Sensitive detection of salbutamol using europium-enhanced fluorescence with trioctylphosphine oxide (TOPO) as coligand

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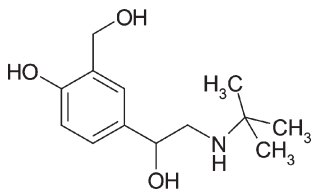
In this work a simple and sensitive fluorimetric method for determination of salbutamol (4-[2-(*tert*-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol) using an Eu enhanced signal was developed. The employed methodology is based on the formation of a ternary complex formed with Eu, salbutamol and trioctylphosphine oxide (TOPO). Intermolecular transfer of energy from the excited organic molecule to the lanthanide followed by lanthanide emission is responsible for excitation of the lanthanide ion in complex solutions and fluorescent enhancement. The luminescence properties of the ternary complex formed with TOPO and optimum formation conditions were investigated. The calibration curve is linear in the range between 6.92–180 μg l^{-1} of salbutamol. The detection limit was 2.31 μg l^{-1}. Common excipients for these formulations were not found to interfere. A proposed method for the assay in commercial aerosols and nebulizer solutions containing salbutamol was applied with very good precision.

1. Introduction

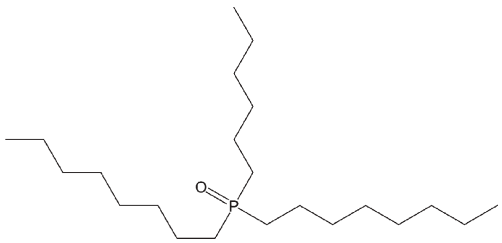
Salbutamol (Scheme 1) is a selective beta 2-adrenergic agonist and represents an effective drug for the treatment of asthma and the symptomatic alleviation of bronchospasm. Aerosols for clinical use are the most common pharmaceutical formulation, containing 100 μg $dose^{-1}$ of this drug. It is interesting and appropriate to note that a sensitive analytical method is required for the quality control of this pharmaceutical form.

The salts and complex compounds of europium ions in solution have fluorescence properties due to, principally, the transition within the 4f-shell.[1] Intermolecular transfer of energy from the excited organic molecule to the lanthanide followed by the lanthanide emission is responsible for excitation of the lanthanide ion in complex solution and fluorescent enhancement.[2,3] If there is efficient intermolecular energy transfer, the upper emitting levels of the lanthanides are more effectively excited by this technique than by the direct form, producing an enhanced fluorescence of the lanthanide.[3,4] However, in aqueous solutions the quenching of the emitting lanthanide by water molecules produces a lower quantum yield of the lanthanides. In order to reduce this radiationless energy transfer, organic molecules like trioctylphosphine oxide (TOPO) or surfactant agents (Scheme 2), have been used to provide an insulating sheath around emitting species, improving the fluorescence intensity for the determination of the lanthanide.[5,6]

The literature revealed that many methods have been proposed for the determination of salbutamol alone or as a mixture with other drugs in pharmaceutical formulations and physiological liquids; spectrophotometry,[7–15] amperometry,[16] voltametry,[17–19] HPLC,[20–31] capillary electrophoresis,[32–35] gas chromatography,[36–38] and many others. Some of these lack the proper detectability or require a large volume of sample. US and British pharmacopoeia describe volumetric acid–base assays that permit the determination of salbutamol in pharmaceutical preparations.[39–41] Luminescence properties of the ternary complex have been studied and the sensitivity and detection limit determined. The new proposed method has then been applied to the determination of salbutamol in commercial pharmaceutical formulations.



Scheme 1



Scheme 2

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The purpose of this work was to develop a sensitive fluorimetric method for the determination of salbutamol based on improving europium-sensitized fluorescence using TOPO as the coligand. Sensitive fluorimetric determination of salbutamol constitutes a relevant subject for investigation because it represents a rapid method which involves fluorimetry, providing a higher sensitivity compared to other methodologies, as well as optimum detection limits and better selectivity. The above mentioned technique is also easy to carry out and inexpensive. In this way, it may be very useful in order to apply and to develop high performance analytical procedures.

2. Experimental

2.1. Apparatus

All fluorescence measurements were made on a Shimadzu RF-5301 PC spectrofluorophotometer with an excitation and emission band pass of 5 nm using 1.0 cm quartz cells. The pH of the solutions was measured using an Orion 701-A pH meter with a Ag/AgCl electrode. A Beckman DU 520 UV-Visible spectrometer with quartz cells of 10 mm path length was used for absorption measurements.

