

Selectivity of Liquid Membrane Cadmium Microelectrodes Based on the Ionophore *N,N,N',N'*-Tetrabutyl-3,6-dioxaoctanedithioamide

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Received: May 26, 1998

Final version: July 8, 1998

Abstract

The selectivity of heavy metal ion-selective electrodes has often been inadequately characterized in the past. Only recently, alternative methods have become available to assess the response toward highly discriminated ion solutions. In this article, the selectivity of vibrating liquid membrane cadmium-selective microelectrodes was characterized using the separate solution method, the fixed interference method, the matched potential method, and Bakker's method which demands one to condition the electrode membrane in discriminated ion solutions prior to measurement. In this study, the matched potential method gave selectivity values for the two least discriminated ions, lead(II) and copper(II), that were comparable to the ones obtained with SSM. Bakker's method, on the other hand, was the only method that reveals the extremely high ion selectivity over all other discriminated ions. This study shows that carrier-based cadmium microelectrodes possess sufficient selectivity for many in situ applications, and that experimental biases may sometimes lead to experimental selectivity coefficient variations of about 12 orders of magnitude depending on the chosen method.

Keywords: Selectivity, Vibrating liquid membrane, Cadmium microelectrode, Separate solution method, Fixed interference method, Matched potential method, Bakker method

1. Introduction

Ion-selective electrodes (ISEs) have achieved a high level of sophistication that allows rapid spatially resolved concentration mapping of a series of ionic analytes [1, 2]. These electrodes are well established for measurements of extra- and intracellular fluids, and account for more than 95% of all blood electrolyte measurements. They are an extremely versatile tool to chemical sensing since the selectivity can be chemically adjusted by incorporating different ionophores into the membrane phase. Consequently, the number of analytes that can be detected with this technique is still increasing. The direct, spatially resolved detection of heavy metals, including cadmium, is important for understanding the mechanisms by which certain plants may accumulate massive amounts of heavy metals in their shoots. These plants have great promise as inexpensive decontaminants for heavy-metal infested soils, and studying the biological processes relevant to this uptake is important.

Direct potentiometric in situ heavy metal mapping requires sensors with extremely high selectivity. However, there is evidence that heavy metal ion-selective electrodes have been inadequately characterized in the past. Corresponding optical sensors have been found to show subnanomolar detection limit and extremely small selectivity coefficients [3, 4]. Very recently, lead-selective membrane electrodes have been reported to possess extremely low potentiometric detection limits and corresponding small selectivity coefficients by proper choice of inner electrolytes. These last findings are still part of important current research [5].

In recent years, there have been debates on how the selectivities of ionophore-based ion-selective electrodes should be reported [6]. Traditional IUPAC recommendations suggest the use of the so-called matched potential method (SSM) and the fixed interference method (FIM) [7]. While SSM was recommended as a rapid screening method, FIM was usually the preferred choice since it mimics the final analytical measurement more closely. More recently, IUPAC has also endorsed the so-called matched potential method (MPM) [8] first introduced by Gadzekpo and Christian [9], which is an empirical method without the constraints

from theoretical predictions or models. On the other hand, a new conditioning procedure for highly selective electrodes has recently been introduced by Bakker [10, 11]. This technique yields Nernstian response slopes even for heavily discriminated ions, and gives theoretically well-founded selectivity coefficients. This article compares for the first time these four techniques for a cadmium-selective system based on a neutral dithioamide ionophore introduced by Schneider et al. [12] (see insert in Fig. 1), and may be a further step towards clarifying the question on how ISE selectivity should be reported. A vibrating microelectrode was chosen for this work since it has been shown that it possesses

