Metal Indicators

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Test Strips for Heavy-Metal Ions Fabricated from Nanosized Dye Compounds**

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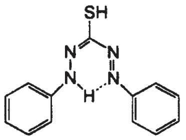
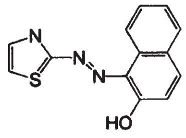
Analytical test strips for heavy-metal ions can provide simple and convenient procedures for on-site analysis and daily monitoring of water quality without using costly instruments. However, since the standard limit values of heavy metals in the environment and drinking water are remarkably low (ppb level), the detection limits of commercial test strips (ppm level) are insufficient to satisfy the required criteria. In addition, since they are generally prepared by soaking paper in the signaling reagent, leakage of reagent is facile, and hence reliability and sensitivity of detection are poor. Firm fixation of colorimetric and fluorometric reagents for metals on solid substrates has been attempted by incorporation into PVC-based liquid membranes,[1] covalent anchoring with cross-linked copolymers,[2] and layer-by-layer accumulation methods such as Langmuir–Blodgett films[3,4] and alternate deposition of oppositely charged polyelectrolytes.[5,6] A wealth of information on optical sensors and probes for simple determination of heavy metals has been reviewed.[7] However, drawbacks include complicated synthetic procedures, insufficient sensitivity, requirements for auxiliary additives, and difficulty in controlling the concentration of the reagent and its uniformity. Hence, we sought a simple procedure for firm and uniform coating of signaling reagents on an appropriate substrate without any additional matrix.

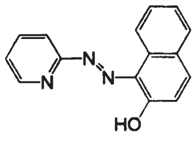
Recently, a simple and versatile process known as the "reprecipitation method" was proposed for the preparation of aqueous dispersions of organic nanoparticles.[8] The process involves injection of a water-miscible organic solution into water with vigorous stirring. We found that various organic nanoparticles can be uniformly coated onto a membrane filter (cellulose ester type) to form a thin layer simply by filtration of the nanoparticle dispersion. We attempted to apply this simple procedure to the fabrication of analytical test strips from a selection of signaling reagents. We examined popular indicator dyes having an aromatic conjugated system: dithizone (Dith), 1-(2-thiazolylazo)-2-naphthol (TAN), 1-(2-pyridylazo)-2-naphthol (PAN), tetraphenylporphin (TPP), and bathophenanthroline (Bathophen).



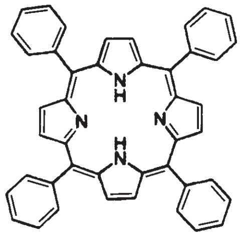
Dith



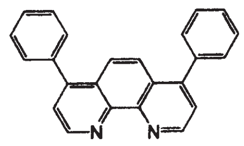
TAN



PAN



TPP



Bathophen

Optimized preparative conditions for aqueous dispersions of the indicator dyes are listed in Table 1. Water-miscible acetone and THF were used for dissolution of the dyes. The pH of the aqueous phase was controlled to maintain electroneutrality of the dye species by considering the pK_a values of the respective compounds. Smaller particles are formed with increasing temperature of the aqueous phase and with decreasing dye concentration in the organic phase.[9] Figure 1 shows scanning electron microscopy (SEM) images of TPP nanoparticles and PAN nanofibers. Round particles (50–100 nm) were mainly formed from TPP and Bathophen. In contrast, fibrous products were formed in the case of PAN, TAN, and Dith. These dyes commonly have dissociative protons capable of forming intermolecular hydrogen bonds which assist formation of three-dimensional fibers.

These dye particles/fibers were firmly coated onto the membrane filter as a thin layer simply by filtering the dispersion. Figure 2 A shows membrane filters loaded with typical analytical dye particles/fibers. It is noteworthy that the present membranes were prepared without any additives such as coating polymers or modifiers. The color intensity was almost constant across the membrane according to linear scanning of the dye zone by a TLC scanner (Figure 2 B), and this indicated uniform dispersion of the reagent (PAN) on the membrane surface. On the contrary, 1–2-μm crystals of PAN prepared by milling formed irregular patches that gave a rough surface on the filter and were easily removed by rubbing with a finger (Figure 2 B).

The cross-sectional SEM image (Figure 3 A) indicates that the thickness of the dye particle/fiber layer is less than 1 μm. Thus, the membrane provides a remarkably concentrated signaling surface composed of 100 % pure indicator dye. In

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Table 1: Preparation conditions and properties of nanosized dyes.

