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The inhibition by amines and amino acids of bleach-induced luminol chemiluminescence during forensic screening for blood

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Abstract

Sprays containing alkaline solutions of peroxide and luminol are used as presumptive screens for bloodstains at crime scenes. These sprays can be subject to interference from hypochlorite-based cleaning agents (bleaches), leading to false positive results. This paper reports the screening of amines for their ability to decrease the interference by bleach while not greatly affecting the reaction with blood. The addition of glycine (0.05 mol L^{-1}) to the Grodsky formulation of luminol spray, together with an adjustment of the pH to 12, gave good discrimination between blood and bleach, and has the advantage that glycine is non-toxic compared to many other amines. The modified spray gave similar chemiluminescence intensity and duration as the unmodified Grodsky spray. However, it is recommended that this modification only be used when there is evidence that hypochlorite bleach may have been used at a scene. The amines triethylamine and sulfamate led to enhanced chemiluminescence in the presence of hypochlorite.

Keywords: Bleach; Blood; Chemiluminescence; Forensic; Luminol

1. Introduction

The use of alkaline luminol-peroxide sprays for the chemiluminescent detection of blood at crime-scenes is well established. These sprays show very good sensitivity towards blood, but are subject to positive interference from peroxidases, some metal salts, and hypochlorite-based cleaning agents [1–3]. Since crime scenes may be subjected to cleaning prior to forensic examination, the false positives from hypochlorite bleach can be of concern. On impervious substrates it is sometimes possible to visually differentiate the chemiluminescence from bleach (bright flashes) from the chemiluminescence from blood (longer lived). However, this is not necessarily true on pervious surfaces or if the bleach is not in droplets. Also, the long-exposure photography often used to capture images after luminol spraying cannot discriminate between the two sources of chemiluminescence. A recent paper by Quickenden et al. reported a slight shift in the luminescence spectrum upon spraying luminol-peroxide onto blood (maximum 455 nm) compared to bleach (maximum 430 nm) [4], the shift most likely being due to the strong absorption of blood at ca. 420 nm providing an inner filter effect on the chemiluminescence. However, the magnitude of the shift is insufficient for ready spectral discrimination. We have instead examined the use of chemical reagents to effect discrimination between blood and bleach. In a previous paper we reported preliminary results that showed that amines could reduce bleach-induced luminol chemiluminescence in basic solution due to their rapid reaction with hypochlorite [5]. That study noted that addition of 0.1 mol L^{-1} 1,2-diaminoethane to a solution of peroxide-luminol could greatly reduce the chemiluminescence due to bleach while only slightly (by 30%) reducing the chemiluminescence due

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to blood. However, 1,2-diaminoethane is toxic and volatile, and this presents problems in terms of storage, transport, and usage. Therefore we have examined the reactivity of amines with bleach, blood, and luminol sprays in more detail, and have identified less-toxic amines that have similar efficacy in this system. Since there is always a possibility that application of chemicals at a scene could affect subsequent processing or expose those present to hazards, we also reiterate our earlier finding [5] that if a scene can be allowed to air for 1–2 days most of the hypochlorite present will decompose so that standard processing can be performed. However, if such a time delay is not possible then treatments such as those discussed in this paper become necessary.

Amines react with hypochlorite-containing solutions according to the reaction

$$RR'NH + HOCl \rightarrow RR'NCl + H_2O \quad (1)$$

with the reaction occurring between the deprotonated amine and hypochlorous acid [6,7]. In basic solution the reaction stops at the chloroamine shown, while in acidic conditions further chlorination and hydrolysis may occur. The above reaction has been used as the basis for analytical procedures for the quantitation of amines. Thus, both primary and secondary amines lead to a quantifiable decrease in the chemiluminescence from the bleach–peroxide–luminol system [8,9]. However, some tertiary amines increase the observed chemiluminescence [10,11], presumably because the *N*-chlorotrialkylammonium ions formed upon reaction of the amines with hypochlorous acid are reactive oxidants [12].

Margerum [6,7] and Antelo [13,14] have studied the reaction of hypochlorite solutions with a range of primary and secondary amines, and have found that the rate constants show a general trend with amine basicity, given by [13]

$$\text{rate} = k_{HOCl}[HOCl][RR'NH^{n+}] \quad (2)$$

$$\log(k_{HOCl}) = (2.9 \pm 0.5) + (0.48 \pm 0.05) \times pK_a(RR'NH_2{}^{(n+1)+}) \quad (3)$$