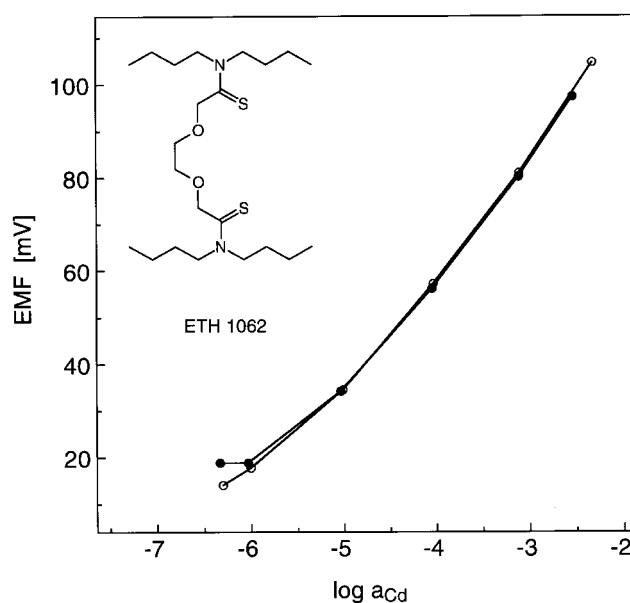


Fig. 1. Calibration curves of a vibrating cadmium-selective microelectrode in different cadmium nitrate solutions (●) with and (○) without an ionic background consisting of 100 μ M $\text{Ca}(\text{NO}_3)_2$. Insert, cadmium ionophore *N,N,N',N'*-tetrabutyl-3,6-dioxaoctanedithioamide (ETH 1062).

superior potential stability characteristics relative to a static microelectrode, i.e., repeatabilities in the microvolt readings are reliably achievable by vibrating the tip of the electrode. Further characterization of these sensors toward selective detection of cadmium in plant roots is published elsewhere [13].

2. Experimental

2.1. Cd²⁺-Selective Microelectrodes Construction

Liquid-membrane ISEs were constructed as previously described in detail [14–16]. Borosilicate glass capillaries (1.5 mm in diameter, without filament, World Precision Instruments, Inc., Sarasota, FL) were cleaned in a mixture of 95 % (v/v) concentrated H₂SO₄ and 5 % (v/v) of 70 % HClO₄. Capillaries were pulled using a two-stage Flaming-Brown horizontal electrode puller (Model P-87, Sutter Instrument Co., Novato, CA), generating a microelectrode with a relatively short shank and a tip diameter of approximately 1 to 2 μm. Microelectrodes were heated (200 °C, ≥3 h), silanized with tri(*n*-butyl)chlorosilane (200 °C, 30 min), cooled, and then stored in an evacuated desiccator. Microelectrodes were initially completely backfilled with a 10 mM Cd(NO₃)₂ + 100 mM KCl solution. The microelectrode tip was then frontfilled with a short column (50 μm in length) of the Cd²⁺ responsive membrane cocktail, which consisted of 10 % (w/w) Cd²⁺ ionophore I (*N,N,N',N'*-tetrabutyl-3,6-dioxaoctanedithioamide; ETH 1062, Fluka), 10 % (w/w) potassium tetrakis(3,5 bis-(trifluoromethyl)phenyl)borate (Fluka), and 80 % (w/w) 2-nitrophenyl octyl ether (Fluka). Subsequently, the backfilling buffer was reduced to a column length of approximately 1.5 cm to minimize parasitic capacitance. Electrical contact between the microelectrode and the head stage of the vibrating probe system was made through a 0.25 mm Ag/AgCl wire, and a single junction reference electrode (Model MI-409F, Microelectrodes, Inc., Londonderry, NH) was connected to the reference input of the head stage.

2.2. Vibrating-Microelectrode System

A detailed description of the technique, the theoretical aspects of the device, and the calculations involved in the ion-selective system have been previously described [14–16]. The system consists of three piezoelectric microstages (PZS-100; Burleigh Instruments, Inc., Fishers, NY) stacked in orthogonal directions and held by translation stages (Newport Corp., Fountain Valley, CA). The stepper motors of the translation stages allow coarse positioning of the microelectrode, and the piezoelectric pushers control the electrode's vibration. The piezoelectric pushers are driven by a damped, squared wave at low frequency (0.3 Hz), vibrating the microelectrode at any desired angle and amplitude in a two-dimensional plane. A 486-PC computer running DVIS6 software (Biocurrents Research Center, Marine Biology Lab, Woods Hole, MA) controlled the movement of the microelectrode between the two preset positions (i.e. vibration amplitude) such that the excursion of the electrode was damped. The vibration amplitude was set at 30 μm. The software also allows for the visual display of the voltage difference, which is calculated by measuring the microelectrode output at each extreme position of the vibration excursion (1000 data points/s), pooling these data into two separate buffers representing the two extremes of vibration, and then subtracting the averaged data of one buffer from the other. The sensitivity of the system permits the measurement of voltage differences in the microvolt range.

