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Talanta 60 (2003) 405–414
Talanta
www.elsevier.com/locate/talanta

FI-on line photochemical reaction for direct chemiluminescence determination of photodegradated chloramphenicol

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Received 17 June 2002; received in revised form 8 November 2002; accepted 18 November 2002

Abstract

A new, simple, clean and selective flow injection strategy based on the tandem photochemical reaction-chemiluminescence detection was applied to the determination of chloramphenicol. The determination is based on the on-line photodegradation of the drug in a glycine buffer at pH 8.8 by using a photoreactor consisting of 697 cm × 0.5 mm PTFE tubing helically coiled around an 8 W low-pressure mercury lamp. Photodegradated chloramphenicol is detected by direct chemiluminescence of resulting photo-fragments and their subsequent reaction with potassium permanganate in sulfuric acid medium as oxidant. The method allows the chemiluminescence determination of compounds which do not exhibit native chemiluminescence. The calibration graph was linear up to 14 μg ml^{-1} chloramphenicol, the limit of detection was 30 ng ml^{-1}, the relative standard deviation was 2.4% for 7 μg ml^{-1} of the drug and the sample throughput was 60 h^{-1}. Taking into account the importance of the medium of photodegradation on the mechanism of photodegradation a comparative study in terms of selective was performed for different chemical media employed in the procedure of photodegradation. The proposed method was applied to the determination of chloramphenicol in commercially available pharmaceutical formulations.

Keywords: Chemiluminescence; Photolysis; Chloramphenicol; Pharmaceuticals; FIA

1. Introduction

At present, chemiluminescence research is in continuous expansion by virtue of the search for new processes allowing the direct chemiluminescence-based determination of substances of phar-

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doi:10.1016/S0039-9140(03)00074-2

maceutical, clinical or environmental interest [1–3]. However, the direct chemiluminescence detection approach can lack throught selectivity and is restricted either to compounds which present 'native' chemiluminescence or can be coupled to a chemiluminescent target by means of an additional chemical reaction increasing the complexity of the analytical procedure [4]. This can be overcome introducing a photochemical/photolytic step to generate a chemical form, mainly an oxidative one which can be determined by means of classical and high sensitive chemiluminescent reactions involving luminol [5–10].

The use of photolytic procedures have been succesfully applied to chromatography by Birks and coworkers [11–14]. Although the viability of the integration of photolytic strategies or chemiluminescence detection in-line with HPLC has been confirmed by the numerous published articles, only a few papers are devoted to the tandem of both stragies, photodegradation and chemiluminometric detection combined with HPLC [15–18].

More recently, photochemical reaction and chemiluminescence detection have been applied to the determination of aromatic amines [19], photoinduction of glucose [20], determination of citrate [21], nitrate [22,23]and generation of singlet molecular oxygen [24,25].

Extending this principle of photochemical reaction, we have investigated whether by on-line irradiation drugs can modify the molecular structure yielding photofragments suitable for direct chemiluminescence detection in the search for a more specific or sensitive determination for compounds which do not exhibit 'native' chemiluminescence.

The viability of the proposed method (tandem photodegradation-direct chemiluminescence detection of photodegradated analyte) was confirmed using chloramphenicol as test substance. Chloramphenicol is a broad-spectrum antibiotic commonly used to fight infections for which penicillins and tetracyclines have proved ineffective. The toxicity of chloramphenicol is derived from its action on the mitocondrial synthesis of proteins and can cause serious secondary effects. To the authors' knowledge, there is only three references to the indirect flow-injection determination of this drug [6,26,27].

The proposed procedure is based on the photolysis of the drug by a coiled PTFE tubing around a low-pressure mercury lamp for germicidal used and the subsequent direct chemiluminescence determination of the chemiluminescent photofragment using potassium permanganate as oxidant.