at 25 °C, where k_{HOCl} has units of $L\,mol^{-1}\,s^{-1}$, although the actual rate constants show some scatter from this relationship [15] presumably because the rates also depend slightly on steric factors. Ammonia deviated significantly from this line, having a rate nine times lower than is predicted by the Eq. (3) [7]. Hydrogen peroxide ($pK_a = 11.65$, $k_{HOCl} = 4.4 \times 10^7\,L\,mol^{-1}\,s^{-1}$ [16]) and luminol ($pK_{a1} = 6.74$ [17], $k_{HOCl} = 5 \times 10^5\,L\,mol^{-1}\,s^{-1}$ [18], $pK_{a2} = 15$ [19]) also react with hypochlorite in a pH dependent manner, so that it is predicted that an amine will best compete for hypochlorite if (1) the amine is strongly basic and (2) the solution is very alkaline (pH > 10) [5]. For a given solution pH the optimum amine is one for which the pK_a of its conjugate acid is approximately equal to the solution pH. The two common forensic luminol spray formulations (reported by Grodsky and Weber [1]) have pH > 10, so we have only examined amines with pK_a (conjugate acid) > 8 based on the above comments. It should be noted that most biological studies of the reaction of hypochlorous acid and luminol are performed at close to neutral pH, and under these conditions amines are very poor competitors for hypochlorite and therefore do not significantly affect the chemiluminescence [20,21], although hypochlorite scavenging by taurine has been reported [22].

2. Experimental

The aim of this study was to develop a system which led to suppression of hypochlorite-induced luminol chemiluminescence in solution, and then to apply this system to visualization of blood on surfaces. Initial experiments were performed in flowing solutions using spectrophotometric measurements for objective assessment of inhibition. Later studies used reproducible spraying techniques and fixed camera exposures, again for increased objectivity. The study did not extend to the effect of interferents other than bleach because it was focused specifically on the common need for identifying blood traces in the presence of hypochlorite-based bleach.

Bovine blood obtained from a local abbatoir was preserved with $2\,g\,L^{-1}$ disodium EDTA and was stored at 4 °C. Luminol, glycine, L-alanine, taurine, tris(hydroxymethyl)methylamine (TRIS), 1,2-diaminoethane, sulfamic acid, aqueous ammonia, and disodium EDTA were used as received. Solutions were prepared using milli-Q water (18 MΩ cm). Domestic sodium hypochlorite bleach solution, 30% hydrogen peroxide, and sodium perborate solutions were standardised by titration with standardised thiosulfate solution [23].

The luminol-peroxide sprays were based on Grodsky's formulation: A freshly-prepared solution containing $1.0\,g\,L^{-1}$ luminol and $50\,g\,L^{-1}$ sodium carbonate was mixed with an equal volume of a freshly-prepared solution containing $7.0\,g\,L^{-1}$ sodium perborate tetrahydrate immediately before taking measurements. Titration of the perborate solutions showed that the concentration was about 78% of the nominal value, so that the final concentrations of luminol, carbonate, and perborate were $2.82 \times 10^{-3}\,mol\,L^{-1}$, $0.24\,mol\,L^{-1}$, and $1.78 \times 10^{-2}\,mol\,L^{-1}$, respectively.

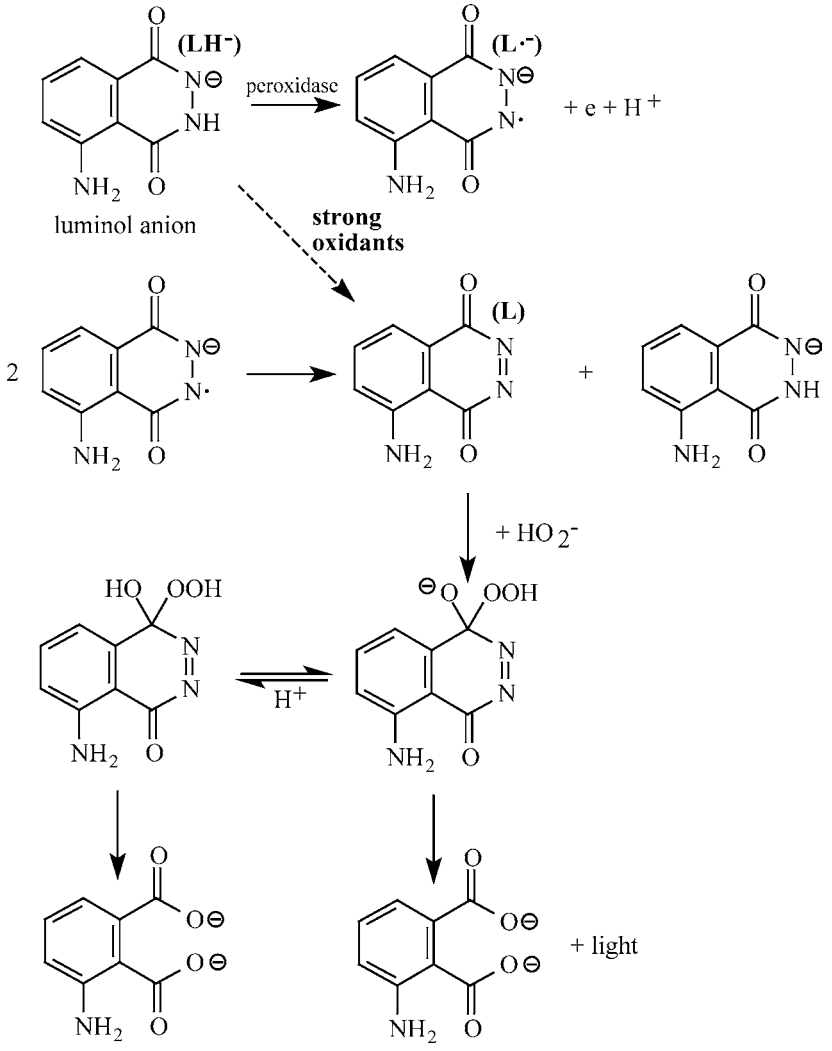
The instrumental chemiluminescence measurements were performed using an Ocean Optics S2000 fibre optic diode array spectrophotometer. Solutions of hypochlorite and luminol-perborate were pumped at $10\,mL\,min^{-1}$ (each reagent stream, peristaltic pump) into a 2 mm i.d. T-junction cell with two right-angle turns 1 cm after the T-junction mixing point. Light emission in the 2 cm of tube immediately after the mixing point was monitored either under constant flow conditions or immediately after the flow was stopped. Studies of the effects of amines on blood-induced chemiluminescence were investigated using a 1 cm flow cell connected to the same spectrophotometer. The light was collected using a collimating lens connected to a 600 mm fibre optic. The light

