Immobilized Poly(aryleneethynylene) pH-Strips Discriminate Different Brands of Cola

Markus Bender,†[a] N. Maximilian Bojanowski,†[a] Kai Seehafer[a] and Uwe H. F. Bunz[a,b]*

Abstract: Fluorescent, water-soluble poly(*p*-aryleneethynylene)s (PAE) were immobilized on commercially available nylon membranes using a slot plotter, creating fluorescent, barcode-like sensor strips. Digital imaging, using a standard digital camera, before and after immersion of the strips in buffers of different pH, displays a unique color for each pH value. Statistical evaluation - MANOVA and PCA - of the acquired data reveals that the immobilized PAEs are superior to the identical fluorophores when dissolved. The pH sensor-array discriminates 20 different brands of commercial cola soft drinks through differences in pH and colorant-PAE-interactions.

Water soluble poly(aryleneethynylene)s (PAE) are useful for the assembly of hypothesis free sensor arrays.[1,2,3,4] Ionic PAEs and poly(phenyleneethynylene)s PPEs change fluorescence quantum yield and/or emission color with the pH-value.[5] Negatively charged PAEs/PPEs show quenching when going to < pH 4, due to protonation and concomitant aggregation of the now neutral chains.[6] Here we immobilized the PAEs (Figure 2) and employ these as pH and cola-sensors.

In a first experiment we immobilized the PAEs onto cellulose strips; upon immersion into water, the PAEs leached out. Slot-casted PAEs on neutral nylon membrane (20 µL of a solution of 100 mg/L, Figure 1, SI) following established procedures afforded barcode-like PAE strips which do not dissolve upon immersion into water. Under daylight the strips appear yellow, but under UV-light all of the PAEs are brightly fluorescent *even the ones that are non-fluorescent in water such as* ***P7***.

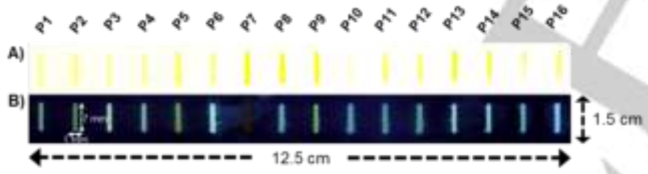

Figure 1. A) PAE strip (DI water), illuminated under daylight and **B)** illuminated under UV-light at $\lambda = 365$ nm and recorded with a digital camera at a shutter speed of 1/20 s, aperture of 2.8 and film sensitivity ISO 100. Images (file format: RAW, color space: ProPhotoRGB) were processed using Adobe Photoshop® software. For camera setup see the Supporting Information.

In Figure 1 the emission of **P7** appears weak, an artefact of the photography. To the eye, the emission appears bright orange. We exposed aqueous solutions and the strips of the 16 PAEs to buffers of different pH-values (SI, Figure S7). In the acidic range the changes in the emission color of the PAEs are distinct, much more so than if we look at the emission change in solution. The scree-plot (Figure 4) shows the increased variance of the immobilized sensor elements, compared to that of the solution-based system, quantifying our observation that the immobilized species are excellent, reversible pH-sensors.

