Metal Ion-Sensitive Holographic Sensors

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Holographic sensors for Na^+ and K^+ have been fabricated from crown ethers incorporated into polymeric hydrogels. The methacrylate esters of a homologous series of hydroxyether crown ethers were synthesized and copolymerized with hydroxyethyl methacrylate and the cross-linker ethylene dimethacrylate (3 mol %) to form stable hydrogel films (~10 μm thick) containing covalently bound (0–97 mol %) 12-crown-4, 15-crown-5, and 18-crown-6 pendant functionalities. The films were transformed into silver-based volume holograms using a diffusion method coupled with a holographic recording using a frequency-doubled Nd:YAG laser. The resulting holographic reflection spectrum was used to characterize the shrinkage and swelling behavior of the holograms as a function of polymer composition and the nature and concentration of alkali, alkaline earth, and NH_4^+ ions in the test media. Optimized film compositions containing 50 mol % crown ether showed substantial responses ($\leq$200 nm) within 30 s at ion concentrations of $\leq$30 mM, which could be rationalized on the basis of the known complexation behavior of the crown ethers. An 18-crown-6 holographic film was shown to be able to quantitate K^+ concentrations over the physiologically relevant range. It was virtually unaffected by variations in the Na^+ background concentration within the normal physiological variation (~0.13–0.15 M) and shows promise for developing simple, low-cost K^+ sensors for medical applications.

There is an urgent requirement for inexpensive mass-producible clinical diagnostics that allow measurements to be made at the bedside, in the physician's office, or at home and empower prospective patients to monitor their own clinical needs. In this respect, the level of blood electrolytes, particularly H^+, Na^+, K^+, HCO_3^-, and Cl^-, is widely used to monitor aberrant physiologies associated with pulmonary emphysema, congestive heart failure, diarrhea, dehydration, diabetes, and acute and chronic renal failure.[1] Among these important ions, strategies for K^+ analysis attract particular attention due to interference from high concentrations of Na^+ in blood. Normal levels of Na^+ and K^+ in human serum lie within the ranges 135–148 and 3.5–5.3 mM, respectively.[2,3] Thus, the development of selective alkali metal ion sensors is a subject of much current interest. A clinically useful detection technology should satisfy two key criteria: It should comprise a recognition moiety with adequate ionic selectivity and an integral transducer to convert the concentration of the target electrolyte into a measurable change. Ionophores in various guises are probably the most important class of ion-selective recognition molecules since they were introduced in the pioneering work of Pedersen.[4,5] For most optical and visual detection strategies, the ionophore is usually coupled to a molecular chromophore, whose absorbance or fluorescence properties are determined by occupancy of the receptor with the appropriate ion.[6–10] More recently, nanosized metal particles have been exploited as colorimetric reporters since the extinction coefficient is several orders of magnitude greater than organic dyes[6] and the transition from dispersion to aggregation results in a distinct color change.[11–16] The phenomenon is termed surface plasmon absorbance, and the color change on aggregation is attributable to the coupling of proximal plasmon absorbances.[17,18] A strategy for the determina-

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tion of K^+ by exploiting crown ethers attached to gold nanoparticles to trigger aggregation by host−guest interaction and induction of color change has recently been reported.[19] A colloidal solution of 15-crown-5 functionalized gold nanoparticles responded to K^+ in the concentration range 0.1−0.48 mM, with the formation of a presumed 2:1 complex between the ion and the 15-crown-5 moiety.

An alternative approach that exploits a change in diffraction rather than absorption and that generates a visually perceptible color change has been reported.[20,21] The system comprises a crystalline colloidal array of polymer spheres (~100-nm diameter) polymerized within a hydrogel that swells or shrinks reversibly in the presence of appropriate analytes.[22] The crystalline colloidal array diffracts light at visible wavelengths determined by the lattice spacing and is governed approximately by Bragg's law:[21]

$$m\lambda = 2nd \sin \theta$$

where m is the diffraction order, λ is the wavelength of light in vacuo, n is the average refractive index of the system, d is the diffracting plane spacing, and θ is the glancing angle between the incident light propagation direction and the diffracting planes. The crystalline colloidal arrays were fabricated by dissolving nonionic polymerizable monomers within the suspension and photopolymerizing into a hydrogel, which entraps the colloid lattice.[23] A change of 0.5% in the hydrogel volume shifts the diffraction wavelength by ~1 nm. This colloidal array has been made sensitive to Pb^{2+}, Ba^{2+}, and K^+ by copolymerizing 4-acryloylaminobenzo-18-crown-6 into the hydrogel.[23] More recently, the technique has been refined for Pb^{2+}, pH, and temperature by using intelligent polymerized crystalline colloidal arrays.[24]

While the use of crystalline colloidal arrays is an attractive approach for the fabrication of facile visual sensors, the preparation of the iridescent charged array is time-consuming. An alternative, and more generic technique, exploits the concept of a simple reflection hologram as the interactive element in an inexpensive, mass-producible biochemical sensor.[25−31] In this approach, the holographic element per se provides not only the analyte-selective

"smart" polymer matrix but also the optical interrogation and reporting transducer.

When holographic diffraction gratings are illuminated by white light, they act as sensitive wavelength filters. Conventionally, the gratings comprise a gelatin−silver halide photographic emulsion coated onto glass or plastic substrates and are fabricated by passing a single collimated laser beam through a holographic plate backed by a mirror. Interference between the incident and reflected beams, followed by development, fixing, and bleaching, creates a modulated refractive index in the form of fringes lying in planes parallel to the gelatin surface and approximately half a wavelength apart within the 7-μm thickness of the gelatin film. Under white light illumination, the developed grating acts as a reflector of the light for a specific narrow band of wavelengths and holographically recreates the monochromatic image of the original mirror used in its construction (Figure 1a). The constructive interference between partial reflections from each fringe plane gives a characteristic spectral peak with a wavelength governed by the Bragg equation ($\lambda_{pk} = 2nd \cos \theta$), where d is the fringe separation distance, n is the average refractive index, and θ is the angle of illumination to the normal. The peak reflectivity is dependent on the number of fringe planes and the modulation depth of the refractive index. Any physical, chemical, or biological mechanism that changes the spacing of the fringes (d) or the average refractive index (n) will generate observable changes in the wavelength (color) or intensity (brightness) of the reflection hologram. As an example of the application of this new technology, if a holographic grating is immersed in a test sample comprising a "wet" hydrophobic organic solvent, absorption of water by the hydrogel layer causes the grating to swell perpendicular to the plane of the polymer layer. This increases the fringe separation and causes longer wavelengths to be selected for reflection from the holographic mirror.[26] Further work has shown that holograms can be fabricated in a very wide range of polymer matrixes, natural, synthetic, or rationally designed, to create inexpensive optical sensors that respond to a range of putative analytes.[23−31] We report here a further development of the holographic sensing technology by the fabrication of sensors for monovalent cations using crown ether-substituted "smart" polymers.

