Polyvinylpyrrolidone: The Electromigration Characteristics of the Blood Plasma Expander

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Before the metabolic role of the blood plasma expander, polyvinylpyrrolidone (PVP) can be studied by the technic of ionography, some fundamental ionographic characteristics of the polymer, per se, must be determined. This study is concerned with the influence of such variable factors as time, potential gradient, temperature, the pH of the buffer and its ionic strength on the mobility of the migrant when under the influence of an electrical field, and an attempt has been made to correlate them. This work, then, may serve as a basis for later investigations to be made on the combining power of PVP for certain physiologic products with which it comes into intimate contact when used as a plasma expander.

Many substances have been suggested as possessing satisfactory properties for use as plasma expanders; among these is polyvinylpyrrolidone (PVP). The literature on this substance has been reviewed1, 2 and the material is currently undergoing thorough clinical evaluation.

During the investigation of the metabolic fate of PVP in laboratory animals, it was realized that a semimicro method of analysis was needed in order to facilitate progress. It appeared that the technic of ionography,3-6 which is based on differences in the electromigration rate of charged particles on wet surfaces of paper or similar materials, could fill this need. Before the course of the PVP in animals could be elucidated, however, it was necessary to know the fundamental ionographic characteristics of the polymer itself. In this paper, a report is given on a study of the following phases of the problem: (1) The method of staining PVP; (2) the interrelationship between the movement of the polymer, on the one hand, and of time and potential gradient on the other; (3) the effect of ionic strength of the buffer used to saturate the paper strips on the mobility of the substance; (4) the mobility as a function of the pH of the buffer solution; and (5) the effect of temperature on mobility.

Materials and Methods

The PVP used was of two types: (1) 3.5 per cent PVP in isotonic saline solution with a K-value of 29.3 (a viscosity constant related to molecular weight), and (2) the polymer in powder form which had a K-value of 32.*

In the first case, the PVP was diluted with buffer to obtain the migrant at a desired pH, for if the PVP saline material were dialyzed against the buffer some of the smaller molecules apparently were lost and the heterogeneity could vary.

It was noticed that the degree of dilution apparently had no effect on later mobility determinations. The powdered sample of PVP was dissolved in a sufficient volume of veronal buffer to cause complete solution but without a change in the pH of the buffer. The specific pH and ionic strength of the buffer used to perform this operation were identical to the pH and ionic strength subsequently used to saturate the paper strips in that particular determination. In isolated experiments run at 2 C. and at 25 C., no noticeable differences were observed in the mobility characteristics of the two forms of PVP. Although either form could therefore have been chosen, the PVP in saline solution was used throughout this work, simply for convenience. The apparatus used was the Precision Ionograph,† using Eaton-Dikeman #613 filter paper in rolled strip form, 8 mm. in width. In all experiments, a helium atmosphere was used, the horizontality of

* Both types were obtained from Abbott Laboratories, North Chicago, Illinois.
† Manufactured by Precision Scientific Co., Chicago, Illinois.
the strips was checked with a spirit level, and a small-bore syphon was maintained in position during the experiments to hold constant the liquid level in the buffer troughs. In all experiments reported on here, a strip length of 50 cm. was used. No auxiliary buffer feeders were employed, that is, the strips were supported in the frame only at the ends by the draw-bolts, as previously described.

The veronal buffer solution used in all experiments (except where otherwise noted) was made up according to the specifications given by Michaelis, with the exception that, since low ionic strengths were used, the sodium chloride was omitted. Approximately 1 lambda of the migrant solution was applied by means of a micropipet as a thin streak across each strip, midway between the ends. On completion of a run, the filter paper strips were quickly oven-dried for 10 minutes at 100 to 105 C. They were dipped into Lugol's solution (5 Gm. iodine and 10 Gm. potassium iodide in 100 ml. of distilled water) and washed with tap water until the migrant appeared as a yellow-brown field against the bluish background of the rest of the strip. These ionograms were then dried at room temperature to prolong the color-retention of the PVP-iodine complex, and the migrant movement was measured directly. In replicate experiments, under identical conditions, the displacement of the forward edge of the migrant zone from the forward edge of the initial zone of application (4 to 5 cm. in most cases) varied from the average of such displacements by 1 to 2 mm.

