lithium Citrate ($4H_2O$) (m. w. 281.98)	1543.59	1563.29	1574.55	1625.13

** Note: Concentrations used for Lithium Citrate differ from the others due to availability.

TABLE 3: AMINO ACIDS: Speed of sound (meters per second) versus molar concentration at 37°C. Note that due to saturation the concentrations used for L-Histidine Hydrochloride differ from the others.

Compound	.125M	.25M	.5M	1.0M
L-Alanine (m. w. 89.10)	1530.88	1538.48	1553.36	1583.27
L-Arginine Hydrochloride (m. w. 210.67)	1538.70	1554.04	1583.05	1640 71
L-Cysteine Hydrochloride ($1H_2O$) (m. w. 175.63)	1532.26	1540.91	1558.74	1591.92
L-Glutamic Acid (Sodium Salt) (m. w. 191.10)	1539.26	1554.51	1585.47	1648.27
Glycine (m. w. 75.07)	1529.82	1536.08	1548.71	1572.15
L-Lysine Monohydrochloride (m. w. 182.65)	1539.93	1555.93	1587.50	1649.77
L-Methionine (m. w. 149.21)	1534.30	1545.57	1569.25	1608.55

	.0625M	.125M	.25M	.5M
L-Histidine Hydrochloride ($1H_2O$) (m. w. 209.63)	1530.20	1536.40	1549.13	1574.14

TABLE 4: OTHERS: Speed of sound (meters per second) versus molar concentration at 37°C. Note that due to saturation the concentrations used for Calcium Gluconate differ from the others.

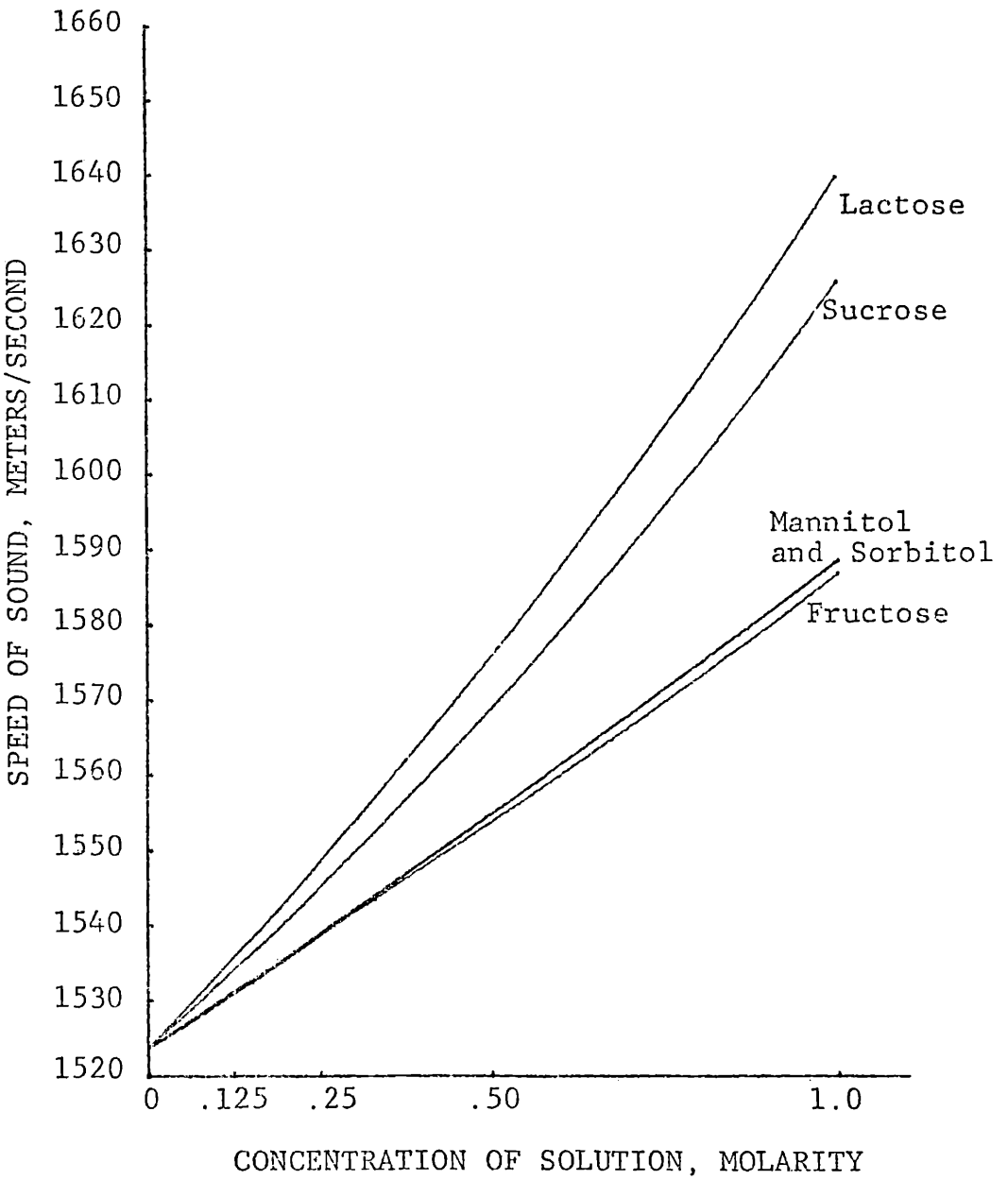
Compound	.125M	.25M	.5M	1.0M
Sodium Acetate ($3H_2O$) (m. w. 136.08)	1534.05	1544.51	1564.15	1604.02
Sodium Bicarbonate (m. w. 84.01)	1532.84	1541.86	1559.69	1592.87
Sodium Lactate (m. w. 112.06)	1535.17	1546.50	1568.39	1610.75
Urea (m. w. 60.06)	1526.37	1528.57	1533.98	1544.48