EXPERIMENTAL SECTION

Materials. 2-Hydroxyethyl methacrylate (HEMA; 97%), ethylene dimethacrylate (EDMA), dimethoxyphenylacetophenone (DMAP), methanol, propan-1-ol, 1,1′-diethyl-2,2′-cyanine iodide, triethylamine, (methacryloxypropyl)triethoxysilane, and neutral alumina were supplied by Aldrich Chemical Co. (Gillingham, U.K.). HEMA was purified prior to use by passage of ~1 mL of HEMA through a column of neutral alumina packed into a Pasteur pipet in order to remove residual methacrylic acid. The 1-hydroxymethyl derivatives of 12-crown-4, 15-crown-5, and 18-crown-6 and methacryloyl chloride (97%) were from Fluka (Gillingham, U.K.). All other chemicals were of analytical grade and were supplied by Sigma or Aldrich. Aluminized 100-μm-thick polyester film (grade MET401) was purchased from HiFi Industrial Film Ltd. (Stevenage, U.K.).

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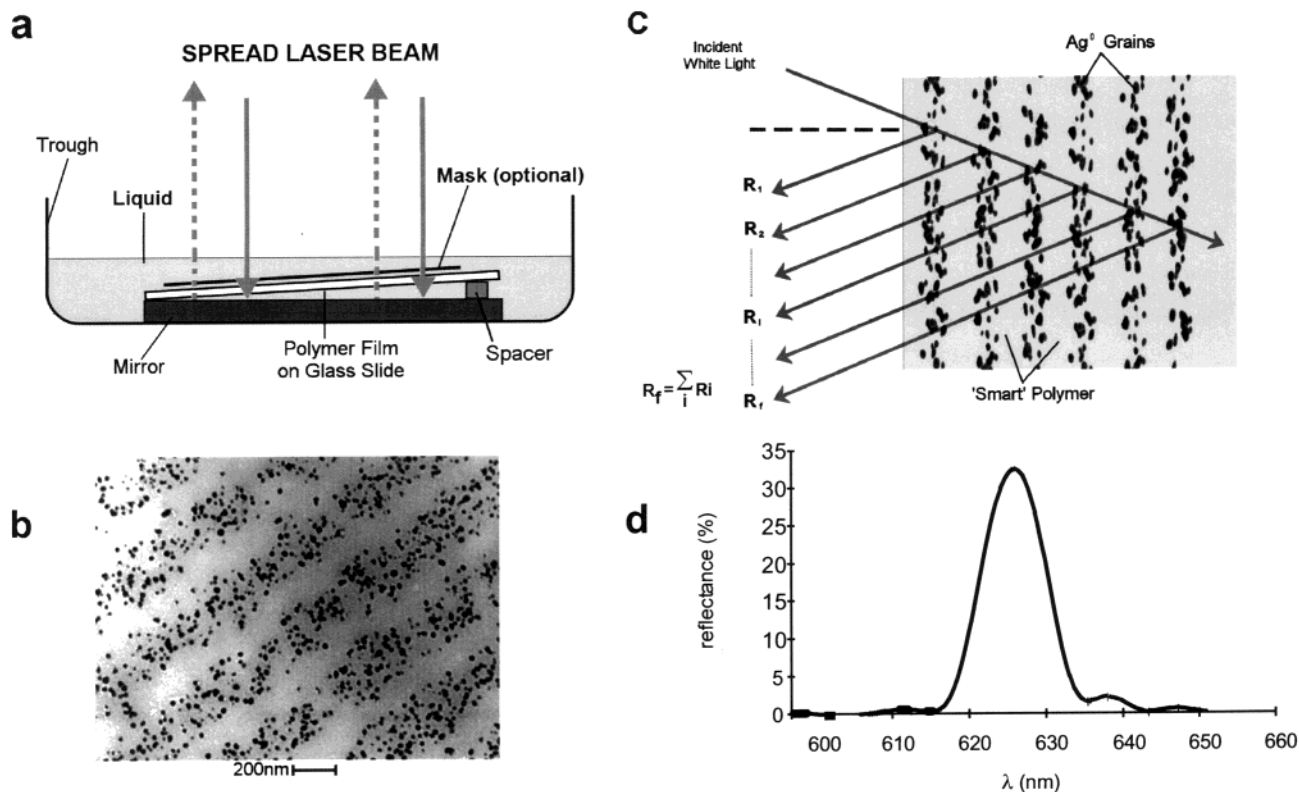
Figure 1. Methodology for constructing holographic diffraction gratings. (a) The spread laser beam interferes with light returning from the mirror in the trough to form a standing wave pattern with the antinodes or fringes spaced $\lambda/2$ apart. The pattern is recorded in a polymer-coated microscope slide resting on a small spacer to ensure that the finished hologram diffracts the viewing light at an angle different from that reflected specularly; (b) a transmission electron micrograph of a fragment from a hologram created in the setup and constructed using the diffusion method;[30] the silver grains are approximately spherical with an average diameter of ≤ 20 nm; (c) schematic of holographic fringe planes and Bragg parameters determined by the expression $\lambda_{pk} = 2nd \cos \theta$, where d is the fringe separation distance, n is the average refractive index, and θ is the angle of illumination to the normal; (d) a typical replay spectrum for the volume hologram.

