*Anal. Chem.* **2010,** *82,* 8402–8405

Letters to *Analytical Chemistry*

# Chromogenic Sensing of Biogenic Amines Using a Chameleon Probe and the Red−Green−Blue Readout of Digital Camera Images

**Mark-Steven Steiner, Robert J. Meier, Axel Duerkop, and Otto S. Wolfbeis***

*Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, 93040 Regensburg, Germany*

**We report on sensing spots containing an amine reactive chromogenic probe and a green fluorescent (amine insensitive) reference dye incorporated in a hydrogel matrix on a solid support. Such spots enable rapid and direct determination of primary amines and, especially, biogenic amines (BA). A distinct color change from blue to red occurs on dipping the test spots into a pH 9.0 sample containing primary amines. BAs can be determined in the concentration range from 0.01 to 10 mM within 15 min, enabling rapid, qualitative, and semiquantitative evaluation. In the "photographic" approach, the typically 4−7.5-fold increase in fluorescence intensity of the probe at 620 nm along with the constant green fluorescence at 515 nm of a reference dye are used for quantitation of BAs. The sensing spots are photoexcited with high-power 505 nm light-emitting diodes (LEDs) in a black box. A digital picture is acquired with a commercially available digital camera, and the color information is extracted via red−green−blue (RGB) readout. The ratio of the intensities of the red (signal) channel and the green (reference) channel yields pseudocolor pictures and calibration plots.**

Biogenic amines (BAs) are defined as low-molecular organic bases with aliphatic, aromatic, or heterocyclic groups and, in contrast to alkaloids, have at least one nonheterocyclic amino group. They are mainly generated during storage or processing of protein-rich seafood, fish, meat, and fermented products by thermal or bacterial enzymatic decarboxylation of the respective amino acids.[1] Histamine, tyramine, putrescine, cadaverine, spermidine, spermine, and ethanolamine are primarily found in foods. Hence, they are important indicators of food quality and hygiene.[2,3] Histamine poisoning with its allergy-like symptoms is a health risk to sensitive individuals. Its adverse effects are even potentiated in the presence of other BAs due to synergistic effects.[4−6]

Nevertheless, BAs occur regularly in metabolic pathways in low concentrations and have numerous functions in physiology and in the cell. They are found in ribosomes (cadaverine, putrescine) and sperm (spermine, spermidine) and form building blocks of phosphatides (ethanolamine), vitamins, and coenzymes (aminopropanol being a building block of vitamin B12 and cysteamine; $\beta$-alanine being a building block of coenzyme A). Elevated levels of biogenic amines are presumed biomarkers for certain tumors[7−10] and for a number of diseases.[11,12]

Hence, research on the simultaneous and rapid analysis of BAs in a variety of biological matrixes is of widest interest, and inexpensive, rapid, and simple methods are sought. High-performance liquid chromatography (HPLC),[13] capillary electrophoresis (CE),[14] and gas chromatography (GC)[15] in combination with various derivatization procedures are among the most important instrumental methods for precise quantitative analysis of biogenic amines. However, these methods often are time-consuming and require considerable skill. Thin-layer chromatography (TLC)[16] and enzymatic[17] and immuno-enzymatic[18] methods are quite common and suitable for routine analysis with semiquantitative and quantitative determination of BAs. Most commercially available test kits for histamine rely

* To whom correspondence should be addressed. E-mail: Otto.Wolfbeis@chemie.uni-r.de. Fax: +49-941-943-4064.

