Solid-Phase Colorimetric Determination of Potassium

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A nonpolar organic film (plasticized polyvinyl chloride) containing the ionophore valinomycin was incubated with an aqueous solution containing potassium ion and a detectable anion (erythrosin B). The amount of erythrosin B retained by the film after washing could be measured by absorbance or reflectance, and was directly related to the potassium concentration. This dye-binding method is quantitative for potassium and is suitable for both aqueous and serum-based solutions. There was no interference by sodium in the range found in serum. Several polyvinyl chloride plasticizers and anionic dyes and some other ionophores were found to be useful. The anion binding is thought to be restricted to the surface.

Additional Keyphrases: polyvinyl chloride membrane · valinomycin · ionophores · erythrosin · film reflectance · K-sensitive film · Langmuir binding isotherm

Monitoring renal function or secondary manifestations of other disease states frequently necessitates determination of serum potassium concentration. A normal range of only 3.6 to 5.5 mmol/L in healthy individuals (1) is indicative of how precisely the concentration of this ion is regulated. A plasma potassium concentration ≤1.5 mmol/L is often fatal, owing to respiratory depression and cardiac arrhythmia. Conversely, characteristic electrocardiographic changes and even cardiac arrest occur when potassium exceeds 10 mmol/L (2). Because of the limited normal range and the effects of relatively small excursions from it, assays for potassium must be highly accurate.

In the clinical laboratory, potassium is usually determined by flame photometry or ion-specific potentiometry. In ion-specific potentiometry the electrical potential developed across a special membrane separating the sample and a reference solution is measured. The special membrane, which responds only to potassium, contains a catalytic amount of an ionophore, a molecule that specifically binds the ion under examination and transports it across a hydrophobic membrane. Thus a conductive path is produced only for this one ion and the membrane effectively ignores concentration gradients of all other ions. Valinomycin is commonly used as the ionophore (3, 4). The conformation of this macrocyclic compound is such that it forms a three-dimensional cavity, lined with oxygen and nitrogen atoms and giving a polarizable interior suitable for the ligand-solvation requirements of potassium ions (5, 6). Valinomycin shows a very high specificity for potassium binding over sodium and divalent cations. The methyl and methylene groups forming the hydrophobic exterior of the molecule allow it to be freely soluble in solvents of low polarity. When potassium from aqueous solution binds to valinomycin at the membrane interface, the complex diffuses across the membrane and the trapped potassium is released on the other side (6), thus establishing a conductive path across the membrane.

A similar mechanism is thought to be involved in the partitioning of metal cations between organic and aqueous phases in the presence of an ionophore (here acting as a phase-transfer catalyst) (7). In the presence of such a compound, typically a cyclic polyether, a metal ion can be transported into the organic phase. By suitable choice of anions, some of the anion may accompany the metal–ionophore complex into the organic liquid (8), presumably so that buildup of the charge there from the metal ion would be neutralized. The anions expected to partition easily would be “soft”—polarizable molecules able to delocalize the negative charge. Theoretical expressions have been developed for the concentration of anion expected in the organic phase as a function of cation and the ionophore concentrations (9).

The basis for the solid-phase colorimetric potassium test described here is our surprising observation that partitioning of an organic anion into a valinomycin-containing solid phase can proceed readily and quantitatively as a function of potassium concentration. Specifically, the solid nonpolar phase is a plasticized polyvinyl chloride (PVC) membrane containing the potassium-sensitive ionophore, valinomycin. A drop of the aqueous phase, which contains potassium ion and a detectable anion, erythrosin B, is placed on the organic phase and allowed to incubate for several minutes. During this time, both potassium and erythrosin B partition between the aqueous and organic phases. After the aqueous drop is removed, the amount of erythrosin B retained by the solid phase is measured spectrophotometrically. Others have shown (10) that when radioactive chloride ion (36Cl-) was incubated with a similar membrane, uptake of this added anion was minimal; instead, membrane-bound water deprotonated to supply a balancing negative charge to the film. In contrast, we will show that by suitable choice of anion, this uptake can be made rapid and quantitative and is a precise measure of potassium concentration of the solution in contact with the membrane.