Synthesis of Crown Ether Methacrylates. The methacrylates of 12-crown-4 (**1**), 15-crown-5 (**2**), and 18-crown-6 (**3**) were
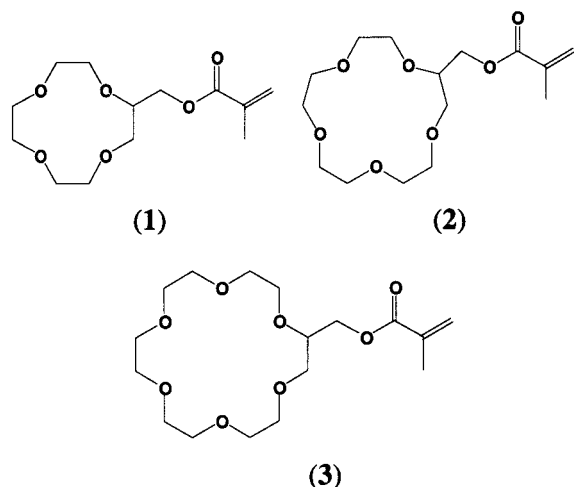
synthesized as follows: The appropriate 1-hydroxymethyl crown was mixed with 1.1 equiv of triethylamine in dichloromethane (DCM), cooled on ice, and 1.1 equiv of methacryloyl chloride was added dropwise. The mixture was stirred for 1 h at 4 °C and overnight at 20 °C; the triethylammonium chloride was filtered off and the filtrate concentrated under vacuum to a pale yellow

oil and subsequently purified by chromatography on neutral alumina using acetone/DCM/methanol (200:450:1, v/v/v) as eluent. Fractions containing the product were pooled, and the solvent was removed by rotary evaporation followed by drying under high vacuum to constant weight. The products were all viscous transparent oils, which showed as single spots on TLC. The structures were confirmed by ^{1}H NMR and ESI-MS. 2-Methylacrylic acid-1,4,7,10-tetraoxacyclododec-2-yl methyl ester (**1**): ^{1}H NMR (CDCl$_3$, 400 MHz) δ 6.09 (s, 1H), 5.56 (d, $J = 1.6$ Hz, 1H), 4.15 (m, 2H), 3.86 (m, 1H), 3.71 (m, 14H), 1.92 (s, 3H); MS-ESI (C$_{13}$H$_{22}$O$_6$) m/z 275.1. 2-Methylacrylic acid-1,4,7,10,13-pentaoxacyclopentadec-2-yl methyl ester (**2**): ^{1}H NMR (CDCl$_3$, 400 MHz) δ 6.09 (s, 1H), 5.54 (s, 1H), 4.24 (m, 2H), 3.84 (m, 1H), 3.67 (m, 18H), 1.92 (s, 3H); MS-ESI (C$_{15}$H$_{26}$O$_7$) m/z 318.2. 2-Methylacrylic acid-1,4,7,10,13,16-hexaoxacyclooctadec-2-yl methyl ester: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 6.09 (s, 1H), 5.55 (t, $J = 1.6$ Hz, 1H), 4.25 (m, 2H), 3.84 (m, 1H), 3.65 (m, 22H), 1.92 (s, 3H); MS-ESI (C$_{17}$H$_{30}$O$_8$) m/z 363.2.

Synthesis of Polymer Films. Polymer films were prepared by UV-induced radical polymerization of appropriate mixtures of monomers, sandwiched between a methacryloylsilane-treated microscope slide and the metallized side of a sheet of aluminized polyester.[26,28,30] Slides (76 mm × 26 mm) were laid out in an aluminum tray, and a minimal quantity of a freshly prepared 1% (v/v) solution of 3-(trimethoxysilyl)propyl methacrylate in

acetone was poured over them and immediately decanted off. The slides were incubated at 20 °C overnight in darkness before being rinsed in methanol, dried, and stored in a lighttight box. Functional monomers were polymerized by a UV-initiated free-radical reaction. Monomer solutions comprised HEMA with various molar proportions of functional monomers with EDMA as cross-linker at a concentration of 5 mol % total monomer and were diluted with 50% (v/v) propan-1-ol containing 2% (w/v) dimethoxyphenylacetophenone (DMPA) as photosensitizer. Silica gel particles (10-μm diameter; HPLC sorbent) were added to control the thickness of the final polymer film of ~1 mg/mL monomer solution. A 100-μL sample of the suspension of silica particles in the monomer solution was placed on the aluminum surface of a flat sheet of 100−μm-thick aluminized polyester. A silanized microscope slide was then laid on the droplet, causing liquid to spread across it uniformly by surface tension, and then a tray of five slides was subjected to 1-h UV radiation in a Stratagene UV Stratlinker 2400 cabinet. The aluminum mirror surface acted as a release layer after the polymerization step and imparted the desired glossy surface to the polymer film. Slides were gently peeled off the aluminized polyester and rinsed thoroughly in methanol.

Fabrication of Holographic Films. Holograms were fabricated in the film by depositing colloidal silver bromide using a two-step diffusion method,[30] followed by exposure using a single pulse from a frequency-doubled Nd:YAG laser. A 200-μL droplet of 0.3 M silver perchlorate in propan-1-ol/water (1:1 v/v) was placed on a flat clean glass sheet, and the polymer-coated side of a slide was laid on it, whereupon the liquid covered the polymer face naturally by surface tension. Work from this point on was carried out under moderately bright safelighting, a red safelight for the green-sensitive slides and vice versa. The silver solution was left in contact with polymer for 3 min, and then surface droplets on the polymer were gently removed by a wipe from a clean very soft rubber squeegee blade. The slide was then dropped, polymer face up, into a shaking Petri dish (140-mm diameter) containing 100 mL of the following agitated solution: 70 mL of methanol, 30 mL of water, 3 g of lithium bromide, 20 mg of sodium ascorbate, and 2.5 mL of 0.2% (w/v) methanol solution of the appropriate sensitizing dye, 1,1′-diethyl-2,2′-cyanine iodide for photosensitizing to the 532-nm laser line and pinacyanol chloride for the 633−650-nm laser line. Time spent in the shaken bromide ion bath was typically 3 min. Slides were then immediately rinsed in running deionized water and either used at once or dried in air and stored in a lighttight box.

