Sensors

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Identification of White Wines by using Two Oppositely Charged Poly(*p*-phenyleneethynylene)s Individually and in Complex

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Abstract: *We present a simple array composed of an anionic and a cationic poly(para-phenyleneethynylene) (PPE), together with an electrostatic complex between the two of them. The individual PPEs and the PPE complex were employed in the sensing of white wines at pH 13; the complex was also successfully employed as a sensor element at pH 3. The sensing mechanism is fluorescence quenching. Thirteen different wines were differentiated by this chemical tongue, which consists of four elements. The fluorescence quenching is not induced by the major components of the wines. Compounds such as acids, sugars, and alcohols alone do not quench the fluorescence, but rather the colored tannins and other polyphenols contained in wine are the main quenchers. However, the major constituents of wine significantly modulate the quenching of the PPEs by the tannins.*

In this contribution we disclose a simple array formed from two conjugated polyelectrolytes (one polyanionic, one polycationic) and an electrostatic complex between the two; these three elements can be used to differentiate white wines at pH 13 and pH 3 in a fluorescence-quenching-based assay.

Wine, which is fermented grape juice, is a complex mixture of sugars, acids, minerals, proteins, and natural dyes in a composition that varies but resembles the values shown in Table 1. Alcohol (10–16.5 vol%) and sugar content vary greatly, as do the amount and type of acids present in wines. Typical white wines are acidic with a pH range of 3.0–3.3.

Wines are a perfect test bed for the power of small arrays of colorimetric or fluorescence sensor arrays because:
1) There are thousands of different wines.
2) Wine as an analyte is available in abundance.
3) Wines can be grouped by grape varietal/blends of grapes, country and area of origin, producer, (designer) yeast, vintage, cooperage, etc.
4) Wine is a complex mixture of compounds, a significant number of which are present in trace amounts—perhaps not even known. They are metabolites of the yeast and probably reach into the thousands, giving the wine its specific body, taste, and smell.

Table 1: Composition of the artificial wine.

Type	Ingredient	Content
alcohols	ethanol	11.5%
	methanol	0.01%
saccharides	fructose	7 $g\,L^{-1}$
	glucose	3 $g\,L^{-1}$
	glycerol	7 $g\,L^{-1}$
acids	malic acid	2 $g\,L^{-1}$
	tartaric acid	3 $g\,L^{-1}$
	lactic acid	1 $g\,L^{-1}$
	citric acid	1 $g\,L^{-1}$
	acetic acid	1 $g\,L^{-1}$
vitamins	vitamin C	0.05 $g\,L^{-1}$
mineral nutrients	K^+	1 $g\,L^{-1}$
	Ca^{2+}	0.1 $g\,L^{-1}$
	Mg^{2+}	0.1 $g\,L^{-1}$

This complexity renders wines different from each other, and consequently one should be able to “fingerprint” wines with respect to their composition. High-priced wines have been counterfeited and relabeled, an annoying problem, particularly for cult wines. Well-known fakes are the Jefferson bottles of Bordeaux wines, purportedly produced for the third president of the US.[1] The addition of cheaper wines or juice from non-allowed grape varietals to fermenting wines of the Brunello or Burgundy type are tricks of the trade to increase the profit (Brunellopoli scandal, or Brunellogate)[2] of the producers and scam unsuspecting consumers. Consequently, simple fingerprint tests that use small amounts of wine (less than 5 mL) would be attractive.

Anslyn et al.[3] have developed a ternary colorimetric wine-sensor array, consisting of copper (II) and pyrocatechol violet (CPV) in the presence of different oligopeptides. The addition of flavonoids to these ternary complexes led to a change in the absorbance at 444 nm; a handful of the histidine-rich peptide/CPV complexes distinguish between the flavonoids. The same complexes can be used to differentiate different red wines, depending upon their grape varietals. In a newer publication, Anslyn et al. even present a method to make predictions about the composition of binary blends of grapes in wines.[4]

We are interested in conjugated, charged, water soluble polymers of the poly(*para*-phenyleneethynylene) (PPE) type[5] and their use in sensory applications for biological species, metal ions, and other analytes.[6] Very recently, we demonstrated that simple polyelectrolyte complexes formed from a cationic and an anionic PPE could be used to detect and identify the anions of carboxylic acids, diacids, and hydroxy acids.[7] The tested carboxylic acids are major components in (white) wines. As a consequence, we set out

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to test whether PPEs or their complexes could also differentiate white wines. In a first experiment, we employed the same set of complexes as we did for the sensing of carboxylic acids, but found that only the PPEs and their complexes shown in Figure 1 A were reactive towards wine 3 (Table 2), which we used as preliminary test sample. We checked the pH-dependence of the fluorescence responses of the three sensor species and found that PPE 1 and PPE 2 were best used