2.2. Reagents

A 3.47×10^{-4} mol l^{-1} standard solution of salbutamol was prepared by dissolving 10 mg of the reagent in water and diluting up to 100 ml. A 1×10^{-3} mol l^{-1} TOPO solution was prepared by dissolving 38.66 mg of the reagent in 100 ml of absolute ethanol. Europium solutions, 3.28×10^{-3} mol l^{-1}, were prepared by dissolving 1.155 g of Eu_2O_3 in HNO_3(c) and diluted to 100 ml with water. The oxide was purchased from Aldrich Co, 99.9% purity (Milwaukee, WI, USA). All solvents used were HPLC grade and all other reagents employed were of analytical grade and were used without further purifications. The water used in all studies was ultrapure water (18 MW cm) obtained from a Barnstead Easy Pure RF compact ultrapure water system.

2.3. Sample treatment

In order to determine the salbutamol concentration in the aerosol, a dose of 100 μg (one aerosol discharge) was collected in a closed vessel and dissolved in 100 ml of ultrapure water. With respect to the nebulizer solutions, 240 μl of the solution was diluted to 10 ml with water. The solution contains 375 mg base salbutamol per 100 ml.

3. Results and discussion

The lanthanides are unique fluorescent metals which display emission in aqueous solutions and decay times of 0.5–3 ms. The emission results from transitions involving 4f orbitals, which are forbidden transitions. Therefore, the absorption coefficient is low and emission ratios are slow. As a consequence of this, they are not usually directly excited. Rather, they can be excited through chelated organic ligands.[42] The fluorescence intensity of the lanthanide ion is markedly increased upon complex formation as well as upon the introduction of surfactants.

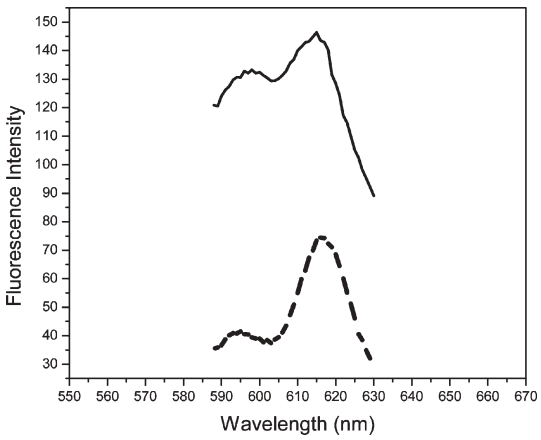


Fig. 1 Emission spectra of Eu at 615 nm in salbutamol–Eu (– – –) and salbutamol–Eu–TOPO (—) systems at pH 10.

Fig. 1 shows the emission spectra of Eu in salbutamol–Eu and salbutamol–Eu–TOPO systems at pH 10. The salbutamol maximum excitation and emission are at 279 and 308 nm, respectively. As can be seen the Eu emission is weaker in the salbutamol–Eu system than in the salbutamol–Eu–TOPO system. Obviously, the addition of TOPO caused a fluorescence intensity enhancement, improving the fluorescence signal. TOPO is a synergic agent, of high molecular weight which presumably replaces water molecules coordinated to the Eu ion, reducing solute–solvent interactions and collisional deactivation.[43]

3.1. Effect of pH on salbutamol complexation

The effect of pH on complexation and fluorescence intensity was studied in the pH range of 5 and 12. Solutions containing 1×10^{-5} mol l^{-1} Eu and 3.47×10^{-6} mol l^{-1} salbutamol were adjusted to different pH values with HCl or NaOH. The solution was excited at 279 nm and the Eu fluorescence emission was measured at 615 nm. The effect of pH on the complexation can be observed in Fig. 2. The maximum fluorescence intensity was obtained in the pH range between 9.5 and 10. At lower pH values the complex is not formed.

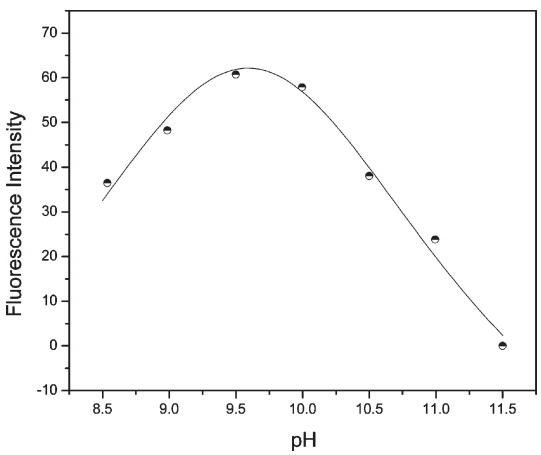


Fig. 2 pH influence of ternary complex formation measured at 615 nm.