The laser source was a frequency-doubled Nd:YAG ("BrilliantB" Quantel) giving a 350-mJ single pulse at 532 nm. HeNe lasers and inexpensive 5-mW laser pointers operating at 635−650 nm with the button batteries replaced by larger capacity dry cells for wavelength stability may also be used. However, slides exposed to continuous wave lasers usually needed to have a prolonged settling time (~60 min) before exposure for 15 s to a beam adequately covering a 76-mm-long slide. The degree of swelling prior to exposure in the trough governs the difference between the replay wavelength of the finished hologram and that of the laser. To suit the available wavelength range of our spectrometers, various methods for making swelling adjustments before exposure were used, such as using various alcohol/water ratios, buffer solutions with various pH values, or high concentra-

tions of inert salt such as sodium nitrate. About 0.1% (w/v) sodium ascorbate was usually included in the trough liquid to maintain the reducing environment needed for photosensitivity. The exposure was usually at a sufficient level to enable the time in the development bath to be less than 60 s at 23 °C. Generally, the shorter the development time needed to obtain a given optical density of silver, the greater the final diffraction efficiency of the resulting hologram.

Equal volumes of 5% (w/v) hydroquinone in 1:1 (v/v) methanol/water and 2% (w/v) sodium hydroxide in 1:1 (v/v) methanol/water allowed development to an optical density level between 1 and 2. Testing for a suitable development time started with finding the maximum density obtainable when developing the material totally exposed to white light. Thereafter, with laser exposure, development was taken to roughly half that maximum level before stopping development in 5% (v/v) acetic acid solution. After a brief rinse in deionized water, the undeveloped silver bromide and the sensitizing dye were removed by 10% (w/v) sodium thiosulfate in 4:1 methanol/water (v/v) on an orbital shaker for 9 min. The finished gratings were washed extensively in deionized water. The water content was reduced in a flow of warm air prior to the slide being cut into sections for insertion into cuvettes.

Monitoring Hologram Responses. All holograms were monitored at ~8° to the normal in a thermostated cell at 25 ± 0.1 °C in a reflection spectrometer as described previously.[26−28] Most measurements were made in Tris buffers, prepared by titrating Tris base with HCl to the required pH value. Phosphate buffers were prepared by titrating sodium dihydrogen phosphate with NaOH to the required pH, with or without the addition of NaCl. Solutions of the test cations (as chlorides) were made up as 0.2 M stock solutions in the required buffers. Small volumes of these stock solutions were added incrementally to 1 mL of buffer in the cuvette. After each addition, the hologram was allowed to equilibrate and the wavelength recorded, before the addition of the next increment. At the higher concentrations, appropriate volumes of buffer and 1 M salt in buffer were mixed and added to the cuvette. After each reading, the solution was removed and the next higher concentration solution added. After each run, the hologram was washed with buffer until the replay wavelength returned to its starting wavelength.

RESULTS AND DISCUSSION

Hologram Fabrication. Photographic emulsions containing ultrafine grains of silver bromide dispersed in gelatin are known as "Lippmann emulsions", after Lippmann was able to produce photographic negative transparencies capable of regenerating full color positives when viewed by reflected diffuse white light, entirely as a result of light interference effects, rather than from the effects of dyes or pigments.[32,33] In later years, Lippmann emulsions were used to record holograms[34] in emulsions made by playing separate jets of silver nitrate and potassium bromide solution into stirred warm gelatin solutions.[35] However, this technique suffered from the consequences created by conflicting

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physical laws; there was a requirement for small grains to prevent Rayleigh scatter,[36] despite their natural tendency to grow, while conversely, the individual grains had to have sufficient surface area to enable them to trap photons to ensure adequate photosensitivity.[36] The method described here involves coating a thin layer ($\sim$10 μm) of unsensitized polymer film on a glass slide and then immersing the slide, consecutively, in solutions of silver nitrate and lithium bromide containing a photosensitizing dye. This protocol causes ultrafine spherical grains of photosensitive AgBr of average diameter <20 nm to be precipitated within the polymer volume. Figure 1a illustrates the experimental setup used to create the holograms. Laser light returning from a plane mirror creates a classical standing wave pattern of nodes and antinodes or interference fringes spaced $\lambda/2$ apart. After a conventional photographic development step, the fringe pattern is represented as a distribution of ultrafine (<20-nm-diameter) grains of silver (Ag^0). A transmission electron micrograph of these patterned silver grains is shown in Figure 1b.

Under white light illumination, the developed grating acts as a reflector of the light for a specific narrow band of wavelengths and holographically recreates the monochromatic image of the original mirror used in its construction (Figure 1c). The constructive interference between partial reflections from each fringe plane gives a characteristic spectral peak with a wavelength described by the Bragg equation. Any physical, chemical, or biological mechanism that changes the spacing of the fringes (d) or the average refractive index (n) will generate observable changes in the wavelength (color) or intensity (brightness) of the reflection hologram (Figure 1d). Since a reflection format is used, the sample solution can be opaque without impairing the optical properties of the hologram. Swelling of the polymeric matrix stabilizing the silver grains registers as a peak wavelength shift on the CCD camera and is recorded on the computer monitor screen.

The presence of crown ethers in the polymer structure produced bright high-efficiency holograms containing fine colloidal silver particles (Ag^0) within the volume of the polymer layer. Films containing high crown ether contents (>50 mol %) appeared thinner and more glassy than HEMA-containing films and generated weaker holograms with lower silver contents. Presumably, the higher content of apolar crown ether functionalities reduced polymer swelling compared to the parent HEMA-containing hydrogel and, hence, reduced the ability to absorb Ag^+ ions during the diffusion steps used for holographic film synthesis.

Optimization of Polymer Film Composition. The starting point for the optimization of the composition of the $\sim$10-μm-thick polymer film was based on the preferred conditions for making the holograms in the parent matrix, poly-HEMA.[31] To achieve a responsive hologram, the polymer must be able to expand and contract as much as possible, suggesting that a soft, lightly cross-linked matrix would be preferred. However, the conditions required for recording bright holograms require a degree of polymer rigidity, adequate to prevent significant structural rearrangement during or after hologram recording. The optimal tradeoff between these opposing requirements was found to be $\sim$3 mol % cross-linker (EDMA) in a poly-HEMA film, and this value was used throughout the current work.

