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Validation of Data from an Artificial Sniffer Dog by Common Analytical Techniques

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Abstract

CRIM-TRACK, an artificial sniffer dog, employs a colourimetric sensor system to monitor the colour change of chromic dyes when in contact with the vapours of illicit molecules (analytes) for detection and identification of substances. Within, the interaction of illicit chemicals and chromic dyes have been studied in solution using Proton Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy and Ultraviolet-Visible (UV-Vis) spectrophotometry, to validate data generated from detection experiments using CRIM-TRACK sniffer. ^{1}H-NMR revealed the colour change mechanism induced by benzyl methyl ketone (BMK), a precursor chemical of methamphetamines, was hydrogen bonding between the BMK and specific dye molecules. It also revealed that hexamine (HEX), an explosives precursor, induced a colour change by formation of ion pairs with the specific dye molecules. The colour changes detected by CRIM-TRACK were confirmed by UV-Vis where a shift in absorption wavelength and/or a change in absorbance occurred.

Keywords: chemical precursors, detection, colourimetric sensor, CRIM-TRACK sniffer system, NMR, UV-Vis

1. Introduction

The manufacture, trafficking, selling and use of illicit drugs, explosives and their chemical precursors are controlled internationally to try and reduce their socio-economic impact on society. Examples of these impacts include the €30 billion a year European Union illicit drug market [1], and the Manchester Arena bombing that killed 22 [2]. To minimise the impact of these substances, instrumental techniques such as ion mobility spectroscopy, and trained dogs are the main methods currently employed by law enforcement agencies to detect explosive and illicit drug materials [3].

A complimentary detection device, the CRIM-TRACK sniffer device, is currently in development [4], [5]. This device utilises a colourimetric sensor system to detect and identify illicit substance by monitoring and analysing colour change of chromic dyes printed on a chip during contact with the substance's (analyte's) vapour. Data obtained using the CRIM-TRACK sniffer device with the analytes benzyl methyl ketone (BMK), a methamphetamine precursor and controlled chemical, and hexamine (HEX), an explosives chemical precursor. It reported that 4-methoxy-4'-hydroxyazobenzene (DAB4) and bromocresol green (SP6) dyes respond – change colour – to BMK and HEX, whilst 1-hydroxyanthraquinone (AQ2) does not.

Validation of sniffer results was performed by characterising selected dyes from the chip and analytes in solution using NMR spectroscopy and UV-Vis spectrophotometry, then characterising analyte:dye mixtures at different ratios, and comparing the mixtures' data to the single components'. NMR spectroscopy revealed structure changes and UV-Vis to colour changes through wavelength and absorbance variations of the analyte:dye mixtures. This allows investigation into the analyte-dye interactions and confirmation of colour changes detected by CRIM-TRACK sniffer, something that has not been previously investigated by other research groups who also study colourimetric sensor systems using chromic dyes for detection purposes [6].

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2. Experimental

2.1 Materials

4-Methoxy-4'-hydroxyazobenzene (97% purity, DAB4) was synthesised in house at Cranfield University [7]. Bromocresol green (95%, SP6) benzyl methyl ketone (99%, (BMK)) and hexamine (99%, HEX) were obtained from Sigma-Aldrich, 1-hydroxyanthraquinone (>95%, AQ2) from Tokyo Chemical Industry, Ltd. Chloroform ($CHCl_3$) (stabilised with 0.6% ethanol) and deuterated chloroform ($CDCl_3$) (with molecular sieves; D, 99.8% and 0.025% v/v tetramethylsilane (TMS)) were obtained from Sigma-Aldrich and Cambridge Isotope Laboratories, Inc respectively. All commercially sourced chemicals were used as provided.

2.2 Methods

^{1}H NMR spectra were recorded on a Bruker Ascend 400 MHz spectrometer in $CDCl_3$, with TMS as reference, at ambient temperature for 32 scans. 2 mM dye and 0.25 M analyte solutions in $CDCl_3$ were freshly prepared before the experiments. 750 µl dye solution was placed in a standard 5 mm NMR tube and the spectra recorded. 6-300 µl aliquots of the analyte solution were then added, and the NMR spectra recorded at 1:1, 5:1, 10:1, 50:1 and 100:1 mol/mol analyte:dye ratios. Processing and analysis of data was carried out using Bruker's TopSpin software.

UV-vis spectra were recorded using a Cary 50 & Thermo Scientific Evolution 220 UV-VIS spectrophotometer and a far UV silica cuvette with 10 mm path length at room temperature. 0.025 mM and 0.05 mM 2 mM dye solutions and 2.5 mM and 5 mM analyte solutions in $CHCl_3$ were freshly prepared before the experiments.

Mixtures at 50:1, 100:1, 150:1 and 200:1 mol/mol analyte:dye ratios were prepared using the 0.05 mM dye and 5 mM analyte solutions. The 0.025 mM dye and 2.5 mM analyte solutions were recorded to provide the curves of the single components for the creation of a predicted 100:1 curve. These are the same concentrations that are present in 100:1 mixture. The predicted 100:1 is the sum of these curves, assuming additive behaviour. All UV spectra were recorded in triplicates and the data averaged.