2.3. Selectivity Measurements

The ability of the Cd²⁺ electrode to discriminate against other ions was evaluated using four methods. Since preliminary work on selectivity suggested that the Cd²⁺ electrode was highly discriminatory against other cations, we used 2 established and 2 novel techniques. Bakker's method [6, 10, 11] involved evaluating the electrode's performance in the absence of Cd²⁺. The electrode was first backfilled with the cation salt of the tetraphenylborate derivative used in making the Cd²⁺ sensor (in this case K⁺ as 10 mM KCl) and then the electrode was frontfilled with the Cd²⁺ responsive cocktail as described above. The liquid membrane was then conditioned for several hours in a solution identical to the backfilling solution. Calibration plots for the individual interfering ions were generated by measuring the electrode millivolt outputs in a series of solutions of varying activities of the interfering ion. Only after these calibration plots were generated for the interfering ions was the electrode exposed to the primary ion (Cd²⁺) and a similar calibration plot was then generated for varying Cd²⁺ activities. The selectivity coefficients were then calculated using the formula:

$$\log K_{Cd,J}^{pot} = \frac{2F\{E_J - E_{Cd}\}}{2.303RT} + \log \left(\frac{a_{Cd}}{a_J^{2/z_J}} \right) \quad (1)$$

where J represents the interfering ion, $K_{Cd,J}^{pot}$ is the selectivity coefficient, z_J is the valency of the interfering ion tested, E_{Cd} and E_J are the electrode millivolt outputs in the testing solutions, a is the activity of the interfering and primary ion, R is the gas constant, F is the Faraday constant, and T is the absolute temperature. For selectivity coefficients determined according to the separate solution method (SSM) and fixed interference method (FIM), electrode cocktails were backfilled with 10 mM Cd(NO₃)₂ + 100 μM KCl and conditioned overnight in a solution of identical composition. For SSM measurements, calibration plots were then determined in pure cadmium and interfering electrolytes with varying concentrations. Selectivity coefficients were calculated according to Equation 1 from a cadmium and an interfering sample of indicated, equal concentration. For FIM measurements, calibration plots were determined in solutions that contained a constant interfering ion background and varying cadmium concentrations. The selectivity coefficient was calculated from the cadmium activity a_{Cd} at the crosssection of the two extrapolated linear segments of the calibration plot, and by the background interfering ion activity a_J as follows:

$$\log K_{Cd,J}^{pot} = \log \left(\frac{a_{Cd}}{a_J^{2/z_J}} \right) \quad (2)$$

The fourth approach used the matched potential method (MPM) [8, 9], which is often the method of choice for calculating selectivity coefficients when the electrode does not exhibit a Nernstian response to changes in interfering ion activity. This method involved adding a specific activity (final activity 25 μM) of the primary ion Cd²⁺ to a reference solution already containing 10 μM Cd²⁺ activity and subsequently measuring the millivolt output. In a separate test, interfering ions were added to an identical Cd²⁺ reference solution until the change in membrane potential matched the previous one obtained by adding primary ions (Cd²⁺) to the reference solution. The matched potential selectivity coefficient was then calculated from the ratio of the activity change of the primary ion to that of the interfering ion:

$$\log K_{Cd,J}^{MPM} = \log \left(\frac{\Delta a_{Cd}}{\Delta a_J} \right) \quad (3)$$

where J again represents the interfering ion and $K_{Cd,J}^{MPM}$ is the matched potential method selectivity coefficient.

3. Results and Discussion

This study characterizes the selectivity of cadmium-selective vibrating liquid membrane microelectrodes containing the ionophore *N,N,N',N'*-tetrabutyl-3,6-dioxaoctanedithioamide (see insert in Fig. 1), in view of their application for root measurements of plants that show hyperaccumulation characteristics. Target concentrations of cadmium and possible interferents are moderately low (i.e., on the order of 100 μM). It is therefore an important goal to characterize these electrodes under conditions that closely mimic their final application. Heavy metal uptake studies are usually performed *in vitro* within an artificial nutrient solution, and 100 μM levels are realistic for this purpose. In addition, the K^+ concentrations in soil solutions vary quite widely from the micromolar to the millimolar range depending on the soil type and the quality of the water. For many soils, therefore, the K^+ and Na^+ activities in the soil solutions are also within the micromolar range as well. Four different methods for determining selectivity coefficients were used, and the experiments are discussed in the following sections.