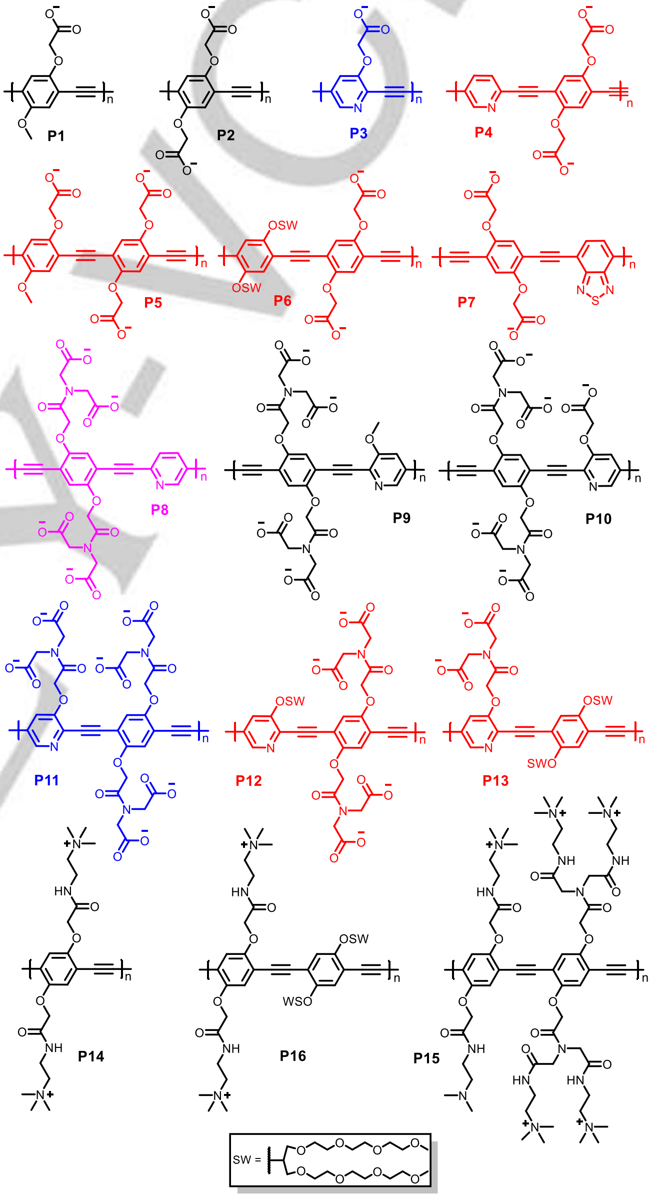

Figure 2. PAEs prepared according to standard Sonogashira protocols and used in this study for slot-die coated fluorophore strips. Red and purple: Elements for soft drink recognition, Blue and purple: Elements for pH-strips.

The color differences are small at pH>7. The data were analyzed by MANOVA[7] leading to autocorrelation plots (SI, Figure S8,S9), which at pH 7, acidified to pH 1 and regenerated to pH 7 shows blacked diagonal squares, and corners (SI, Figure S10), the system should work as a reversible pH-sensor.

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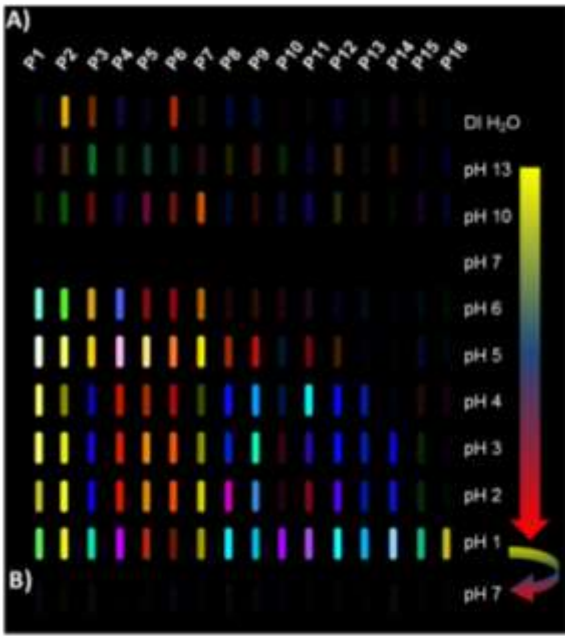

Figure 3. A) Color difference map, obtained by subtracting (absolute values) the red, green and blue color values of the strips after exposure to different buffer solutions from initial strips at pH7. **B)** Color difference map of a PAE strip after the recovery of its initial fluorescence at pH7. For effective visualization, the color range shown in these representations is expanded from RGB values of 20-83 (i.e., 6 bit) to 0-255 (i.e., 8 bit); the complete digital data is provided in the Supporting Information.