	.0625M	.125M	
Calcium Gluconate (m. w. 430.38)	1532.44	1540.55	--saturates--

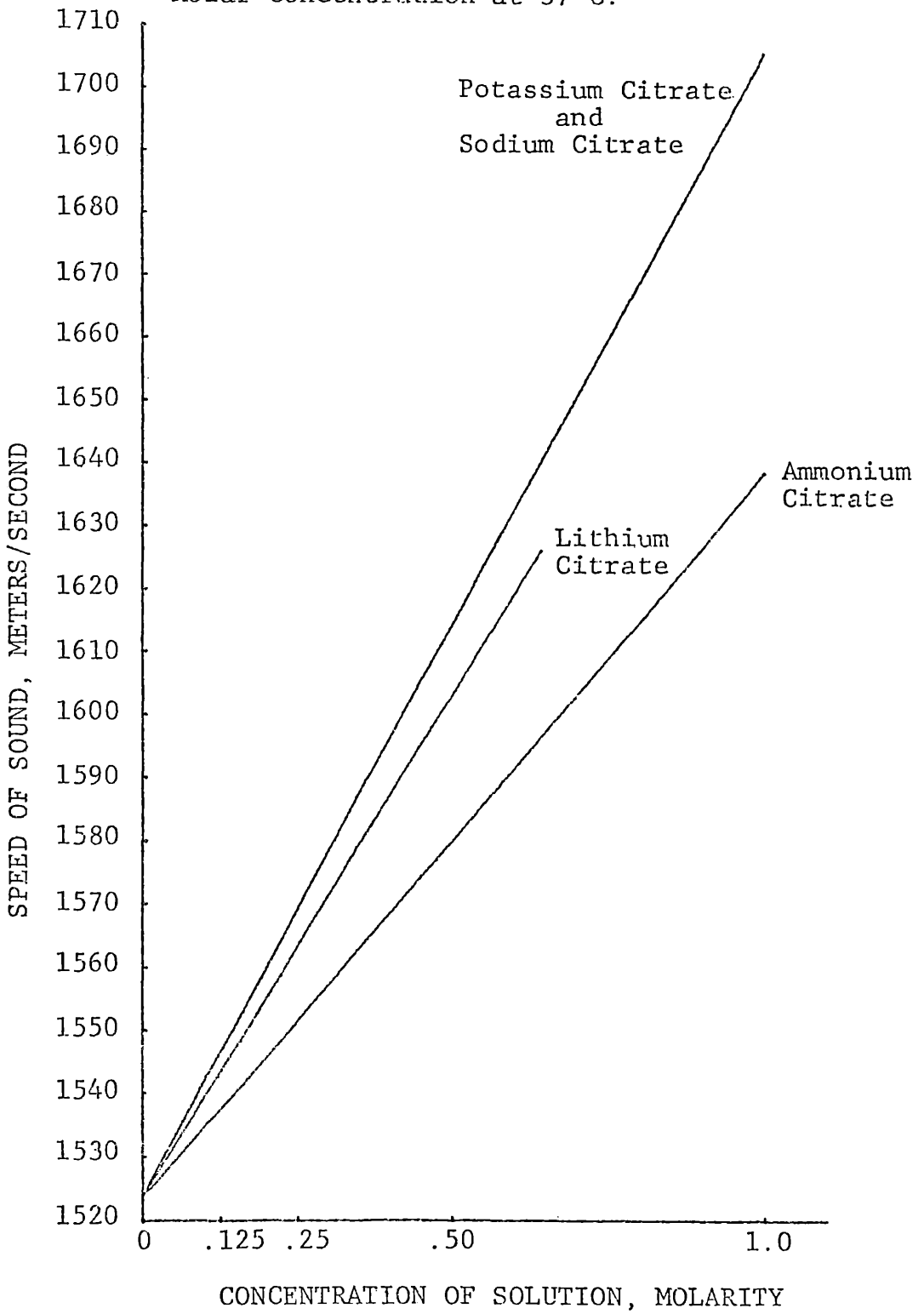
TABLE 5: Parameters for Linear Least-Square Fits, column 2 and 3, and Normalized Slope, column 4. Values for the intercept and slope are in m/s and m/s/mole, respectively.

