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# Flow injection analysis of oxymetazoline hydrochloride with inhibited chemiluminescent detection

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**Abstract**

A rapid and sensitive chemiluminescence method for the quantitation of oxymetazoline hydrochloride in pharmaceutical formulations has been developed. Oxymetazoline hydrochloride inhibits strongly the chemiluminescence from the oxidation of luminol in alkaline medium. Based on this effect, a simple method is proposed for the determination of this imidazoline derivative by flow injection analysis employing chemiluminescence detection. The optimization of the experimental and instrumental variables such as, selection of the oxidant, concentration of reagents, flow rates, injection volume, etc., has been carried out using the proposed flow injection manifold. The performance characteristics have been established, showing a wide linear response in the range of 1.88–200 ng/ml and a detection limit of 1.21 ng/ml. This is the first chemiluminescent method for the determination of oxymetazoline which has been satisfactorily applied to the analysis of the drug in intranasal pharmaceutical formulations.


*Keywords:* Chemiluminescent inhibition; Luminol; Oxymetazoline hydrochloride; FIA

## 1. Introduction

Oxymetazoline hydrochloride (3-[(4,5-dihydro-1*H*-imidazol-2-yl)methyl]-6-(1,1-dimethylethyl)-2,4-dimethylphenol hydrochloride) is an imidazoline derivative (Fig. 1) that is usually found as a decongestant in various pharmaceutical preparations used in the treatment of eye-irritation and nasal-congestion derived of cold, rhinosinusitis and/or allergic symptoms.

To date, a few number of analytical methods have been reported for the determination of oxymetazoline, i.e., in pharmaceutical preparations. HPLC has been used with UV detection at 280 nm [1] and, in biological fluids, only two methods have been developed, one of this based on the determination of radiolabeled oxymetazoline, using fraction collection–liquid scintillation counting detection [2] and an HPLC-electrospray mass spectrometric assay for the determination of the oxymetazoline pharmacokinetics in blood rats [3]. The study of its stability in aqueous solution by HPLC-UV showed a minimal rate of hydrolysis occurring in the pH range from 2.0 to 5.0 [4].

In this paper, we have investigated the possibility to apply chemiluminescence (CL) as detection method in the analysis of oxymetazoline, considering the inherent high sensitivity and high versatility of this technique, which is being increasingly applied to the analysis of many biomedically important analytes [5].

The production of strong chemiluminescent emission by the oxidation of luminol in alkaline medium is one of the best known and more efficient CL reactions. Different oxidants can be used, such as, hydrogen peroxide, molecular oxygen, hypochlorite, iodide or permanganate, mainly in the presence of some type of initiator or catalyst such as peroxidase, ferricyanide, heme compounds or metal ions (Co(II), Cu(II), Cr(III), Ni(II), etc.). In this respect, this CL system has been applied for the direct determination of luminol derivatives, catalysts, oxidants or inhibitors of the cited CL reaction [6]. This very well-known CL reaction has been used to develop

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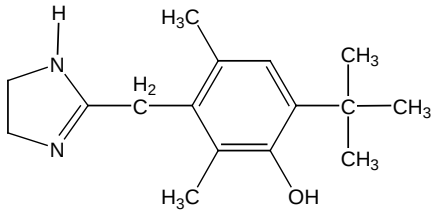


Fig. 1. Chemical structure of oxymetazoline.

indirect analytical determinations based on the interaction of the analytes with different oxidants, producing a proportional decrease of the CL intensity. Hypochlorite for thiol-containing drugs [7], hexacyanoferrate(III) for ascorbic acid [8], paracetamol [9] and chlorogenic acid [10], hydrogen peroxide in the presence of Cu(II) for tannic acid [11] or in the presence of manganese-tetrasulfonatophthalocyanine for proteins [12] or potassium permanganate for paracetamol [13] have been cited in the literature.

In this study, we have found that oxymetazoline hydrochloride strongly inhibits the luminol–permanganate CL reaction under certain conditions, causing an analytically proportional decrease of the CL emission in alkaline medium. Due to the transient characteristics of the CL emission, the method can be satisfactorily incorporated in flow injection manifolds, providing a rapid and sensitive determination method. Based on these observations, a new analytical procedure is being proposed for the determination of oxymetazoline hydrochloride by flow injection analysis (FIA). This relatively simple and easy method provides a low detection limit and a wide dynamic range and could be satisfactorily applied to the determination of this compound in pharmaceutical formulations. To our knowledge, this is the first method for the analysis of oxymetazoline based on CL detection.

## 2. Experimental

### 2.1. Chemical

All the reagents used were of analytical grade and the water used to prepare the solutions was purified with a Milli-Q Plus water system (Millipore).

A stock solution of oxymetazoline hydrochloride (Sigma) (100 μg/ml) was prepared by dissolving 100 mg in deionized water and then diluting to 1000 ml with deionized water. Working solutions were prepared by dilution.