RESULTS AND INTERPRETATIONS

In figures 1 and 2 are shown plots of the movement of PVP versus time and potential gradient, respectively. Each point represents 5 runs of 7 strips each, that is, a total of 35 mobility determinations per point. From the figures it is apparent that the movement is a linear function of the time and potential gradient. This may be interpreted to mean that equilibrium conditions are being maintained with respect to moisture in the paper strip and in the atmosphere within the apparatus.

Durrum and Schulz and Wegner have reported a nonlinear relationship between movement and time. In Durrum's experiments, of course, the center of the paper ribbon was elevated above the ends which dipped into the buffer solution. On this basis alone one should...
not expect a linear relationship and experiments conducted in the manner described, that is, utilizing the ridge-pole type of apparatus, are not suitable for mobility determinations, although, when all procedures are properly standardized, they are satisfactory for fractionation and preparative work. On the basis of experience in the authors' laboratory, it would appear that in the work of Schulz and Wegner the conditions under which the experiments were run may have exceeded the limits within which linearity of movement with time can be expected. It should be stressed that a nonlinear relationship can readily be obtained, using any variations of the electrophoretic apparatus utilizing paper which have been proposed thus far if precautions are not taken to minimize water shifts about the paper. Some of these unsatisfactory experimental conditions which lead to water shift through the paper are the employment of too high an ionic strength of the buffer solution or too high a potential gradient across the strips, both of which result in too large an expenditure of energy with its concomitant loss of water. Other unsatisfactory conditions would include nonhorizontality of the paper strips throughout their length, lack of maintenance of constant liquid level in the vessels into which the ends of the strips dip, and similar factors. The real objective, in quantitative experiments involving mobilities, as opposed to simple fractionation procedures, must be to obtain a linear relationship between the movement of the migrant on one hand, and the time and potential gradient, respectively, on the other, under conditions that are otherwise constant. That this is an achievable goal, has been demonstrated in every case so far investigated in this laboratory, and the list of substances includes amino acids, proteins, lipoproteins, PVP, and carbohydrate-borate complexes.

The movement of the polymer was in every case towards the negative pole and under the stated conditions it migrated without any observable fractionation into discrete zones. From a consideration of the method of synthesis of the polymer a sample would not be expected to contain molecules of a unique molecular weight, but there will be molecules of a range of different molecular weights, as found with most synthetic polymers. This being the case, no clear-cut fractionation into distinct and separate zones would be expected, but rather a band on the filter paper whose breadth would increase with increased distance of polymer migration.

**Mobility of PVP as a Function of Ionic Strength**

The mobility of PVP was determined at five different ionic strength levels of the veronal buffer system, ranging from values of 0.005 to 0.045 ionic strength. The reasons for the limitation on the ionic strength range are simply (1) that at very high ionic strength values, evaporation of water from the filter paper strips becomes a problem regardless of other variables such as potential gradient, time, and buffer temperature, and (2) that at ionic strength values above 0.045 the PVP had too low a mobility to migrate a sufficient distance in a convenient length of time. Using too low a buffer concentration may cause insufficient buffering of the migrant with resultant inaccuracies in the mobility determinations.

Figure 3 gives the curve obtained on plotting the mobility of the polymer in microns per second per volt per centimeter as a function of the ionic strength of the buffer. It is seen that the mobility increases with decreasing ionic strength in such a way that the rate of increase of migration velocity becomes greater as the ionic strength of the buffer is reduced. Thus, apparently the polymer behaves accord-
ing to the known effect of electrolyte concentration on the ion atmosphere of an ion in solution, or on the zeta potential of a colloidal particle. Viewing the migration of PVP with reference to the Debye-Hückel theory of strong electrolytes, and using the relationship:

\[
\text{Mobility} = K \frac{\sigma}{D} \times \frac{1}{0.33 \times 10^5 \sqrt{\mu}}
\]

where \(\sigma\) is the surface charge density of the migrating substance; \(D\) is, to a fair approximation, the dielectric constant; \(\mu\) is the ionic strength, and \(K\) is the constant of proportionality between the mobility and the zeta potential, it is seen that if the mobility of the migrant is plotted against the reciprocal of the square root of ionic strength, a straight line relationship should result. Figure 4 represents such a graph. It should, however, be emphasized that this curve was obtained directly from the data of figure 3, using points from the best curve drawn through the experimentally determined points. It was found that in the region from the lower ionic strengths used, up to that of 0.03, a straight line relationship was evident, indicating that the polymer migrated much as it would be expected to out in the body of the liquid.