Compound	Intercept	Slope	Normalized Slope
Potassium Citrate	1524.14	180.21	1.00
Sodium Citrate	1524.14	180.20	1.00
Lithium Citrate	1523.89	157.60	.87
Calcium Gluconate	1524.33	129.76	.72
Lysine Monohydrochloride	1524.49	125.42	.70
Glutamic Acid (Sodium Salt)	1523.78	123.31	.68
Arginine Hydrochloride	1524.20	117.95	.65
Ammonium Citrate	1523.40	112.94	.63
Lactose	1523.18	101.52	.56
Histidine Hydrochloride	1523.91	100.52	.56
Sucrose	1523.12	88.16	.49
Sodium Lactate	1524.81	86.17	.48
Methionine	1524.69	84.75	.47
Sodium Acetate	1524.30	79.75	.44
Cysteine Hydrochloride	1523.58	69.20	.38
Sodium Bicarbonate	1524.72	68.47	.38
Sorbitol	1522.97	63.80	.35
Mannitol	1523.34	63.18	.35
Fructose	1523.73	60.33	.33
Alanine	1523.46	59.82	.33
Glycine	1524.04	48.33	.27
Urea	1523.56	20.88	.12

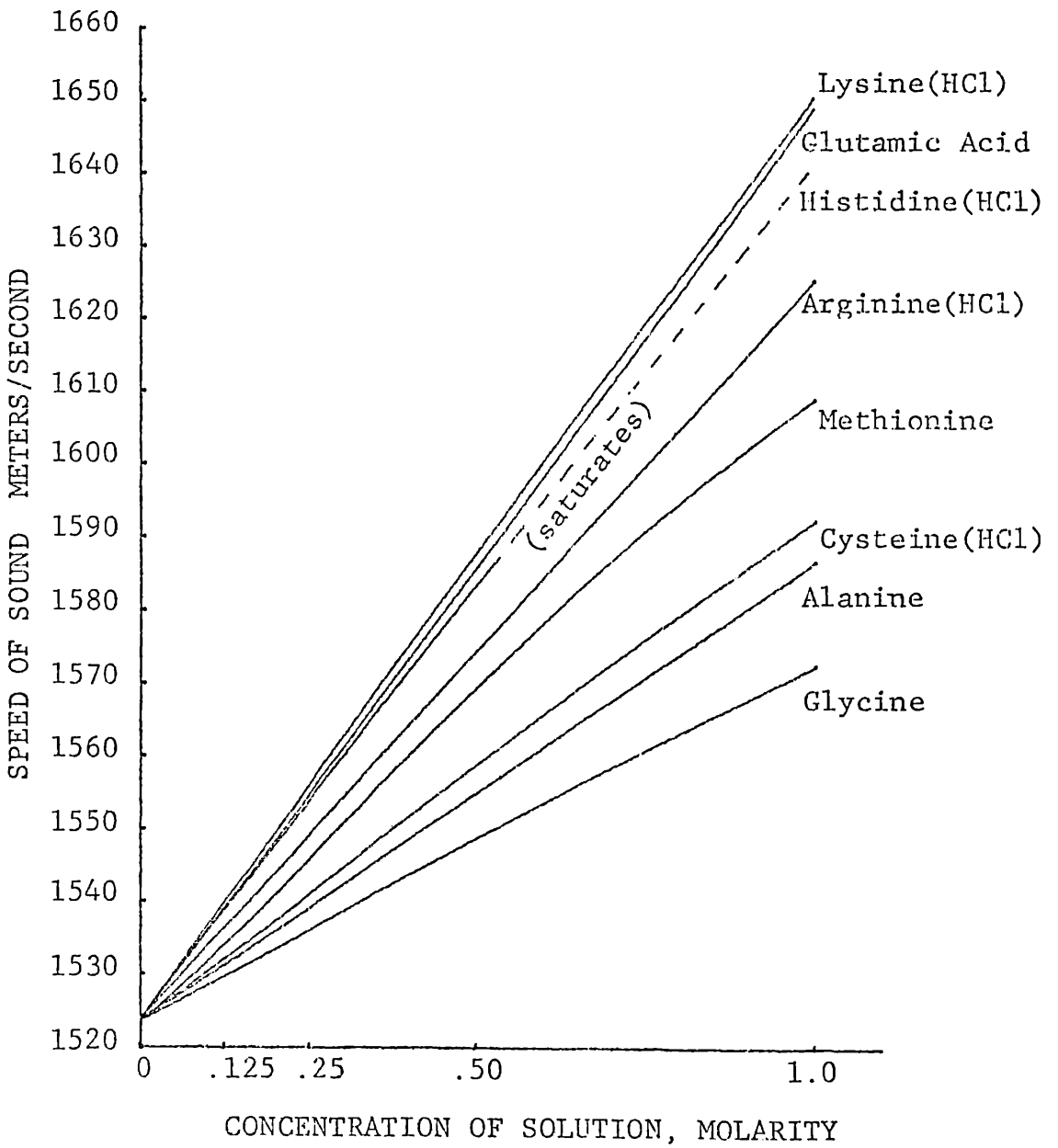
GRAPH 1: SUGARS: Speed of Sound versus Molar Concentration at 37°C.



