

# Calixarene-based Sensing Agents

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## 1 Introduction

The development of new and more efficient means of performing real-time monitoring of chemical and biochemical species through the use of sensors is among the most significant challenges facing modern science. The problems involved are multifaceted requiring a broad understanding of many areas ranging from synthesis to thin layer deposition and surface analysis technologies, and involving computer-based data acquisition and signal processing. The nature of the component used to generate the diagnostic signal is central to determining the overall performance of any chemical sensor as this will largely, though not absolutely, define the critical characteristics of the device, namely its selectivity, lifetime and response time. However, despite much effort over the past 30 or so years, the number of really efficient individual sensors remains disappointingly small, probably reflecting the *ad hoc* nature of the design and synthesis of potential sensing agents. To be fair, the difference between a really efficient sensing agent and a hopeless one is very difficult to predict since, on a molecular basis, the processes which together define the overall preference for a target substance, in preference to all interferences, interact in subtle ways. However, recent improvements in the power of computer systems and refinements in the algorithms used to minimise molecular energies in solution have enabled more accurate predictions of structures and conformations to be made, and it is now possible in advance to probe how a sensor might interact in a dynamic sense with certain target species in different solvents. In addition, the large amount of information now available should enable statistical tools and pattern recognition techniques to provide more insight into the factors which determine selectivity.

## 2 Transduction Modes

The role of the sensing agent in a chemical sensor is to provide a transduction mechanism which enables an analytical signal to be

obtained. The vast majority of calixarenes investigated as potential chemical sensors have employed an electrochemical transduction mechanism, either potentiometric or voltammetric–amperometric, although recently there has been a strong movement towards optical transduction. These studies are the subject of this brief review.

## 3 Electrochemical Transduction

There are two principal methods of electrochemical transduction, potentiometric and voltammetric. Both involve the use of electrodes to probe the sample and return an analytical signal. In the potentiometric method spontaneous processes occur at both electrodes in the electrochemical cell leading to the creation of a cell potential which reaches a steady state when the net current flowing in the cell and measurement circuitry is zero, *i.e.* the processes occurring at the electrodes are at equilibrium. In the voltammetric method, in contrast, electrochemical reactions are forced to happen at the working electrode under the influence of an externally poised potential, controlled by a potentiostat.

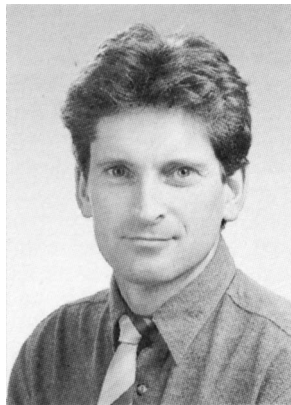
### Potentiometric Sensors: Ion-selective Electrodes

Prior to the development of calixarenes as selective sensing agents for potentiometric sensors, more popularly known now as ion-selective electrodes (ISE), pioneering work by Simon and his coworkers<sup>1</sup> over a period of about 20 years had identified groups of compounds whose complexation characteristics, particularly towards alkali and alkaline earth cations, made them suitable candidates for use as ionophores in ISEs aimed at cation analysis. These compounds included several acyclic and macrocyclic antibiotics, the most prominent being valinomycin for K<sup>+</sup> analysis, a number of crown ethers and a family of acyclic amides.<sup>2</sup>

Simon's studies identified a set of criteria which any ionophore should meet if it is to function as an efficient sensor. Although

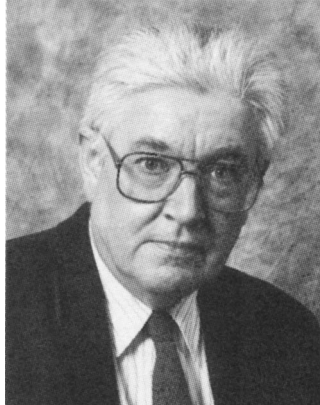
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most modern ISEs utilise polyvinyl chloride (PVC), or some similar support material, to give a 'pseudo-solid' sensing membrane, the signal generation process still involves partitioning the primary ion into a non-polar sensing liquid membrane phase. Hence, the criteria for a successful ionophore can be summarised as follows:<sup>2</sup>

- 1 It should be capable of selective complex formation with the primary or target ion.
- 2 It should be unresponsive to other cations and anions.
- 3 The ionophore must be retained within the membrane phase.
- 4 The complex must be able to diffuse freely in the direction of the potential gradient.
- 5 The stability constant of the complex,  $\beta$ , defined by equation 1, should not be too large, or too small.

$$\beta = \frac{[LM^+_{(m)}]}{[L_{(m)}][M^+_{(aq)}]} \quad (1)$$

- 6 The kinetics of ion transfer between the aqueous and membrane phases, and of complexation with the ligand (equation 2) should be fast and reversible,



where L is the ionophore or ligand,  $M^+$  the cation,  $LM^+$  the complex, (m) denotes the sensor membrane phase, and (aq) the aqueous phase.

Criteria 1 and 2 are vital in determining the selectivity of the sensor, whereas criterion 3 ensures an adequate lifetime. Criterion 4 is necessary to provide a mechanism for charge transfer through the membrane, while criterion 5 is a requirement for ensuring a constant concentration of free ion in the membrane over the measuring range, a prerequisite for Nernstian behaviour. Criterion 6 is necessary to ensure an acceptable response time to fluctuations in the primary ion concentration during continuous monitoring situations, and to ensure that the signal obtained is reversible.

These criteria, in turn, influence the structural and functionality features of ionophores required to make them effective in ISE membranes. These features include the presence of polar ligating groups arranged spatially in such a way that they can interact strongly with selected ions. The alkali and alkaline earth cations have been particularly attractive targets over the past 30 years with the development of sensing technology for blood analysis as the commercial driving force. For cations suitable ligating groups include ethereal and/or carbonyl (in the form of amide, ester, ketone or carboxylic acid) oxygen atoms, arranged so as to define a polar cavity of sufficient rigidity to maximise selectivity and provide a level of ion-dipole attraction and solvation consistent with the stability requirements of the complex formed.

These considerations notwithstanding, in order to comply with criterion 3 the ionophores, and the resulting positively charged complexes, will have to be retained in the non-polar membrane phase of the sensor. This may be achieved by adding large non-polar groups around the ligating binding sites so as to shield the effect of the polar groups and the charged complex from the non-membrane environment. However, the molecule should not become too bulky or diffusion of the complex through the membrane will be hampered (criterion 4). The processes occurring in our idealised ISE membrane for cation detection are summarised in Figure 1. The aqueous analyte ions  $[M^+_{(aq)}]$  in the sample phase are in equilibrium with the electrode membrane phase under the control of the ion-ionophore complexation reaction, generating a boundary potential  $E''_m$ . Likewise, the same ions present in the internal electrolyte generate an internal boundary potential  $E'_m$ . A diffusion potential ( $E_d$ ) also occurs across the membrane, and complexes ( $LM^+$ ) are able to diffuse in the direction of the concentration gradient until an equilibrium is established. Of these three potentials,  $E'_m$  is fixed and  $E_d$  is normally constant or zero. Hence, any changes in the overall membrane potential arise solely from fluctuations in  $E''_m$ , which is related to the activity of the analyte ions *via* the well-known Nernst equation.

