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Fuzzy Liquid Analysis by an Array of Nonspecifically Interacting Reagents: The Taste of Fluorescence

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Supporting Information

ABSTRACT: Complex or unknown liquid analysis requires extensive instrumentation and laboratory work; simple field devices usually have serious limitations in functionality, sensitivity, and applicability. This communication presents a novel, effective, and simple approach to fingerprinting liquids. The method is based on nonspecific interactions of the sample liquid, a long lifetime luminescent europium label, and various surface modulators in an array form that is readily converted to a field analysis μTAS system. As compared to existing e-nose or e-tongue techniques, the method is unique both in terms of sensitivity and usability, mainly due to the well-known unique properties of the europium label. This communication demonstrates the use of this new method in distinguishing different wines, waters, alcohols, and artificially modified berry juices.

Liquid fingerprinting with an array of probes that have cross-reactivity (nonspecificity) has been demonstrated in the literature;[1,2] this communication demonstrates the combination of nonspecificity and time-resolved luminescence detection in a "fuzzy", simple, and low-cost disposable array as a tool for generic liquid analysis. The method is based on cooperative nonspecific interactions of the sample liquid, a long lifetime luminescent europium chelate label, and selected luminescence−modulator chemistry in an array form. On the contrary to typical e-nose and e-tongue techniques, the approach is not limited to detection of small molecules and ions but due to the cooperative action of the components; the whole composition of the sample affects the sensor fingerprint. With such an array almost anything from water to complex liquids and liquefiable solids may be analyzed by utilizing standard chemometric tools for the interpretation of the array signal.

The use of specific luminescent probes has the inherent sensitivity of a single molecule or aM in the molar scale, depending on the assay setup and reading instrument.[3,4] These assays are common in all fields of biosciences, but their advantage of specificity and sensitivity is also their pitfall—unknown (unexpectedly appearing) entities remain undetected. Further, in bioaffinity assays, each specific interaction is accompanied by nonspecific interaction, normally a nuisance that limits both specificity and sensitivity. On the other hand, nonspecific interactions have been utilized in bioanalysis, for example, to block other nonspecific interactions in assays by introducing a universal nonspecific reagent, such as BSA to the assay buffer and coatings, to detect total amount of DNA by the use of an intercalating dye.[5] Nonspecificity has also been used in recent works to create new types of bioassays for classification or concentration determination of cells, proteins, or detergents or for simplified time-resolved photoluminescence assays by the use of a nonspecific quencher.[6] Such nonspecific assay components are targeted to a broad range of molecular species, e.g. the total DNA content or entire population of unbound fluorophore. A nonspecific interaction may also be targeted to a certain feature of molecules or the sample carrying liquid, e.g. charge, hydrophobicity, and pH.

In pursuit of analyzing an unknown sample, instead of asking specific questions, one could also ask several "property-class" questions to clarify the sample content. If several of these generic features of sample liquid are probed, the sample begins to have form and shape. Anzenbacher et al. also discussed this same notion in their recent reviews.[1,7] We go beyond this and claim that we do not even need to know the property-class questions and can probe randomly. Let us assume that we wish to map an N-dimensional space uniquely. In any N-dimensional space it is sufficient to have N random axes to define any point in this N-space—the directions of these N axes and their scale are irrelevant as long as no plane is formed by more than two axes. Similarly we can probe the (bio)chemical space unambiguously by probing with random probes as long as each random probe obeys the above condition.

We have opted to probe this chemical "N-space" with an array of wells with semirandomly coated surfaces (modulators) and a nonspecific, long lifetime luminescent lanthanide chelate label. The unknown sample interacts nonspecifically in each well of this array with the modulators and the label (Scheme 1).

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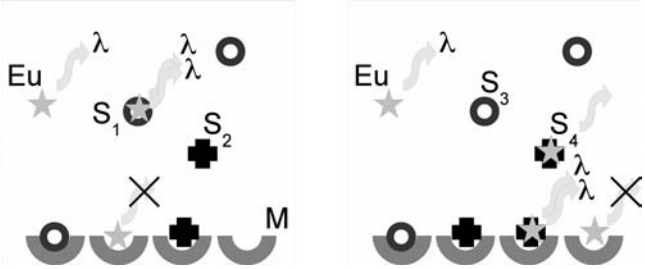
Scheme 1[a]



[a]The principles of the nonspecific array: Depending on the used modulator (M), the Eu-luminescence signal is modulated differently for each given sample (S_n). Possible mechanisms are: (1) Quenching (M or S_n quench the luminescence). (2) Protection from quenching (M or S_n protect the luminescence from quenching). (3) Enhancement (M or S_n stabilize the label and increase the luminescence quantum yield).

