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TECHNICAL NOTE

Increasing the specificity of the forensic luminol test for blood

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ABSTRACT: It is shown that the presumptive luminol chemiluminescence test for the presence of traces of blood can be made more determinative by measuring the peak emission wavelength of the luminol chemiluminescence. When sprayed onto a surface containing traces of human haemoglobin, a 1 g/L solution of aqueous luminol containing 7 g/L sodium perborate gives an emission peak at 455 ± 2 nm, whereas the same mixture gives an emission peak at 430 ± 3 nm when sprayed onto a surface containing traces of sodium hypochlorite (household bleach). This spectral difference can readily be determined using spectroscopic equipment that either scans the spectrum before significant luminescence decay occurs or corrects the spectrum for the effects of any decay. It was found that bovine haemoglobin and human haemoglobin showed no significant spectral differences. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: chemiluminescence; luminol; presumptive blood test; emission spectrum; haemoglobin; sodium hypochlorite

INTRODUCTION

The chemiluminescent reaction of luminol with an oxidizing agent in the presence of haemoglobin was first proposed by Specht (1) in 1937 as a method for detecting trace amounts of blood at a crime scene. The test is particularly sensitive, with a detection limit in the nanogram region (2). Although the test is now well established as a presumptive test for blood, it is prone to produce false positive indications of the presence of blood because some household and industrial chemicals are able to catalyse the chemiluminescence as effectively as does the haemoglobin. Hypochlorite ions are one of the most important examples of such substances, as they are commonly available as solutions of household and industrial bleach and, to add a further complication, may even be used to clean up and remove blood evidence. Other substances are animal haemoglobin, plant peroxidases (particularly as found in fibrous plant material from fruits and vegetables) (3) and disinfectants or antiseptics containing potassium permanganate (4) or iodine (5). In addition, copper(II) ions (6) from water pipes or wires and Fe(II) (7) and Fe(III) (5) ions from rusting iron are other domestic and commercial sources of luminol reaction catalysts.

Although such potential interferences with the luminol test for blood have been known for many years, there has been a notable absence of research on the various ways in which the luminol test could be modified to distinguish such sources of false positive results.

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The aim of the present study was to focus on the interference provided by hypochlorite ions catalysing the emission of chemiluminescence and find a way to distinguish that emission from the chemiluminescence catalysed by human haemoglobin. The approach used was to search for any spectral shifts that occurred when hypochlorite ion was replaced by haemoglobin.

MATERIALS AND METHODS

The luminol solution was prepared using the following; 0.1 g luminol (Aldrich), 5.0 g Na_2CO_3 (Ajax), 0.7 g $NaBO_3.4H_2O$ (Aldrich) and 100 mL distilled water.

The human haemoglobin (Sigma), bovine haemoglobin (Sigma) and sodium hypochlorite samples were made by preparing solutions of the species, applying these solutions to a sheet of absorbent paper and drying them overnight. These were then placed inside a light-tight box and sprayed with the luminol solution. All spectral measurements were made using a calibrated Barr and Stroud (Glasgow, UK) CGS2 circular graded interference filter, rotated by a microprocessor-driven motor, to monochromate the emitted light. An EMI (Middlesex, UK) 9635 QAM photomultiplier tube, operated in the DC mode, was used as the light detector. The DC photocurrent from the tube was passed through a load resistor and the potential difference generated across the latter was applied to a polarity inverter, a Pico Technology ADC-10 (Cambridge, UK) analogue-to-digital converter, and then recorded on a digital computer.