A second consideration was the observation that the use of HEMA as received from the supplier generated poly-HEMA films containing residual methacrylic acid functionalities. Ionization of the carboxylic acid groups at the pH used for ion binding (pH 7.0) will concentrate the ion in the polymer film by the Donnan effect and alter the apparent equilibrium position for ion complexation to the crown ether. However, studies on the pH behavior of poly-HEMA films prepared from purified HEMA with small amounts of methacrylic acid quantitatively reintroduced suggested that the impurity content was equivalent to $\sim$0.5 mol % of total monomers. At this level, it was observed that the polyelectrolyte gels swell appreciably under the conditions used in the present studies.[37] Consequently, all work reported in this paper was obtained with films fabricated with alumina-purified HEMA monomer to eliminate this effect and simplify any interpretation of the responses of the crown ether holograms.

It was also noted that the presence of solvents during the film polymerization step affected the brightness and the subsequent swellability of the polymer film in response to the analyte of interest. This effect was investigated for the crown ether-functionalized films by arbitrarily fixing the content of 12-crown-4 at 30 mol % and synthesizing films either with no solvent or with 25 or 50% (v/v) methanol or propan-1-ol included in the monomer mixture. The resulting films were used for hologram recording and the resulting holograms tested for their responses to Li^+, Na^+, and K^+ ions. Films synthesized in the absence of solvent take up less silver, make weaker holograms, and have poor responses to challenge with the ions. Addition of solvent ensures that the monomers are solvated during film formation and leads to a "porimer" effect, which enhances the response to ions.[38,39] Thus, addition of 25% (v/v) solvent approximately doubled the response to any given ion, while at 50% (v/v) solvent, the wavelength shift was almost 4 times that seen for films in the absence of solvent (Figure 2). The difference between methanol and propan-1-ol was minimal, and 50% (v/v) propan-1-ol was used as solvent in all subsequent film syntheses.

Crown ethers are known for their unusual property of forming stable complexes with alkali metal ions and correlates with the close fit of the cation to the crown cavity where, on complexation, the oxygen atoms lie in a nearly planar configuration about the central cation. Inspection of Figure 2 shows that the 12-crown-4 hologram markedly contracts on exposure to increasing Na^+ ion concentrations, while the hologram showed little response to Li^+ or K^+. At first sight this is surprising, since the Li^+ ion has the ionic radius (0.76 Å) best matching the cavity size (0.6−0.75 Å) of 12-crown-4.[40] It appears that, in this case, the response is dominated by a dimerization reaction between immobilized crowns, brought together into a sandwich structure by the presence of the Na^+ ion, which has an ionic radius (1.02 Å) too large to fit snugly inside the cavity. The formation of the 2:1 crown/Na^+ complex has the effect of forming a cross-link, and entropic constraints on the chain length increase the elastic modulus of the hydrogel and thereby cause the observed contrac-

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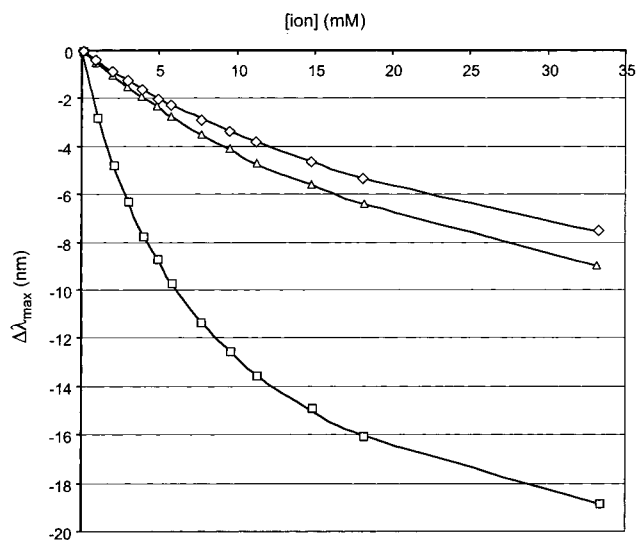**Figure 2.** Response of a poly-HEMA hologram containing 50 mol % 12-crown-4 to Li$^+$ ($\diamond$), Na$^+$ ($\square$), and K$^+$ ($\triangle$) over the concentration range 0−33 mM in 20 mM Tris-HCl buffer, pH 7.4 at 25 ± 0.1 °C.

tion in the spacing of the holographic fringes. Dimerization of 12-crown-4 in the presence of Na$^+$ and 15-crown-5 in the presence of K$^+$ is well documented[41] and is the basis for the well-known sodium ionophore bis[(12-crown-4) methyl] dodecylmethyl malonate[42] and the recognition of K$^+$ by 15-crown-5-functionalized gold nanoparticles.[19] The low affinity constant for Li$^+$ binding inside the cavity of a single 12-crown-4 ring, with log K being reported as ≤1 even in apolar solvents,[43] would make 1:1 complexation unfavorable in water over the concentration range of Li$^+$ used and thus no swelling due to Li$^+$ binding is observed.