The long lifetime luminescence of the label is by its nature sensitive to these chemical interactions, thus providing the sensor signal; the modulated intensity of luminescence is recorded and analyzed from all the different wells. In the development phase of such an assay, microtiter plates and a standard time-resolved plate reader may be used. In the actual application the format is then transferred to a micro-total analysis system (μTAS) chip (Figure 1) containing the

Figure 1. Developed μTAS chip for the method. After coating, the chip was covered with a transparent foil-tape.

equivalent wells and the required liquid channels for sample input. In the standard protocol of the method, the modulators are dispensed into the wells and dried for future use. These dried arrays can then be used at any time to fingerprint samples. The selection procedure for the chemistry is also rather straightforward. At first, different modulator candidates are selected, e.g. on the basis of availability, solubility, or chemical properties. These candidates can be almost anything, e.g. detergents, polymers, metal salts, peptides, proteins. The candidates are then coated to the assay plates and tested in application simulation. The number of candidates is then reduced in an optimization round that aims for a low coefficient of variation, high modulation, and optimal concentration of the coating agent. A further selection parameter is the correlation with other candidates to obey the rules of mapping the "*N*-space". The protocol for actual coating and the modulators used in this communication are given in Table 1 of the Supporting Information [SI].

The fingerprinting assay protocol is simple: the prepared sample is diluted with europium-containing solution and dispensed into the wells, incubated for a few minutes, and read by a TRF-reader (see SI for the exact details). In the assay development phase this reader can be any TRF plate reader. For the application with μTAS chips we have developed a simple and low-cost TRF reader. The data from the measurements are then analyzed. In the example cases of this communication we have calculated the principal component analysis (PCA) to be able to display the data in two dimensions. In all examples, the two first principal components represent more than 90% of all the variance within the wells. Depending on the actual application, the data may also be analyzed by, for example, regression methods and different clustering or classification algorithms.[8]

The basic idea of fingerprinting is to compare known samples against unknowns. A good method is able to detect both minute differences and substitutions as well as tolerate significant changes in the sample without method saturation; these types of comparisons can aid, for example, in detection of quality problems or adulteration to ensure safety of foodstuff and beverages. With this in mind, the performance of the new method was studied in differentiating red wines, bottled waters, cola-drinks, vodkas, and artificially modified chokeberry juices. Although the testing materials were genuine and legally marketed products, we believe that our demonstration with the new nonspecific fingerprinting method shows the potential of the method for quality control and detection of adulteration.

In the first test, 20 different red wines were compared. The wines were chosen from the selection of the Finnish alcohol monopoly, ALKO (Table 2, SI), to cover a broad range of taste preferences from lighter to stronger wines, different grape compositions, and different producers from around the world. The purpose of this test was to see if the method is able to differentiate these wines. Figure 2 shows the separation achieved by PCA. For the exact measurement protocol of wines see the SI.

Following the complex wines, one of the most popular drinks worldwide was selected: cola soft drinks. Around the globe several "imitations" of the market leaders can be found in stores. We chose two local variants and also compared the

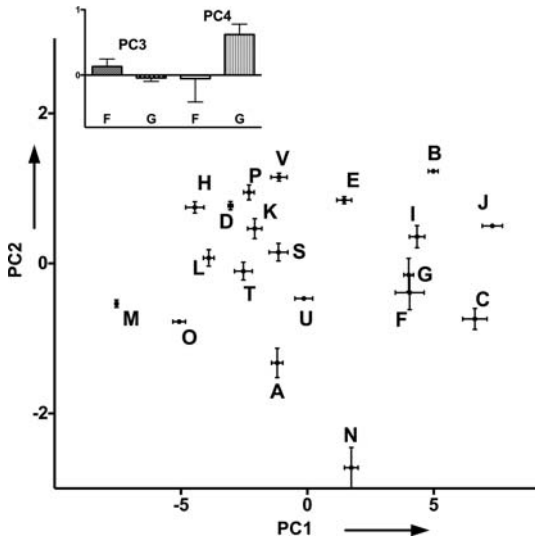


Figure 2. Data principal components (PC1 and PC2) for the wines listed in Table 2 of the SI. For the overlapping wines (G and F) the higher-order principal components (PC3 and PC4) are plotted in the inset. PCA was carried for the whole data set of the 20 wines, each measured in 6 replicates. The error bars for each sample denote the standard deviation of these 6 replicates. The data of the measurements were processed with the PCA tool of Molegro Data Modeler (version 2.1.0) and subsequently plotted with Prism 6 (version 6.0b).