Sodium hydrogen carbonate and sodium hydroxide were purchased from Merck to prepare a pH 11.5 carbonate buffer solution ($NaHCO_3/Na_2CO_3$, buffer concentration 0.05 M) A stock solution of luminol (3-aminophthalhydrazide), purchased from Sigma ($1 \times 10^{-3}$ M) was prepared by dissolving 0.0885 g in 50 ml of a pH 11.5 carbonate buffer solution. Working solutions were prepared daily by appropriate dilutions in the same buffer.

Potassium permanganate (98%) was obtained from Panreac. A stock solution ($1 \times 10^{-3}$ M) was prepared by dissolving the product in deionized water and the corresponding working solutions were prepared daily by appropriate dilutions.

### 2.2. Apparatus

CL measurements were carried out on a Jasco CL 1525 detector (Jasco, Japan), equipped with a PTFE spiral detection cell with a volume of 200 μL, data control and acquisition program. A peristaltic pump (Gilson Minipulse-3, France), a Rheodyne 5020 manual injection valve, and Omnifit tubing and connexions were used for constructing the FIA manifold.

### 2.3. Procedure

Prior to sample analysis, buffered solutions of alkaline luminol were mixed with the permanganate solution and with a buffer stream, the CL resulting from the oxidation of luminol was measured and recorded as the background signal. The determination was based on the net CL intensity $\Delta I = I_0 - I$, where $I$ is the decreased CL signal due to the presence of oxymetazoline and $I_0$ the background signal. Using the acquisition software, it was possible to remove this background signal from the "auto zero" option, allowing the direct acquisition of the negative CL intensity corresponding to $I$. For sample analysis, standard solutions of oxymetazoline hydrochloride were injected in the buffer stream and allowed to mix with the luminol–$KMnO_4$ mixture before passing through the CL detection region of the cell. As oxymetazoline hydrochloride is reacting with $KMnO_4$, the $KMnO_4$ concentration in the flowing stream will be decreased. A negative CL peak results from the injection of standard or sample solutions containing the oxymetazoline hydrochloride analyte.

For the analysis of the selected pharmaceutical preparations, appropriately diluted solutions were directly prepared in deionized water without any previous treatment and directly injected into the carrier stream in the FIA manifold.

## 3. Results and discussion

### 3.1. Selection of the continuous-flow assembly

Several reactions were checked to study the behaviour of oxymetazoline hydrochloride in relation to the production of chemiluminescence emission: direct oxidations with permanganate, hydrogen peroxide, or Ce(IV) in acid medium, direct oxidations with hexacyanoferrate(III) in alkaline medium and its effect in the luminol reaction using as oxidant permanganate, hydrogen peroxide or hexacyanoferrate(III) in alkaline medium. From this screening, it was found that the presence of oxymetazoline produced

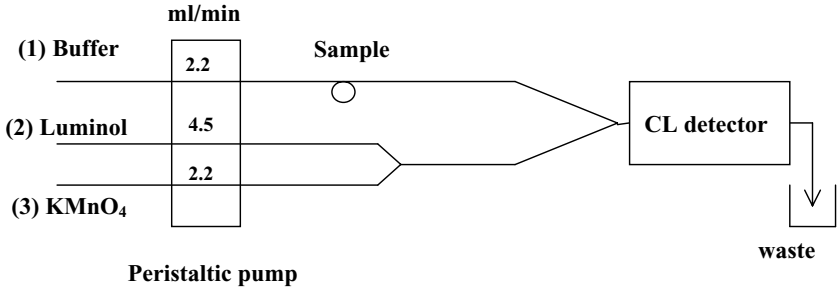


Fig. 2. FIA manifold for the determination of oxymetazoline using the luminol–$KMnO_4$ reaction. Standard and sample solutions of oxymetazoline hydrochloride were injected in the buffer stream (1). Luminol (2) and $KMnO_4$ (3) solutions are mixed before passed through the CL detection region. Both streams are mixed by means of a "T" connector just before the detection cell, in which the reaction takes place. The flow rate for each stream is indicated on the diagram.

a substantial inhibition of the CL intensity from the oxidation of luminol using permanganate as an oxidant, in basic medium. Permanganate is an oxidant applicable in a luminal-based CL system [14], recently its analytical usefulness was demonstrated in the analysis of paracetamol by inhibited CL [13]. In this study, we observed a negative CL peak from the injection of oxymetazoline standard solutions as this molecule is being oxidized by permanganate in this medium, producing a decrease of the background CL signal due to the permanganate concentration decrease. A FIA method for the determination of oxymetazoline hydrochloride based on its inhibition effect upon the CL intensity of the luminol–permanganate system could hence be developed.