Dye	pH buffer[a]	Organic solvent (concentration)	Target ions	Properties of nanosized dye
Dith	0.01 M HCl (pH 2)	acetone (2 mM)[b]	Hg^{II}, Ag^{I}	fibers: width 25–70 nm, length >1 µm
TAN	MES (pH 6.15)	acetone (2 mM)[b]	Cu^{II}, Ni^{II}, Pd^{II}	mixture of fibers and particles fibers: width 50–150 nm, length >1 µm particles: 25–70 nm
PAN	TAPS (pH 8.4)	acetone (2 mM)[c]	Zn^{II}, Ni^{II}, Co^{III}	mixture of fibers and particles fibers: width 50–150 nm, length >1 µm particles: 25–70 nm
TPP	TAPS (pH 8.4)	THF (5 mM)[b]	Zn^{II}, Cu^{II}	round particles 50–100 nm
Bathophen	TAPS (pH 8.4)	acetone (5 mM)[b]	Fe^{II}	round particles 50–200 nm

[a] MES = 2-morpholinoethanesulfonic acid, TAPS = *N*-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid. [b] 100 µL of organic solvent was injected into 10 mL of water containing pH buffer. [c] 200 µL of organic solvent was injected into 20 mL of water containing pH buffer

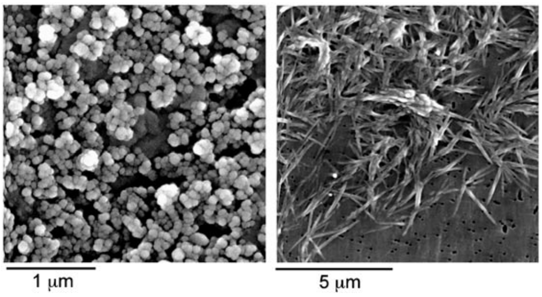

Figure 1. SEM images of TPP nanoparticles and PAN nanofibers on a flat polycarbonate membrane.

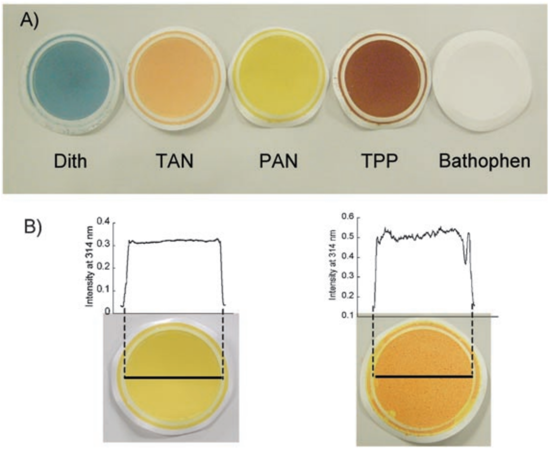

Figure 2. A) Photographs of dye-loaded membranes. B) Color intensity profiles by linear TLC scanning across a PAN nanofiber membrane prepared by the present method (left) and a membrane prepared from PAN microcrystals (right).

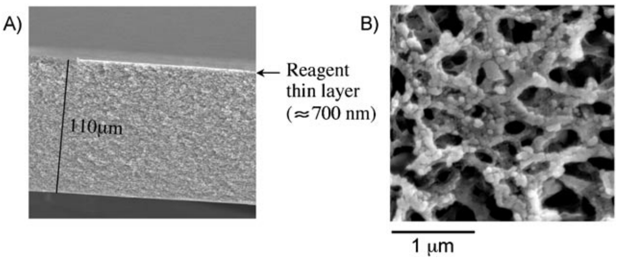

Figure 3. SEM images of Bathophen nanoparticles retained on a mixed cellulose ester membrane filter. A) Cross section. B) Top view.

addition, reagents are not removed from the support membrane even by rubbing with a finger or by immersion in water. As shown in the top-view SEM image of the membrane (Figure 3B), the Bathophen nanoparticles stick to the cellulose fiber network of the support. Nanofibers of PAN, TAN, and Dith were also firmly captured on the membrane by clinging to cellulose fiber. Since more than 99.5% of dye particles/fibers are retained on the membrane, the present method permits simple control of dye concentration on the filter surface.

The membranes are primarily useful as dip test strips for screening of metal ions. In addition, they are water-permeable, and hence trace metals are enriched on them by filtration of the sample, so that much lower detection limits are achieved. Dissolution of reagents in the sample solution was practically negligible during dip testing and sample filtration, presumably because the dyes are firmly retained on the fiber network of the membrane filter.