(1) Silla Santos, M. H. *Int. J. Food Microbiol.* **1996**, *29*, 213–231.
(2) Paleologos, E. K.; Kontominas, M. G. *Anal. Chem.* **2004**, *76*, 1289–1294.
(3) Önal, A. *Food Chem.* **2007**, *103*, 1475–1486.
(4) Taylor, S. L.; Stratton, J. E.; Nordlee, J. A. *Clin. Toxicol.* **1989**, *27*, 225–240.
(5) García-Acosta, B.; Comes, M.; Bricks, J. L.; Kudinova, M. A.; Kurdyukov, V. V.; Tolmachev, A. I.; Descalzo, A. B.; Marcos, M. D.; Martínez-Máñez, R.; Moreno, A.; Sancenón, F.; Soto, J.; Villaescusa, L. A.; Rurack, K.; Barat, J. M.; Escriche, I.; Amorós, P. *Chem. Commun.* **2006**, *21*, 2239–2241.
(6) Latorre-Moratalla, M. L.; Bosch-Fusté, J.; Lavizzari, T.; Bover-Cid, S.; Veciana-Nogués, M. T.; Vidal-Carou, M. C. *J. Chromatogr., A* **2009**, *1216*, 7715–7720.
(7) Eccleston, D.; Crawford, T. B. B.; Ashcroft, G. W. *Nature* **1963**, *197*, 502–503.
(8) Toninello, A.; Pietrangeli, P.; De Marchi, U.; Salvi, M.; Mondovi, B. *Biochim. Biophys. Acta* **2006**, *1765*, 1–13.
(9) Kubota, S.; Okada, M.; Imahori, K.; Ohsawa, N. *Cancer Res.* **1983**, *43*, 2363–2367.
(10) Medina, M. A.; Urdiales, J. L.; Rodríguez-Caso, C.; Javier Ramírez, F.; Sánchez-Jiménez, F. *Crit. Rev. Biochem. Mol. Biol.* **2003**, *38*, 23–59.
(11) Xu, C. X.; Marzouk, S. A. M.; Cosofret, V. V.; Buck, R. P.; Neuman, M. R.; Sprinkle, R. H. *Talanta* **1997**, *44*, 1625–1632.
(12) Banchaabouchi, M. A.; Marescau, B.; D'Hooge, R.; Engelborghs, S.; De Deyn, P. P. *Amino Acids* **2000**, *18*, 265–277.
(13) Özdestan, Ö; Uren, A. *Talanta* **2009**, *78*, 1321–1326.
(14) Steiner, M.-S.; Meier, R. J.; Spangler, C.; Duerkop, A.; Wolfbeis, O. S. *Microchim. Acta* **2009**, *167*, 259–266.
(15) Awan, M. A.; I. Fleet, I.; Thomas, C. L. P. *Food Chem.* **2008**, *111*, 462–468.
(16) Lapa-Guimarães, J.; Pickova, J. *J. Chromatogr., A* **2004**, *1045*, 223–232.



10.1021/ac102029j 
Published on Web 09/17/2010

on immuno-enzymatic techniques. Recently, an interesting concept using polymer layers with different changes in absorption due to interaction with aliphatic, aromatic, and polyamines was reported.[19] Few colorimetric methods using test spots are known.[20,21] These can be semiquantitatively evaluated by visual readout of the originated color in comparison to a reference color scale. However, quantitative determination of BAs with this method requires sophisticated (reflectance-based) readout. Colorimetric sensor arrays consisting either of several amine sensitive dyes[22,23] or organic liquid crystals[24] also have been used for the determination of volatile amines. Computer-based methods have been applied for qualitative estimation (via pattern recognition) and quantitative determination (via principal component analysis). One recent trend in optical sensor technology involves the use of simple and readily accessible light sources like computer screens in combination with low-tech mobile phone cameras[25−27] or light-emitting diodes (LEDs) and commercially available digital cameras with subsequent readout of the red−green−blue (RGB) information.[28]

We are presenting here new chromogenic and fluorogenic dry-chemistry sensing spots based on filter paper containing an amine-reactive chromogenic probe and fluorescein as a green fluorescent (but amine insensitive) reference dye incorporated in a hydrogel matrix. These test spots can quantitate biogenic amines upon dipping into the sample. The test spots were evaluated with six different biogenic amines at concentrations between 0.01 and 10 mM using the RGB readout option of a digital camera.