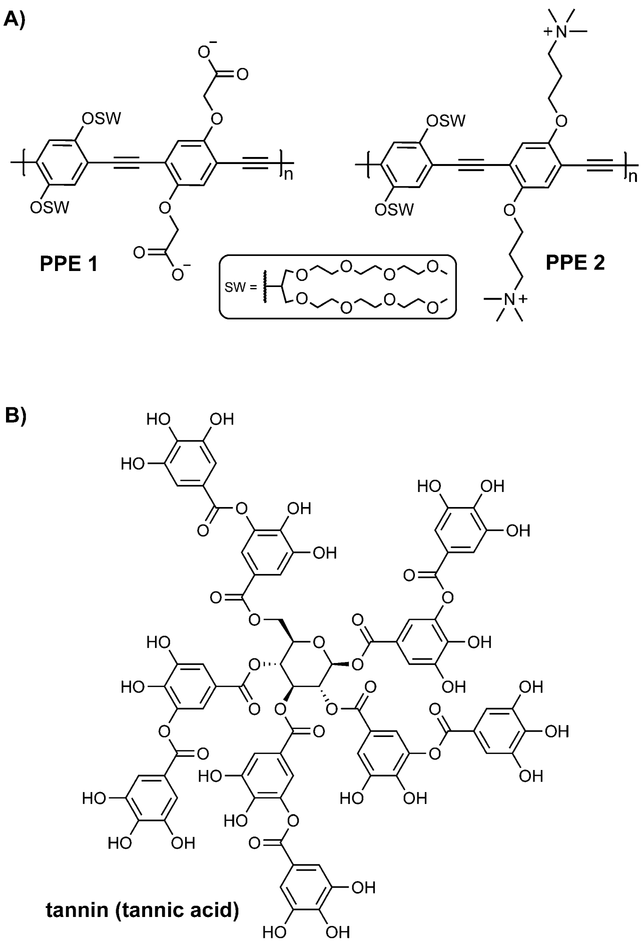

Figure 1. A) Structures of the negatively charged PPE 1 and positively charged PPE 2, which were used for white wine sensing. B) Structure of the tannin used (tannic acid).

at pH13, while a complex between the two worked both at pH3 and pH13. Consequently, we have a small sensor field consisting of four elements (Figure 2). In all cases we observed fluorescence quenching. Fluorescence enhancements were not observed with white wines, contrary to our experience when sensing carboxylic acids.

With the four sensor elements in place, we investigated a group of 13 different wines (Figure 3). While wine 1 was a red wine, all of the other wines were white wines. The wines 7 and 9–13 were Riesling wines (according to their label); the other wine samples were obtained from other grapes. An inherent difficulty is the use of different strains of *S. cerevisiae*, the metabolomes of which will strongly influence the sensory and analytical properties of the wines.

Table 2: Detailed information on the thirteen white wines used in this study.

Wine	Varietal	Origin	Vintage	pH	Sugar	EtOH content [%]
1	Spätburgunder	Baden Germany	2014	3.3	semidry	11.5
2	Pinot Grigio	Valdadige Italy	2014	3.2	dry	12.0
3	Müller Thurgau	Baden Germany	2014	3.3	semidry	11.0
4	Sauvignon blanc	Western Cape, South Africa	2015	3.1	dry	12.5
5	Chardonnay	Valdadige Italy	2014	3.0	dry	12.0
6	Grüner Veltliner	Burgenland Austria	2015	3.1	dry	11.5
7	Riesling	Pfalz Germany	2014	3.0	dry	11.5
8	Weißburgunder	Baden Germany	2014	3.2	dry	12.5
9	Riesling	Rheinhessen Germany	2014	3.0	dry	11.5
10	Riesling	Pfalz Germany	2014	3.1	semidry	11.5
11	Riesling	Baden Germany	2014	3.2	dry	12.0
12	Riesling	Baden Germany	2014	3.1	dry	11.5
13	Riesling	Pfalz Germany	2012	3.1	smooth/ sweet	10.0

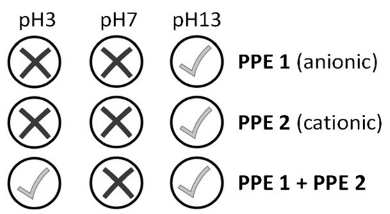

Figure 2. Screening of the previously selected PPEs at different pH values. The single PPEs PPE 1 and PPE 2 work best at pH13, while the electrostatic complex (PPE 1 + PPE 2) is successful at pH3 and pH13.

Figure 3 shows the results of the fluorescence quenching for the wines 1–13. Furthermore we successfully tested 6 different bottles of the same wine (wine 10) to ensure that the quenching behavior was not subject to artifacts (see the Supporting Information).

