Supporting Information

Leveraging the analytical chemistry primary literature for authentic, integrated content knowledge and process skill development

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Article: Andres W. Martinez, Scott T. Phillips, Emanuel Carrilho, Samuel W. Thomas, III, Hayat Sindi, and George M. Whitesides. "Simple Telemedicine for Developing Regions: Camera Phones and Paper-Based Microfluidic Devices for Real-Time, Off-Site Diagnosis." *Analytical Chemistry,* **2008,** *80,* 3699–3707.
Link: https://doi.org/10.1021/ac800112r
Topics: Calibration Types of Error
Figures of Merit
Question Author: RAH

Questions:

1) This manuscript describes the design of a prototype paper-based microfluidic device that quantifies two analytes in human urine simultaneously. The following excerpt describes the use of paper-based microfluidic devices for quantitative analysis:
We prepared solutions with known concentrations of glucose and protein (BSA) in artificial urine. We then dipped the bottom of the reagent-loaded device into a drop (5.0 μL) of artificial urine and allowed the analytes to move up the paper by capillary action. The patterned paper absorbed the 5.0 μL sample within 10 min, but another 20 min was required for the color to develop fully.
   a) Is the method described considered to be an external standard or standard addition calibration? Explain what information you used to determine this.
   b) List advantages and disadvantages of this calibration method (at least 2 of each).
2) Table 1 (below) summarizes the quantitative data obtained using these prototype devices. Would you consider the assay described in the paper to be accurate? What about precise? Describe the evidence that supports your answer.

Table 1. Observed Quantitative Detection of 5 μL Samples Containing Glucose (2.5, 3.5, and 4.5 mM) and Protein (25, 35, and 45 μM)[a]

	[glucose], mM			[BSA], μM		
	known concentration			known concentration		
detection method	2.5	3.5	4.5	25	35	45
	observed concentration			observed concentration		
camera phone (with auto focus)	2.3 ± 0.5	3.9 ± 0.7	4.5 ± 0.7	24 ± 7	33 ± 8	42 ± 7
camera phone (with PDMS lens)[b,c]	2.6 ± 0.6	3.2 ± 0.7	4.8 ± 0.5	26 ± 6	34 ± 8	45 ± 7
portable scanner	2.6 ± 0.5	3.4 ± 0.6	4.7 ± 0.4	24 ± 6	35 ± 6	44 ± 5
portable scanner (unknown volume)	2.3 ± 0.6	3.8 ± 0.7	4.6 ± 0.7	27 ± 7	34 ± 4	43 ± 7
desktop scanner	2.5 ± 0.4	3.5 ± 0.6	4.3 ± 0.4	25 ± 3	36 ± 6	45 ± 6
digital camera	2.4 ± 0.4	3.8 ± 0.5	4.5 ± 0.8	24 ± 6	35 ± 6	48 ± 8

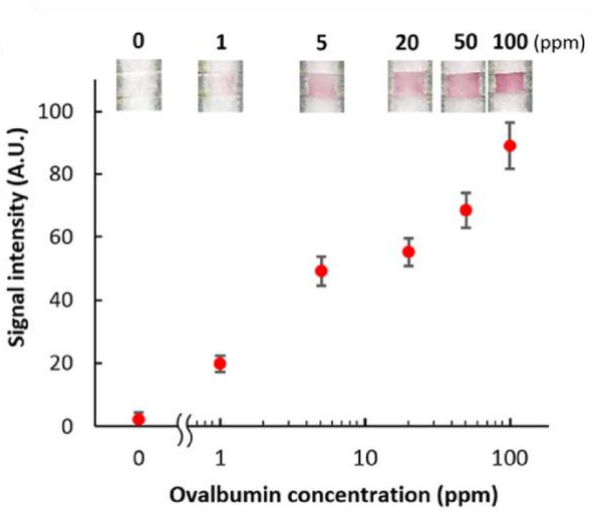
[a] The values are the average and 1 standard deviation of 12 experiments. [b] We used the calibration curve for the camera phone with automatic focus to quantify these results. [c] Experimental details on the fabrication and use of the PDMS lens are given in the Supporting Information.

3) For the scenarios described below, would systematic or random error be introduced into the measurement? Explain your answers. Propose how you might correct for this error experimentally.
   a) The authors state that one major disadvantage of paper-based devices is that "the analytes may stick to the paper and not distribute equally into the test zones."
   b) Accurate analysis with this device requires a 5 μL sample, and any significant evaporation may introduce inaccuracies. The calibration standards are run in a climate-controlled environment, and the samples are run outdoors where the humidity levels are much higher.
   c) A set of expired devices, with diminished sensitivity, are used to analyze a set of samples.

Article: Marti Z. Hua and Xiaonan Lu. "Development of a Microfluidic Paper-Based Immunoassay for Rapid Detection of Allergic Protein in Foods" *ACS Sensors,* **2020,** *5,* 4048–4056.
Link: https://dx.doi.org/10.1021/acssensors.0c02044
Topics: Calibration Types of Error
Sample Preparation
Question Author: RAH

Questions:

1) The filter paper used for this device is modified by soaking in a blocking buffer (5% BSA, 0.5% PVA, PBS) for 20 min and drying at 37 °C for 30 min.
   a) Explain the role of BSA in the blocking buffer.
   b) If BSA were not added, predict how the sensitivity of the device would be impacted. Be sure to explain your answer.
2) The manuscript provides the following details about sample preparation: *Food sample pretreatment was developed as follows. In a 50 mL centrifuge tube, 1 g of spiked sample was mixed with 19 mL of extraction solution containing 1% sodium dodecyl sulphate and 7% 2-mercaptoethanol in phosphate buffered saline. The tube was shaken for 30 s and heated at 90 °C for 8–10 min, followed a 10x dilution with phosphate buffered saline.*
   a) Describe how you would prepare an ideal method blank for this analysis. Be sure to include all required components and their concentrations and/or volumes.
   b) Imagine you are using this device to test various baking mixes in the facility in which they are manufactured. Would use of a method blank or field blank be more appropriate? Explain.
3) The calibration curve (Figure 4) is provided at right. Is this an example of an external standard or standard addition calibration? Explain how you know.
4) What did the authors determine to be the limit of detection of this device? Explain whether this LOD is acceptable for the intended application.
5) Predict what type of error (random or systematic) each of the following might introduce and explain how you might be able to correct for it (experimentally or during data processing).
   a) the contrast between the pink color and background becomes less recognizable when the µPAD dries out
   b) a variety of food samples have slightly varied viscosities after sample prep, and the sensitivity of the device is heavily dependent on flow rate



Article: These practice exercises are based on the article "Analysis of technetium species and fractions in natural seaweed using biochemical separation and ICP-MS measurement" by Keliang Shi, Xiaolin Hou, Jixin Qiao, Xuejie Sun, Per Roos, and Wangsuo Wu. *Analytical Chemistry*, **2016**, *88*, 11931-11937.
Link: https://pubs.acs.org/doi/10.1021/acs.analchem.6b03837
Topic: Sample preparation
Question Author: MLK

1. List the steps in the analytical process.

2. Read the paper's abstract, given below, and explain why sample preparation was a particularly important part of this paper.

   An extremely high accumulation and retention of technetium in marine plants, especially brown seaweed, makes it a unique bioindicator of technetium. In the present work, a novel approach was developed for the speciation analysis of technetium in seaweed, wherein a series of biochemical separations was exploited to isolate different species of technetium. Inductively coupled plasma mass spectrometry (ICP-MS) was applied for the measurement of ^{99}Tc after thorough radiochemical preconcentration and purification. The results show that the distribution of technetium species in seaweed is relatively dispersive. Besides the inorganic species of TcO_4^-, most of technetium (>75%) combined with organic components of seaweed such as algin, cellulose, and pigment. This investigation could provide important fundamental knowledge for studying the processes and mechanisms of ^{99}Tc accumulation in the natural seaweed.

3. Consider Figure 1 from the paper below, and identify at least 3 factors/conditions that were varied during sample prep to separate the Tc species.

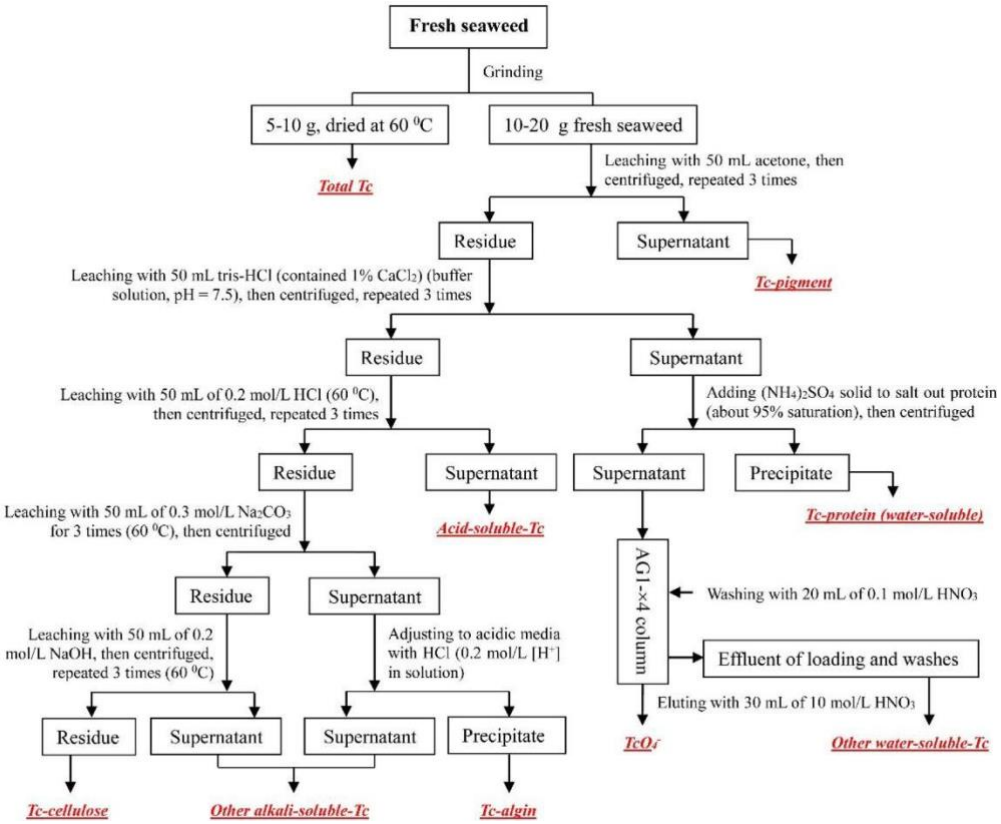


Figure 1. Operational procedure for the separation of different technetium species/fractions in natural seaweed.

4. Using Figure 1, compare and contrast sample prep steps used to analyze inorganic TcO_4^- vs Tc bound to proteins.

5. Consider Table 3 below. Determine how these data would change if the hydrochloric acid treatment did not precipitate all of the Tc-algin from the samples.

Table 3. Concentration and Distribution of Technetium-99 in Various Species/Fractions in *Fucus vesiculosus*[a]

species/ fractions of Tc	conc of Tc (mBq/g dry weight (total mass))	percentage of each Tc species/ fractions in whole sample (%)
Tc-pigment	0.5 ± 0.1	2.8 ± 0.3
TcO_4^-	2.7 ± 0.3	14.6 ± 1.5
water-soluble Tc-protein	ND	
other water-soluble Tc	5.5 ± 0.6	29.1 ± 2.9
acid-soluble Tc	2.6 ± 0.3	13.9 ± 1.4
Tc-algin	2.0 ± 0.2	10.7 ± 1.1
other alkali-soluble Tc	4.8 ± 0.5	25.4 ± 2.5
Tc-cellulose	0.6 ± 0.1	3.2 ± 0.3
sum	18.7 ± 2.0	99.6 ± 10.1
total	18.7 ± 0.9	

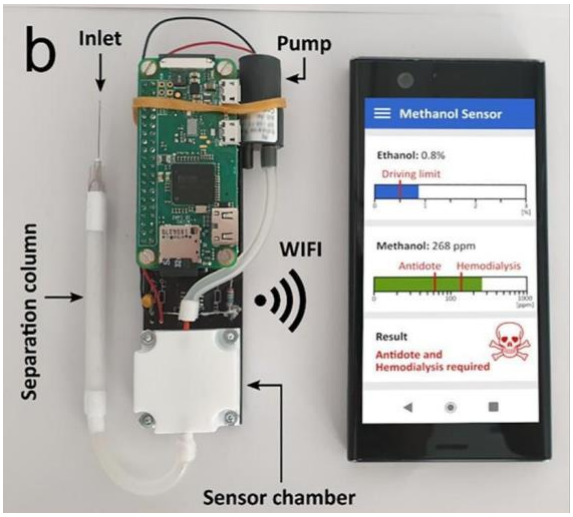
[a]Technetium-99 was not detected in water-soluble protein. Except total concentration of technetium (2SD of uncertainty), all other results are presented as the measured values ± 10% uncertainties estimated in whole analytical procedure.

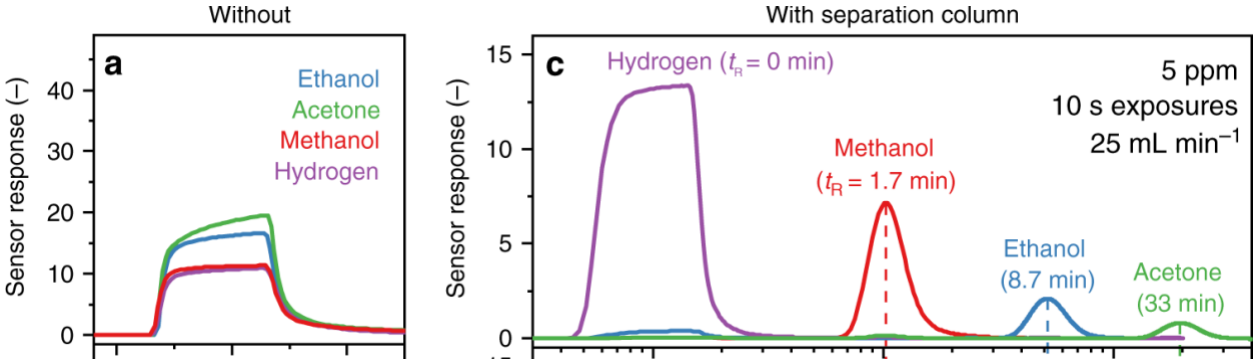
6. Predict how you might need to adapt this sample preparation protocol for use with sea water instead of seaweed.

Article: Jan van den Broek, Dario Bischof, Nina Derron, Sebastian Abegg, Philipp A. Gerber, Andreas T. Güntner, and Sotiris E. Pratsinis. "Screening Methanol Poisoning with a Portable Breath Detector" *Analytical Chemistry,* **2021,** *93,* 1170–1178.
Link: https://doi.org/10.1021/acs.analchem.0c04230
Topics: Separation Science, Fundamentals
Question Author: RAH

Questions:

1) A portable device (shown at right) to measure methanol poisoning and contamination in alcoholic beverages was recently developed. It can also work as a portable breathalyzer. The separation column contains a nonpolar adsorbent material known at Tenax TA (particles are (~200 µm in diameter). Unlike traditional gas chromatography, separation of analytes is based on size rather than boiling point – heavier molecules are retained longer due to stronger adsorption via van der Waals forces. A sample chromatogram is shown below (frame C), demonstrating sufficient resolution between methanol and ethanol, as well as other analytes. Without the separation column (frame A), no analyte selectivity is possible.





a) You'll notice the run time for this separation is quite long (33 min, the x-axis does not scale linearly). Propose one way you could change the column to decrease analysis time for these compounds while still maintaining sufficient resolution. Explain the fundamental theory behind why this change would work and describe any downsides that may be associated with this change.
b) Propose a parameter you could change to decrease analysis time that is NOT related to the column itself. Explain the fundamental theory behind why this change would work and describe any downsides that may be associated with this change.
c) The detector used for this analysis is very sensitive to fluctuations in temperature and humidity. Describe how you could correct for these fluctuations experimentally.

Article: "New horizons for ninhydrin: Colorimetric determination of gender from fingerprints," by Erica Brunelle, Crystal Huynh, Anh Minh Le, Lenka Halámková, Juliana Agudelo, and Jan Halámek, *Analytical Chemistry*, **2016**, *88*, 2413-2420.
Link: https://pubs.acs.org/doi/10.1021/acs.analchem.5b04473
Topics: Interpreting statistical test results
Question Author: MLK

1. A statistical test based on *F* or *t* values compares the magnitude of the difference between groups to _____ ?

2. Read the abstract of the paper below, then in your own words, summarize how the authors propose to distinguish fingerprints from males versus females.

   In the past century, forensic investigators have universally accepted fingerprinting as a reliable identification method via pictorial comparison. One of the most traditional detection methods uses ninhydrin, a chemical that reacts with amino acids in the fingerprint content to produce the blue-purple color known as Ruhemann's purple. It has recently been demonstrated that the amino acid content in fingerprints can be used to differentiate between male and female fingerprints. Here, we present a modified approach to the traditional ninhydrin method. This new approach for using ninhydrin is combined with an optimized extraction protocol and the concept of determining gender from fingerprints. In doing so, we are able to focus on the biochemical material rather than exclusively the physical image.

3. Using GraphPad's free online t-test calculator, determine whether the data in Figure 4B show a statistically significant difference between amino acid levels in fingerprints from men and women. Estimate any values you need from the figure, and record them with your answer.

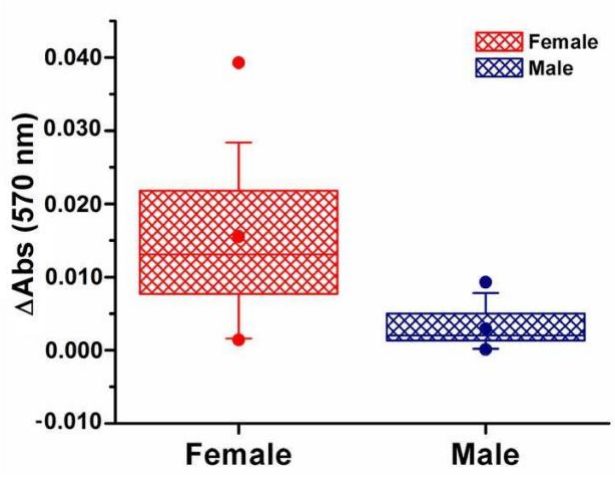


Figure 4b. Box and whisker plots of change in absorbance (ΔAbs) for female and male groups; n = 5 for each of the five surfaces. The boxes represent the range of values from the 25th percentile to the 75th percentile and the ends of the whiskers represent 5th and the 95th percentile of values. The median value for each group is denoted by the horizontal line within each box and the three dots are the maximum, mean, and minimum values, respectively.

4. Compare the concentrations of amino acids for males and females in Table 1. Which amino acids contribute most to the difference in Ruhemann's purple produced by fingerprints for the two groups? Explain.

Table 1. 40 Average Concentrations (mM) of Amino Acids (AA) for Females and Males Derived from Sweat[a]

AA	female concn (mM)	male concn (mM)
Thr	0.2090	0.1121
Ser	0.9840	0.5208
Glu	0.1780	0.1109
Gly	0.6463	0.3418
Ala	0.3870	0.1968
Cit	0.1967	0.1267
Asp	0.1196	0.0638
Asn	0.0380	0.0161
Gln	0.0178	0.0120
Pro	0.0728	0.0349
Val	0.0919	0.0459
Cys	0.0012	0.0009
Met	0.0085	0.0034
Iso	0.0494	0.0229
Leu	0.0625	0.0324
Tyr	0.0559	0.0303
Phe	0.0378	0.0172
β-ala	0.0128	0.0034
Orn	0.1361	0.0684
Lys	0.0528	0.0285
Trp	0.0151	0.0071
His	0.1790	0.0804
Arg	0.0948	0.0540

[a]These values have been previously reported and were used to prepare mimicked fingerprint samples.

5. Justify the choice of *t*-test that you used in #3.

6. Propose further experiments needed to address *at least two additional variables or scientific issues* that you feel the authors have failed to address fully based on the data shown here. Explain your answers.

Article: "Furfural determination with disposable polymer films and smartphone-based colorimetry for beer freshness assessment," by Alberto Rico-Yuste, Victoria González-Vallejo, Elena Benito-Peña, Tomás de las Casas Engel, Guillermo Orellana, and María Cruz Moreno-Bondi, *Analytical Chemistry*, **2016**, *88*, 3959-3966.
Link: https://pubs.acs.org/doi/abs/10.1021/acs.analchem.6b00167
Topics: Figures of Merit
Question Author: MLK

Read the abstract below, then answer the questions that follow.

We have developed disposable color-changing polymeric films for quantification of furfural – a freshness indicator – in beer using a smartphone-based reader. The films are prepared by radical polymerization of 4-vinylaniline, as a furfural-sensitive indicator monomer, 2-hydroxymethyl methacrylate as a co-monomer, and ethylene dimethyl methacrylate (EDMA) as a cross-linker. The sensing mechanism is based on the Stenhouse reaction in which aniline and furfural react in acidic media with the generation of a deep red cyanine derivative, absorbing at 537 nm, which is visible to the naked eye. The colorimetric response has been monitored using either a portable fiber-optic spectrophotometer or the built-in camera of a smartphone. Under the optimized conditions, a linear response to furfural in beer was obtained in the 39 to 500 μg L^{-1} range, with a detection limit of 12 μg L^{-1}, thus improving the performance of other well-established colorimetric or chromatographic methods. The novel films are highly selective to furfural, and no cross-reactivity has been observed from other volatile compounds generated during beer aging. A smartphone application (app), developed for Android platforms, measures the RGB color coordinates of the sensing membranes after exposure to the analyte. Following data processing, the signals are converted into concentration values by preloaded calibration curves. The method has been applied to determination of furfural in pale lager beers with different storage times at room temperature. A linear correlation ($r > 0.995$) between the storage time and the furfural concentration in the samples has been confirmed; our results have been validated by HPLC with diode-array detection.

1. List the figures of merit the authors could use to evaluate their method.

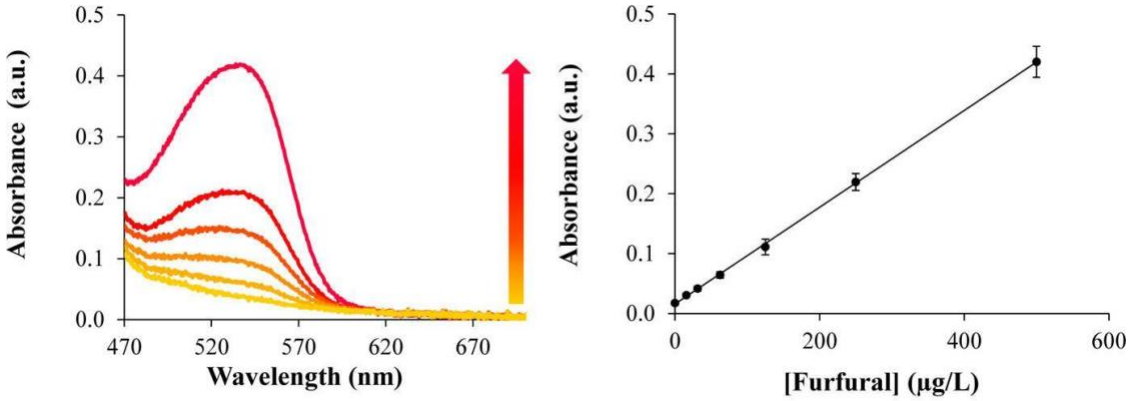
2. Based on Table 2 below, rank the calibration sensitivities of three film compositions tested by the authors, named NVE-1, NVE-2, and NVE-3.

Table 2. Analytical Figures of Merit for the Furfural-Sensitive Films Prepared with Different HEMA Concentrations[a]

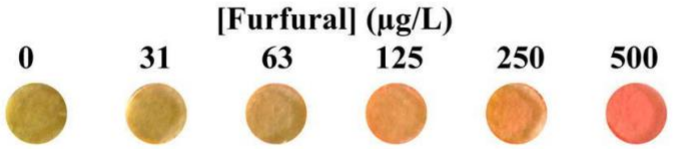
formulation	NVE-1	NVE-2	NVE-3
slope × 10^4 (L μg^{-1})	4.1 ± 0.3	9.4 ± 0.5	27 ± 1
intercept × 10^3 (a.u.)	3 ± 7	10 ± 10	10 ± 30
LOD (μg L^{-1})	19	12	14
LR (μg L^{-1})	62–500	41–500	46–500
r	0.9986	0.9994	0.9992

[a]Calibration standards of furfural (0–500 μg L^{-1}) were prepared in water–ethanol (80:20, v/v). Incubation time: 1 h (n = 3). The actual calibration curves are shown in Figure S3, Supporting Information).

3. Using Figure 3 below, calculate the analytical sensitivity of the method using the data point for 250 µg/L furfural. Use a ruler to estimate any necessary values.



(a)



(b)

Figure 3. (a) Left, absorption spectra of the polymeric films in water/ethanol (94.6:5.4, v/v) mixtures containing furfural at (yellow) 0, (yellow orange) 31, (orange) 63, (red-orange) 125, (red) 250, and (bright red) 500 µg L^{-1}. Right, representative calibration plot (n = 9; λabs = 537 nm). (b) Images of the polymeric films obtained with the smartphone.

4. Using the excerpts provided here, calculate the standard deviation of the blank measurement for the NVE-1, NVE-2, and NVE-3 films. Explain why higher calibration sensitivity for the NVE-3 film does not result in the lowest limit of detection.

5. Evaluate the overall performance of the method for this application, using multiple pieces of data from the excerpts provided here.