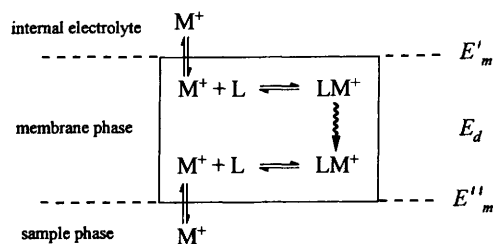
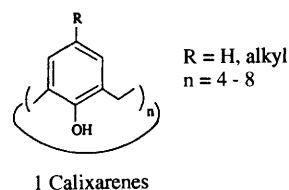


Figure 1 Processes occurring in an idealised ion-selective electrode membrane

### Synthetic Ionophores for ISEs Based on Calixarenes

Simon and his coworkers<sup>1</sup> led the way in the design of synthetic receptors, primarily based on amides, for metal ions suitable for incorporation into ISEs. Later, other workers looked for alternatives among the various groups of neutral ligating families such as crowns and cryptands which were being developed during the 1970s and 1980s.<sup>3</sup> Given this activity, it was inevitable that calixarenes would eventually be examined as their structural features met many of the requirements outlined above. The first publication in the area appeared in the 1986<sup>4</sup> and in the intervening years there have been numerous papers highlighting this aspect of the use of calixarenes, not just in ISE devices but in analytical science as a whole. Soon after the discovery in 1985 that several calixarene ester derivatives possessed ionophoric activity towards metallic cations, notably the alkali metals, projects were initiated by Svehla and McKervey to incorporate these esters into sensing devices, mainly ISEs. It is appropriate, however, before reviewing these investigations, to summarise briefly those features of calixarenes that make them attractive as potential sensing components in analytical devices.

The calixarenes<sup>5</sup> are a family of oligophenols linked in macrocyclic arrays by methylene bridges. They are formed by base-promoted condensation of *para*-alkylphenols with formaldehyde and are available on a multi-gram scale from 'one-pot' procedures. The most accessible calixarenes are tetramers, hexamers and octamers, **1**,  $n = 4, 6$  and  $8$ , respectively. Pentamers are rather less accessible, though preliminary indications of useful ionophoric activity of some pentamer derivatives are beginning to emerge. One of the most attractive features of calixarenes is the ease of chemical modification, making possible changes in ion-complexing selectivity, simply by switching from one ligating functional group to another.<sup>5</sup> The name 'calixarene' was originally coined by Gutsche to evoke the potential of these molecules to function as molecular cups or baskets for guest molecules or ions.<sup>5a</sup>



Calixarenes possess an upper rim, defined by the *para*-substituents of the phenolic rings, and a lower rim defined by the phenolic hydroxy groups. Between the two lies a hydrophobic cavity whose boundaries are the inside  $\pi$ -surfaces of the constituent aromatic rings. By appropriate substitution at the hydroxy groups it is possible to create a second actual or potential cavity on the lower rim. Similarly, by attaching additional substituents to the *para*-positions a further cavity can be constructed on the upper rim. However, this description, while helpful in visualising the receptor potential of these molecules, disguises the fact that all the parent calixarenes, *i.e.* those with free hydroxy groups, are conformationally mobile at ordinary temperatures in solution. By a series of ring flips about the  $CH_2$ -Ar- $CH_2$  bonds the phenolic rings have the freedom to rotate through the annulus of the macrocyclic making numerous conformations accessible.<sup>5a</sup> At sufficiently low temperatures, however, ring mobility can be

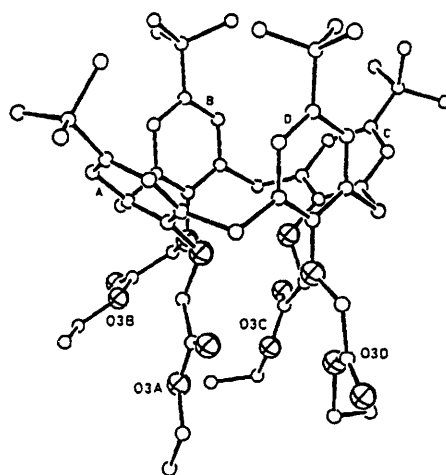
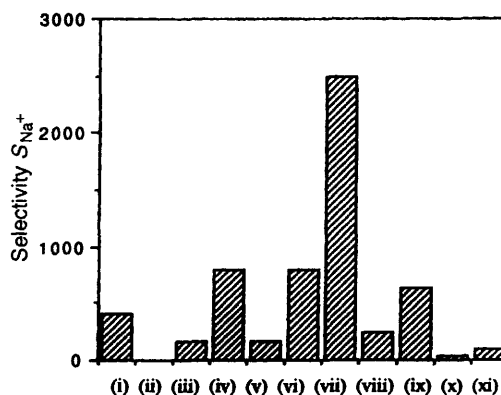
**Table 1** Calixarene derivatives used in sensors

Compound No.	R	R'	n
2	Bu <sup>t</sup>	CH <sub>2</sub> CO <sub>2</sub> Me	4
3	Bu <sup>t</sup>	CH <sub>2</sub> CO <sub>2</sub> Et	4
4	Bu <sup>t</sup>	CH <sub>2</sub> CO <sub>2</sub> C <sub>12</sub> H <sub>25</sub>	4
5	Bu <sup>t</sup>	CH <sub>2</sub> CO <sub>2</sub> Ph	4
6	Bu <sup>t</sup>	CH <sub>2</sub> COMe	4
7	Bu <sup>t</sup>	CH <sub>2</sub> COAd	4
8	Bu <sup>t</sup>	CH <sub>2</sub> COBu <sup>t</sup>	4
9	Bu <sup>t</sup>	CH <sub>2</sub> COPh	4
11	Bu <sup>t</sup>	CH <sub>2</sub> CO <sub>2</sub> Et	6
12	H	CH <sub>2</sub> CO <sub>2</sub> Et	6
13	Bu <sup>t</sup>	CH <sub>2</sub> CO <sub>2</sub> CH <sub>2</sub> N[(CH <sub>2</sub> ) <sub>3</sub> C=O	4
14	Bu <sup>t</sup>	CH <sub>2</sub> C(S)NEt <sub>2</sub>	4
15	Bu <sup>t</sup>	CH <sub>2</sub> CO <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> SMe	4
16	Bu <sup>t</sup>	CH <sub>2</sub> CH <sub>2</sub> SMe	4
17	Bu <sup>t</sup>	CH <sub>2</sub> CH <sub>2</sub> C(S)NMe <sub>2</sub>	4

suppressed to the point where NMR spectroscopy reveals the presence of distinct conformations.