The proportion of the methacryloyl-crown ether incorporated into the HEMA copolymer film to elicit an optimal response with the monovalent cation Na$^+$ was determined by varying the amount of methacryloyl-12-crown-4 used in the polymer film within the range 0 (poly-HEMA) and 97% (0 mol % HEMA, 3 mol % crosslinker). Figure 3a shows the response curves to ion concentration in the range 0−33.3 mM Na$^+$ at various ratios of 12-crown-4 to HEMA in the range 0−97 mol %. These data are replotted in Figure 3b as wavelength response as a function of mole percent 12-crown-4 at two arbitrary concentrations of Na$^+$ ion, 5.8 and 18.2 mM, respectively. It is evident that the optimum response to Na$^+$ occurs at ∼40 mol % crown, although the shape of the curve is similar and broadly flat over the range 30−50 mol % crown, regardless of the Na$^+$ concentration selected for comparison. A plausible explanation for this behavior lies in the fact that if the crown content is low, the receptors are too far apart in the polymer network to form productive 2:1 molar complexes, while, at the higher crown contents, the polymer becomes much more hydrophobic in nature and swells less in response to ion binding. The latter proposal is supported by the reduced silver content of the hydrogels at high crown contents. The crown content of holograms used in all subsequent experiments was between 30 and 50 mol %.
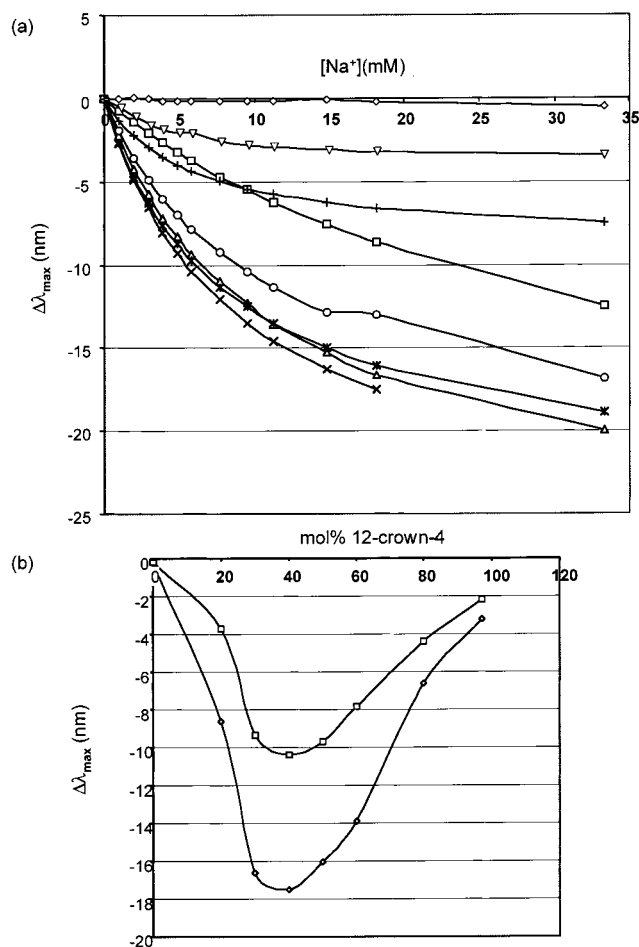
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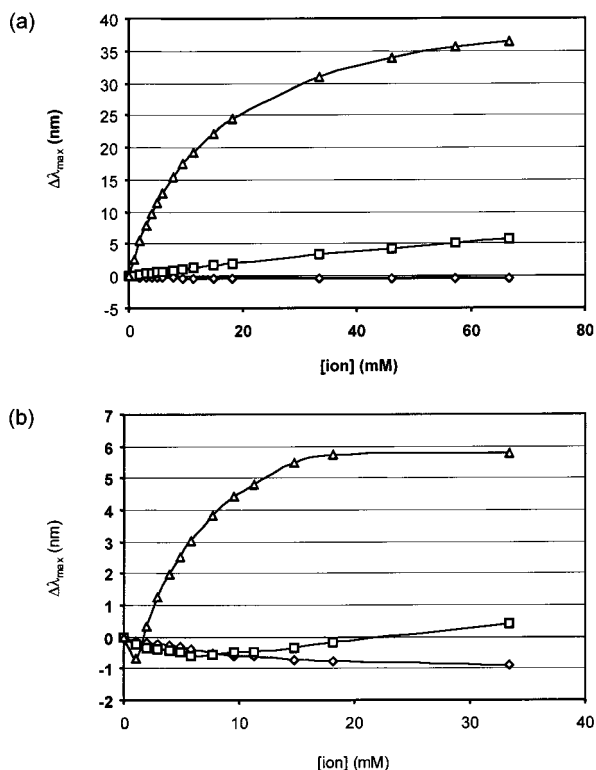(43) Hirotsu, S.; Hirokawa, Y.; Tanaka, T. *J. Chem. Phys.* **1987**, *87*, 1392.

Figure 3. Responses to [Na$^+$] in the range 0−33 mM poly-HEMA 12-crown-4 holograms in 20 mM Tris-HCl buffer, pH 7.4 at 25 ± 0.1 °C at various mole percent crown ether contents: (a) as a function of [Na$^+$] for polymers containing various mole percent 12-crown-4: 0 ($\diamond$), 20 ($\square$), 30 ($\triangle$), 40 ($\times$), 50 (*), 60 ($\bigcirc$), 80 (+) and 97 ($\triangledown$); (b) as a function of mole percent 12-crown-4 at 5.82 ($\square$) and 18.18 mM ($\diamond$) Na$^+$.

The selectivity of the holograms toward Li$^+$, Na$^+$, and K$^+$ ions varied slightly with the crown ether content of the hydrogels. For example, at 20 mol % 12-crown-4, differentiation between all three ions was at a minimum, with the response to Li$^+$ and K$^+$ being almost indistinguishable and notably less that that for Na$^+$. In contrast, at a crown content of 50 mol %, there was a distinctly higher response to K$^+$ than Li$^+$ and a response to Na$^+$ of over 2-fold higher than K$^+$.