Table 3. Method Accuracy and Precision

	repeatability			reproducibility		
[furfural] added (µg L^{-1})	found (µg L^{-1})	*R* (%)	RSD (%)	found (µg L^{-1})	*R* (%)	RSD (%)
500	540	108	5	530	106	3
300	297	99	1	285	95	3
150	150	100	2	145	97	5

Table 4. Method Validation (*n* = 3)

[furfural] (µg L^{-1})		
added	found (smartphone)[a]	found (HPLC-DAD)[a]
50	55 (5)	53 (1)
100	89 (10)	92 (2)
200	225 (24)	194 (4)
300	344 (49)	298 (6)
500	483 (48)	494 (5)

[a](SD) = Standard deviation.

6. Design marketing materials for this method with a target audience of home brewers. Consider which figures of merit or other qualities will be of most interest and how you will describe them to this audience.

Article: Alina N. Sekretaryova, Valerio Beni, Mats Eriksson, Arkady A. Karyakin, Anthony P.F. Turner, and Mikhail Yu. Vagin. "Cholesterol self-powered biosensor," *Analytical Chemistry* **2014**, *86*, 9540-9547.
Link: https://pubs.acs.org/doi/abs/10.1021/ac501699p
Topics: Figures of merit Statistics
Question Author: MLK

The table below shows figures of merit for 8 different sensors designed to measure cholesterol. Review the data, then answer the questions which follow.

Table 2. Analytical Characteristics of Reagentless Cholesterol Electrochemical Biosensors Based on Mediated ChOx

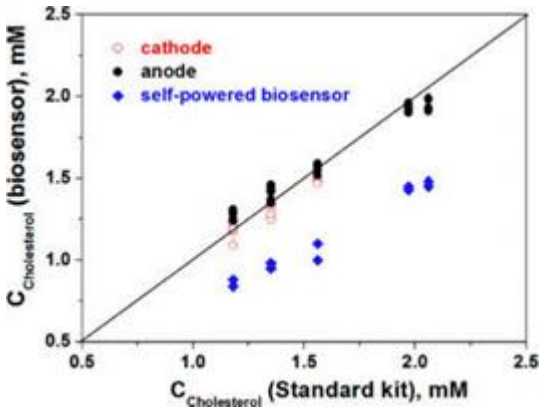
biosensor configuration	linear range, mM	detection limit, μM	sensitivity mA M^{-1} cm^{-2}	ref
ChOx/dodecylbenzenesulfonate/Ppy/ITO	2–8			5
ChOx/ferrocene monocarboxylic acid/PPy/Pt/Pt	up to 0.3	12.4	1.25	9
ChOx/Poly(vinylferrocenium)perchlorate/Pt	0.1–0.5		0.14	6
ChOx/AuNPs/polythionine/GCE	0.002–1	0.6	2.8	7
ChOx/1,10-phenanthroline-5,6-dione in ink of SPE	1–10	1000	8	8
ChOx/poly(neutral red)/carbon film electrode	up to 0.22	1.9	0.018	48
ChOx/PEDOT^{ox}/poly(methylene blue)/GCE	0.01–0.22	1.6	79	49
PTZ-ChOx-modified SPE	0.015–0.15	2.3	33.1	this work

1. Which sensor is best for distinguishing between two cholesterol levels that are very similar? Justify your answer using the data from the table.

2. Blood cholesterol levels in humans range from healthy levels below 5 mM up to unhealthy levels of 9 mM or more. Recommend one or more of the sensor(s) for development into a medical device for at-home cholesterol monitoring. Justify your answer using multiple pieces of data from the table. Suggest at least 2 other pieces of information about a sensor that are not in the table that you would want to consider.

3. The figure below compares readings for several sensors to a standard kit used to measure cholesterol. Each data point represents a single sample. Consider the data for the self-powered biosensor (blue data points). If the readings from the standard kit are considered the "true" cholesterol values, do the data suggest the presence of determinate (systematic) error, indeterminate (random) error, both, or neither? Explain briefly.

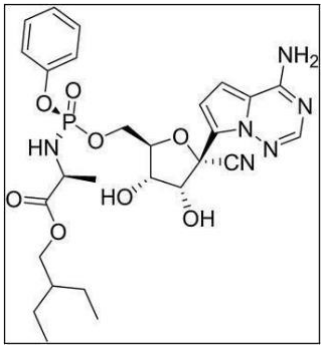
4. What type of statistical test would you use on the blue data points to determine whether there is a significant difference between the data acquired with the biosensor and data from the standard kit? Explain briefly.



Article: Republished with permission of Elsevier Science & Technology Journals, from Raghavendra Rao Pasupuleti, Pei-Chien Tsai, Vinoth Kumar Ponnusamy, and Arivalagan Pugazhendhi. "Rapid determination of remdesivir (SARS-CoV-2 drug) in human plasma for therapeutic drug monitoring in COVID-19-Patients." *Process Biochemistry*, **2021,** *102,* 150–156.; permission conveyed through Copyright Clearance Center, Inc.
Link: https://doi.org/10.1016/j.procbio.2020.12.014
Topics: Calibration Separation Science, Fundamentals
Question Author: RAH

Questions:

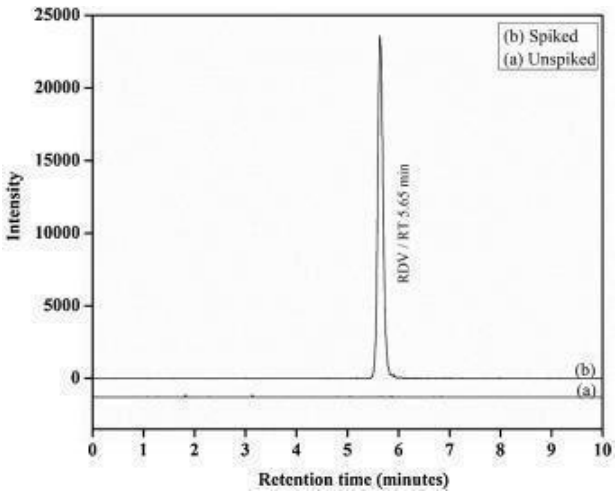
1) Liquid chromatography has been used to track the concentration of remdesivir (a broad-spectrum antiviral drug, structure shown at right) in COVID patients undergoing experimental treatments.



The authors provide the following details regarding standard solutions preparation:
Remdesivir (RDV) stock solution (5000 µg/mL) was prepared by dissolving RDV drug powder using the mixture of DMSO:MeOH (30:70 v/v). The RDV working standard solutions for calibration and quality controls were prepared using methanol in concentrations of 100, 10, 1, 0.1, 0.01 µg/mL. 1, 2.5, 5, 7.5, 10, 25, 50, 75, 100, 250, 500, 1000, and 5000 ng/mL sample solutions were prepared freshly by spiking calibration standard solutions into the blank human plasma samples for method calibration.

   a) What type of calibration method is being described? Why do you think the authors chose this method as opposed to another?
   b) Based on the details provided in part a, describe an appropriate method blank for this analysis.

The authors provide the following details regarding the chromatographic analysis of remdesivir:



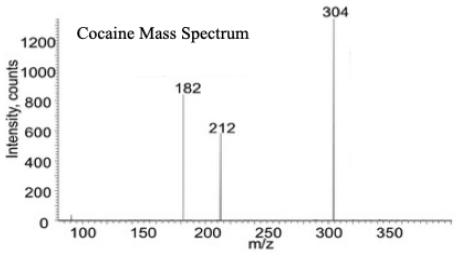
A C18 column (150 × 4.6 mm, 3 µm stationary phase particles) was utilized for the separation of RDV. The mobile phase A used was 0.05 % (v/v) formic acid in ultrapure water, and mobile phase B was 100 % ACN with isocratic elution (A: B) 52:48 % with the total analysis time of 10 min. The column flow rate was set at 0.5 mL/min with 35 °C as the ideal column temperature, and the sample injection volume was 5 µL.

   c) What is one parameter you could change to decrease the retention time of RDV? Explain the theory that supports why this would work.
   d) What is one parameter you could change that would decrease the peak width of RDV? Explain the theory that supports why this would work.

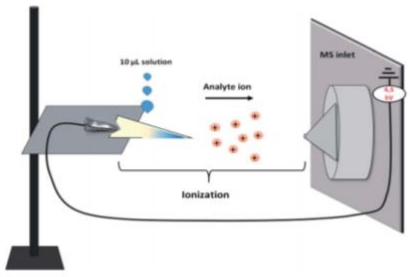
Topics: Mass Spectrometry, Ionization Calibration
Question Author: RAH

Questions:

1) Drug testing is usually carried out by taking a sample of blood or urine from a suspect and using either an antibody assay or chromatographic analysis to detect the relevant drug and/or its metabolites. Recently, a rapid, point-of-care fingerprint drug test has been developed, based on the discovery that excreted metabolites of drugs of abuse can be detected in fingerprints. The authors were successfully able to analyze latent fingerprints collected on glass slides using both matrix-assisted laser desorption ionization (MALDI) and desorption electrospray ionization (DESI) techniques.



   a) Briefly explain how MALDI and DESI work, highlighting the similarities and differences between the two techniques. Are these considered "hard" or "soft" ionization techniques?
   b) A mass spectrum obtained for cocaine is provided above. Which peak represents the molecular ion? Explain your choice.
   c) A technique known as paper spray ionization (PSI, setup shown below) is a variant to electrospray ionization (ESI) that can also be coupled to mass spectrometry. Briefly explain how ESI works and discuss its major advantages relative to traditional hard ionization techniques such as electron impact ionization.
   d) A major advantage of the paper platform is its amenability to "point-of-care" sample collection, as recently demonstrated with the analysis of simulated seized cocaine samples, which often contain a mixture of other substances such as wheat flour, boric acid, and sodium bicarbonate.[5] The paper spray analysis method for this sample is described and shown below:



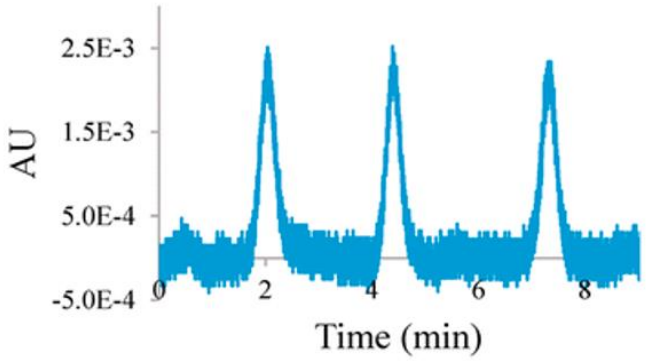
The sample was prepared by dissolving 1 mg into 10.0 mL of methanol, followed by vigorous mixing and filtration. The final extract was analyzed by PSI-MS. For the PSI-MS experiments, a triangular piece of chromatographic paper (10 mm height and 5 mm base width) was positioned approximately 5 mm away from the MS entrance using a copper clip fixed to a platform with three-dimensional motion. Subsequently, 10 µL of each sample extract was dropped onto the paper surface and a voltage of 4.5 kV was applied on the paper base through the metal clip.

The authors chose to validate their method using an internal standard calibration. Explain how this type of calibration works and why this was an appropriate choice for this specific analysis.

Article: Sonika Sharma, H. Dennis Tolley, Paul B. Farnsworth, and Milton L. Lee. "LED-Based UV Absorption Detector with Low Detection Limits for Capillary Liquid Chromatography" *Analytical Chemistry,* **2015,** *87,* 1381–1386.
Link: https://doi.org/10.1021/ac504275m
Topics: Signal-to-noise ratio
Question Author: RAH

Questions:
In designing portable instruments for chromatographic separations, it is often important to simplify the detector being used. One way to simplify and miniaturize absorbance-based detectors for liquid chromatography is to replace the traditional light source with a small light-emitting diode (LED) that requires very little power to operate. Compared to a traditional light source, however, the power output from a LED is much lower.

1) Explain how the lower intensity light from the LED might affect the signal-to-noise ratio of the instrument (compared to a traditional light source).



2) Use a ruler to estimate the signal-to-noise ratio of the detector used to generate the figure at left.

3) Describe one way you could improve the S/N of the series of absorbance measurements shown at left. Describe any detrimental effects to the data that may result by improving S/N in this way.

4) Describe how an improvement in S/N will impact the limit of detection of this measurement.

Article: "Automated microfluidic droplet-based sample chopper for detection of small fluorescence differences using lock-in analysis," by Jean T. Negou, L. Adriana Avila, Xiangpeng Li, Tesfagebriel M. Hagos, and Christopher J. Easley. *Analytical Chemistry*, **2017**, *89*, 6153.
Link: https://pubs.acs.org/doi/10.1021/acs.analchem.7b00991
Topics: Hardware methods of noise reduction
Question Author: MLK

Researchers from Auburn University demonstrated a device they called the "µChopper". They were using microfluidics, which are tiny channels for chemical analysis, and they wanted to improve the signal-to-noise ratio of their fluorescence data. A major advantage of microfluidics is that they are small and portable, so the authors did not want to add expensive or complex optical or mechanical components to their device. Instead, they used a network of channels to create alternating droplets of aqueous sample and a constant reference standard separated by oil, as in Figure 1 below. The authors collected the fluorescence signals as a function of time, then processed the data by fast Fourier transform in MatLab to keep only data from the sample that were phase-locked to the reference.

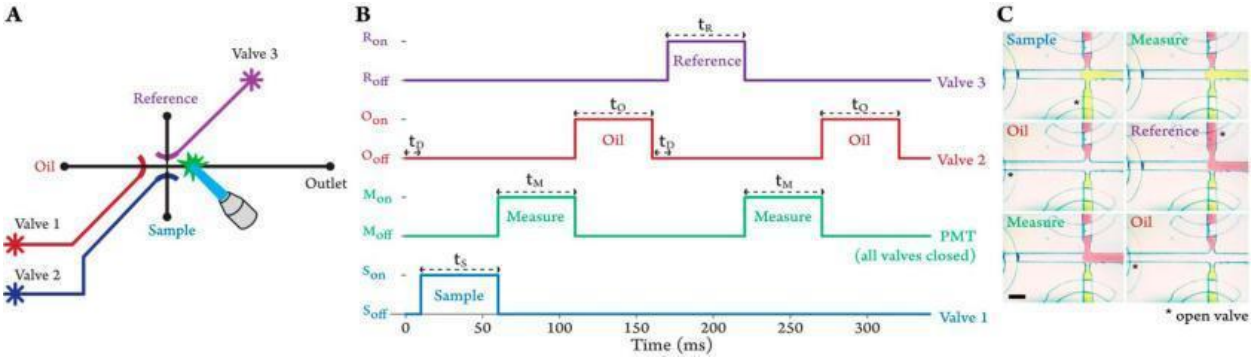
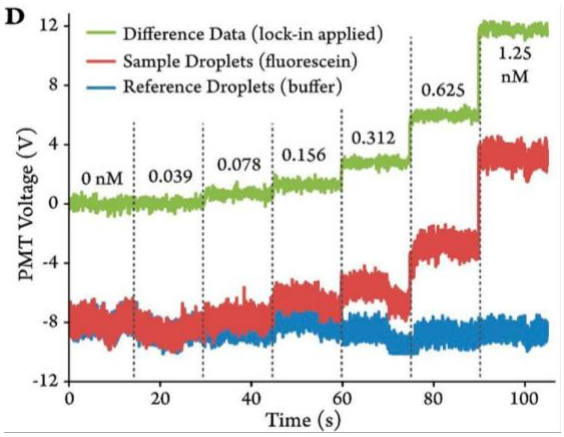


Figure 1. µChopper device design and operation. (A) Channel layout depicts flow channels (black), control channels (blue, red, and purple), and the fluorescence detection point. (B) Schematic of valve-automated chip operation cycle: t_D is the delay time, t_S is the sample flow time, t_M is the measuring time, t_O is the oil flow time, and t_R is the reference flow time. Droplet position was phase-locked with the detector collection cycle. (C) An image montage showing segments of a video collected during automated droplet formation on the µChopper (full video file, Video S-1). Open valves are marked with asterisks. Scale bar represents 200 µm.

1. A chopper is one hardware-based method to improve signal-to-noise ratio. List all the other hardware-based methods of signal-to-noise enhancement that we discussed in class.

2. Explain how the µChopper reduces 1/*f* noise compared to a measurement of a constant stream of sample.

3. Estimate the signal-to-noise ratio for the 0.625 nM sample in Figure 2D (shown below) before and after the lock-in amplification was applied. Use a ruler if needed to improve your estimate, do not forget to blank-subtract the data, and show your work.



4. Compare and contrast this method of "chopping" the signal to a traditional chopper. What aspects are similar? What aspects are different?

5. In this paper, droplets were generated at 3.5 Hz. The authors suggest that kHz droplet generation may be possible in the future. In the meantime, outline some considerations if the authors try to work in the intermediate frequency range (10-1000 Hz).

6. Imagine that this µChopper were applied to a sample stream that was the eluent from a separation step, such as a liquid chromatography separation. As a result, the sample stream contains two peaks, corresponding to two analytes. Design a new version of Figure 1B that includes the elution of the two analyte peaks in time and shows how the width of these two chromatographic peaks should compare to the valving cycles used to operate the µChopper.

Article: "Study of extraction and recycling of switchable hydrophilicity solvents in an oscillatory microfluidic platform," by Gabriella Lestari, Moien Alizadehgiashi, Milad Abolhasani, and Eugenia Kumacheva, *ACS Sustainable Chemistry & Engineering*, **2017**, *5*, 4304-4310.
Link: https://pubs.acs.org/doi/10.1021/acssuschemeng.7b00339
Topic: Equilibrium, partition coefficients
Question Author: MLK

Consider Figure 1 which illustrates how a switchable hydrophilicity solvent (SHS) extraction works, then answer the questions that follow. Note that in this example the SHS is a tertiary amine, represented in Reaction 1 as NR_3.

(Reaction 1) $$NR_3 + H_2O + CO_2 \leftrightharpoons NR_3H^+ + HCO_3^-$$

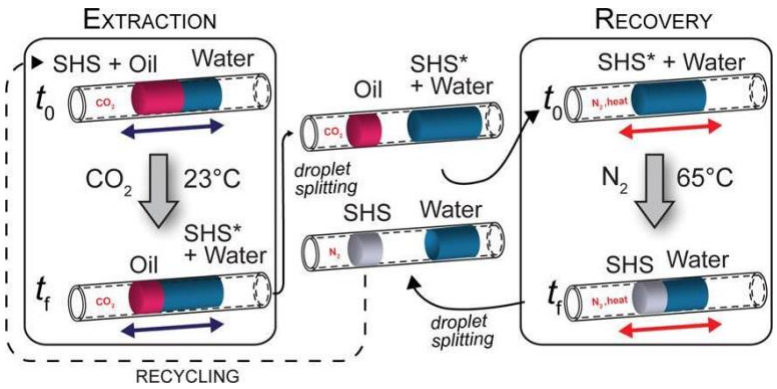


Figure 1. Schematic of the complete SHS extraction-recovery cycle.

Left panel: Extraction experiment. At time t_0, the SHS (not shown) is miscible with oil. Using CO_2 as a carrier gas, the two-droplet segment containing deionized water (blue droplet), and a solution of SHS in oil (red droplet) is subjected to an oscillatory motion at room temperature. The SHS becomes protonated (Reaction 1) and in its hydrophilic state transfers to the aqueous droplet.

Middle panel, top: The oil droplet is separated from the droplet of the aqueous solution of SHS* by applying carrier gas volume displacement at a velocity of 100 mm/s.

Right panel: Recovery experiment. The initial state of the system is the aqueous SHS* solution. Reaction 1 is reversed by removing CO_2 from the aqueous droplet. Under oscillatory motion, an aqueous solution of SHS* phase-separates into a hydrophobic SHS (colorless droplet) and water.

Middle panel, bottom: The recovered SHS droplet is detached from the aqueous droplet by applying carrier gas volume displacement at a velocity of 100 mm/s and separated for further use.

1. Define partition coefficient and distribution coefficient with words and an equation.

2. Explain the purpose of oscillating the liquid droplets during these experiments.

3. The authors used their device to extract 2-(dibutylethanol) amine (DBAE). Calculate the distribution coefficient for the DBAE between the oil (phase 1) and aqueous (phase 2) phases based on the information for Figure 3.

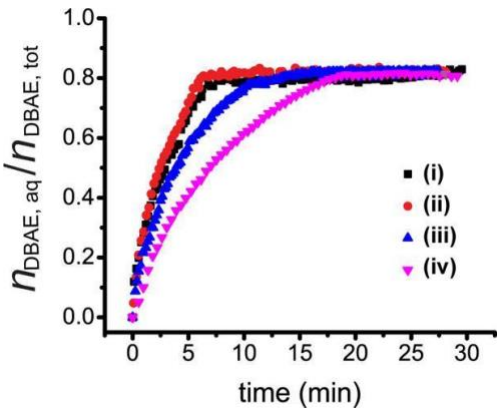


Figure 3. Time-dependent extraction of DBAE from the oil phase at varying initial DBAE concentration in the oil phase. The initial concentrations of DBAE in the oil phase were (i): 3.2 M (■), (ii): 4.0 M (●, red), (iii): 4.3 M (▲, blue), and (iv): 4.5 M (▼, purple). The values converged to 0.78 ± 0.05 at equilibrium. The experiments were conducted for 30 min at P_{CO2} = 206 kPa at the oscillation amplitude of 10 cm, and the oscillation flow velocity of 50 mm s^{-1}. The volume of the oil mixture was 6 μL; the total volume of the two-phase liquid segment was 36 μL.

4. List three factors that will affect the efficiency of this extraction when this method is applied to new SHS analytes.

5. Assess and sketch how Figure 3 would change if the distribution coefficient for the charged SHS more strongly favored the aqueous phase.

6. Propose a method to use this device to separate two different SHS species with the same partition coefficients but different pK_a values for their protonated, hydrophilic forms.

Article: Sergio Armenta, Miguel de la Guardia, and Francesc A. Esteve-Turrillas, "Hard Cap Espresso Machines in Analytical Chemistry: What Else?" *Analytical Chemistry* **2016**, *88*, 6570-6576.
Link: https://pubs.acs.org/doi/10.1021/acs.analchem.6b01400
Topics: Sample Preparation Extraction
Types of Error
Question Author: MLK

In 2016, researchers at the University of Valencia demonstrated that an espresso machine can be used to extract trace amounts of polycyclic aromatic hydrocarbons (PAHs) from contaminated soil samples by replacing the coffee grounds with soil and filling the espresso maker with a mixture of acetonitrile and water.

1. Suggest at least two sample preparation steps that you would suggest analysts perform before loading the soil samples into an espresso machine. Justify your choices.

2. Table 3 compares the results for two certified reference samples of soil using the espresso maker method (HCE) and a reference method, which is known to give accurate results. Calculate the percent error for the naphthalene (Nap) in the CRM 170 clay soil.

3. Which method is more precise, the reference method or HCE? Justify your response using data from Table 3.

4. Does Table 3 show any evidence of systematic error? Explain.

5. What statistical test would you use to determine whether PAH concentrations measured by the two methods are statistically different? Justify your choice.

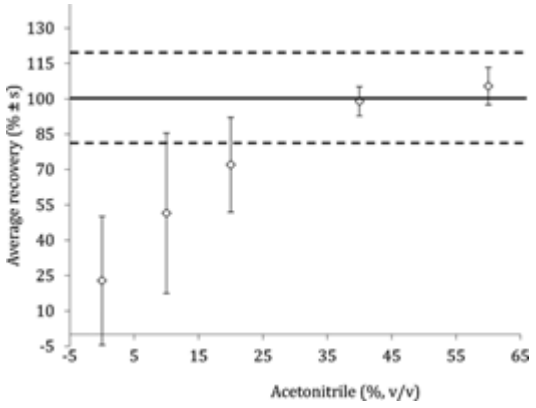
6. The authors filled the espresso machine with a mixture of acetonitrile (CH_3CN) and water and evaluated how the percent recovery of PAHs varied with the % acetonitrile in the extracting solvent. Evaluate how %acetonitrile affected systematic error, and propose a chemical explanation for this result.

7. Imagine you are an author of this espresso machine paper, and a reviewer who is evaluating it for publication suggests that you should be using an internal standard. Do you agree? Justify your opinion.

Table 3. Determination of PAHs in Certified Reference Materials by the Proposed Hard Cap Espresso Extraction (HCE)

	PAHs concn ($\mu g\ kg^{-1} \pm s$, $n = 3$)			
	CRM170 clay soil		CRM104 sediment	
cmpd	reference[a]	HCE	reference[a]	HCE
Nap	480 ± 148	473 ± 54	504 ± 215	508 ± 44
Ace	293 ± 68	359 ± 28	575 ± 195	538 ± 25
Flu	267 ± 17	287 ± 5	270 ± 79	234 ± 6
Phe	231 ± 54	190 ± 7	202 ± 58	181 ± 9
Ant	574 ± 124	642 ± 54	701 ± 175	703 ± 22
Fln	349 ± 78	347 ± 38	542 ± 171	444 ± 50
Pyr	283 ± 86	262 ± 20	375 ± 105	361 ± 11
BaA+Chr	612 ± 70	689 ± 72	493 ± 56	514 ± 22
BbF	118 ± 34	141 ± 9	283 ± 74	283 ± 33
BkF	114 ± 31	117 ± 37	296 ± 64	295 ± 10
BaP	197 ± 59	165 ± 34	164 ± 52	165 ± 9
DahA+BghiP	519 ± 80	411 ± 108	792 ± 89	676 ± 95
InP	168 ± 71	146 ± 21	215 ± 42	226 ± 11

[a]Certified values were obtained from the certification organization.