Notwithstanding the mobility of the parent calixarenes, it is possible by appropriate substitution at the hydroxy groups to produce conformationally fixed derivatives. Two such compounds are the tetramethyl calix[4]arene ester **2** and its ethyl analogue **3**<sup>6</sup> (see Table 1). Originally these compounds were synthesised in stable cone conformations. The intention in preparing esters of type **2** and **3** was to explore the possibility of using calix[4]arenes as semi-rigid platforms or substructures on which to assemble convergent or potentially convergent ligating functional groups, in this case ester carbonyls, so as to form flexible hydrophilic cavities suitable for encapsulating guest cations. The crystal structure of **3** (Figure 2) does indeed reveal such a cavity on the lower rim with an appreciable degree of preorganisation of the ester podands.<sup>6</sup>

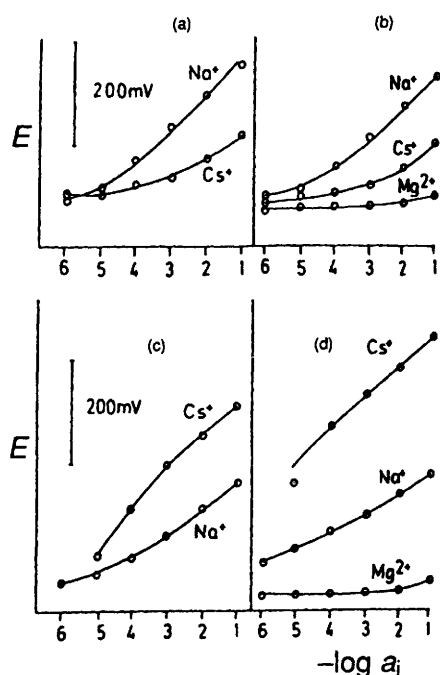
Physicochemical measurements<sup>6</sup> quickly confirmed that esters **2** and **3**, and later many other ester derivatives, do indeed possess significant affinity for alkali metal salts in biphasic extraction from water into dichloromethane, complexation in single solvents, and transport through liquid membranes. The most significant conclusions from these early studies with **2** and **3** were: (a) the medium complexing power towards Na<sup>+</sup>, and (b) the preferences for this cation over the other alkali cations.<sup>6</sup> In complexation in methanol the stability constant,  $\beta(\text{Na}^+)$ , has a value  $\log \beta = 5.18$  and  $S_{\text{Na}^+}$ , the selectivity over K<sup>+</sup>, expressed as the ratio  $\beta(\text{Na}^+)/\beta(\text{K}^+)$ , approximately 400. This selectivity compares very favourably with that of cryptand 221 ( $S_{\text{Na}^+} = 1.2$ ), the member of that series best adapted for Na<sup>+</sup>. Tetraester **3** was also more selective than the naturally occurring ionophore monensin ( $S_{\text{Na}^+} = 2-6$ , depending on the literature source). These studies later revealed that Na<sup>+</sup> selectivity could be modulated quite dramatically simply by changing the alkyl residue in the ester groups of **3**. This is shown graphically for a series of eleven esters in Figure 3 where  $S_{\text{Na}^+}$  reaches a maximum of 2500 for the phenacyl derivative.<sup>7</sup> The combination of high selectivity for Na<sup>+</sup> and medium complexing power proved to be important features of the ionophore profiles of these calixarene esters apropos of their potential as selective sensing agents for this cation, since the stability constant for the **3** (Na<sup>+</sup>) complex lies in the optimum range, *i.e.*  $\log \beta = ca. 5$  in methanol, expressed in equation 1. The optimum range was quantified by the work of Lehn and Kirch<sup>8</sup> who established the existence of a correlation between the value of  $\beta$  and the transport rate and found that maximum transport rate of alkali picrates by cryptands occurred when  $\log \beta$  (methanol) was about 5 units. Thus, it is perhaps not surprising that the efficient natural K<sup>+</sup> carrier valinomycin shows just about these same properties.

**Figure 2** X-Ray structure of tetraester **3** showing the cone conformation and the disposition of the ester podands about the hydrophilic cavity.<sup>6</sup>**Figure 3** Variation of selectivity Na<sup>+</sup>/K<sup>+</sup> within the tetraester series calix-[OCH<sub>2</sub>CO<sub>2</sub>R]<sub>4</sub>,<sup>7</sup> R = (i) Et, (ii) Bu<sup>t</sup>, (iii) Me, (iv) Bu<sup>n</sup>, (v) Bn, (vi) Ph, (vii) CH<sub>2</sub>COPh, (viii) [CH<sub>2</sub>]<sub>2</sub>OMe, (ix) [CH<sub>2</sub>]<sub>2</sub>SMe, (x) CH<sub>2</sub>CF<sub>3</sub>, (xi) CH<sub>2</sub>C≡CH.

Initial screening experiments carried out with liquid membrane sensors confirmed that excellent sensors for sodium could be produced with esters **2** and **3** (Figures 4a and 4b respectively). These results were published in 1986 as part of the proceedings of an international conference held in Dublin.<sup>4</sup> They represent the first use of calixarenes as sensing agents. A second publication in 1987 by Diamond and Svehla<sup>9</sup> again highlighted the excellent selectivity of ester **2** for Na<sup>+</sup> against K<sup>+</sup> and a range of other interferents which can affect the estimation of Na<sup>+</sup> in blood, the most important commercial application for Na<sup>+</sup> measurements. Detailed studies on the properties of PVC membrane ISEs based on esters **2** and **3** confirmed their usefulness as Na<sup>+</sup> sensors.<sup>9</sup> The related calix[4]arene derivatives, the dodecyl and phenyl esters **4** and **5**, show comparable behaviour.<sup>10</sup> Studies recently completed in which a range of twelve or more tetraesters were screened in PVC membrane electrodes indicate that the 2-methoxyethyl ester analogue of **2** produces the most selective Na<sup>+</sup> electrode.<sup>11</sup>

An obvious application of these sodium sensors is in the clinical analysis of sodium in body fluids. Although sodium is present in blood at elevated levels (typically 120–150 mmol l<sup>-1</sup>), the range over which the sodium concentration extends is relatively limited, compared to potassium (1–4 mmol l<sup>-1</sup>). It follows that the signal obtained will have a limited range of a few mV over which the entire normal sodium distribution will occur. Hence, careful experimental design and attention to sampling and signal processing is required in order to obtain acceptable accuracy and precision in the analytical results.

Initial studies on the performance of mini-PVC membrane



**Figure 4** Response of liquid membrane electrodes based on (a) 2, (b) 3, (c) 11 and (d) 12. These were the first results demonstrating the selectivity of ISEs based on calixarene esters. The tetramers 2 and 3 are clearly Na<sup>+</sup>-selective while the hexamers 11 and 12 are Cs<sup>+</sup>-selective.<sup>9,24</sup>

electrodes for blood analysis were encouraging. In this study, 44 plasma samples were analysed for sodium with the PVC electrodes based on tetramethyl ester 2, and the results compared with those obtained with a SMAC-Technicon Analyser. Good correlation was found ( $r = 0.95$ ), but a systematic bias was apparent due to the calibration regime used in the study. A more detailed report of these investigations published the following year confirmed the utility of applying the sensors based on 2 to the analysis of sodium in blood.<sup>12</sup>

