

Video Article

Fluorescent Paper Strips for the Detection of Diesel Adulteration with Smartphone Read-out

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Abstract

Three fluorescent molecular rotors of 4-dimethylamino-4-nitrostilbene (4-DNS) were investigated for their potential use as viscosity probes to indicate the content of kerosene in diesel/kerosene blends, a wide-spread activity to adulterate fuel. In solvents with low viscosity, the dyes rapidly deactivate via a so-called twisted intramolecular charge transfer state, efficiently quenching the fluorescence. Measurements of diesel/kerosene blends revealed a good linear correlation between the decrease in fluorescence and the increase of the fraction of the less viscous kerosene in diesel/kerosene blends. Immobilization of the hydroxy derivative 4-DNS-OH in cellulose paper yielded test strips that preserve the fluorescent indicator's behavior. Combination of the strips with a reader based on a smartphone and a controlling app allowed to create a simple field test. The method can reliably detect the presence of kerosene in diesel from 7 to 100%, outperforming present standard methods for diesel adulteration.

Video Link

The video component of this article can be found at <https://www.jove.com/video/58019/>

Introduction

Fuel adulteration is a serious problem in many different parts of the world, simply due to the enormous relevance of fuel as an energy source. Running engines on adulterated fuel reduces their performance, leads to earlier engine failure and entails environmental pollution¹. Increased SO_x emissions occur if diesel is adulterated with kerosene that usually contains a higher amount of sulfur^{2,3}. Although the problem exists for decades, sustainable fuel management that uncovers such criminal activity at its point of origin is still rare, because simple and reliable tests for fuel adulteration are largely lacking⁴. Despite substantial progress in laboratory-based mineral oil analysis in the past decades^{5,6,7}, approaches to on-site measurements are still scarce. Various methods for the use outside of the laboratory have recently been devised, using fiber optics⁸, field-effect transistors⁹ or mechano-chromic materials¹⁰. Although they overcome some of the drawbacks of conventional methods, robust, user-friendly and portable methods are still lacking largely. Fluorescent viscosity probes based on molecular rotors are an interesting alternative^{11,12}, because mineral oils are comprised of a great variety of hydrocarbons that differ in chain length and cyclicity, being often reflected in different viscosities. Because fuels are complex mixtures without specific lead compounds to act as tracers, the measurement of the change of a macroscopic property like viscosity or polarity seems very promising. The latter can be addressed by fluorescent molecular rotors for which the fluorescence quantum yields depend on environmental viscosity. After photoexcitation, deactivation commonly involves a twisted intramolecular charge transfer (TICT) state, the population of which is determined by the viscosity of its surrounding microenvironment¹³. Highly viscous solvents hinder molecular rotors to adopt a TICT state, entailing bright emission. In low-viscous solvents, the rotor can much better access the TICT state, accelerating non-radiative decay and thus quenched fluorescence. The addition of kerosene, with a viscosity of 1.64 mm²·s⁻¹ at 27 °C, to diesel, with respective viscosities of 1.3–2.4, 1.9–4.1, 2.0–4.5 or 5.5–24.0 mm²·s⁻¹ at 40 °C for grades 1D, 2D, EN 950 and 4D^{14,15,16}, reduces the kinematic viscosity of the mixture and potentially leads to a proportional quenching of the fluorescence of a molecular rotor probe. The family of 4-dimethylamino-4-nitrostilbenes (4-DNS) seemed most promising to us because of their strong fluorescence variation over a kinematic viscosity range of 0.74–70.6 mm²·s⁻¹. This range matches well with the known values of kerosene and diesel.