2. Experimental

2.1. Reagents

All reagents were analytically pure unless stated otherwise and prepared in deionized water (18 MΩ cm) using a Sybron/Barnstead Nanopure II water purification system provided with a fiber filter of 0.2 μm pore-size. Aqueous solution of chloramphenicol were prepared by dissolving the drug in deionized water and diluting in glycine buffer (glycine 0.1 M, NaCl 0.1 M at pH 8.8 by drooping NaOH 0.1 M). Other reagents used were: H_2SO_4, $KMnO_4$, NaClO, $K_2S_2O_8$, KIO_4, $Ce(SO_4)_2 \cdot 4H_2O$, NH_4Cl, H_2O_2, H_3PO_4, NaOH, $Na_2B_4O_7 \cdot 10H_2O$, $K_3Fe(CN)_6$, CH_3COOH, HNO_3, HCl, $HClO_4$, dioxane, 8-hydroxyquinoline, KCl, NaCl, dimethylformamide and formic acid from Panreac (Moncada y Reixac, Barcelona, Spain); NH_3 from Merck (Darmstadt, Germany); chloramphenicol, ethanol, acetone, quinine sulfate, 1,2-polyethylenglycol, hydrocortisone, erythromicin, polysorbate 20, glucose, glycerol, prednisolone, lactose and lidocaine chlorhydrate from Guinama (Alboraya, Valencia, Spain); sodium dodecyl sulfate from Fluka (Buchs, Switzerland); acetonitrile; Triton X-100 from Scharlau (Barcelona, Spain); Rhodamine B and Rhodamine 6G from Sigma (Alcobendas, Madrid, Spain); EDTA and $MgCl_2 \cdot 6H_2O$ from Prolabo (Barcelona, Spain); benzocaine and saccharose from Acofarma (Terrassa, Barcelona, Spain); acridine yellow from Aldrich (Alcobendas, Madrid, Spain); soluble starch (Probus, Barcelona, Spain), hexadecylpyridinium chloride (Fluka, Buchs, Switzerland).

2.2. Apparatus

The flow manifold consisted of a PTFE coil of 0.5 mm id, a Rheodyne (Cotati, CA, USA) Model 5041 injection valve, a Gilson (Worthington, OH, USA) Minipuls 2 peristaltic pump provided with pump tubes from Omnifit and an 8 W low-pressure mercury lamp (Zalux) for germicidal use. The flow cell was a flat-spiral quartz tube of 1 mm id and 3 cm total diameter backed by a mirror for maximum light collection. The photodetector package was a P30CWAD5F-29 Type 9125 photomultiplier tube (PMT) supplied by Electron Tubes operating at 1280 V and was located in a laboratory-made light-tight box. The output was fed to a computer equipped with a counter-timer, also supplied by Electron Tubes. The peak heights were measured in kHz.

2.3. Flow-injection assembly

The proposed FIA-manifold is depicted in Fig. 1a. The sample solution (chloramphenicol in glycine buffer at pH 8.8) was pumped at 0.8 ml min^{-1} through the photoreactor consisting of 697 cm × 0.5 mm PTFE tubing helically coiled around an 8 W low-pressure mercury lamp. Then 524 μl of irradiated sample were heated in a thermostated bath at 70 °C and injected into a pure water carrier stream flowing at 6 ml min^{-1}. The photodegradated drug reacted with a oxidant solution (2×10^{-4} mol l^{-1} $KMnO_4$ in 2 mol l^{-1} H_2SO_4) flowing at 1.6 ml min^{-1}. The resulting mixture reached the flat spiral quartz flow cell and the total chemiluminescence emission was detected by the photomultiplaier tube working at 1280 V.

2.4. Procedures for preparation of buffers

2.4.1. Phosphate buffer

Solutions of KH_2PO_4 and Na_2HPO_4 both at a 0.067 mol l^{-1} concentration were mixed in suitable proportions to obtain solutions of pH throughout the interval 6–8.