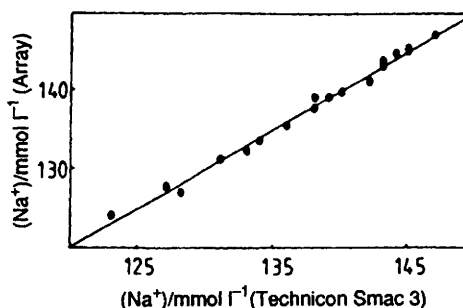
In parallel with these studies, other ligands were assessed for use in sodium-selective electrodes, including the *p*-tert-butylcalix[4]arene alkyl ketones 6–8. However, only the methyl ketone derivative 6 produced satisfactory PVC membrane electrodes.<sup>11</sup> These were subsequently applied to the analysis of sodium in plasma samples.<sup>13</sup> Excellent correlations ( $r = 0.979, 0.987$  and  $0.951, n = 10$ ) were found in comparative tests with three reference instruments (Hitachi 704 Analyser, Flame Photometer, SMAC Technicon Analyser, respectively). However, as before, a bias in the results was apparent in each case. Interestingly, in a paper by Kimura and coworkers, PVC electrodes based on 4 were also applied to the determination of blood sodium. Although only five samples were processed, a positive bias of around 2–3 mmol l<sup>-1</sup> was evident in all but one sample.<sup>14</sup>

Obviously, when trying to establish a new analytical device in the face of existing technology, any bias in the results is unacceptable. Bias in analytical determinations commonly arises from systematic errors in calibration. In the above investigations, drift during the calibration and analytical measurements was problematic. Its effect was further magnified by the very restricted range found in blood sodium samples, which leads to a narrow voltage range over which the measurements must be made, and, perhaps more importantly, significant 'bunching' of the concentration distribution in the samples. Hence most concentrations focused in a very narrow range (135–140 mmol l<sup>-1</sup>) with a few outliers on either side which extend the range to perhaps 120–150 mmol l<sup>-1</sup> sodium. These outliers have a significant influence on the slope of the regression line, and must therefore be determined with particular care.

One way to reduce the effect of drift and give very reproducible sample handling is to use flow-injection analysis (FIA). PVC membranes incorporating the methyl ketone 6 and methyl ester 2

derivatives were assessed as detectors in an FIA system for blood sodium analysis and the results demonstrated that the bias described above could be greatly reduced while still maintaining excellent correlation.<sup>15</sup>

However, the best results were obtained when the tetramethyl ester 2 was used as an element in an ISE array both in conventional dip-type measurements and in a flow-injection analysis system.<sup>16</sup> Using sophisticated calibration and sensor modelling techniques, these papers rigorously demonstrated that 2 could be applied to blood sodium analysis with excellent results (Figure 5). Furthermore, the same ISE was shown to be suitable for the analysis of sodium in mineral water samples.



**Figure 5** Plasma sodium analysis results obtained with a PVC membrane electrode based on tetraester compound 2 with the results obtained with a SMAC analyser.

More recently, sodium-selective PVC membrane electrodes incorporating the ester 2 have been assessed using batch injection analysis (BIA). This technique differs from FIA in that the sample is injected directly onto the sensor surface, and a dilution/mixing effect sweeps the sample quickly away, resulting in high-speed transient signals which can be used for analytical measurements. Initially, a single sodium electrode was investigated and shown to have excellent characteristics for this technique. Subsequently, the electrode was used in a 3 × ISE array (Na, K, Ca) and successfully applied to the analysis of these ions in mineral water samples.<sup>17</sup> In the array study, the excellent selectivity of the calixarene-PVC membrane was apparent in carryover studies performed during the evaluation of the array, as virtually no response to the interfering ions was indicated.

From the above, it is clear that calixarene tetraesters and related derivatives can form the basis of excellent sodium ISEs. Studies on device lifetime showed that the sensors can be expected to be used for months at a time<sup>18</sup> and are able to analyse several thousand blood samples before the signal becomes unacceptably affected by membrane coating or leaching of membrane components. Significantly higher sodium selectivity has been claimed with calix[4]arene ionophores other than esters and ketones. Yamamoto and Shinkai combined a crown ether with the lower rim of a calix[4]arene dialkyl ether to produce an electrode showing a Na<sup>+</sup> selectivity of 10<sup>5</sup> (relative to K<sup>+</sup>).<sup>19</sup> Membranes containing this ionophore have recently been assessed as the detector in a flow-analysis system and successfully applied to the determination of sodium in blood samples.

### Solid-state Sodium-selective Sensors

In addition to the traditional ISE configuration discussed above, researchers are interested in solid-state designs of these sensors, such as ISFETs (ion-selective field-effect transistors) or coated wire electrodes (CWEs), as these are expected to be easier to mass produce and will be more compatible with the planar fabrication technologies used in the semiconductor and related industries. It is not surprising, therefore, that studies on the performance of ISFETs incorporating calix[4]arene derivatives have recently appeared in the literature. One paper<sup>20</sup> describes the characteristics of ISFETs based on the ketones 8 and 9 (Table 1). These gave Nernstian slopes and good selectivity against other group 1 and group 2 cations. A well known problem with these devices is the lack of a well-defined

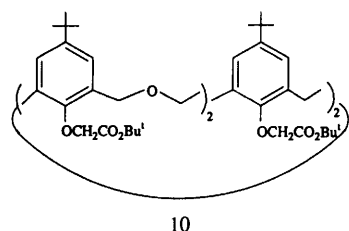
internal boundary potential (*i.e.*  $E'_m$  in Figure 1) due to the absence of an internal filling solution or compensating mechanism by which charge can be exchanged across the internal boundary. The same problem occurs with CWEs, which differ from ISEs in that the sensing membrane is deposited directly onto a metallic conductor. This leads to a blocked internal interface between the membrane and the metal, as the former conducts only by means of ion movement, while the latter is an electronic conductor. Hence CWEs, while simpler in make up than equivalent ISEs, are generally much less stable, and exhibit greatly reduced effective lifetimes. The design proposed by Brunink *et al.*<sup>20</sup> involved using a poly(2-hydroxyethyl methacrylate) (polyHEMA) hydrogel layer to help anchor the PVC membrane on the gate region of the device and simultaneously reduce the effect of interferents such as  $\text{CO}_2$  which can diffuse through the PVC layer and affect the internal boundary potential. An alternative proposed by Tsujimura *et al.*<sup>21</sup> was to use calix[4]arenes bearing oligosiloxane moieties in the esters in silicone rubber membrane ISFETs. These groups promoted the dispersibility of the ligands within the rubber membrane leading to more stable responses compared to similar devices based on the ethyl ester tetramer **3**. However, no data on the performance of the device in real samples such as plasma are given.

One strategy which might overcome this limitation is to substitute a conductor of mixed character which is capable of transferring charge by means of either ion or electron movement. With this in mind, PVC membranes incorporating ligand **3** have been deposited on polypyrrole which was electrochemically formed on platinum substrates.<sup>22</sup> The resulting sodium-selective solid-state sensors were shown to be much more stable than CWE equivalents, and were unaffected by the presence or absence of redox-active species in the sample solution which react on polypyrrole surfaces. Impedance studies confirmed a dramatic reduction in the charge transfer resistance through the device compared to CWE devices which had no polypyrrole layer between the Pt layer and the PVC.

### Potentiometric Sensors for Other Ions

One of the main reasons for the great interest in calixarenes as synthetic ionophores is the scope for structural modification and elaboration, not just of the calix itself, but of the pendant binding sites. Since the complexation selectivity rests largely on a best-match relationship between receptor, substrate and solvent, which ideally should maximise the complexation free energy of the primary ion compared with that of interfering ions, the ability to vary the cavity size offers the prospect of developing ligands suitable for use in sensors for ions other than sodium.