We therefore explored the ability of 4DNS, 2-[ethyl[4-[2-(4-nitrophenyl)ethenyl]phenyl]amino]ethanol (4DNSOH) and (*E*)-4-(2-(ethyl(4-(4-nitrostyryl)phenyl)amino)ethoxy)-4-oxobutanoic acid (4DNSCOOH) to indicate the viscosity of diesel-kerosene mixtures through their fluorescence, depending on intramolecular rotation and finally yielding a rapid test for diesel adulteration with kerosene. The disposable test is easy to use, precise, reliable, cost-effective and dimensionally small. The adsorption of the probes onto the filter paper as a solid support was investigated and the analysis was accomplished with an embedded smartphone-based fluorescence reader. Today, ubiquitously available smartphones are equipped with high-quality cameras, rendering the detection of optical changes such as color and fluorescence straightforward, and paving the way for powerful on-site analyses. We demonstrate here that the measurement of the emission of fluorescent probes adsorbed on paper strips with a smartphone can be used for fraud detection on combustion fuels in a reliable manner¹⁷.

Protocol

1. Fluorescent Dyes (Figure 1A)

1. Purchase commercially available 4-DNS and 4-DNS-OH.
Note: 4-DNS-COOH is not commercially available and is prepared from 4-DNS-OH as described hereafter.
2. Place 50 mg (0.16 mmol) of 2-[ethyl[4-[2-(4-nitrophenyl)ethenyl]phenyl]amino]ethanol, 2 mg (0.016 mmol) of 4-dimethylaminopyridine and 19.2 mg (0.192 mmol) of succinic anhydride in a 10 mL round bottom flask.
3. Dissolve the reagents in 2 mL of dry dichloromethane under argon atmosphere.
4. Add 11.6 μ L (0.08 mmol) of triethylamine and let the mixture react for 20 h.
5. Monitor the reaction by thin layer chromatography until quantitative conversion of the starting materials (R_f = 0.61) into the product (R_f = 0.27) is indicated (hexane/EtOAc, 4/6, v/v).
6. Add 2 mL of water to the mixture before acidification to pH 2 with acetic acid (approx. 10 μ L).
7. Extract the mixture by performing two successive liquid-liquid extractions, with 10 mL of dichloromethane each time.
8. Wash once the reunited organic phases with 10 mL of saturated NaCl ($> 359 \text{ g L}^{-1}$).
9. Dry the organic phases by adding Na_2SO_4 powder until some fine drying agent powder remains visible.
10. Purify the crude product by flash silica column chromatography with petroleum ether:ethylacetate 1:9 as the eluent.
Note: The yield achieved were 49 mg (74%) of the desired product.
11. Perform ^1H NMR analysis of the purified product in $\text{DMSO}-d_6$ to validate the structure (δ 8.17 (d, J = 8.8 Hz, 2H), 7.75 (d, J = 8.8 Hz, 2H), 7.49 (d, J = 8.8 Hz, 2H), 7.41 (d, J = 16.3 Hz, 1H), 7.10 (d, J = 16.3 Hz, 1H), 6.75 (d, J = 8.9 Hz, 2H), 4.18 (t, J = 6.0 Hz, 2H), 3.58 (t, J = 6.0 Hz, 2H), 3.43 (q, J = 7.0 Hz, 2H), 2.50 – 2.45 (m, 4H), 1.10 (t, J = 7.0 Hz, 3H) ppm).
12. Perform ^{13}C NMR analysis of the purified product in $\text{DMSO}-d_6$ to validate the structure (δ 173.36, 172.20, 147.99, 145.23, 145.13, 133.89, 128.76, 126.30, 124.03, 123.67, 120.95, 111.58, 61.52, 48.05, 44.57, 28.73, 28.63, 12.00 ppm).
13. Perform high resolution mass spectrometry with positive electro spray ionization of the purified product, corresponding to the calculated value ($\text{C}_{22}\text{H}_{25}\text{N}_2\text{O}_6$ $[\text{M}+\text{H}]^+$: 413.1707) m/z ratio of 413.1713.

2. Synthesis of the Reference Dye

Note: The synthetic procedure of 8-(phenyl)-1,3,5,7-tetramethyl-2,6-diethyl-4,4-difluoro-4 bora-3a,4a-diaza-s-indacene was adopted from Coskun *et al.*¹⁸.

