*Journal of Pharmaceutical & Biomedical Analysis*
Vol. 7, No. 8, pp. 947–954, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00


# An ion-selective microelectrode for bile salts

LISA J. KUNZE and SIEGFRIED LINDENBAUM*

*Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045-2504, USA*

**Abstract**: The development of a prototype ion-selective microelectrode for bile salts is described. The microelectrodes demonstrated Nernstian response (59 ± 10 mV $dec^{-1}$) with sodium deoxycholate in Tris (0.2 M, pH 9.0, 15–30°C), HEPES (O.13 M, pH 7.5), and bicarbonate buffers (0.1 M, pH 7.5) (25 ± 0.1°C) were stable for several days, and the responses were highly reproducible. The microelectrodes were selective for bile salts over the physiologically important inorganic anions, bicarbonate and chloride. The response to sodium cholate (42 mV $dec^{-1}$) was consistently lower than the ideal response (59 mV $dec^{-1}$). This ion-selective microelectrode may show promise as a useful tool for the determination of intracellular bile salt activity.

**Keywords**: *Bile salts; microelectrode; ion-selective electrode; deoxycholate; cholate; chenodeoxycholate.*

## Introduction

Bile salts are transported in the liver from the sinusoids to the bile ducts via paracellular and intracellular processes [1]. Intracellular transport involves three phases including transport from the sinusoid into the hepatocyte, transport within the hepatocyte, and transport across the apical canalicular membrane into the canalicular lumen. The mechanisms of transport of bile salts across the apical canalicular lumen have not been well elucidated due to the lack of a technique capable of monitoring bile salts within the intact hepatocyte and the canalicular lumen [1]. Mechanisms that have been proposed [1] include exocytosis, microfilament involvement, and electrochemical potential differences. An ion-selective microelectrode with specific selectivity for bile salts could be utilized to monitor the activity of bile salts within the hepatocyte and the canalicular lumen and, thus, might be a useful tool for determining the importance of the electrochemical potential mechanism. The purpose of this research was to design and develop a prototype of a bile salt-specific ion-selective microelectrode.

The precedence of the development and use of ion-selective microelectrodes for *in vitro* and *in vivo* measurements is tremendous and beyond the scope of this paper, but some of the references relevant to the development of the bile salt-selective microelectrode have been listed [2–12]. Likewise, the historical literature relevant to the development and use of ion-selective electrodes for determining organic ions and ionic surfactants, including the development of electrodes capable of measuring the physiologically important ionic surfactants, the bile salts, have been included [13–23].

*To whom correspondence should be addressed.

This primary focus of this research is to describe the construction of the bile salt-selective microelectrodes and to demonstrate how the response to different bile salts compares with the ideal Nernstian behaviour (59 mV $dec^{-1}$) under differing pH and temperature conditions. The selectivity for bile salts over the predominant inorganic anions in bile is also shown.

## Experimental

### *Chemicals*

The bile salts (Calbiochem, San Diego, CA) and other reagents used in these experiments were analytical grade or better and were used without further purification. The ion-pairing agent, hexadecyltributylammonium bromide (HDTBAB), was synthesized using a procedure from the literature [19] and recrystallized from ethanol–ether mixtures.

### *Instrumentation*

The instrumentation was contained within a Faraday cage, constructed of copper screen and included a Corning 112 digital pH-meter (Corning Scientific Instruments, Medfield, MA), a magnetic stirrer, and the electrodes. The temperature was controlled (±0.1°C) with a waterbath. A separate silver–silver chloride electrode (Cole-Parmer, Chicago, IL) from a glass electrode was utilized as the reference electrode unless otherwise noted.

### *Preparation of the ion-selective solution*

Equimolar concentrations (approximately 5 mM) of HDTBAB and sodium deoxycholate (NaDC) were combined in distilled, deionized water. The resulting ion-pairs were extracted with an equal volume of 1-octanol. A solution containing polyvinylchloride (PVC; 0.3 g) and sebacic acid dioctylester (1 ml) was prepared in THF (9 ml). Equal volumes of the PVC solution and the 1-octanol solutions containing the HDTBAB–NaDC ion-pair were combined and stored in a tightly sealed glass vial.

