Sensor systems
The dramatic development of sensor technology during the past decade has focused chiefly on the problem of detecting one or more chemicals. In many instances, in both medicine and industry, the problem is to detect small amounts of a chemical in a complex mixture of chemicals. The detection of glucose in blood plasma is an archetypal problem of this kind. Enzyme-based technology has provided elegant solutions to such problems. The high substrate specificity of enzymes has enabled the design and construction of simple, inexpensive instruments which are having a major impact on key, routine analyses in medicine. The specificity of monoclonal antibodies also offers a related solution using the intrinsic properties of natural biosensors.

We are interested in solving problems at the opposite end of the spectrum of sensor technology, namely the problem of an integrated measurement of a complex stimulus consisting of some tens to hundreds of different chemicals. The problem of flavour and odour, both of which are key variables in the production of a consistent quality of comestibles, beverages and perfumes, is an example of the problems we have chosen.

It is reasonable to enquire about the wisdom of tackling such complex problems at this stage of sensor development. After all, in the fullness of time, biosensor methodology can be expected to advance to a stage at which it is appropriate to address the more complex problems. Our justification for tackling flavour assessment and allied areas is that these important problems are solved daily in a variety of industries through the simple expedient of having a sniff. With a single sniff the sense of smell can reach important commercial decisions such as the fitness of a batch of beer, whisky or wine.

Receptor mechanisms in the vertebrate olfactory system
The olfactory epithelium, like the related sensory tissue, the retina, is constant in its essential morphological features in vertebrates. The tissue is located in the upper part of the nasal tract and consists of about equal numbers of two principal types of cell, the primary neurones and the supporting sustentacular cells, lying above undifferentiated basal cells. The secretions from glands, including the Bowman's glands of the olfactory mucosa, cover the top of the tissue with mucus.

The first biochemical studies on the tissue were carried out only two decades ago, but recent work has provided insight into some of the molecular mechanisms. At least some classes of odorants modulate an adenylate cyclase in the membranes of the olfactory neurones [1–3]. The properties of the
Table I

Some typical odorants

<table>
<thead>
<tr>
<th>Odorant</th>
<th>Odor</th>
<th>Threshold (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nerol</td>
<td>Rose</td>
<td>290</td>
</tr>
<tr>
<td>Thymol</td>
<td>Thyme</td>
<td>86</td>
</tr>
<tr>
<td>Limonene</td>
<td>Lemon</td>
<td>10</td>
</tr>
<tr>
<td>Cineole</td>
<td>Eucalyptus</td>
<td>1.3</td>
</tr>
<tr>
<td>β-Damascone</td>
<td>Rose</td>
<td>0.09</td>
</tr>
<tr>
<td>Octadienone</td>
<td>Butter off-flavour</td>
<td>0.01</td>
</tr>
<tr>
<td>Menthenethiol</td>
<td>Grapefruit</td>
<td>$2 \times 10^{-5}$</td>
</tr>
<tr>
<td>Thiamine smell</td>
<td>Off-odour of thiamine</td>
<td>$2 \times 10^{-6}$</td>
</tr>
</tbody>
</table>

The threshold values are given as part per billion in water.

system have recently been reviewed [4]. Thus olfaction, at least in part, has a transduction element in common with several well-studied receptors. There is also evidence for the involvement of a phosphatidylinositol-based mechanism, but the exact role of this type of receptor mechanism is currently unclear.

The nature of the olfactory receptor is unclear. Typical odorants are small lipophilic molecules which can penetrate the lipid phase of a membrane [5, 6]. Thus, for at least some types of odorants, the receptor may be the phospholipid moiety of the sensory membrane. The tissue contains a high concentration of unsaturated fatty acids [7] and the odorants may act by modulating the dynamic properties of the lipids.

The sensory membrane contains a number of glycoproteins which are possible candidates for olfactory receptor proteins [8]. Superfusion of rat olfactory mucosa with the lectin concanavalin A causes a reduction in the amplitude of the electro-olfactogram to certain odours [9]. The responses to aliphatic acids, aldehydes, thiols and hydrocarbons were especially affected [10]. Maximum inhibition was found for the C₅-C₉ homologues in the n-alkyl series of each of these functional groups. A recent study using alkane odorants [11] concluded that concanavalin A disables an olfactory receptor molecule which normally responds to the alkyl moiety of odorants in a particular size range. That moiety may represent a 'primary' odour quality-determining component in odour discrimination. Interestingly, the data on the concanavalin A inhibition of the response to aliphatic acids in rat olfactory mucosa showed a high correlation with the data on specific anosmia to these odorants in humans.

A number of odorant-binding proteins have been discovered [12]. These are small (18–20 kDa) water-soluble proteins. They may act as odour-collecting proteins but their role in olfactory mechanisms is unclear. A particularly puzzling aspect of these proteins is that they are not found in all vertebrates.

A highly active P-450 mono-oxygenase enzyme system is located in the olfactory epithelium [13]. This system may metabolize odorants and so assist in their removal from the tissue. This type of mechanism could help explain how we can continue to smell under constant odour stimulation. Several odorants from different odour categories have recently been found to act as inhibitors of the olfactory P-450 enzymes. Effects of this kind may explain the observed reduction in olfactory thresholds both with age and with exposure to polluted air.