**Effect of Temperature on the Mobility of PVP**

The mobility of PVP was determined at five different temperatures over the range 2 to 29 °C and it was found that the mobility increased with increasing temperature. As the electromigration of the migrant through the buffer solution saturating the paper strip is fundamentally a simple rate process, it would be expected that when the logarithm of the mobility was plotted as a function of the reciprocal of the absolute temperature a straight line would be obtained. In a similar way, when the reciprocal of the viscosity of the buffer solution was plotted as a function of the reciprocal of the absolute temperature a straight line was obtained. The viscosity measurements were determined by means of a simple Ostwald viscosity pipet at four different temperatures. Now the slope and the energy of activation can serve as a basis of comparison for the two processes. Actually, the curves have comparable slopes and thus the energies of activation are similar, being approximately 3500 calories. It would appear on this basis that the two processes are probably fundamentally related, that is, the shear of the liquid layers that occurs during the movement of PVP may be similar to the shear of the liquid that occurs when the viscosity of the buffer solution is measured.

**Mobility of PVP as a Function of the pH of the Buffer System**

The mobility of PVP was determined over a considerable pH range of the veronal buffer system and also at pH 2.4 using HCl. The dotted extension of the curve in figure 6 illustrates this change in buffer system. From this lowest pH to one of pH 6, the mobility of the polymer increases from 0.09 to 0.30 microns per second per volt per centimeter, while over the range of pH 6 to 9, the mobility remains reasonably constant at a value of 0.30 microns per second per volt per centimeter. In all cases, the polymer migrated to the cathode, and attempts to obtain either an isoelectric point or reversal of migration, failed. An experimental point, not shown on the graph, obtained at a pH of 11.2 using NaOH solution as the buffer, gave the relatively high mobility of 0.49 microns per second per volt per centimeter. The work of Oster and Frank is interesting in this connection. They observed a considerable shift of the ultraviolet absorption peaks below pH values of 1 to 2 and above 11 to 12.
During free electrophoresis, using the moving boundary method, the polymer has been found to exhibit characteristics different from those described above. Ott states that PVP migrated to the anode at pH 8.6 with a mobility similar to that of human serum gamma globulin. Weese has stated, as an interpretation of action by acidic groups, it has been observed in this laboratory and elsewhere, that the polymer powder when dissolved in water does yield an acidic solution.

Further work, in free solution done in another laboratory using a moving boundary type of apparatus manufactured by Pearson Associates gave the following results: Using a veronal buffer, at a pH of 7.7 and an ionic strength of 0.1, the PVP behaved as if it bore a negative charge, and a mobility of $1.03 \times 10^{-6}$ cm$^2$ per volt second was obtained, while using a lactate buffer at a pH of 3.4 and an ionic strength of 0.03, the mobility was computed to be $0.59 \times 10^{-5}$ cm$^2$ per volt second.
Caution is then obviously necessary when mobilities and charge are to be compared with those obtained from observations made in nonstabilized electrolytes. This is especially so when the migrant exhibits a small charge, as does PVP and the plasma substitute, dextran. The latter substance has a mobility, in free solution electrophoresis, of \(-0.16 \times 10^{-5}\) cm\(^2\) per volt second, when the buffer has a pH of 8.8 and an ionic strength of 0.1. Its ionographic mobility, under similar conditions was approximately \(+1.7 \times 10^{-6}\) cm\(^2\) per volt second.

To explain the differences in mobility obtained using these two technics, the phenomenon of electro-osmosis\(^3\), \(^5\) may be introduced. It may be postulated that the filter paper, which appears to acquire a negative charge when in contact with aqueous solutions of most electrolytes, does so, perhaps by selective adsorption of negative ions from the solution or by ionization of individual functional groups, such as the hydrogen from the hydroxyl groups on the cellulose fibers. Now, when a potential gradient is impressed across the paper, it is unable to move, despite its net negative charge. Instead, the layers of buffer solution adjacent to the charged paper surface move in the opposite direction, that is, toward the negatively charged electrode or cathode. Qualitatively, this classic picture of the phenomenon of electro-osmosis accounts for the apparent reversal of charge on the PVP molecule, but it is difficult to make a quantitative correction for the phenomenon.