3.1. Separate Solution Method

This method requires that ion-selective electrodes are immersed in separate solutions of primary and interfering ions with typically equal concentrations. In view of the target application of these sensors, 100 μM solutions were the preferred choice, although for comparison purposes measurements at 10 mM levels were also performed. Ideally, selectivity coefficients should be independent of the measured concentration. As Table 1 shows, however, the values vary widely for the two different concentrations used, indicating non-Nernstian slopes toward the discriminated ions. The selectivity values for the more dilute measurement, which should reflect the final application most closely, would actually indicate that alkali metal ions may interfere heavily in a practical application. Since the measurements performed with more concentrated solutions yield apparently higher selectivities, this indicates the presence of an experimental bias in the measurements. This bias is very likely dictated by the intrinsic lower sensitivity limit of these electrodes [5, 17]. Accordingly, potentials at the detection limit are assumed to originate from a response toward these interfering ions, which does clearly not hold. While the separate solution method is a popular and rapid one-point screening method, it is clearly an inadequate method for the current application. The ions Cu^{2+} and Pb^{2+} seem to interfere substantially enough that the reporting of SSM selectivity values seems warranted. Further studies were however necessary to more clearly characterize the selectivity characteristics of the membrane.

3.2. Fixed Interference Method

According to IUPAC, the fixed interference method is the preferred experimental choice for characterizing selectivity since it closely

Table 1. Selectivity determinations according to the separate solution method.

Ion:	$\log K_{Cd,J}^{pot}$ (10 mM solutions)	$\log K_{Cd,J}^{pot}$ (100 μM solutions)
Cd^{2+}	0.0	0.0
Na^+	-2.6	2.3
K^+	-2.8	1.1
NH_4^+	-1.2	2.1
Ca^{2+}	-3.6	-3.3
Zn^{2+}	-3.0	-2.4
Cu^{2+}	-1.5	-0.1
Pb^{2+}	-0.5	-0.2

Table 2. Selectivity determinations according to the fixed interference method in 100 μM interfering ion solutions.

Interfering ion:	$\log a_{Cd}$ (DL)	$\log K_{Cd,J}^{pot}$
none (Cd^{2+} only)	-5.6	N/A
Na^+	-5.8	2.2
K^+	-5.9	2.1
NH_4^+	-5.9	2.1
Ca^{2+}	-5.4	-1.4

reflects the final application of the electrode [18]. In view of the target application, therefore, cadmium calibration plots were performed in 100 μM interfering ion solutions. It can be seen in Table 2 and Figure 1 that the detection limits in these measurements are not significantly different from calibration plots in pure cadmium solutions without any interferent, which in part explains the heavy bias observed with the separate solution method discussed above. Hence, it is inappropriate to assign the background potential to the response of the electrode to the interfering ion alone, and Equation 2 is invalid for this activity range. Instead, the inherent detection limit of the sensor at low primary ion activities dictates the lowest measurable potential. It seems clear from these experiments that the background ions studied here do not substantially interfere with the measurements. Oddly, however, the calculated selectivity coefficients are rather large and again suggest that alkali metal ions may interfere strongly in a final measurement. Clearly, the fixed interference method yields meaningless data if the background interfering ion does not induce a potential that is significantly higher than the one at the inherent detection limit of the sensor.

3.3. Matched Potential Method

A recent IUPAC recommendation endorsed the matched potential method, which seems to alleviate the problem mentioned above [8]. This method requires the addition of interfering ions to a reference solution, typically containing a fixed activity of primary ions, until the same potential change is recorded as with a given primary ion activity increase. The matched potential method selectivity coefficient is given by this primary to interfering ion activity ratio. Cadmium-selective microelectrodes were characterized by this method in 10 μM cadmium solutions. With the exception of the ions lead(II) and copper(II), the addition of interfering ions caused the membrane potential to drop, not to increase (see Table 3). The indicated millivolt change is already corrected for dilution effects and influences on the activity coefficients of cadmium from adding additional electrolyte to the sample. This potential drop may be in part caused by small variations in the liquid junction potential at the reference electrode side. These results are a further important indication that most ions

Table 3. Selectivity determinations according to the matched potential method.