1. Purify the crude product by column chromatography on silica with toluene as eluent.
Note: The yield achieved were 441 mg (29 %) of bright reddish crystals.
2. Perform ^1H NMR analysis of the pure product at 600 MHz in $\text{DMSO}-d_6$ to validate the structure (δ 0.98 (t, 6H, J = 7.6 Hz), 1.27 (s, 6H), 2.29 (q, 4H, J = 7.6 Hz), 2.53 (s, 6H), 7.27-7.29 (m, 2H), 7.46-7.48 (m, 3H) ppm).
3. Perform high resolution mass spectrometry with positive electro spray ionization of the purified product, corresponding to the calculated value ($\text{C}_{23}\text{H}_{28}\text{BF}_2\text{N}_2$ $[\text{M}+\text{H}]^+$: 381.2314) m/z ratio of 381.2267.

3. TEST STRIP FABRICATION, METHOD 1.

1. Prepare 1 mM solutions of the reference dye and dyes 4-DNS, 4-DNS-OH and 4-DNS-COOH in toluene.
2. Cut cellulose strips of 30×5 mm from filter paper.
3. Place approximately 50 of those strips (611 mg) in a sealable 5 mL vial together with 4.5 mL of the desired dye solution from step 3.1.
4. Shake the strips inside the vial with a vertical rotator for 20 min at 30 rpm.
5. Pour the toluene solution out of the vial, and immediately fill with 4 mL of cyclohexane and rotate for 1 min at 30 rpm to wash off excess dyes.
6. Repeat the washing operation from Step 3.5 three times.
7. Dry the obtained test strips on a filter paper for 10 min in air at room temperature.

4. Test Strip Fabrication, Method 2.

1. Amination of the paper strips.
 1. Cut cellulose strips of 30×5 mm from filter paper.
 2. Under a fume hood, place approximately 20 of those strips (308 mg) in a flask containing 40 mL of toluene.
 3. Add 960 μ L of 3-aminopropyltriethoxysilane (APTES) in the flask and stir the mixture for 24 h at 80 $^\circ\text{C}$.
 4. Remove the strips from the flask and wash thoroughly with 50 mL of ethanol.
 5. Dry the strips for 2 h at 50 $^\circ\text{C}$.
2. Grafting of the dye.
 1. Under a fume hood, dissolve 5 mg of 4-DNS-COOH (13 μ mol) in 10 mL of dry dichloromethane under argon atmosphere in a 25 mL flask.
 2. Add N,N' -dicyclohexylcarbodiimide (DCC, 3.3 mg, 16 μ mol) and allow the carboxylic acid to be activated for 15 min.
 3. Add triethylamine (2.2 μ L, 16 μ mol) and 18 aminated paper strips (278 mg).
 4. Stir the mixture for additional 2 h.
 5. Remove the strips from the solution and wash with 25 mL of dichloromethane and 25 mL of ethanol.

5. Sample Pre-Treatment.

1. Laboratory treatment
 1. Place 10 mL of a fresh diesel/kerosene blend in a 25 mL vial.
 2. Suspend 10 wt% of active charcoal in the blend.
 3. Stir the vial for 1 h, centrifuge (400 x g, 10 min) and filter to remove the charcoal.
2. On-site treatment
 1. Purchase circular activated carbon loaded filters of 47 mm diameter.
 2. Place four of the filters in a 47 mm stainless steel in-line filter holder.
 3. Flush 5 mL of a fresh diesel/kerosene blend through the filters with a standard 10 mL syringe; approximately 2 mL of polycyclic aromatic hydrocarbon-free solution was obtained.