At higher values of pH the Eu begins to form its hydroxide (solubility product = 3.4×10^{-22}),[44] so the fluorescence of the complex decreases. In further experiments the pH was adjusted to 10 with NaOH (1 M).

3.2. Determination of europium–salbutamol mol ratio

Stock standard solutions of salbutamol–Eu (continuous variation method) were prepared and a sufficient NaOH volume (1 M) added to the solution to adjust the pH to 10. The continuous variation method implies a mixture of metal and ligand solutions with identical formal concentrations and thus the total volume of each mixture must be the same. The absorbance of the appropriate wavelength is determined and corrected in accordance to the absorbance of white sample and, finally, the respective corrected absorbance *versus* molar fraction of one of the reagents is made.[45]

The absorbance of the complex was measured at 293 nm. The formation and stoichiometry of the complex was investigated using the above mentioned method. The absorption spectra of reagent show that salbutamol absorbs in the 285 to 300 nm wavelength range with maximum absorbance at 293 nm. Eu does not absorb at these wavelengths, therefore absorption spectra were recorded against a reference cell containing water and buffer. The plot of complex absorbance at pH 10 gave two straight lines intersecting at the stoichiometry ratio Eu : salbutamol 1 : 2 (Fig. 3)

3.3. Effect of TOPO on fluorescence intensity

In order to establish the concentration range of TOPO for total salbutamol complexation inside the linear calibration range, it was necessary to determine the stoichiometry of the ternary complex (minimal concentration) and the effect on fluorescence for maximal concentration. The same method as mentioned above was used for determination of the ternary complex stoichiometry, but in this case the Eu–salbutamol ratio was maintained at 1 : 2. For this purpose, different volumes of TOPO, 1×10^{-3} mol l^{-1}, were added to solutions containing 3.47×10^{-6} mol l^{-1} salbutamol and 1.34×10^{-4} mol l^{-1} Eu and adjusted to pH 10 with NaOH (1 M). The fluorescence intensity was measured at 615 nm. The complex was formed, fixing the salbutamol–Eu ratio, with different

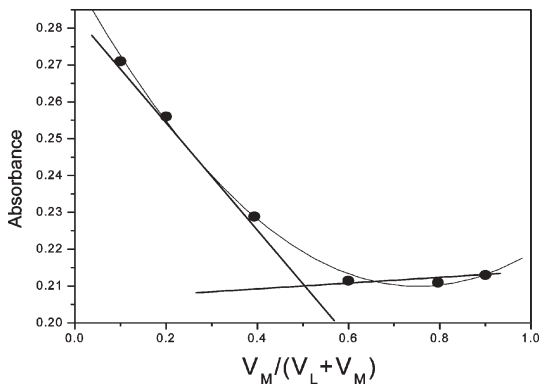


Fig. 3 Continuous variation method applied to the determination of europium–salbutamol mol ratio, measuring the absorbance at 298 nm. (V_M = volume of Eu solution, V_L= volume of salbutamol solution).

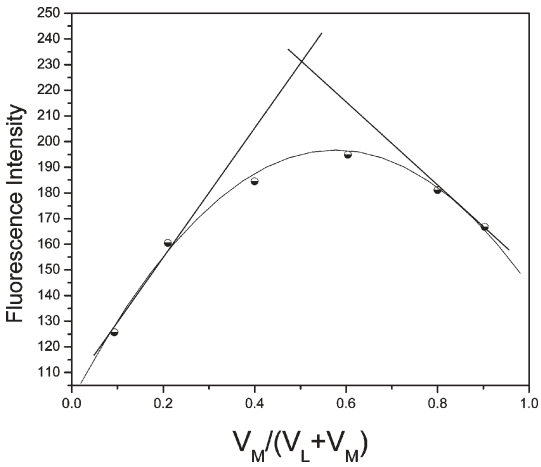


Fig. 4 Continuous variation method applied to the determination of europium–salbutamol–TOPO mol ratio, measuring the absorbance at λ_{exc} = 279 nm and λ_{em} = 615 nm.

TOPO concentrations. The stoichiometry of the complex was determined to be Eu(salbutamol)$_2$(TOPO)$_2$ using the continuous variation method (Fig. 4).