### *Electrode fabrication*

The microelectrodes were constructed from borosilicate glass capillary tubing (Kimax, Kimble Glass Inc., Vineland, NV, 1.6–1.8 × 100 mm). The tubing was cleaned with hot nitric acid and acetone prior to use. The electrode bodies were prepared by drawing the tubing out with a vertical pipette-puller (Narishige, USA, Greenvale, NY) to yield electrodes with tips measuring <1–20 μm. (The size constraints for electrodes for actual intracellular measurements would be controlled by the diameter of the cells (30 μm) and the canaliculi (1 μm) [24].) The retention of this hydrophic ion-selective solution was enhanced by silanizing the inverted electrode bodies over dichlorodimethylsilane for 5 min, drying them at 180°C, and storing them in a desiccator.

The ion-selective microelectrode was prepared by introducing the PVC–octanol solution into the tip via a fine needle. The height of this solution was 3 ± 1 mm. Insertion of a glass fibre facilitated the flow of the solution into the tip. This solution was allowed to turn slightly opaque before an aqueous solution containing NaDC (0.1 mM) and NaCl (100 mM) was backfilled on the top. Air bubbles were teased out with a rabbit whisker. The diameter of the microelectrode tip was measured using a microscope with an ocular micrometer.

The reference microelectrode was prepared by filling the tip with a sodium chloride solution (0.15 M) and filling the body with a thick agar solution containing potassium chloride (1 M). The internal references for both electrodes were Ag–AgCl wires obtained from glass electrodes.

*Procedures*

All experiments were conducted in 50 ml of buffer contained in a jacketed beaker. Standard curves were prepared in Tris buffer (0.2 M, pH 9.0 with sodium hydroxide) at constant temperature (25 ± 0.1°C). Aliquots of an NaDC solution (0.075 M) in Tris (0.02 M) were added with an automatic pipette (Pipettman, Gilson Medical Electronics, Middleton, WI) and the EMF was recorded after each addition. Regression analyses of the EMF versus concentration of NaDC curves were completed using the data in the linear portion of the curves. Microelectrodes exhibiting deviation from the Nernstian response (59 mV $dec^{-1}$) of more than ±10 mV $dec^{-1}$ concentration were discarded. The detection limits were determined by observing the concentration at the break in the EMF versus concentration curves [25].

The selectivity of an ion-selective electrode has been described previously [26] (equation 1).

$$E = E^\circ + RT/\ln(a_m + \Sigma K_i a_i) + E_j, \tag{1}$$

where $K$ is the selectivity coefficient, $E_j$ is the liquid junction potential, $a_i$ is the activity of the interfering ion, and $a_m$ is the activity of the measured ion. The selectivity of the microelectrode for bile salts was determined using two methods. The method recommended by IUPAC [25] involved quantitating the ion of interest (in this case NaDC) in the presence of a constant concentration of a potential interfering ion. The selectivity constant is calculated (equation 2) using the concentration of the measured ion (NaDC) at the break in the EMF versus concentration curve:

$$K = a_m/a_i. \tag{2}$$

Selectivity experiments following the IUPAC protocol involved observing the EMF resulting from the addition of aliquots of NaDC (0.075 M) to solutions of Tris buffer (0.2 M, pH 9.0, 25 ± 0.1°C) containing either sodium chenodeoxycholate (NaCDC; 0.38 M), sodium taurochenodeoxycholate (NaTCDC; 0.37 M), sodium cholate (NaC; 1 mM), sodium chloride (0.15 M), or sodium bicarbonate (0.05 M). The other method, the method of separately measured solutions [7, 25], involved measuring the EMF as a function of the concentration of each interfering ion. The selectivity constant was then calculated (equation 3) where the slopes for the ions being compared are equal:

$$\log K = \Delta\text{EMF/slope}. \tag{3}$$

Utilizing this method, the EMF was measured as a function of the concentration resulting from the addition of aliquots of NaC, NaCDC, NaTCDC (0.075 M), sodium chloride, potassium chloride (1 M), sodium bicarbonate (0.5 M), trimethylamine (TMA; 0.07 M) or HDTBAB (0.02 M), into 50 ml of Tris buffer (0.2 M, pH 9.0, 25 ± 0.1°C).