Materials and Methods

Materials. Valinomycin, 18-crown-6 (1,4,7,10,13,16-hexacyclooctadecane), polyvinyl chloride (very high molecular mass), 2,6-dichloroindophenol, dimethylphthalate, and dioctylphthalate were from Aldrich Chemical Co., Milwaukee, WI 53233. Tetrahydrofuran, 1,4-dioxane (“Photrex”; reagent grade), acetone (HPLC reagent grade), potassium chloride, and phloxine B (reagent grade), were from J. T. Baker Chemical Co., Phillipsburg, NJ 08865. Erythrosin B, orange G, phenolphthalein, hematein, and dipentyl-, dibutyl-, diethyl-, and dinonylphthalates were from Eastman Kodak Co., Rochester, NY 14650. From MCB Manufacturing Chemists, Inc., Cincinnati, OH 45212 we obtained the dyes eosin Y, phenol red, and Kryptofix K-221 and K-222. Orange IV and Alizarin Red S came from Allied Chemical Corp., Morrisstown, NJ 07960, and 3-(N-morpholino)propanesulfonic acid, sodium salt, from Calbiochem-Behring Corp., La Jolla, CA 92037. Bis-2-ethylhexylsulfate and polyvinyl fluoride were from Scientific Polymer Products, Ontario, NY 14519, and tris-2-ethylhexyl phosphate was from ChemServices, West Chester, PA 19380. The polyester film base (Scotchpak) was obtained from the Film and Allied Products Division of 3M Co., St. Paul, MN 55144.

Reflectance measurements were taken with a Seralyzer.
Potassium, 3R) 

We used a flame photometer (Model IL 443; Instrumentation Laboratory, Inc., Lexington, MA 02173) for comparison determinations of potassium, calibrating at 5 mmol of potassium and 140 mmol of sodium per liter. Nonpolar solid phases were prepared by dissolving PVC, dipentylphthalate (DPP) plasticizer, and ionophore (typical concentrations of each were 40, 100, and 1.5 g/L, respectively) in a suitable organic solvent (tetrahydrofuran or a 1,4-dioxane/acetone solution, 810 g of dioxane per liter of acetone), and spreading a uniform layer of this solution onto an inert transparent polyester support. To dry this film, we exposed it to a stream of air for 5 min. As prepared, this material was suitable for absorbance measurements because both support and film were transparent. For reflectance measurements we laminated a uniformly scattering white material to the uncoated side of the polyester support with double-sided adhesive tape.

Procedure. A general assay procedure was to dilute the sample such that the potassium concentration was in the range 0.2 to 1.1 mmol/L (equivalent to a ninefold dilution of serum), with a buffered solution of a counterion such as erythrosin B. Unless mentioned otherwise, after dilution, the buffer concentration was 125 mmol of the sodium salt of 3-(N-morpholino)propanesulfonic acid per liter and the dye concentration was 10 mmol/L. We incubated a drop of this solution (140 µL) for 4 min on a piece of prepared film (0.5 × 1 cm), then washed the surface for 5 s with a jet of water. After blotting the film dry, its absorbance or reflectance could be measured with use of a suitable mounting device if required. The absorption maximum at 528 nm of erythrosin B in water shifted to 552 nm when retained in the film. We usually measured absorbance at 550 nm. Reflectance measurements (R) were made with a Seralyzer reflectance spectrophotometer also at 550 nm.

We used the simplified form of the Kubelka-Munk (11, 12) equation

\[ \text{K/S} = (1 - R)^2 / 2R \]

to transform reflectance data to a function (K/S) that is proportional to the chromophore concentration. K/S is the ratio of the absorption (K) to the scattering (S) coefficient.

To convert reflectance measurements into potassium concentration, we used a two-point calibration, measuring two samples with known potassium concentrations bracketing the normal range. The reflectances were measured and converted to K/S values, which were used to generate a calibration line of K/S vs potassium concentration. Ordinarily, these computations and other functions are done automatically by the Seralyzer instrument.

Absorbance measurements were made by suitably mounting an analytical element in the light path of a spectrophotometer. As before, we used a two-point calibration procedure to transform the measured absorbance of a test solution to potassium concentration.

The slope of the calibration lines could also be used to express the film reactivity—in terms of absorbance units or K/S per mole of potassium per liter.

All samples were diluted ninefold before assay, and all cation concentrations shown correspond to the concentration before dilution.

Results

Response to aqueous potassium. Test solutions having nominal potassium concentrations ranging from 1.8 to 10 mmol/L were prepared by adding potassium chloride to a buffer-dye solution. After reaction, we measured the reflectance of the film at 550 nm. Potassium concentrations were

![Fig. 1. Response of analytical elements to a range of potassium concentration (mmol/L) as measured by reflectance (A) or absorbance (B). Reflectance was transformed to K/S; absorbance was plotted in double-reciprocal form. Each value was determined after calibration with duplicate measurements of potassium solutions at 3.3 and 6.2 mmol/L.](image-url)
compared with those measured by the flame photometer. Figure 1A shows that the K/S response to potassium is linear. Conversion of K/S units to those of potassium concentration gave correlation statistics vs the flame photometer of slope 1.058, intercept -0.222, correlation coefficient 0.9988, and standard error 0.128.