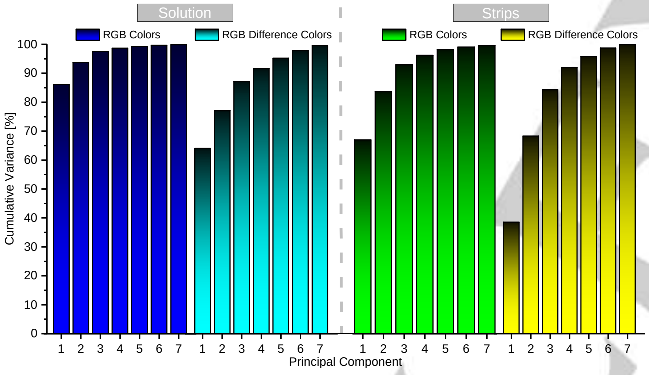

Figure 4. Scree plot, obtained by PCA (covariance) of the PAE solutions response **(left)** and of the strip based response **(right)**. The underlying dataset corresponds to the absolute RGB color values and the color difference map by subtracting the red, green and blue color values of the strips from initial strips at pH7 towards different pH-values. 48 elements, each r,g,b-value of **P1-16** is individually represented as an element.

Upon performing PCA[8,9] (48 elements, each r,g,b-value of P1-16 is individually represented as an element) using a Scree plot we determined that three different PAEs, all pyridine based, suffice to perform pH-sensing. Pyridine-PAEs (SI, Figure S7 A) show a red-shift in emission when going from neutral to acidic conditions in the strips. Pyridine units are protonated and increase the donor-acceptor character of the PAE with red shift in emission. In water one only observes quenching, but in the strips, emission persists. The same three PAEs have a considerably worse pH recognition when employed in water, both for low but also for high pH (Figure 5).

An excellent visualization of pH-induced color change is achieved by a color difference map, by taking the difference in color-channel data (RGB-values).[10,11] A color difference map before (control) and after exposure (initial) of images of the array discriminates all of the pH-values from pH1-13 (Figure 3 A). The pH-values are discriminated by eye, when using a PAE strip, but not when looking at the PAEs in solution (SI, Figure S11), and the scree plot indicates that the difference color map of the strips works best.

Comparison of the raw data with the information gleaned from the false color approach, employing the loading plot-coefficients via PCA, demonstrates that for the raw data PC 1 accounts for 67% of the variance, i.e. most of the signal is generated by quenching. PC 2 is weighed with only 17% (SI, Table S17). If the elements are selected according to the color variance, the analysis reflects the colorimetric analysis and the first three PCs are weighted with 39, 30 and 16% respectively (SI, Table S18). This finding supports the colorimetric approach over the analysis of the raw data, which are dominated by the quenching. A three-PAE-sensor array thus discriminates (Figure 5) the whole pH-spectrum and can be re-used multiple times after careful washing with water.

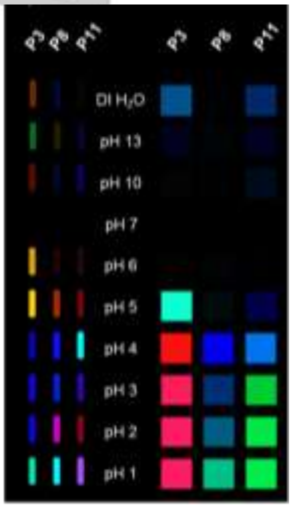

Figure 5. Three-element color difference map maintaining full discriminatory power of the pH-value of buffer solutions for the PAE strips (left) and the PAEs in solution (right).

An attractive application of our sensor strips was the discrimination of cola-type soft drinks, mainly consisting of water, sugars, natural flavoring, caffeine, phosphoric acid and colorants e.g. caramel color (E150d). Table 1 shows the investigated brands. pH alone does not suffice but is significantly different in the tested brands so that our full array might be able to discriminate successfully as all of the colas are deeply colored.

Table 1: Selected cola-type soft drinks and their pH-values.

Brand[a]	pH-Value[b]	Brand[a]	pH-Value[b]
Afri Cola	2.56	Dr. Pepper Cherry	3.08
Afri Cola Sugarfree	3.43	Dr. Pepper Light	3.02
Club-Mate Cola	2.48	Fritz-Kola	2.52
Coca-Cola	2.48	Fritz-Kola Sugarfree	2.94
Coca-Cola Cherry	2.47	Ja! Cola	2.39
Coca-Cola Life	2.58	Mio Mio Cola	2.42
Coca-Cola Light	2.56	Pepsi Cola	2.34
Coca-Cola Vanilla	2.58	Pepsi Light	2.61
Coca-Cola Zero	2.81	Red Bull Cola	3.27
Dr. Pepper Cola	2.73	Sinalco Cola	2.53

[a] Purchased at local store. For pictures, see SI. [b] Measured directly after opening.