emitted was either monitored in the range 400–440 nm (for bleach reactions) or 460–480 nm (for blood), the latter range being used to avoid the effects due to absorption of light by blood at shorter wavelengths. For the steady-state reactions the integration time was 750 ms, while for the stopped-flow reactions the integration time was set at 50 ms for the bleach reactions and 500 ms for the blood reactions. All experiments were performed at room temperature. Multiple runs were performed under each reaction condition and the results averaged prior to comparison.

A standardised method of applying the luminol spray using a hand-pressurised sprayer was used to ensure uniform delivery of the luminol reagent. Vinyl floor tiles and unbleached cotton were used as substrates. In both cases 10–20 mL of bovine blood was placed on a damp cloth which was then wiped across the lower half of the square substrate and allowed to dry for 3–4 h. Bleach (1:20 dilution) was then applied in a perpendicular direction to the right half of each sample immediately before spraying and imaging. This gave a sample with blank, blood, bleach, and blood + bleach in different quarters. The samples were sprayed with freshly-prepared Grodsky's luminol formulation (pH 11), the same formulation adjusted to pH 12, and the Grodsky formulation containing 0.05 mol L^{-1} glycine and adjusted to pH 12. In all cases the luminol solution and perborate solutions were mixed within 5 min of the spraying and photography. Photography was performed with a Canon D-30 digital SLR camera with a 25–80 mm zoom lens. The camera was computer-controlled using the program D30 Remote (Breeze Systems) with settings ASA 400, f4.5, exposure times 6 s or 10 s, and the white balance set at "flash". The images have been contrast-adjusted to highlight the difference between the blood and bleach areas

3. Results and discussion

The chemiluminescent reaction sequence between luminol, hypochlorite, and hydrogen peroxide can be rationalized by the mechanism given in Scheme 1 [24,25],



Scheme 1. Mechanism for reaction of luminol to produce chemiluminescence, based on that proposed by Merenyi et al. [24].

Table 1
Relative chemiluminescence observed when equal flow rates of solutions containing (i) 2.28×10^{-2} mol L^{-1} OCl^- and (ii) luminol (2.82×10^{-3} mol L^{-1}), perborate (1.78×10^{-2} mol L^{-1}), carbonate (0.24 mol L^{-1}), and amine as shown, are mixed at pH 12

Amine	pK_a[a]	Concentration of amine (mol L^{-1})				
		0	0.008	0.020	0.040	0.080
Alanine	9.87	1.00[b]	0.74	0.45	0.23	0.08 (0.01)[c]
Ammonia	9.25	1.00	0.95	0.90	0.80	0.68
1,2-Diaminoethane	10.71	1.0 (0.1)	0.50 (0.05)	0.24 (0.03)	0.09 (0.01)	0.030 (0.006)
Glycine	9.78	1.00	0.74	0.46	0.24	0.070
Sulfamate	[d]	1.00	1.31	1.9	2.7	10.8 (0.7)
Taurine	9.06	1.00	0.75	0.51	0.27	0.11 (0.01)
Triethylamine	10.75	1.00 (0.07)	3.3 (0.3)	3.8 (0.4)	4.1 (0.4)	–