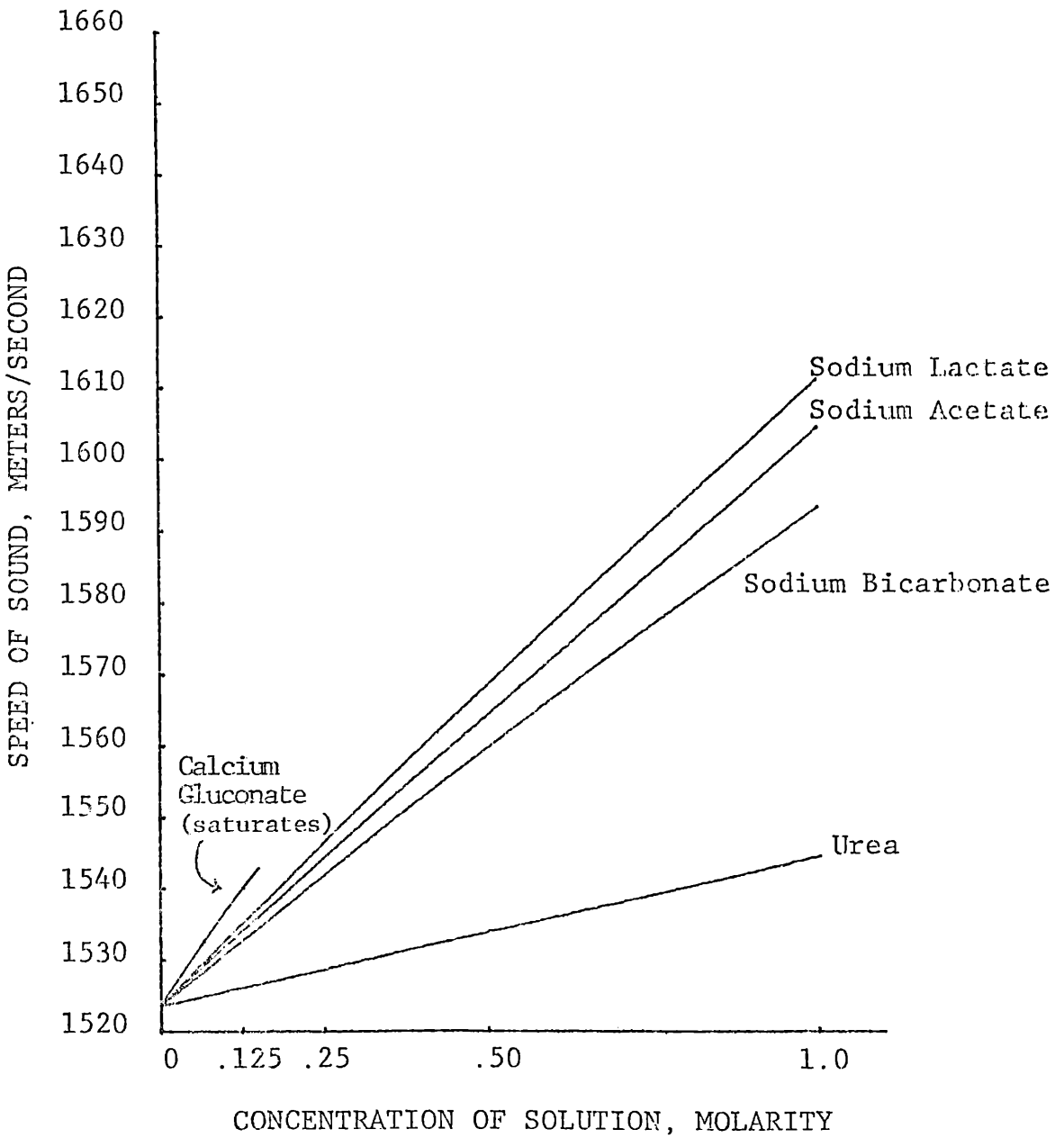
GRAPH 2: SALTS OF CITRIC ACID: Speed of Sound versus Molar Concentration at 37°C.