## EXPERIMENTAL SECTION

**Preparation of Sensor Spots.** The general procedure for the preparation of the paper strips is described in detail in the Supporting Information. In short, a "sensor cocktail" was prepared by adding the probe Py-1 and fluorescein to a solution of the polymer Hypan (a polyacrylamide-co-polyacrylonitrile copolymer) in dimethylsulfoxide (DMSO). The mixture was shaken for 20 min at 60 °C. Square pieces of filter paper were then immersed in this solution until saturation and dried at 80 °C in an oven for 45 min to evaporate the DMSO. The paper strip was then washed with distilled water to remove an excess of Py-1 that may adhere to the surface of the sensing paper. After drying, round sensing spots with a diameter of 6 mm were cut from the square filter paper via a metal hole puncher.


(17) Alonso-Lomillo, M. A.; Domínguez-Renedo, O.; Matos, P.; Arcos-Martínez, M. J. *Anal. Chim. Acta* **2010**, *665*, 26–31.
(18) Rogers, P. L.; Staruszkiewicz, W. F. *J. Aquat. Food Prod. Technol.* **2000**, *9*, 5–17.
(19) Nelson, T. L.; Tran, I.; Ingallinera, T. G.; Maynor, M., S.; Lavigne, J. J. *Analyst* **2007**, *132*, 1024–1030.
(20) Hall, M.; Eldridge, D. B.; Saunders, R. D.; Fairclough, D. L.; Bateman, R. C., Jr. *Food Biotech.* **1995**, *9*, 39–57.
(21) Patange, S. B.; Mukundan, M. K.; Ashok Kumar, K. *Food Control* **2005**, *16*, 465–472.
(22) Rakow, N. A.; Avijit, S.; Janzen, M. C.; Ponder, J. B.; Suslick, K. S. *Angew. Chem., Int. Ed.* **2005**, *44*, 4528–4532.
(23) Tang, Z.; Yang, J.; Yu, J.; Cui, B. *Sensors* **2010**, *10*, 6463–6476.
(24) Sutarlie, L.; Quin, H.; Yang, K.-L. *Analyst* **2010**, *135*, 1691–1696.
(25) Filippini, D.; Alimelli, A.; Di Natale, C.; Paolesse, R.; D'amico, A.; Lundström, I. *Angew. Chem., Int. Ed.* **2006**, *45*, 3800–3803.
(26) Lim, S. H.; Feng, L.; Kemling, J. W.; Musto, C. J.; Suslick, K. S. *Nature Chem.* **2009**, *1*, 562–567.
(27) Rakow, N. A.; Suslick, K. S. *Nature* **2000**, *406*, 710–713.
(28) Wang, X.-D.; Meier, R. J.; Link, M.; Wolfbeis, O. S. *Angew. Chem., Int. Ed.* **2010**, *49*, 4907–4909.


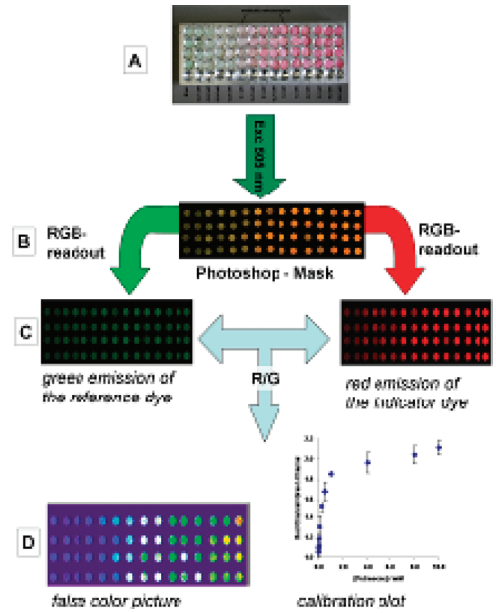


**Figure 1.** Readout scheme. (A) Photograph of sensing paper. Calibration of tyramine (TY; $n = 4$); 15 concentrations from 0 to 10 mM; photo taken after 20 min. (B) Photo taken with illumination via 505 nm LEDs and orange glass filter. Adjustment of color temperature to 2550 K and masking. (C) RGB readout; the green channel contains the intensity information of fluorescein, while the red channel contains that of the Py-1 TY conjugate. (D) Direct picture calculation (red channel picture divided by green channel picture) delivers pseudocolor picture (bottom left). Evaluation of the histographic information of each sensor spot of the red and green channel picture yields calibration plots (bottom right).