An attempt to reconcile results obtained from paper-stabilized electrolytes with those obtained from electrophoretic measurements in free solution has been made by Kunkel and Tiselius.\(^10\) These investigators view the path of ion or particle migration as a sort of meandering tunnel or worm-hole through the paper (fig. 7). It is then assumed, for a given paper, that all migrants follow along similar paths and that since the path is much longer than the actual length of the paper, the potential gradient actuating the migrating particle will be less than that given by simply dividing the potential difference impressed across the paper strips by their length. On this basis, then, and after applying a correction factor for electro-osmosis (using dextran as an indicator of the amount of electro-osmotic movement), they apply a second correction factor for "added migration path length" which appears to bring the mobility of the migrant up to that observed in free solution. They state that this latter correction factor is characteristic for a given type of paper and can be used for mobility calculations under widely varying conditions. In other words, this correction factor (for "added migration path length") is a function principally of the paper and not of the migrant.
varieties of paper, the percentage change in migration velocity, for say, migrant X, from say, paper A to paper B to paper C, should be the same as for a different migrant, Y. Experimental evidence (as shown in table 1) does not support this conclusion. As a particular example, consider aspartic acid and PVP. The mobility of aspartic acid is practically the same on the different papers, while that of PVP varies from 0.19 on Schleicher and Schuell no. 413 paper, to 0.30 on Munktells Cremer-Tiselius paper. In the case of the aspartic acid, the average deviation of the mobility for a given paper, from the average of the measurements on all papers, was 7.4 per cent, while in the case of PVP, it was 20 per cent. This experimental data can be interpreted to mean that different migrants apparently may follow different migration paths and that therefore no single correction for “added migration path length” can be applied for different migrants, even when the same type of paper is used to stabilize the electrolyte.

The authors have suggested another picture of the mechanics of electromigration, in buffer solutions stabilized with paper, which is consistent with the experimental facts; for purposes of reference it will be referred to as the “barrier theory.” According to this latter view, the paper fibers act as barriers in the path of migration and their effect will differ with migrants of different molecular volumes, and will also depend to some extent on the shape of the migrating particles. While the particle is undergoing its forward movement, however, it is doing so essentially in the buffer solution trapped in the interstices between the paper fibers, and the potential gradient which is actuating its movement is given directly by the potential difference impressed across the paper’s length, divided by the length of the paper. According to the barrier theory, then, the correction which is applied must be a function of both the paper and the migrant. The lower mobility observed on paper (as compared with that found in nonstabilized electrolytes) for migrants of larger molecular weight (and volume), for example, bovine serum albumin, is then due not so much to an increased length of migration path over that of the actual length of the paper itself as it is due to the fact that the individual molecules of the migrant suffer collision with the paper fibers and therefore are momentarily slowed down. On the barrier theory, then, the movement of the migrant is pictured as a halting one, but nevertheless, when the migrant does move, it does so under the impetus of the full potential gradient given directly by the voltage impressed across the paper divided by the paper’s length.

It can be shown17 in the light of the barrier theory, that an electro-osmotic indicator should have a molecular volume and shape close to that of the migrant whose mobility is being studied. There would seem, therefore, to be some question as to the advisability of using dextran as an indicator for migrants of widely varying molecular volume and shape. It would seem, perhaps, sounder policy for an investigator, at the present time, simply to report the experimental mobility data, as obtained, rather than to apply a “correction factor or factors” of questionable validity. If the time comes when a range of electro-osmotic indicators of varying molecular volumes are available, then corrections may be made, if found necessary, to the previously published values. For the above reasons, the authors have not attempted to apply any correction factor for electro-osmosis or for so-called “increased migration path length” to the measurements reported in this paper. The whole problem, of “correction factors” applicable to paper-stabilized electrolytes will be reviewed in a more quantitative fashion in a forthcoming publication.18

"TABLE 1.  Effect of Molecular Volume of Migrant on Its Mobility on Different Types of Filter Paper"