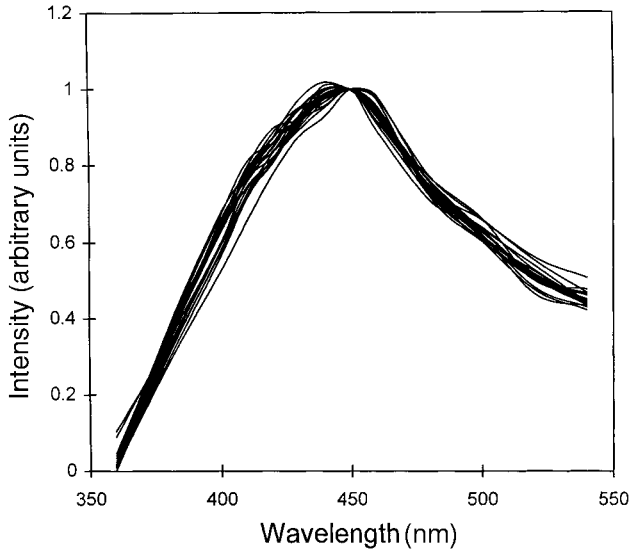

Figure 1. Twenty replicate determinations of the chemiluminescence spectrum of luminol with 15 g/L human haemoglobin. Ten of the replicates were scanned in the direction of increasing wavelength, while the remaining 10 were scanned in the opposite direction.

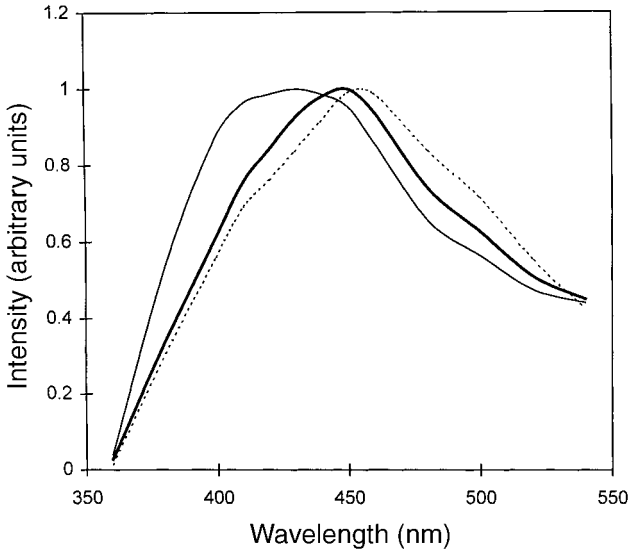

Figure 2. A comparison of the emission spectrum of the chemiluminescent luminol reaction in the presence of 150 g/L human haemoglobin (·····), 15 g/L human haemoglobin (——) and 1.25 g/L sodium hypochlorite (——). Note that relative intensities have not been shown, to aid comparison.

Correction for the fall of chemiluminescence intensity with time

As the chemiluminescence from the luminol reaction decays quite quickly with time, any viable system for measuring the chemiluminescence spectrum must involve scan times faster than 1 or 2 s or involve some correction for spectral distortion caused by luminescence decay during the scan period of ca. 50 s. This was done by obtaining mean values for a number of replicate luminescence decay curves and using the mean to correct for spectral distortion. Scanning the spectrum in opposite directions and applying the corrections verified the validity of this method. The corrected spectra obtained from the oppositely scanned data agreed well with one another, their maxima agreeing within ca. 1 nm.

RESULTS

The spectra in Fig. 1 represent 20 replicate determinations, 10 scans being carried out in the direction of increasing wavelength and 10 being carried out in the opposite direction. Each replicate involved the use of a fresh sample of catalyst (15 g/L haemoglobin) of ca. 3 mL, deposited on a new piece of absorbent paper using a Pasteur pipette and then sprayed with the luminol–perborate solution for ca. 1 s. The good agreement in Fig. 1, despite the inevitable variability of the manual procedure and of the heterogeneous surface emission, indicates that the method provides robust data.

Fig. 2 shows the mean of the replicate spectra from Fig. 1 for 15 g/L human haemoglobin and similar mean spectra for more concentrated 150 g/L human haemoglobin and for 1.25 g/L sodium hypochlorite. The two concentrations of human haemoglobin were chosen to represent blood diluted 10-fold and undiluted blood, respectively. The diluted haemoglobin provided a simulation of blood droplets that had been diluted say 10-fold by an attempt to clean up the blood spill.

