Solution-Based Analysis of Multiple Analytes by a Sensor Array: Toward the Development of an "Electronic Tongue"

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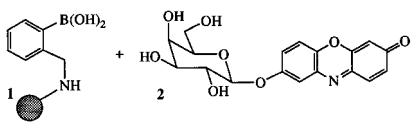
The sensations of smell and taste result from a series of specific and nonspecific molecular recognition events that take place in parallel.[1] In a few cases there are receptors that are specific for individual analytes. However, most tastants and odorants are identified through a composite of responses from nonspecific interactions. The pattern created by the simultaneous response of these receptors is specific for a particular set of stimuli. For example, the mammalian tongue has "taste buds" (Figure 1A)[2] which respond to four taste catagories: sweet, sour, salty, and bitter. The combination of only these four signals, along with olfactory information, creates a distinct pattern for each tastant.

The sense of smell has recently been mimicked using a variety of different transduction schemes.[3] In one case, an "electronic nose" creates patterns that result from small changes in the resistivity of a series of different conducting polymers when the polymers differentially adsorb volatile molecules.[4] The lack of suitability for solution-phase analysis and the ultimate desire to have biologically relevant sensors has driven scientists to search for alternative mimics of biological sensory systems.[5]

Herein, we describe a new sensor methodology which allows for the simultaneous identification of multiple analytes in solution. Advances in micromachining techniques and efficient/rapid data acquisition using a charge-coupled device (CCD) were combined with known chemical indicators to create a single sensor suite that may be described as a primitive "electronic tongue". Poly(ethylene glycol)−polystyrene (PEG-PS) resin beads[6] that were derivatized with a variety of indicator molecules were exploited to mimic "taste buds". These indicators are selective for individual analytes but, importantly, are not specific in their recognition properties. To mimic the cavities in which natural taste buds reside, we positioned the resin beads within micromachined wells formed in Si/SiN wafers, thus confining the beads to individually addressable positions on a multicomponent chip (Figure 1B).[7,8] The size of the wells was chosen so that they hold the beads in swollen and unswollen states.[9] For demonstration purposes, a 3 × 3 array of beads was created to mimic the capacity of the mammalian tongue to simultaneously identify a variety of analytes. Signal transduction was accomplished by analysis of the absorption properties of the beads using a CCD that was interfaced with the sensor array (Figure 1C).[10] Upon exposure to analytes, color changes for the beads were found to be 90% complete within 1 min, although only seconds were typically required (i.e., 3.5 s for changes in pH). Data streams composed of red, green, and blue (RGB) light intensities were acquired for each of the individual beads. The resulting patterns were stored in a computer for analyte identification and ultimate quantification.

In our proof of concept experiments, responses recorded at one specific and three nonspecific sensors were compared to a control. The sensors are fluorescein for pH,[11a] *o*-cresolphthalein complexone for Ca^{2+} and pH,[11b] alizarin complexone for Ce^{3+}, Ca^{2+}, and pH,[11c] and finally a boronic ester of resorufin-derivatized galactose for simple sugars.[12,13] The control was simply a resin bead with the terminal amines acetylated.

A demonstration of the capacity of four of the five different beads to complete simultaneous detection is provided in Figure 2. In this example, the presence of Ca^{2+} (0.1 M $Ca(NO_3)_2$) was analyzed under conditions of varying pH (pHs 3, 5, 7, 9, and 11 all buffered by a mixture of 0.04 M phosphate, 0.04 M acetate, and 0.04 M borate[14]). Similar analyses for 0.1 M $Ce(NO_3)_3$ and



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(9) Dry PEG-PS bead was measured to be on average 130 μm diameter, while the wet diameter measured about 250 μm using SEM. Novabiochem Corp. suggests swelling volumes on the order of 4 times the dry diameter which is approximately 210 μm.