<table>
<thead>
<tr>
<th>Migrant</th>
<th>Eaton &amp; Dikeman #603</th>
<th>Cremer-Tiselius</th>
<th>Schleicher &amp; Schuell #413</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nickel ion</td>
<td>$-1.43 \pm 0.10$</td>
<td>$1.31 \pm 0.13$</td>
<td>$1.61 \pm 0.07$</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>$-1.05 \pm 0.11$</td>
<td>$0.90 \pm 0.06$</td>
<td>$0.99 \pm 0.03$</td>
</tr>
<tr>
<td>Polyethylene glycol</td>
<td>$-0.35 \pm 0.01$</td>
<td>$0.32 \pm 0.01$</td>
<td>$0.21 \pm 0.01$</td>
</tr>
<tr>
<td>Polyvinyl pyrrolidone</td>
<td>$-0.00 \pm 0.02$</td>
<td>$0.30 \pm 0.01$</td>
<td>$0.19 \pm 0.02$</td>
</tr>
</tbody>
</table>

Conditions during experiments: potential gradient, 5 volts/cm.; pH, 8.3; temperature, 2.0°C; buffer, sodium acetate-sodium veronal-hydrochloric acid; ionic strength of the buffer, 0.0075. Each value listed in the table represents three runs of seven strips each, that is, a total of 21 measurements.
With the help of the data and graphs published here, it is now possible for an investigator to proceed in an orderly way, utilizing the technic of ionography, with investigations involving the separation of PVP from other complex materials with which it is associated when used as a blood plasma expander. In preliminary experiments on the actual separation of PVP from serum proteins using the ionographic technic (using 1 lambda samples of serum; buffer, veronal-acetate-HCl; ionic strength, 0.0075; pH 9.3; room temperature; filter paper, E. and D., no. 613; potential gradient 4 volts per centimeter; time of separation, 5 to 6 hours), the separation of the PVP from albumin and alpha and beta globulin was satisfactory while a good separation from the gamma globulin fraction has still to be developed. This last result does not come as a surprise since the mobility of gamma globulin in free solution electrophoresis does not differ much from that observed for PVP. Both appear to undergo a reversal in movement of a similar magnitude during ionographic studies, due apparently to electro-osmosis. The problem of a satisfactory fractionation of these substances is therefore not an easy one. Further experiments are underway to determine the conditions for a satisfactory fractionation.

**SUMMARY**

1. Using the technic of ionography, the fundamental electromigration characteristics of the blood plasma expander, polyvinylpyrrolidone (PVP) were determined. Conditions necessary for obtaining reproducible results are discussed.

2. A satisfactory color test for locating PVP on filter paper was found. The compound is stained with Lugol’s “iodine-potassium iodide” solution. A resulting yellow-brown colored derivative of PVP is obtained after washing with water.

3. The movement of PVP was found to be a linear function of both time and potential gradient when all other factors affecting mobility were maintained constant.

4. The mobility of PVP increased with decreasing buffer ionic strength in such a way that the rate of increase of migration velocity becomes greater as the ionic strength of the buffer is reduced.

5. The mobility of PVP was found to increase with an increase in the buffer temperature. Increasing the temperature of the buffer solution decreases its viscosity. The energies of activation for these two processes are similar, and the inference is made that they may be fundamentally related.

6. The pH-mobility curve for PVP was determined. From pH 2.4 to pH 6, the mobility increased, while over the pH range 6 to 9 the mobility was reasonably constant. In all cases, the polymer migrated to the cathode.

7. A possible explanation for the fact that the polymer exhibited electromigration properties on paper different from those observed using nonstabilized electrolytes, is presented. This is in part due to the role played by electro-osmosis in the former process. It was found, also, that the molecular volume becomes an important factor when its mobility is determined on different filter papers. To substantiate this fact, the mobilities of various molecules having wide differences in molecular weight (and volume) were obtained on three types of filter paper.

It was found that the percentage change of these migrants on changing the type of filter paper was not equal. The results were correlated into a picture and theory of the mechanics of electromigration in buffer solutions stabilized with filter paper. This concept, the barrier theory, can aid in comparing ionographic results with those obtained in nonstabilized electrolytes.

**REFERENCES**

1. PVP—Polyvinylpyrrolidone—Preparation, Properties and Applications in the Blood Field and in Other Branches of Medicine. An Annotated Bibliography (to 1950); Compiled and published by General Aniline and Film Corporation, New York, N. Y., 1951.


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10. OTT, H.: Private communication. Medizinische Universitäts Klinik und Poliklinik, Tübingen, Germany; Laboratory of H. Bennhold.
14. SPITZER, R. H.: The Ionographic Characteristics of Polyvinylpyrrolidone. M.S. Thesis, Department of Biochemistry, Stritch School of Medicine, Loyola University, Chicago, June, 1953.
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