Cd^{2+} Conc.	Interferent	Final Conc.	EMF Change	$\log K_{Cd,J}^{MPM}$
10 μM	K^+	4.7×10^{-3} M	-0.1 mV	N/A
10 μM	NH_4^+	5.0×10^{-3} M	-0.3 mV	N/A
10 μM	Ca^{2+}	2.9×10^{-3} M	-1.0 mV	N/A
10 μM	Zn^{2+}	3.6×10^{-3} M	-1.3 mV	N/A
10 μM	Mg^{2+}	3.6×10^{-3} M	-1.0 mV	N/A
10 μM	Fe^{2+}	2.4×10^{-3} M	-0.4 mV	N/A
20 μM	Cu^{2+}	5.0×10^{-4} M	11.5 mV	-1.3
20 μM	Pb^{2+}	7.9×10^{-5} M	12.5 mV	-0.6
10 μM	Ni^{2+}	2.7×10^{-3} M	-0.4 mV	N/A
10 μM	Mn^{2+}	3.8×10^{-3} M	-0.2 mV	N/A

under study do not interfere with cadmium determinations, and that the electrode is indeed very selective. Although the membrane selectivity cannot be quantified with heavily discriminated ions, the matched potential method does not give wrong predictions as compared to the two classical methods described above. As seen in Table 3, Pb^{2+} and Cu^{2+} each gave a positive response, even in $20\ \mu\text{M}$ cadmium solutions. This potential change was used to calculate matched potential selectivity coefficients. Since both of these ions interfere substantially, and the ions carry the same charge as Cd^{2+} [6], these selectivity coefficients are very close to those obtained by the separate solution method in the more concentrated solutions (see Table 1). This indicates that, at high concentrations, these interfering ions are fully potential determining and show near-Nernstian response slopes.

3.4. Bakker's Method: Using Liquid Membranes Conditioned in Discriminated Ion Solutions

To eliminate the influence of the inherent sensitivity limit on the response toward discriminated ions, the membranes were conditioned in $10\ \text{mM}$ KCl solutions without any contact to cadmium ions. These ions initially contained in the membrane are not tightly bound by the ionophore and can now easily ion-exchange with other sample ions. Only at the very end of the experiment is the electrode exposed to the most preferred ion, in this case cadmium. As reported in the original work [10, 11], such a treatment may lead to observable Nernstian electrode slopes and to an unbiased quantification of the inherent ion-exchange selectivity of the membrane. Figure 2 shows that most measured ions indeed show a near-Nernstian response behavior, with alkali metal ions being extremely discriminated. Table 4 quantifies this data for the same concentrations as shown before in Table 1 for the SSM values. It is evident that the selectivity coefficients deviate much less than the ones shown in Table 1 as the sample concentrations are varied. Apparently, this method seems suitable to the quantification of selectivity coefficients toward highly suppressed ions. The observation that calcium and other ions are discriminated by