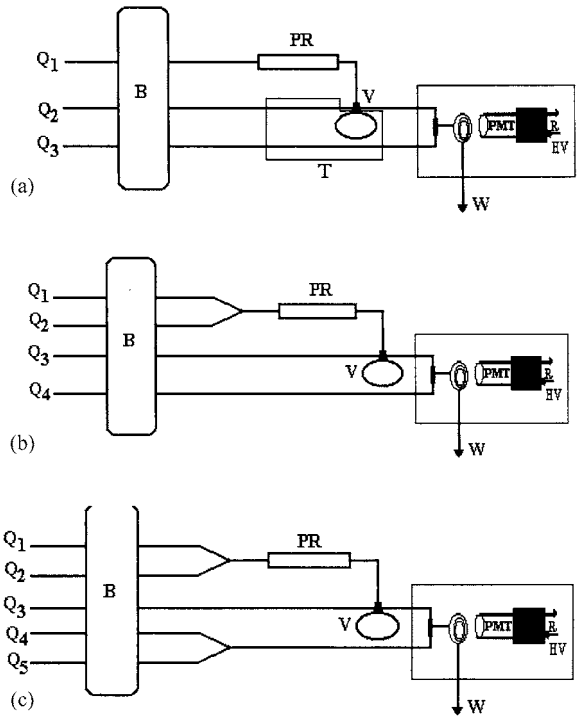

Fig. 1. (a) Optimised flow-assembly. Q1: 0.8 ml min^{-1}, chloramphenicol in 0.1 mol l^{-1} glycine buffer at pH 8.8; Q2: 6 ml min^{-1}, water; Q3: 1.6 ml min^{-1}, 2×10^{-4} mol l^{-1} $KMnO_4$ in 2 mol l^{-1} H_2SO_4; V: 524 μl; T: 70 °C. (b) and (c) Flow assemblies for preliminary studies. Q_1 1.1 ml min^{-1} sample solution; Q_2 1.1 ml min^{-1} medium of photodegradation; Q_3 4.1 ml min^{-1} carrier solution (water); Q_4 1.3 ml min^{-1} oxidant solution (fig. b); $Q_4 = Q_5 = 0.65$ ml min^{-1} (fig. c); V, 408 μl of sample injected; B, peristaltic pump; PR, photoreactor; PMT, photomultiplier tube; W, waste.

2.4.2. Glycine buffer

The pH of a solution 0.1 mol l^{-1} in glycine and 0.1 mol l^{-1} in NaCl was ajusted by means of the addition of small amounts of 0.1 mol l^{-1} NaOH.

2.4.3. Acetic/acetate buffer

Solutions of CH_3COOH and CH_3COONa both at a 0.2 mol l^{-1} concentration were mixed in suitable proportions to obtain solutions of pH throughout the interval 3.7–5.7.

2.4.4. Borax buffer

A borax solution 0.0125 M was dropped with 0.1 M NaOH to obtained the appropiated pH.

2.4.5. NH_4^+/NH_3 buffer

Identical procedure was followed that with the previous buffer to obtain buffer solutions at pH throughout the interval 8.7–10.5.

2.5. Procedures for preparation of samples

2.5.1. Colircusí Cloramfenicol (Laboratorios Cusi, S.A., Barcelona, Spain), Anco nil Antidiarreico (Productos ANCO S.L., Madrid, Spain) and Oftalmocan Biótico (Laboratorios IVEN, Madrid, Spain)

No pre-treatment other than dilution and buffering was required.

2.5.2. Colircusí Medrivás (Laboratorios Cusi S.A., Barcelona, Spain)

This preparation is available as suspension. The suspension was filtered through a nylon mesh of 0.45 μm pore size and the filtrate was diluted and buffered to obtain a solution containing ca. 8 mg l^{-1} chloramphenicol.

2.5.3. Cortison Chemicetina (Farma Astra S.A., Barcelona, Spain)

An amount of 0.6 g of the pomade was disolved in 50 ml of petroleum ether at 60 °C and extracted with three 50 ml portions of water. The extract was made up to 200 ml with deionised water to obtain a solution containing ca. 60 mg l^{-1} of chloramphenciol. Following buffering, aliquots of this solution were used to obtain more diluted solutions containing ca. 8 mg l^{-1} chloramphenicol.

2.6. Reference method

The results obtained with the proposed method were assessed by comparison with the manufacturer's stated content and with those provided by the official method of the Spanish Ministry of Health and Consumer Affairs as published in the Royal Spanish Pharmacopoeia [28]. For this purpose an appropriated amount of sample containing 0.1000 g of the drug was weight and diluted with deionized water to obtain a solution containing chloramphenicol in the vicinity of 20 μg ml^{-1}. The absorbance of the resulting solution was measured at 276 nm. The content of chloramphenicol was calculated considering as specific absorbance the value 297.

2.7. Procedure

From the chemical and FIA point of view the proposed method can be divided in two distinct and independent systems: (a) the photodegradation of the chloramphenicol; and (b) the so-called 'indicator reaction' (oxidation of the photodegradation products employing $KMnO_4$ as oxidant in H_2SO_4 medium and chemiluminescence detection). The studies were focused on system (a), for which the optimised chemical variables were the medium and the time of irradiation. The optimisation of the chemical and FI parameters for the redox reaction (system (b)) included the study of different oxidants and chemical hydrodynamic variables. They were performed by a sequential combined methodology, by using a multivariate method, the modified simplex method (MSM) [29–31], for the FIA parameters.