[a] pK_a values taken from ref. [42].
[b] All chemiluminescence values are normalized to the chemiluminescence seen in the absence of amine.
[c] Relative errors in relative chemiluminescence values are ±5%, except where *absolute* error in relative chemiluminescence is given in parentheses.
[d] The pK_a for sulfamic acid (NH_2SO_3H) has been given as 1.13 [43]. The pK_a for propylsulfamate ($CH_3CH_2CH_2NHSO_3^-$) has been given as 11.89 [43], suggesting that $NH_2SO_3^-$ can also be deprotonated in this pH range.

although as stated earlier there is also a competing reaction between hypochlorite and hydrogen peroxide [16]. In this scheme it is predicted that HOCl can oxidize the luminol anion to the diazaquinone (L) which can then react with hydroperoxide to initiate the pathway which finally results in the emission of light. Hypochlorous acid can react with luminol to give chemiluminescence in the absence of hydrogen peroxide, but the chemiluminescent intensity is much decreased compared to that in the presence of hydrogen peroxide unless a catalyst is present. Mixing equal volumes of a solution containing hypochlorite (1:20 diluted domestic bleach, 2.28×10^{-2} mol L^{-1} OCl^-) with a solution containing luminol (2.82×10^{-3} mol L^{-1}), perborate (1.78×10^{-2} mol L^{-1}), and carbonate (0.24 mol L^{-1}) at pH 12 in a flow system leads to a steady-state emission of chemiluminescence. This chemiluminescence scaled linearly with the concentration of the hypochlorite solution over the range 4.6×10^{-3} to 3.65×10^{-3} mol L^{-1} when the perborate concentration in the luminol solution was held at 1.78×10^{-2} mol L^{-1}, showing that the chemiluminescence reaction is first order in hypochlorite over this range of conditions. At higher concentrations (4.6×10^{-2} mol L^{-1}) the increase with [OCl^-] became less than linear. The chemiluminescence intensity was almost independent of perborate concentration over the range 4.4×10^{-3} to 1.78×10^{-2} mol L^{-1}, and decreased by 10% when the concentration was 3.6×10^{-2} mol L^{-1} (all concentrations refer to the luminol solution).

The addition of primary and secondary organic amines (0.02–0.08 mol L^{-1}) to the luminol/perborate/carbonate solution caused suppression of the chemiluminescence observed when this solution was mixed with a hypochlorite solution, with the suppression depending on the amine and the concentration, Table 1. If the reactions of amine with hypochlorite, luminol with hypochlorite, and hydrogen peroxide with hypochlorite are all first order in hypochlorite, and if the only function of the amine is to react with the hypochlorite according to Eq. (1), then it can be predicted that the light observed in the presence of amine, $\text{emission}_{\text{amine}}$, will be related to the light emission in the absence of amine $\text{emission}_{\text{no amine}}$, by the following equation:

$$\frac{\text{emission}_{\text{no amine}}}{\text{emission}_{\text{amine}}} = 1 + \frac{k_{\text{HOCl}}}{k_1}[\text{amine}] \quad (4)$$

where k_1 is the apparent rate constant for the reaction of hypochlorite with luminol to produce chemiluminescence, and k_{HOCl} is the rate constant for the reaction of hypochlorite with the amine. This predicts a linear dependence of the ratio on the left hand side of the equation with [amine]. A linear relationship was indeed followed at low amine concentrations (< 0.02 mol L^{-1}), but at higher concentrations the decrease in light emission was greater than linear in [amine]. Studies with glycine showed that the deviation from linearity became greater as the perborate concentration decreased, and that linearity could be restored by increasing the perborate concentration from 1.78×10^{-2} to 3.5×10^{-2} mol L^{-1} (Fig. 1). The most likely explanation for this behaviour is that some amines can also compete with HO_2^- for the diazaquinone

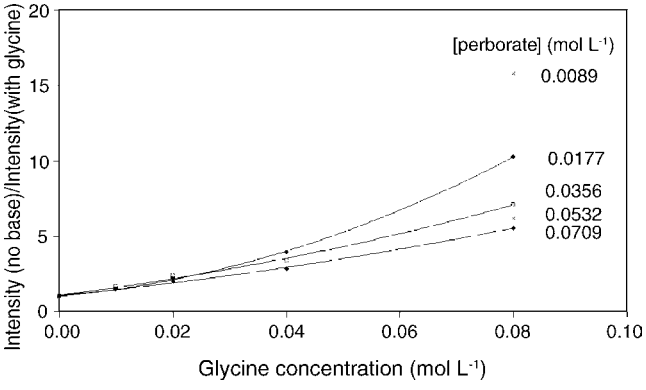


Fig. 1. Plot showing effect of glycine and perborate concentrations on the relative chemiluminescent light intensity resulting from the reaction of hypochlorite with luminol. The curves are quadratic fits through the data at perborate concentrations of 0.0709, 0.0356, and 0.0709 mol L^{-1}; measurements at 0.0089 and 0.0533 mol L^{-1} perborate were only made for 0 and 0.08 mol L^{-1} glycine. Perborate concentrations are corrected to the titratable peroxide content (=0.78 of nominal concentration).