**Standard Procedure for Determination of Biogenic Amines.** The dry, blue colored round sensing spots were dipped for 30 s into 200 $\mu$L of a pH 9.0 sample containing the BA. The color was allowed to develop for 15 min on a glass plate. The spots were dried on a paper towel for ca. 5 min and then immediately submitted to photography. Stable fluorescence signals are obtained after this period.

A commercially available digital camera (Canon EOS 350D) equipped with a standard 18−55 mm objective with orange glass filter was used for sample determination throughout. More precise information about the camera adjustment can be found in the Supporting Information. The camera was fixed on top of a black box. A template to hold the test spots was placed in the box and illuminated from the top with six turquoise high power (1 W) LEDs with peak emissions at 505 nm. Both the amine−Py-1 conjugate and the reference dye (fluorescein) are excited at these wavelengths, but their Stokes' shifts are highly different in that their emission peaks at 620 and 510 nm, respectively (Figure S-2, Supporting Information).

**Data Evaluation.** The general procedure for data evaluation is schematically given in Figure 1, and more detailed information is in the Supporting Information and in the literature.[28] Briefly, photos were taken of the LED-illuminated sensing spots in RAW format (Figure 1A). A black mask layer was added around the sensing spots to suppress undesired light scattering, and the image was saved as 16-bit color TIF file (Figure 1B). The red, green, and blue channel information was extracted via ImageJ software (NIH, Bethesda, MD) available free of charge at http://rsbweb.nih.gov/ij/. The blue channel contained virtually no

**Table 1. Regression (Expressed as the Ratio R/G) of the Calibration Plots ($n = 4$), Limits of Detection (LODs, in mM), and Linear Ranges (in mM)**

| amine | regression | $R$ | LOD | linear range |
|---|---|---|---|---|
| TY | 0.93 L mmol$^{-1}$ [TY] + 1.30 | 0.975 | 0.02 | 0.04−1.0 |
| HI | 0.35 L mmol$^{-1}$ [HI] + 1.41 | 0.970 | 0.10 | 0.25−2.0 |
| PU | 0.60 L mmol$^{-1}$ [PU] + 1.24 | 0.959 | 0.04 | 0.05−1.0 |
| EA | 0.31 L mmol$^{-1}$ [EA] + 1.04 | 0.970 | 0.25 | 0.5−2.0 |
| $\beta$AL | 0.07 L mmol$^{-1}$ [$\beta$AL] + 1.23 | 0.965 | 0.75 | 0.5−2.0 |
| SP | 0.57 L mmol$^{-1}$ [SP] + 1.49 | 0.962 | 0.05 | 0.1−1.0 |

information due to the transmittance of the orange glass filter. Data from the red channel contained the emission intensity information of the amine−Py-1 conjugate. The green channel contained the emission intensity information of the reference dye (fluorescein; Figure 1C). Finally, the red channel was divided by the green channel delivering a pseudocolor picture that is easily evaluated semiquantitatively by the eye (Figure 1D, left). Additionally, histogram information from the red and green channel was used for quantitative determinations and calibration plots (panel D in Figure 1).

## RESULTS AND DISCUSSION

The amine reactive probe Py-1 was chosen because of its chromogenic and fluorogenic properties. It is blue and virtually nonfluorescent in its nonconjugated form but shows a dramatic color change to red accompanied by a strong increase in fluorescence intensity when covalently reacted with primary amino groups. Its reactivity toward proteins was studied in great detail.[29−31] In order to overcome this "interference problem" with proteins on determination of biogenic amines, we investigated several hydrogels on their protein-shielding or BA-selective-filtering features, respectively. Finally, the poly(acrylonitrile)-based hydrogel Hypan was chosen as polymer matrix, first, because it is a good solvent for the amine probe and the reference dye. Second, and most importantly, there is virtually no undesired reaction of proteins with the amino reactive probe when combining Hypan and Py-1 as shown with HSA as model protein (vide infra).