3. Results & Discussion

BMK and HEX were chosen as analytes as they have ketone and amine functional groups respectively, so were expected to potentially interact differently with the dyes. They are common chemical precursors for the manufacturing of methamphetamine and explosives respectively. DAB4, SP6 were chosen to represent two different families of responsive dyes on the CRIMTRACK microchip (colour changing) while AQ2 dye was chosen as negative non-responsive dye on the microchip. The chemical structures of these chemicals are shown in Figure 1.

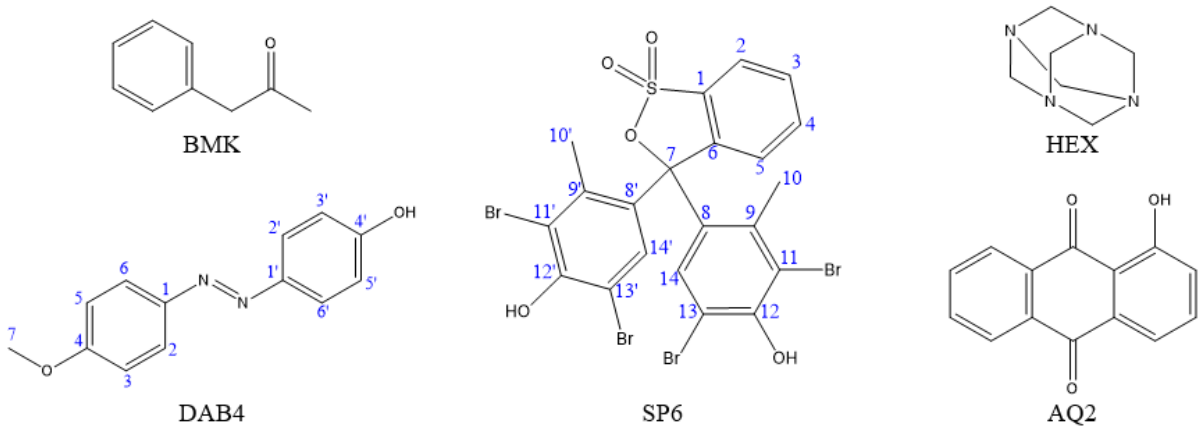

Figure 1: Structures of analytes (BMK and HEX) and dyes (DAB4, SP6 and AQ2) with carbons numbered for DAB4 and SP6

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3.1 BMK: Dye Interactions

During preparation of NMR mixture samples, no visible colour change was observed. The dye solutions remained same the pale yellows on addition of BMK. No formation of a new product is seen in the NMR spectra of the BMK:dye mixtures, nor any changes in the chemical shift of the BMK peaks. Downfield shift of the hydroxyl peaks of DAB4 and SP6 by 0.01 ppm at 1:1 BMK:dye ratio was discerned which increased in magnitude with increase in the ratio of BMK (+0.6 ppm and +0.4 ppm at 100:1 respectively). No effect on the chemical shifts of AQ2's protons was seen at any ratio, confirming AQ2's assignment as a non-responsive dye from the sniffing data.

No visible colour change was observed during the preparation of the UV-Vis mixture samples. Non-additive behaviour was indicated in both DAB4 and SP6 mixtures as the experimental 100:1 and predicted 100:1 curves did not align - there was a hyperchromic shift (to greater absorbance). Additive behaviour was however observed with AQ2. Isosbestic points (where curves of mixtures at different ratios cross, Table 1) in all BMK:dye mixtures confirms that only the single components are present in the solution, and that there is not a third covalently formed product. There was no shift in λ_{max} of DAB4 or AQ2, but for SP6 there was a hypsochromic shift (to shorter wavelength) by 3 nm.

The NMR and UV-Vis data combined indicates weak electrostatic interactions between BMK and DAB4, and BMK and SP6 in solution. After consideration of the structures (Figure 1), it is believed that there is weak, non-covalent and reversible hydrogen bonding interaction between hydroxyl groups of dyes and ketone of BMK. This would account for the deshielding of the hydroxyl protons. This does not occur with AQ2 as it has a stronger, intramolecular hydrogen bond in place already between the ketone and adjacent hydroxyl group (Figure 1).

	Wavelength, λ (nm)								
	DAB4	BMK:DAB4	HEX:DAB4	SP6	BMK:SP6	HEX:SP6	AQ2	BMK:AQ2	HEX:AQ2
λ_{max}	357	357	357	412	409	415	406	406	406
Isosbestic point		308	245		318	252		317	241

Table 1: UV-Vis Spectrophotometry Data

3.2 HEX: Dye Interactions

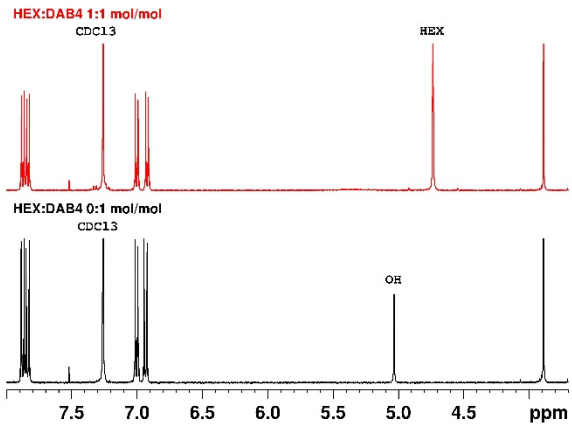

Figure 2: ^{1}H-NMR ($CDCl_3$, 400 MHz) spectra of HEX:DAB4 0:1 (black line, bottom) and 1:1 (red line, top).