Standard curves were prepared in HEPES (0.13 M) and bicarbonate buffer (0.1 M) at pH 7.5, and in Tris buffer (0.2 M, pH 9.0) at 15 and 30°C.

Ultimately, the bile salt-selective microelectrode was to be used for intracellular measurements, so the ion-selective and reference electrodes would have to be combined into a double-channel electrode with a tip of <1 μm [24]. Due to technical difficulties encountered during the preparation and use of double-channel microelectrodes, the ion-selective and reference microelectrodes utilized in this study were prepared as separate microelectrodes. The response of the bile salt-selective microelectrodes used in conjunction with the reference from a glass electrode to NaDC, was compared with the response used in conjunction with a microreference electrode. Standard curves were prepared for each electrode following the protocol described previously using NaDC as the titrant and Tris buffer (0.2 M, pH 9.0, 25 ± 0.1°C).

## Results

The bile salt-selective microelectrodes demonstrated linear responses to NaDC within the concentration range 0.075–3.1 mM (Fig. 1). However, there was occasionally a slightly convex trend in the curve. The average slope (±SD) was 58 ± 4.9 mV $dec^{-1}$ for 33 standard curves prepared using seven microelectrodes. The detection limit in 0.2 M Tris was 44 ± 7.0 μM ($n$ = 5, one electrode). The maximum lifetimes of the

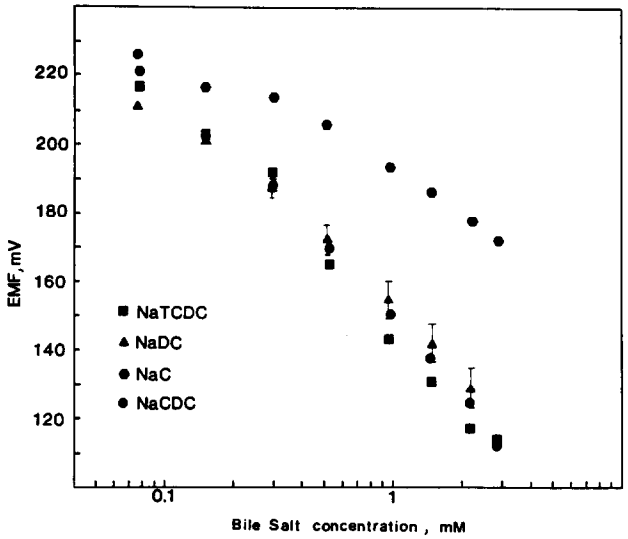


**Figure 1**
The response of a microelectrode to the bile salts, NaC, NaDC, NaCDC and NaTCDC, using the method of separately measured solutions

| Bile salt | Slope (mV $dec^{-1}$)* |
|---|---|
| NaDC 1 | 51 ± 3.7 |
| NaDC 2 | 62 ± 2.1 |
| NaDC 3 | 60 ± 3.1 |
| NaC | 42 ± 1.3 |
| NaCDC | 72 ± 1.1 |
| NaTCDC | 69 ± 1.7 |

*Mean ± SD.

microelectrodes were not determined, but they were used repeatedly over a period of at least 20 days without showing significant deviations of $>\pm 10$ mV dec$^{-1}$ from Nernstian response (Table 1). Furthermore, an analysis of variance (ANOVA) of the data in Table 1 indicated that the differences in the responses among electrodes, between days for each electrode, and between experiments within any day were statistically insignificant.

The selectivity of the microelectrode determined using the IUPAC recommended method is summarized in Table 2. The approach of the $1/K$ values to 1 for the bile salts, NaC, NaCDC, NaTCDC with respect to NaDC, indicates that the microelectrode had little selectivity among the bile salts but was highly selective for bile salts over the inorganic ions, chloride and bicarbonate. The results of the selectivity studies conducted using the method of separately measured solutions (Fig. 1) shows that the responses of the electrode for NaDC, NaCDC, and NaTCDC were practically indistinguishable whilst the response for NaC was obviously lower. There was essentially no response to