Upon exposure of the cola-brands to our PAE-strips (SI, Figure S13) and a color difference map from difference in color-channel data (RGB values) before (control) and after exposure (initial) of images (SI, Figure S14), all brands are discriminated, despite the relatively small range of pH-values from 2.3-3.4.

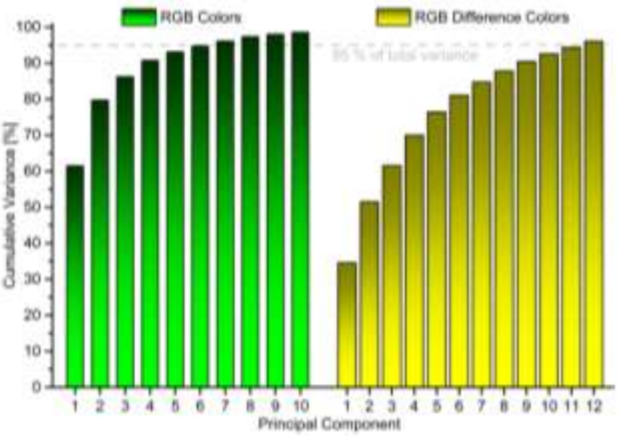

Figure 6. Scree plot, obtained by PCA (covariance) **(green)** of the strip based response (absolute RGB values) and **(yellow)** of the color difference map obtained by subtracting the red, green and blue color values of the strips from initial strips at pH7 towards different colas. (48 elements, each r,g,b-value of **P1-16** is individually represented as an element).

While the number of eigenvalues to achieve 95% discrimination of the colas is increased in comparison to that the of pH values, the main variance in this case is still the pH-value. Yet the broader distribution of the PCs is attributable to the complex nature of the cola analytes (Figure 6).

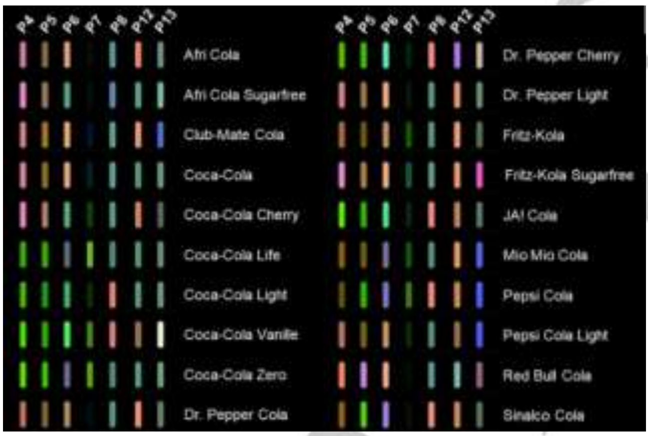

Figure 7. Reduced color difference map maintaining full discriminatory power, obtained by subtracting (absolute values) the red, green and blue color values of the strips immersed in different caffeine containing soft drinks for 30 seconds, from initial strips at pH7. For effective visualization, the color range is expanded from RGB values of 20-83 (i.e., 6 bit) to 0-255 (i.e., 8 bit); the complete digital data is provided in the Supporting Information.

Figure 7 shows the reduced color difference map containing seven elements with which the different cola brands are discriminated. It is noteworthy that some of the products of a specific brand are grouped together, while other products of the same brand display a significantly different false color response. That suggests that the recipes for different cola types (light, sugar free...) from one brand are more different than similar types from different producers. The colas contain – besides sugar, natural flavoring and phosphoric acid particularly caramel color (E150d); phosphoric acid and E150d are probably the main ingredients responsible for the color response of the strips. We assume that in these complex liquids the presence of sugar might also modulate the response of the strips.