(L in Scheme 1) formed from the reaction of luminol with hypochlorite. This is consistent with earlier observations of amines and other nucleophiles such as OH^- competing effectively with HO_2^- for this intermediate, although the extent of competition observed here suggests that the reported extrapolated rate constant for a primary alkyl amine may be low [26].

There was a correlation between the rate of reaction between hypochlorite and the different amines and the pK_a of the conjugate acid of each amine, in agreement with the earlier studies by Margerum [6,7] and Antelo [13,14]. Thus, a plot of $\log(k_{HOCl}/k_1)$ against pK_a for the five primary amines tested had a slope of 0.47 ± 0.14, in comparison to the value reported by Antelo et al of 0.48 ± 0.05. The plot showed significant scatter around the best-fit line, showing that other factors (presumably steric and/or charge) are also important, in agreement with the observation by Armesto [15]. Based on these results, glycine was chosen for further study based on its reasonable reaction rate, low toxicity, and ready availability at low cost.

Two amines tested caused an increase of the hypochlorite-induced chemiluminescence. The tertiary amine triethylamine increased the chemiluminescence greatly at low concentrations, but then reached a saturation value corresponding to a four-fold increase at 0.04 mol L^{-1}. If this corresponds to complete formation of chlorotriethylammonium ions from hypochlorite, then the reaction resulting in the chemiluminescence of luminol is about four times faster for chlorotriethylammonium ion that it is for hypochlorite under these conditions. Alternatively, a previous study on chemiluminescent determination of triethylamine noted non-linearity in the calibration plot at concentrations above 1×10^{-3} mol L^{-1} [10], and both these observations may be due to a side reaction of the chlorotriethylammonium ion occurring at higher concentrations. The other tertiary amine studied, ethylenediaminetetraacetate (EDTA), had no discernable effect on the chemiluminescence at pH 12, in agreement with our earlier study [5]. An increase in chemiluminescence was also seen for sulfamate at pH 12, with the intensity increasing tenfold when the sulfamate concentration was increased to 0.08 mol L^{-1}. This result was surprising, since sulfamate is reported in the open [27] and patent [28,29] literature to stabilize bromine- and chlorine-containing solutions, and it was for this reason that it was included in this study. However, there is a report of sulfamate being used to catalyse the chlorination of cyclopentadiene by hypochlorite [30], so it is possible that under some conditions it can activate hypochlorite-induced oxidation.

Study of the reaction of Grodsky luminol/carbonate/perborate (pH ca. 11) with hypochlorite in stopped-flow mode showed that the reaction was very rapid, with a half-life of approximately 0.5 s under the concentration conditions used. This time was similar to the stopping time of the flow system, so detailed kinetics were not performed. Increasing the solution pH from 11 to 12 led to a 44% decrease in the hypochlorite-induced chemiluminescence, in qualitative agreement with Isaacson [17]. The addition of 0.05 mol L^{-1} glycine at pH 12 caused a further large decrease (70%) in the intensity of the hypochlorite-induced chemiluminescence. In both cases the half life for the chemiluminescence decay did not change significantly. This decrease in chemiluminescence upon increasing the pH from 11 to 12 is less than the 10-fold reduction predicted (and observed with low analyte concentrations [31]) if the only route to chemiluminescence is via Scheme 1. This suggests either that the second deprotonation of luminol [19] might be producing a more active nucleophile than the luminol monoanion [32], or that an additional reaction path is occurring. The reaction of hypochlorite with peroxide produces singlet oxygen [16] and based on the known rate constants for singlet oxygen production [16] and reaction with luminol [33] a significant fraction of the luminol may react with singlet oxygen rather than with hypochlorous acid under the conditions present when forensic luminol sprays contact hypochlorite residues. It has been suggested that the reaction with singlet oxygen leads to the intermediate azaquinone (L in Scheme 1) [33], which can then react with peroxide to cause chemiluminescence.

