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Continuous-Flow Serum Albumin Determination by Reaction with Picrate Ions, with Use of a Flow-Through Picrate Ion Electrode

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An automated potentiometric method for serum albumin determination by use of the picrate/albumin reaction is described. A continuous-flow system and a specially designed flow-through picrate ion electrode were used in making the measurements. Various factors affecting the reaction, such as pH, picrate ion concentration, and reaction time, were studied. Peak height in millivolts and albumin concentration were linearly related in the range 10–70 g/L. Both within-run and day-to-day, the CV for the method was about 2%. Analytical recovery of albumin added to serum samples ranged from 97.0 to 110.3%, averaging 102.2%. Results compare favorably with those by the established bromcresol green method. The proposed method is suitable for routine use and for screening tests.

Additional Keyphrases: *ion-selective electrodes · potentiometry*

The main source of human albumin is the liver, and disease of this organ leads to abnormally low serum albumin concentration. In nephrosis, serum albumin concentration is lowered because of massive losses via the kidneys. Also, protein deficiency or malabsorption are important causes of hypoalbuminemia. Many methods have already been described for serum albumin determination. Several of them are based on dye-binding reactions involving indicator dyes such as 2-(4′-hydroxyazobenzene)benzoic acid (HABA) (*1–3*), methyl orange (*4, 5*), bromcresol green (BCG) (*6–17*), and bromcresol purple (*18–20*). These methods have been subject to some criticism, because there is a competition for albumin binding sites between indicator dye and other plasma constituents such as bilirubin and drugs (salicylates) and because of the binding of the indicator dye to plasma proteins other than albumin.

We have attempted to assess the suitability of the reaction between serum albumin and picrate ions (*21*) for the automated potentiometric determination of albumin in human serum. The reaction is carried out in a continuous-flow system consisting of the Technicon AutoAnalyzer Sampler II and Proportioning Pump. The samples are treated with a standard amount of picrate ions for a fixed time interval, while a specially designed flow-through picrate ion-membrane electrode (22) is used to monitor the excess of unreacted picrate ions. Under controlled conditions of pH, sampling rate, and reaction time there is a direct linear relation between the potentiometric signal and the albumin concentration in the range 10–70 g/L.

The proposed method for serum albumin determination is suitable for routine clinical measurements and screening tests with a day-to-day and within-run precision (CV) of about 1–2%. Recovery and comparison studies carried out on actual clinical serum samples show a recovery of 97.0 to 110.3% (average 102.2%) and acceptable agreement with the conventional BCG method.

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Materials and Methods

Apparatus

Figure 1 is a schematic diagram of the automated albumin analysis system used in this study. Sampler II and the Proportioning Pump III were of the AutoAnalyzer type II (Technicon Instruments Corp., Tarrytown, NY 10591). The specially designed flow-through picrate ion membrane electrode, constructed in our laboratory as previously described (*22*), was used in conjunction with an Orion 90-01 single-junction reference electrode. The liquid ion-exchanger for the picrate electrode consisted of tetrapentylammonium picrate in 2-nitrotoluene (*23*). The potentiometric signals were monitored with a Corning Model 12 pH/mV meter and displayed in the form of peaks on a Sargent-Welch Model XKR potentiometric recorder. The timing functions (sampling rate and sample-to-wash ratio) of the Sampler II were controlled by a "digital sample-wash timer," designed and constructed in our laboratory as described elsewhere (*24*). Regular timing cams supplied with the sampler can also be used for the same purpose.

For manual measurement of albumin with the BCG spectrophotometric method, we used a Beckman Model DK-1A spectrophotometer. The comparison studies with a BCG automated procedure were done with a Gilford 3500 computer-directed analyzer.

Reagents

All solutions were prepared in de-ionized–twice distilled water from reagent-grade materials, unless otherwise stated.

Sodium picrate: A stock solution was prepared from picric acid (Fluka, "purum" grade) standardized with standard sodium hydroxide solution. An appropriate volume of solution containing 0.100 mol of picric acid was neutralized with NaOH to a pH of about 6 and diluted to 1 L. More dilute standard solutions were prepared by appropriate dilutions and stored at room temperature.

Buffer solutions: Acetate, pH 4 and 5; carbonate, pH 10; chloroacetate, pH 3; citrate, pH 5; formate, pH 3; and phosphate, pH 7 and 8. Concentrations of all buffers were 0.50 mol/L, and they were prepared from the corresponding acids or acid salts and 18 mol/L NaOH solution.