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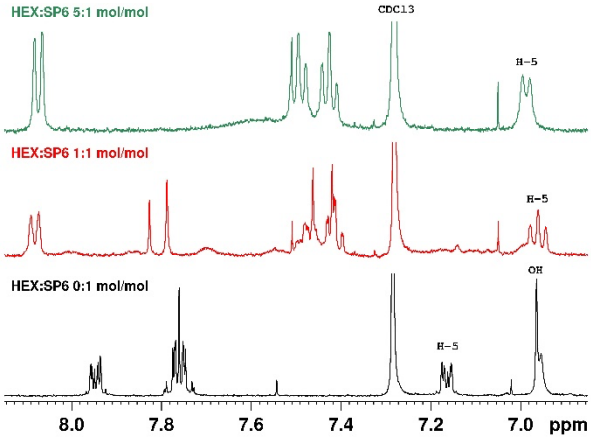

Figure 3: Aromatic region of ^{1}H-NMR ($CDCl_3$, 400 MHz) spectra of HEX:SP6 0:1 (black line, bottom), 1:1 (red line, middle) and 5:1 (green line, top).

The pale yellow SP6 chloroformic solution became a bright yellow mixture upon addition of HEX, but no colour changes of DAB4 and AQ2 mixtures were visible by eye. As in the BMK:dye mixtures, the NMR data illustrated that there was no formation of a new product and no changes to the chemical shift of the analyte, HEX. On addition of HEX to DAB4 from 1:1 and SP6 from 2:1 onwards, the hydroxyl proton peaks' intensity decreases to 0. In DAB4, a minor upfield shift (-0.01 ppm) of the chemical shifts of aromatic protons attached to carbon atoms 3' and 5' is observed (Figure 2), determined to be due to greater inductive effect by O^- than OH, caused by deprotonation by HEX. The chemical structure of SP6 changes on addition of HEX – the sulphonate ring opens. This is clearly indicated by the additional peaks and changes in the chemical shifts and multiplicities of the aromatic peaks at 1:1 and 5:1 ratios (Figure 3). In the same manner of BMK:AQ2, no effect on the chemical shifts of AQ2's protons was seen at any ratio.

The same colour change of pale yellow to bright yellow was observed during the preparation of the HEX:SP6 mixtures for UV-Vis as was observed during the NMR experiments. As with the BMK:dye mixtures, HEX:DAB4 and HEX:SP6 indicated non-additive behaviour due to hyperchromic shift, and HEX:AQ2 displayed additive behaviour. Isosbestic points (Table 1) in all HEX:dye mixtures confirms that only the HEX and dye are present in the solution and that there is not a third product. Once more, there was no shift in λ_{max} of DAB4 or AQ2, but for SP6 there was a bathochromic shift (to longer wavelength) by 3 nm.

The observations gained from NMR and UV-Vis together indicates that chemical interaction, in solution, occurs between HEX and DAB4 and HEX and SP6, but not between HEX and AQ2. It is believed that there is strong, non-covalent, irreversible ion-pair formation by deprotonation of the hydroxyl protons which cannot occur with AQ2 due to its strong, intramolecular hydrogen bonding.

4. Conclusions

The interactions of analytes, benzyl methyl ketone (BMK) and hexamine (HEX) with three dyes, 4-methoxy-4'-hydroxyazobenzene (DAB4), bromocresol green (SP6) and 1-hydroxyanthraquinone (AQ2) were investigated in chloroformic solutions. Proton NMR spectroscopy of the mixtures determined the detection mechanism of BMK to be hydrogen bonding and the detection mechanism of HEX to be ion-pair formation by deprotonation when using DAB4 and SP6 dyes. UV-Vis spectrophotometry of the mixtures recorded colour changes not seen by eye and determined cause of colour change: change in absorption amount and/or change of wavelength absorbed. BMK did not induce a visible colour change in the dyes but had a slight increase in absorption whereas HEX had both, changing from pale yellow to bright yellow at a different wavelength in solution. This has validated data from CRIM-TRACK sniffer experiments that reported that DAB4 and SP6 dyes responded to BMK and HEX, whilst AQ2 did not.

5. Future Work

Using the methodologies laid out in these proceedings, it is planned to analyse the interactions of other illicit substances such as methamphetamine hydrochloride, cocaine, and precursor chemicals with responding chromic dyes to further validate sniffer data.

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