In another experiment we looked at the absorbance response to aqueous potassium solutions, measuring the film absorbance after treatment with potassium/erythrosin B solutions. The dependence of the absorbance on potassium was best described by a hyperbola

$$K = S \cdot A / (1 - I \cdot A)$$

where K is the potassium concentration, A is the measured absorbance, and S and I are constants of the hyperbola. The double-reciprocal plot of Figure 1B is therefore linear, and constants S and I are determined by two-point calibration. Against the comparison method, the strip gave a slope of 0.987, intercept of 0.107, correlation coefficient 0.9997, and standard error 0.11.

Response to serum solutions. To a serum pool with low potassium concentration we added potassium to give a range of concentrations up to 9 mmol/L, then applied samples to the film and measured the film reflectance at 550 nm. As shown in Figure 1A, the transformed reflectance was linearly dependent on the analyte concentration as determined by flame photometry. The positive intercept at zero potassium concentration corresponds to a small degree of nonspecific binding. After conversion to potassium concentration units, the correlation statistics were: slope 1.058, intercept -0.222, correlation coefficient 0.9988, and standard error 0.13.

Effect of sodium. A viable potassium test must be free of interference by sodium at the 140 mmol/L concentration found in plasma, 30-fold that of potassium. We incubated the potassium test strips with solutions corresponding to sample sodium concentrations ranging from 0 to 900 mmol/L and then measured the absorbance. At zero potassium concentration, there was a blank reaction corresponding to 0.2 mmol/L potassium. Sodium-containing samples gave a small increase in apparent potassium concentration, but the increase was random and not dependent on the metal cation concentration.

**Effect of lithium.** When strips were incubated with lithium solutions equivalent to sample concentrations up to 10 mmol/L, the apparent potassium ion concentration was less than 0.1 mmol/L more than the blank.

**Reaction conditions.** The amount of color retained by the film is a function of the time during which test liquid and solid are in contact. Solutions containing two concentrations of potassium were incubated on potassium-sensitive film for 0.5 to 8 min. As shown in Figure 2A, the amount of dye retained increases with time. We chose to use a reaction time of 4 min, to give a balance between speed and the imprecision that arises from small discrepancies in test timing. Increasing the washing time decreased the amount of color somewhat (Figure 2B). A wash time of 5 s was found to be adequate. Acceptable reactivity was found with a dye concentration of 10 mmol/L, although the reactivity did increase with higher dye concentration.

**Film composition.** We examined several different film compositions in order to optimize the formulation. With fixed amounts of PVC and DPP and varied valinomycin concentration, reactivity was proportional to the valinomycin:plasticizer ratio (Figure 3A). We used a ratio of 14.4 mg/g to give a balance between reactivity and economy.

Plotting reactivity against DPP/PVC ratio (and adjusting reactivities for changes in valinomycin:plasticizer ratio) showed that reactivity increased with an increasing DPP/PVC ratio, but became saturated at ratios greater than 4 (Figure 3B). At these high ratios, however, the film deteriorates, becoming sticky and quite fragile; again, a compromise was in order and we chose a DPP/PVC ratio of 2.6.

**Film thickness.** Coatings of fixed composition but variable thicknesses were prepared by adjusting the solids content of the spreading solution or the thickness of the wet layer. Evaluations of film performance at two concentrations of potassium (Figure 4) show clearly that the reactivity is almost independent of film thickness in the range 24 to 260 μm.

**Alternative plasticizers.** Compounds generally used to plasticize PVC are the high-boiling-point members of the phosphate, sebacate, and phthalate families (13). Films prepared by substituting tris-2-ethylhexylphosphate or bis-2-ethylhexylsebacate for DPP were both responsive to potassium (Figure 5). Homologs to DPP, substituted at fixed weight percentage, were also reactive, in the order: dimethyl > diethyl

![Fig. 2.](image-url) **Fig. 2.** (A) Effect of varying incubation time of the potassium-dye reagent and a 5-s wash, and (B) effect of varying wash time after incubation for 4 min, at two concentrations of potassium.
> dibutyl > dipentyl > dioctyl > dinonyl, i.e., in order of molecular mass. This is also the order of decreasing mole fraction, which may be the basis of the effect. We also found (data not shown) that polyvinyl fluoride could be substituted for PVC as the structural polymer.