Albumin standards: Human and bovine albumin, used in this study as standards, were obtained from various sources:

(*a*) Human serum albumin (Worthington Diagnostics, Freehold, NJ 07728) prepared by ion-exchange chromatography from human serum. We dissolved 1 g of the preparation in 10.0 mL of water.

(*b*) Human albumin (cat. no. B 5158; DADE Division, American Hospital Supply Corp., Miami, FL 33152) specified as 100% albumin and assayed by the macro-Kjeldahl method. The contents of the vacuum-sealed ampoule were reconsti-

tuted in 3.0 mL of water according to the manufacturer's instructions, to give a concentration of 79 g/L.

(*c*) Human albumin solution 20% (Institut Merieux, 17 rue Bourgelat, 69002 Lyon, France) containing sodium caprylate as stabilizer. Because the solution in this form could not be used for the albumin standards preparation, the stabilizer was removed by treating it with activated charcoal according to Chen (*25*). The resulting albumin solution was ready for use after adjustment of the pH to 7 with 1 mol/L NaOH.

(*d*) Bovine albumin (Cohn Fraction V, powder 96–99%, cat. no. A 4503; Sigma Chemical Co., St. Louis, MO 63178), 1 g in 10.0 mL of water.

(*e*) Bovine albumin solution, 300 g/L (cat. no. 84840; DADE Biochemicals) was prepared from bovine plasma containing stabilizer and preservative.

Albumin working solutions of various concentrations were prepared from the above commercial preparations by appropriate dilution with water and stored under refrigeration. All solutions were standardized colorimetrically by the BCG method before use.

Control sera: Lyophilized control sera ("Validate"; General Diagnostics Division, Warner-Lambert Co., Morris Plains, NJ 07950) and horse-serum-based "Wellcomtrol Two" (Wellcome Reagents Ltd., Beckenham BR3 3BS, U.K.) were used for the precision studies. These controls were reconstituted according to the manufacturer's instructions.

BCG method reagent and standards: A commercial preparation of the BCG reagent (Sclavo, Inc., Wayne, NJ 07470) was used during this study, containing (per liter) 358 μmol of BCG, 61 mmol of succinate buffer (pH 3.9), 9 mL of polyoxyethylene sorbitan, and a nonreactive stabilizer. The reagent was used as supplied. Commercially prepared bovine albumin (Cohn Fraction V) standards (Sclavo Inc.) were also used for the BCG method.

Serum samples obtained from patients were used in evaluating the method.

Procedures

Automated serum albumin determinations. The operating conditions for the analysis are indicated in Figure 1. The albumin standards and the serum samples were loaded in 2-mL plastic cups and the system was set to operate at a sampling rate of 50 samples per hour (sample consumption ≃300 μL) with a sample-to-wash ratio of 1:2. The albumin/picrate reaction was permitted to proceed in a seven-turn mixing coil (approx. delay time, 25 s) at room temperature, before the debubbled stream reached the flow-through sensing electrode.

The standard curves were prepared by plotting the potential difference (peak height, in mV) between the reagent (picrate) baseline and the peak maxima vs. albumin concentration in grams per liter.

BCG spectrophotometric method. The analyses for the standardization of albumin working solutions were performed manually with the commercial preparation of BCG reagent. Ten microliters of serum or albumin standard solution was mixed with 1.5 mL of BCG reagent, the mixture was allowed to stand for 10 min at room temperature, and the absorbance was then measured at 630 nm vs the reagent blank. For the comparison studies, the automated BCG procedure was carried out by the Gilford 3500 computer-directed analyzer under the same conditions described in the manual BCG procedure.

Results and Discussion

Flow-through electrode response. The flow-through electrode unit specially designed in our laboratory was used in preparing the liquid membrane picrate-ion electrode. This