The home-built setup and the sensing spots were tested with the following six biogenic amines: ethanolamine (EA), histamine (HI), tyramine (TY), putrescine (PU), spermidine (SP), and $\beta$-alanine ($\beta$AL); each at 14 different concentrations (0.01, 0.02. 0.04, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, and 10 mM); and with four test spots for each concentration. All measurements were conducted as described in the Experimental Section and the Supporting Information. The particular amines were chosen to cover mono- and polyamines from the aliphatic, aromatic, and heteroaromatic class of BAs to show the applicability of the method toward various kinds of biogenic primary amines. Table 1 shows that, not unexpectedly, there are distinct differences in the sensitivity of the probe Py-1 toward various BAs (as can be seen from the differences in the slope of the regression equation). The clinically relevant concentration range of BAs is between 0.3 and 1.0 mM.[19] Biogenic amines in concentrations below 1.0 mM are not detectable by the human nose in most cases but may be indicative of food spoilage and, hence, represent a health risk. Table 1 shows that the linear range for the quantitative determination of the amines tested with our setup matches this critical range of interest. Figure S-3 in the Supporting Information shows a calibration plot of TY obtained with the standard operational protocol.

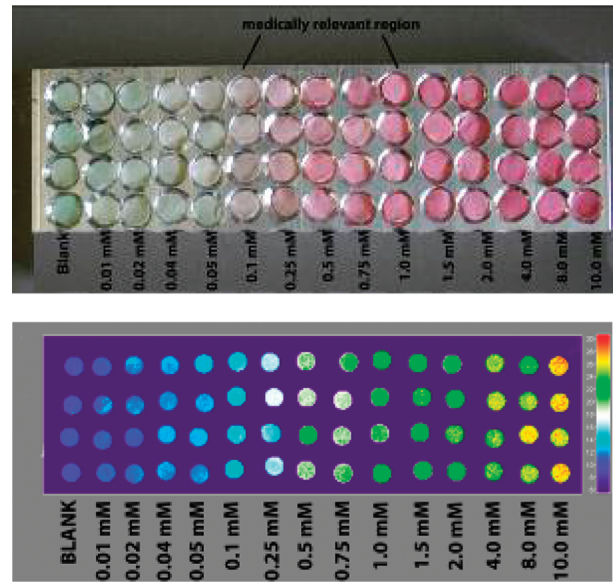


**Figure 2.** (Top) Photographs of the sensing spots aligned in the black box and showing calibration series of TY from 0 to 10 mM ($n = 4$). (Bottom) Pseudocolor picture of the photo after RGB readout and digital processing.

The sensing spots are greenish (the prevailing color of fluorescein) at the lowest amine concentrations of this range. At a concentration of 1 mM of amine, however, the spots show a pink color, and at even higher concentrations of BA, the color changes to a deep red (Figure 2A). This would clearly indicate a potentially toxic amine concentration, even to the untrained home user. Most biogenic amines are visually verifiable down to 0.25 mM. The BAs are instrumentally detectable with high sensitivity and limits of detection (LOD) below 0.1 mM, except for $\beta$-alanine. This amine has a carboxy group that probably interferes with the binding of its amino group to Py-1. This observation is similar to those of the interference tests with amino acids (vide infra).

The LOD is defined as the analyte concentration yielding a red/green (R/G) signal equal or higher than the average value produced by the blank sample, plus three standard deviations. The linear calibration range covers 1 to 2.5 decades of [BA]. Saturation of the red color is observed at concentrations of BA above 2 mM. This is most likely due to the limited quantity of Py dye available for the reaction. At least four test spots from the same batch were used in each experiment. The overall accuracy was 10% ± 2.5%.

Next, we have investigated the reaction of the sensor spots with amino acids, thiols, proteins, secondary amines, tertiary amines, and ammonia in a 1 and 10 mM concentration, each. Additionally, the response of the spots was also tested in the presence of 1 mM EA and 10 mM of the above-mentioned potential interfering agents. The results are summarized in Table S-1,

(29) Hoefelschweiger, B. K.; Duerkop, A.; Wolfbeis, O. S. *Anal. Biochem.* **2005**, *344*, 122–129.
(30) Craig, D. B.; Wetzl, B. K.; Duerkop, A.; Wolfbeis, O. S. *Electrophoresis* **2005**, *26*, 2208–2213.
(31) Wetzl, B. K.; Yarmoluk, S. M.; Craig, D.; Wolfbeis, O. S. *Angew. Chem., Int. Ed.* **2004**, *43*, 5400–5402.