**Table 1**
The variability of the response of the microelectrodes

| Electrode | Trial | Age (days) | Slope (mV dec$^{-1}$) | EMF(mV) at 0.5 mM NaDC |
|---|---|---|---|---|
| 1 | 1 | 2 | 56 | 128 |
| | 2 | 2 | 51 | 127 |
| | 3 | 2 | 50 | 140 |
| | 1 | 3 | 59 | 122 |
| | 2 | 3 | 54 | 126 |
| 2 | 1 | 1 | 57 | 96 |
| | 2 | 1 | 59 | 95 |
| | 3 | 1 | 63 | 112 |
| | 1 | 5 | 61 | 129 |
| | 2 | 5 | 55 | 122 |
| | 1 | 6 | 70 | 136 |
| | 2 | 6 | 61 | 126 |
| | 3 | 6 | 58 | 128 |
| 3 | 1 | 1 | 55 | 142 |
| | 2 | 1 | 63 | 132 |
| | 3 | 1 | 65 | 149 |
| | 4 | 1 | 57 | 143 |
| | 5 | 1 | 55 | 135 |
| | 1 | 20 | 64 | 147 |
| | 2 | 20 | 60 | 152 |
| | 3 | 20 | 62 | 162 |

**Table 2**
The selectivity constants calculated using the IUPAC method $K = a_m/a_i$

| Interfering ion | Concentration (mM) | $K_i^{pot}$* | $n$ | $1/K$ |
|---|---|---|---|---|
| Chloride | 150 | $1.0 \times 10^{-3} \pm 9 \times 10^{-5}$ | 3 | 977 |
| Bicarbonate | 50 | $1.7 \times 10^{-3} \pm 7 \times 10^{-5}$ | 3 | 602 |
| NaC | 1 | $0.14 \pm$ — | 2 | 7.4 |
| NaCDC | 0.37 | $0.68 \pm 0.188$ | 3 | 1.5 |
| NaTCDC | 0.37 | $0.37 \pm 0.076$ | 3 | 2.7 |

*Mean ± SD.

physiological concentrations of chloride ion or bicarbonate ion [24, 27]. Lack of selectivity due to the counterion of the ion-pair acting as an ion-exchanger has been implicated as a potential problem with this type of electrode [21]. In this case that would mean that the bile salt acts as an ion-exchange substrate for organic cations. The results from these studies demonstrated that the microelectrode was not sensitive to the organic cations TMA or HDTBAB.

Neither the buffer, the pH or the temperature caused the microelectrode to demonstrate erratic behaviour. The responses of the electrode in HEPES and sodium bicarbonate buffers at pH 7.5 were indistinguishable from the response in Tris at pH 9.0. The slopes observed at 15 and 30°C were not significantly different (according to a student's *t*-test) from the Nernstian slopes at these temperatures (Table 3).

The behaviour of the microelectrodes using the large glass reference and the microreference is compared in Fig. 2. The slopes for the EMF versus concentration curves were similar, but the absolute EMF values were consistently lower when the microreference electrode was used. The overall tip size of the ion-selective and the microreference, or of double-channel electrodes, affected the stability of the EMF readings. Electrical interferences limited the use of electrodes when the total tip diameters were <10 μm in this system.

**Table 3**
The effect of the temperature of the medium on the slope of the EMF versus concentration curve

| Temperature (°C) | Slope* (mV $dec^{-1}$) | *n* | Expected slope (mV $dec^{-1}$) |
|---|---|---|---|
| 15 | 60 ± 2.1 | 3 | 57 |
| 25 | 62 ± 2.0 | 3 | 59 |
| 37 | 71 ± 4.9 | 4 | 62 |

*Mean ± SD.

## Discussion and Conclusions

The results obtained from the preparation of the standard curves indicate that direct determination of intracellular bile salt activity may be possible with these bile salt-selective microelectrodes. Providing that protein binding or intracellular compartmentalization does not occur to a significant extent, the sensitivity is sufficient to quantitate amounts in the range of the expected intracellular concentration of bile salt (0.1–0.3 mM) [1]. The microelectrode was highly selective for the bile salts over the predominant inorganic salt species in bile, sodium chloride and sodium bicarbonate. The stability and reproducibility exhibited by the electrode were reasonable. There were no obvious effects of the buffer or the pH of the medium, and the differences in the slopes at 15 and 30°C (Table 3) were consistent with the Nernst relationship [28]. However, no explanation can be offered at this time for the much higher slope (71 ± 4.9) observed at 37°C.