The fluorescence of the sensor elements is most strongly quenched by the red wine (1). But the white wines also show quenching. In the case of anions of lactic acid, mandelic acid, and tartaric acid (major components of wine), fluorescence turn-on of the PPEs was observed for most of the employed sensor-elements. As a consequence, we were surprised that there was no fluorescence turn-on of the sensor elements in any of the white wines. To find out whether the major components of the white wines would elicit any response towards the sensor elements, we created an artificial, colorless test wine with a composition described in Table 1. Exposure

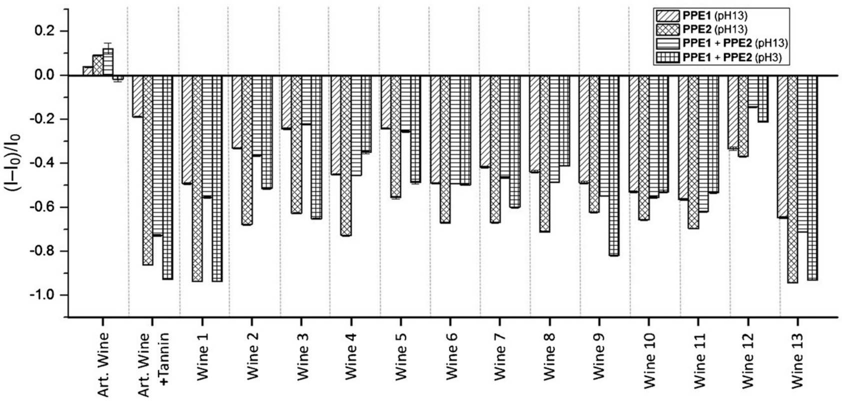

Figure 3. Fluorescence-response pattern ($(I-I_0/I_0)$) obtained with PPE 1, PPE 2 (2 μм, each at pH13, buffered), and the PPE 1/2 complex (each PPE 2 μм, at pH3 and pH13, buffered) treated with artificial (art.) wine (7 vol% for PPE 2, 33 vol% for the others, see Table 1), artificial wine plus tannin (0.1 $mg\,mL^{-1}$), and thirteen different commercially available white wines (7 vol% for PPE 2, 33 vol% for the others, see Table 2). Each value is the average of six independent measurements; each error bar shows the standard error of these measurements.

our sensor elements to this test wine (Figure 3) shows that a combination of the major components gives a small turn-on effect for three of the four sensor elements.

Artificial colorants for wines are commercially available. We spiked our artificial wine with commercial tannins (tannic acid, 0.1 $mg\,mL^{-1}$) and the artificial wine showed fluorescence quenching on a level quite similar to that observed for the tested red wine 1 (Figure 3). Our artificial wine resembled real wine in terms of sensory response once the tannin was added. We were interested to see whether the major components of the wine, shown in Table 1, would modulate the fluorescence response of the tannin-containing artificial wine. As can be seen in Figure 4, the fundamental quenching of the fluorescence of the PPEs in a simple water/ethanol/tannic acid mixture by the tannic acid is modulated by the added components that are present in white wines. If we look at Figure 3, we see similar patterns in the commercially available white wines. Even if we assume that the tannins and their related flavones all lead to fairly similar quenching, then differences in the major components modulate the quenching properties of the white wines. Glucose, fructose, and malic acid are the most potent modifiers of the fluorescence quenching properties of the water/ethanol/tannic acid solution in the presence of the PPEs.

Figure 5 contains the linear discriminant analysis (LDA) plots of all of the investigated wines 1–13; LDA converts the training matrix (4 sensor elements × 13 wines × 6 replicates) into canonical scores according to their Mahalanobis distance (see the Supporting Information). The jackknifed classification matrix with cross-validation reveals 100% accuracy. As a result, all of the wines are reliably differentiated by this simple four-element sensory array.

To further validate the efficiency of our sensing system, we established tests with randomly chosen white wines of our training set. The new cases were classified into groups generated from the training matrix, based on the shortest Mahalanobis distance to the respective group. Only 1 of 52 unknown wines was misclassified, representing an accuracy of 98% (also see the Supporting Information). The 3D LDA results from wines made from identical grape varietals (in this case the family of Riesling wines) weakly group together, as visualized by the Factor 2 and particularly the Factor 3 values (Figure 5). This is consistent with the results from Anslyn et al., who could show that LDA analysis for the discrimination of red wines would cluster, depending upon the grape varietals.[8]

In our case, the varietals only cluster to a moderate extent (wine 6 is similar to the Riesling wines). There could be a number of reasons for this. The most probable explanation is that the metabolome of the yeast and the cooperage change the composition of the white wines, such that the nature of the grape varietal loses importance in white wines and is "washed out" in the sensing results.