6. Smartphone Reader Implementation

Note: An Android based smartphone with a centered front camera was used as the core of the smartphone measurement system. All the necessary optical elements and 3D-printed accessory were custom-made for this device. However, any other smartphone with a CMOS (Complementary Metal Oxide Semiconductor) camera can be used.^{19,20}

1. Purchase a standard 5 mm epoxy LED at 460 nm, a 100 Ω resistor and a USB on-the-go (OTG) cable with an ON/OFF switch and a micro USB port.
2. Cut the USB cable on the opposite of the OTG side to isolate the red wire powering +5 V (up to 300 mA) and the black wire corresponding to the ground.
3. Cut the black wire of the USB cable and solder the 100 Ω resistor on the back of the switch. Solder the LED anode to the +5V red wire and the LED cathode to the ground black wire.
4. Purchase a diffuser and two filters for the LED and the camera, typically a short pass filter for the excitation channel (LED) and a band pass filter for the emission collection (camera).
5. 3D-print a smartphone case that fits on the smartphone and integrates the different optical parts consisting of a black chamber (20 x 30 x 40 mm)²¹ as described in **Figure 2**.
6. 3D-print a strip holder as described in **Figure 2** to hold a reference and a test strip.
7. Implement the excitation channel by placing the LED, the diffuser and the filter to illuminate the paper strips at an angle of 60°.
8. Implement the reading channel by placing the filter in front of the smartphone CMOS camera.
9. Insert the test strip holder containing the strips to start a measurement.

7. Sample Analysis Using the Smartphone-Based Detector

Note: Analyses were carried out by running a Java app(lication) for Android that finally displayed the adulteration level on the screen. Without the app, pictures can be taken, exported to a computer and analyzed with a standard image analysis software.

1. Select the adequate calibration file, here diesel/kerosene, from the software memory by clicking on the **Menu** button in the upper-right corner of the software window.
2. Dip the test strip into the diesel sample for a couple of seconds by holding the test strip with tweezers.
3. Remove excess fuel by simple patting with a drying paper.
4. Place the test strip inside the strip holder besides the reference strip and introduce the holder into the smartphone case.
Note: An image of the strips' fluorescence is then immediately displayed on the smartphone's screen.
5. Press the **SHOOT** button to record the fluorescence intensities of test and reference strips.
Note: The degree of adulteration is immediately calculated by the internal algorithm and displayed on the screen.

Representative Results

The three structures of the two commercial dyes 4-DNS and 4-DNS-OH and the synthesized dye 4-DNS-COOH contain a stilbene core element substituted with a donor (-NR₂) and an acceptor (-NO₂) group at both ends, the central double bond constituting the hinge of the so called 'molecular rotor' (**Figure 1A**). The structures differ in amino group substitution pattern with short alkyl groups for 4-DNS, two slightly longer groups including an alcohol moiety for 4-DNS-OH and an ester linker terminating with a carboxylic acid function for 4-DNS-COOH (**Figure 1A**).

Although the three dyes showed similar fluorescence properties in solution, adsorption on cellulose (paper) induced different behavior. When the polarity of the terminal group on the amino substituent (-Me < -OH < -COOH) increased, a bathochromic shift and a quenching of the emission was observed (**Figure 1B**). The deposition of diesel or kerosene samples on the paper strips further enhanced the fluorescence. The latter is ascribed to an increasing affinity of the dyes for the cellulose fibers, reducing micro-solvation and thus the fluorescence quantum yields (**Figure 1A**).

Upon an increase of kerosene in the blend, the fluorescence of the 4-DNS-OH test strips was reduced, shifted hypsochromically from 550 to 515 nm and the band became more structured (**Figure 1C**)¹⁷. Different from the behavior in solution, the fluorescence intensity of 4-DNS-OH correlated linearly with kerosene when adsorbed in the strip, revealing a correlation coefficient of 0.997 and a low standard deviation of 2.5% (**Figure 1C**).

The fluorescence of the strips was determined with a 3D printed smartphone case integrating a strip holder and all necessary optical elements such as an LED powered directly by the smartphone USB port, filters and a diffusor (**Figure 2A, 2B**).

The assay procedure remained as simple as possible with 6 main steps: dipping, placing the strip in the holder, starting the LED, positioning the holder in the case and analyzing the fluorescence signal with an application and process the data with an application (**Figure 2C**). The analysis software averaged all the RGB values of the pixels in predefined spatial areas corresponding to the strips and converted them to fluorescence intensities. The accuracy of 3% found for the determination of diesel content was even better than the accuracy of the standard method and also as the uncertainties reported for other sensors.