Article: "Dynamic control of gas chromatographic selectivity during the analysis of organic bases," by Ernest Darko and Kevin B. Thurbide, *Analytical Chemistry*, **2019**, *91*, 6682-6688.
Link: https://pubs.acs.org/doi/10.1021/acs.analchem.9b00703
Topic: Equilibrium, partition coefficients
Separation science, fundamentals
Separation science, instruments
Question Author: MLK

Read the abstract and then answer the questions that follow.

A novel method for controlling selectivity during the gas chromatographic (GC) analysis of organic bases is presented. The technique employs tandem stainless steel capillary columns, each coated with a pH adjusted water stationary phase. The first is a 0.5 m trap column coated with a pH 2.2 phase, while the second is an 11 m analytical column coated with a pH 11.4 phase. The first column traps basic analytes from injected samples, while the remaining components continue to elute and separate. Then, upon injection of a volatile aqueous ammonia solution, the basic analytes are released as desired to the analytical column where they are separated and analyzed. Separations are quite reproducible and demonstrate an average RSD of 1.2% for analyte retention times in consecutive trials. Using this approach, the retention of such analytes can be readily controlled and they can be held in the system for periods of up to 1 h without significant erosion of peak shape. As such, it can provide considerable control over analyte selectivity and resolution compared to conventional separations. Further, by employing a third conventional GC column to the series, both traditional hydrocarbon and enhanced organic base separations can be performed. The method is applied to the analysis of complex mixtures, such as gasoline, and much less matrix interference is observed as a result. The findings indicate that this approach could be a useful alternative for analyzing such samples.

1. List the physical or chemical characteristics of the analytes that typically form the basis of a GC separation.

2. Explain why organic base analytes are trapped on the pH 2.2 column and then released by addition of aqueous ammonia.

3. The separations on these columns were run at 80-100 °C. How does this compare to the typical temperature of a GC oven? Identify the reason for the difference.

4. Consider Figure 5. Contrast the behavior of peak 2 (acetone) with the behavior of peaks 1 and 3 (pentylamine and butylamine) when the dual column system is used compared to when it is not.

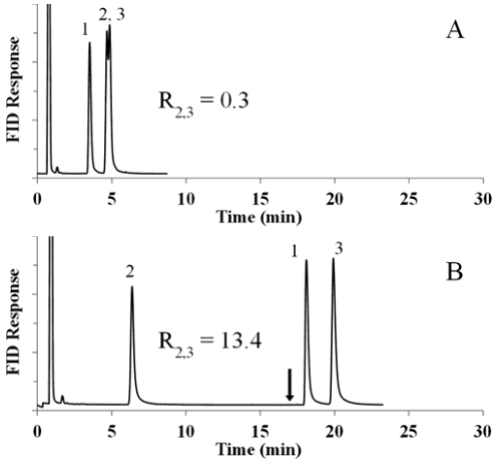


Figure 5. Chromatograms of (1) pentylamine, (2) acetone, and (3) butylamine on (A) single analytical column (11 m, pH 11.4 NaOH coating) and (B) dual column system (0.5 m trap with pH 2.2 sulfamic acid coating and 11 m analytical column with pH 11.4 NaOH coating). Analytes in B are released at the 17 min mark. Column temperature is 80 °C. Other conditions are as in Figure 2. [The arrow in B indicates addition of 3 μL of 10 M aqueous NH_3.]

5. Justify the authors' choice to use NH_3 to release the analytes and formic acid (HCOOH) to regenerate the acidic trapping column between runs. Why use these compounds rather than, for example, NaOH and HCl?

6. Based on Figure 7, propose a set of guidelines describing when it would be best to use (A) a conventional DB-5 GC column versus (B) the dual-column system versus (C) a combination of both.

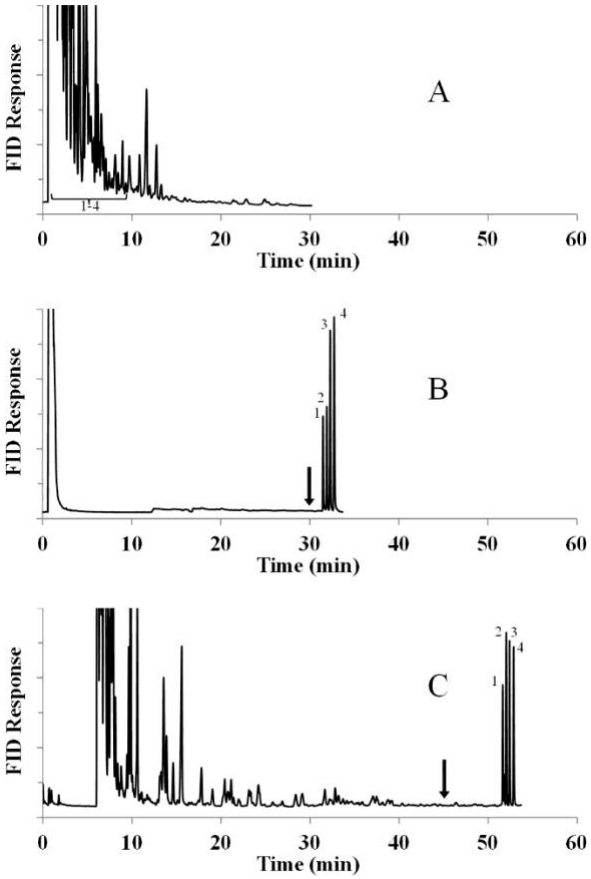


Figure 7. Separations of a gasoline sample containing (1) heptylamine, (2) pentylamine, (3) butylamine, and (4) propylamine on (A) conventional DB-5 column (80 °C, 50 cm/s carrier gas), (B) dual column system (1 m trap with pH 2.2 sulfamic acid coating and 11 m analytical column with pH 11.4 NaOH coating; 100 °C, 18 cm/s carrier gas, analytes released at 30 min), and (C) tandem system with DB-5 column from A joined with dual column from B (80 °C for 30 min then increased to 100 °C, 13 cm/s carrier gas, analytes released at 45 min). Other conditions as in Figure 2. [The arrow in B and C indicates addition of 3 μL of 10 M aqueous NH_3.]

Article: "Raman microscopy for pH-gradient preconcentration and quantitative analyte detection in optically trapped phospholipid vesicles" by Chris D. Hardcastle and Joel M. Harris, *Analytical Chemistry*, **2015**, *87*, 7979-7986.
Link: https://pubs.acs.org/doi/10.1021/acs.analchem.5b01905
Topics: Ionization and pH
Question Author: MLK

1. Write the reaction and equilibrium constant expression for protonation of a neutral base.

2. Review Figure 1 below and explain in your own words how the pH gradient causes the weak base to become more concentrated inside the vesicle.

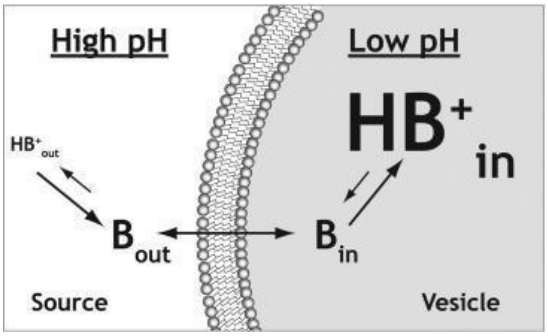


Figure 1. pH-gradient loading of a weak base into a phospholipid vesicle. The neutral base, B, can permeate the lipid membrane, while the protonated acid form, HB^+, cannot.

3. The authors used benzyldimethylamine (BDMA, pK_a = 8.91) as their weak base. Calculate the percent ionization of BDMA inside the vesicle (pH 2.8) versus outside (pH 10.0) when the formal concentration of BDMA in each location is 10.0 µM.

4. Consider Figure 2 in light of questions #2-3. The equation for enrichment is given below.

$$E = \frac{[B]_{in}^{total}}{[B]_{out}^{total}}$$

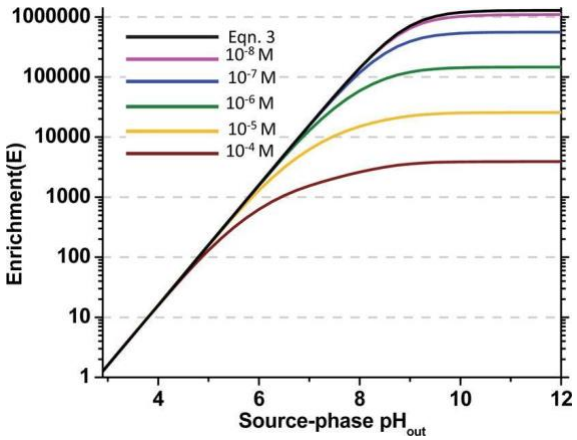


Figure 2. Predicted concentration enrichment of an amine analyte having a pK_a = 8.91 within a vesicle containing 250 mM internal citrate buffer at an initial pH = 2.8, for varying source-phase concentrations of the amine.

Identify the variables affecting analyte enrichment and the conditions that maximize enrichment. In particular,

a) Why does the *x*-intercept (when enrichment =1) occur at $pH_{outside}$ = 2.8?
b) Why does enrichment level off when the $pH_{outside}$ gets higher than about 9?

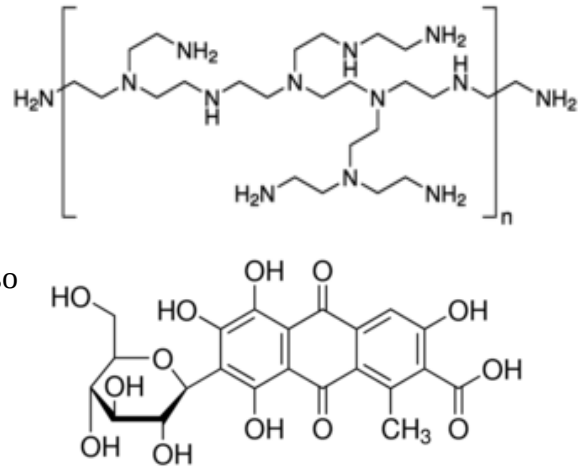
c) Why does the enrichment value plateau at a lower level when the initial concentration of base outside the vesicle is higher?

5. Using your answers to #4, discuss how and why Figure 2 would change if the amine analyte had a lower pK_a.

6. Propose a method to pre-concentrate a weak acid. Explain which aspects of this method would be the same and what would need to change.

Article: Kasinee Katelakha, Vanida Nopponpunth, Watcharee Boonlue, and Wanida Laiwattanapaisal. "A Simple Distance Paper-Based Analytical Device for the Screening of Lead in Food Matrices." *Biosensors,* **2021,** *11,* 90. Used with permission under a Creative Commons license.
Link: https://doi.org/10.3390/bios11030090
Topics: Equilibrium, pH Ionic Strength
Question Author: RAH

Questions:
A new paper-based analytical device (PAD) has been developed for the detection of lead in food. The channels of the paper device are coated with polyethyleneimine (PEI), a polymer whose structure is shown at right. To use the device, food samples are homogenized in a blender, filtered, and then mixed with a reagent buffer that contains the red-colored carminic acid (structure also shown at right).



Chemical structures for PEI (top) and carminic acid (bottom).

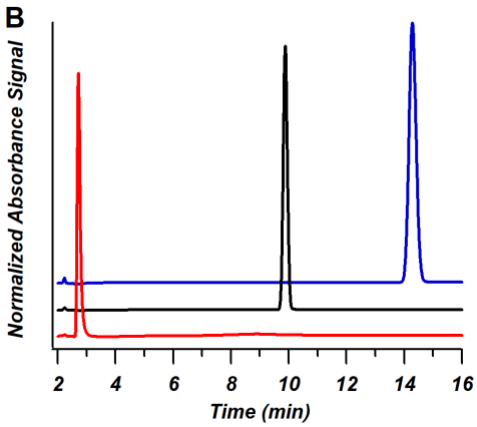
If lead contamination is present, the lead ions will bind strongly with carminic acid, causing its flow through the PAD to decrease due to the increased mass. Simply measuring the distance of carminic acid flow through the PAD channel allows for the determination of lead concentration in the sample.

1) Consider the impact of pH on various functional groups when answering the following questions:
   a) Predict whether the binding affinity between lead ions and carminic acid will be greater at a low pH (< 7) or a high pH (>7). Explain your answer.
   b) PEI serves as an "immobilizing agent" to keep the dye from spreading out on the paper too much. Predict whether the immobilization of carminic acid by PEI will be greater at a low pH (< 7) or a high pH (>7). Explain your answer.
2) Imagine you wanted to use this assay to measure lead in seawater, which has a very high salt concentration. What impact will a high salt concentration have on the pH of the reagent buffer? Use any relevant theory and/or equations to explain your answer.
3) Based on your answers to the previous questions, do you predict the high salt sample will increase, decrease, or not affect the distance that carminic acid will travel in the paper device channel? Explain your answer.

Article: Michelle Pham, Samuel W. Foster, Sangeeta Kurre, Rebecca A. Hunter, and James P. Grinias. "The Use of Portable Capillary Liquid Chromatography for Common Educational Demonstrations Involving Separations" *Journal of Chemical Education,* **2021,** *98,* 2444–2448.
Link: https://doi.org/10.1021/acs.jchemed.1c00342
Topics: Equilibrium, pH Separation Science, Fundamentals
Question Author: RAH

Questions:

1) The figure at right depicts an observed retention time of benzoic acid (pK_a = 4.2) as the mobile phase pH is changed (blue trace is pH = 2.1, black trace is pH = 4.2, and red trace is pH = 6.1). A nonpolar (C_{18}) stationary phase was used.

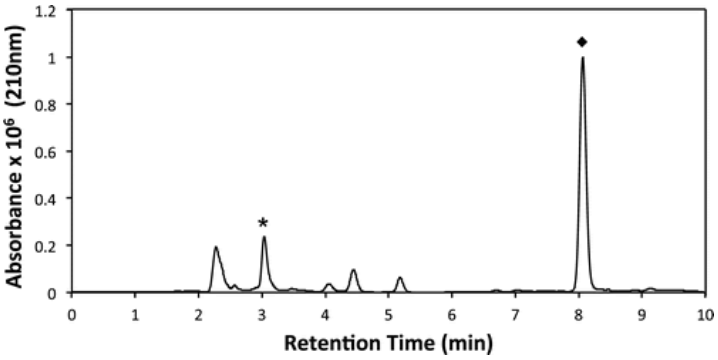


   a) Explain why increasing the mobile phase pH causes a decrease in retention time. Be sure to include any important fundamental theory or equations that support your answer.
   b) Explain why the benzoic acid peak in the blue trace is wider than those in the red and black traces. Be sure to include any important fundamental theory or equations that support your answer.
   c) Besides changing the pH, what are <u>two</u> other ways you could decrease the retention time of benzoic acid. Explain the theory behind how each of these changes works.
   d) What is one drawback of <u>each</u> proposed change you mentioned in part c?

Article: Breanna Miranda, Nicole M. Lawton, Sean R. Tachibana, Natasja A. Swartz, and W. Paige Hall. "Titration and HPLC Characterization of Kombucha Fermentation: A Laboratory Experiment in Food Analysis." *Journal of Chemical Education,* **2016,** *93,* 1770–1775.
Link: https://doi.org/10.1021/acs.jchemed.6b00329
Topics: Acid-base, Titration Separation Science, Fundamentals
Question Author: RAH

Questions:

1) Kombucha is made via the fermentation of sweetened black tea using a symbiotic culture of bacteria and yeast, which produces acetic acid in addition to a variety of other organic acids and vitamins. A 50 mL sample of kombucha was diluted via addition to 50 mL of water and titrated using a standardized 0.0987 M solution of sodium hydroxide to determine the total acidity.
   a) The equivalence point of the titration was found to occur when 20.8 mL of sodium hydroxide was added. Determine the concentration of acid in the original kombucha sample. Be sure to show your work.
   b) Explain how the volume of NaOH required to reach the endpoint/equivalence point would change if you were to titrate an undiluted kombucha sample.
2) Different components of kombucha can also be analyzed using chromatography. The figure at right shows the chromatogram collected for a sample of kombucha. The first starred compound at ~3 min is acetic acid, while the second starred compound at ~8 min is caffeine. The other peaks represent various other components of the kombucha, including other organic acids.



   a) Calculate the resolution between the caffeine peak and the prior peak.
   b) Propose a method by which you could decrease the retention time of caffeine while keeping the retention time of the other components unchanged. Explain the theory behind why this change would work.
   c) Do you expect the concentration of acetic acid determined using chromatography to be lower, equal to, or higher than the concentration of acid determined using the titration method? Explain your answer.

Article: Republished with permission of John Wiley & Sons – Books, from "A chemical basis for sour taste perception of acid solutions and fresh-pack dill pickles," by E.R. Da Conceicao Neta, S.D. Johanningsmeier, M.A. Drake, and R.F. McFeeters, *Journal of Food Science*, **2007**, *72*, S352-S359.; permission conveyed through Copyright Clearance Center, Inc.
Link: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1750-3841.2007.00400.x
Topics: Equilibrium, pH Applications of acid-base chemistry
Question Author: MLK

Read the abstract below, then answer the questions that follow.

Sour taste is influenced by pH and acids present in foods. It is not currently possible, however, to accurately predict and modify sour taste intensity in foods containing organic acids. The objective of this study was to investigate the roles of protonated (undissociated) organic acid species and hydrogen ions in evoking sour taste. Sour taste intensity increased linearly with hydrogen ion concentration (R^2 =0.995), and with the concentration of protonated organic acid species at pH 3.5 (R^2=0.884), 4.0 (R^2=0.929), and 4.5 (R^2=0.975). The structures of organic acid molecules did not affect sour taste after adjusting for the effects of protonated organic acid species and hydrogen ions. Sour taste intensity was also linearly related to the total concentration of protonated organic acid species in fresh-pack dill pickles (R^2 =0.957). This study showed that the sour taste of organic acids was directly related to the number of molecules with at least 1 protonated carboxyl group plus the hydrogen ions in solution. Conclusions from these results are that all protonated organic acids are equally sour on a molar basis, that all protonated species of a given organic acid are equally sour, and that hydrogen ions and protonated organic acids are approximately equal in sour taste on a molar basis. This study provides a new understanding of the chemical species that are able to elicit sour taste and reveals a basis for predicting sour taste intensity in the formulation of acidified foods.

1. Write the equations that relate (a) $[H^+]$ and pH and (b) K_a and pK_a. Also write (c) the equation for K_a in terms of the concentrations of hydrogen ions, protonated organic acid (HA), and deprotonated organic acid (A^-).

2. Look up the structure of fumaric acid and sketch its acid-base equilibrium reactions. Label each equilibrium with its pK_a value from Table 5, and circle the forms expected to contribute to sour taste based on this paper.

Table 5—Dissociation constants (pK_a) and hydrophobicities (log P) for organic acids used for sour taste evaluations

Acid	pK^a_{a1}	pK^a_{a2}	pK^a_{a3}	log P^b
Acetic	4.75			−0.17
Lactic	3.86			−0.62
Adipic	4.43	5.41		0.08
Fumaric	3.03	4.44		0.27
Malic	3.40	5.11		−1.26
Tartaric	2.98	4.34		−1.84
Succinic	4.19	5.50		−0.59
Citric	3.14	4.77	6.39	−1.72

[a]Gardner (1977).
[b]Gardner (1980).

3. Convert the maximum and minimum hydrogen ion concentrations on the *x*-axis of Figure 1 (reproduced below) to pH values, pOH values, and $[OH^-]$. Assume that $\gamma \approx 1$ for these samples. Show your work for at least one sample calculation for each.

4. Rank the acids in Table 5 from most acidic (strongest acid) to least acidic (weakest acid).

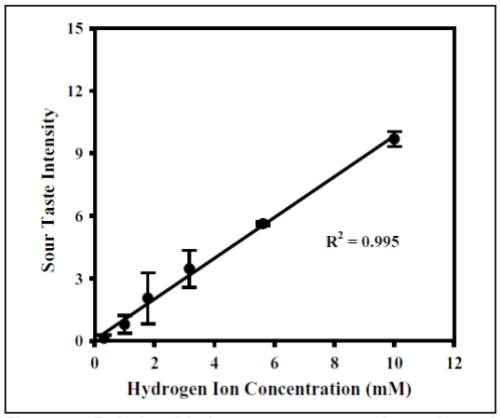


Figure 1—Relationship between sour taste intensity and hydrogen ion concentration

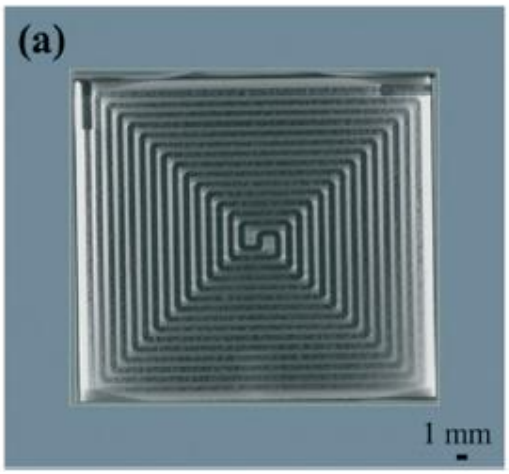
5. The authors varied both the pH and the concentration of protonated organic acid molecules in solution for this study. If the formal (total) concentration of an acid is held constant, determine how the concentration of protonated organic acid will change as the pH is increased. Explain your thinking.

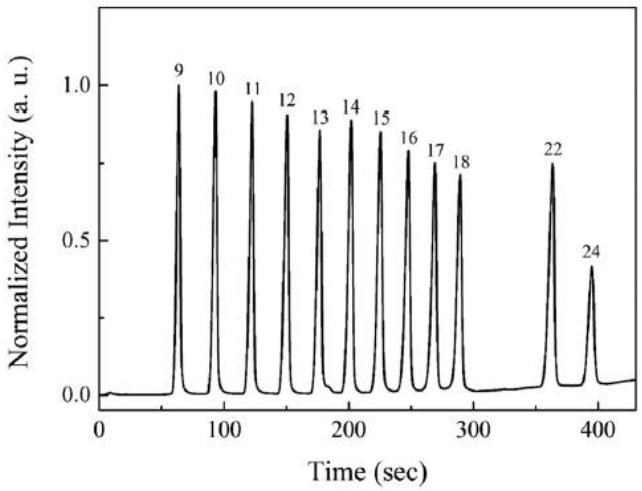
6. In this paper, the authors used sodium hydroxide to adjust the pH of their solutions, meaning that higher pH solutions also had higher sodium ion concentrations. Propose a series of solutions that could be used to test whether this difference in sodium ion concentration affected sour taste perception.

Article: Republished with permission of the Royal Society of Chemistry, from Sooyeol Phyo, Sung Choi, Jaeheok Jang, Sun Choi, and Jiwon Lee. "A 3D-printed metal column for micro gas chromatography" *Lab on a Chip,* **2020,** *20,* 3435–3444.; permission conveyed through Copyright Clearance Center, Inc.
Link: https://doi.org/10.1039/D0LC00540A
Topics: Separation Science, Fundamentals
Separation Science, Instruments
Question Author: RAH

Questions:

1) A 3D-printed GC column (shown below) was created for use with "micro" gas chromatography applications. To prove its utility, it was used to separate a mixture of alkanes (C9–C18, C22, C24). For the separation shown below, the column temperature was ramped from 40 °C to 250 °C at a rate of 30 °C per minute.



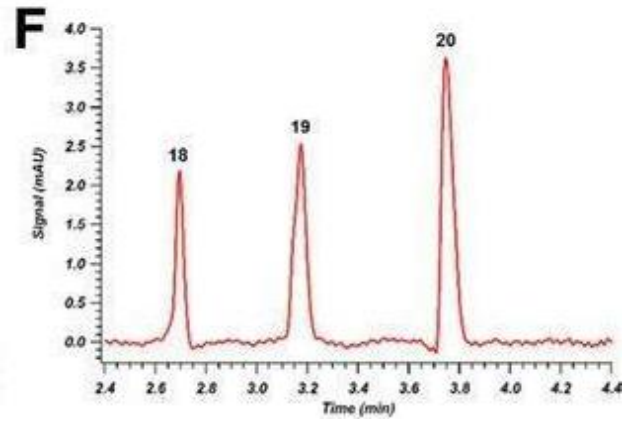


a) What detector would you use for this analysis? Justify your selection.
b) Explain how the chromatogram would change if the separation was run isothermally.
c) Explain how the chromatogram would change if the temperature ramp were increased to 50 °C per minute.

Article: Republished with permission of John Wiley & Sons - Books, from Samuel W. Foster, Xiaofeng Xie, Michelle Pham, Paul A. Peaden, Leena M. Patil, Luke T. Tolley, Paul B. Farnsworth, H. Dennis Tolley, Milton L. Lee, and James P. Grinias. "Portable capillary liquid chromatography for pharmaceutical and illicit drug analysis." *Journal of Separation Science*, **2020,** *43*, 1623–1627.; permission conveyed through Copyright Clearance Center, Inc.
Link: https://doi.org/10.1002/jssc.201901276
Topics: Separation Science, Fundamentals
Separation Science, Instruments
Question Author: RAH

Questions:

1) Recent advancements in liquid chromatography include the development of ultrahigh pressure liquid chromatography (UHPLC) and in increased use of capillary columns that had previously only been used with gas chromatography. Both of these advances have made the development of portable LC systems possible. For example, Axcend Corp. makes a portable system that uses a capillary column – with an internal diameter of 150-µm – that is packed with 1.7-µm stationary phase particles. In contrast, a traditional LC column has a 4.6 mm internal diameter and utilizes 5-µm stationary phase particles.
   a) Explain one advantage that is afforded by the use of a capillary column in liquid chromatographic separation. Explain one disadvantage of capillary columns.
   b) Explain how the use of smaller stationary phase particles can improve the resolution of a separation. Include any relevant equations that support your explanation.
   c) My friend at Rowan University is using the Axcend LC to conduct analyses of pharmaceutical and illicit drugs. Figure 3F from his paper (copied here) shows the separation of morphine (peak 18) and its metabolites: 6-acetylmorphine (peak 19) and heroin (peak 20). Look up the structures of these molecules. Based on the structures of these compounds, do you believe this method used reversed- or normal-phase partition chromatography? Be sure to explain how you came to this conclusion.