3.1. Effect of glycine on the luminol–blood reaction at pH 12

The reaction of the Grodsky luminol/peroxide/carbonate solution with an equivalent volume of diluted blood (1:50) showed an induction time of 10–15 s before reaching peak intensity, and the chemiluminescence then decayed over a 200 s period. Addition of glycine (0.08 mol L^{-1}) to the Grodsky luminol/peroxide/carbonate solution at pH 12 resulted in a slight (15%) decrease in emitted light intensity when the solution was mixed 1:1 with 50-fold diluted blood. When the same experiment was performed with diluted old blood (stored for 2 years in liquid form at 4 °C), glycine caused a slight increase in the initial chemiluminescence and a slightly more rapid decay, compared to the reaction in the absence of glycine. This suggests that degradation can lead to alteration in the reaction behaviour of the heme in blood. The observed reduction in blood-induced chemiluminescence at 0.08 mol L^{-1} glycine and the evidence for competition of amines for the reactive intermediate (L) reported above led us to use 0.05 mol L^{-1} glycine in all further trials.

Increasing the alkalinity of the Grodsky luminol/peroxide solution from the initial pH 11 to pH 12 prior to mixing with diluted blood led to an 80% increase in the peak chemiluminescence intensity and a slowing of the chemiluminescence decay rate, while the addition of 0.05 mol L^{-1} glycine at pH 12 had little additional effect on the chemiluminescence (Fig. 2). This is in marked contrast to the behaviour of the hypochlorite reaction stated earlier, so that the increase of the solution pH from 11 to 12 combined with the addition of 0.05 mol L^{-1} glycine has led to a greater than 11-fold increase of the specificity of the chemiluminescence for blood relative to that for hypochlorite based on peak intensities and

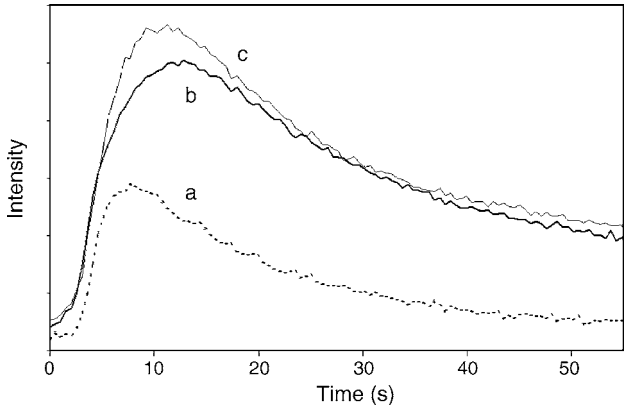


Fig. 2. Plot showing the measured light intensity for luminol-blood against time. (a) pH 11 no glycine (- - -), (b) pH 12 no glycine (—), (c) pH 12 glycine 0.05 mol L^{-1} (___).

a 15-fold increase based on the intensity integrated over 30 s from time of mixing.

3.2. Photographic imaging of luminol spray on solid substrates

The above experiments were all performed in solution, whereas luminol sprays are used in forensic science by spraying onto two-dimensional or three-dimensional objects. We therefore performed several experiments where luminol sprays were applied to blood, bleach, and mixed stains on cotton cloth and on vinyl floor tiles. The experiments were either performed by spraying the substrates and then photographing, or by opening the camera shutter prior to spraying. This latter method will provide the greatest light intensity but will also be most susceptible to chemiluminescence from the hypochlorite reaction, and so is a more stringent test of the methodology. The experiments were performed on cloth patches (Fig. 3) and tiles (Fig. 4), both types of substrates being prepared with horizontal blood bands and a vertical bleach region such that they had areas containing blood alone (lower left), blood + bleach (lower right), bleach only (upper right), and nothing (upper left). The increase in pH of the Grodsky luminol/peroxide solution from 11 to 12 led to a small decrease in the chemiluminescence detected upon spraying bleach-treated cotton and no detectable change to the blood chemiluminescence (Fig. 3a–d). The addition of 0.05 mol L^{-1} glycine at pH 12 greatly reduced the hypochlorite-induced chemiluminescence, while only slightly decreasing the chemiluminescence due to blood (Fig. 3e–f). The figure shows 10 s exposure time images taken as spraying was performed (a, c, e), and then starting 20 s after spraying had commenced (b, d, f). The spraying itself took about 4 s. A comparison of the effects of luminol spray at pH 12 with and without glycine on vinyl floor tiles (mounted vertically) is shown in Fig. 4. In this case, the first photograph, taken when the spray with no glycine was used (Fig. 4a), shows bright chemiluminescence from the region with bleach (right hand side), while the second photograph (Fig. 4b)

