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Previous misleading report and present
approach

Takashi Katsu*	Yumi Tsunamoto [†]	Nobumitsu Hanioka [‡]
Keiko Komagoe**	Kazufumi Masuda ^{††}	Shizuo Narimatsu ^{‡‡}

*Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, katsu@pharm.okayama-u.ac.jp

[†]Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University

[‡]Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, hanioka@pharm.okayama-u.ac.jp

**Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University

^{††}Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University

^{‡‡}Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, shizuo@pharm.okayama-u.ac.jp

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**A caffeine-sensitive membrane electrode: Previous misleading report
and present approach**

Takashi Katsu, Yumi Tsunamoto, Nobumitsu Hanioka, Keiko Komagoe,
Kazufumi Masuda, Shizuo Narimatsu*

*Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama
University, Tsushima, Okayama 700-8530, Japan*

**Corresponding author. Tel.: +81 86 2517954; fax: +81 86 2517926.*

E-mail address: katsu@pharm.okayama-u.ac.jp (T. Katsu).

Abstract

Although a previous study [S. S. M. Hassan, M. A. Ahmed, M. M. Saoudi, *Anal. Chem.* 57 (1985) 1126.] had shown that a caffeine-sensitive electrode made with picrylsulfonate and 1-octanol as a cation-exchanger and a solvent mediator, respectively, had a wide working pH range (5.5–9.5) and exhibited a Nernstian response, we could not find such response in this electrode. The present result was reasonable, because the pK_a value of caffeinium ion was reported to be around 0.7 and the neutral form of caffeine was predominant in the pH range examined. Thus, we reinvestigated the response characteristics of a caffeine electrode, taking into consideration the pK_a value, and constructed a new electrode with a combination of the lipophilic cation-exchanger, tetrakis[3,5-bis(2-methoxyhexafluoro-2-propyl)phenyl]borate (HFPB), and the solvent mediator with high degree of dielectric constant, 2-fluoro-2'-nitrodiphenyl ether (FNDPE). This electrode showed a pH-dependent response to caffeinium ion and gave a detection limit of 50 μM with a slope of 55 mV per concentration decade at pH 2. The use of other solvent mediators was less effective than that of FNDPE. The electrode was applied for the determination of caffeine in some central stimulants.

Keywords: Ion-selective electrode, Caffeinium ion, Solvent mediator, 2-Fluoro-2'-nitrodiphenyl ether, Caffeine determination

1. Introduction

In 1985, Hassan et al. [1] constructed a caffeine-selective liquid membrane electrode using picrylsulfonate as a cation-exchanger and 1-octanol as solvent mediator. The electrode had a wide working pH range (5.5–9.5) and exhibited a Nernstian response in the range of 10^{-6} to 10^{-2} M caffeine with a slope of 59 mV per concentration decade. This result was very strange, because the pK_a value of caffeinium ion was reported to be around 0.7 [2–4], and thus the neutral form was dominant in neutral pH region, according to an equilibrium equation between the protonated and the neutral form of caffeine shown in Fig. 1. An ion-selective electrode, of course, should only respond to the ionic form and the result reported by Hassan et al. [1], showing a response to the neutral form of caffeine, was questionable. Hence, we reinvestigated in detail the response characteristics of a caffeine-sensitive electrode, taking into consideration the pK_a value. Although ion-selective electrodes respond to only ionic analytes as described above, the name of the neutral form of caffeine is conventionally used in this paper to describe the name of electrode, i.e., caffeine electrode [5].

While developing electrodes that respond to various biologically active amines, a combination of the lipophilic cation-exchanger, tetrakis[3,5-bis(2-methoxyhexafluoro-2-propyl)phenyl]borate (HFPB), and the solvent mediator, 2-fluoro-2'-nitrodiphenyl ether (FNDPE), was quite suitable for constructing for these electrodes [6–12]. Thus, we used this combination in a caffeine electrode, and found that it exactly responded to caffeinium ion according to the pK_a value. Determination of caffeine in some central

stimulants using the present electrode gives results in good agreement with those obtained by high-performance liquid chromatography (HPLC).