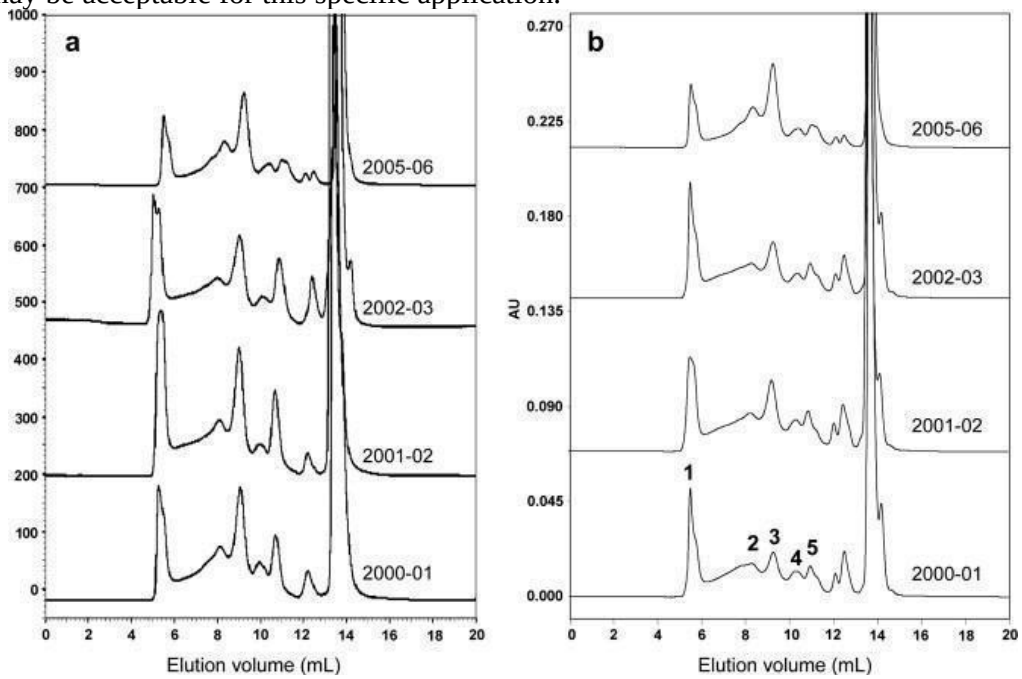


   d) Use peaks 19 and 20 of the above chromatogram to calculate the resolution (R_s) and the number of theoretical plates (N) for this column.
   e) In the analysis of morphine and its metabolites using this portable LC, there is a lot of "dead time" between the elution of each analyte. Propose two parameters that could be adjusted to reduce wasted time without compromising the quality of the separation (as shown, the method is being run as a gradient elution with a flow rate of 2 µL/min). Explain the theory that supports your proposed changes.

Article: Republished with permission of Elsevier Science & Technology Journals, from Virginia Garcia-Cañas, Barry Lorbetskie, Terry D. Cyr, Mary A. Hefford, Sophie Smith, and Michel Girard. "Approach to the profiling and characterization of influenza vaccine constituents by the combined use of size-exclusion chromatography, gel electrophoresis and mass spectrometry" *Biologicals*, **2010**, *38*, 294–302.; permission conveyed through Copyright Clearance Center, Inc.
Link: https://doi.org/10.1016/j.biologicals.2009.12.005
Topics: Separation Science, Instruments Size Exclusion Chromatography
Question Author: RAH

Questions:

1) Many influenza vaccines are created using purified surface antigen preparations. The major antigens in influenza vaccines are the surface membrane glycoproteins: hemagglutinin (MW ≈ 77,000 Da) and neuraminidase (MW ≈ 60,000 Da). Other protein constituents of the influenza virus include matrix protein (MP1, MW ≈ 28,000 Da; MP2, MW ≈ 12,000 Da) and nucleoprotein (MW ≈ 55,000 Da), which are found at varying levels in vaccine preparations. Size exclusion liquid chromatography is frequently used for quality control monitoring of these types of vaccines
   a) Briefly explain how size exclusion chromatography works, and why it is an appropriate separation method for this sample.
   b) Predict the elution order of the 5 compounds named above, listing them from first to last eluted. Explain how you came up with your prediction.
   c) Eluent from the size exclusion column can be analyzed using an absorbance measurement or mass spectrometry. Explain the advantages and disadvantages of each of approach.
   d) Figure 5 from the paper (provided below) shows representative chromatograms from size-exclusion-HPLC analysis of (a) lots of vaccine A analyzed during the production year and (b) the same lots re-analyzed in 2005–2006. Hypothesize why the poor resolution between peaks may be acceptable for this specific application.



Article: "Characterization of fullerene-modified silica as a complement to graphite-like phases for use in two-dimensional high performance liquid chromatography" by Tuan A. Tran, Ian Gibbs-Hall, Paul J. Young, Jonathan D. Thompson, and Dwight R. Stoll, *Analytical Chemistry*, **2013**, *85*, 11817-11825.
Link: https://pubs.acs.org/doi/abs/10.1021/ac4023428
Topics: Separation Science, Fundamentals
Question Author: MLK

This paper describes the use of fullerene molecules, also known as buckyballs, as a stationary phase for liquid chromatography. The performance of the fullerene-modified stationary phase (FMS) is compared to that of a more common C18 stationary phase and to two other carbon-based stationary phases, PGC and COZ.

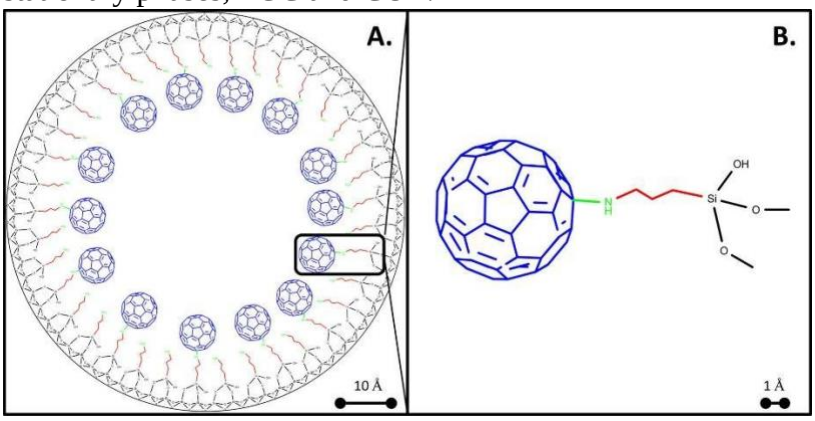


Figure 1. Idealized drawing of the cross-section of a pore inside a silica particle, showing the relative densities of aminopropylsilyl (red/green) and fullerene (blue) groups: (A) full cross-section; (B) detailed view of covalent bonding of fullerene to the silica surface. Surface densities of silyl and fullerene groups were inferred from elemental composition results obtained at each stage of the synthesis (see Table 1).

1. Define selectivity, α, with words and an equation.

2. Explain how the choice of stationary phase affects selectivity.

3. Calculate the resolution of the nitrobenzene and toluene peaks in the top trace of Figure 2. (Estimate any values you need from the graph, and show your work.)

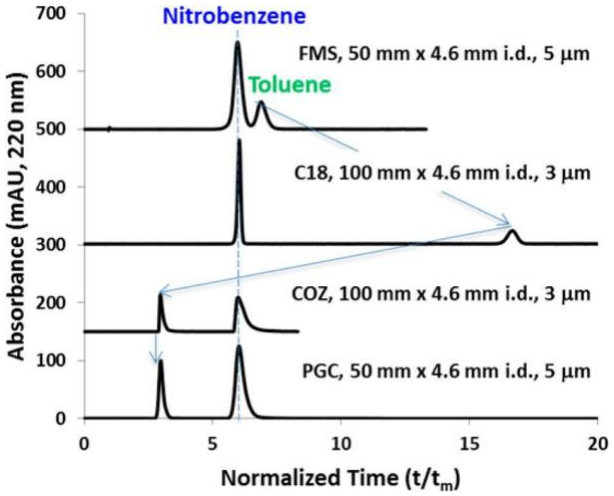


Figure 2. Normalized retention of toluene and nitrobenzene on a conventional C18 bonded phase compared to FMS, PGC, and COZ. Eluent compositions [acetonitrile/water (A/W) v/v] were adjusted to obtain a retention factor of about 6.0 for nitrobenzene in each case: FMS (23.5/76.5 A/W), C18 (35/65 A/W), COZ (43/57 A/W), and PGC (54/46 A/W). All other conditions were constant: flow rate 2 mL/min, column temperature 40 °C, and injection volume 1 µL.

4. In Figure 4, each stationary phase shows some negative correlation between plate count and retention factor. In other words, as *k*' increases, *N* decreases. Explain this relationship between *k*' and *N*.

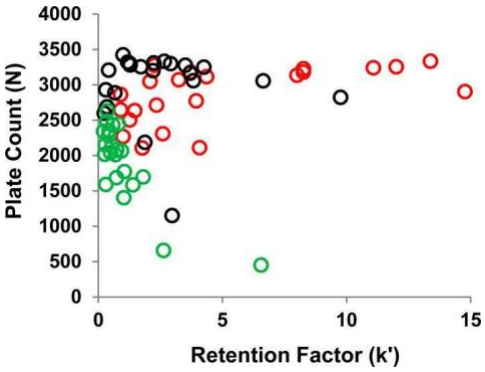


Figure 4. Column efficiency (N) vs retention factor (k′) for 22 nonionizable solutes on FMS (red), PGC (black), and COZ (green). Eluent compositions (acetonitrile/water, A/W) were adjusted to obtain k′ less than 15, which was achieved for most solutes as follows: FMS (30/70 A/W), PGC (60/40), COZ (80/20). Slightly different compositions were used for the most highly retained solutes. All columns were 50 mm × 4.6 mm i.d. and packed with 5 μm particles, except for COZ, which was packed with 3 μm particles. All other chromatographic conditions were constant: column length 5 cm, column i.d. 4.6 mm, flow rate 2 mL/min, column temperature 40 °C, and injection volume 0.5 μL.

5. Figure 5 shows retention factor data for 16 solutes used in the hydrophobic subtraction model (HSM), which is used to characterize how ionizable solutes will behave on a new stationary phase. (You will notice that many of the HSM solutes are weak acids and/or weak bases.) Which stationary phase (FMS or C18) is better at retaining the HSM solutes? Why is this the case?

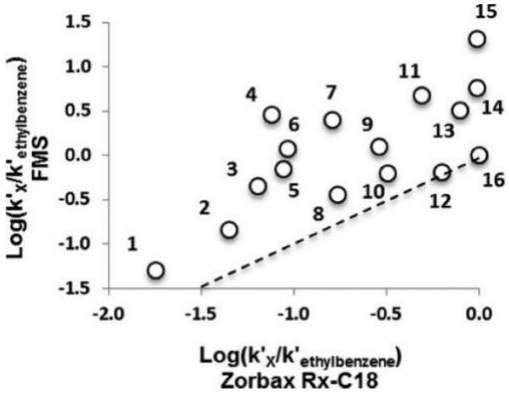


Figure 5. Retention of 16 HSM solutes relative to ethylbenzene on the FMS phase vs Zorbax Rx-C18. Data for the Rx-C18 phase were from Lloyd Snyder (personal communication). The dashed line has a slope of +1 and a y-intercept of 0. The R^2 value for this plot is 0.51. The Rx-C18 column was 150 mm × 4.6 mm i.d. with 5 μm particles, whereas the FMS column was 50 mm × 4.6 mm i.d. with 5 μm particles. All other chromatographic conditions were constant: mobile phase 50/50 ACN/60 mM potassium phosphate buffer at pH 2.8, flow rate 2 mL/min, column temperature 35 °C, and injection volume 10 μL.

Solutes: (1) N,N-dimethylacetamide, (2) N,N-diethylacetamide, (3) nortriptyline, (4) amitriptyline, (5) p-nitrophenol, (6) 5,5-diphenylhydantoin, (7) acetophenone, (8) benzonitrile, (9) 5-phenylpentanol, (10) anisole, (11) 4-n-butylbenzoic acid, (12) toluene, (13) cis-chalcone, (14) trans-chalcone, (15) mefenamic acid, and (16) ethylbenzene.

6. Predict how the van Deemter curve in Figure 7 would change if the temperature were raised from 40 °C to 55 °C.

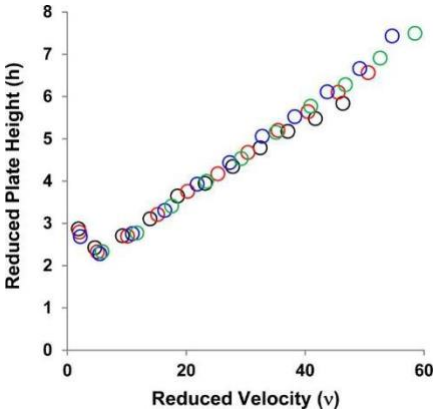
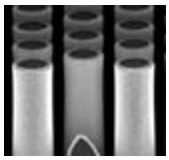


Figure 7. van Deemter curves in reduced coordinates for four nitroalkane homologues (nitropropane, black; nitrobutane, red; nitropentane, blue; and nitrohexane, green) separated on the FMS phase. Chromatographic conditions: column dimensions 50 mm × 4.6 mm i.d., eluent 30/70 ACN/water, flow rates 0.2−5.0 mL/min, injection volume 0.5 μL, and column temperature 40 °C. No corrections to the plate heights have been made to account for extracolumn dispersion.

Article: Martyna Baca, Gert Desmet, Heidi Ottevaere, and Wim De Malsche. "Achieving a Peak Capacity of 1800 Using an 8 m Long Pillar Array Column, *Analytical Chemistry* **2019**, *91*, 10932-10936.
Link: https://pubs.acs.org/doi/abs/10.1021/acs.analchem.9b02236
Topics: Separation Science, Fundamentals
Question Author: MLK



In 2019, Belgian researchers demonstrated an 8-m long HPLC column filled with an ordered array of 5 µm diameter pillars coated with C18 stationary phase, as shown. The column was 315 µm wide and 430 nm deep.

1. Compare and contrast this column with a traditional HPLC column.

2. Using the chromatogram below, calculate estimates of *k'*, *H*, and *N* for peak 3 (valerophenone).

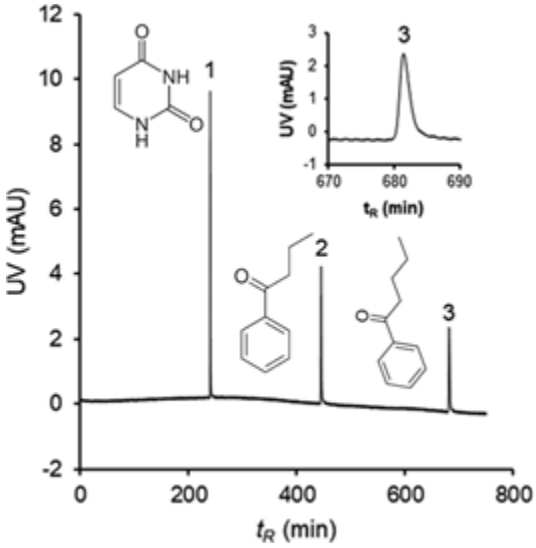


Figure 1. Isocratic separation of an unretained compound (uracil) and two alkylophenones on an 8 m long column using 30% ACN in water with 0.1% formic acid at the flow rate of 150 nL/min. Peak identification: (1) uracil, (2) butyrophenone, and (3) valerophenone.

3. Compare and contrast the values you calculated in the previous question to typical values for HPLC.

4. Explain the elution order of the three compounds in the figure.

5. This separation lasted more than 12 hours. Suggest a method by which the authors could reduce the retention times of the peaks. Predict how this change would affect the selectivity and the efficiency of the separation.

Article: Bingcheng Yang, Min Zhang, Tinakorn Kanyanee, Brian N. Stamos, and Purnendu K. Dasgupta. "An Open Tubular Ion Chromatograph," *Analytical Chemistry*, **2014**, *86*, 11554-11561.
Link: https://pubs.acs.org/doi/abs/10.1021/ac503249t
Topics: Separation Science, Fundamentals Ion Chromatography
Question Author: MLK

Read the abstract below, then answer the questions that follow.

We describe an open tubular ion chromatograph (OTIC) that uses anion exchange latex coated 5 μm radius silica and 9.8 μm radius poly(methyl methacrylate) tubes and automated time/pressure based hydrodynamic injection for pL–nL scale injections. It is routinely possible to generate 50 000 plates or more (up to 150 000 plates/m, columns between 0.3 and 0.8 m have been used), and as such, fast separations are possible, comparable to or in some cases better than the current practice of IC. With an optimized admittance detector, nonsuppressed detection permits LODs of submicromolar to double digit micromolar for a variety of analytes. However, large volume injections are possible and can significantly improve on this. A variety of eluents, the use of organic modifiers, and variations of eluent pH can be used to tailor a given separation. The approach is discussed in the context of extraterrestrial exploration, especially Mars, where the existence of large amounts of perchlorate in the soil needs to be confirmed. These columns can survive drying and freezing, and small footprint, low power consumption, and simplicity make OTIC a good candidate for such a mission.

1. Consider the van Deemter curves in Figure 3. Label the optimum velocity for the separations on the figure.

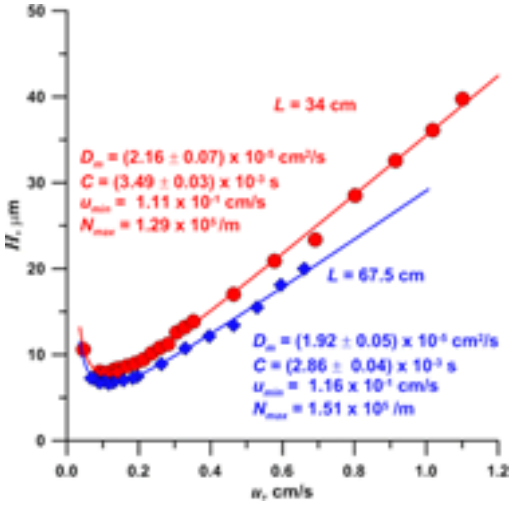


Figure 3. Van Deemter plots of OTCs. Conditions: analyte, 500 μM Cl^-; injection volume, ~43 pL for 34 cm OTC, 90 pL for 67.5 cm OTC; other conditions as in Figure 2.

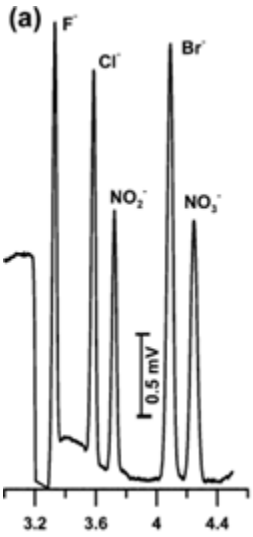


Figure 4a. Typical anion chromatogram on 70 cm PMMA column (r = 9.8 μm): sample, 100 μM F^-; 50 μM Cl^-, NO_2^-, Br^-, NO_3^-; 90 pL injection; 1 mM NaBz eluent, 30 psi, linear velocity, 0.35 cm/s (63 nL/min).

2. Based on the information provided in the abstract and the figure, which terms of the van Deemter equation apply to this separation. Explain your answer.

3. Briefly explain the ions' order of elution in Figure 4a.

4. In Figure 5a, the authors used varying concentrations of sodium bicarbonate as the eluent. Assuming complete dissociation and no significant protonation or deprotonation of the bicarbonate, calculate the ionic strength of the 2 mM $NaHCO_3$ eluent.

5. Explain how and why changing ionic strength of the eluent affects the retention of the analytes in Figure 5a.

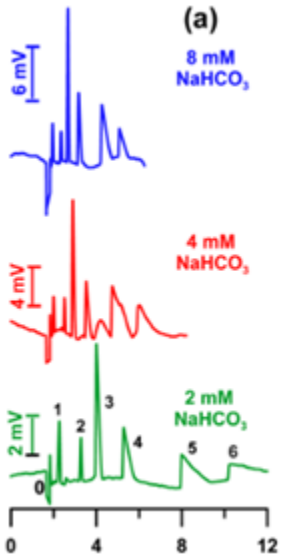


Figure 5a. Illustrative chromatograms. (a) Various concentrations of $NaHCO_3$ as eluent, 80 psi (19.2 nL/min): (0) system peak, (1) F^-, (2) ClO_2^-, (3) Cl^-, (4) NO_2^-, (5) Br^-, (6) NO_3^-, 1.9 nL.

Article: "Novel Instrument for Automated pK_a Determination by Internal Standard Capillary Electrophoresis," by Joan M. Cabot, Elisabet Fuguet, Martí Rosés, Petr Smejkal, and Michael C. Breadmore, *Analytical Chemistry*, **2015**, *87*, 6165-6172.
Link: https://pubs.acs.org/doi/10.1021/acs.analchem.5b00845
Topic: Capillary electrophoresis
Question Author: MLK

Read the abstract below and answer the questions that follow.

The internal standard capillary electrophoresis method (IS-CE) has been implemented in a novel sequential injection−capillary electrophoresis instrument for the high-throughput determination of acidity constants (pK_a) regardless of aqueous solubility, number of pK_a values, or structure. This instrument comprises a buffer creation system that automatically mixes within a few seconds four reagents for *in situ* creation of the separation electrolyte with a pH range of 2−13, ionic strength of 10−100 mM and organic solvent content from 0% to 40%. Combined with 1.2 kV/cm and a short effective length (15 cm to the UV detector) fast 20 s electrophoretic separations can be obtained. The low standard deviation of the replicates and the low variation compared to reference values show that this system can accurately determine acidity constants of drugs by IS-CE. A single pK_a can be determined in 2 min and a set of 20 measurements in half an hour, allowing rapid, simple, and flexible determination of pK_a values of pharmaceutical targets.

1. What properties of the analyte molecules are the basis of a CE separation?

2. Explain why the electrophoretic mobility of acids and bases depends on pH.

3. Based on Figure 5 below, calculate the electroosmotic mobility and the electrokinetic mobility of the analyte (ibuprofen) at 2% methanol.

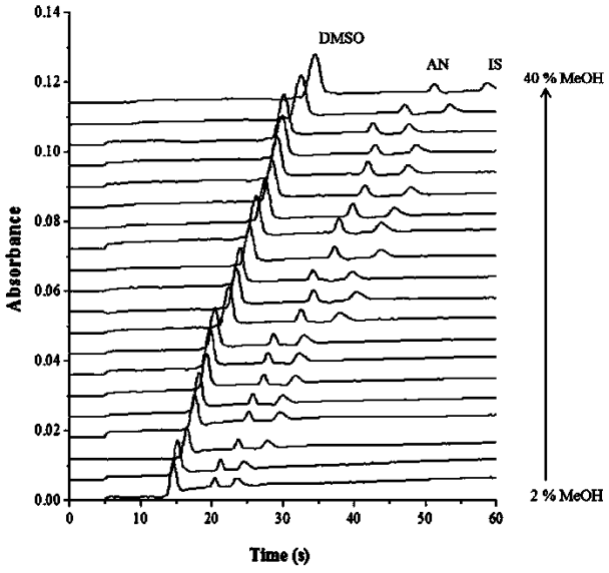


Figure 5. Electropherograms from 2% to 40% of MeOH (v/v) of ibuprofen (AN, analyte) and benzoic acid (IS, internal standard) using DMSO as electroosmotic flow marker. Conditions: Ionic strength of 0.05 M (CHES/CHES$^-$), -30 kV, BGE flow rate 0.3 $\mu L \cdot s^{-1}$ during separation (isolation valve closed).

4. Consider Figure 4. The acidic analytes transition from a mobility of 0 at low pH to a negative mobility at higher pH values. The basic analytes go from a positive mobility at low pH to 0 mobility at high pH. Explain these observations.

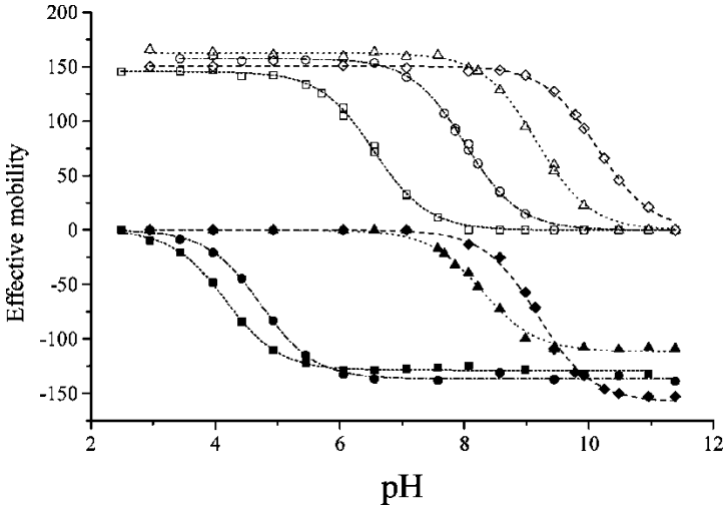


Figure 4. Electrophoretic mobilities ($\times 10^4$ cm^2 V^{-1} min^{-1}) of basic (white) and acidic (black) internal standards versus pH. Markers are experimental mobilities and fitted lines are obtained by eq 3; (□) papaverine, (○) lidocaine, (Δ) diphenhydramine, (◇) nortryptyline, (■) benzoic acid, (●) nicotinic acid, (▲) methylparaben, (◆) 4-bromophenol. Electrophoretic conditions: 50 mM ionic strength, normal polarity at 30 kV, 25 °C.