In conclusion, we were able to discriminate and differentiate white wines using a small array of two ionic PPEs and a complex between the two ionic PPEs. The PPEs function best at pH13, while the complex generates responses at pH 3 and at pH 13. The fluorescence response of the sensor elements to the wines is primarily due to the wine colorant, as demonstrated by the quenching behavior of a water/

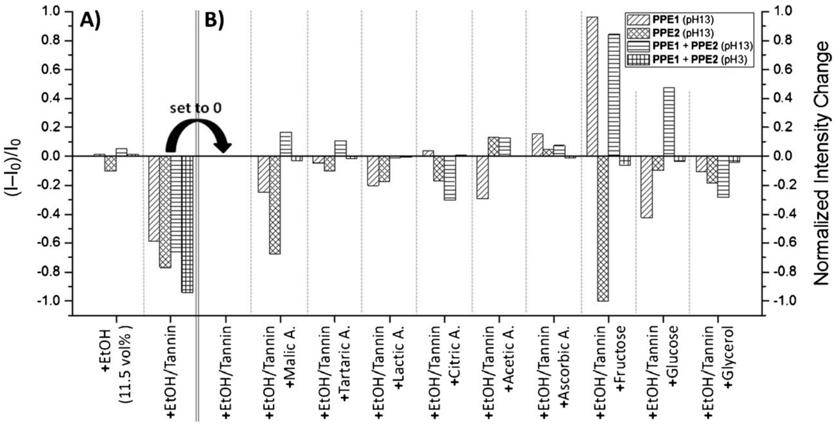

Figure 4. A) Fluorescence-response pattern ($(I-I_0/I_0)$) obtained with PPE 1, PPE 2 (2 μм, each at pH13, buffered), and the PPE 1/2 complex (each PPE 2 μм, at pH3 and pH13, buffered) treated with EtOH (11.5 vol%; first array). To this solution, tannin (0.1 $mg\,mL^{-1}$, second array) was added. B) First array: the results from (A), second array were set to 0. Remaining arrays: additional indicated ingredients (for final concentrations see Table 1, acid = A.) were added and the data shown (normalized) are the raw results minus the results from (A), second array. Each value is the average of three independent measurements.

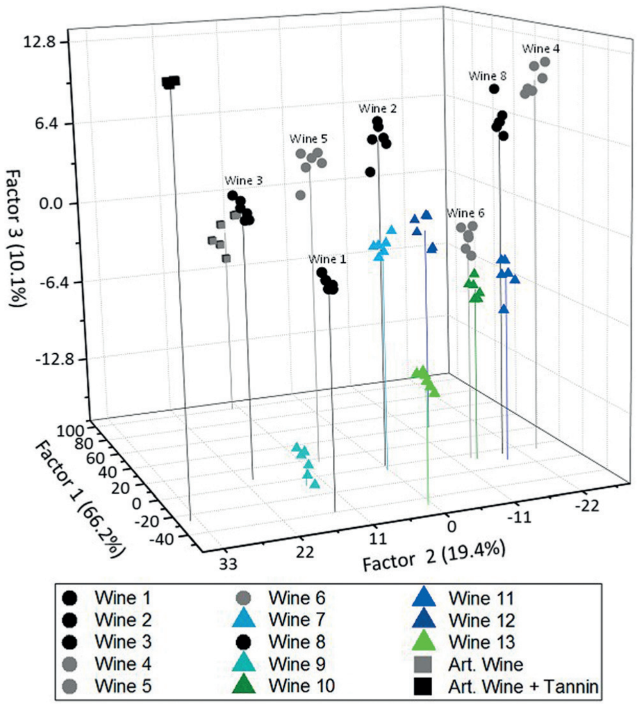

Figure 5. 3D canonical score plot for the first three factors of simplified fluorescence-response patterns obtained with an array of PPE 1, PPE 2 (each at pH13, buffered), and the PPE 1/2 complex (at pH3 and pH13, buffered). Each point represents the response pattern for a single wine to the array. The blue/green triangles represent the Riesling wines, the circles correspond to the other wines, and the squares indicate the artificial wine (with or without tannin).

ethanol/tannic acid mixture. The mixture quenches the fluorescence of the sensor elements like real wines do. The fluorescence quenching is modulated by the presence of the major components of the wines, such as sugars and acids. Particularly, fructose and malic acid are active, even though on their own, they do not modulate the fluorescence of the PPEs to any significant extent.

Our continuing commitment to conjugated polymers and their electrostatic complexes as sensory systems stems from their powerful sensing performance, facile, highly modular, and scalable synthesis, their great stability and relatively low cost.

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Keywords: fluorescence · linear discriminant analysis · poly(*para*-phenyleneethynylene)s · sensors · wine

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