3. Results and discussion

3.1. Preliminary tests

First, the viability of the photodegradation of chloramphenicol in different media and combined with chemiluminescence detection of the photodegradated analyte was studied. For this proposal oxidant systems usually employed in direct chemiluminescent procedures were used. The unsegmented continuous-flow assemblies used are depicted in Fig. 1b and c. The ligth source was placed inline with the manifold and the sample was irradiated as it flowed through a PTFE tube helically coiled around the UV lamp. The sample solution (drug aqueous solution, 100 μg ml^{-1}) was mixed with photodegradation medium solution (10^{-3} and 0.1 mol l^{-1} H_2SO_4, H_2O, 10^{-3} and 0.1 mol l^{-1} NaOH, 0.01 mol l^{-1} NH_4^+/NH_3 buffer at pH 9.5, borax buffer at pH 9.5) in an Y-shaped piece. This solution was pumped through the photoreactor and was injected into a carrier of water. The resulting solution merged with the oxidant solution in a T-piece much close to the

flow-cell. Drug insertions were performed with the lamp on and off.

The tested oxidants were: $KMnO_4$, Ce(IV), KIO_4 and $K_2S_2O_8$ 5×10^{-3} mol l^{-1} in medium H_2SO_4 1 mol l^{-1}; $K_3Fe(CN)_6$ 5×10^{-3} mol l^{-1} in NaOH 1 mol l^{-1}; H_2O_2 and NaClO 5×10^{-3} mol l^{-1} in NaOH 1 mol l^{-1}; and, H_2O_2 5×10^{-3} mol l^{-1} with NaClO 0.1%. For the last three oxidants, the FI-manifold was modified because when they are prepared together they are unstable. So, the oxidant and the medium were mixed in an Y-shaped piece (Fig. 1c). All the condition were like in Fig. 1b, except $Q_4 = Q_5 = 0.65$ ml min^{-1} for the oxidant and medium for the chemiluminescent reaction.

The results obtained can be summarized as follows:

a) No signal was obtained with KIO_4 or $K_2S_2O_8$, whether or not UV radiation was applied.
b) The $K_3Fe(CN)_6$/NaOH and H_2O_2/NaClO systems exhibited the same response in all media tested, whether or not the lamp was ON. The slight differences observed can be ascribed to the increased temperature of the photodegraded solution by effect of the applied radiation and its influence on the chemiluminescent reaction. The signals obtained were smaller than 1 kHz in all instances.
c) The NaClO/NaOH system provided a decreased analytical signal upon irradiation of the sample except in the presence of H_2SO_4 at a 0.1 mol l^{-1} concentration, where, however, the response also increased significantly with the lamp OFF.
d) The H_2O_2/NaOH system allowed discriminating between the effects of the lamp ON and OFF (zero signal) in 10^{-3} M H_2SO_4, H_2O and the NH_4^+/NH_3 buffer. However, it was discarded on the grounds of the instability of the oxidizing solution and the weak chemiluminescence signal obtained.
e) The $KMnO_4/H_2SO_4$ and Ce(IV)/H_2SO_4 systems provided no chemiluminescence signal in any of the photodegradation media tested with the lamp OFF. With both oxidants, photodegraded chloramphenicol exhibited chemiluminescence in all media except Ce(IV) in dilute H_2SO_4. Also, both systems yielded a signal peak in 10^{-3} mol l^{-1} NaOH; however, $KMnO_4$ provided stronger peaks by a factor of 5–7. The results obtained with the borax buffer were similar to those provided by $KMnO_4$ in 10^{-3} mol l^{-1} NaOH.

Based on these results, the $KMnO_4/H_2SO_4$ system was chosen as oxidant, and 10^{-3} mol l^{-1} NaOH and borax buffer as media for the photodegradation of chloramphenicol, with a view to developing a chemiluminescence-based method for the direct determination of a non-chemiluminescent compound.

3.2. Study of the chemiluminescent reaction

The chemiluminescent reaction was studied simultaneously in the two selected photodegradation media, using the assembly depicted in Fig. 1b and a 100 μg ml^{-1} chloramphenicol solution.

The effect of the $KMnO_4$ concentration was examined over the range 10^{-1}–10^{-6} mol l^{-1}. The results are shown in Fig. 2. An identical trend was observed in both photodegradation media, with an emission peak at a 4×10^{-4} mol l^{-1} $KMnO_4$ concentration.