5. Compare this method of determining pK_a to a potentiometric titration. Suggest advantages and disadvantages of each method.

6. Predict the appearance of an electropherograms of the eight compounds shown in Figure 4 if the separation were conducted under the conditions shown and a pH of 9.0. Label axes and peaks.

Article: "Multiscattering-enhanced absorption spectroscopy," by Volodymyr B. Koman, Christian Santschi, and Olivier J.F. Martin, *Analytical Chemistry*, **2015**, *87*, 1536-1543.
Link: https://pubs.acs.org/doi/10.1021/ac502267q
Topic: Spectroscopy, Beer's Law
Question Author: MLK

Read the abstract and review Figure 1 below, then answer the questions that follow:

An original scheme for sensitive absorption measurements, particularly well-suited for low analyte concentrations, is presented. The technique is based on multiscattering-enhanced absorption spectroscopy (MEAS) and benefits from the advantages of conventional absorption spectroscopy: simplicity, rapidity, and low costs. The technique relies on extending the optical path through the sensing volume by suspending dielectric beads in the solution containing the analytes of interest, resulting in multiple scattering of light, which increases the optical path length through the sample. This way, a higher sensitivity and lower limit of detection, compared to those of conventional absorption spectroscopy, can be achieved. The approach is versatile and can be used for a broad variety of analytes. Here, it is applied to the detection of phenol red, 10 nm gold nanoparticles, and envy green fluorescence dye; the limit of detection is decreased by a factor of 7.2 for phenol red and a factor of 3.3 for nanoparticles and dye. The versatility of this approach is illustrated by its application in increasing the sensitivity of colorimetric detection with gold nanoparticle probes and a commercially available hydrogen peroxide bioassay. The influence of different parameters describing the scattering medium is investigated in detail experimentally and numerically, with very good agreement between the two. Those parameters can be effectively used to tailor the enhancement for specific applications and analytes.

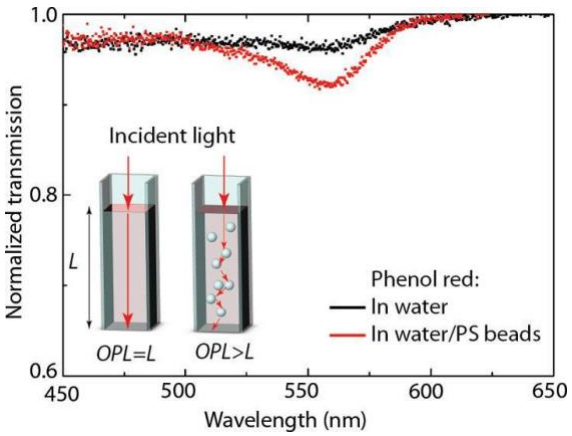
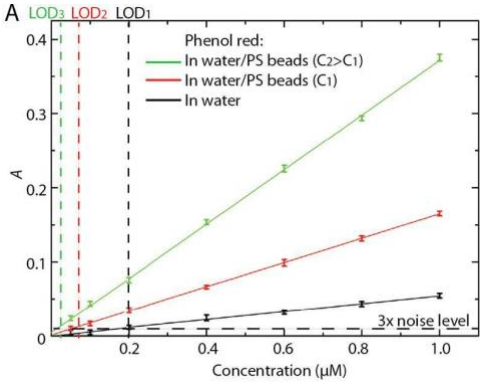


Figure 1. Normalized transmission measurements for the two configurations: 0.6 μM phenol red in water and 0.6 μM phenol red with 0.6 nM PS beads (d = 0.5 μm) in water. The inset shows a schematic drawing of the absorption spectroscopy and the MEAS configurations. The optical path length (OPL) is increased by the presence of the scatterers in the MEAS configuration.

1. Write Beer's law and identify all the variables and their units.

2. Identify the term in Beer's law that is modified by the multiscattering method described in this paper, and explain how and why the change in this term affects sensitivity.



3. Sigma-Aldrich reports the molar absorptivity of phenol red as 31, 620 M^{-1} cm^{-1}. Using Figure 2A at right, estimate the path length of the sample cuvette used to collect these data. Show your work.
4. Figure 6A shows the effect of filling factor, *F*, on the enhancement of the sample absorbance, *k*. *F* represents what percent of the sample solution is filled with beads. *k*

represents how many times higher the absorbance is than it would be with no beads present. Explain why *k* increases with *F*.

5. Based on Figure 6, recommend conditions (bead diameter, bead concentration, and measurement cell thickness) for a researcher who would like to try this technique. Justify your choices using data from the article.

6. Look up the UV-Vis absorbance spectrum of polystyrene. Over what wavelength range is this new technique useful? Predict what would happen at very low wavelengths.

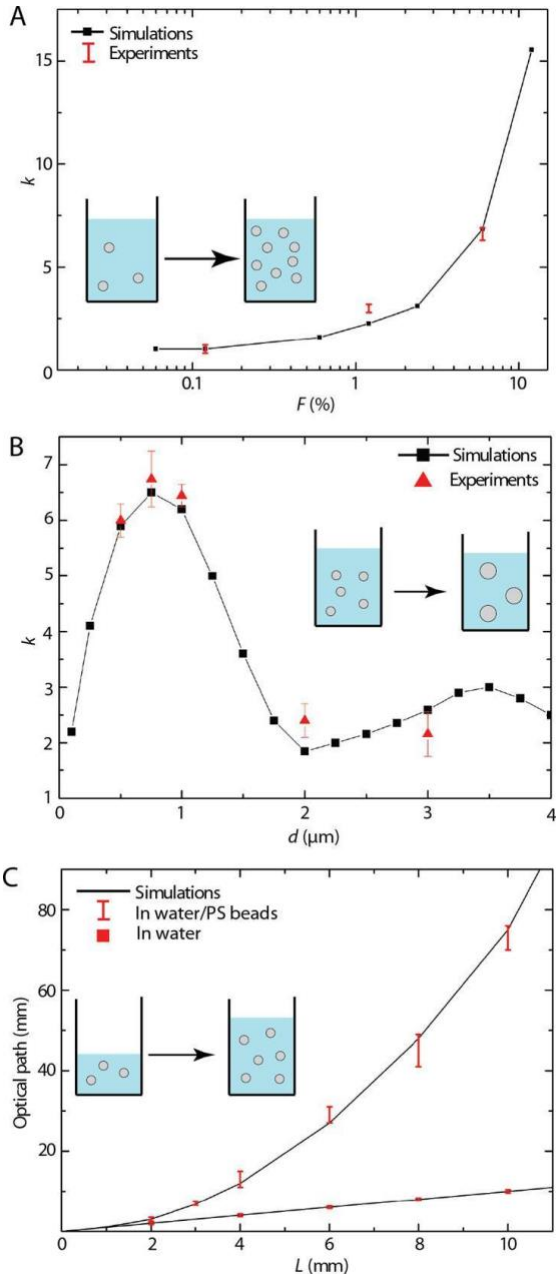
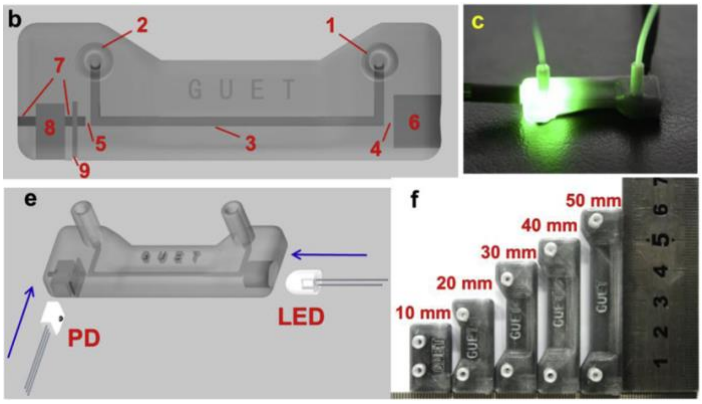


Figure 6. (A) Optical path length (OPL) enhancement as a function of PS bead filling factor F for a 40 μL mixture, when changing the concentration of the PS beads (d = 0.5 μm). (B) OPL enhancement for PS beads with different sizes for a 40 μL mixture, keeping filling factor F equal to 0.052. (C) Optical path for phenol red in water and in a water/PS bead dispersion (d = 0.5 μm) (calculated using eq 5) as a function of measurement cell thickness L. The PS bead concentration (Cps) is kept constant at 0.6 nM. Error bars represent the standard deviation for five samples.

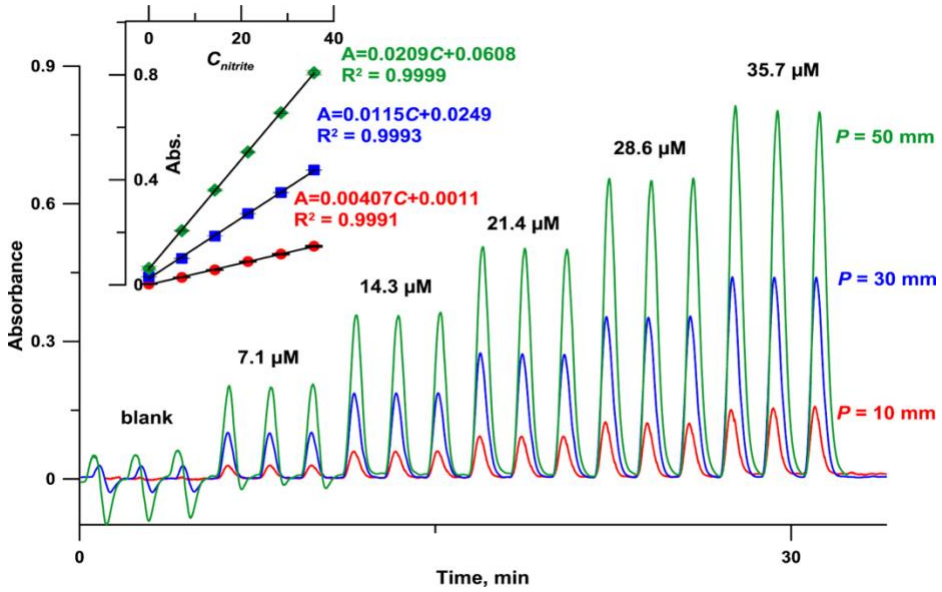
Article: Republished with permission of Elsevier Science & Technology Journals, from Ying Liang, Qiang Liu, Shuai Liu, Xiaoyu Li, YanLi, and Min Zhang. "One-step 3D printed flow cells using single transparent material for flow injection spectrophotometry." *Talanta,* **2019,** *201,* 460–464.; permission conveyed through Copyright Clearance Center, Inc.
Link: https://doi.org/10.1016/j.talanta.2019.04.009
Topics: Spectroscopy, Beer's Law
Question Author: RAH

Questions:

1) In an effort to reduce costs and increase the accessibility of instruments that utilize spectrophotometric detection, some researchers are beginning to experiment with 3D-printed parts. One example of this is the 3D-printed flow cell, shown at right. This device was made using polylactic acid and accommodates a LED at one end and a detector at the other. It can be used for standalone flow injection spectrophotometry or coupled to a chromatographic separation to be used as a detector.



   a) Explain why the sensitivity varies with the length of the flow cell, as shown in the data below.



   b) Could this setup be used for fluorescence analysis? Why or why not?

Article: "Fraunhofer effect atomic absorption spectrometry" by Jennifer A. Rust, Joaquim A. Nobrega, Clifton P. Calloway, Jr., and Bradley T. Jones, *Analytical Chemistry*, **2005**, *77*, 1060-1067.
Link: https://pubs.acs.org/doi/10.1021/ac048917u
Topic: Spectroscopy, atomic absorption
Question Author: MLK

Read the abstract and review the figures, then answer the questions that follow.

The dark lines in the solar spectrum were discovered by Wollaston and cataloged by Fraunhofer in the early days of the 19th century. Some years later, Kirchhoff explained the appearance of the dark lines: the sun was acting as a continuum light source and metals in the ground state in its atmosphere were absorbing characteristic narrow regions of the spectrum. This discovery eventually spawned atomic absorption spectrometry, which became a routine technique for chemical analysis in the mid-20th century. Laboratory-based atomic absorption spectrometers differ from the original observation of the Fraunhofer lines because they have always employed a separate light source and atomizer. This article describes a novel atomic absorption device that employs a single source, the tungsten coil, as both the generator of continuum radiation and the atomizer of the analytes. A 25-μL aliquot of sample is placed on the tungsten filament removed from a commercially available 150-W light bulb. The solution is dried and ashed by applying low currents to the coil in a three-step procedure. Full power is then applied to the coil for a brief period. During this time, the coil produces white light, which may be absorbed by any metals present in the atomization cloud produced by the sample. A high-resolution spectrometer with a charge-coupled device detector monitors the emission spectrum of the coil, which includes the dark lines from the metals. Detection limits are reported for seven elements: 5 pg of Ca (422.7 nm); 2 ng of Co (352.7 nm); 200 pg of Cr (425.4 nm); 7 pg of Sr (460.7 nm); 100 pg of Yb (398.8 nm); 500 pg of Mn (403.1 nm); and 500 pg of K (404.4 nm). Simultaneous multielement analyses are possible within a 4-nm spectral window. The relative standard deviations for the seven metals are below 8% for all metals except for Ca (10.7%), which was present in the blank at measurable levels. Analysis of a standard reference material (drinking water) resulted in a mean percent recovery of 91%. This report attempts to give an historical perspective on the development of a novel atomic spectrometer based on the Fraunhofer effect.

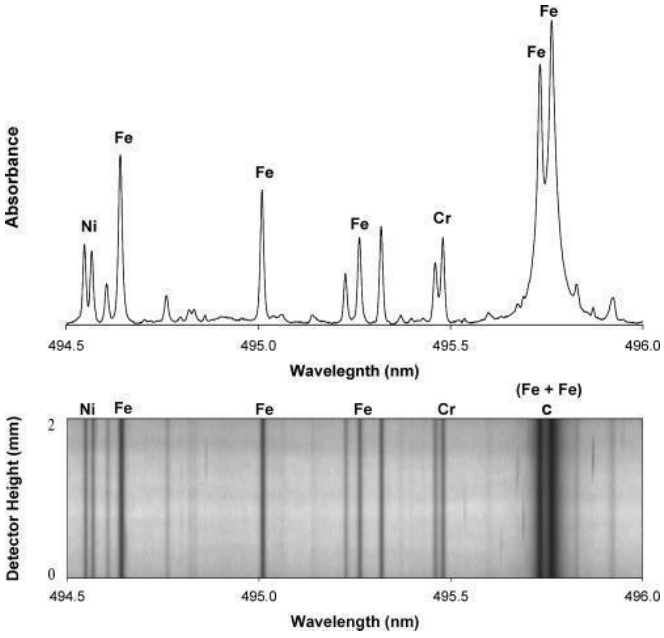


Figure 2. Fraunhofer effect spectrum of the sun including the c band.

1. List the most common methods of atomization in commercial AA instrumentation.

2. Explain why atomic absorption bands are so narrow.

3. Estimate the spectral bandwidth of the monochromator used to collect the data in Figure 5. Show your work.

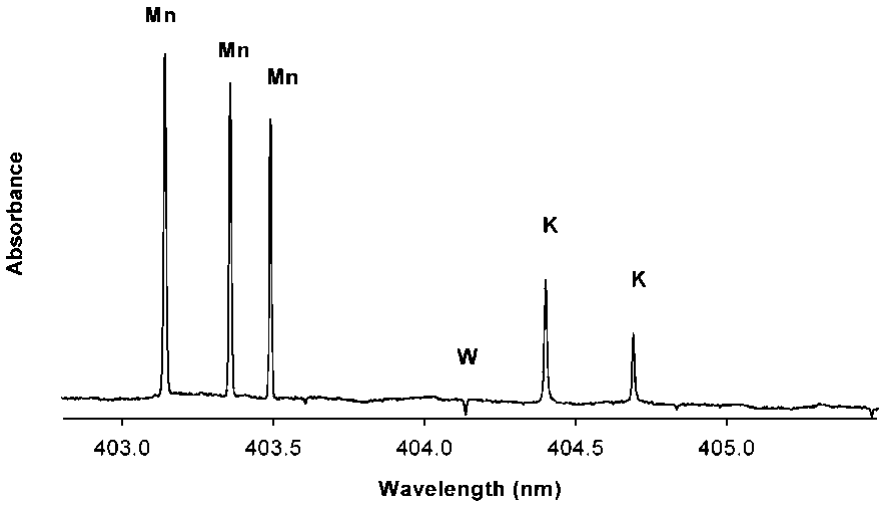


Figure 5. FEAAS spectrum of 10 mg/L Mn and 10 mg/L K. The total time represented by the spectrum is 12 ms, and the spectrum was collected 150 ms after the onset of atomization.

4. Using the abstract, Table 2, and your notes, compare and contrast the figures of merit for this method, flame AA, and graphite furnace AA (GF-AAS).

Table 2. Analytical Figures of Merit for FE-AAS

	Co	Yb	Sr	Ca	Cr	Mn	K
absorption wavelength (nm)	352.7	398.8	460.7	422.7	425.4	403.1	404.4
peak FE-AAS absorbance time (ms)[a]	375	340	200	270	200	348	300
boiling point (K)	3143	1466	1657	1757	2945	2235	1047
relative atomic mass	58.93	173.04	87.62	40.08	52.00	54.94	39.10
recommended GFAAS atomization temp (°C)[b]	2400	2300	2400	2500	2300	1900	1500
FE-AAS LOD (ng)[c]	2	0.1	0.007	0.005	0.2	0.5	0.5
GFAAS LOD (ng)[d]	0.1	0.0005	0.001	0.0005	0.003	0.02	0.1
upper limit of linear range (ng)	400	3	1	0.1	30	500	600
precision (% RSD)[e]	3.8	6.1	1.8	10.7	6.7	4.5	7.8
certified value for drinking water std (mg/L)	5.1[f]		0.015	0.50	0.50		---
FE-AAS determined value (mg/L)	5.1		0.0090	0.60	0.57		---

[a] See Figure 6. [b] Recommended by The Perkin-Elmer Corp. [c] Based on 3 standard deviations in the blank signal and a 25-μL sample volume. [d] Based on 3 standard deviations in the blank signal and a 50-μL sample volume (see refs 48 and 49). [e] The precision was measured at the following analyte concentrations: 0.1 mg/L Yb, 10 mg/L Co, 0.05 mg/L Sr, 3.0 μg/L Ca, 1.0 mg/L Cr, 1.0 mg/L Mn, and 15 mg/L K. [f] This sample was spiked with 5.1 mg/L Co.

5. Assess how the use of a continuum spectrum source in this method would affect the results compared to those obtained with a hollow cathode lamp. Describe the advantages and disadvantages of each light source.

6. Suggest a potential application for this technique and describe how this method would be advantageous compared to more traditional AA instrumentation for this application.

Article: "Spatial Emission Profiles for Flagging Matrix Interferences in Axial-Viewing Inductively Coupled Plasma-Atomic Emission Spectrometry: 1. Profile Characteristics and Flagging Efficiency" by George C. Y. Chan and Gary M. Hieftje, *Analytical Chemistry*, **2013**, *85*, 50-57.
Link: https://pubs.acs.org/doi/10.1021/ac302095w
Topic: Spectroscopy, Atomic emission
Question Author: MLK

Read the abstract and review the figures, then answer the questions that follow.

Spatially resolved measurements of analyte emission along the cross-sectional axis of an axially viewed inductively coupled plasma (ICP) are utilized to indicate the presence of any of the three major categories of matrix interferences (i.e., plasma-related, sample introduction-related, and spectral interferences). Barium at concentrations of 0.05 or 0.1 M was chosen as a prototype element for plasma-related matrix effects, whereas common mineral acids (nitric, hydrochloric, sulfuric, and phosphoric) at volumetric concentrations from 1% to 20% were used to simulate sample introduction-related matrix effects. Three spectrally interfering line pairs (As and Cd at 228.81 nm, Er and Co at 239.73 nm, and Er and Ce at 302.27 nm) were selected for the study of spectral interferences. Due to dependence on the nature of the interference, the analytical bias at the center of the cross-sectional profile varied between −40% and +50%. In all matrix-interference categories, because plasma characteristics and excitation conditions are heterogeneous along this cross-sectional axis, matrix-induced shifts in analyte emission vary accordingly. As a result, the concentrations determined for an analyte along the cross-sectional plasma axis are not constant but exhibit a position dependence that allows the interference to be flagged. With the exception of spectral interference from emission lines whose total excitation potentials (i.e., the sum of ionization and excitation energies of an ionic emission line) are very close, the spatially resolved concentrations provide an effective indicator for flagging any other matrix interference in axial-viewing ICP-emission spectrometry. The method can be employed under the plasma forward power and carrier-gas flow conditions that are common for robust plasma operation.

1. Label the RF coil, the wavelength selector, and the detector in Figure 1 below.

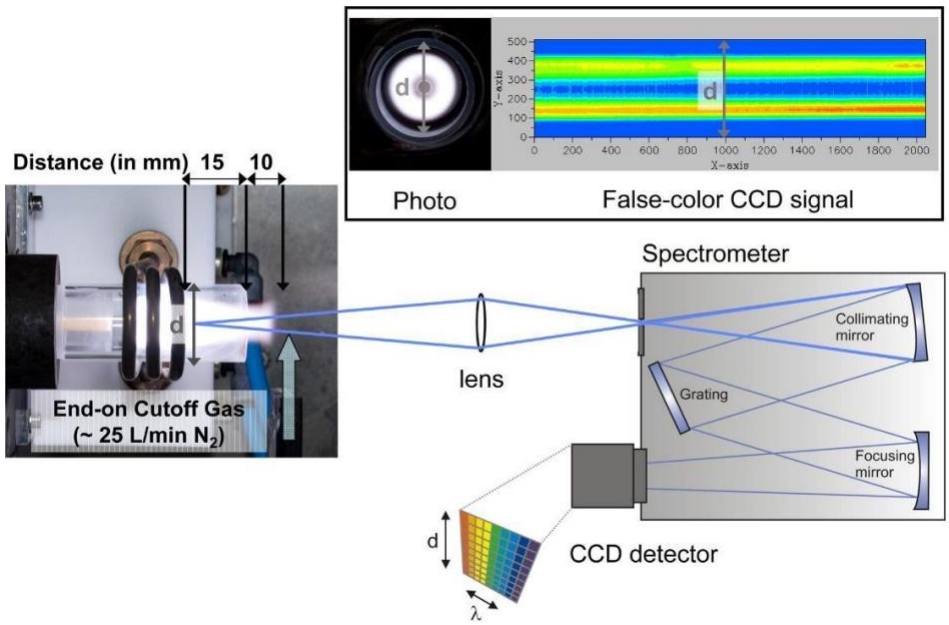


Figure 1. Schematic diagram of the optical arrangement for axial-viewing ICP-AES used in this study. The inset shows a photograph of the plasma when viewed axially and a false-color spatial map of the plasma continuum recorded by the CCD.

2. Describe the three sources of matrix effects listed in the abstract in your own words.

3. Based on the abstract and applying concepts from class, explain in your own words why the effect of matrix components on analyte signal might vary across the diameter of the ICP torch.

4. Explain the abstract's statement that this method cannot effectively detect "spectral interference from emission lines whose total excitation potentials (i.e., the sum of ionization and excitation energies of an ionic emission line) are very close". Why is this interference harder to detect than interference of the matrix with the plasma or sample introduction?

5. Based on Figure 2a below, evaluate the effect of 0.1 M barium on the determination of Mg by ICP-AES. What type of error(s) will be introduced by barium in the sample matrix? How will the results of the analysis by affected?

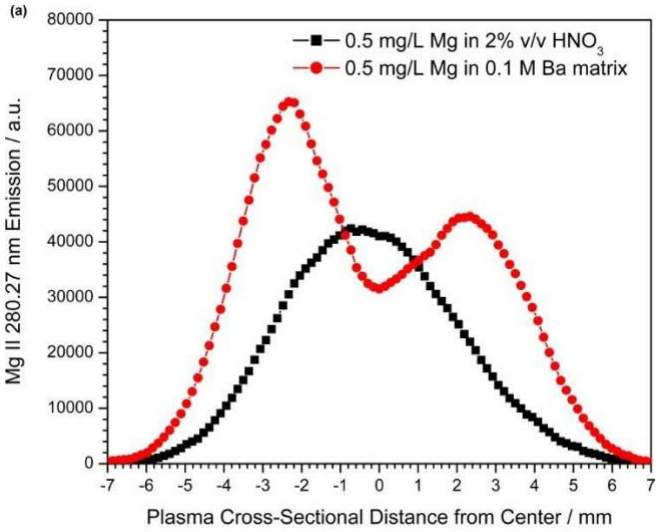


Figure 2. Cross-sectional spatial emission profiles of the Mg II 280.27 nm line in the absence and presence of a 0.1 M Ba matrix at a total central-channel gas flow (including the 0.10 L/min sheath gas flow) of (a) 1.50 L/min. The plasma power was 1100 W, and the injector diameter was 3 mm.

6. Based on Figures 2a and 3, propose a method using ICP-AES to determine accurately the concentration of Mg in a sample that contains Ba.