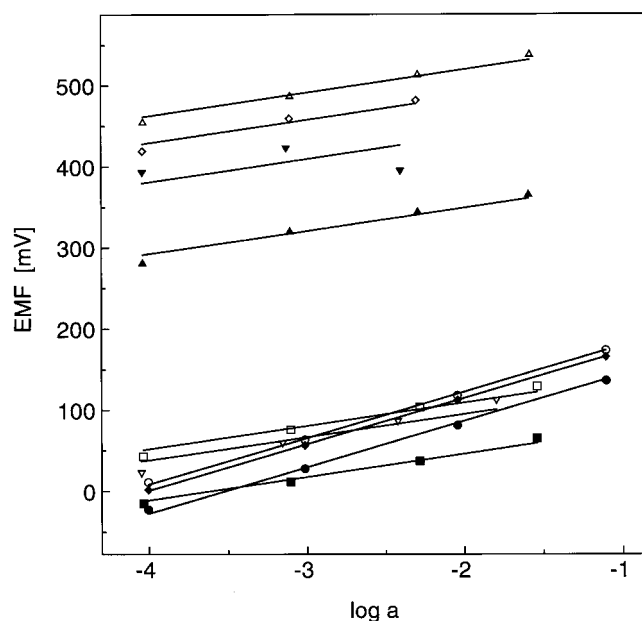


Fig. 2. Normalized response curves obtained according to Bakker's method toward the ions (Δ) Cd^{2+} , (\diamond) Cu^{2+} , (∇) Pb^{2+} , (\blacktriangle) Zn^{2+} , (\triangledown) Ni^{2+} , (\square) Ca^{2+} , (\blacksquare) Mg^{2+} , (\bullet) Na^{+} , (\circ) K^{+} , (\blacklozenge) NH_4^+ . The electrodes were conditioned in $10\ \text{mM}$ KCl solutions before measurement (see Sec. 2).

Table 4. Selectivity determinations according to Bakker's method.

Ion:	$\log K_{Cd,J}^{\text{pot}}$ (10 mM solutions)	$\log K_{Cd,J}^{\text{pot}}$ (100 μM solutions)
Cd^{2+}	0.0	0.0
Na^{+}	-12.5	-12.2
K^{+}	-11.7	-11.5
NH_4^+	-11.8	-11.4
Mg^{2+}	-16.2	-16.0
Ca^{2+}	-13.9	-14.0
Ni^{2+}	-14.4	-14.7
Zn^{2+}	-5.7	-5.9
Cu^{2+}	-1.1	-1.1
Pb^{2+}	-3.9	-2.1

more than 10 orders of magnitude explains why the other methods are inadequate to quantify the extent of interference. These results are not overly surprising, since similar findings were reported earlier for lead-selective optodes [3] and electrodes [5] on the basis of a structurally related ionophore. The selectivity over copper was satisfactorily reproduced relative to the other 2 methods (see Table 1 and 2). However, measurements in lead ion solutions showed poorly reproducible and sometimes unstable potential readings. An apparent super-Nernstian response toward lead was reported in the literature before with similar ionophores, which was explained by the extraction of PbA^+ ions, with A^- being a sample anion such as OH^- [19]. Electrode instabilities or this latter effect may explain the apparent non-Nernstian behavior of lead (see Table 4 and Fig. 2) and the poor correlation with the SSM and MPM results. While this finding may not be generally valid, it does indicate that the new conditioning procedure seems primarily suitable for the selectivity characterization of highly discriminated ions. In effect, this procedure demands a different reference ion to be initially present in the membrane, and the resulting selectivity coefficient of cadmium over the interferent is obtained by subtracting two large potential changes. If interference is strong the other methods reflect more closely the final application and are, therefore, preferred.

4. Conclusions

The selectivity of heavy-metal ion-selective electrodes has often been inadequately reported in the past. This article has shown that a variety of methods may be used to characterize selectivity more properly. The main drawback of the separate solution and fixed interference methods is that they often yield numerical selectivity values that have no direct selectivity meaning. Traditionally, the interfering ion concentration is not varied with these methods, so that no information about the electrode slope, which is indicative whether the measured ion is truly potential determining, is obtained. The matched potential method identifies potential sample ions that could interfere significantly. For heavily discriminated ions, negative potential changes are obtained, so that this method does not introduce meaningless selectivity values as the other two do. Bakker's method avoids initial contact of the liquid membrane with primary ion solutions, and is therefore primarily suitable for the characterization of the ion exchange selectivity toward highly discriminated ions. This method gives less reliable selectivity values with strong interferents and is therefore in many ways complementary to the other three methods reported in this article. This finding indicates that a range of different methods, rather than one method alone, may be most suitable for the selectivity characterization of heavy-metal ion-selective electrodes. The cadmium selective system studied in this work appears to possess extremely high selectivities over a wide range of potential interferents. This makes it a potentially powerful tool for accurate

cadmium profile mapping in the vicinity of plant roots. In view of the final application of these sensors, all selectivity measurements were performed with vibrating microelectrodes with tip sizes on the order of 1–2 μm and vibrating frequencies of 0.3 Hz. Although no direct comparative experiments were performed, selectivities of corresponding macroelectrodes are expected to be similar.

5. Acknowledgements

This work was supported by a grant from the U.S. Department of Energy-Division of Energy Biosciences (Interagency Agreement DE-A I02-95ER 21097) to L.K. and by a type G grant to E.B. from the Petroleum Research Fund (administered by the American Chemical Society).

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