(10) Color attenuation values are measured using the eight intensity values recorded by the CCD. Data are measured relative to the average throughput for a series of blank beads. Values are expressed in terms of a linear scale, although the linear response of the CCD has yet to be studied carefully. For a reference on CCDs, see: Borman, S. Array Detectors are Transforming Optical Spectroscopy. *Chem. Eng. News* **1996**, *74*, 33.

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(12) A resin bound boronic acid (**1**) was saturated with a fluorescently tagged sugar, resorufin-β-D-galactopyranoside (**2**). The addition of D-fructose, which has a higher affinity than **2** for the boronic acid, results in a displacement of **2**. Upon washing the released tag away from the resin, there is colorimetric modulation.

(13) The first three sensors were immobilized on a PEG-PS bead via a dicyclohexylcarbodiimide coupling between a terminal resin-bound amine and a carboxylic acid on the sensor.[8] The boronic ester was linked to the resin via an amine linkage. This linkage was created by a substitution reaction of a resin-bound amine on a bromobenzyl boronic ester. The ester was then hydrolyzed to the acid with dilute aqueous acid.

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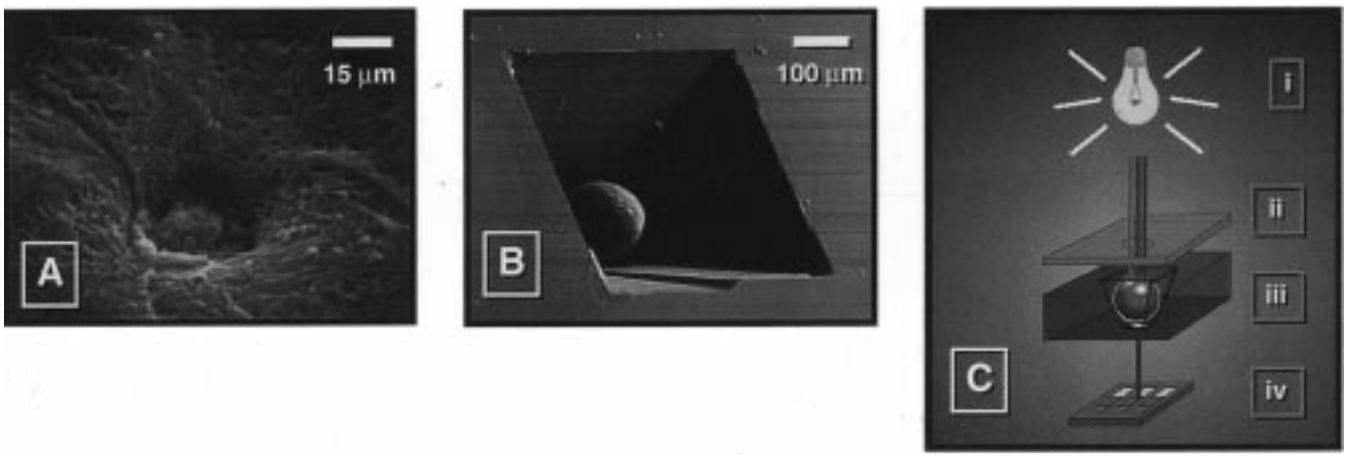

Figure 1. (A) Scanning electron micrograph (SEM) of a rat fungiform papilla (taste bud, adapted with permission from ref 2). (B) An SEM image of a PEG-PS bead immobilized in a micromachined Si well. (C) Schematic representation of the "electronic tongue" device. A light source (i) irradiates a series of beads held in pits (iii) through a cover plate (ii). The transmitted light is analyzed with a CCD array (iv).