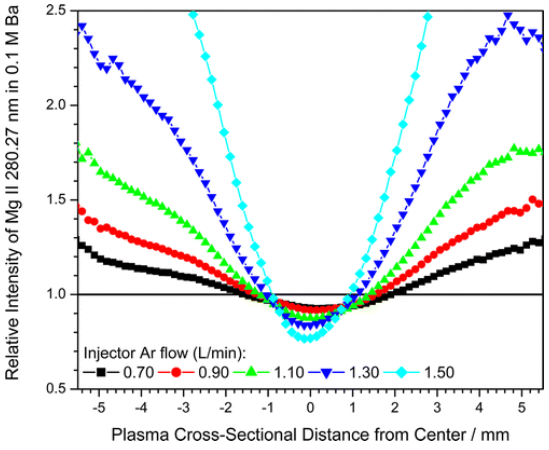


Figure 3. Cross-sectional relative-intensity profiles of Mg II 280.27 nm emission in the presence of a 0.1 M Ba matrix as a function of the total central-channel gas flow at 1100 W plasma power. Injector diameter was 3 mm.

Article: "Lab-on-Chip Measurement of Nitrate and Nitrite for In Situ Analysis of Natural Waters" by Alexander D. Beaton, Christopher L. Cardwell, Rupert S. Thomas, Vincent J. Sieben, François-Eric Legiret, Edward M. Waugh, Peter J. Statham, Matthew C. Mowlem, and Hywel Morgan, *Environmental Science & Technology*, **2012**, *46*, 9548-9556.
Link: https://pubs.acs.org/doi/10.1021/es300419u
Topic: Spectroscopy, UV-Vis
Question Author: MLK

Read the abstract, examine the figure below, then answer the questions that follow.

Microfluidic technology permits the miniaturization of chemical analytical methods that are traditionally undertaken using benchtop equipment in the laboratory environment. When applied to environmental monitoring, these "lab-on-chip" systems could allow high-performance chemical analysis methods to be performed in situ over distributed sensor networks with large numbers of measurement nodes. Here we present the first of a new generation of microfluidic chemical analysis systems with sufficient analytical performance and robustness for deployment in natural waters. The system detects nitrate and nitrite (up to 350 μM, 21.7 mg/L as NO_3^-) with a limit of detection (LOD) of 0.025 μM for nitrate (0.0016 mg/L as NO_3^-) and 0.02 μM for nitrite (0.00092 mg/L as NO_2^-). This performance is suitable for almost all natural waters (apart from the oligotrophic open ocean), and the device was deployed in an estuarine environment (Southampton Water) to monitor nitrate+nitrite concentrations in waters of varying salinity. The system was able to track changes in the nitrate–salinity relationship of estuarine waters due to increased river flow after a period of high rainfall. Laboratory characterization and deployment data are presented, demonstrating the ability of the system to acquire data with high temporal resolution.

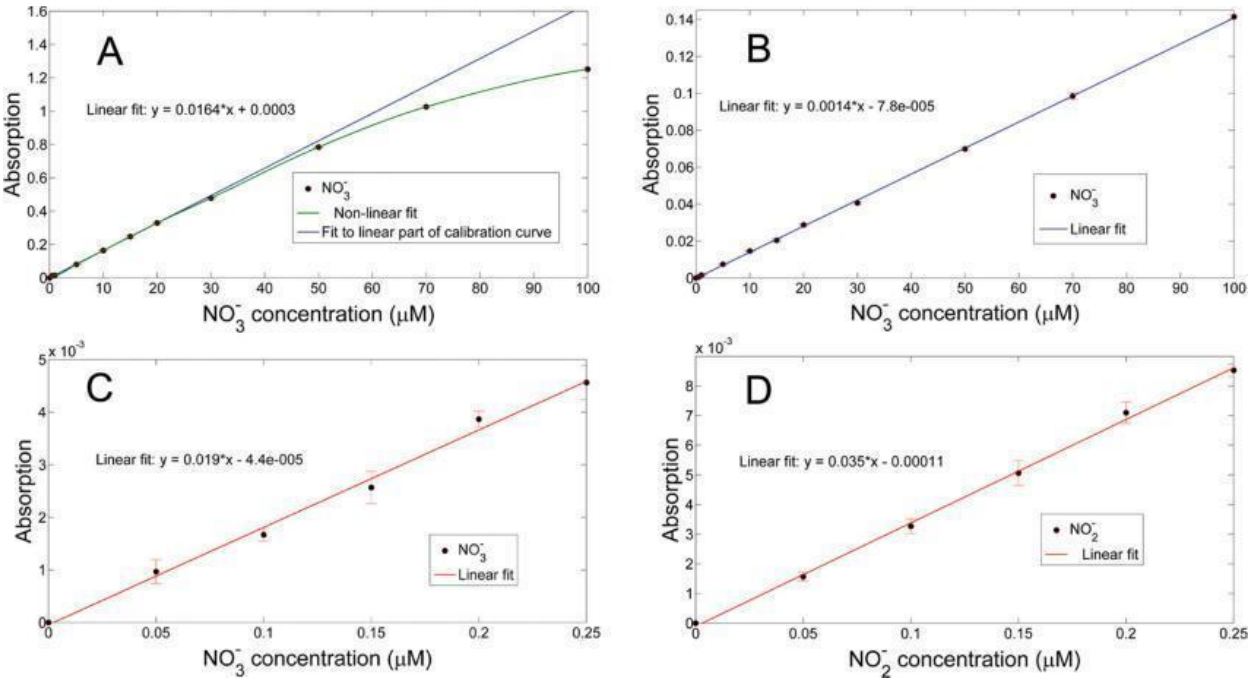


Figure 2. Calibration curves with associated error bars (± 1σ) based on three repeats. (A) Calibration data for the long (25 mm) cell for nitrate standards up to 100 μM. Points up to 20 μM are fitted with a linear regression line. (B) Calibration data for the short (2.5 mm) cell for nitrate standards, which remains linear up to 100 μM. (C) Calibration data for the 25-mm cell for low nitrate standards. (D) Calibration data for the 25-mm cell for low nitrite standards. A flow rate of 300 μL/min (per syringe) was used for data in graphs A and B, and a flow rate of 150 μL/min (per syringe) was used for the data in graphs C and D (to maximize reduction efficiency at low concentrations).

1. Identify the equation that relates absorbance and path length.

2. The microfluidic device included two different absorbance cells with different path lengths. Using Figure 2, report the calibration sensitivity for nitrate for each absorbance cell.

3. Using Figure 2, estimate the concentration limits of detection and limits of linearity for each cell. Show your work, and check your answers against the values reported in the abstract.

4. Compare and contrast the figures of merit you determined in questions #2-3 for the two cells. Explain any differences.

5. Instead of using a chopper to divert a single light source to the reference cell and two measurement cells, the authors used three separate LEDs for the reference cell and each of the two sample cells. Assess how this instrument design might affect the measurement.

6. Describe how this device would need to be modified for a new application: point-of-production testing of nitrate levels in cured meats.

Article: Republished with permission of Elsevier Science & Technology Journals, from Jian Ma, Bo Yang, and Robert H. Byrne, "Determination of nanomolar chromate in drinking water with solid phase extraction and a portable spectrophotometer," *J. Haz. Mat.* **2012**, *219-220*, 247-252.; permission conveyed through Copyright Clearance Center, Inc.
Link: https://www.sciencedirect.com/science/article/pii/S0304389412003792
Topics: Figures of merit UV-Vis
Error analysis
Question Author: MLK

The UV-Vis absorbance spectra and calibration curve at 550 nm below are for detection of hexavalent chromium in drinking water. The Cr^{6+} ion is reacted with 1,5-diphenylcarbazide to form a colored complex, then extracted by solid phase extraction (SPE).

1. Calculate an estimate of the percent of the incident light is transmitted through the 0.2 µM solution. Show your work.

2. The pathlength of the cell used was 1 cm. Calculate an estimate of the molar absorptivity of the chromium complex at 550 nm. Show your work, and justify your choice of data used in the calculation.

3. The authors compared several SPE methods to extract the colored complex. Based on their table below, which method would you recommend? Justify your choice.

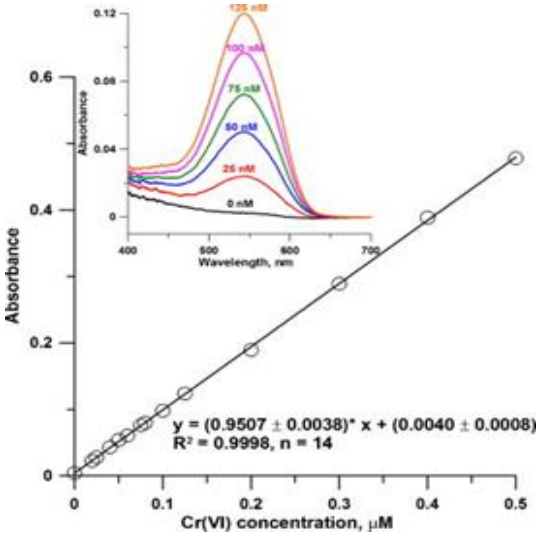


Table 2
Comparison of Cr–DPC extraction methods.

SPE material	Eluent	Enrichment factor	Sample volume, mL	Detection limit, nM	Reusable times of SPE	Analysis time, min	Linear range, nM	RSD, %
Amverlite XAD-4 resin	Acetone–H_2SO_4	27	400	115	7	>100[a]	N.D.[b]	3.6
Mixed-bed adsorbent	Polyethylene glycol–H_2SO_4	25	250	115	10	>62[a]	0–19,000	4.0
Alumina	H_2SO_4	125	5000	77	5	>1000[a]	0–19,000	2.5
Ambersorb 563 resin	Acetone	30	150	65	N.D.[b]	>75[a]	0–4800	<6.0
C18	Acetone–H_2SO_4	10	50	16	N.D.[b]	>20[a]	0–23,000	2.5
C18	Methanol	25	50	3	60	10	0–500	3.4

[a] Calculated from the reported sample loading volume and maximum flow rate.
[b] No data.

4. Mercury, molybdenum, and vanadium ions also react with 1,5-diphenylcarbazide to form complexes with similar absorbance spectra. What type of error (random or systematic) would result if the water samples were contaminated with one or more of these metals ions? Explain briefly.

5. Suggest a method that could be used to separate Cr(VI) ions or the 1,5-diphenylcarbazide complexes from the contaminants mentioned above prior to detection. Justify your choice.

Article: "Development of a Novel Lysosome-Targeted Ruthenium(II) Complex for Phosphorescence/Time-Gated Luminescence Assay of Biothiols," by Quankun Gao, Wenzhu Zhang, Bo Song, Run Zhang, Weihua Guo, and Jingli Yuan, *Analytical Chemistry*, **2017**, *89*, 4517-4524.
Link: https://pubs.acs.org/doi/abs/10.1021/acs.analchem.6b04925
Topic: Spectroscopy, luminescence methods
Question Author: MLK

Read the abstract, then answer the questions that follow.

Considering the important roles of biothiols in lysosomes of live organisms, and unique photophysical / photochemical properties of ruthenium(II) complexes, a novel ruthenium(II) complex, **Ru-2**, has been developed as a molecular probe for phosphorescence and time-gated luminescence assay of biothiols in human sera, live cells, and *in vivo*. **Ru-2** is weakly luminescent due to the effective photoinduced electron transfer (PET) from Ru(II) luminophore to electron acceptor, 2,4-dinitrobenzene-sulfonyl (DNBS). In the presence of biothiols, such as glutathione (GSH), cysteine (Cys), and homocysteine (Hcy), the emission of **Ru-2** solution was switched ON, as a result of the cleavage of quencher to form the product, **Ru-1**. **Ru-2** showed high selectivity and sensitivity for the detection of biothiols under physiological conditions, with detection limits of 62 nM, 146 nM, and 115 nM for GSH, Cys, and Hcy, respectively. The emission lifetimes of **Ru-1** and **Ru-2** were measured to be 405 and 474 ns, respectively, which enabled them to be used for the background-free time-gated luminescence detection even in the presence of strongly fluorescent dye, rhodamine B. On the basis of this mode, the quantification of biothiols in human serum samples was achieved without interference of background autofluorescence. A morpholine moiety was introduced into the complex to ensure **Ru-2** molecules to be driven into lysosomes of live cells. **Ru-2** showed low cytotoxicity and excellent membrane permeability toward live cells. Using **Ru-2** as an imaging agent, visualizations of biothiols in lysosomes of live cells and in *Daphnia magna* were successfully demonstrated. The results suggested the potential of **Ru-2** for the biomedical diagnosis of biothiol-related human diseases.

1. Define "quencher".

2. Based on Figure 1A, what is the excitation maximum? What is the emission maximum? Explain how you know which is which.

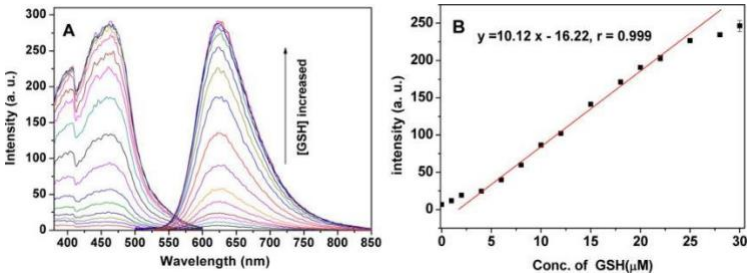


Figure 1. (A) Excitation and emission spectra of **Ru-2** (10 μM) in the presence of different concentrations of GSH (0.0, 1.0, 2.0, 4.0, 6.0, 8.0, 10, 15, 20, 25, 30, 35, 40, 45, 50, and 55 μM) in 50 mM HEPES-DMSO buffer (1:1, pH 7.0). (B) Linear correlation of emission intensity against GSH concentration.

3. Using data from Table S1, explain why Ru-1 is more luminescent than Ru-2.

Table S1. Phosphorescence properties[a] of **Ru-1** and **Ru-2**.

Complex	$\lambda_{ex,\,max}$ (nm)	ε_{459nm} ($cm^{-1}\,M^{-1}$)	$\lambda_{em,\,max}$ (nm)	ϕ^{b} (%)	τ^{c} (ns)
Ru-1	459	2.09×10^4	620	1.94	405
Ru-2	459	2.15×10^4	620	0.12	474

4. Consider the data in Figure S19, which shows time-dependent signals for mixtures of Ru-2 (the indicator molecule), glutathione (GSH, a biothiol), and Rhodamine B (RB, a highly fluorescent dye).
   a) What effect does GSH have on the Ru-2 signal?
   b) How does the RB signal change with time? How does the Ru-2 signal change with time? Explain this difference.

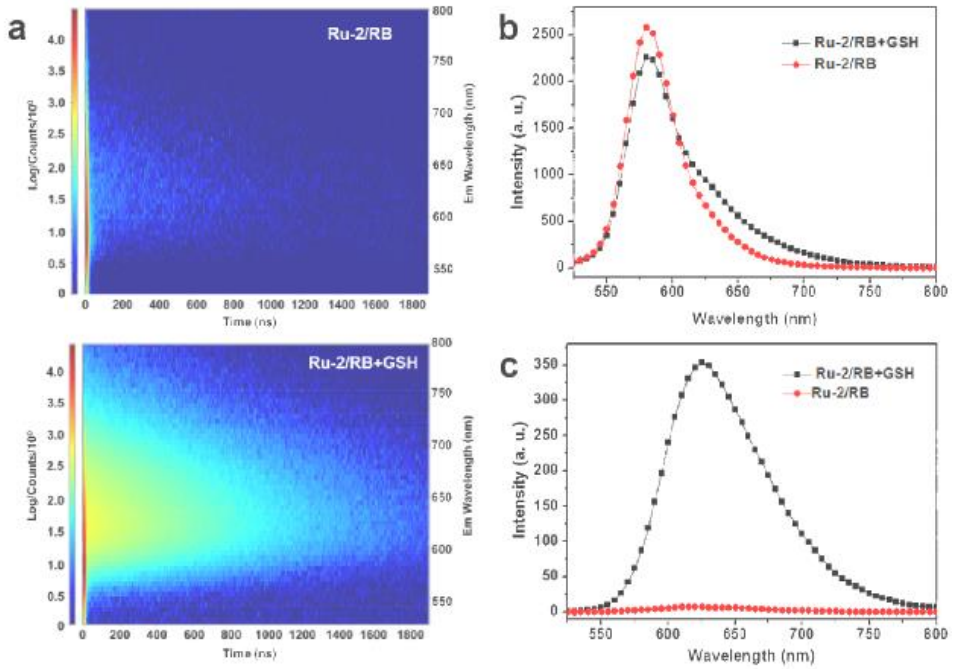


Figure S19. Time-gated emission spectra of the mixture of Ru-2 (10 μM) and rhodamine B (5.0 μM) in the absence and presence of GSH (50 μM) (a), and emission spectra of the mixture acquired after 0 ns delay (b) and 100 ns delay (c). λex = 450 nm.

5. Ru-2 includes a functional group that attracts the molecule to the lysosomal compartment of cells, which is an organelle which performs enzymatic digestion of unwanted cellular components. The pH inside the lysosome is 4.5-5.0. The pH inside the cytoplasm and nucleus of the cell is 7.0-7.4. Biothiols are found in all of these compartments. Based on this information and Figure S17, predict the relative intensity of the signal in the lysosomal compartments, cytoplasm, and nucleus of a cell loaded with Ru-2.

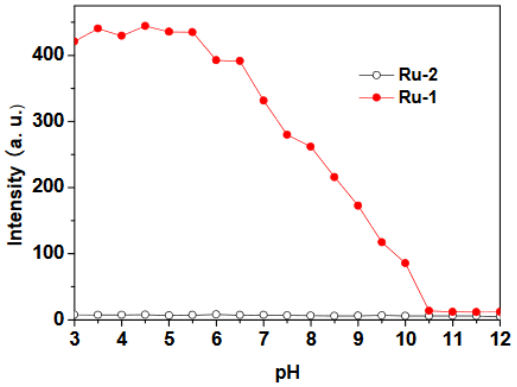


Figure S17. Effects of pH on the phosphorescence intensities of Ru-2 (10 μM, ○) and Ru-1 (10 μM, ●) in 50 mM HEPES-DMSO (1:1) buffers with different pH values.

Article: "Fingerprinting Oils in Water via Their Dissolved VOC Pattern Using Mid-Infrared Sensors" by Thomas Schädle, Bobby Pejcic, Matthew Myers, and Boris Mizaikoff, *Analytical Chemistry*, **2014**, *86*, 9512-9517.
Link: https://pubs.acs.org/doi/10.1021/ac5015029
Topic: Spectroscopy, IR application
Question Author: MLK

Read the abstract below, then answer the questions that follow.

An infrared attenuated total reflection (IR-ATR) method for detecting, differentiating, and quantifying hydrocarbons dissolved in water relevant for oil spills by evaluating the "fingerprint" of the volatile organic compounds (VOCs) associated with individual oil types in the mid-infrared spectral range (i.e., 800–600 cm^{-1}) is presented. In this spectral regime, these hydrocarbons provide distinctive absorption features, which may be used to identify specific hydrocarbon patterns that are characteristic for different crude and refined oils. For analyzing the "VOC fingerprint" resulting from various oil samples, aqueous solutions containing the dissolved hydrocarbons from different crude oils (i.e., types "*Barrow*", "*Goodwyn*", and "*Saladin*") and refined oils (i.e., "*Petrol*" and "*Diesel*") were analyzed using a ZnSe ATR waveguide as the optical sensing element. To minimize interferences from the surrounding water matrix and for amplifying the VOC signatures by enrichment, a thin layer of poly(ethylene-*co*-propylene) was coated onto the ATR waveguide surface, thereby enabling the establishment of suitable calibration functions for the quantification of characteristic concentration patterns of the detected VOCs. Multivariate data analysis was then used for a preliminary classification of various oil-types via their VOC patterns.

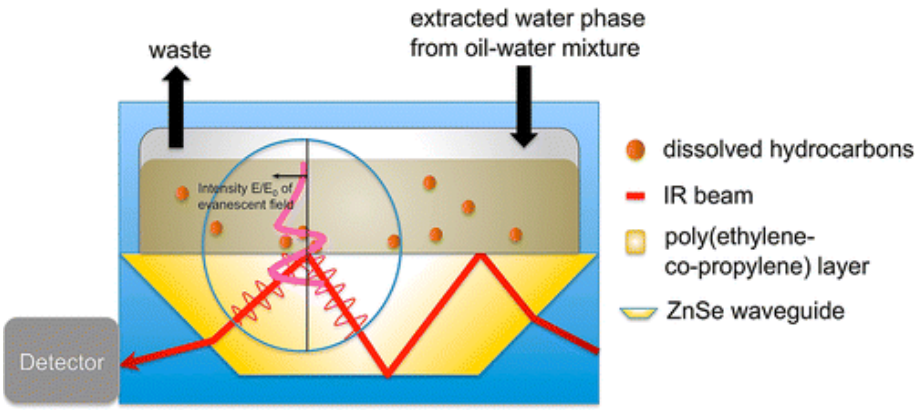


Figure 1. Schematic of the measurement principle. The water phase from the respective oil–water mixture containing the associated dissolved hydrocarbons at a characteristic concentration pattern is flown across a polymer-coated ZnSe IR-ATR waveguide, and the hydrocarbons are detected via evanescent field absorption spectroscopy after enrichment within the polymer membrane.

1. What is the response measured in IR spectroscopy?

2. Explain the concept of an evanescent wave.

3. As noted in the abstract, the poly(ethylene-co-propylene) layer serves two functions in this experiment. List these two functions, and identify which is most critical for an ATR-IR experiment.

4. Figure 3 shows the effect of enrichment time on the spectra for a mixture of water and Saladin, a type of crude oil. Contrast the effect of increased enrichment time on the signal-to-noise ratio and its effect on the resolution of the spectra. Briefly explain your observations.

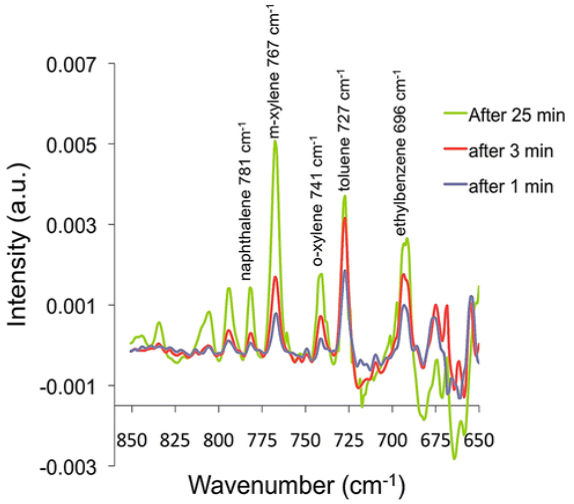


Figure 3. IR-spectra of the aqueous phase from a *Saladin*–water mixture using a polymer coated ZnSe waveguide after enrichment periods of 1, 3, and 25 min exposure/partitioning time. (a.u. ... absorbance units).

5. Consider the Figure 6 below, which shows calibration curves for several volatile organic compounds found in oil samples. Review the challenges of using IR for quantitative analysis and evaluate whether or how this study has addressed these challenges.

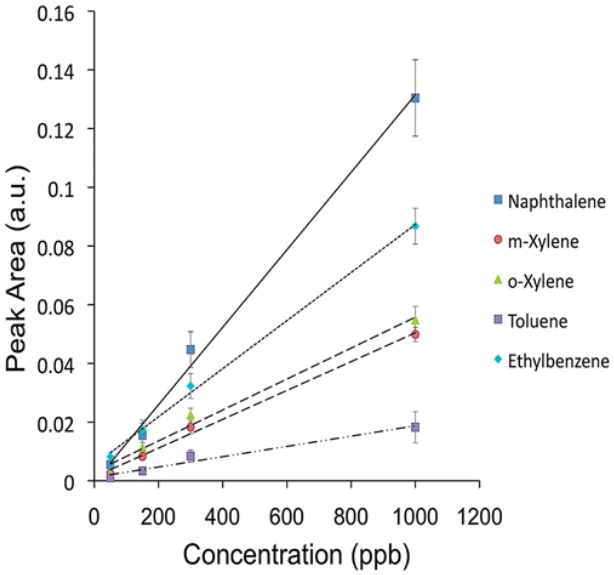


Figure 6. Calibration functions derived from the associated IR-ATR spectra recorded after enrichment at a poly(ethylene-co-propylene) coated ZnSe ATR waveguide exposed to various hydrocarbon mixtures dissolved in water. All absorbance values were plotted after 1.5 h of exposure time ensuring equilibrium conditions. The error bars were calculated as the standard deviation of 5 independent measurements.

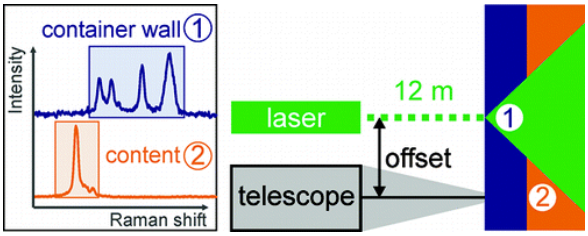
6. Imagine you are writing a press release for the authors of this paper. Compose a 3–5 sentence summary, suitable for a general (non-scientist) audience, that describes how this specific methodology could be used in the aftermath of an oil spill.

Article: "Stand-Off Spatial Offset Raman Spectroscopy for the Detection of Concealed Content in Distant Objects," by Bernhard Zachhuber, Christoph Gasser, Engelene T.H. Chrysostom, and Bernhard Lendl, *Analytical Chemistry*, **2011**, *83*, 9438-9442.
Link: https://pubs.acs.org/doi/10.1021/ac2021008
Topic: Spectroscopy, Raman application
Question Author: MLK

Consider the abstract, excerpt, and figures below, then answer the following questions.