A major concern with utilizing this microelectrode to monitor the total bile salt activity within hepatocyte, was the low response observed when NaC was used as the titrant. Ideally, the microelectrode would have shown equal response to all of the bile acids so

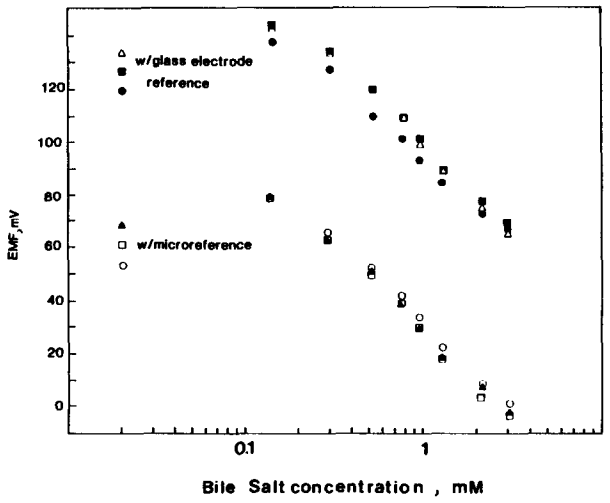


**Figure 2**
A comparison of the response to deoxycholate of single- and double-barrel microelectrodes. The reference for the single-barrel electrode was the Ag–AgCl chloride reference part of a glass electrode

| Electrode | | Slope (mV $dec^{-1}$)* |
|---|---|---|
| Single | 1 | 56 ± 1.9 |
| | 2 | 56 ± 3.7 |
| | 3 | 55 ± 2.8 |
| Double | 1 | 60 ± 2.1 |
| | 2 | 63 ± 2.2 |
| | 3 | 62 ± 2.3 |

*Mean ± SD.

that solutions containing mixtures of bile salts produce the same EMF values as pure solutions. Identical responses had been reported for the trihydroxy- and dihydroxycholanate salts (such as NaC and NaDC, respectively) using a similar liquid membrane electrode [22]. However, the lower response observed for NaC using the microelectrode developed in this research indicates that the total activity of a solution containing significant amounts of NaC would be lower than solutions containing NaDC alone. Since human bile consists of 35–70% trihydroxycholanic acids (NaC) and 30–65% dihydroxycholanic acids (NaCDC) [29–31], the response observed for measurements in biological samples might not accurately reflect the total activity of the bile acids. Before an attempt is made to use the microelectrodes to measure intracellular activities, the anomalous behaviour of the electrodes to NaC needs to be investigated further. The problem might be alleviated by utilizing a modified ion-selective medium.

Double-barrel microelectrodes with tip diameters $<1$ μm must be perfected before the bile salt-selective microelectrode could be utilized for intracellular measurements. Microelectrodes of this size are necessary to minimize cellular disruption and to be small enough to fit within the hepatic canaliculi [24]. The electrical interferences observed with the combined tip diameter of the ion-selective and microreference of $<10$ μm, could probably be alleviated by utilizing a more sophisticated electronic signal amplifier and high impedance leads [6–8, 11, 32].

*Acknowledgements* — The authors express their thanks to Dr Ralph Adams for providing the use of his laboratory facilities and valuable advice and help in the preparation of microelectrodes. This work was supported by NCI training grant number 09242.