A calixarene with a cavity intermediate in size between that of a tetramer and a hexamer has been used in an electrode with  $\text{K}^+$  selectivity.<sup>23</sup> Although this ionophore **10** is a tetraester, the dioxa-



calix[4]arene substructure in which two of the four bridging methylene units are expanded by additional oxygen atoms, has a cavity size larger than that of a normal tetramer. The resulting electrode has good sensitivity, though over a narrow working range with a somewhat limited selectivity. The selectivity is inferior to that of the well known  $\text{K}^+$  electrode based on valinomycin.

Calix[6]arene derivatives show selectivity towards the larger alkali cations in complexation and extraction and this is reflected in their suitability as ionophores for caesium ion in ISEs. Four PVC membrane electrodes based on hexaesters **11** and **12** have been found to be caesium-selective against a wide range of possible

interfering ions<sup>24</sup> (see Figures 4c and 4d). X-Ray diffraction studies confirm that these ligands are more open and define much larger cavities than sodium-selective tetramers. Initial studies with **12** have indicated that nitrophenyl/octyl ether is the most effective plasticizer to employ for long-life electrodes.

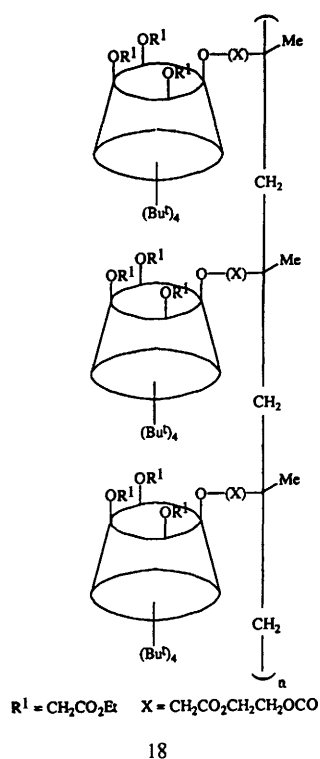
The use of calixarenes with soft donor atoms as binding sites in ISEs for heavy metal cations provides another illustration of their versatility in sensing devices. This was first demonstrated by O'Connor *et al.*<sup>25</sup> who quantified the performance of calix[4]arene derivatives **13**, **14** and **15** with sulfur and nitrogen groups in ISEs sensitive to silver(I), copper(II) and lead(II), these ionophores having previously been found to be efficient extractants for heavy metals from aqueous solution into dichloromethane. With the appropriate number and disposition of soft donor atoms selectivity for heavy metal ions over alkali cations can be realised. Calixarenes **13** and **14** show sensitivity for silver(I) but still have some response to alkali cations. Of the three, the thioalkyl ester **15** displayed the best performance in selectivity against sodium with  $\log K_{\text{Ag Na}}^{\text{Pot}} = -1.16$ . A glassy carbon electrode coated with PVC containing **15** was subsequently used to follow potentiometric titrations of mixtures of  $\text{I}^-$ ,  $\text{Br}^-$ , and  $\text{Cl}^-$ . Later similar studies by Malinowska *et al.*<sup>26</sup> using sulfur-functionalised calix[4]arenes including thioamide **14** confirmed these results with heavy metal ions. However, mercury(II) interference is a problem with these thio-calixarene-based ISEs.

Cobben *et al.*<sup>27</sup> have described calixarene-based ISFETs targeted at silver(I), copper(II), cadmium(II) and lead(II) using 29 calixarene derivatives including those described above. ISFETs employing thioethers **16** and **17** exhibited excellent silver(I) sensitivity and good selectivity, though no mention is made of selectivity over sodium ions. Ligand **17** resulted in devices more selective for copper(II), although limited data are given, and significantly more for the selectivity against silver(II) or mercury(II), both of which would be probable interferents for this type of membrane sensor. Preliminary data are also reported for cadmium(II) and lead(II)-selective ISFETs. The behaviour of the latter is curious, showing a slope of nearly 60 mV/decade change in  $[\text{Pb}^{II}]$  (twice the theoretical value) and the calibration curve shows a response linearly decreasing below  $10^{-6} \text{ mol l}^{-1}$ , in contrast to that observed with most conventional PVC membrane ISEs (and other ISFETs reported by Cobbin). Unfortunately, no data regarding the lifetimes of the devices are given.

### Voltammetric Sensors for Ions

Chemically modified electrodes (CMEs) can be made by immobilisation of organic molecules at the electrode surface. Although CMEs containing calixarenes are much less well developed than their ISE counterparts, they do offer benefits in electroanalysis because the analytical reagent (the sensor) is confined to the electrode surface where a reaction of chemical interest occurs. Among the properties of CMEs that render them attractive is the ability to accumulate trace analytes from solution into the modifying layer, resulting in an increased analyte concentration at the electrode surface. This accumulation process is similar to the electrolytic accumulation employed in anodic and cathodic stripping voltammetry, except that in a CME accumulation is achieved by the immobilised receptor, with a selectivity for the target analyte, at the electrode surface.

Arrigan *et al.*<sup>28</sup> have examined the use of the polymeric calixarene ester **18** as a modifier of CMEs for voltammetric analysis of lead(II), copper(II) and mercury(II) ions in dilute aqueous solution. In this study, the calixarene was incorporated into a carbon paste, prepared from carbon powder and a suitable binding agent such as Nujol, and then packed into the electrode body. The electrode was left on open circuit in the presence of an aqueous solution of the analyte for various times to allow encapsulation of the metal ions at the electrode surface (accumulation cycle). The collected ions were then stripped off reductively and determined by anodic differential pulse voltammetry. The overall analytical cycle consisted of encapsulation of the ion by the calixarene, electrochemical



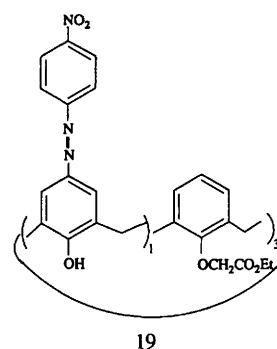
reduction, and anodic stripping measurement. Typically, peak currents for metal anodic stripping increased with accumulation time up to about 5 minutes, after which a plateau region indicated either the attainment of equilibrium or saturation of the calixarene binding sites. This CME showed good selectivity for lead in particular, with LODs of 0.2, 1.0 and 5.0 mmol l<sup>-1</sup> for lead(II), copper(II) and mercury(II), respectively. However, a predictable limitation of the electrode, which stems from the use of a calix[4]arene tetraester as the sensing agent, was interference from alkali cations, notably Na<sup>+</sup> (*vide supra*). The presence of Na<sup>+</sup> or K<sup>+</sup> caused competition for the binding sites, thus reducing the lead(II) signal. Clearly, for optimum behaviour the calixarene binding sites need to be matched with target analyte ions. Preliminary attempts to achieve better selectivity matching, through the use of softer binding sites of thioamide **14** in a CME for Ag<sup>+</sup>, were not successful. The presence of **14** did not significantly enhance Ag<sup>+</sup> ion uptake. The use of calixarenes as electrode-surface coatings in amperometric detectors has been described by Wang *et al.*<sup>29</sup> who found enhanced selectivity towards neurotransmitters such as dopamine and epinephrine while excluding common electroactive interferences such as ascorbic acid, uric acid and amphetamines.<sup>30</sup>