A pulsed (4.4 ns pulse length) frequency-doubled Nd:YAG laser operated at 10 Hz was used to generate Raman scattering of samples at a distance of 12 m. The scattered light was collected by a 6 in. telescope, and the Raman spectrum was recorded using an Acton SP-2750 spectrograph coupled to a gated intensified charge-coupled device (ICCD) detector. Applying a spatial offset between the point where the laser hit the sample and the focus of the telescope on the sample enabled collection of Raman photons that were predominantly generated inside the sample and not from its surface. This is especially effective when the content of concealed objects should be analyzed. High-quality Raman spectra could be recorded, within 10 s of data acquisition, from a solid ($NaClO_3$) as well as a liquid (isopropyl alcohol) placed inside a 1.5 mm thick opaque low-density polyethylene (LDPE) plastic bottle. The applied spatial offset was also advantageous in cases where the surface of the container was highly fluorescent. In such a situation, Raman spectra of the sample could not be recorded when the sampling volume (telescope observation field) coincided with the focus of the excitation laser. However, with the use of a spatial offset of some millimeters, a clear Raman spectrum of the content (isopropyl alcohol) in a strongly fluorescent plastic container was obtained.

Due to random scattering of the photons in the sample material, the illuminated area in the sample increases with increasing depth. In conventional Raman spectroscopy, the detector is placed in line with the laser. However, with SORS the detector is spatially displaced from the laser spot. With increasing offset between laser and detector the ratio of content-to-surface signal rises.



1. List the ways in which matter can interact with light. Which interaction is measured in Raman spectroscopy?

2. Explain why fluorescence signals often interfere with Raman scattering signals.

3. The frequency doubled Nd:YAG laser used in this experiment has a wavelength of 532 nm. Calculate the wavelength in nm of the characteristic Stokes line for sodium chlorate highlighted in Figure 4.

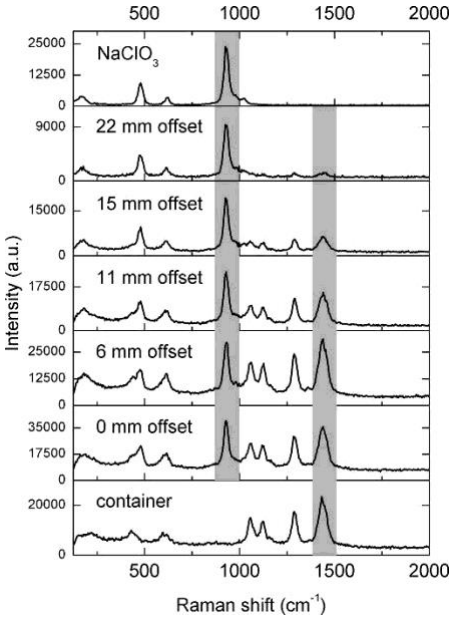


Figure 4. Stand-off SORS spectra of sodium chlorate ($NaClO_3$) in a white HDPE container at 12 m distance; the top and bottom show reference spectra of pure $NaClO_3$ and the container, respectively; other spectra show stand-off SORS spectra; marked bands were used to calculate normalized band intensities.

4. Compare and contrast the block diagram of this instrument (Figure 2) with the block diagram of a standard Raman instrument. Which components are the same? Which components are different? How do the differing components affect the data obtained?

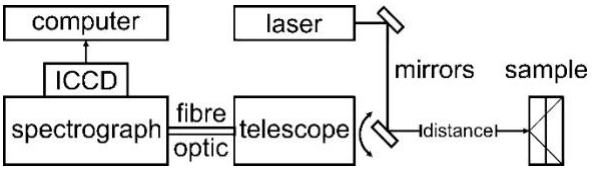


Figure 2. Spatial offset stand-off Raman setup; the laser offset is adjusted with a rotating mirror.

5. Raman and IR are often complementary techniques. Evaluate whether IR spectroscopy could be used for this application, i.e. to determine the chemical contents of a container from a distance of several meters. Justify your answer using content from class discussions.

6. Propose at least 3 possible applications for this technique. For at least one application, list an advantage and a disadvantage of this technique compared to alternatives.

Article: "Capillary HPLC−NMR Coupling: High-Resolution 1H NMR Spectroscopy in the Nanoliter Scale," by Beate Behnke, Götz Schlotterbeck, Ulrich Tallarek, Sabine Strohschein, Li-Hong Tseng, Tony Keller, Klaus Albert, and Ernst Bayer, *Analytical Chemistry*, **1996**, *68*, 1110-1115.
Link: https://pubs.acs.org/doi/10.1021/ac950925a
Topic: Spectroscopy, Nuclear magnetic resonance
Question Author: MLK

Consider the abstract, excerpt, and figures below, then answer the following questions.

Coupling HPLC and NMR is one of the most powerful techniques for simultaneous separation and structural elucidation of unknown compounds in mixtures. To date, however, minimizing the detection volume, as is required when coupling NMR with miniaturized separation techniques, has been accompanied by a dramatic loss in resolution of the NMR spectra. Here, we report on the coupling of gradient capillary HPLC with on-column, high-resolution NMR detection. On-line stopped-flow and static ^{1}H NMR spectra were acquired with capillary columns of 75−315 μm i.d. With detection over a length of 1.2 cm, cell volumes cover a range of 50−900 nL. An on-line-detected NMR separation of dansylated amino acids was carried out in a 315 μm i.d. fused silica capillary packed to a length of 12 cm with C_{18} stationary phase. The low solvent consumption makes the use of fully deuterated solvents economically feasible. NMR spectra with resolution on the order of 3 Hz were obtained using a 50 nL detection cell to measure 1.1 nmol of dansylated γ-aminobutyric acid under static conditions in a 75 μm i.d. capillary.

1. List the major components of a NMR spectrophotometer.

2. Explain why deuterated solvents are preferred for NMR spectroscopy, even though this makes the HPLC step more expensive.

3. Consider Figure 1. Briefly describe the purpose(s) of the NMR probe. Why does the probe include an RF coil?

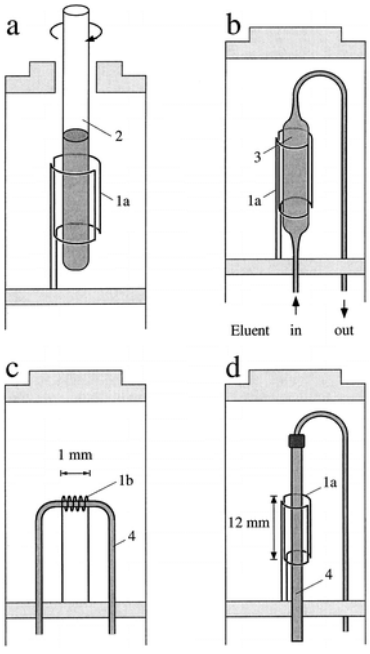


Figure 1. Different geometries of NMR probes. (a) Conventional probe with rotating NMR tube. (b−d) NMR probes with continuous-flow cells for (b) HPLC−NMR and SFC−NMR coupling, (c) CE−NMR coupling, and (d) capillary HPLC−NMR coupling. 1a, Saddle-type rf coil; 1b, solenoidal rf coil; 2, NMR tube (5 mm diameter); 3, flow cell; 4, separation columns with on-column detection.

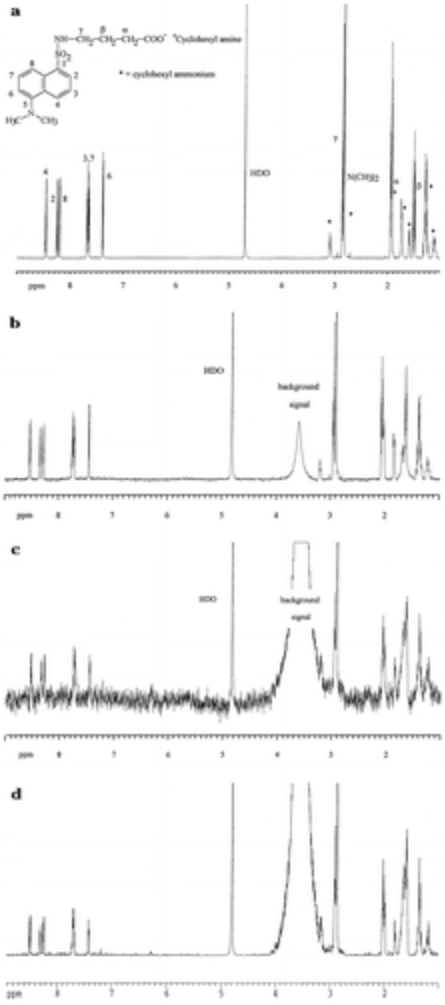


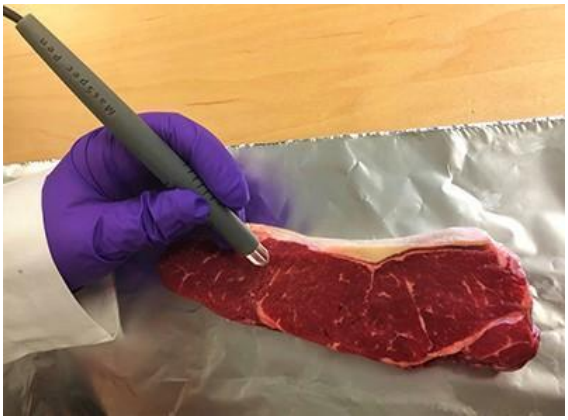
Figure 4. Comparison of static ^{1}H NMR measurements of the cyclohexylammonium salt of dansylated γ-aminobutyric acid in D_2O: (a) 10 nmol in a 240 μL detection cell of a conventional 5 mm tube (64 scans); (b) 21 nmol in a 900 nL detection cell of a 315 μm i.d. capillary (128 scans); (c) 1.1 nmol in a 50 nL detection cell of a 75 μm i.d. capillary (512 scans); and (d) same conditions as in (c), but postacquisition data processing with the program MaxEnt results in an improved presentation of the spectrum.

4. What experimental factors account for the differences in signal to noise ratio for the NMR spectra in Figure 4?

Article: Abigail N. Gatmaitan, John Q. Lin, Jialing Zhang, and Livia S. Eberlin. "Rapid Analysis and Authentication of Meat Using the MasSpec Pen Technology." *Journal of Agricultural and Food Chemistry,* **2021,** *69,* 3527–3536.
Links: https://doi.org/10.1021/acs.jafc.0c07830; https://www.acs.org/content/acs/en/pressroom/presspacs/2021/acs-presspac-march-31-2021/revealing-meat-and-fish-fraud-with-a-handheld-masspec-pen-in-seconds.html
Topics: Mass Spectrometry, Ionization
Mass Spectrometry, Mass Analyzer
Question Author: RAH

Questions:

1) The MasSpec Pen is a new "handheld mass spectrometry device" that has the potential to revolutionize a number of diagnostic methods. However, the MasSpec Pen is currently more of a sample collection device than a handheld mass spectrometer. When using this this device for identifying potentially fraudulent meat products, a small volume (20 μL) of water is delivered to the suspicious tissue surface where metabolites, lipids, and proteins. This device then needs to be coupled to a more traditional mass spectrometer (ionization source, mass analyzer, and detector) for analysis. What ionization source and mass analyzer would you choose to be coupled with this device for the identification of fraudulent meat (e.g., horse meat labeled as beef). Justify your choice and explain any limitations of the analysis.



Article: "Direct Analysis of Large Living Organism by Megavolt Electrostatic Ionization Mass Spectrometry," by Kwan-Ming Ng, Ho-Wai Tang, Sin-Heng Man, Pui-Yuk Mak, Yi-Ching Choi, and Melody Yee-Man Wong, *Journal of the American Society for Mass Spectrometry*, **2014**, *25*, 1515-1520.
Link: https://pubs.acs.org/doi/10.1007/s13361-014-0932-x
Topic: Mass spectrometry, ionization
Question Author: MLK

Review the abstract and figures below, then answer the questions that follow.

A new ambient ionization method allowing the direct chemical analysis of living human body by mass spectrometry (MS) was developed. This MS method, namely Megavolt Electrostatic Ionization Mass Spectrometry, is based on electrostatic charging of a living individual to megavolt (MV) potential, illicit drugs, and explosives on skin/glove, flammable solvent on cloth/tissue paper, and volatile food substances in breath were readily ionized and detected by a mass spectrometer.

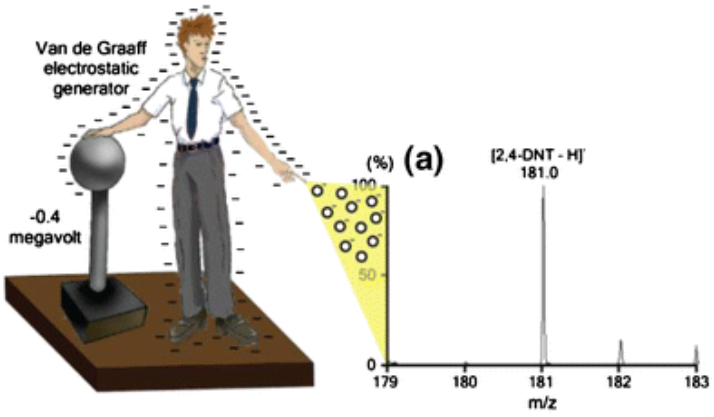


Figure 2. Schematic shows the use of MV electrostatic charging for ionization and mass spectrometric analysis of a human body. The living individual was charged electrostatically to MV potential (0.4 MV) at negative polarity by touching a Van de Graaff generator. An explosive compound (<1 mg of 2,4-dinitrotoluene, 2,4-DNT) deposited on his latex glove was readily ionized and desorbed, and then detected by a mass spectrometer. (**a**) Mass spectrum showing the detection of 2,4-DNT in the form of deprotonated ion at *m/z* 181.0.

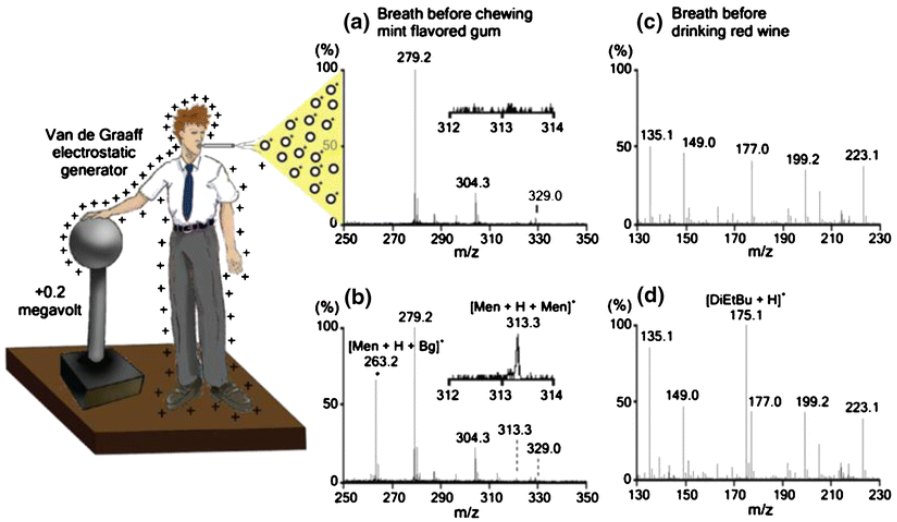


Figure 4. Direct detection of exogenous compounds in the breath of a healthy individual, after chewing mint-flavored gum and drinking a cup of red wine, respectively. Mass spectra of the breath background before (**a**) chewing of mint-flavored chewing gum and (**c**) drinking red wine. (**b**) Mass spectrum of breath after chewing mint-flavored gum for 5 min. *Ions at *m/z* 313.3 in (**b**) were assigned to be the protonated menthol dimer $[Men + H + Men]^+$, ions at *m/z* 263.2 were assigned to be the protonated adduct of menthol with a background molecule (i.e., $[Men + H + Bg]^+$). (**d**) Mass spectrum of breath after drinking red wine. Ions at *m/z* 175.1 were assigned to be protonated diethyl butanedioate, which is a volatile component in red wine.

1. List five common ionization methods for MS.

2. Explain how the polarity of the Van de Graaff generator affects the charge on the analyte ions.

3. Identify the most likely source of the peak at *m/*z = 182 in Figure 2a.

4. Compare and contrast this ionization method with one other ionization method with respect to hard/soft, mass range (atoms, small molecules, macromolecules), and sample phase (gas/condensed).

5. The authors propose that this method could be used for security screening, for example at airports. Assess the potential of this method for that application, including a list of any additional data that would be needed to make a decision.

Article: "Mini 12, Miniature Mass Spectrometer for Clinical and Other Applications — Introduction and Characterization," by Linfan Li, Tsung-Chi Chen, Yue Ren, Paul I. Hendricks, R. Graham Cooks, and Zheng Ouyang. *Analytical Chemistry*, **2014**, *86*, 2909-2916.
Link: https://pubs.acs.org/doi/10.1021/ac403766c
Topic: Mass spectrometry, tandem MS
Question Author: MLK

Review the abstract and figures below, then answer the questions that follow.

A benchtop miniature mass spectrometer system, Mini 12, with ambient ionization source and tandem mass spectrometry capabilities has been developed and characterized. This instrument was developed as a self-contained system to produce quantitative results for unprocessed samples of small volumes including nonvolatile analytes. The ion processing system, vacuum system, and control system are detailed. An integrated sample loading system facilitates automated operation. A user interface has been developed to acquire and to interpret analytical results for personnel who have limited mass spectrometry knowledge. Peak widths of $\Delta m/z$ 0.6 Th (full width at half-maximum) and a mass range of up to m/z 900 are demonstrated with the rectilinear ion trap mass analyzer. Multistage experiments up to MS^5 are accomplished. Consumable cartridges have been designed for use in ambient paper spray ionization, and the recently developed extraction spray ionization method has been employed to improve the quantitative performance. Monitoring of trace-levels of chemicals in therapeutic drugs, as well as in food safety and environmental protection operations is demonstrated. Dual MS/MS scans are implemented to obtain the intensities of the fragment ions from the analyte and its internal standard, and the ratio is used in quantitative analysis of complex samples. Limits of quantitation (LOQ) of 7.5 ng/mL, with relative standard deviations below 10%, have been obtained for selected therapeutic drugs in whole blood throughout their individual therapeutic ranges.

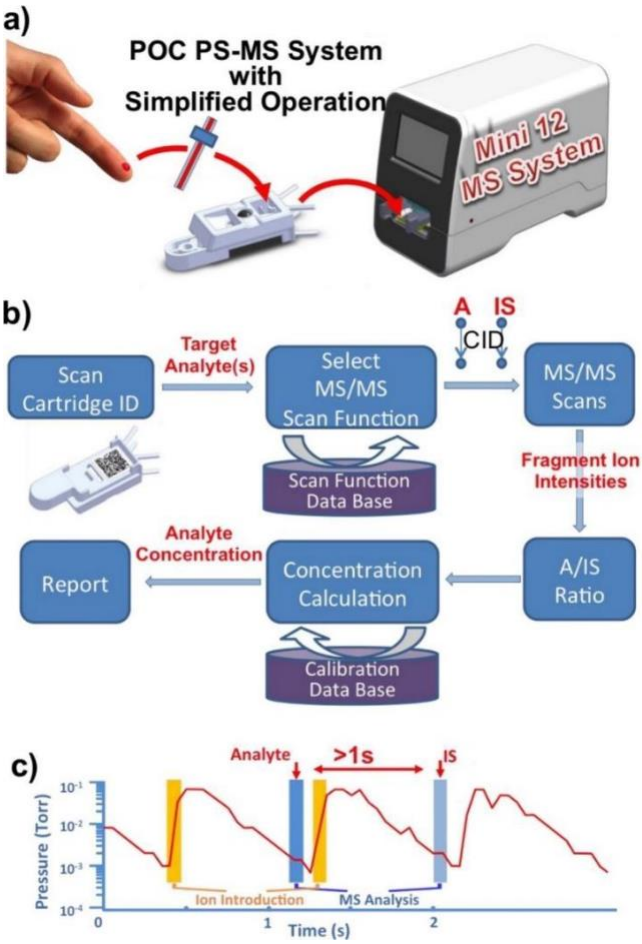


Figure 1. (a) Mini 12 system that provides a simplified operational protocol. (b) Flowchart for automated quantitative analysis using an internal standard. (c) Operation of the dual MS/MS scan function in the time domain.

1. Define precursor ion and product ion.

2. Explain how this mass spectrometer is able to perform tandem MS experiments.

3. Calculate the mean free path of an ion in the mass analyzer at the end of ion introduction. Estimate any values you need from Figure 1c.

4. In Figure 3a and 3d, the authors show an MS spectrum of clenbuterol and an MS^5 spectrum of the same molecule. What reasons might the authors have for performing an MS^5 analysis?

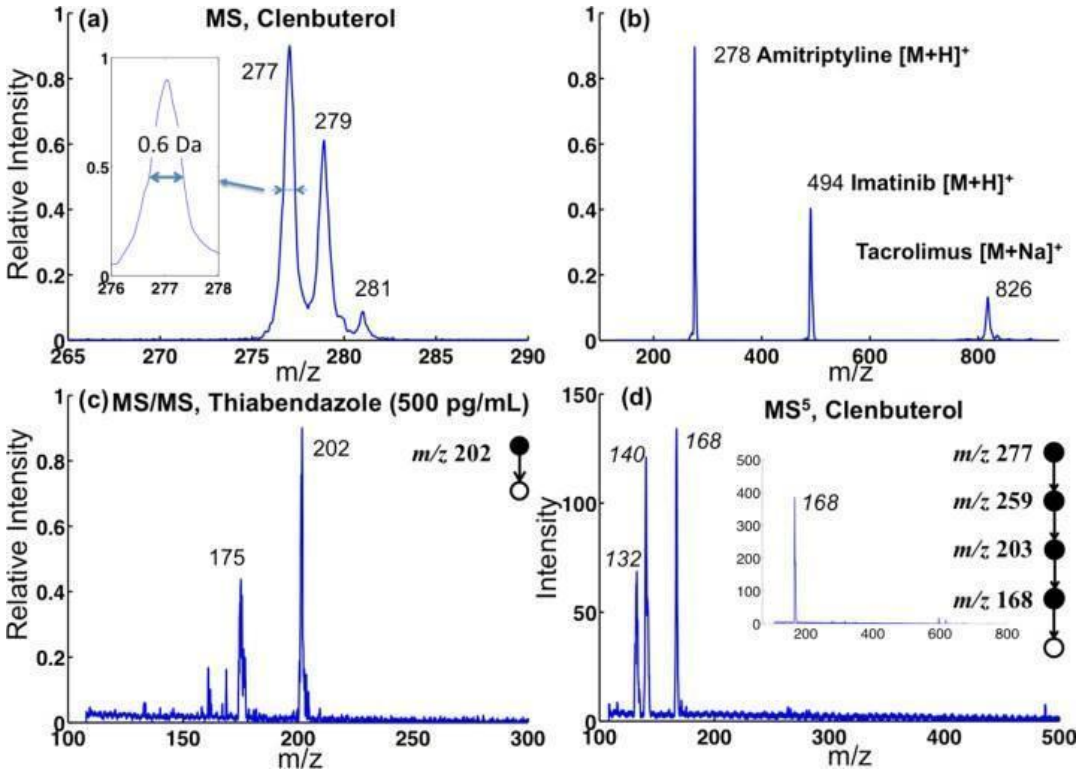


Figure 3. (a) Mass spectrum of 1 ppm clenbuterol recorded using nanoESI with RIT operated at rf frequency 1000 kHz with resonance ejection at 350 kHz (q = 0.80) and ac amplitude ramped from 1.5 to 3.0 V_{p-p}. (b) Mass spectrum of 5 ppm tacrolimus, 1 ppm imatinib, and 1 ppm amitriptyline mixture recorded using nanoESI and RIT rf frequency 1000 kHz and resonance ejection at 300 kHz and 3.5–7.5 V_{p-p}. (c) Mass spectrum of MS^2 of 500 ppt thiabendazole in 50/50 MeOH/H_2O using nanoESI. (d) Mass spectrum of MS^5 of 20 ppm clenbuterol in 50/50 MeOH/H_2O using nanoESI (inset shows the isolated peak of ions with m/z 168).

5. The authors use a rectilinear ion trap for this study. This is similar to the 3D or Paul trap we discussed in class, but the electrodes are all shaped as rectangular plates. Justify why this mass analyzer is a good choice for a miniaturized instrument.

6. The authors suggest that this system could be used to analyze blood, food, or environmental samples. Choose a specific application that requires analysis of one of these types of samples and discuss how this method could be used. Be sure to consider every step of the analysis, not just the mass spectrometer.

Article: "Determination of lead with a copper-based electrochemical sensor" by Wenjing Kang, Xing Pei, Cory A. Rusinek, Adam Bange, Erin N. Haynes, William R. Heineman, and Ian Papautsky, *Analytical Chemistry*, **2017**, *89*, 3345-3352.
Link: https://pubs.acs.org/doi/10.1021/acs.analchem.6b03894
Topics: Electrochemistry, electrochemical methods
Question Author: MLK

Review the abstract and figures below, then answer the questions that follow.

This work demonstrates determination of lead (Pb) in surface water samples using a low-cost copper (Cu)-based electrochemical sensor. Heavy metals require careful monitoring due to their toxicity, yet current methods are too complex or bulky for point-of-care (POC) use. Electrochemistry offers a convenient alternative for metal determination, but the traditional electrodes, such as carbon or gold/platinum, are costly and difficult to microfabricate. Our copper-based sensor features a low-cost electrode material – copper – that offers simple fabrication and competitive performance in electrochemical detection. For anodic stripping voltammetry (ASV) of Pb, our sensor shows 21 nM (4.4 ppb) limit of detection, resistance to interfering metals such as cadmium (Cd) and zinc (Zn), and stable response in natural water samples with minimum sample pretreatment. These results suggest this electrochemical sensor is suitable for environmental and potentially biological applications, where accurate and rapid, yet inexpensive, on-site monitoring is necessary.