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"light" versions of the market leaders. Figure 3A shows the respective first two principal components calculated from the

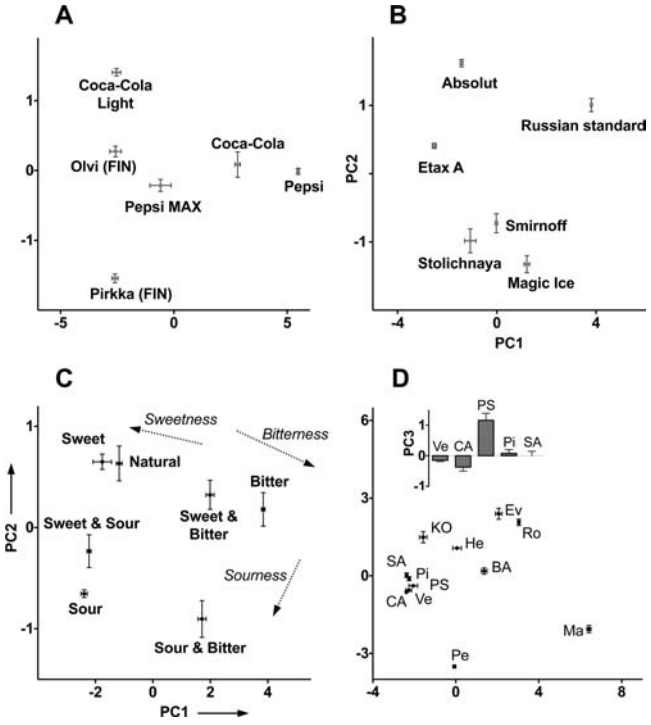

Figure 3. First two principal components of the experimental data from (A) cola drinks, (B) vodkas, (C) modified juices, and (D) water samples (see also Table 2 of the SI). Experiments A–C were measured on microtiter plates and D on the custom chip. The error bars for all experiments (A–D) represent standard deviation of three replicates. (A) Clear distinction between the different cola brands, (B) difference between brands of vodka and ethanol (>94%, Etax A, Altia Oy) diluted to 40% (vol) in water before the measurement protocol. The juices were modified artificially for sweetness, bitterness, and sourness (B). The dotted arrow approximates the directions of the effects on the PC1/PC2 plot of the artificial taste modifiers. In (D) (Waters on chip), the third principal component is plotted for the overlapping waters; the waters "Pi" and "SA" are from the same source but marketed with different brand names.

measured signals of these six cola drinks. As before, the results suggest that the method is well suited for tracing the source of the sample.

Figure 3B shows the results for five well-known vodka brands compared with diluted pure alcohol. As with other drinks, vodkas are produced under a vast number of brands and are also forged—a simple method for identification of brands could be advantageous. The most likely source for the differentiation with rather "pure" drink as vodka is the water used at the manufacturing site; thus, a simple method that is sensitive to water quality is advantageous in this type of testing.

Natural pressed chokeberry (*Aronia mitschurinii*) juices were modified along the taste sensation "axis" of humans: bitterness, sweetness, and sourness, and the results are plotted in Figure 3C (see also Table 3, SI). Although the design of the sensor tried in no way to imitate human taste, the artificial taste modifications "tuned" the PCA to detect these changes. The fact that there are clear separating trends from each other with increasing bitterness, sweetness, and sourness leads us to believe that the sensor could be further developed and algorithms trained to be used to assess taste parameters.

For the next test, the newly developed μTAS chip was tested with bottled waters from worldwide known brands and local waters (Table 3 SI). This was considered as a rather "simple" test for the method, since waters vary "only" in their ionic and mineral content, something that one would expect to be able to detect rather easily. Thus, testing the chip solution along with the newly developed custom reader was expected to be relatively straightforward (see also the SI). The results are plotted in Figure 2D. The study shows that the waters from the same source but from different manufacturers have similar luminescent fingerprints (see Pirkka (Pi) vs Spring Aqua (SA), Figure 3D and inset) suggesting that the origin of the water can be traced. For the preparation and water testing protocol see the SI.

To make the sensor simple, a lab-on-a-chip type device was developed on the basis of experience using the microtiter format. Since the sensor dimensionality can, in many applications, be relatively low, the chip was designed accordingly. The design has 18 wells, each 3 mm in diameter with connecting channels and vent channels (in collaboration with Aqsens Inc., IMM/Mainz, and MiniFAB/Melbourne; see Figure 1, and the SI). This simple, low-density design allowed avoiding one of the major caveats of miniaturized assays: the repeatability. In our experience, the results with the chip of these dimensions are comparable to those acquired with microtiter plates. Figure 3D, as outlined above, shows our experiment analyzing different bottled waters with the lab-on-a-chip solution with remarkably good separation and repeatability. With the microchip design, due to its fast deployment, it is also possible to utilize time dependence of the signal as additional information to increase the dimensionality and reliability of the results; in our experience, utilization of two time points with water testing increased the separation and precision of the results (comparison data not shown). Although the use of these prototype chips was relatively reliable with occasional failures in filling (air bubbles; the manufacturing by hand was tedious and error prone), the obvious next step will be the automation of this task.

Although the results of this paper are still preliminary and the microchip design and manufacturing processes are yet to be completed, they are extremely encouraging in view of a simple and universally applicable liquid fingerprinting tool. Long lifetime luminescence, by default, is sensitive to the environment; with properly selected conditions and environmentally sensitive modulators it can be utilized in assessing water-based liquids for origin, authenticity, and quality.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed protocols for coating, modulators and materials used, along with description of the instrumental setup. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): P.H. has shares in Aqsens Inc. No other competing interests have been declared.

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