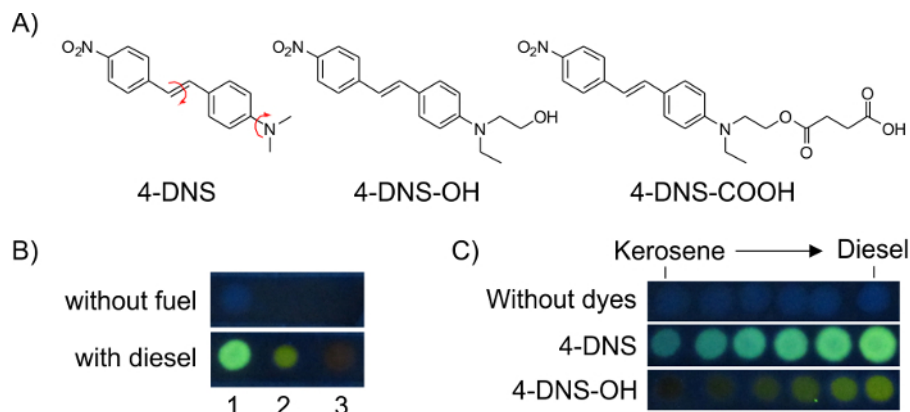


Figure 1. Chemical and photophysical properties of the molecular viscosity probes 4-DNS, 4-DNS-OH and 4-DNS-COOH. (A) Chemical structures. (B) Fluorescence of the dyes adsorbed on paper strips upon UV excitation (365 nm) before adding fuel and after adding some diesel (1 = 4-DNS, 2 = 4-DNS-OH and 3 = 4-DNS-COOH). (C) Evolution of the test strips' fluorescence with a fuel gradient from kerosene to diesel. [Please click here to view a larger version of this figure.](#)

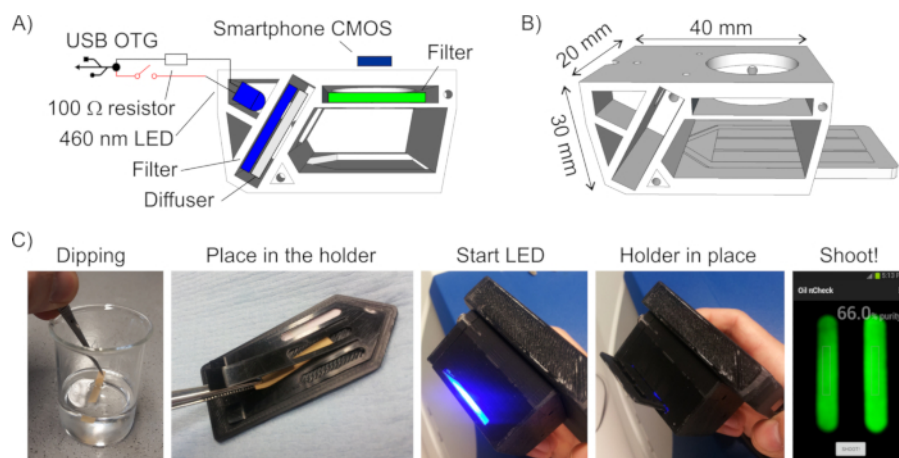


Figure 2. Embedded system to analyze the purity of a diesel blend. (A) Scheme of the smartphone case containing all the necessary optical and electronic parts. (B) Perspective view of the smartphone case with the strip holder in place and without the additional elements (a cover is coming on the side to close the chamber). (C) The successive steps: dip in sample, place in the holder, start the LED, place the holder and press the shoot button to obtain directly the diesel purity on screen. [Please click here to view a larger version of this figure.](#)

Discussion

A fluorescent probe, based on a molecular rotor dye that is sensitive to viscosities in the range of those measured for diesel and its different blends with kerosene, was used to obtain simple and efficient test strips for the detection of diesel fuel adulteration. The emission intensity of 4-DNS at 550 nm in various diesel/kerosene blends correlates with a reduction in viscosity when the proportion of kerosene increases. At a temperature of 24 °C, a nonlinear fluorescence quenching of up to 55% was observed for up to 100% adulteration with kerosene, allowing for reliable quantification of the adulteration with a low standard deviation of 1.70%.