We used the PAN-coated membrane as test strip for the detection of Zn^{II}. The pH of the test solution was optimized to 8.4, at which the most intense red color is developed and the reaction rate is optimum.[10] We monitored the color change and the relative color intensity (Figure 4) with reflectance-absorption spectrometry. The peak at $\lambda_{max} = 550$ nm indicates that neutral $[Zn(PAN)_2]$ is formed on the membrane filter as the major species. The increasing peak intensity with increasing Zn^{II} concentration enables quantitative determination of test samples by comparison with the calibration curve (inset). Figure 5A shows the color changes of the PAN strip in dip testing. Notably, as little as 65 ppb (1×10^{-7} mol dm^{-3}) of Zn^{II} was detected by naked-eye color test after dipping the test strip for 15 min. Alternatively, sub-ppb concentrations of Zn^{II} were successfully detected by filtration enrichment of 100 mL of sample solution at a filtration rate of about 6.9 mL min^{-1} (Figure 5B).

Dithizone forms a remarkably stable Hg^{II} complex and gives the intense orange-yellow color of $[Hg(HDith)_2]$ under slightly acidic conditions.[11] By simple filtration of the sample solution (100 mL) at 9 mL min^{-1}, detection down to 10 ppb was successfully achieved (see Supporting Information). Interference was studied by filtration of simulated wastewater containing 780 ppm of Na^+, 1000 ppm of K^+, 400 ppm of Ca^{II}, 5.6 ppm of Fe^{II}, 6.4 ppm of Cu^{II}, 6.5 ppm of Zn^{II}, 0.5 ppm of Cd^{II}, 0.5 ppm of Pb^{II}, and 20 ppb of Hg^{II} at pH 2. Addition of ethylenediaminetetraacetic acid (10^{-4} mol dm^{-3}) to the sample solution effectively masked interferences from these ions in the detection of 20 ppb of Hg^{II}.

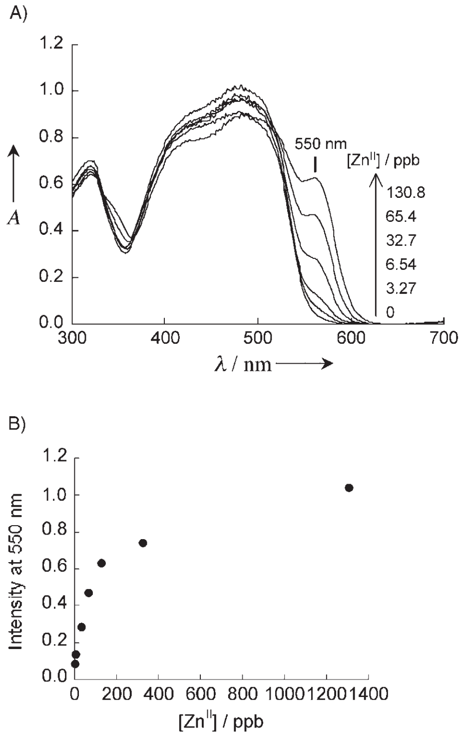

Figure 4. A) Change in reflectance-absorption spectra of a PAN-coated membrane on dipping into Zn^{II} solutions of various concentrations (pH 8.4). B) Calibration curve obtained by plotting the intensity at 550 nm.

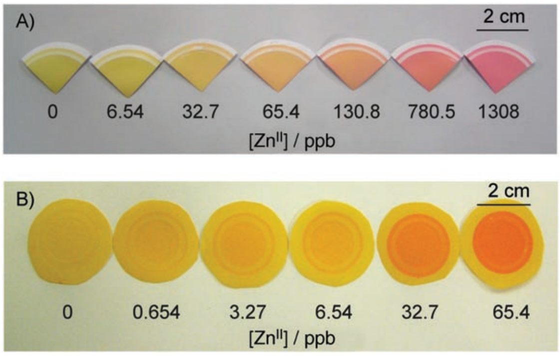

Figure 5. Detection of Zn^{II} by PAN-loaded membrane by the dip and filtration methods. A) Dip method: a test strip was dipped into 10 mL of aqueous Zn^{II} solution at pH 8.4 for 15 min. B) Filtration method: 100 mL of aqueous Zn^{II} solution at pH 8.4 was filtered through the PAN-coated membrane at a flow rate of 6.9 mL min^{-1}.

We also examined a Bathophen-coated membrane for Fe^{II} detection. The test strip showed a marked red color in dip testing of Fe^{II} that was detectable by the naked eye down to 5.6 ppb (see Supporting Information).

In summary, firmly attached and uniform coatings of organic dye nanoparticles/nanofibers on a membrane filter were obtained without using an additional matrix. Key features of this method are its simplicity and applicability to a wide variety of hydrophobic organic dyes. Very thin and pure dye membranes provide a highly concentrated signaling surface, which leads to high sensitivity of this system. Moreover, water-permeable dye-coated membranes allowed naked-eye detection down to ppb concentrations of metal ions by combining filtration enrichment of samples with color signaling.