The study of the influence of the medium on the oxidizing solution included H_2SO_4, HNO_3, HCl, $HClO_4$, H_3PO_4 and acetic acid (all at a 2 mol l^{-1} concentration), in addition to H_2O and NaOH at pH 9.5 and 11 (viz the pH values corresponding to the borax buffer and NaOH solutions used as photodegradation media) (see Table 1). Using an acid medium proved essential with a view to ensuring acceptable yields in the chemiluminescent reaction. The influence of the concentration of

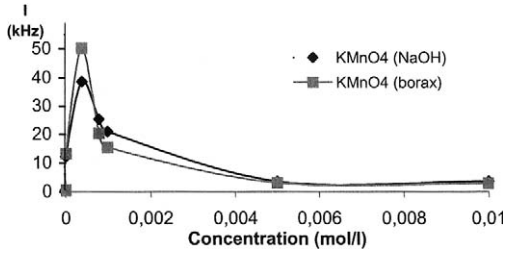

Fig. 2. Influence of concentration of $KMnO_4$ oxidant solution.

Table 1
Influence of acidic media on the chemiluminescence reaction (for 100 μg ml^{-1} of chloramphenicol); I: average intensity ($n = 5$)

Medium	H_2SO_4	HNO_3	HCl	$HClO_4$	H_3PO_4	CH_3COOH	H_2O	pH 9.5	pH 11
I (kHz)	58.85	41.40	57.07	49.04	28.88	10.84	2.22	1.36	4.41
rsd %	2.2	2.0	1.6	1.5	3.4	2.9	21.9	4.0	1.6

H_2SO_4 and HCl, which were found to be the best suited to the intended purpose, was studied over the ranges 0.1–5 and 0.1–3 mol l^{-1}, respectively. On equal concentrations, H_2SO_4 yielded stronger signals. In the presence of HCl, some oxidant was consumed and chlorine produced as a result. Increasing the H_2SO_4 concentration increased the analytical signal, probably through increased viscosity and structural rigidity of the medium, which favoured the production of and stabilized the excited chemiluminescent species. A H_2SO_4 concentration of 2 mol l^{-1} was chosen as optimal.

The effect of temperature was studied by immersing different parts of the continuous-flow manifold (viz the sample, carrier and oxidant lines, and the sample loop) in a thermostated bath. The photodegradation media studied behaved identically. Thus, the maximum signal was obtained when all manifold lines were heated: the signal doubled on heating from 25 (33.2 kHz) to 80 °C (63.3 kHz). On the other hand, the weakest signals were obtained when only the sample and oxidant streams were heated; this was a result of the temperature decrease caused by the carrier solution flowing at a rate of 4.1 ml min^{-1}.

3.3. Optimization of FI parameters

The influence of FI variables on the chemiluminescent reaction were optimized by applying a multivariate method (viz the Modified Simplex Method, MSM) to both photodegradation media. The parameters included in the Simplex and the ranges examined were as follows: carrier flow-rate (1.5–8.8 ml min^{-1}), oxidant flow-rate (0.3–2.6 ml min^{-1}), inserted sample volume (151–610 μl).

The best results were obtained with similar conditions in both photodegradation media, which experienced rapid contraction of the Simplex to a peak (apex 11 with NaOH and 15 with the borax buffer). The optimum oxidant and carrier flow-rates were 6.0 and 1.6 ml min^{-1} for both systems, and the optimum sample volumes 515 and 525 μl with 10^{-3} mol l^{-1} NaOH and the borax buffer, respectively.

3.4. Study of the photodegradation reaction

The simultaneous study of the physico–chemical and hydrodynamic variables associated to the chemiluminescent reaction and the consistency between the results obtained in 10^{-3} mol l^{-1} NaOH and the borax buffer revealed the photodegradation and chemiluminescence processes to be mutually independent, and hence the suitability of the sequential approach adopted in the optimization process. The optimum values of the chemiluminescent reaction were thus used to examine the influence of some variables potentially affecting the photodegradation mechanism and yield.

The photodegradation of chloramphenicol involves oxidation, reduction and condensation reactions. Partial information about these processes and the different photolysis products obtained has been reported by several authors [27,32–35]. The preferential photodegradation mechanisms and their yields are dictated by the spectrum of the radiation source, the irradiation time and, especially, the photodegradation medium.