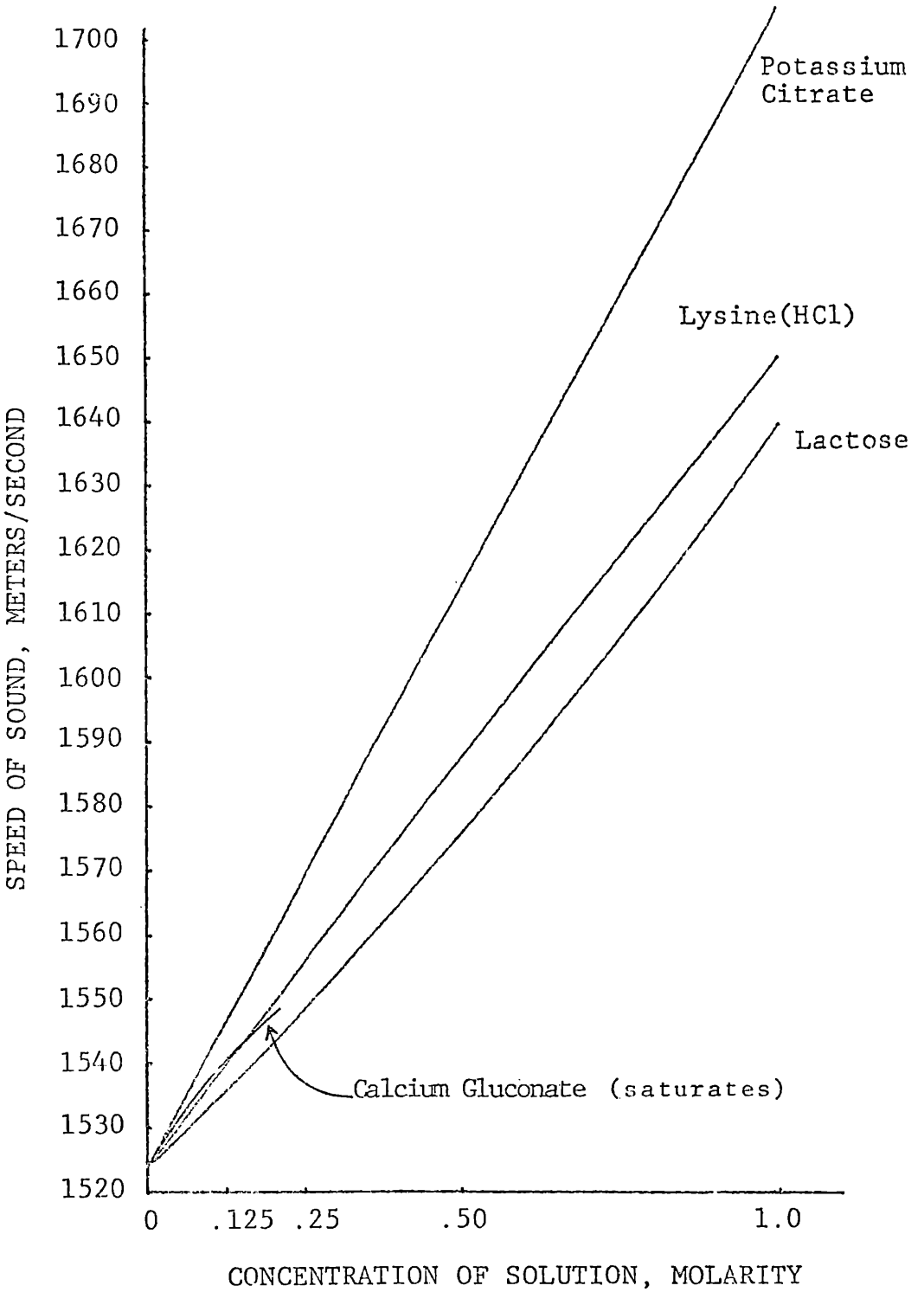
GRAPH 3: AMINO ACIDS: Speed of Sound versus Molar Concentration at 37°C.



GRAPH 4: OTHERS: Speed of Sound versus Molar Concentration at 37°C.



GRAPH 5: BEST CANDIDATE FROM EACH GROUP: Speed of Sound versus Molar Concentration at 37°C.



V. ACCURACY.

The equation used to determine the speed of sound in solution was:

$$U_s = \frac{n_o}{n_s} U_o \qquad (1),$$

where U_s is the speed of sound in the solution, U_o is the speed of sound in pure water at 37°C and n_s and n_o are the number of clock pulses measured for the solution and pure water respectively. The speed of sound at this temperature for pure water was reported accurate to ±.05 meters per second or better than 33 ppm (18). The number of clock pulses (both n_s and n_o) were taken accurate to ±1 pulse. This represented the resolution for the electronics which was assumed to be 25 nanoseconds (although this was double the true estimated resolution of 12.5 nanoseconds). For the small velocimeter this corresponded to an accuracy of better than 13 parts in 10^5 for all measurements; while, for the large velocimeter, the accuracy was closer to 7 parts in 10^5. Thus, considering only the accuracy of the relative water sound speed and the resolution of the electronics, the optimal certainty was better than 13 parts in 10^5.

Unfortunately, the temperature for these measurements was only controlled to a reasonable certainty of ±.1°C (although ±.05°C was probably maintained). For a temperature

of 37°C, this corresponds to an uncertainty for the speed of sound in pure water of ±.18 meters per second or about 12 parts in 10^5 for both (10) the pure water measurement and the solution measurement.

Concentrations were determined to an accuracy of between 14 parts in 10^4 for the .125 molar solution of Urea and the 3 parts in 10^4 for the .125 molar solution of Calcium Gluconate. For the worst case, this uncertainty in concentration translates in to a speed of sound certainty of better than 4 parts in 10^5.

Taking all these values into account, it appeared reasonable to assume an accuracy estimate of not better than ±.3 meters per second or 2 parts in 10^4 for all speed of sound measurements made.