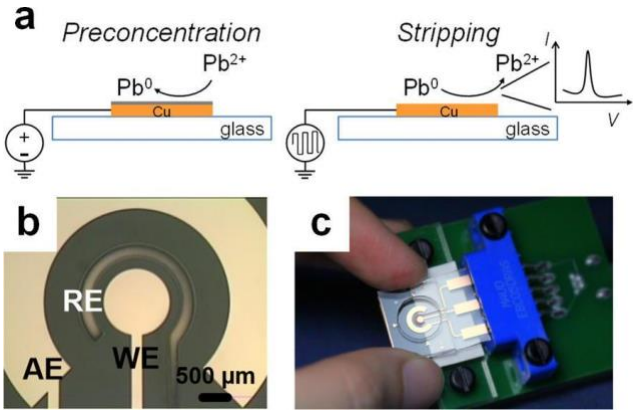


Figure 1. Schematics of the experiment theory and electrode configuration. (a) Illustration of ASV of Pb on a Cu working electrode. (b) Close-up of the electrochemical cell, working electrode (WE), auxiliary electrode (AE), and reference electrode (RE). (c) Photograph of the sensor with an interface connecting to a potentiostat.

1. List the purpose of each of the three electrodes: the working electrode (WE), auxiliary (or counter) electrode (AE), and the reference electrode (RE).

2. In the preconcentration step, is the lead being oxidized or reduced? Explain how this step allows the lead to be concentrated at the working electrode.

3. The authors found that an applied potential of -0.8 V (versus their copper/copper (II) chloride reference electrode) was most effective for the preconcentration step. Calculate this potential versus a more typical silver/silver chloride reference electrode (+0.197 vs SHE). Assume the potential of the $Cu/CuCl_2$ reference electrode is equal to E°.

4. Describe the characteristics of an ideal reference electrode that we discussed in class. Explain the purpose of using both copper and copper (II) chloride in the reference electrode.

5. The authors checked whether cadmium or zinc would interfere or cause error in the determination of lead using their sensor because both of these elements are commonly found in lead contaminated water. Look up and list the standard potentials for cadmium, lead, and zinc. Which do you expect will be more likely to interfere with the lead determination,

cadmium or zinc? Justify your answer. (Values of E° can be found in reputable sources online or in Appendix 3 of the Skoog textbook. Cite your source.)

6. The authors found that samples needed to be deoxygenated to avoid interference from redox chemistry of oxygen (Figure 4 below). Propose at least two different methods that the authors could use to avoid this issue if they wanted to use their portable device to test water samples in the field.

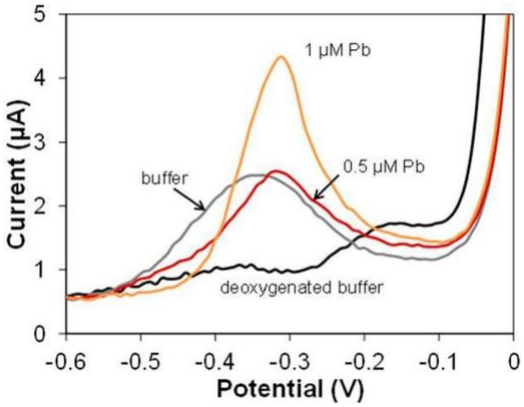


Figure 4. ASV of acetate buffer background (0.2 M, pH 5.5) before (gray) and after (black) deoxygenating. The peak amplitudes are compared with deoxygenated 0.5 and 1 µM Pb.

Article: "An Easily Fabricated Low-Cost Potentiostat Coupled with User-Friendly Software for Introducing Students to Electrochemical Reactions and Electroanalytical Techniques," by Yuguang C. Li, Elizabeth L. Melenbrink, Guy J. Cordonier, Christopher Boggs, Anupama Khan, Morko Kwembur Isaac, Lameck Kabambalika Nkhonjera, David Bahati, Simon J. Billinge, Sossina M. Haile, Rodney A. Kreuter, Robert M. Crable1 and Thomas E. Mallouk, *Journal of Chemical Education*, **2018**, *95*, 1658-1661.
Link: https://pubs.acs.org/doi/10.1021/acs.jchemed.8b00340
Topic: Electrochemistry, potentiostats
Question Author: MLK

Consider Figure S6, then answer the questions that follow.

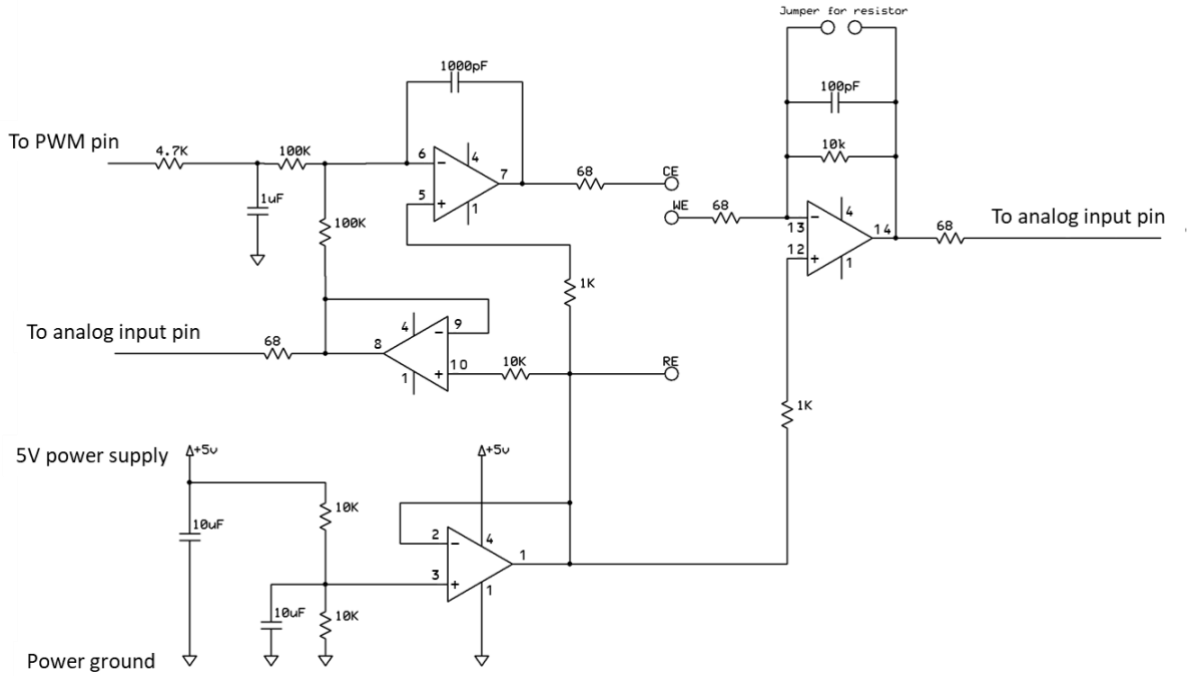


Figure S6. The electronic schematic diagram for the Arduino daughter board. The component parts are listed in Table S1 and the assembly instructions are given in Figure S4 and Figure S5.

1. List the rules for operational amplifier circuits.

2. Explain the purpose of the pins labeled 1 and 4 on each of the op amps in Fig. S6.

3. Circle the voltage follower circuit(s) in the diagram.

4. Contrast the resistance to current flow through the counter electrode (CE) with the resistance to current flow through the reference electrode (RE). How does the resistance associated with each electrode contribute to its function?

5. The upper right op amp has a "Jumper for resistor" as part of its feedback loop. How will adding resistance to this circuit affect the current-to-voltage converter output?

Article: Thanyarat Chaibun, Chan La-o-vorakiat, Anthony P. O'Mullane, Benchaporn Lertanantawong, and Werasak Surareungchai. "Fingerprinting Green Curry: An Electrochemical Approach to Food Quality Control."*ACS Sensors,* **2018,** *3,* 1149–1155.
Link: https://doi.org/10.1021/acssensors.8b00176
Topic: Electrochemistry, voltammetry
Question Author: RAH

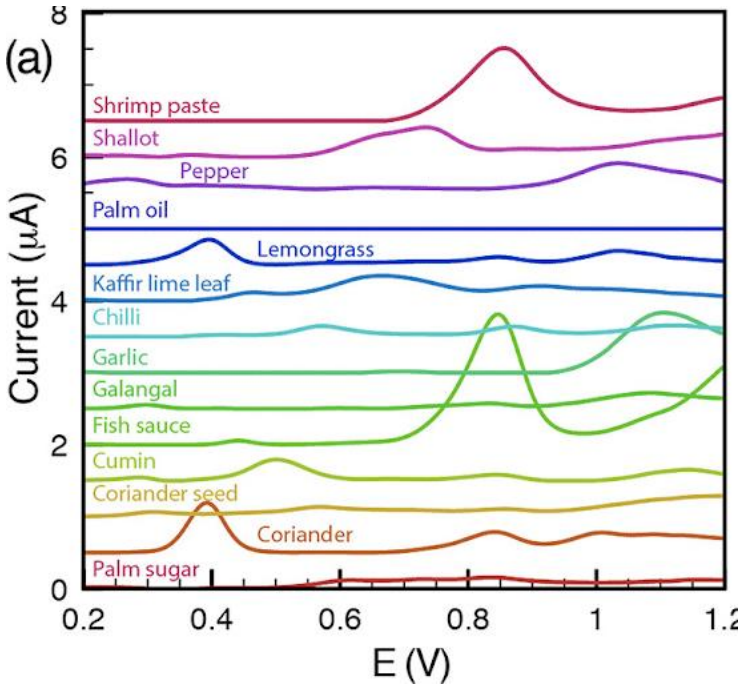
Questions:
Electrochemistry has great potential for applications such as food safety monitoring and quality control, as recently demonstrated in a paper on "fingerprinting" of green curry. The authors used a pulsed voltammetry technique to identify and quantify four key ingredients in Thai curries.

1) Explain two advantages of electrochemical analysis techniques relative to chromatographic or spectroscopic techniques.

2) Explain two disadvantages of electrochemical analysis techniques relative to chromatographic or spectroscopic techniques.

3) When utilizing voltammetric analysis techniques, explain what factors other than concentration may contribute to the observed current.

4) Using the data in the below figure, assess the limitations of this technique for the simultaneous detection (identification and/or quantification) of multiple ingredients in curry.



Article: "Voltammetric pH Nanosensor" by Magdalena Michalak, Malgorzata Kurel, Justyna Jedraszko, Diana Toczydlowska, Gunther Wittstock, Marcin Opallo, and Wojciech Nogala, *Analytical Chemistry*, **2015**, *87*, 11641-11645.
Link: https://pubs.acs.org/doi/10.1021/acs.analchem.5b03482
Topic: Electrochemistry, voltammetry
Question Author: MLK

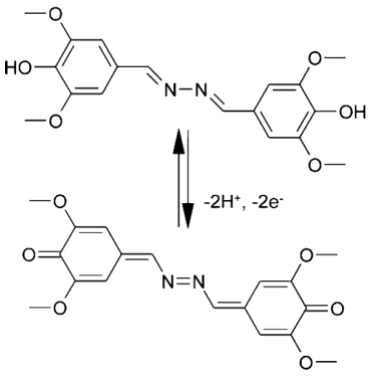
Read the abstract and consider the figures, then answer the questions that follow.

Nanoscale pH evaluation is a prerequisite for understanding the processes and phenomena occurring at solid–liquid, liquid–liquid, and liquid–gas interfaces, e.g., heterogeneous catalysis, extraction, partitioning, and corrosion. Research on the homogeneous processes within small volumes such as intracellular fluids, microdroplets, and microfluidic chips also requires nanometer scale pH assessment. Due to the opacity of numerous systems, optical methods are useless and, if applicable, require addition of a pH-sensitive dye. Potentiometric probes suffer from many drawbacks such as potential drift and lack of selectivity. Here, we present a voltammetric nanosensor for reliable pH assessment between pH 2 and 12 with high spatial resolution. It consists of a pyrolytic carbon nanoelectrode obtained by chemical vapor deposition (CVD) inside a quartz nanopipette. The carbon is modified by adsorption of syringaldazine from its ethanolic solution. It exhibits a stable quasi-reversible cyclic voltammogram with nearly Nernstian dependency of midpeak potentials (−54 mV/pH). This sensor was applied as a probe for scanning electrochemical microscopy (SECM) in order to map pH over a platinum ultramicroelectrode (UME), generating hydroxide ions (OH^-) by the oxygen reduction reaction (ORR) at a diffusion-controlled rate in aerated phosphate buffered saline (PBS). The results reveal the alkalization of the electrolyte close to the oxygen reducing electrode, showing the insufficient buffer capacity of PBS to maintain a stable pH at the given conditions.

1. Sketch the applied potential as a function of time for a cyclic voltammogram.

2. Based on the abstract and our class discussions, use your own words to describe two advantages of this method of pH measurement compared to alternative methods.

3. The pH sensor is based on the reaction shown in Scheme 1. As pH increases, will this reaction favor the oxidized or reduced form of syringaldazine? Explain.



Scheme 1. Mechanism of Electrode Reaction of Syringaldazine

4. Explain the relationship between the data shown in Figure 3 and your answer to Question #3.

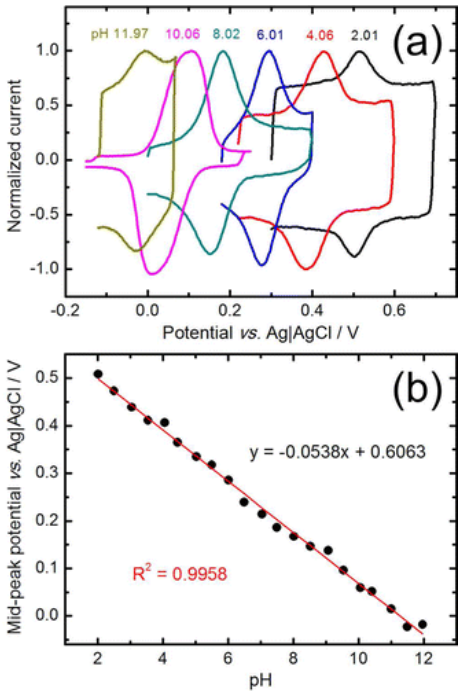


Figure 3. Selected CVs (a) of carbon nanoelectrodes with preadsorbed syringaldazine recorded at various pH values (labeled) of 0.1 M phosphate solutions. Current is normalized versus anodic peak current. (b) Plot of midpeak potential of CVs at various pH values.

5. Evaluate the reversibility of the redox reaction that produces the cyclic voltammograms shown in Figure 2. Cite at least two different pieces of evidence to support your conclusion.

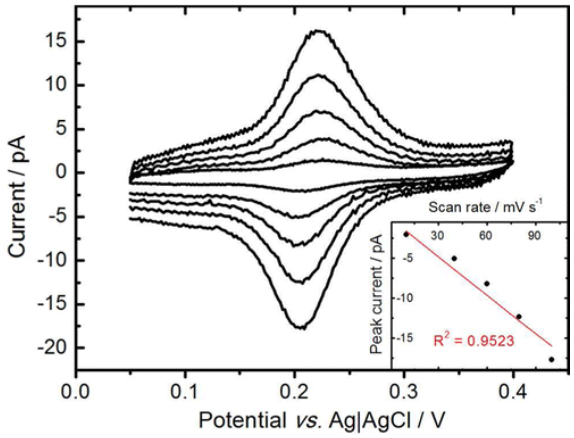
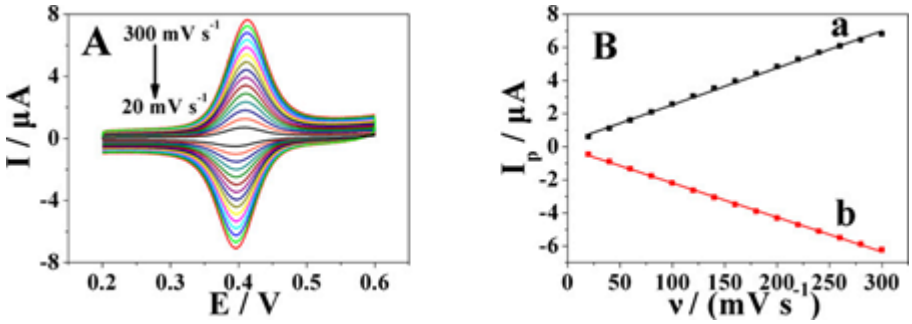


Figure 2. Cyclic voltammograms of carbon nanoelectrode with preadsorbed syringaldazine recorded in 0.1 M phosphate buffer, pH 7.05, at various scan rates: 10, 40, 60, 80, and 100 mV s^{-1}. Inset: plot of cathodic peak current vs scan rate.

6. Describe how this technology could be used to create a map of the pH gradient around a single living cell.

Article: Jianzhi Huang, Qiang Zeng, Silan Bai, and Lishi Wang, "Application of coal in electrochemical sensing," *Analytical Chemistry* **2017**, *89*, 8358-8365.
Link: https://pubs.acs.org/doi/10.1021/acs.analchem.7b01612
Topics: Voltammetry
Question Author: MLK

A 2017 paper demonstrated that annealed coal can be used to make working electrodes as a much less expensive alternative to current carbon-based electrode materials. The figure below shows data for cyclic voltammograms of 10 μM rutin at varying scan rates using a coal-based working electrode.



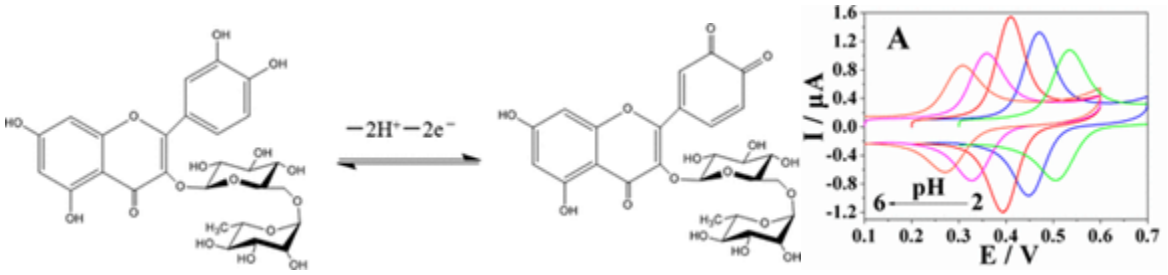
1. Label the oxidation and reduction half-waves in panel A.

2. Is the redox reaction reversible under these conditions? Justify your answer.

3. A saturated calomel electrode (+0.244 V vs SHE) was used as the reference electrode to collect these data. At what voltage would the peak appear if a silver/silver chloride reference electrode (+0.199 V vs SHE) were used?

4. Panel B shows peak current increasing linearly scan rate. Is this the relationship you expect between these two variables? Explain, using one or more equations to support your response.

5. The figures below show the redox behavior of rutin at several pH values. Label the oxidized and reduced forms of the analyte, and explain why the cyclic voltammograms shift to more positive potentials as pH decreases.



Article: "Electrochemical Tattoo Biosensors for Real-Time Noninvasive Lactate Monitoring in Human Perspiration" by Wenzhao Jia, Amay J. Bandodkar, Gabriela Valdés-Ramírez, Joshua R. Windmiller, Zhanjun Yang, Julian Ramírez, Garrett Chan, and Joseph Wang, *Analytical Chemistry*, **2013**, *85*, 6553-6560.
Link: https://pubs.acs.org/doi/10.1021/ac401573r
Topic: Electrochemistry, amperometry
Question Author: MLK

Review the excerpt and figures below, then answer the questions that follow.

The new epidermal lactate biosensor, displayed in Figure 1, consists of a mediated lactate oxidase (LOx) working electrode, prepared by functionalizing the surface of the printed tattoo electrode with tetrathiafulvalene (TTF) and multiwalled carbon nanotubes (CNT), followed by tethering the LOx enzyme, and a biocompatible chitosan overlayer. The latter prevents the efflux of the biochemical backbone from the reagent layer onto the underlying epidermis. The resulting tattoo lactate biosensor was evaluated extensively for its capability to withstand repeated iterations of mechanical deformations relevant to the wearer's daily activity. Additionally, its analytical figures of merit have been characterized. Finally, in order to validate the concept, the lactate biosensor was applied to the skin of human subjects, who were asked to endure prolonged physical exercise (cycling); the corresponding sweat lactate temporal profiles were recorded via amperometric methods. Simultaneous assessment using control epidermal tattoo sensors (lacking the LOx enzyme) confirmed the high specificity toward sweat lactate. The temporal lactate profiles demonstrate that the new wearable lactate biosensor platform performs desirably under fitness routines, thereby substantiating its utility for the noninvasive assessment of lactate levels and degree of physical exertion.

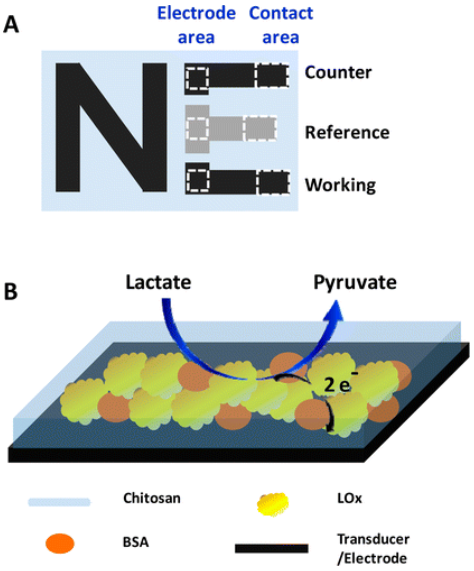


Figure 1. (A) Schematic illustration of a three-electrode "NE" tattoo biosensor for electrochemical epidermal monitoring of lactate. (B) Constituents of the reagent layer of the working electrode which is coated by biocompatible polymer (chitosan). See the text for further details.

1. List the possible reasons why a coating might be applied to a working electrode.

2. Explain why very little signal was obtained for the enzyme-free sensor traces (b) in Figure 5C, D compared to the LOx-containing sensor traces (a).

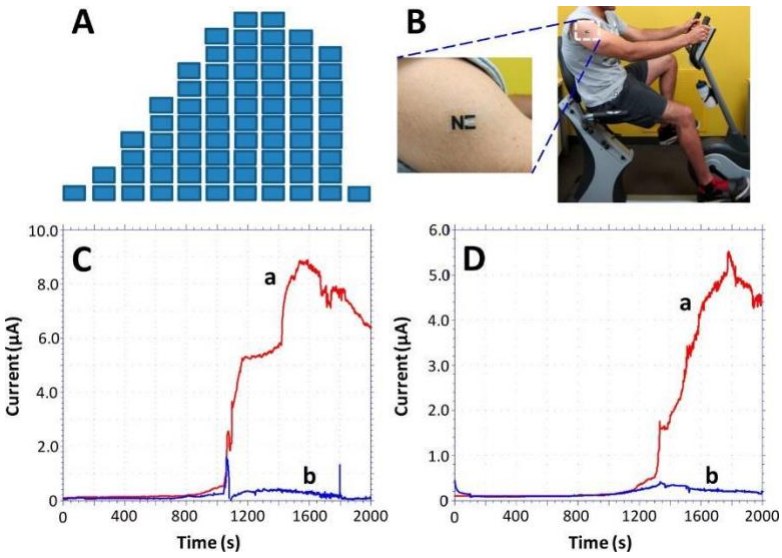


Figure 5. Monitoring of sweat lactate during 33 min of cycling exercise while changing the work intensity. (A) Exercise resistance profile on a stationary cycle. Subjects were asked to maintain a constant cycling rate while the resistance was increased every 3 min for a total evaluation of 30 min. A 3-min cool down period followed the exercise. (B) An "NE" lactate biosensor applied to a male volunteer's deltoid; (C and D) Response of the LOx- (a) and enzyme-free (b) tattoo biosensors during the exercise regimen (shown in part A) using two representative subjects. Constant potential, +0.05 V (vs Ag/AgCl); measurement intervals, 1 s.

3. Identify which parts of the signals in Figure 2A are due to non-Faradaic current versus Faradaic current.

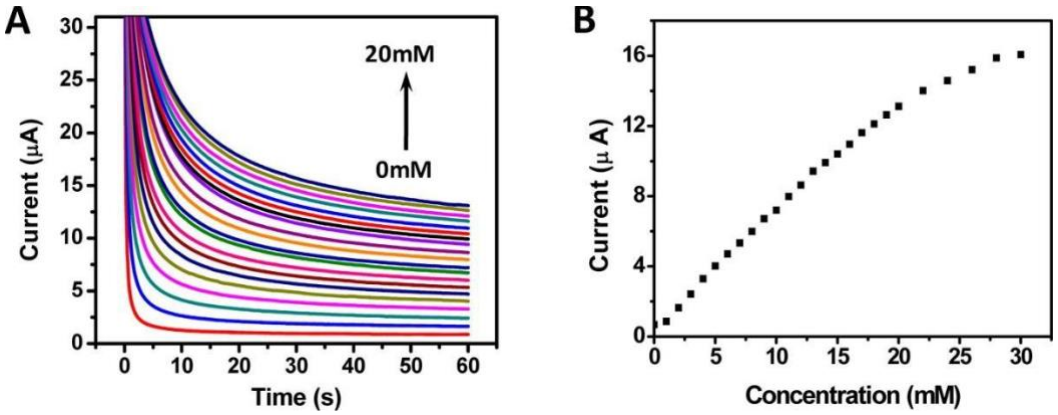


Figure 2. (A) Amperometric response for increasing concentrations of lactate (1 mM increments) over the 0–20 mM range; $E_{applied}$ = +0.05 V (vs Ag/AgCl). (B) Calibration plot up to 30 mM lactate, based on sampling the current at 60 s.

4. Explain why the authors chose to use the current 60 s after voltage was applied to construct the calibration curve in Figure 2B.

5. Justify the choice of amperometric detection for this application based on its advantages compared to alternative techniques we have discussed in class.

6. Compose a list of questions that you would want answered before investing in a company that planned to commercialize this technology.