Hologram Sensitivity and Selectivity. Figures 2 and 4 compare the responses to Li$^+$, Na$^+$, and K$^+$ within the concentration range 0−70 mM holographic sensors containing 50 mol % 12-crown-4 (Figure 2), 18-crown-6 (Figure 4a), and 15-crown-5 (Figure 4b), respectively. The behavior of 12-crown-4 holograms to the ions contrasts sharply with the response of the 18-crown-6 holograms at the same mole percent crown content. Holograms containing 50 mol % 18-crown-6 (Figure 4a) show very little response to Li$^+$, even at concentrations up to 70 mM, produce a weak response to Na$^+$, but swell substantially in the presence of K$^+$. It is presumed that, under these conditions, the K$^+$ ions (ionic radius 1.38 Å) form a 1:1 complex within the cavity of the 18-

(a)

(b)

Figure 4. Responses to Li$^+$ ($\diamond$), Na$^+$ ($\square$), and K$^+$ ($\triangle$) of holographic sensors containing 50 mol % (a) 18-crown-6 and (b) 15-crown-5 in 20 mM Tris-HCl buffer, pH 7.4, at 25 $\pm$ 0.1 °C.
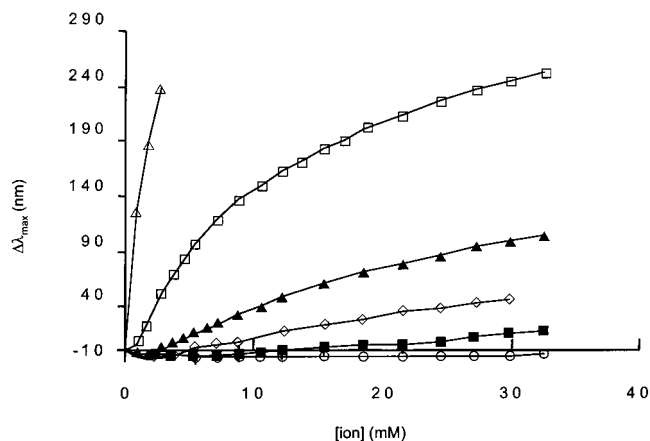

Figure 5. Effect on λ_{max}(nm) of a range of alkali and alkaline earth cations in 20 mM Tris-HCl buffer, pH 7.4 at 25 $\pm$ 0.1 °C, on an 18-crown-6 hologram (47 mol %): Ba^{2+} ($\triangle$); K$^+$ ($\square$); Rb$^+$ ($\blacktriangle$); NH$_4^+$ ($\diamond$); Na$^+$ ($\blacksquare$); Ca^{2+} ($\bigcirc$).

crown-6 ring (cavity radius 1.34–1.43 Å) and thereby induce a positive charge on the holographic polymer matrix. This causes the hydrogel matrix to swell by one or both of the following mechanisms: First, the hydrogel may swell due to direct repulsion of adjacent immobilized positively charged ions. At the high crown ether loadings used in these polymer films, the polyether rings will be statistically close enough in space for significant direct repulsion to occur. Second, and perhaps additionally, immobilization of charges within the polymer gel will increase the osmotic pressure due to a Donnan potential arising from mobile counterions to the crown ether-bound cations and creating a polyelectrolyte gel.[44,45] The charge state of the polyelectrolyte gel is determined by the number of cations bound, which leads proportionately to an influx of water and hence swelling of the hydrogel matrix.[44,45] In this respect, the holographic sensors behave qualitatively and quantitatively like the intelligent polymerized crystalline colloidal arrays.[22,23] In the case of the swelling response of the 18-crown-6 hologram to K$^+$ (Figure 4a), these two effects are likely to complement each other, while in situations where contraction of the holographic matrix occurs due to dimerization, as in the formation of the 2:1 12-crown-4/Na$^+$ complex, the osmotic effect may oppose the contraction due to dimerization and expulsion of water, with the latter being the dominant effect. Figure 4a shows that the 18-crown-6 hologram also swells in the presence of Na$^+$, albeit to a lesser extent than K$^+$, and attributable to the lower stability constant for Na$^+$ (log

$K_s = 0.8$) than K$^+$ (log $K_s = 2.03$) binding to this crown.[41] At very high ion concentrations, the mutual charge repulsion by immobilized charges is masked by the shielding effect of ions in solution and the incremental swelling ($\Delta\lambda$) per unit increase in ion concentration decreases again. Interestingly, Li$^+$ shows little response to this crown hologram, other than a small progressive contraction as the ionic concentration increases. This effect is possibly due to a nonspecific enhancement of hydrophobic interactions in the polymer film, causing the hydrocarbon backbones to interact more strongly. This "salting-out" effect has been noted for many hydrophilic polymers in aqueous solution.[46]

The response of 15-crown-5 holograms to the ions is intermediate between those of 12-crown-4 and 18-crown-6 (Figure 4b). At very low K$^+$ concentrations (<2 mM), the 15-crown-5 holograms contract like the 12-crown-4 holograms, presumably because of the formation of a 2:1 crown/ion complex, while at higher concentrations, the behavior is more akin to the 18-crown-6 holograms, with marked swelling observable at concentrations of >3 mM K$^+$ due to the formation of a 1:1 complex and its sequelae. The process of contraction followed by swelling is also apparent as the 15-crown-5 hologram responds to increasing concentrations of Na$^+$, with the null point ($\Delta\lambda = 0$) occurring at approximately 21–22 mM Na$^+$ under the conditions selected. These observations are a little surprising, given the closely comparable stability constants of Na$^+$ and K$^+$ with 15-crown-5.[43] At concentrations up to 33.3 mM, Li$^+$ is responsible for a contraction of the 15-crown-5-containing hologram.

The selectivity of the response to ions of the 50 mol % 18-crown-6 hologram in 20 mM Tris-HCl buffer (pH 7.4) is shown in Figure 5. The wavelength shifts for this hologram are exactly on the order of what would be predicted from the known stability constants (K_s) for these ions binding in the cavity of the 18-crown-6 ring (cavity radius 1.34–1.43 Å) and which, in turn, mirror the ionic radii and charge densities of the ions.[47] Strongest binding is shown by Ba^{2+} ($r = 1.34$ Å, log $K_s = 3.87$), followed by K$^+$ (r

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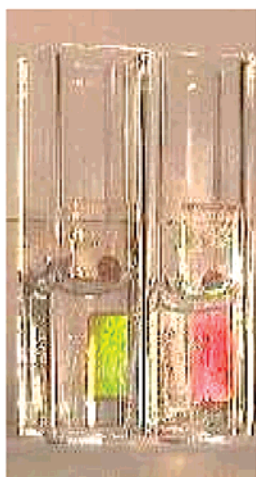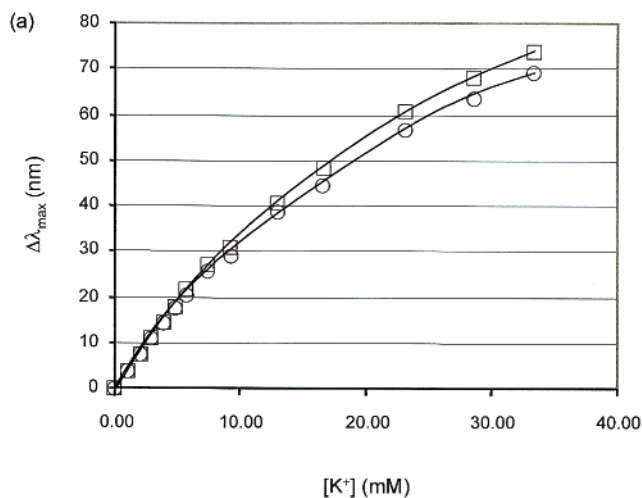
(a)