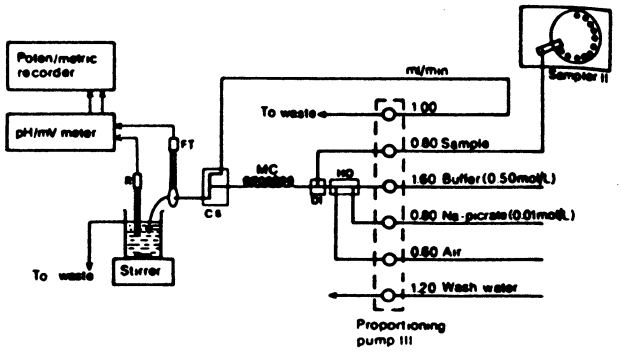

Fig. 1. Flow diagram of the automated analysis system
H0, D1: fitting; *MC*: seven-turn mixing coil; *C5*: debubbler; *FT*: flow-through picrate ion electrode; *R*: reference electrode

design permits the channelling of the measured solution through a glass frit holding the electroactive phase. The prepared electrode exhibits near-Nernstian response to picrate ion activity in the range 10^{-2} to 3×10^{-5} mol/L (*22*). We studied the effect of pH on electrode characteristics under continuous-flow conditions, using the continuous-flow system shown in Figure 1. The measurements were carried out with the sampler in the "wash" position, while the various picrate solutions were aspirated into the system via the corresponding tube from each stock solution bottle. The electrode showed the same response slope and response time against all the buffers used in a pH range of 3 to 10. Figure 2 illustrates a typical example of electrode response curves recorded under such conditions.

The picrate ion concentrations indicated in Figure 2 are the initial ones; the actual reaction concentrations are lower because of the 25% picrate dilution in the manifold. Commercial flow-through electrode bodies (units) such as Orion's Model 92 with the flow-through cup can also be used for this purpose, but these types usually present operational problems owing to the improper geometry of the sensing path (*22*).

pH effect on the picrate/albumin reaction. We studied the effect of pH on the picrate/albumin reaction, using a series of

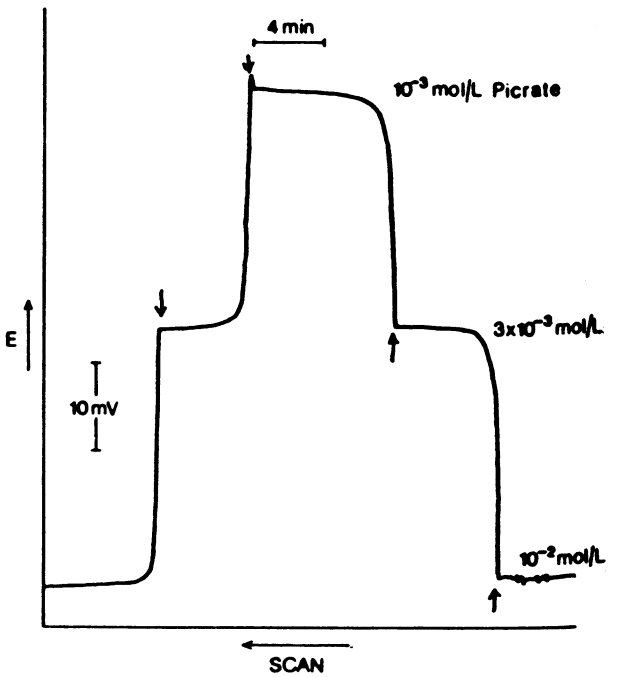

Fig. 2. Flow-through picrate ion-electrode response to various picrate concentrations under continuous-flow conditions
Acetate buffer 0.50 mol/L, pH = 4.0. The *arrows* indicate the point at which the new solution first reaches the electrode