## References

[1] B. L. Blitzer and J. L. Boyer, *Gastroenterology* **82**, 346–357 (1982).
[2] J. L. Walker, *Analyt. Chem.* **43**, 89A–93A (1971).
[3] H. D. Lux, *Neuropharmacology* **13**, 509–517 (1974).
[4] F. Lanter, R. A. Steiner, D. Ammann and W. Simon, *Analytica Chim. Acta* **135**, 51–59 (1982).
[5] R. Y. Tsien and T. J. Rink, *J. Neurosci. Meth.* **4**, 73–86 (1981).
[6] R. C. Thomas, in *Ion-Selective Intracellular Microelectrodes*, pp. 61–103. Academic Press, New York (1978).
[7] K. Cammann, in *Working with Ion-Selective Electrodes*, pp. 21–51, 77–89, 105–145, 174–180, 194–196. Springer, New York (1979).
[8] R. D. Purves, in *Microelectrode Methods for Intracellular Recording and Ionophoresis*. Academic Press, New York (1981).
[9] K. Tasaki, Y. Tsukahara, S. Ito, M. J. Wayner and W. Y. Yu, *Physiol. Behav.* **3**, 1009–1010 (1968).
[10] S. J. Youmans, J. O'Doherty, W. McD. Armstrong and R. J. Stark, *Science* **209**, 510–513 (1980).
[11] E. Fromter, M. Simon and B. Gebler, in *Progress in Enzyme and Ion-Selective Electrodes* (D. W. Lubbers, H. Acker, R. P. Buck, G. Eisenman and M. Kesseler, Eds), pp. 35–44. Springer, New York (1981).
[12] L. B. Haberly and J. M. Bower, *J. Neurosci. Meth.* **3**, 251–254 (1981).
[13] T. Higuchi, C. R. Illian and J. L. Tossounian, *Analyt. Chem.* **42**, 1674–1676 (1970).
[14] C. Gavach and P. Seta, *Analytica Chim. Acta* **50**, 407–412 (1970).
[15] C. Gavach and C. Bertrand, *Analytica Chim. Acta* **55**, 385–393 (1971).
[16] G. Baum, *Analyt. Lett.* **3**, 105–111 (1970).
[17] B. J. Birch and D. E. Clark, *Analytica Chim. Acta* **61**, 159–162 (1972).
[18] B. J. Birch and D. E. Clark, *Analytica Chim. Acta* **67**, 387–393 (1973).
[19] D. F. Evans, K. M. Kale and E. L. Cussler, *J. Phys. Chem.* **84**, 593–598 (1980).
[20] P. Meares, S. G. Cutler and D. G. Hall, *J. Electroanal. Chem.* **85**, 145–161 (1977).
[21] P. Meares, S. G. Cutler and D. G. Hall, *J. Chem. Soc. Faraday Trans. 1* **74**, 1758–1767 (1978).
[22] S. Lindenbaum and M. Vadnere, *Int. J. Pharm.* **11**, 57–69 (1982).
[23] L. Campanella, L. Sorrentino and M. Tomassetti, *Analyst* **108**, 1490–1494 (1983).
[24] H. O. Wheeler, in *Handbook of Physiology* (C. F. Code, Ed.), Section 6, Vol. 5. American Physiological Society, Washington, D.C. (1968).
[25] IUPAC, Recommendations for nomenclature of ion-selective electrodes, *Pure Appl. Chem.* **48**, 127–132 (1976).
[26] G. Eisenman, in *Advances in Analytical Chemistry and Instrumentation* (H. W. Nurnberg, Ed.), Vol. 4, p. 213. Wiley, New York (1965).
[27] R. L. Smith, in *The Excretory Function of Bile*, pp. 12–15. Chapman and Hall, London (1973).
[28] A. Martin, J. Swarbrick and A. Cammarata, in *Physical Pharmacy*, 3rd edn, pp. 179–182, 245–263. Lea and Febiger, Philadelphia (1983).
[29] J. B. Carey Jr, in *The Bile Acids* (P. P. Nair and D. Kritchevsky, Eds), Vol. 2, Chap. 3. Plenum Press, New York (1973).
[30] P. P. Nair and T. Okishio, *Biochemistry* **5**, 3662–3668 (1966).
[31] F. Nakayama, *J. Lab. Clin. Med.* **69**, 594–609 (1967).
[32] A. Haemmerli, J. Janata and H. Mack Brown, in *Progress in Enzyme and Ion-Selective Electrodes* (D. W. Lubbers, H. Acker, R. P. Buck, G. Eisenman, M. Kessler and W. Simon, Eds), pp. 45–51. Springer, New York (1981).

[*Received for review 30 December 1988; revised manuscript received 16 April 1989*]