The effect of pH and the buffer medium was studied by using 20 μg ml^{-1} solutions of chloramphenicol in a 0.0125 mol l^{-1} borax medium at pH 7.6–10.0. Because the signal increased with increasing pH, various buffers allowing the pH to be further reduced without detracting from its buffering capacity were prepared. The following buffers were tested to this end (see Section 2.4): HPO_4^{2-}/HPO_4^-, glycine, HAcO/NaAcO and NH_4^+/NH_3. The results are shown in Figs. 3

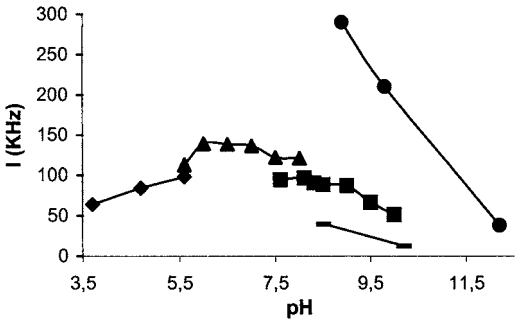

Fig. 3. Influence of pH and buffer medium on the photodegradation. ◆, (HAcO/NaAcO); ■, ($Na_2B_4O_7 \cdot 10H_2O$); ▲, ($HPO_4^{2-}/H_2PO_4^-$); ●, (glycine); —, (NH_4^+/NH_3).

and 4. Glycine doubled the strength of the analytical signal, so it was chosen for further testing, which revealed pH 8.8 to be optimal. Although the glycine buffer also resulted in increased blank signals (6 kHz at pH 8.8) with increase in pH, the limit of detection (calculated as the lowest analyte concentration yielding a signal equal to the blank signal plus three times its standard deviation) remained constant at 30 ng ml^{-1}.

Attending different criteria such as capability for stabilizing free radicals or recognized use as sensitizers to increase the chemiluminescence intensity by energy transfer, a wide variety of potential photosensitizers described in the literature [36–40] were examined. The following substances were tested: 0.5% acetone, 20% acetonitrile, 0.5% acetone+20% acetonitrile, 5% dioxane, 5% dimethylformamide, 5% ethanol, 5×10^{-4} mol l^{-1} Rhodamine B or 6G, 5×10^{-4} mol l^{-1} quinine, 0.5% formic acid, and anionic, cationic and neutral surfactants such as 0.02% SDS, 0.02% hexadecylpyridinium chloride and 0.02% Triton X-100.

A 100 μg ml^{-1} solution of chloramphenicol was merged prior to the photoreactor with each of the above-mentioned sensitizers. The results thus obtained can be summarized as follows:

a) The only sensitizer that provided negligible blank signals in the absence of analyte with the lamp ON was 20% acetonitrile. Also, the only media that resulted in a near-zero blank signal with the lamp OFF were 0.5% acetone, 0.5% acetone+20% acetonitrile, 20% acetonitrile, 5% dimethylformamide, 0.02% Triton X-100, 0.02% hexadecylpyridinium chloride and 0.02% SDS.
b) Both 5% ethanol, 0.5% formic acid, Acridine Yellow and Rhodamine B gave stronger signals—and hence decreased analytical signals—in the absence of chloramphenicol.
c) With no photosensitizer did the chemiluminescence intensity obtained exceed that for chloramphenicol in the absence of one.

Based on the foregoing, the use of a photosensitizer in the photodegradation of chloramphenicol was discarded.

The effect of the time of exposure to UV light was examined by changing the flow-rate of the irradiated solution stream from 0.2 to 1.4 ml min^{-1}. The analytical signal was found to increase with increasing irradiation time as a result of an increased photodegradation yield and hence of the increased concentration in the resulting chemiluminescent species. A flow-rate of 0.4 ml min^{-1} was selected, corresponding to an irradiation time of 410 s, as the best compromise between chemiluminescence intensity and throughput.

Bubbling air through both the sample carrier and oxidant solution was found to decrease the analytical signal by 37%. On the other hand, deaerating the solutions by ultrasonication increased the signal by 1.9%.