The enhancement of fluorescence intensity as a function of TOPO concentration is shown in Fig. 5. The Eu fluorescence intensity is enhanced up to 1.4×10^{-5} mol l^{-1} TOPO. At this concentration the large increase in fluorescence intensity is due to the insulating sheath around the emitting species (europium) and completion of the coordination sphere. At higher TOPO concentrations, the intensity decreases due to an inner filter effect.

3.4. Optimization of the reaction conditions

The first aim was to find out which design variable (salbutamol, TOPO and Eu concentration, pH, *etc*) had an influence on the response (fluorescence intensity) and establish the optimum conditions to understand the interaction between them. Experimental variables such as salbutamol and Eu concentrations were optimized using experimental design (chemometric techniques). The experimental design scheme

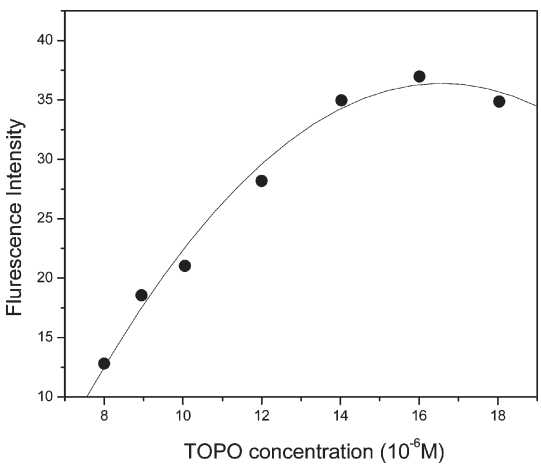
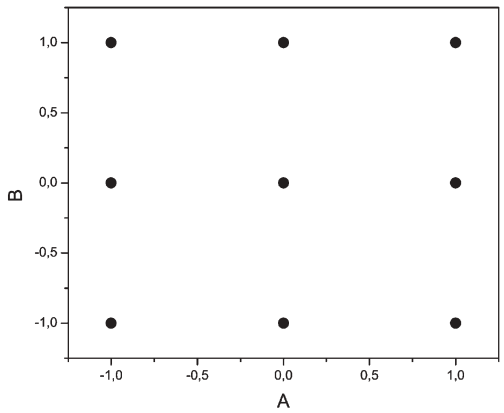


Fig. 5 Fluorescence intensity as a function of TOPO concentration (λ_{exc} = 298 nm and λ_{em} = 615 nm).



Scheme 3

Table 1 Matrix design of two factors and three levels (3^2)

Experimental variables		
Number of experiments	A[a](salb)	B[b](Eu)
1	−1	−1
2	0	−1
3	1	−1
4	−1	0
5	0	0
6	1	0
7	−1	1
8	0	1
9	1	1

[a] Salbutamol concentration: 1×10^{-7} M (−1), 1×10^{-8} M (0), 1×10^{-9} M (1). [b] Eu concentration: 8.25×10^{-5} M (−1), 1.23×10^{-4} M (0), 1.65×10^{-4} M (1).

used is shown in Scheme 3 where a wide concentration range of variables (salbutamol and Eu) were studied (Table 1).

A large dependence of fluorescence intensity on Eu and salbutamol concentration was determined using the experimental design method, as was expected. This relationship is shown in Fig. 6. It can be observed that the curve has a maximal response value which correspond to 4×10^{-9} mol l^{-1} salbutamol and 1×10^{-5} mol l^{-1} Eu. The values obtained after optimization through experimental design were employed as fixed variables.

The salbutamol and Eu concentrations giving an optimum fluorescence signal were employed to carry out the optimization of TOPO. For this purpose, these concentrations remained constant and the surfactant (TOPO) concentration was gradually varied. Then, the TOPO concentration was increased to obtain the best fluorescent signal. In this case, at concentrations higher than 1×10^{-5} mol l^{-1} the fluorescence intensity decreases due to an inner filter effect (Fig. 5).

4. Validation of the method

Validation of the method was checked by the standard addition method (see Table 2).

The proposed method shows good results for the quantification of salbutamol in aerosols and solutions for nebulizer.