**Alternative dyes.** Besides erythrosin B, a fluorescein derivative, we screened several other substituted fluoresceins for use in the assay. Phloxine B and eosin Y both showed quantitative binding in response to potassium. Correlation of strips reacted with phloxine B against the gravimetric potassium concentration gave: slope 0.968, intercept 0.360, correlation coefficient 0.992, and standard error 0.49. Surprisingly, the parent compound, fluorescein, was inactive. 2,6-Dichloroindophenol and Orange IV (molecules quite different from each other and from the fluorescein derivatives) were also useful in the assay method; in a correlation study the former compound gave a slope of 1.101, intercept −1.02, correlation coefficient 0.986, and standard error 0.78. Phenol red, Alizarin Red S, Orange G, phenolphthalein, and hematein were inactive.

**Alternative ionophores.** Separate DPP-plasticized coatings were prepared in which we substituted other ionophores for valinomycin: a monocyclic crown ether, 18-crown-6, and the bicyclic cryptates, Kryptofix K-221 and K-222. Each film was treated with an erythrosin B solution containing a range of sodium or potassium concentrations. As Table 1 illustrates, incorporation of K-222 gives a film that is responsive to potassium but not to sodium, whereas incorporation of K-221 produces a sodium-sensitive film that is insensitive to potassium. This selectivity is as expected (14). Films containing 18-crown-6 showed no dye binding in response to sodium or potassium.
Table 1. Reflectances of Films Containing the Ionophores K-222 or K-221 after Treatment with Potassium or Sodium and Dye-Reagent Solutions

<table>
<thead>
<tr>
<th>Cation</th>
<th>Reflectance, %R</th>
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<tbody>
<tr>
<td></td>
<td>Potassium</td>
</tr>
<tr>
<td>concen.</td>
<td>K-222</td>
</tr>
<tr>
<td>mmol/L</td>
<td>Potassium</td>
</tr>
<tr>
<td>0</td>
<td>56.1</td>
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<tr>
<td>0.9</td>
<td>50.6</td>
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<tr>
<td>2.7</td>
<td>46.1</td>
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<tr>
<td>9.0</td>
<td>28.1</td>
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<tr>
<td>27.0</td>
<td>9.97</td>
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<tr>
<td>90.0</td>
<td>ND</td>
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<td>270.0</td>
<td>ND</td>
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Discussion

Independence of the reactivity to the film thickness suggests that the interaction is restricted to the surface. Surface reactions (and heterogeneous catalysis) have frequently been expressed in terms of the Langmuir binding isotherm, where the amount of a molecule $L$ bound to a surface ($L_B$) is hyperbolically dependent on the free concentration of that molecule ($L_F$), e.g.:

$$L_B = B \times L_F / (C + L_F)$$

where $B$ is the number of binding sites and $C$ is a constant.

A hyperbolic dependence of the amount bound is indeed seen (Figure 1B), as predicted by this model. In addition, the proportionality of reactivity with the ionophore/plasticizer ratio and the plasticizer/polymer ratio is also consistent. If potassium and its counterion partition mainly into the plasticizer part of the solid phase under the influence of the ionophore, then increasing either the fraction of plasticizer or ionophore would have the effect of increasing the number of potential binding sites.

From the studies of solid phases containing a representative of three plasticizer classes of various ionophores, or the different dyes that could be bound to the film, evidently there is no specific matrix-ionophore-dye molecule combination involved, and this kind of test procedure is suitable for any analyte for which a specific ionophore is available.

The amount of dye retained by the film can be measured by absorbance or reflectance (or fluorescence, if a suitable counterion is chosen). The measured absorbance has a hyperbolic dependence on potassium concentration. The K/S transformation, which is used merely as a convenient algorithm, does, however, provide a function linearly dependent on potassium concentration.

The specificity of the film response is excellent and sodium ion is not expected to be an interference in serum measurements for two reasons: principally the excellent selectivity of the ionophore valinomycin but also the pre-existing high concentration of sodium in the dye–buffer solution. The buffer contributes 125 mmol and the dye 20 mmol of sodium per liter. Under these conditions, an increase from 120 to 170 mmol/L produces an increase of only 3.5% in the sodium concentration of the diluted sample. It is a measure of the specificity of the ionophore that at zero potassium concentration the blank is so small. Reactivity is decreased somewhat for serum, as compared with aqueous solutions, but the use of serum-based calibrators obviates this problem. Other systems have been described for analysis of potassium (15) and sodium (16) by partitioning of a detectable counterion between two liquid phases, but are less convenient because of the number of sample manipulations required, including deproteinization. The method described here is considerably more rapid and convenient and has all the advantages associated with use of a solid-phase format.

References