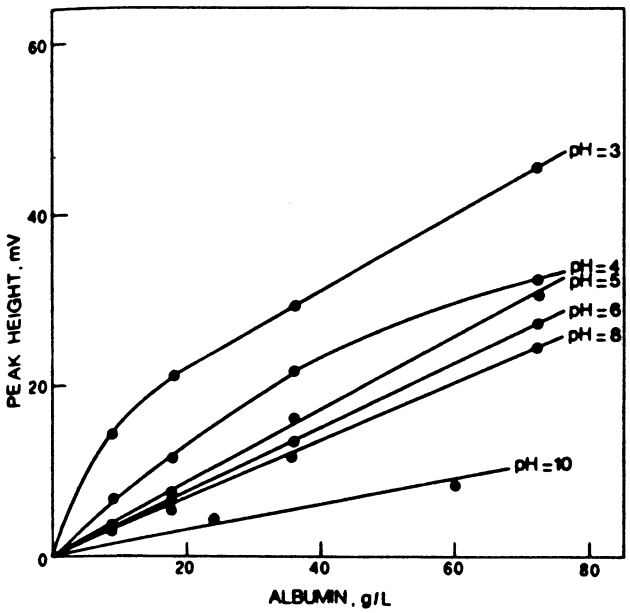

Fig. 3. Effect of pH on the calibration curve for the albumin determination

suitable buffers (see above) covering a pH range from 3 to 10. The experiments were carried out on samples of human and bovine albumin prepared from three different commercial preparations (human albumin from Worthington and Merieux, bovine albumin from Sigma). Results were similar with all preparations. The pH effect on the reaction is shown in Figure 3. The calibration curves (peak height vs. albumin concentration) prepared for each pH value showed that linearity exists at pH values from 5.0 to 10.0. Therefore the 0.50 mol/L acetate buffer, pH 5.0, was chosen as optimum for the method.

Effect of picrate concentration. The effect of picrate concentration on peak height was studied by decreasing the initial picrate concentration from 10 to 1 mmol/L. Calibration curves (Figure 4) obtained with human and bovine albumin standards show that at low picrate concentrations there is a significant deviation from linearity, especially at high albumin concentrations, whereas peak height increases sharply. These deviations at low picrate concentrations may be partially due to deterioration of electrode performance because of the extremely low amount of picrate left.

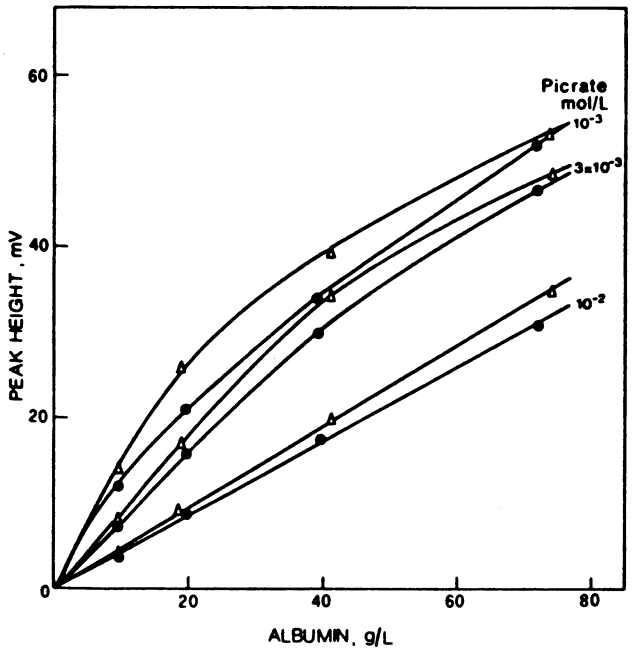

Fig. 4. Effect of picrate ion concentration on the picrate/albumin reaction

Human (●) and bovine (△) albumin standards. The numbers at the right-hand border refer to the *initial* picrate concentration (mol/L)

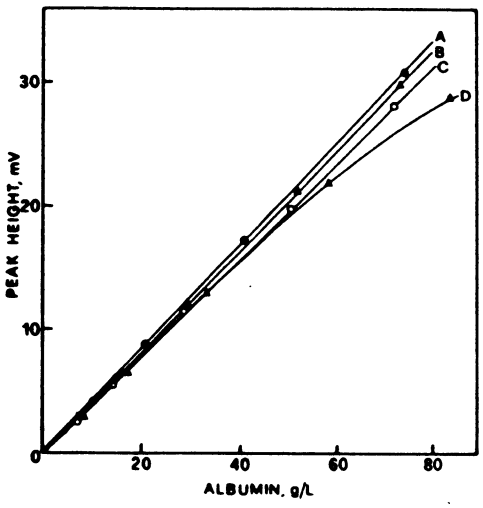

Fig. 5. Calibration curves prepared with albumin standards obtained from various sources

A: human albumin (Merieux); *B*: bovine albumin (Sigma); *C*: human albumin (Worthington); *D*: bovine albumin (DADE)

Picrate/albumin reaction time. Mixing coils of various lengths were used in the manifold for the reaction, to vary the delay time from 30 s to 20 min. This has no effect on the results, suggesting that the reaction between picrate and albumin is very fast. A seven-turn mixing coil with a delay time of about 30 s was chosen, in order to avoid possible slow reactions between picrate and other serum constituents.

