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Potentiometric flow injection determination of serum bromide in patients with epilepsy

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Abstract

A flow injection system was constructed using a bromide-selective electrode and used to determine serum bromide in patients with epilepsy. A 10-μl serum sample was injected into a carrier stream flowing at 0.12 ml min^{-1}. Potential changes and bromide concentrations were linearly related in the range 3–50 mM. The lower limit of detection for serum bromide was 1 mM and this electrode sensitivity spanned the entire concentration range required for bromide therapy (9–24 mM). The results compared favourably with those obtained by colorimetry. © 1997 Elsevier Science B.V.

Keywords: Flow injection analysis; Ion-selective electrode; Bromide; Therapeutic drug monitoring; Epilepsy

1. Introduction

Inorganic bromide salts such as potassium bromide are used to treat refractory epilepsy of early childhood and monitoring the bromide concentration in serum is an accepted measure since the therapeutic and toxic concentration ranges of the drug are very similar [1–5]. Bromide in serum is usually measured by conventional colorimetry [5,6]. However, the procedure requires deproteinization of the serum samples and an additional color reaction. A potentiometric method using a bromide ion-selective electrode has been developed, which allows a simple, rapid and inexpensive assay [7,8]. In the present study, we associated this potentiometric method with flow injection [9]. The latter can process many samples automatically and rapidly and is therefore suitable for analyzing clinical samples. The present results compared favourably with those obtained by established colorimetry.

2. Experimental

2.1. Serum samples

Sera containing bromide were obtained from the venous blood of patients taking potassium bromide (Toyo Seiyaku Kasei, Osaka, Japan) for epilepsy. Control sera were obtained from healthy adult volunteers.

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2.2. Flow injection

The flow injection system for determining serum bromide is shown in Fig. 1. The system consists of a peristaltic pump (SJ-1211L; Atto, Tokyo, Japan), an automatic sample injector (Autosampler Model 33; System Instruments, Tokyo, Japan) equipped with a 10-μl sample loop (7021; Rheodyne, Cotati, CA) and a flow-through type electrode detector including a flow-through cell (FLC 11; DKK, Tokyo, Japan), a bromide electrode (7041 L; DKK), a reference electrode (4401L; DKK), and a voltmeter of high input impedance constructed in house, using a field-effect transistor operational amplifier (LF 356; National Semiconductor; Sunnyvale, CA; input resistance $>10^{12}$ Ω) connected to a recorder (LR4200; Yokogawa Electric, Tokyo, Japan). The flow-through cell consists of methacrylic resin with an effective cell volume of about 20 μl. Samples were injected into a carrier stream (0.1 mM KBr and 100 mM KNO_3), which was pumped through the flow line at a flow rate of 0.12 ml min^{-1}. The addition of a low concentration of bromide in the carrier stream stabilized the base-line potential of the detector electrode. Serum samples (200 μl) were pipetted into a microvial (No. 12962, 100-μl polypropylene screw vial; Alltech, Deerfield, IL), covered with a white Teflon liner (No. 98094; Alltech) attached to an open hole screw cap (No. 73044; Alltech), and placed in an autosampler. The surface of the bromide electrode was polished before use.

2.3. Colorimetry

Bromide concentrations in sera were also determined by colorimetry. Bromide ion is oxidized by chloramine-T to form bromine molecule, which reacts with fluorescein to yield eosin and the concentration of the eosin is then determined spectrophotometrically [10]. In brief, the procedure is as follows [5]. A serum sample (50 μl) was treated with 0.5 M perchloric acid (50 μl), then centrifuged to remove any protein. The supernatant (20 μl) was pipetted into a test tube (10 ml) containing 200 μl of 200 mM acetate buffer (pH 5.5) and 200 μl of 0.625 mM sodium fluorescein. The reaction was started by adding 200 μl of 8 mM chloramine-T. The mixture was incubated for 10 min at room temperature, then the reaction was stopped by adding 3 ml of 40 mM sodium thiosulfate containing 40 mM sodium carbonate. The absorbance at 520 nm was determined using a Hitachi 228 spectrophotometer.