#### 4 Optical Transduction

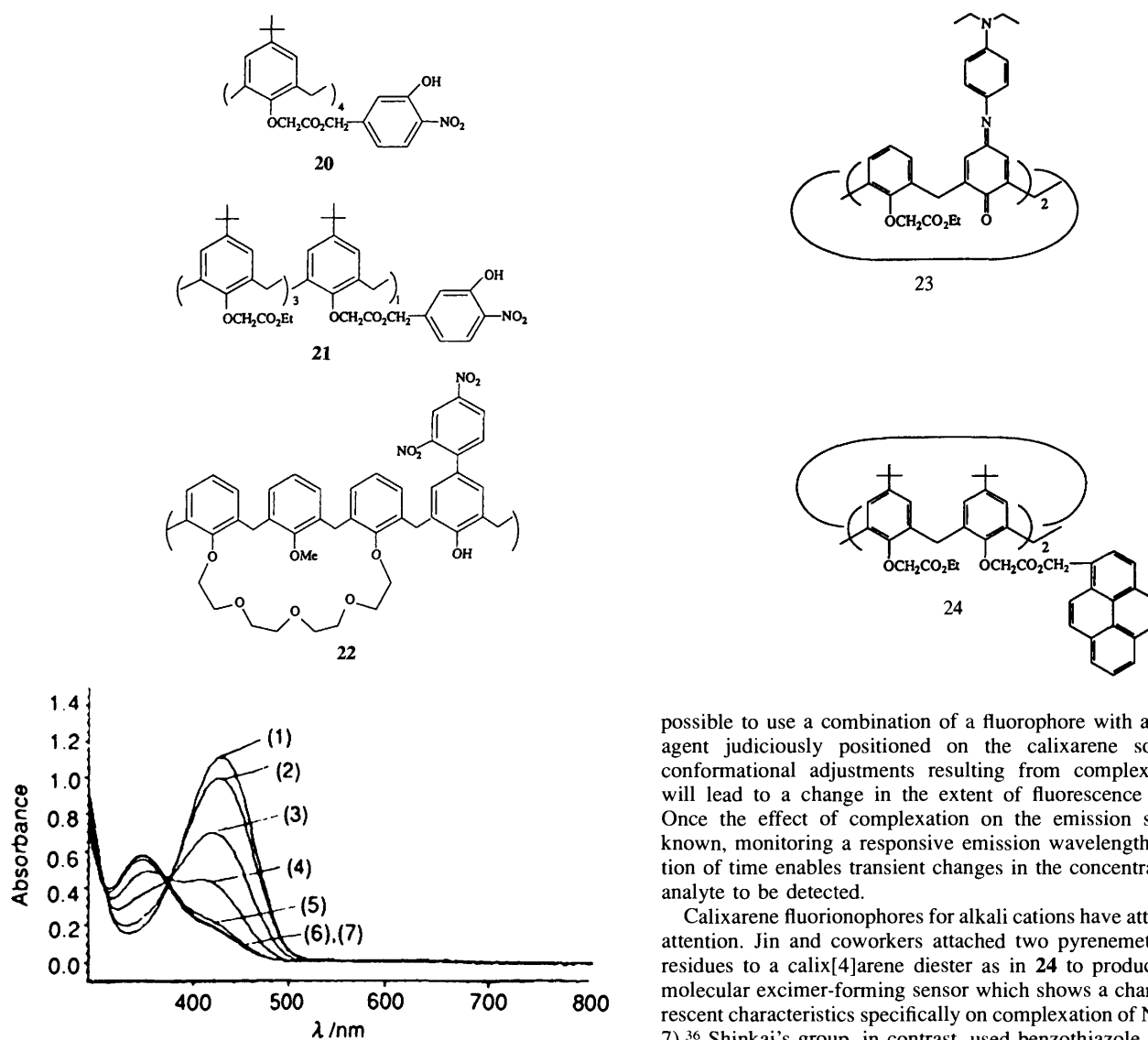
An important trend in sensor research over the past several years has been the development of optical methods of transduction for detection and estimation of clinically important species. The objective here is to transduce a chemical signal, *e.g.* resulting from complexation of a cation, into an optical response, *e.g.* a colour change, ideally in a completely reversible and reproducible way. Optical based sensing is attractive for several reasons which include inherent safety, less noise pickup in signal transmission over long distances, and the possibility of obtaining much more comprehensive information from a single probe (full spectrum *vs.* one channel of electrochemical information). While there have been several recent publications describing calixarene derivatives capable of signalling the presence of metal ions optically, there are as yet no functioning optodes or optical equivalents of the electrochemical sensors described above. Nevertheless, there are a number of chromogenic and fluorogenic calixarene-based receptors which show promise for metal ion detection.

These systems contain a chromophore/fluorophore which can be

either appended to the calixarene at the lower or upper rim or else form an integral part of the molecular substructure. On complexation, the environment of the light-responsive probe may be sufficiently perturbed so as to produce a significant change in the UV-VIS absorption spectrum or the fluorescence emission spectrum. For the former, this can be achieved conveniently by using a calixarene with a pH-dependent chromophore, *e.g.* a phenol, in the presence of a base which alone is insufficiently strong to deprotonate the phenol in the uncomplexed receptor. Complexation of a cation may trigger the release of a proton to the base which in turn is revealed in the bathochromic shift accompanying phenoxide formation. Such spectral shifts are easily monitored and, through judicious choice of substituent on the phenol, will register as a colour change. In practice, however, there are additional considerations which may influence the ability of the system to function as a selective chromoionophore. It is necessary, for example, to establish that the very presence of the probe, possibly in close proximity to the binding sites of the calixarene, does not adversely affect the binding power of the receptor, its ion selectivity, or the stability of the resulting deprotonated complex. A variety of chromophores have been investigated including nitrophenol and azophenol derivatives and in most cases selective transduction of cation complexation has been observed, although the selectivity is somewhat inferior to that realised with equivalent electrochemical devices.



Shinkai and his coworkers<sup>31</sup> have synthesised calix[4]arene **19** with a 4-(4-nitrophenyl) azophenol unit and three ethyl ester residues on the lower rim and found that on cation complexation in the presence of triethylamine the compound exhibits a new absorption maximum at 600 nm which is lithium-selective. Triethylamine alone does not cause any spectral changes, confirming that deprotonation and complexation are integral events in the chromogenic response. Other nitrophenol-based chromogenic calixarenes which show a selective colour response on complexation with a cation in the presence of base include compounds **20**, **21** and **22**. The former two are tetraesters with one and four nitrophenol residues, respectively, incorporated into podands on the lower rim. The chromogenic response of both derivatives in tetrahydrofuran (THF) in the presence of morpholine reveals a Li<sup>+</sup> selectivity with a 10–40-fold response over Na<sup>+</sup> (Figure 6).<sup>32</sup> Compound **22**, designed by Sutherland's group,<sup>33</sup> consists of a calix[4]arene with a triethyleneoxy bridge across two distal phenolic functions to provide binding sites for alkali cations. The chromogenic response is provided by one free phenolic unit with a dinitrophenyl substituent at the *para*-position with the remaining phenolic ring present as its methyl ether. Compound **22** in chloroform extracts K<sup>+</sup> in preference to Na<sup>+</sup> in aqueous solution in the pH range 7–9 with a selectivity of *ca.* 1000 in extraction coefficient and a shift in the absorption spectrum from 437 to 628 nm. Extraction of Mg<sup>2+</sup> or Ca<sup>2+</sup> under these conditions was not observed. Preliminary experiments suggest that **22** may be suitable for use in optical fibre sensors for measuring K<sup>+</sup> concentrations in biological fluids without any significant loss of K<sup>+</sup>/Na<sup>+</sup> selectivity. Kubo and his coworkers<sup>34</sup> have designed a chromogenic Ca<sup>2+</sup> sensor, again with the objective of developing an optical fibre for clinical analysis, by incorporating an indoaniline chromophore into a calix[4]arene ester as in **23**. The optical properties of the