The primary olfactory neurones appear to respond to most odorants. Large number of the primary neurones (1000–20000) synapse with the secondary neurones in the system. This marked convergence leads to the secondary neurones showing a more specific response to odorant stimulation. Odour discrimination appears to be a systems
property of the ensemble of neurones, with many interactions and feed-back loops occurring at different levels of the system [14].

The design of an artificial olfactory system

A number of studies during the past 40 years have contributed to this general area and they are reviewed elsewhere [15]. The first experimental electronic nose based on the likely discrimination principles used by the natural olfactory system, was described in 1982 [16]. This nose has evolved into our present device [15], which can distinguish between odours and flavours of commercial importance, not as well as a trained human nose, but with none of the consequences of boredom which human assessors experience after prolonged bouts of smelling.

The array of primary neurones in the olfactory tissue is mimicked in the electronic nose by an array of sensors. We currently use two types of sensors, inorganic semiconductors and conducting polymers; the properties of both types are described elsewhere [15]. The electroactive polymers are almost ideal materials for a sensor array. A great variety of monomers can be envisaged and these can be polymerized into a very large number of possible polymers.

The polymers are laid down by controlled electrochemical deposition of the polymer across a small (10 μm-wide) gap between gold electrodes on standard microelectronic substrates as outlined in Fig. 1. The conductance of the polymer is altered (usually an increase) when odorants adsorb to the polymer. Thus this type of device is an example of a chemiresistor. Advances in the fabrication techniques now allow an array of up to 20 different types of polymer to be deposited on a chip in an area comparable to the same number of living neurones, but the fabrication methods have not yet been optimized.

Different polymers respond differentially to odorants though no complete structure–odour response relationship has yet been reported. For example, the polymer formed from 5-carboxyindole gives a conductance decrease on exposure to methanol, ethanol and acetone; a small conductance increase with toluene, and no conductivity change with diethyl ether. The polymers have a rise time in seconds at ambient temperature and consume little power.

The polymers hold the promise of allowing a large sensor array comprised of over 50 polymers which will react differentially to the complete range of odorants. Significant technical problems with the reproducibility of the devices is a current limitation which can be expected to diminish as these novel polymers are further characterized.

The next significant problem with the electronic nose is the interpretation of the differential odour response from the array of polymers. We have used several approaches to this problem. One method is to analyse the pattern of responses using conventional multivariate statistical methods. Clustering techniques have enabled us to separate the responses to different brands of one type of flavour.

The natural olfactory system has several striking properties including the apparent parallelism of the sensor neurone array; flexibility of response and fault-tolerance (detection when subject to interfering odours). These are desirable features to be incorporated into an analogue of the real system. Analysis of the responses of the sensor array using neural network methods allow us to include some of these desirable properties. The inherent parallelism of these methods leads to a high speed of operation and this will be a significant advantage as the arrays grow larger. Though it is difficult to either formalize these methods or to interpret the results, the rapid development of neural network methods should lead to the type of high-speed recognition techniques which are required for electronic noses.

Fig. 1

A typical design of an odorant-sensing device based on an electrodeposited conducting polymer film

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Prospects for nucleic acid biosensors

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Introduction

In terms of the active biological element, biosensors can be divided into two broad groups, based on either catalytic or affinity reactions. Reference to the literature clearly shows that the majority of work to date has centred around catalytic biosensors utilizing enzymes as the catalytic recognition element or, occasionally, enzymes in whole cells or tissues. The alternative affinity systems, based mainly on antibodies have been slower to emerge, reflecting in part the number and degree of problems encountered.

Immunosensors, however, are now progressing rapidly and, for some applications, are approaching a stage where commercial devices are a realistic goal. The launch of the first commercial immunosensor will represent a significant milestone in biosensor technology by opening the door to a wider range of analytes and an improvement of the sensitivity limit from to lo-9 M (the typical enzymic range) down to lo-11 M and possibly lower. This will represent a step towards the technology required to produce a nucleic acid biosensor which, as an affinity system, will have many similarities to an immunosensor.

Abbreviations used: p.g.e., pyrolitic graphite electrode; s.p.r., surface plasmon resonance; PCR, polymerase chain reaction.

Markets and applications

As with immunoassay technology (including immunosensors), the existing and potential market for DNA technology and, by inference, DNA sensors is significant. Frost & Sullivan recently estimated the U.S. market for DNA probes in 1989 was $13.8m and forecast a rise to $301m by 1994. Nucleic acid technology (mainly DNA probes but also rRNA and mRNA) is in direct competition with immunoassay technology in many areas of application such as micro-organism identification. Diagnostic systems based on nucleic acids, however, offer the advantage of high sensitivity, often better specificity, and in some cases, such as retroviral infection, offer the ability to distinguish between past and present infection.

Other applications are unique to nucleic acid technology. These include forensic identification of individuals from DNA collected at the scene of a crime, genetic profiling to indicate susceptibility to disease, paternity testing, prenatal diagnosis of genetic disease and a host of research applications.

Many of the above applications are likely to remain in the laboratory and improvements in the technology have, therefore, been aimed at rapid, safe and less labour-intensive laboratory-based analytical systems. On this front a number of significant developments have arisen over the last