Supporting Information. Secondary and tertiary amines like dimethylamine (DMA) and trimethylamine (TMA) are not able to form a red conjugate with Py-1 (as do primary amines). They slowly react (compared to primary amines) to yield a rather sallow colored and nonfluorescent reaction product. A 10-fold molar excess of DMA quenches the R/G value of EA only by 33%. TMA decomposes the label to a higher degree compared to DMA (negative values of the blank-corrected R/G value). Nevertheless, EA can be determined even in the presence of a 10-fold molar excess of TMA.

The amino acids cysteine (CYS), lysine (LYS), and serine (SER) show weak interference on the determination of EA only at concentrations of >10 mM. LYS contains an $\varepsilon$-amino group that conceivably can react like an amino group of a biogenic amine and, therefore, yield the highest fluorescence signal of all three amino acids tested. However, the R/G ratio at a concentration of 10 mM LYS is not as high as the R/G ratio of a 1 mM solution of EA. Dithiothreitol (a dithiol) also showed virtually no reaction with the sensing spots. HSA was used as model protein but gave no color change. Obviously, the polymer matrix is capable of shielding off the protein even at concentrations as high as 1 mg/mL. Finally, ammonia was tested for interference with the sensing spots. The red/green ratios in the presence of 1 and 10 mM ammonia are comparable to those of TMA. However, EA was successfully determined even in the presence of a 10-fold molar excess of ammonia.

The operational stability of the sensing spots is at least 3 months when stored in the dark in sealed glass vials at ambient temperature. The color intensity of reacted sensing spots is stable for 24 h at least.

## CONCLUSIONS

A self-referenced portable and rapid test for biogenic amines is presented here that is based on a hydrogel matrix and an amine-reactive dye. On one hand, the sensing spots can be visually evaluated semiquantitatively via comparison to a calibration color scale. On the other hand, sensitive and more precise quantitative analysis of the spots can be performed via an inexpensive home-built setup using a RGB readout method of a digital image taken with a commercial digital camera. The sensor spots show high selectivity for biogenic amines and suffer from minor interferences only of ammonia and secondary and tertiary amines. The sensing system may be used for rapid screening for total biogenic amines in food or body fluids. This will represent a substantial advantage over the common immuno-enzymatic method that focuses on the determination of a single BA (mostly HI) because the spoilage of food is a process that is not restricted to the formation of only one single BA. Thus, the sensor spots represent an attractive alternative to existing schemes for sensing biogenic amines.[32] Its digital read-out makes it more robust, and the use of conventional cameras goes along current trends toward simplified methods for absorption-based and emission-based detection schemes.[25,33] The method also may enable high-throughput analysis and in-field examinations and does not require sophisticated instrumentation or trained personnel.

(32) Mcdonagh, C.; Burke, C. S.; Maccraith, B. D. *Chem. Rev.* **2008**, *108*, 400–422.
(33) Wang, X.; Chen, H.; Zhao, Y.; Chen, X.; Wang, X. *Trends Anal. Chem.* **2010**, *29*, 319–338.

## ACKNOWLEDGMENT

M.-S.S. thanks the German-Egyptian Research Fund for a grant within the Project EGY 08/004.

## SUPPORTING INFORMATION AVAILABLE

Materials; preparation of filter paper-based sensing spots; home-built setup for fluorescence-readout via RGB-signal with digital camera; data evaluation; optimization of the sensing spots; choice of indicator dye; choice of polymer; Figure S-1. Composition of the sensing paper; Figure S-2. Emission spectra; Figure S-3. Calibration plot; Table S-1. Effect of possible interfering substances on the sensing paper. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review July 30, 2010. Accepted September 14, 2010.

AC102029J