**Figure 6** Typical shifts in UV-VIS absorbance accompanying complexation of a metal cation (in this case  $\text{Li}^+$ ) by calixarene ionophore **20** in the presence of a base. The results show shifts brought about by addition of  $\text{LiClO}_4$  to a  $5 \times 10^{-5} \text{ mol l}^{-1}$  solution of **20** in THF containing  $20 \text{ mm}^3$  morpholine to give final  $\text{Li}^+$  concentrations ( $\text{mol l}^{-1}$ ) of (1) 0.1, (2)  $10^{-2}$ , (3)  $4 \times 10^{-3}$ , (4)  $8 \times 10^{-4}$ , (5)  $2 \times 10^{-5}$ . Curves (6) and (7) illustrate absence of either  $\text{Li}^+$  salt or base.<sup>32</sup>

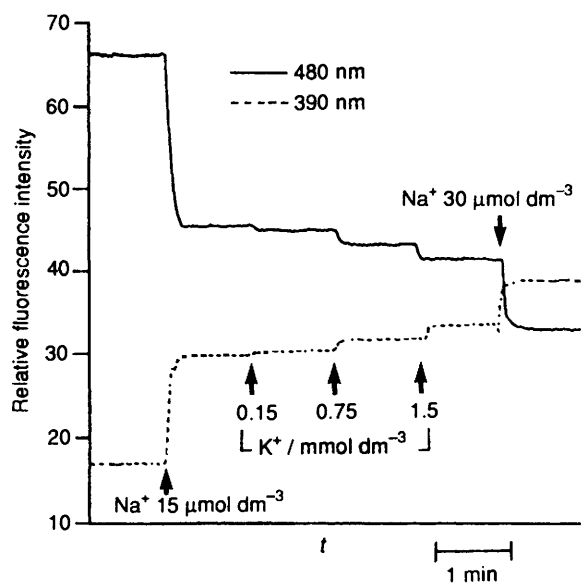
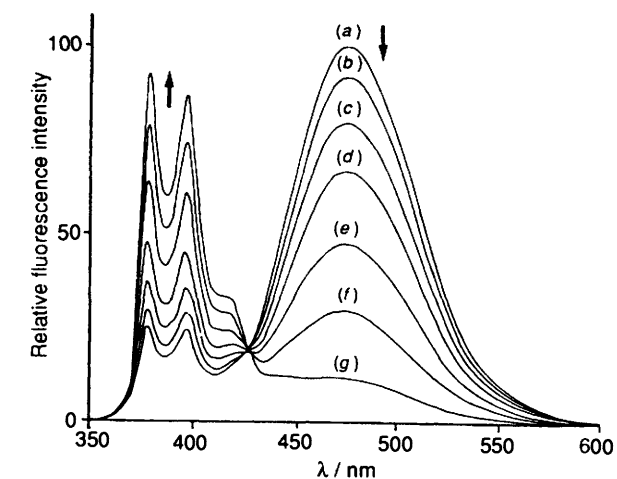
indoaniline probe are easily perturbed by chemical stimuli other than pH change, in this case by interaction of the quinone carbonyl group with divalent cations. Compound **23** is blue; addition of calcium thiocyanate in ethanol causes a large bathochromic shift (*ca.* 100 nm) with a large increase in absorption intensity. Addition of  $\text{NaSCN}$ ,  $\text{KSCN}$  or  $\text{Mg}(\text{ClO}_4)_2$  causes only minor changes in the absorption spectrum of **23** suggesting a significant selectivity for  $\text{Ca}^{2+}$ . IR absorption changes for the C=O (ester) and C=O (quinone) groups in the presence of  $\text{Ca}^{2+}$  suggest that **23** forms an encapsulated complex on the lower rim of the calixarene. The use of indoaniline-derived sensors has been extended to calix[6]arenes to produce a chromoionophore with a selective  $\text{UO}_2^{2+}$  ion-induced pronounced colour change in ethanol. An alternative approach that has been applied successfully to a chromogenic sensor for  $\text{Na}^+$  is to couple a calix[4]arene tetraester ionophore with a Simon-type lipophilic pH-sensitive dye such as ETH 5294.<sup>35</sup>

Selective optical transduction is also possible through the use of fluorescent calixarene receptors. The conformational changes which invariably accompany complex formation can be exploited to advantage to perturb a suitable fluorophore attached to either the upper or lower rim of the calixarene. Alternatively, it may be

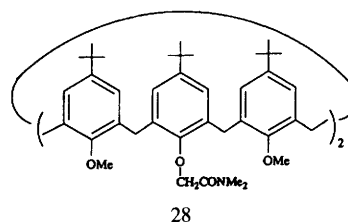
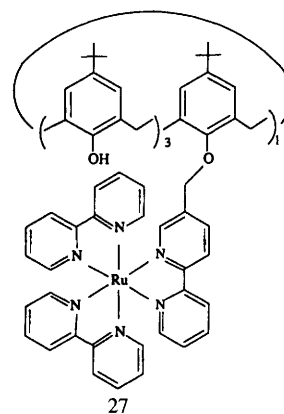
possible to use a combination of a fluorophore with a quenching agent judiciously positioned on the calixarene so that any conformational adjustments resulting from complex formation will lead to a change in the extent of fluorescence quenching. Once the effect of complexation on the emission spectrum is known, monitoring a responsive emission wavelength as a function of time enables transient changes in the concentration of the analyte to be detected.

Calixarene fluorionophores for alkali cations have attracted most attention. Jin and coworkers attached two pyrenemethyl acetate residues to a calix[4]arene diester as in **24** to produce an intramolecular excimer-forming sensor which shows a change in fluorescent characteristics specifically on complexation of  $\text{Na}^+$  (Figure 7).<sup>36</sup> Shinkai's group, in contrast, used benzothiazole as the fluorophore to construct the calix[4]arene sensor **25** which has been described as having 'perfect'  $\text{Li}^+$  selectivity.<sup>37</sup>  $\text{Na}^+$  selectivity has also been observed with calix[4]arene amides and esters containing anthracene residues, *e.g.* **26**, on the lower rim.<sup>38</sup> An  $\text{Na}^+$  sensory system has been devised in which a calix[4]arene carries a pyrene unit (the fluorophore) and a nitrobenzene unit (the fluorescent quencher) on the periphery of the molecular cavity.<sup>39</sup> The conformational changes which accompany complexation of the cation are such that the pyrene and nitrobenzene rings are moved further apart resulting in a dramatic enhancement of fluorescence intensity.