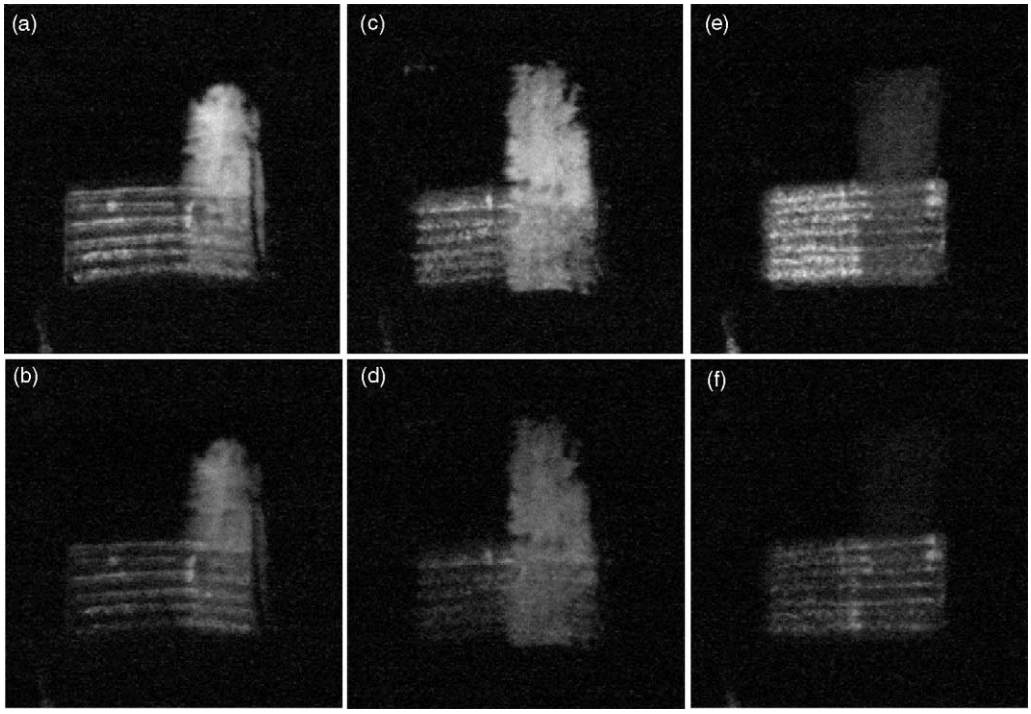


Fig. 3. Cotton calico substrate with blood stains and bleach treated with unmodified and modified Grodsky luminol spray. Photographic exposure time 10 s. (a) pH 11, no glycine at time 0 s, (b) at 20 s, (c) pH 12, no glycine at 0 s, (d) at 20 s, (e) pH 12, glycine 0.05 mol L^{-1} at 0 s, (f) at 20 s. Blood was smeared across the lower two quadrants of the target area and allowed to dry. Bleach was then wiped down the right two quadrants of the target area a few minutes prior to spraying.

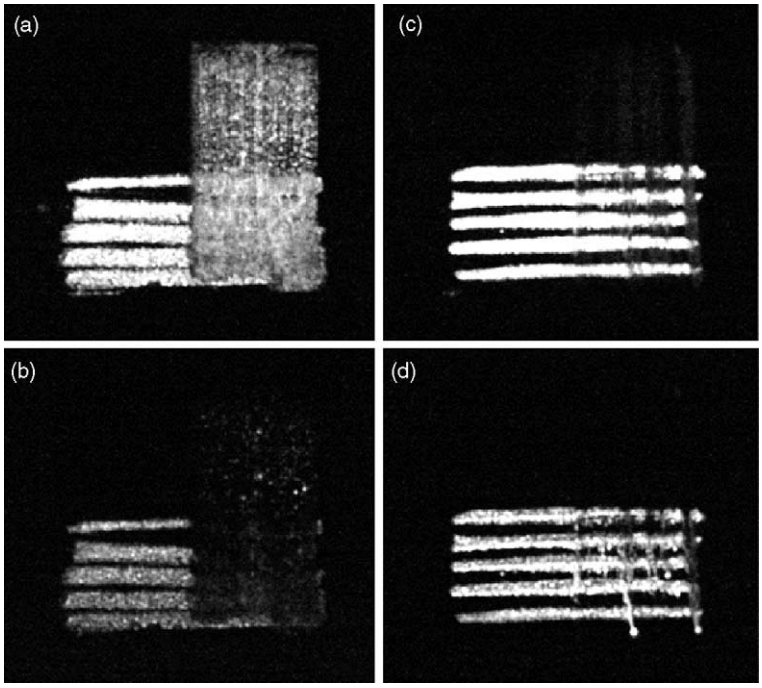


Fig. 4. Vinyl floor tile substrate with blood stains and bleach treated with modified Grodsky luminol spray. Photographic exposure time 6 s. (a) pH 12 no glycine at time 0 s, (b) after 20 s, (c) pH 12 glycine 0.05 mol L^{-1} at time 0 s, (d) at 20 s. Blood was smeared across the lower two quadrants of the target area and allowed to dry. Bleach was then wiped down the right two quadrants of the target area a few minutes prior to spraying.

shows almost no chemiluminescence from the right hand side even where blood is present. This latter observation is probably due to the consumption of the perborate via reaction with hypochlorite. In contrast, the photographs for the spray which contained glycine (Fig. 4c and d) show little effect due to the bleach, so that only the blood chemiluminesces.