As a further check of this assumed accuracy and as a means of detecting any effect of dispersion (19) or attenuation, speed of sound measurements were made using both velocimeters. No significant distortion in the pulse shape due to attenuation was noted and the two values obtained, in all cases, differed by less than 2 parts in 10^4.

VI. DISCUSSION OF RESULTS.

The twenty-two compounds listed in the introduction were divided into four groups: sugars, salts of Citric Acid, amino acids and others. Except for the last group, each grouping represents similar compounds. This approach was chosen to allow easier analysis both within and among groups and to facilitate comparison with results found by others. The speed of sound measurements for each compound as a function of molar concentration appear in tabular and graphical form in Tables 1 through 4 and Graphs 1 through 4, respectively.

Because the curves are seen to be nearly linear in almost all cases and since slopes from straight line fits would provide a convenient measure of the dependence of a compound's speed of sound on concentration (usefulness as a contrast agent requires a marked dependence, see Introduction), linear least-square fits were made for each compound. The results appear in Table 5 in order of decreasing slope. Moreover, to provide for a greater degree of fit and better accuracy in calculating the speeds of sound for any given concentration, least square fits were also obtained using the equation:

$$U_s - U_o = Am + Bm^{3/2} + Cm^2 \qquad (2)$$

Here U_o is the speed of sound in pure water at $37^{\circ}C$, U_s is the speed of sound in the solution and m is the molar con-

centration of the solution (11). Constants A, B and C were determined and appear in the appendix for all compounds except Calcium Gluconate (insufficient datum points).

As is clear from Graph 1, the three low molecular weight sugars, Fructose, Sorbitol and Mannitol, show nearly linear variations in sound speeds out to concentrations of .5 molar. The two high molecular weight sugars, Lactose and Sucrose, however, begin to deviate from linearity at about .25 molar. The deviation from linearity could probably have been reduced if molal concentrations had been used instead of molar (12). Of the sugars, Lactose exhibits the largest slope of the speed of sound versus concentration, 101.52 m/s/mole, while Fructose shows the lowest with 60.33 m/s/mole.

All the salts of Citric Acid exhibit very linear slopes (Graph 2). Potassium Citrate and Sodium Citrate show the largest effect with nearly identical slopes of 180.21 and 180.20 m/s/mole, respectively. Lithium Citrate is not much lower with a slope of 157.60 m/s/mole, while Ammonium Citrate is the lowest of this group with 112.94 m/s/mole. Again the curves are seen to be quite linear.

In the amino acid group, a wide range of slopes is found (Graph 3). The rough correlation between molecular weight and slope which can be inferred losely for both of the previous groups is not as obvious here. While

the lower weight amino acids do have the lowest slopes, the highest slopes were not found to belong to the highest molecular weight group members. Of the amino acids, the simplest and lowest molecular weight amino acid, Glycine, shows the smallest variation with a slope of 48.35 m/s/mole, while the highest slopes, 125.40, 123.75 m/s/mole, were found for Lysine Monohydrochloride and Glutamic Acid (Sodium Salt), respectively.

The remaining group of compounds show variations as indicated by Graph 4. Except for Calcium Gluconate, which saturated at concentrations above .125 molar, the variations in speeds of sound are again nearly linear out to 1.0 molar. Although these compounds are not all similar (except two sugar deriviatives and two sodium salts), the same rough correlation between slopes and molecular weights was seen here. Calcium Gluconate shows the largest slope found in this group (keeping in mind its limited range), measured at 129.76 m/s/mole. The lowest here is that for Urea at 20.88 m/s/mole.

The linear or near linear variation observed for all these compounds at low concentration corresponds to that predicted and found by Barthel (see Literature Review). Moreover, the rough correlation between slopes and molecular weight can be understood from the work of Freyer and Marks (see Literature Review).