For this purpose, different FIA configurations were examined, leading to a three-channel FIA manifold from which optimal signal/noise ratios and good reproducibility were obtained. A schematic diagram of the selected flow system is shown in Fig. 2, in which solutions containing oxymetazoline hydrochloride are injected in the buffer stream. Luminol and $KMnO_4$ solutions are mixed before passing through the CL detection region; the length of the employed tube is not a critical variable. Both streams are mixed by means of a "T" connector just before the detection cell, in which the reaction takes place, considering the CL emission is instantaneously produced once the reagents are mixed.

Employing this configuration, the effect of both experimental (pH and concentrations of permanganate and luminol solutions) and instrumental variables (injected volume of analyte and flow rates) were studied in order to achieve the highest decrease of the CL signal, proportional to the analyte concentration.

### 3.2. Optimization of chemical variables

The pH effect on the analysis of oxymetazoline hydrochloride was studied by varying the pH of the buffer solution in a system containing a luminol concentration of 100 μM in the buffer solution, a $KMnO_4$ concentration of 2.26 μM and 200 ng/ml of oxymetazoline hydrochloride. The buffer solution was prepared using $NaHCO_3/Na_2CO_3$ in a pH range of 8.5–11.5. The largest inhibition effect on CL intensity was found at pH 11.5, hence a buffer solution of pH 11.5 ($NaHCO_3/Na_2CO_3$) was selected as the carrier as well as the medium for the preparation of luminol solutions.

The change of CL intensity with the variation of permanganate concentration was studied in the range of 0.1–6 μM with a similar system using the selected carrier, a 100 μM luminol solution and 200 ng/ml of oxymetazoline hydrochloride. Fig. 3 shows the changes of CL intensity with the variation of oxidant concentration. The CL intensity progressively decreased with increasing $KMnO_4$ concentration, producing a maximum effect for permanganate concentrations between 3.0 and 4.5 μM. In this sense, a 3.5 μM concentration was subsequently chosen for the $KMnO_4$ stream.

To study the influence of luminol concentration, the system utilized the above optimized values for permanganate and buffer solutions, employing a 200 ng/ml concentration of the injected oxymetazoline solution, varying the luminol concentration in the range of 25–150 μM. The inhibitory effect upon the CL signal increased with increasing luminol concentrations. Considering the high emission produced by the CL system before the injection of oxymetazoline and due to the inherent characteristics of an inhibition method, it was not possible to study higher luminol concentrations so as to avoid saturation of the photomultiplier tube (PMT)

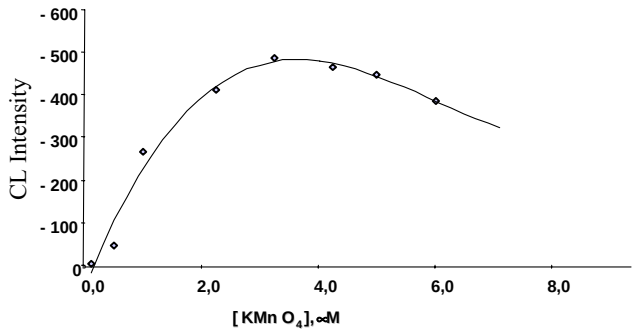


Fig. 3. Change of the CL intensity in function of $KMnO_4$ concentration in the proposed FIA system. A 100 μM luminol solution was used and 200 ng/ml oxymetazoline hydrochloride solution was injected into the carrier (buffer solution pH 11.5 ($NaHCO_3/Na_2CO_3$)).

of the CL detector at the selected gain conditions for the determination of low analyte concentrations. So as to obtain a stable response from the PMT, a 100 μM luminol concentration solution was chosen for further experimental work.

### 3.3. Optimization of flow variables

The flow rates for the different streams considered in the selected manifold and the injected volume for oxymetazoline solutions (loop volume) were studied so as to obtain the best decreasing CL signal/background ratios for the analysis of oxymetazoline. The above optimized values for luminol, potassium permanganate and buffer solutions were used.

The flow rate for the three channels was changed in the range of 0.5–5.0 ml/min. The negative CL intensities became larger when increasing the flow rates of luminol stream, up to 4.5 ml/min. However, higher effects and better stability and repeatability were obtained for flow rates of 2.2 ml/min for both buffer and potassium permanganate streams.

The effect of the injection volume of the oxymetazoline hydrochloride solution was examined over the 20–250 μl range. An injection volume of 20 μl was selected as optimum because of it produced good repeatability, the best analyte/background signal ratios and a fast acquisition of the CL signal.

Under these optimized conditions, the proposed FIA assembly consisted of three different streams, containing buffer solution (pH 11.5), i.e., the carrier channel, a second one for the permanganate solution (3.5 μM), both at flow rates of 2.2 ml/min, and the last one with luminol solution (100 μM), prepared in buffer solution, at a flow rate of 4.5 ml/min. Standard and sample solutions were directly injected into the buffer stream using an injection volume of 20 μl.