2. Experimental

2.1. Reagents

The sources of the reagents were as follows: sodium tetrakis[3,5-bis(2-methoxyhexafluoro-2-propyl)phenyl]borate (NaHFPB) and FNDPE were from Dojindo Laboratories (Kumamoto, Japan); sodium picrylsulfonate and 1-octanol were from Wako (Osaka, Japan); caffeine and theophylline were from Sigma (St Louis, MO, USA); tricresyl phosphate (TCP) and dioctyl phthalate (DOP) were from Tokyo Kasei (Tokyo, Japan); tris(2-ethylhexyl) phosphate (TEHP), bis(2-ethylhexyl) sebacate (BEHS), and σ -nitrophenyl octyl ether (NPOE) were from Fluka (Buchs, Switzerland); and poly(vinyl chloride) (PVC) (degree of polymerization, 1020) was from Nacalai Tesque (Kyoto, Japan). All other chemicals were of analytical reagent grade.

2.2. Electrode system

The caffeine electrode was constructed according to previously described procedures [6–12]. The components of the sensor membrane were NaHFPB (0.5 mg), FNDPE (60 μ L), and PVC (30 mg). The materials were dissolved in tetrahydrofuran (about 1 mL), poured into a flat Petri dish (28 mm in diameter), and the solvent was then evaporated off at room temperature. The resulting membrane was excised and attached to a PVC tube (4 mm o.d., 3 mm i.d.) with

tetrahydrofuran adhesive. The PVC tube was filled with an internal solution composed of 1 mM caffeine hydrochloride and 10 mM HCl and the sensor membrane was conditioned overnight. Because the solvent mediator, 1-octanol, was not effective for plasticizing the PVC membranes, we used an experimental kit for making an ion-selective electrode commercially available from Denki Kagaku Keiki (DKK) (Tokyo, Japan) [11]. This kit had a porous membrane filter, which was infiltrated with the solvent mediator. By using this kit, we prepared the sensor membranes containing 1-octanol as a solvent mediator and sodium picrylsulfonate or NaHFPB as an ion-exchanger. The electrochemical cell arrangement was Ag, AgCl/internal solution/sensor membrane/sample solution/1 M NH_4NO_3 (salt bridge)/10 mM KCl/Ag,AgCl. The internal solution was the same as that used to condition the membrane. Potential measurements were made with a voltmeter produced by a field-effect transistor operational amplifier (LF356; National Semiconductor, Sunnyvale, CA, USA; input resistance $>10^{12} \Omega$) connected to a recorder. To examine the pH-dependence of the electrode, a miniature pH glass electrode (1826A-06T; Horiba, Kyoto, Japan), together with test and reference electrodes, was immersed in each sample solution to simultaneously measure the solution pH.

2.3. Evaluation of the electrode's performance

The detection limit was defined as the intersection of the extrapolated linear regions of the calibration graph [13]. The selectivity coefficients of the electrode ($k_{i,j}^{\text{Pot}}$) were determined by a matched potential method [14–16]. We

used a fixed concentration (1×10^{-4} M) of caffeine as the background. The selectivity coefficients were calculated from the concentration of the interfering ion that induced the same amount of potential change as that induced by increasing the concentration of caffeine to 2×10^{-4} M. This measurement was performed in the presence of 10 mM HCl and 100 mM MgCl₂ to keep the solution pH around 2 and the ionic strength of the solution constant; 100 mM MgCl₂ was chosen because the interference from magnesium was very slight. All measurements were performed at room temperature (about 25°C).

2.4. Determination of caffeine in medicines

Three different kinds of central stimulants containing caffeine and sodium benzoate were used. They were 20% Annaca for injection to animals (Nippon Zenyaku Kogyo, Fukushima, Japan), 20% and 10% Annacas for injection to humans (Fuso Pharmaceutical Industries, Osaka, Japan). These stimulants were diluted 1000-times in a solution adjusted to pH 2 by mixing 0.1 M HCl and 0.1 M MgCl₂. The electrodes were placed in 200 μ L of the solution and constantly stirred with a bar. This electrode system, including the reference electrode [17], is compact. Therefore, a volume as low as 200 μ L can be assayed. When the electrode was applied to Annaca pharmaceutical samples, it was properly exchanged to new ones, because the response of the electrode deteriorated significantly after several measurements of the samples. The concentrations of caffeine in Annacas were determined using the calibration graph of electrode measured in the same solution used for dilution. All

measurements were performed at room temperature (about 25°C).