3.3. Practical and safety issues

The glycine-containing luminol spray formulation developed in this study was designed for preparation immediately prior to use. This is in accord with practice by ESR Ltd. forensic scientists, who take the pre-weighed reagents for the preparation of the Grodsky luminol spray to scenes and prepare the solutions on site [34]. Prolonged storage of luminol and glycine in solution may lead to Schiff base formation, although this is likely to be limited at the high pH of forensic luminol sprays. The increased pH of this spray (pH 12, comparable to that of the Weber luminol spray formulation) compared to that of the Grodsky formulation (pH ca. 11) will lead to slightly increased corrosivity of the spray, and standard protective wear (goggles, spray mask, and gloves) should be worn when using the spray. None of the components are volatile, so that exposure will be due to inhalation of, or contact with, aerosol, or contact with solution. Upon reaction with hypochlorite, the glycine will initially form the conjugate base of *N*-chloroglycine [6,7], a non-volatile organic chloramine. *N*-chloroglycine has some ecotoxicity (EC_{50} (1 h exposure, with mortality assessed 48 h post-exposure) = 575 mg L^{-1} for mosquitofish [35], EC50 (30 min) = 0.11 mg L^{-1} as free chlorine by a Microtox bioassay [36]), although the mosquitofish ecotoxicity is less than that of hypochlorite (EC_{50} (1 h) = 2.21 mg L^{-1} [35]). *N*-chloroglycine has been investigated for chemomechanical caries removal [37], and other *N*-chloroamino acids are presently being evaluated for this application [38], suggesting that small amounts of these compounds are tolerated by humans. Under the basic conditions of the spray, *N*-chloroglycine is likely to decompose via hydrolysis [39], with the initial product being the conjugate base of glyoxylic acid (2-oxoethanoic acid), which is nonvolatile. Glyoxylic acid has low acute toxicity (oral LC50 for rates of >90 mL kg^{-1} [40]), but is classified by the US National Institute for Occupational Safety and Health (NIOSH) Registry of Toxic Effects of Chemical Substances (RTECS) as a mutagen, based on in vitro tests, with similar in vitro mutagenicity to sodium perborate and sodium hypochlorite [41]. No toxicological data appeared to be available on the conjugate bases of *N*-chloroglycine or glyoxylic acid. In summary, in the absence of bleach the modified luminol spray will show similar hazards to the standard Grodsky luminol formulation, with slightly increased corrosivity due to the increased pH of the solution, while in the presence of bleach the conjugate bases of N-chloroglycine and glyoxylic acid may be formed in amounts comparable to the original amount of hypochlorite. Due to their nonvolatile nature these chlorination byproducts would only be present where hypochlorite was previously deposited, and water-based decontami-

nation should remove these from surfaces, so that cleanup should be identical to that currently used for the Grodsky luminol spray. Even though our tests indicate that the addition of glycine and alteration of the pH does not greatly affect the chemiluminescence due to blood, this modification has not been tested under all circumstances, so we recommend that the modification only be used when there is strong likelihood that hypochlorite-based bleach has been used.

4. Conclusions

This study confirms the prior observation that some primary and secondary amines can reduce the interference by hypochlorite bleaches on the forensic luminol/perborate test for blood. It was found that amino acids such as glycine or alanine were almost as efficacious as 1,2-diaminoethane, while having the advantage that they are much less toxic and volatile. Surprisingly, it was found that sulfamate increased the chemiluminescence due to hypochlorite, despite its use as a stabiliser for chlorine- and bromine-containing aqueous solutions.

The optimum concentration of a given amine is controlled by its ability to compete for hypochlorite and its ability to compete for the oxidized luminol intermediate (L). In solution at a concentration of $0.05\,\mathrm{mol\,L^{-1}}$ glycine greatly reduced the chemiluminescence due to $2.28 \times 10^{-2}\,\mathrm{mol\,L^{-1}}$ bleach while not significantly affecting the chemiluminescence due to blood. This concentration of glycine also provided enhanced selectivity for blood over hypochlorite on cotton and vinyl substrates. Thus, if it is desired to use a luminol spray to detect blood and there is evidence to suggest the presence of hypochlorite bleaches it may be advantageous to add glycine to the Grodsky luminol formulation and to increase the pH to 12. As we have noted in a previous paper, an alternative strategy if time is available is to air the item for a few days to allow the hypochlorite to decompose. When this delay is not possible, the addition of amine can allow the successful use of luminol to detect trace blood despite the presence of bleach.

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