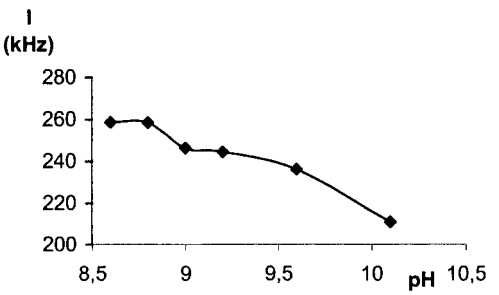

Fig. 4. Influence of glycine buffer on the photodegradation.

3.5. Re-optimization of chemical and physico–chemical variables

In order to examine the effect of temperature on the photodegradation of chloramphenicol, the reactor was heated only in the portion preceding the lamp (length 2 m) and the sample loop immersed in an ice bath to avoid the effect of temperature on the chemiluminescent reaction. Raising the temperature up to 80 °C was found to result in no difference from the signal obtained at room temperature.

Because of its special significance, the oxidant concentration (see preliminary tests) was re-optimized, together with the H_2SO_4 concentration in the oxidant solution and the temperature, after examining the photodegradation reaction. To this end, the concentration of $KMnO_4$ was changed between 10^{-4} and 8×10^{-4} mol l^{-1}, that of H_2SO_4 from 2 to 3 mol l^{-1} and the temperature over the range 20–80 °C (by thermostating the sample loop and the carrier and oxidant solutions). The optimum values of these variables were found to be virtually the same as those found in optimizing the chemiluminescent reaction (viz 2×10^{-4} mol l^{-1} $KMnO_4$, 2 mol l^{-1} H_2SO_4 and 70 °C).

4. Analytical applications

4.1. Analytical characteristics

The calibration graph was applied over the range 0.03–14 μg ml^{-1} and fitted the equation $I=(0.297\pm0.027)c^2+(12.6\pm0.6)c-(5.9\pm1.8)$ with a correlation coefficient of 0.9993, where I is the chemiluminiscent emission in kHz and c the concentration of chloramphenicol in μg ml^{-1}. This range presented also a straight line in a log–log plot. The average equation for seven curves obtained was $\log I=(1.26\pm0.05)\log c-(0.92\pm0.05)$. The response was linear over the range 3–14 μg ml^{-1} and fitted the equation $I=(17.8\pm0.6)c-(25\pm4)$ with a coefficient correlation of $R=0.9993$. The limit of detection (30 ng ml^{-1}) was defined three times the back-ground average and was established by decreasing the concentration of injected chloramphenicol until this relationship was reached. The inter-day reproducibility of the proposed method was estimated by running calibrations with solutions of the reagents freshly prepared each day. The R.S.D. of the slopes of different calibration graphs over 7 days obtained in this way was 3.4% for chloramphenicol. The RSD for a series of 20 injections of a 7 μg ml^{-1} solution of chloramphenicol was 2.4%. The throughput, calculated using the same series, was 60 samples h^{-1}.

With the NaOH system, a quadratic equation was obtained between 0.4 and 35.2 mg l^{-1} ($I=0.1135c^2+4.6802c-4.2333$, correlation coefficient of 0.9995); the response was linear over the range 10–35 μg ml^{-1} chloramphenicol and the regression equation was $I=10.091x-61.316$ ($R^2=0.9988$). The limit of detection ($S/N=3$) 0.04 μg ml^{-1}.

4.2. Study of interferents

The analytical features of the proposed method and its tolerance to potential interferences accompanying chloramphenicol in pharmaceutical preparations were studied for a concentration of chloramphenicol of 7 μg ml^{-1} (glycine buffer system) (see Table 2). Good tolerance was exhibited for sugars and electrolytes. The most critical interference is presented by alcohols which can be easily oxidized by potassium permanganate. As reference, solutions containing only ethanol 5%, polyethilenglycol 0.05% and glycerol 0.05% provided 162, 23 and 15 kHz with the lamp OFF, respectively.

The obtained results for 20 mg l^{-1} of chloramphenicol (5×10^{-3} mol l^{-1} NaOH system) were as follows (concentration in mg l^{-1} and relative error in %): NaCl 120, −1.3%; KCl 100, −4.1%; $MgCl_2$ 30, −2.7%; H_3BO_3 4, +4.1%; $Na_2B_4O_7\cdot10H_2O$ 10, +3.0%; EDTA 1, +4.5%; glucose 5, +4.2%; lactose 2.5, +1.5%, lidocaine 1, +3.9%, benzocaine 2, +3.3 %, starch 10, +4.9%.