2.5. HPLC analysis

Caffeine concentrations in central stimulants were also determined by means of HPLC with several modifications of previous methods [18,19]. The system consisted of a Shimadzu SCL-6B system controller (Kyoto, Japan) equipped with an LC-9A pump, an SPD-6A UV spectrophotometric detector, and a C-R4A chromatopac integrator. The same samples (10 μ L), which were diluted 1000-times for the potentiometric measurements using the caffeine electrode, were injected into an Inertsil ODS-80A column (150 \times 4.6 mm i.d.; GL Sciences, Tokyo, Japan) maintained at 40°C. The elution was performed isocratically with 20 mM sodium perchlorate (pH 2.5)/acetonitrile (84:16, v/v) at a flow rate of 1.0 mL min⁻¹. The UV wavelength was fixed at 280 nm.

3. Results and discussion

Hassan et al. [1] used a combination of picrylsulfonate and octanol as a cation-exchanger and solvent mediator, respectively, to construct a caffeine-selective liquid membrane electrode. This electrode had a wide working pH range (5.5–9.5) and exhibited a Nernstian response in the range of 10⁻⁶ to 10⁻² M caffeine with a slope of 59 mV per concentration decade. We constructed a caffeine electrode composed of the same components and examined the response characteristics over a wide pH range, as shown in Fig. 2. In this

experiment, we adjusted the caffeine concentrations using caffeine hydrochloride (containing equimolar mixture of caffeine and hydrochloric acid) and changed the pH by adding an appropriate amount of dilute sodium hydroxide, as in the case of the previous study [1]. However, we could not observe any clear caffeine concentration-dependent response in the wide pH range examined. Hassan et al. showed a Nernstian response to caffeine at around neutral pH in their report (Fig. 2 in the reference [1]), but we could not observe such a response, as is also shown in Fig. 2. The present result was quite reasonable, because the pK_a value of caffeinium ion was around 0.7 [2–4], and the neutral form was dominant in around pH 4–10 region examined.

In order to develop an effective caffeine electrode, it is important to find certain materials giving a stable electrode response even in the strongly acidic region. A cation-exchanger, HFPB, is quite suitable for this purpose, because such lipophilic borate analogs were reported to be very stable in the acidic region [20]. Furthermore, the use of a solvent mediator with a high degree of polarity, such as FNDPE, is known to increase the response to organic ammonium ions [6–12]. Thus, we used a combination of HFPB and FNDPE to construct a caffeine electrode. Fig. 3 shows the pH dependence of this electrode in examining the response to caffeine. In order to see directly the electrode's response to the protonated form of caffeine, we adjusted the initial caffeine concentrations using caffeine and changed the pH by adding appropriate amounts of dilute hydrochloric acid. This measurement was also performed in 0.1 M $MgCl_2$ to make the ionic strength of the solution nearly constant, as in the case of Fig. 2. The response to caffeine markedly increased in the acidic region,

and increasing the concentration of caffeine enhanced the magnitude of the response. These results indicated that the electrode responded to the protonated form of caffeine, whose concentration increased corresponding to the pK_a value of caffeinium ion (around 0.7). However, even without caffeine, a large degree of response was observed below pH 4, showing that the electrode suffered interference from H^+ . To find a suitable pH for determining caffeine, we constructed calibration graphs at various pHs. The graphs were obtained by measuring known amounts of caffeine hydrochloride added to appropriate buffer solutions, and plotting the concentration against the corresponding e.m.f. values. The measurements were performed in the concentration range of 1×10^{-8} to 1×10^{-2} M caffeine. As shown in Fig. 4, the calibration graph at pH 2 showed the best response characteristic to caffeine. The graphs obtained in the range above pH 2 afforded lower sensitivity to caffeine because of a large decrease in the concentration of ionic form of caffeine, while the graph at pH 1 gave a higher degree of interference from H^+ to decrease the sensitivity to caffeine. At pH 2, the slope of the electrode afforded 55 mV per concentration decade and the limit of the detection was 50 μ M. The response time (90% final signal) of this electrode was below 10 s when the concentration of caffeine was changed from 100 to 200 μ M. Such fast response was the same as usually observed with organic ammonium ion-sensitive electrodes made using HFPB and FNDPE [6–12].