Although a detailed analysis of the observed behavior of each compound is beyond the scope of this work, an analysis of the general behavior within each group and between each group is necessary. The dominant factors involved for the sugars are apparently molecular size and symmetry, and association (9, 10). Each of these affect the degree of hydrogen bonding and therefore the compressibility and density of the solution. Since speed of sound varies inversely with the square root of the compressibility and density product, the speed of sound is affected. For the salts of Citric Acid the dominant factors appear to be the high ionic valance and ionic size. The larger the ionic size the larger the speed of sound for identical ionic valances; hence, Potassium Citrate has the largest slope. Since these salts have the highest ionic valances of the compounds studied, they exhibit the largest slopes. As for the amino acids, the same reasoning holds and the presence of differing ionic valances, sizes, symmetries and degree of association for these compounds adequately accounts for the observed slopes. The same reasoning applies to the last group of compounds, also. Every compound studied exhibited a positive slope for the speed of sound versus concentration (12), although the spread in slopes was large (Table 5).

In order to obtain a relative measure of the usefulness of the compounds as contrast agents wholly on the

basis of variation of sound speed with concentration, the slopes from the straight line fits for each compound were normalized to the highest slope observed, that of Potassium Citrate. The relative ranking thus obtained appears in Table 5. Graph 5, which shows the curves for the best candidate from each family, allows another relative ranking.

It is obvious from the above that Potassium and Sodium Citrate are the best candidates. However, it is feasible that any compound ranking high in this study (greater than .5, for example) should be considered as a good candidate for further consideration and study as a potential contrast agent.

It is fortunate, though, that Potassium and Sodium Citrate rank highest in this study since both of these have other positive attributes. Both have low toxicity (Sodium has much lower toxicity than Potassium) and are, in a general comparison with the other compounds studied here, more routinely used in the clinic (especially Sodium Citrate)

The results of this study are potentially useful for purposes other than just indicating contrast agent feasibility. The precision of the measurements allow for their use in comparisons with theorectical results and with the results of other similar measurements.

VII. CONCLUSIONS.

The necessity that the acoustic properties of ultrasonic contrast agents exhibit marked dependence upon concentration at normal body temperature is seen not to be a deterrent to the feasibility of obtaining such contrast agents. Several compounds, Potassium and Sodium Citrate, Calcium Gluconate and Lysine Monohydrochloride, for example, exhibit large variations in their sound speeds as a function of concentration.

The results obtained also indicate that the reasons proposed by others for the variation of sound speed as a function of concentration for aqueous solutions hold and, more importantly, could possibly serve as the criteria for predicting the usefulness of other compounds as potential ultrasonic contrast agents.

APPENDIX

Parameters for Equation 2, pg. 27.

Ammonium Citrate
A = 111.100144948
B = -0.4762169556
C = 3.56039715815

Alanine
A = 59.9666718955
B = 5.94021104157
C = -3.2589040561

Arginine Hydrochloride
A = 126.462322890
B = -13.300879593
C = 3.91966282769

Cysteine Hydrochloride
A = 61.9514624845
B = 24.2992416189
C = -17.944819554

Fructose
A = 66.7736985624
B = -20.641340315
C = 16.7632994650

Glutamic Acid
A = 127.732938956
B = -12.308492540
C = 9.23125112229

Glycine
A = 45.3319385654
B = 15.5338039377
C = -12.333987591

Histidine Hydrochloride
A = 107.086654404
B = -14.719405744
C = 8.74173928711

Lactose
A = 99.8937193743
B = -16.421906670
C = 32.0999188685

Lithium Citrate
A = 160.647983037
B = -4.1540113380
C = 2.01587512271

Lysine Monohydrochloride
A = 134.025712249
B = -11.238370990
C = 3.36519402057

Mannitol
A = 61.0305431148
B = -0.9813047863
C = 4.66389270503

Methionine
A = 60.1891810803
B = 89.4657158211
C = -64.715455076

Potassium Citrate
A = 192.240599253
B = -26.997437223
C = 15.5455168633

Parameters for Equation 2, continued.

Sodium Acetate

A = 90.9234373431

B = -21.626051486

C = 11.0958489572

Sodium Bicarbonate

A = 72.3703772548

B = 6.25103417964

C = -9.3694747385

Sodium Citrate

A = 191.748627032

B = -26.283306038

C = 15.4529770658

Sodium Lactate

A = 95.9701016668

B = -9.5710356222

C = 0.72940047727

Sorbitol

A = 53.7356450143

B = 16.4113553807

C = -5.5145002443

Sucrose

A = 86.6184451870

B = -17.456534545

C = 32.3582656383

Urea

A = 21.0798467596

B = -1.8715978638

C = 1.65975650587

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