Of the two systems studied, the glycine buffer was found to be more tolerant than 5×10^{-3} mol l^{-1} NaOH to all the interferents tested. As a rule, the glycine buffer was much more selective and allowed the determination of chloramphenicol free

Table 2
Influence of potential interfering compounds on the determination of chloramphenicol

Interferent	Concentration	Error (%)
NaCl	200 mg l^{-1}	−0.9
KCl	200 mg l^{-1}	−1.8
$MgCl_2$	200 mg l^{-1}	+0.7
H_3BO_3	200 mg l^{-1}	−0.5
$Na_2B_4O_7 \cdot 10H_2O$	200 mg l^{-1}	+1.0
EDTA	1 mg l^{-1}	+3.8
Glucose	90 mg l^{-1}	+4.6
Saccharose	70 mg l^{-1}	+1.7
Lactose	100 mg l^{-1}	−4.1
Lidocaine	40 mg l^{-1}	−0.1
Benzocaine	40 mg l^{-1}	−3.1
Hydrocortisone	5 mg l^{-1}	+4.5
Ethanol	0.4%	+4.8
Polyethilenglycol	0.0005%	+4.6
Glycerol	0.001%	+3.9
Erythromicin	9 mg l^{-1}	+4.1
Polysorbate 20	0.18%	+4.8
Prednisolone	10 mg l^{-1}	−1.6
Starch	150 mg l^{-1}	−2.6

of major errors in the presence of interferent concentrations well above those typically encountered in pharmaceutical formulations. On the other hand, the NaOH medium was found to be much less tolerant to sugars and ordinary excipients such as ethanol, starch and boric acid, which reveals a marked difference between the photodegradation mechanisms for chloramphenicol in the two media.

4.3. Applications to pharmaceutical samples

The proposed method employing glycine buffer as photodegradation medium was applied to the determination of chloramphenicol in collyria for conjunctivitis (Colircusí Medrivás Antibiótico and Colircusí Cloramfenicol), a pomade for external use against dermatitis (Cortisón Chemicetina) and an antibiotic given to canary birds (Anco nil Antidiarreico). The results are compared with those of the reference method in Table 3. Good agreements were found against the reference method [28] and the label claims even for the samples containing compounds interfering strongly, as hydrocortisone. The results obtained for Oftalmocan Biotico were assesed with those provided by a second reference method consisting of the photodegradation of the drug and the biamperometric determination of the photofragments using I_3^-/I_2 as indicating redox system [27]. Coincident results with those obtained with the proposed method were observed.

5. Conclusions

A continuous-flow method for the determination of choramphenicol in pharmaceutical preparations based on photodegradation and direct chemiluminescence detection of its photolytic fragments is proposed.

The photolysis provided by low pressure mercury lamps of germicidal use permits to increase the number of compounds to be determined by direct chemiluminescence (even compounds without chemiluminiscent behaviour) thanks to the chemiluminescence properties of the resulting photofragments.

Many properties of an ideal system are exhibited in on-line photochemical reactions, namely, no excess reach the detector (‘cleaning’ of photoreac-

Table 3
Determination of chloramphenicol in pharmaceutical formulations

Commercial preparation	Label claim	FIA method	Reference method	Error[a] (%)
Colircusí Cloramfenicol	5 mg/ml	(5.61 ± 0.10) mg/ml	(5.58 ± 0.04) mg/ml	+0.5
Colircusí Medrivás Antibiótico	7.3 mg/ml	(7.36 ± 0.09) mg/ml	(7.55 ± 0.07) mg/ml	−2.5
Cortisón Chemicetina	10 mg/g	(8.65 ± 0.23) mg/g	(8.9 ± 0.4) mg/g	−2.8
Anconil	100 mg/ml	(100.0 ± 1.7) mg/ml	(98.1 ± 1.1) mg/ml	+1.9
Oftalmocan	3 mg/ml	(2.73 ± 0.03) mg/ml	(3.58 ± 0.03) mg/ml	–

[a] Error against the reference method.

tors), and selectivity is highly improved thanks to the pathway involving the photodegradation step which is strongly determined by the power, irradiation time of the source and chemical media.

The proposed method increases the selectivity of the officially recommended methods in pharmacopoeias for determining chloramphenicol [28,41–43] which are scarcely selective as they rely on direct measurements of the absorbance at 276 nm of an aqueous solution of the drug.

Acknowledgements

The authors are grateful to the University Cardenal Herrera-CEU for financial support.

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