The sodium hypochlorite solution placed on the

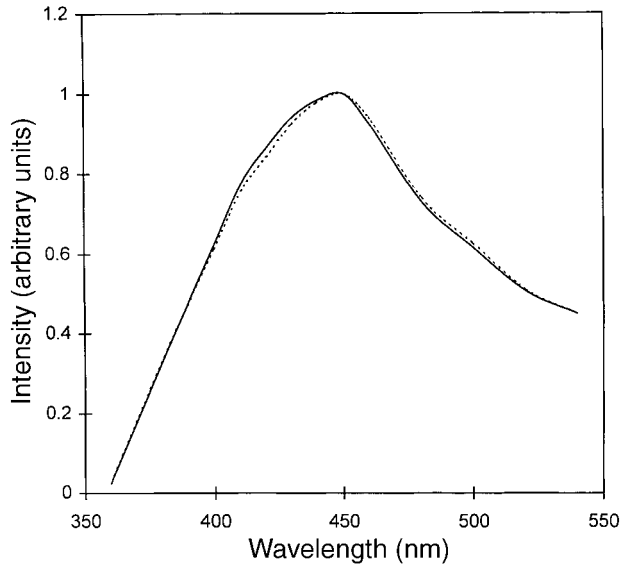

Figure 3. A comparison of the emission spectrum of the chemiluminescent luminol reaction in the presence of 15 g/L human haemoglobin (——) and 15 g/L bovine haemoglobin (·····).

absorbent paper contained 1.25 g/L of solute and was chosen to simulate approximately a typical amount that might be left on a surface after washing it with household bleach, which generally contains ca 50 g/L sodium hypochlorite.

DISCUSSION

The spectra in Fig. 2 show that the spectral maxima for sodium hypochlorite and for haemoglobin are clearly different and would readily be distinguishable using relatively straightforward spectroscopic equipment. The spectral peak for the concentrated (150 g/L) haemoglobin is at 455 ± 2 nm which is red-shifted by ca. 25 nm from the sodium hypochlorite peak at 430 ± 3 nm. The concentrated haemoglobin is at about the same concentration as found in human blood (8) and should (9) provide a good simulation of the behaviour of blood.

It is interesting to note that the spectral peak at 449 ± 1 nm for the dilute (15 g/L) haemoglobin is red-shifted by a little less (ca. 19 nm) from the hypochlorite peak. This suggests that simple optical filtering of the chemiluminescence by the red haemoglobin is responsible for the wavelength shift.

It is also important to note that changing the concentration of sodium hypochlorite by three times does not cause significant spectral shifts. Thus, Arnhold *et al.* (10) used 0.37 g/L sodium hypochlorite and obtained virtually the same spectral maximum of 431 nm that is obtained in the present work. If the filter effect of coloured substances is the main cause of spectral shifts, then it is not surprising that the colourless sodium hypochlorite samples do not cause any spectral shift.

It is clear from the above results that the spectral shift provides a clear diagnosis of whether sodium hypochlorite or human blood is catalysing the luminol reaction. Fig. 3 shows the results of an attempt to distinguish bovine from human haemoglobin using the luminol reaction, but it is clear that the spectra (including the positions of the spectral peaks) are not distinguishable by this method.

An interesting forensic question arises from this work. If a person at a crime scene attempted to remove blood stains by washing the area with sodium hypochlorite solution (household bleach), what would be the effect on the luminescence spectrum, given that both blood and hypochlorite can initiate chemiluminescence from the luminol reagent used in blood detection? Several possibilities arise. If the person cleaning the surface adopted a thorough approach and removed all blood via oxidation by the hypochlorite, washing of the surface with the hypochlorite solution and final washing with water, no luminol chemiluminescence should be emitted. Various combinations of blood-initiated emission and hypochlorite-initiated emission (each peaking at its separate respective wavelength) might be expected if the cleaning process were not complete. A practical study of this matter might be a useful field for future forensic research.

CONCLUSIONS

It should be possible to construct a portable chemiluminescence spectrometer which could distinguish the presence of haemoglobin from that of household bleach on surfaces at a crime scene. Provided that a fast scanning system (ca. 100 nm/s) or a diode array were used, the rather more tedious correction of the spectrum for luminol decay used in the present study would not have to be adopted.

This method would not distinguish between human and animal blood samples, but further work should be carried out in order to assess whether the other catalytic substances mentioned in the Introduction could be distinguished from blood in this manner.

Acknowledgement

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