However, the simple adsorption of 4-DNS on filter paper strips led to an elution of the dye when dipped into liquid samples, because of hydrophobic and π - π interactions between hydrophobic dye and hydrophobic solvent. Fortunately, the introduction of a hydroxyl (in 4-DNS-OH) or carboxyl group (in 4-DNS-COOH) circumvented this detrimental behavior and led to a steric anchoring of these polar derivatives in the cellulose fibers via hydrogen bonds. As an alternative approach, the grafting of 4-DNS-COOH to a linker-functionalized substrate was also considered to avoid elution, and filter paper previously aminated with 3-aminopropyltriethoxysilane (APTES) was chosen as substrate here. Unfortunately, this material was only weakly emissive even in the presence of viscous substances, like 4-DNS-COOH in a paper matrix. Among the three rotor dye derivatives with various terminal functional groups tested, only 4-DNS-OH was found to be suitable for the current test. This dye combined interactions with cellulose that are strong enough to avoid elution with a solvent shell that allows for the access of the fuel sample.

It was not leaking out of the paper strip when dipped into fuels and presented a strong enough fluorescence emission in the visible range for recording with a smartphone.

With such 4-DNS-OH-coated test strips, titration experiments employing real samples were also performed. However, the use of raw fuels was problematic because of the presence of polycyclic aromatic hydrocarbons (PAH), entailing an intolerably high background signal. A simple filtration step of the diesel/kerosene blends through activated carbon was thus implemented, efficiently removing those compounds as well as potential fuel marker dyes, which are frequently added by manufacturers, leading to convincing results.

For the sake of straightforward use on-site, the smartphone detection system was designed. An Android based platform was chosen as it offers more flexible development features in terms of application deployment and connectors (lightning port requires an extra adaptor for OTG cable). All electronics and optics are widely available off-the-shelf components and the smartphone case can be made by a standard 3D printer. Even though recent high-end smartphones run with the newest operating systems allow users to obtain RAW pictures from camera acquisition, a vast majority of the mobile devices currently on the market or in use are equipped with a hardware-based auto-exposure compensation algorithm directly within the CMOS chip. This feature, handy for consumer uses, is an important problem when smartphone chemometric systems are concerned, as lux amount received by the camera's CMOS is automatically tuned to match certain lux criteria. Using these values as absolute measurement readings can thus easily yield misleading and false results. Measurement of a reference strip side by side to a test strip is thus essential to account for such auto-exposure compensation. In future, with the importance of smartphone-based analysis dramatically increasing, this feature can significantly simplify the method by having to analyze only the test strip like realized in today's commercial test strip readers found on the market.

The smartphone and printed case embedded sensory system was compared to a standard method based on GC-FID for validation¹⁷, revealing excellent agreement with linear responses and low limits of detection down to 7% for the mobile system. To enhance the precision of the method, a pure diesel and a pure kerosene reference solution can be analyzed (in analogy to the common two-point calibration of any conventional pH electrode) to obtain calibration files for the fuels, especially when diesel fuels of different grades are encountered that have specific viscosities and respond specifically on the strip. Such calibration files can be readily entered and stored in the app. Such cost-effective, precise and rapid tests are an interesting forensic solution for fraud detection by consumers or untrained authority personnel.

Further developments of rapid tests for fuels based on test strips and smartphone read-out are currently ongoing, noteworthy for the adulteration of gasoline with alcohol or other petroleum products like kerosene. Naturally, the smartphone system for fluorescence read-out can be easily adapted to other fluorescent indicator systems.

Disclosures

The authors have nothing to disclose.

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