Calibration of the method with albumin from various sources. Albumin from various sources was used to calibrate the method. The calibration curves obtained are presented in Figure 5. All albumin preparations provided almost the same linearity up to 70 g/L, except for a bovine albumin preparation (curve D) obtained from DADE, which shows a deviation beyond 50 g/L. This may be caused by the presence of stabilizer and preservative in this preparation. Figure 6 shows a typical recording for a series of albumin determinations carried out by the proposed procedure.

Linearity and stability of the calibration curve. The linearity and stability of the calibration curve were checked by analyzing continuously a series of four standard samples of human albumin for a period of 100 min. The results are summarized in Table 1. Regression analysis of the data proved the excellent linearity of the calibration curve over the range of 10 to 70 g/L albumin ($y = 0.413x + 0.10$, $r = 0.9996$).

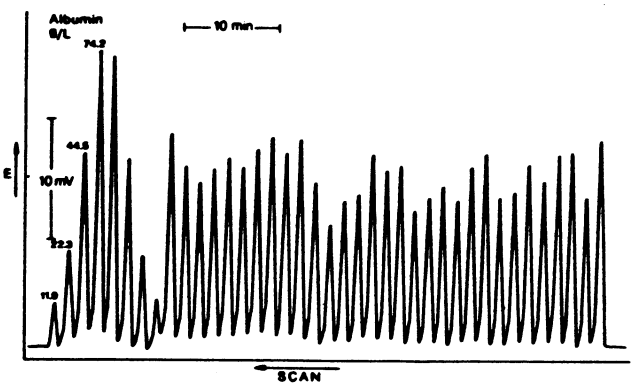

Fig. 6. Recorder trace of analyses for albumin

Table 1. Linearity of the Picrate/Albumin Method

Control[a]	1	2	3	4
Mean, g/L	11.9	22.3	44.5	74.2
No. detns.[b]	20	20	20	20
Av peak height, mV	4.84	9.24	18.92	30.49
SD, mV	0.13	0.27	0.45	0.41
CV, %	2.7	2.9	2.4	1.3

[a] Human albumin, Merieux.
[b] Sampling rate, 50 per/h; sample-to-wash ratio, 1:2.

Table 2. Within-Run and Day-to-Day Precision Data

	Within-Run			Day-to-Day		
Control, g/L	30[a]	46[b]	U[c]	U	U	U
Mean, g/L	29.1	44.3	53.6	30.1	43.2	61.6
No. detns.	15	17	17	10	10	10
CV, %	0.83	0.54	0.61	3.2	2.2	1.2

[a] "Wellcomtrol two." [b] "Validate." [c] U: Unassayed pooled sera.

The stability of the calibration curve was also tested on a day-to-day basis, with use of two albumin standards (25 and 50 g/L). We found that the coefficient of variation (CV) for the slope of the calibration curve was ±2.8% during 10 days, while in all cases the intercept was practically zero (<0.3 mV). Therefore, it seems to be unnecessary to prepare a calibration curve every day when the required accuracy is not better than 4%.

Precision of the method. The within-run and day-to-day precision of the method was studied by analyzing albumin samples from various sources (Table 2).

Analytical recovery. The accuracy of the proposed method was checked by adding 0.500 mL of a 44.5 or 74.2 g/L human albumin standard solution to 1.000 mL of serum sample and assaying. The albumin recovery ranged from 97.0 to 110.3% (average, 102.2%).

Interferents. The possibility of interference by those serum constituents that commonly interfere (*18, 19*) with other procedures was also checked. We found that bilirubin up to 370 mg/L, heparin up to 577 mg/L, or sodium salicylate up to 462 mg/L does not interfere.

Comparison with the BGC method. Sera from 138 patients, with values ranging from 25 to 52 g/L, were analyzed by the proposed method and an automated BCG method. The regression line equation was: y (electrode) = 0.956x (BGC) + 0.035 (r = 0.902). The proposed method gives slightly lower results than the BCG method, probably because most of the BCG methods overestimate serum albumin concentration, as is well known (*10–15, 18, 19*).

We conclude that the proposed method is simple, reliable, and provides a satisfactory potentiometric alternative for the albumin determination in serum. The use of the picrate ion-selective electrode offers competitive advantages over other optical methods, such as freedom from interferences, stability during the measurement, and easy adaptability to automated systems. The method can also be performed manually, e.g., by using an Orion liquid membrane electrode body and the picrate liquid ion exchanger (*23*). The measurements can be carried out in a 4-mL reaction cell with use of the reagents described above.

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