Experimental Section

Distilled and deionized water was used throughout this work. All analytical reagents were converted to homogeneously dispersed nanoparticles by the reprecipitation method. Typically, 100 μL of a 2 mmol dm^{-3} solution of TAN in acetone was injected into vigorously stirred (1000 rpm) water (10 mL). The pH of the aqueous phase was adjusted to 6.15 with MES buffer. The detection membranes were fabricated by filtration of the aqueous dispersion through a mixed cellulose ester type membrane filter (pore size: 0.1 μm, thickness: 150 μm, ∅ 47 mm, Advantec Co.) under suction. Then the membrane was washed with water and air-dried. The surface concentration of the dye was estimated to be 7×10^{-7} mol cm^{-2}. The concentration of dye can be adjusted in the range of 5×10^{-9} to 7×10^{-7} mol cm^{-2}. The particle size of the aqueous dispersion was analyzed by dynamic light scattering (DLS) before filtration. SEM observation was carried out after loading on a flat polycarbonate membrane filter (pore size: 0.1 μm, Advantec or pore size: 0.05 μm, Millipore).

Dip tests: The detection membranes were cut into 4–10 pieces, and then the test strips were immersed in a buffered (0.01 mol dm^{-3}) solution of appropriate pH. After change in color, the absorption spectra were measured by reflection-absorption spectrophotometry.

Filtration method tests: The detection membrane (∅ 25 mm) was sandwiched between separable filter holders (effective area: 3.14 cm^2). Sample solution (100 mL) containing pH-buffered metal ions was passed through the filter under suction. The color change was monitored by spectroscopy.

The size of nanosized dyes in dispersed solution was determined by DLS (Zetasizer Nano-S, Malvern). The size and morphology were observed by SEM (S-800, Hitachi) and XRD (M18XHF22, Mac Science). Reflection-absorption spectra were measured by a multichannel spectrophotometer (MCPD-3000, Photal Co.) equipped with an optical fiber as light source and signal receiver. The reflection absorbance A is defined by $A = -\log R/R_o$, where R is the reflectance of the colored sample and R_o is that of colorless substrate.[12] The relative intensity of the reflectance signal was determined by scanning the wavelength at appropriate sample point.

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[1] K. Seiler, *Ion-selective Optode Membranes*, Fluka, Buchs, Switzerland, **1993**, pp. 91–97.
[2] G. Orellana, A. M. Gomez-Carneros, C. D. Dios, A. A. Garcia-Martinez, M. C. Moreno-Bondi, *Anal. Chem.* **1995**, *67*, 2231.
[3] G. Caminati, E. Margheri, G. Gabrielli, *Thin Solid Films* **1994**, *244*, 905.
[4] Y. Zheng, J. Orbulescu, X. Ji, M. F. Andreopoulos, M. S. Pham, M. R. Leblanc, *J. Am. Chem. Soc.* **2003**, *125*, 2680.
[5] S.-H. Lee, J. Kumar, S. K. Tripathy, *Langmuir* **2000**, *16*, 10482.
[6] C. Pearson, J. Nagel, M. C. Petty, *J. Phys. D: Appl. Phys.* **2001**, *34*, 285.
[7] I. Oehme, O. S. Wolfbeis, *Mikrochim. Acta* **1997**, *126*, 177–192.
[8] H. Kasai, H. S. Nalwa, H. Oikawa, S. Okada, H. Matsuda, N. Minami, A. Kakuta, K. Ono, A. Mukoh, H. Nakanishi, *Jpn. J. Appl. Phys.* **1992**, *31*, L1132.

[9] H. Oikawa, H. Kasai, H. Kamatani, H. S. Nalwa, S. Okada, H. Matsuda, N. Minami, A. Kakuta, K. Ono, A. Mukoh, H. Nakanishi, *Chemistry of Functional Dyes, Vol. 2* (Eds.: Z. Yoshida, Y. Shirota), Mita Press, Tokyo, **1993**, pp. 755–758.

[10] K. Ueno, T. Imamura, K. L. Cheng, *Handbook of Organic Analytical reagents*, 2nd ed., CRC, Boca Raton, **1992**, pp. 209–214.

[11] K. Ueno, T. Imamura, K. L. Cheng, *Handbook of Organic Analytical Reagents*, 2nd ed., CRC, Boca Raton, **1992**, pp. 431–439.

[12] H. Yamamoto, T. Kurita, J. Suzuki, R. Hira, K. Nakano, H. Makabe, *J. Chromatogr.* **1976**, *116*, 29.