3. Results and discussion

In designing a flow injection system, it is important to consider the following: injected sample volume, tube length between the sample injector and the flow-through cell, and the flow-rate. We selected a sample volume of 10 μl, which decreases the burden on patients. The tube length was as short as possible (5 cm) to prevent dispersion of sample. A flow-rate of 0.12 ml min^{-1} accommodated the slow response time of the electrode, particularly at higher concentrations. The peristaltic pump was reliably operated at this flow-rate.

We added known amounts of bromide to normal serum samples, then injected them into the carrier stream to determine the linearity of the calibration curve in the present system. A typical result is shown in Fig. 2. Since the bromide electrode is known to be subject to interference from chloride [7,11], the electrode responded to a serum sample without bromide. The calibration graph based on peak heights (mV) was linear from 3 to 50 mM, with a slope of 51.3 mV per concentration decade (Fig. 3). The lower limit of

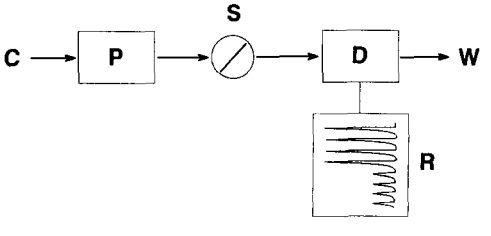

Fig. 1. Arrangement for potentiometric flow injection. C, carrier stream; P, peristaltic pump; S, automatic sample injector; D, flow-through type electrode detector; R, recorder; W, waste. The length and inner diameter of the tube from S to D was 5 cm and 1 mm, respectively.

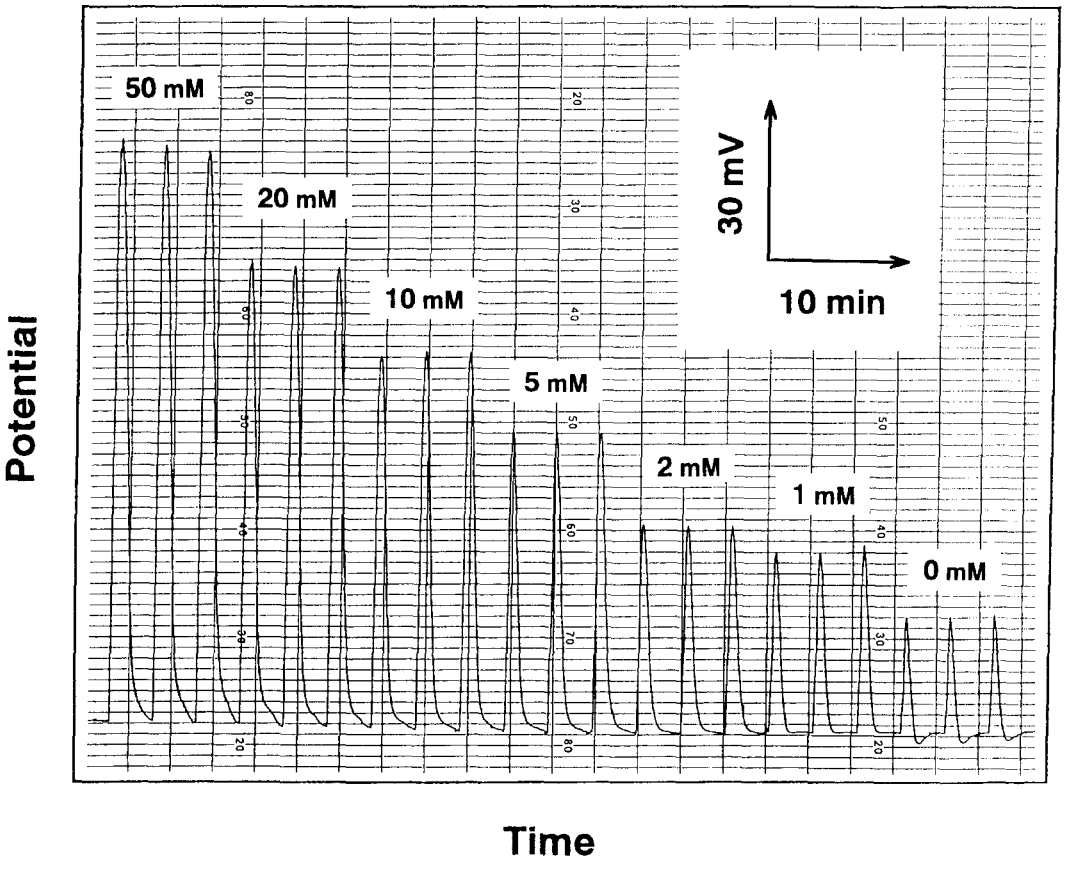

Fig. 2. Typical response to bromide in serum samples.