We then evaluated the selectivity coefficients of the electrodes (expressed as $\log k_{i,j}^{\text{Pot}}$). They were: H^+ , -2.1; Na^+ , -2.7; K^+ , -1.0; NH_4^+ , -1.3; theophylline, -1.1;

CH_3NH_3^+ , -0.3 ; $(\text{CH}_3)_4\text{N}^+$, 1.4 . The electrode suppressed the response to inorganic cations such as Na^+ ; however, the response to lipophilic quaternary ammonium ion was much greater than that of caffeine. Theophylline, corresponding to demethylated compound of caffeine, decreased the response. These results indicate that the lipophilicity of the organic ammonium ions determines the selectivity sequence, which was characteristic of ion-exchanger-based ion-selective electrodes [21,22].

We examined the effects of solvent mediators (Fig. 5). Firstly, we focused on 1-octanol, the solvent mediator previously applied by Hassan et al. [1]. This mediator has hydrogen bonding ability [11], which may enhance the response to caffeine ion. However, it did not show a good response to caffeine ion, similar to the results of phosphate-type mediators with hydrogen-bonding ability [11], TEHP and TCP. The use of BEHS and DOP, which possess low values of dielectric constants ($\varepsilon = 4.0$ and 5.1 [23], respectively), yielded poor responses. 1-Octanol, TEHP, and TCP also had low values ($\varepsilon = 10$ for 1-octanol [24], $\varepsilon = 4.9$ for TEHP [25], and $\varepsilon = 6.1$ for TCP [26]). In contrast, the phenyl ether-type mediators with higher values of dielectric constants, NPOE ($\varepsilon = 24$ [23,25]) and FNDPE ($\varepsilon = 50$ [23,25]), showed enhanced responses; that of FNDPE was remarkable. In general, the selectivity of the electrode using ion-exchanger and solvent mediator is governed only by the partition coefficients of the ions between sensor membrane and sample solution, and the partition coefficients of organic ions can be increased by using solvent mediators with higher degrees of polarity, enhancing the response [21,22]. Thus, the use of FNDPE was appropriate for improving the sensitivity to caffeine ion, decreasing the interference from

inorganic ions.

We applied the proposed caffeine electrode for the direct determination of caffeine in a central stimulant composed of a mixture of caffeine (48.0–50.0 wt%) and sodium benzoate (50.0–52.0 wt%) [27], named Annaca in Japan. These stimulants were diluted 1000-times in a solution adjusted to pH 2 by mixing 0.1 M HCl and 0.1 M MgCl₂. The measurement at pH 2 was the best for the caffeine electrode as mentioned above, and the calibration graph is shown in Fig. 4. The results of three replicate determinations of caffeine in three different samples with the present electrode and HPLC method are summarized in Table 1. Good agreement was seen between the results obtained by both methods, indicating that the caffeine electrodes developed in this work can perform satisfactorily.

4. Conclusions

A new caffeine-sensitive electrode was constructed using a combination of the cation-exchanger HFPB and the solvent mediator FNDPE. The electrode responded to caffeine, according to the pK_a value. Using the electrode, we determined the caffeine concentrations in some central stimulants.

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Figure Legends

Fig. 1 – Ionization equilibrium of caffeine.

Fig. 2 – Effects of pH on the response to caffeine of the electrode made using picrylsulfonate and 1-octanol: (●) 10^{-2} M, (□) 10^{-3} M, and (▲) 10^{-4} M caffeine.

Insert shows the calibration graph at pH 7, in which the values represent the mean \pm standard deviation of four separate electrodes. The concentrations of caffeine were adjusted using caffeine hydrochloride, as in the case of the previous report [1], and the pH of the solution was adjusted by adding an appropriate amount of dilute sodium hydroxide. The measurement was performed in the presence of 0.1 M MgCl_2 to make the ionic strength of the solution constant.

Fig. 3 – Effects of pH on the response to caffeine of the electrode made using HFPB and FNDPE in the presence of 0.1 M MgCl_2 . See text for experimental details.

Fig. 4 – Calibration graphs of the electrode made using HFPB and FNDPE to caffeine in solutions at various pHs. The following combinations of solutions were used for obtaining specific pHs: pHs 1 and 2, 0.1 M HCl and 0.1 M MgCl_2 ; pH 3, 0.1 M lactic acid and 0.1 M magnesium lactate; pHs 4, 5, and 6, 0.1 M acetic acid and 0.1 M magnesium acetate.

Fig. 5 – Comparison of the calibration graphs of electrodes made changing solvent mediator.