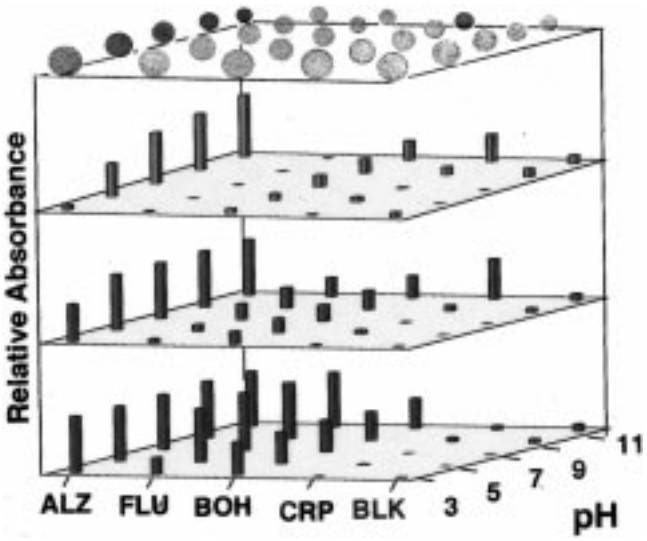

Figure 2. At the top portion of the illustration, a series of PEG-PS polymer beads derivatized with indicators are shown. These images were obtained with optical microscopy. At the lower portion, a series of bar graphs serve to quantify the extent of color attenuation recorded for red ($\lambda = 700$ nm), green ($\lambda = 550$ nm), and blue ($\lambda = 435$ nm), as obtained with the CCD. Color attenuation values are expressed in a linear scale.[10] Various PEG-PS beads are shown responding to Ca^{2+} at various pHs: ALZ = alizarin complexone, FLU = fluorescein, BOH = boronic acid/galactose−resorufin, CRP = *o*-cresolphthalein complexone, BLK = blank.

mixtures of Ca^{2+} and Ce^{3+} were performed. The bead derivatized with *o*-cresolphthalein complexone responds to Ca^{2+} at pH values of ~11.4, as noted by the purple bead seen at the top of the illustration. Similarly, in the absence of Ca^{2+} the same color was found at pHs of 12.5 and above. The figure also shows that during the same measurements, the fluorescein derivatized bead acts as a pH sensor. At pHs below 6, the fluorescein bead exhibits a light yellow color, but at higher pH values it turns orange. In other studies (see the Supporting Information), we find that the alizarin complexone plays a variety of roles. First, it acts as a proton sensor yielding a yellow color at pHs below 4.5, orange-red colors at pHs between 4.5 and 10, and deep purple hues at pHs above 11.5. Second, it functions as a sensor for Ce^{3+} at relatively low pHs by turning orange to red. Third, the alizarin complexone also responds to Ca^{2+}, but the color change is less dramatic than with Ce^{3+}. In separate experiments, the boronic acid-based sensor was analyzed along with the other sensors. This sensor responds to the presence of sugars as expected, but also responds to pH. The beads turned from dark orange to yellow in solutions containing fructose. Interestingly, when the pH was increased and this sensor was monitored, a change in color undectable by the human eye was revealed by the RGB analysis as an increase in absorbance of red.

In summary, three important factors have been demonstrated which relate to the design, testing, and functionality of micromachined sensor arrays for solution phase analyses. First, polymer beads derivatized with indicators give response times well under 1 min when incorporated in micromachined platforms. Second, the arrays can be integrated with commercially available CCDs allowing for parrallel access of spectral data from multiple beads. Third, simultaneous detection of several analytes in a mixture is possible by analysis of the RGB color patterns. The power of the approach partially resides in the fact that many methods exist for the attachment of single analyte detectors, biosensors, and combinatorial libraries to resin beads. Therefore, although relatively primitive from a receptor perspective, our initial experiments reported here demonstrate the principles of a new generation of sensors. Work is currently underway to delineate detection thresholds, develop more sophisticated receptors, and apply pattern recognition protocols to the RGB analyses.

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Supporting Information Available: Complete legend of patterns created for the simultaneous detection of pH, Ca^{2+}, and Ce^{3+} and RGB patterns for a series of arrayed sensors under different chemical environments (4 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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