A luminescent pH sensor based on the *p-tert*-butylcalix[4]arene-linked ruthenium(II) trisbipyridyl complex **27** has been devised by Grigg *et al.*<sup>40</sup> The trisbipyridylruthenium(II) moiety was chosen as the luminophore with the three free phenolic units of the calixarene acting as acid-base sites. Formation of the phenolate anion(s) causes photoinduced intramolecular electron transfer to occur from the phenoxide ion to the trisbipyridyl ruthenium(II), thus quenching the luminescence. Once the phenolate ions are protonated, electron transfer is prevented and luminescence is thus restored. The luminescence properties of lanthanide ions have been of much interest because of their potential use as probes and labels for a variety of chemical and biochemical applications. Although working sensors have yet to be constructed, it is known that calix[4]arene amides form strong complexes with  $\text{Eu}^{\text{III}}$ ,  $\text{Tb}^{\text{III}}$  and  $\text{Gd}^{\text{III}}$  ions.<sup>41</sup> The  $\text{Tb}^{\text{III}}$  complex shows a high luminescence quantum yield and a long luminescence lifetime suggesting that it may be useful for time-resolved fluoroimmunoassay.



**Figure 7** (a) Changes in fluorescence emission spectra obtained with fluorescent calixarene derivative **24** on addition of NaSCN. (b) Selectivity of fluorescent response measured at 480 and 390 nm on addition of Na<sup>+</sup> and K<sup>+</sup> ions.<sup>36</sup>



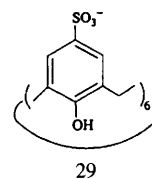
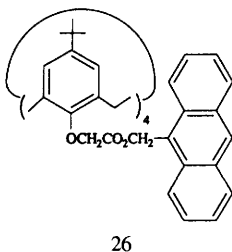
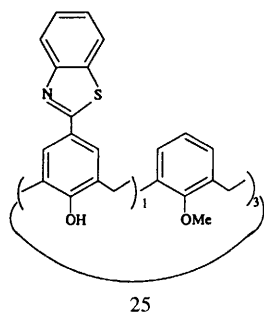
## 5 Sensors for Organic Guests

The design of receptors for use as sensors for organic guest molecules or ions poses new challenges. Unlike many inorganic ions, organic guests are likely to be non-spherical and thus the geometric and stereochemical features essential to effective mutual recognition will need to be accommodated. Furthermore, when neutral analytes are the targets, attractive forces other than purely electrostatic will have to be exploited to ensure adequate binding. Nevertheless, the calixarenes do offer opportunities and some calixarene-based sensors for organic compounds are beginning to appear with cases of potentiometric, voltammetric and optical transduction.

Chan and Odashima and their respective coworkers have independently developed ISEs for organic amines, in protonated form, using calix[6]arene esters **11** and **12**. These derivatives function as ionophores in organic potentiometric sensors.<sup>30</sup> The electrodes are selective for primary amines against secondary or tertiary amines. Best responses with primary amines are observed for compounds not having a chain branch adjacent to the amino group as, for example, in hexylamine, octylamine and dopamine. The electrode response is presumed to result from formation of an inclusion complex with hydrogen bonding between the protonated amino group of the analyte and the carbonyl groups of the calixarene ester. Chan *et al.*<sup>42</sup> have extended this investigation to include the coupling of a lipophilic hexaester with a pH-sensitive chromoionophore developed earlier by Simon (ETH 5294) to produce an amine sensor.

The guanidinium ion is an important analyte target for sensing devices because of its relevance to biological systems. A good receptor for guanidinium should possess multiple hydrogen-bond acceptor sites located in a single plane. A PVC membrane CHEMFET incorporating calixarene receptor **28** capable of multiple hydrogen-bond formation has been constructed by Kremer *et al.*<sup>43</sup> This device displays an excellent response to guanidinium ions and although potassium, sodium and ammonium are the strongest interfering ions, there is good selectivity for the guanidinium ion even in the presence of these ions.

Cyclic voltammetry has been employed by Gokel and his coworkers to study complex formation between the water-soluble sulfonated calix[6]arene derivative **29** and protonated amines and





neutral ferrocene derivatives<sup>30</sup> The voltammetric response is presented in terms of shifts in half-wave potential and current variation, with the protonated analytes exhibiting the larger effects due to electrostatic effects in the binding Although a voltammetric sensor for neutral organics has yet to emerge, on the basis of the binding mechanism likely to operate here, one might anticipate that the binding of suitable electroactive amines to a calixarene host could be detected voltammetrically and form the basis of such a sensor Voltammetric studies with calix[4]arenequinone–hydroquinone systems indicate that these systems may serve as redox-switchable metal-ion binding sensors for a chemical sensor<sup>44</sup> Beer *et al* have described a system based on a calix[4]arene diquinone which is capable of complexation and electrochemical recognition of ammonium and alkylammonium ions<sup>45</sup>

A gas sensor for colorimetric determination of trimethylamine has been devised by McCarrick *et al*<sup>46</sup> using a calix[4]arene bearing four nitrophenylazophenol residues similar to those present in **19** This material when complexed to lithium and immobilised onto filter paper undergoes a dramatic colour change from yellow to red in the presence of gaseous trimethylamine at concentration above 20 ppb within minutes The intensity of the colour change is amine dependent and the device may be applicable to the monitoring of fish freshness More recently, Chawla and Srinivas<sup>47</sup> have described an entire series of doubly bridged calix[4]arenes with azophenol moieties which provide visual detection of amines including ethylenediamine *In situ* formation of an ionic lipophilic hydrazone forms the basis of a calixarene ISE for determination of formaldehyde<sup>48</sup>

## 6 Patents

A search of the patent literature has revealed almost 100 patents filed which include the use of the keyword 'calixarene' These patents cover methods of preparation/synthesis of certain calixarene derivatives, or their use in a wide variety of applications including corrosion inhibitors, fuel additives, hair dyes, charge-controlling agents for developing electrostatic images, additives in epoxy resins and adhesives, developer solution for photographic negatives, extraction of uranium ions, deodorant additive, and stabilisers in rubbers Very recently, the first chemical sensor-related patents have begun to appear, and this trend will probably continue, given the obvious commercial importance of these devices Of the four sensor patents, three relate to electrochemical sensors for alkali metal ions, and one to the use of cation complexing dyes (chromoionophores) in optical sensors

## 7 The Future

Sensing applications of calixarene derivatives are only beginning to develop The potentiometric and other electrochemical sensors for metal ions can be regarded as the first generation of these sensors Calixarenes capable of optically signalling complexation with metal ions, while valuable in their own right, could be the precursors of much more interesting sensing materials (see for example the fluorescence signalling of encapsulation of a flavin, pteridine, by calix[4]arene host capable of changing from a 'closed' to an 'open' form<sup>49</sup>) It is now recognised that calixarenes can be used as building blocks of much more substantial structures which could be used to sense a huge number of potential hosts, ranging from neutral gaseous molecules (*e.g.* toxic solvent vapours) to amino acids or more complex biological molecules Working sensors for anions have also yet to emerge

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