detection was 1 mM. The linear response region of the calibration graph and the base-line corresponding to the peak height induced by a serum sample without bromide, were extrapolated and the intersection was defined as the detection limit. The sensitivity of the system was sufficient to measure the bromide concentration range required for pharmacotherapy. The clinical range of bromide in serum required for antiepileptic therapy is reported to be 750–1250 μg ml^{-1} (9.4–15.6 mM) [3], 960–1400 μg ml^{-1} (12.0–17.5 mM) [2] or 1160–1940 μg ml^{-1} (14.5–24.3 mM) [1], while the toxic range is thought to be above 1500 μg ml^{-1} (18.8 mM) [3], which partly overlaps the clinical range. Thus, care is needed when concentrations above 1500 μg ml^{-1} (18.8 mM) are applied. The sampling rate was about 20 samples h^{-1} under the present flow conditions. A series of 10 successive determinations of serum samples containing 10 or 20 mM potassium bromide was used to evaluate the precision of the measurement. The mean peak height at 10 mM was 70.0 mV (0.9% R.S.D.) with a range of 68.9–70.7 mV, while that at 20 mM was 85.5 mV (1.5% R.S.D.) with a range of 83.6–87.4 mV.

We determined serum bromide concentrations in patients with epilepsy using the calibration graph shown in Fig. 3 and compared the results with those determined by colorimetry [5]. Fig. 4 shows good correlation over the concentration range of 1–25 mM. The colorimetric method required deproteinization and further reaction to estimate the bromide concentrations in serum samples, whereas the present potentiometric flow injection does not need sample preparation and is automated. Bromide levels can also be monitored

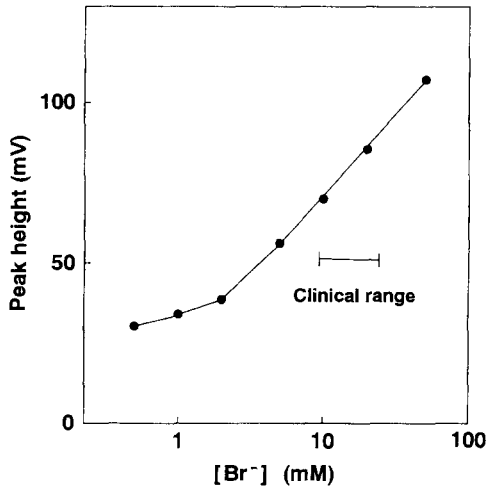

Fig. 3. Calibration graph for serum bromide assay. The clinical concentration range required for antiepileptic therapy is also shown.

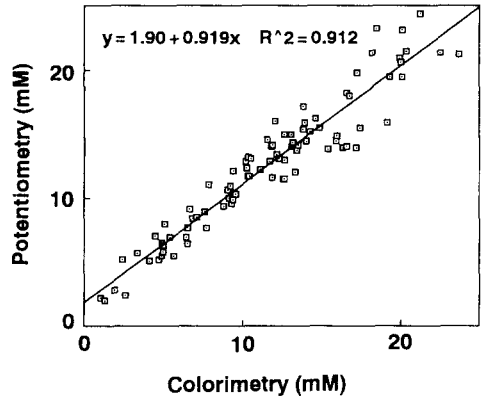

Fig. 4. Correlation of bromide concentrations in 88 serum samples from patients with epilepsy determined by a flow injection analysis using a bromide ion-selective electrode and by colorimetry.

in whole blood, as the potentiometric procedure is not affected by sample color or turbidity. This potentiometric flow injection will markedly reduce the workload involved in therapeutic drug monitoring of bromide in a clinical setting.

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