(b)

Figure 6. (a) Response of a 47 mol % 18-crown-6 polymer hologram to [K$^+$] over the concentration range 0–33 mM in 20 mM Tris-HCl buffer, pH 7.4 at 25 $\pm$ 0.1 °C, in solutions containing the two extremes of the physiological [Na$^+$] range: 130 (□) and 150 mM (○). (b) Two cuvettes each containing a strip of holographic grating fabricated from a 47 mol % 18-crown-6/HEMA (50 mol %)/EDMA (3 mol %) copolymer are shown side by side. Each cuvette initially contained 1 mL of 150 mM NaCl, to which 20 μL of 1 M KCl solution was added to the cuvette on the right. The replay color originating from a carefully angled small white light source shows that 20 mM K$^+$ has caused appreciable swelling in the presence of 7 times the concentration of Na$^+$ and altered to grating color.

= 1.33 Å, log K_s = 2.03), Rb$^+$ (r = 1.47 Å; log K_s = 1.56), NH$_4^+$ (r = 1.43 Å; log K_s = 1.23), Na$^+$ (r = 0.97 Å; log K_s = 0.8), and Ca^{2+} (r = 0.99 Å; log K_s < 0.5). The values for log K_s are for complexation of the ions with 18-crown-6 in aqueous solution.[43] It is likely that the polarity of the polymer environment is considerably lower that that of water, since it is known that hydrogels contain both free and complexed water[48] and also the alcoholic side-chain functionalities of the poly-HEMA matrix. These effects should favor ion complexation and enhance the response of the holographic sensor at low ion concentrations. Typically, stability constants are ∼3 orders of magnitude higher in methanol than in water.[47]

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Holographic Sensor Response in Physiological Saline. The relative selectivity for K$^+$ ions compared to other ions likely to be found in human blood, and the large response over the concentration range relevant for physiological measurements, suggested that 18-crown-6 holograms may be suitable for measuring K$^+$ ions in blood. To test whether this could be achieved, measurements with 50 mol % 18-crown-6/HEMA copolymer holograms as a function of K$^+$ ion concentration were repeated in a phosphate buffer containing a physiological sodium background. Figure 6a shows that the difference caused by varying the Na$^+$ background level between the high (153 mM) and low (130 mM) extremes of the physiological range[2,3] had relatively little effect on the K$^+$ determination. Figure 6b shows two cuvettes each containing a strip of holographic grating fabricated from 47 mol % 18-crown-6/HEMA copolymer. The cuvette on the left contains 150 mM NaCl, while the cuvette on the right is supplemented with 20 mM KCl. A carefully angled white light source shows that the presence of the K$^+$ ions have caused appreciable swelling of the hologram in the presence of 7 times the concentration of Na$^+$ ions and altered the replay color of the grating. A detection limit of ∼1 mM K$^+$ was observed under these conditions. In principle, a correction could be made for the variance in the Na$^+$ background using a 15-crown-5 hologram to determine the Na$^+$ concentration. Thus, it seems likely that the sensor could be developed to yield accurate K$^+$ concentrations in serum or whole blood. The polymer hydrogel can act as a size-selective semipermeable membrane and allow only small molecules and ions, but not whole blood cells, to penetrate the volume of the holographic matrix and thus bind to the receptor. It has previously been shown that holograms, unlike other types of optical sensors, function effectively in turbid and highly colored solutions, properties that should make the holographic sensor ideal for measurements in whole blood.

Conclusions. A variety of different polymer hydrogel compositions containing pendant crown ether functionalities have been synthesized as ∼10-μm-thick films and transformed into reflection holograms. These have been used to characterize the volume changes when the films bind a variety of different monovalent and divalent metal ions. Film composition has been optimized and the behavior of holograms containing 12-crown-4, 15-crown-5, and 18-crown-6 to binding Li$^+$, Na$^+$, and K$^+$ ions examined over wide concentration ranges. Typically, these films respond within 30 s with up to a 200-nm wavelength change when challenged with 33 mM ion at 20 °C. An optimized 18-crown-6 hologram has been characterized with respect to its ion selectivity for a group of alkali and alkaline earth metal ions. and the responses have been found to be in accord with the known ion-complexing characteristics of the free crown ether in solution.[43] These properties are similar to those reported for sensors based on intelligent polymerized crystalline colloidal arrays (IPCCA).[22,23] However, in the IPCCA approach, it is reported that the response to K$^+$ ions was nonlinear and that the saturation limit for the acryloylamidobenzo-18-crown-6 sensor was 20 mM K$^+$, based on a K_s for K$^+$ of 107 M^{-1}. The holographic sensor responds linearly to K$^+$ concentrations up to ∼10 mM and continues to generate a change in wavelength at concentrations above 30 mM. The diffraction peak maximums of individual holographic sensors were reproducible within the resolution capability of the spectrophotometer ($\pm$0.3 nm). Fur-

thermore, the holographic sensors are relatively facile to fabricate and do not require the elaborate dialysis and deionization procedure required to remove excess ions and surfactants for preparation of the highly charged monodisperse polystyrenecolloids. The 18-crown-6 holograms reported here can be used as chemical sensors to determine the concentration of K^+ ions in a sample and appear to be virtually unaffected by variations in the Na^+ ion background that would be encountered in blood or serum samples. This response to K^+ at high ionic strengths differs to the responses reported for the otherwise similar IPCCA technology.[22,23,49]

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