### 3.4. Calibration curve and performance characteristics

A calibration curve for the determination of oxymetazoline hydrochloride was established from the inhibitory effect upon injection of increasing analyte concentrations in the range from 0 to 200 ng/ml, using the proposed FIA manifold and the optimized chemical and instrumental variables. Three injections were performed for each standard of oxymetazoline hydrochloride solution, including a blank solution. In the concentration range studied, the inhibition effect increased proportionally with the increment of oxymetazoline hydrochloride concentration, providing a good linear relationship between the logarithm of oxymetazoline concentration ($C$) and the decrease of CL intensity ($I_{CL}$), the regression equation being $I_{CL}$ (mV) = $-383.60 + 537.14 \log C$ (ng/ml). The performance characteristics calculated from the calibration data set using the ALAMIN software [15], learned a linear dynamic linear range from 1.88 to 200 ng/ml (lack-of-fit $P$-value = 32.4%), an analytical resolution of 1.079 ng/ml, a linearity (expressed as relative standard deviation of slope) of 99.01%, a relative standard deviation of 1.60% (for 50 ng/ml oxymetazoline) and IUPAC detection and determination limits [16] of 1.21 and 1.86 ng/ml, respectively.

The linear dynamic range established for the proposed method can be correlated with the reaction characteristics of oxymetazoline in the presence of permanganate. The linear dependence occurs when the concentration of oxymetazoline is much smaller than that of permanganate, indicating a first-order reaction. As the inhibition of CL intensity is closely related to the amount of $KMnO_4$ reacting with oxymetazoline hydrochloride in the flowing stream, the application of a higher concentration of permanganate would facilitate the determination of the analyte in a higher concentration range. By keeping the concentration ratio of oxymetazoline to permanganate in an optimal range, the detection limit and dynamic range for oxymetazoline could be varied by changing the concentration of permanganate used in the system.

### 3.5. Real sample analysis and interferences

The developed procedure was applied to the analysis of oxymetazoline hydrochloride in two available pharmaceutical preparations so as to prove the usefulness of the proposed method to real sample analysis. These pharmaceutical formulations are:

1. Oximetazolina Edigen (drops, from Edigen S.A. Laboratories): oxymetazoline hydrochloride (0.5 mg/ml), excipients (ethanol and benzalkonium chloride).
2. Respir (drops, from Schering-Plough S.A.): oxymetazoline hydrochloride (0.5 mg/ml), excipients (propylene glycol).

The influence of foreign compounds and excipients found in these preparations was also studied. The effects of increasing concentrations of different substances upon the determination of 50 ng/ml of oxymetazoline was evaluated, setting the tolerance limit at an error of $\pm ts_R$ over the predicted value [17] for the peak height (decrease in the CL intensity) ($t$ = one-tail Student $t$-value for $n - 2$ degrees of freedom and a value of 0.05; $s_R$, standard deviation on the analytical response, predicted for the tested analyte concentration, obtained from the calibration data set), which correspond to a tolerance limit of 4%. The first level of interference proved to be a ratio of 200 (w/w) with respect to the oxymetazoline hydrochloride concentration. In all cases, no interference was observed because the signal produced was included in the corresponding confidence interval.

For the analysis of the pharmaceutical formulations only appropriate dilutions were carried out. The results (average of 10 determinations) were compared with the values as claimed on the formulation label. The results are shown in Table 1. As can be seen, no significant differences were found between the compared values.

Table 1
Analysis of two pharmaceutical formulations by the proposed method

| Pharmaceutical formulation | Declared concentration (mg/ml) | Found concentration[a] (mg/ml) | *P*-value[b] (%) |
|---|---|---|---|
| Oximetazolina Edigen | 0.50 | 0.490 ± 0.025 | 21.4 |
| Respir | 0.50 | 0.472 ± 0.042 | 11.4 |

[a] Mean of $n = 10$ determinations (for $\alpha = 5\%$; $n - 1 = 9$ degrees of freedom, $t = 2.26$).
[b] From the student *t*-test.

## 4. Conclusions

The inhibitory effect of oxymetazoline upon the permanganate–luminol CL reaction presently constitutes the basis of a simple and sensitive method, which features important advantages in the analysis of pharmaceuticals based on its easy coupling to a flow injection manifold for continuous analysis, low cost of instrumentation and reagents, satisfactory precision and high sensitivity. To our knowledge, this is a scarcely exploited procedure in pharmaceutical quality control. In addition, the proposed procedure exhibits an adequate selectivity avoiding pre-treatment of the matrices and previous chromatography-based separation steps. Also, the easy coupling of this CL system based to the luminol reaction to HPLC will allow the analysis of the compound in more complex matrices such as biological fluids. Further work is being developed in this sense.

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