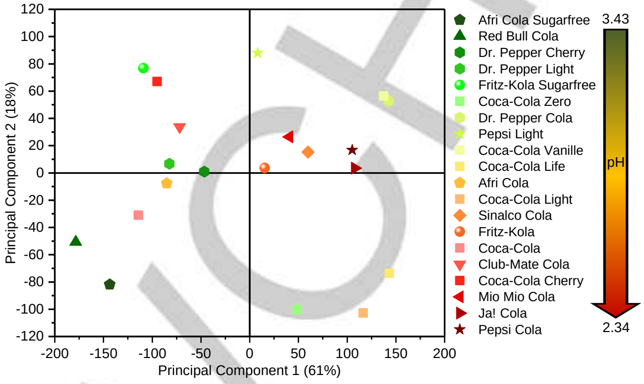

Figure 8. PCA score plot using the two most important principal components based on the absolute RGB color values of all colas. PC1 and PC 2 account for 79% of the total variance. As shown on the right the color code from green (pH3.5) to red (pH2.34) represents the acidity of the colas.

How is the signal for the colas generated – besides the pH? We produced and artificial cola (AC) by mixing water, caramel color, caffeine (10 mg/mL) and adjusted the pH to 2.7 – the average pH of all investigated colas; this AC leads to some fluorescence quenching of the strips. We added ingredients (sugars, sweeteners and vanillin) to the AC and compared the fluorescence response of AC with that of the AC plus the added ingredients. Figure 9 shows – in a color difference map that all of the additives modulate the quenching behavior of AC but each in a different way, therefore allows to discriminate them. This suggest that the response of the strips to the caramel color in the colas is modulated by the added sweeteners in addition to the change in pH.

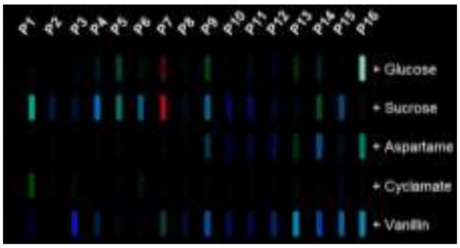

Figure 9. Color difference map obtained by subtracting (absolute values) the red, green and blue color values of the strips immersed in an artificial cola (caramel color, 10 mg/ml caffeine and phosphoric acid pH2.7) with separately added ingredients for 30 seconds, from initial strips solely immersed in artificial cola. For effective visualization, the color range is expanded from RGB values of 10-73 (i.e., 6 bit) to 0-255 (i.e., 8 bit); the complete digital data is provided in the Supporting Information.

In conclusion we have prepared nylon-membrane-based slot-die cast sensor strips, consisting of charged and pyridine-containing PAEs. Strips with three elements act as elegant and reversible pH-sensors, while strip-arrays made from seven PAEs reliably discriminate cola-type soft drinks without any problem. For the pH-strips, the presence of the protonatable pyridine unit is important, while for the colas a wider range of PAE-based elements (Chart 1, red and purple elements) are necessary. The

signal generation for the soft drinks is partly due to the pH-differences and part due to the caramel color and the other ingredients (sweeteners, sugars, etc.) that interact with the sensor strips via electrostatic or hydrophobic interactions.[12]
Overall the immobilization of the PAEs and the employ of a simple camera[13] is a significant step towards more practical applications of PAE-based arrays in hypothesis-free sensor applications.[14]

Experimental Section

The protocols to prepare all PAEs, more experimental details (UV, measurements, photography setup, etc.) and a full digital database for the analytes is provided in the Supporting Information.

Acknowledgements

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Keywords: sensing, slot-die coating, pH-sensing, PAEs

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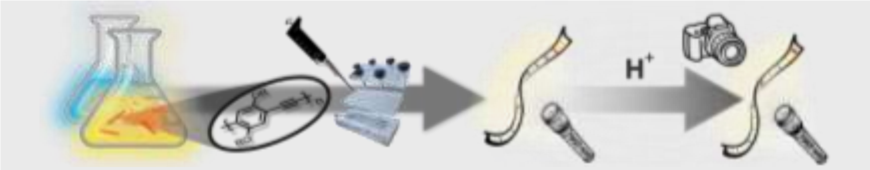
COMMUNICATION



Poly(aryleneethynylene)s were immobilized on nylon membranes to afford cost effective, reusable strips, which were successfully applied as pH sensor and discriminate different brands of cola.

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