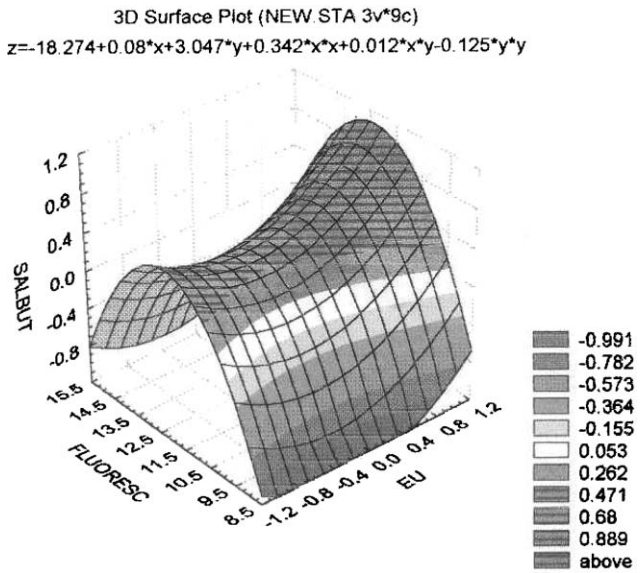


Fig. 6 Optimization of the variables affecting the fluorescence intensity using experimental design.

4.1. Linearity and range

Linearity and range of the method were performed by analysing 10 different concentrations ($n = 6$) of the standard solution containing 0.3–346 μg l^{-1}. The calibration curve was

Table 2 Validation of proposed method by the standard addition method

Commercial sample: aerosol inhaler ($n = 6$) containing 100 μg l^{-1} of salbutamol	Quantity of salbutamol added/μg l^{-1}	Quantity of salbutamol found/μg l^{-1} (average)	Precision/μg l^{-1}
1	0	108.57	X[a] = 108.57 ± 0.47 SD[b] = 1.16 VC[c] = 1.07%
2	25	128.3	X = 128.3 ± 1.06 SD = 2.61 VC = 2.03%
3	50	153.7	X = 153.7 ± 1.03 SD= 2.54 VC = 1.65%
4	75	176.2	X = 176.2 ± 0.77 SD = 1.88 VC = 1.07%
Solution for nebulizer			
1	0	97	X = 97 ± 0.69 SD = 1.7 VC = 1.75%
2	25	123.5	X = 123.5 ± 0.99 SD = 2.44 VC = 1.97%
3	50	147.8	X = 147.8 ± 1.05 SD = 2.58 VC = 1.74%
4	75	168.6	X = 168.6 ± 2.01 SD = 4.91 VC = 2.9%

[a] X = mean. [b] SD = standard deviation. [c] VC = variation coefficient.

Table 3 Parameters determined for calibration curves of salbutamol obtained from the method developed

Parameter	$n = 6$
Linear dynamic range/μg l^{-1}	6.92–180
Regression equation[a]	
Slope (b)	0.495
Error	0.24
Intercept (a)	10.37
Error	0.30
Standard deviation	1.45
LOQ/μg l^{-1}	7.6
LOD/μg l^{-1}	2.31

[a] $y = a + bx$, where x is the concentration in μg l^{-1}.

plotted using relative fluorescence intensity *versus* concentration of the standard solutions. The calibration curve was found to be linear over the concentration range 6.92–180 μg ml^{-1}. The data were analysed by the linear regression least-squares fit method (Table 3).

4.2. Repeatability and reproducibility

The mean of the relative fluorescence intensity of five separate sample solutions of the commercial formulations of salbutamol of the same batch number gave a relative standard deviation lower than 0.0175. This level of precision is suitable for the routine quality control analysis of pharmaceutical dosages.

4.3. Stability

The stability of ternary complexes in standard solutions, and in matrices used for salbutamol in aerosols and solutions for nebulizer was investigated. This indicated that ternary complex solutions (standard or synthetic matrices) are stable within 30 min and can be used without having any significant effects on the results.

4.4. Selectivity, detection and quantification limits

The selectivity of the method was investigated and no interferences were observed between salbutamol and common excipients for these formulations. The detection limit (DL) is the lowest concentration that can be distinguished from the noise level. In this study, the DL was 2.31 μg l^{-1}. The detection limit is lower than other known techniques for its determination.[39–41] The quantification limit (QL) is generally for samples with known concentrations of analyte and for establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision. In this study, the QL was 7.6 μg l^{-1}.

5. Analysis of commercial formulations

The method developed for salbutamol determination was applied to four commercial preparations. Table 4 gives the analysis results for commercial preparations. The standard addition method was employed as a comparison to evaluate the validity of the developed method.

The results were obtained using the two methods for six separate determinations starting from different groups of commercial formulations containing salbutamol. The results were compared and there was no significant difference between the methods. The results obtained from this study showed that the proposed methods can be recommended for determination of salbutamol in commercial formulations.

6. Conclusions

The fluorescence method proposed in this paper can be considered as a precise, economical, rapid, selective and sensitive procedure for the determination of salbutamol in pharmaceutical formulations. This method does not require previous separation techniques due to there being no interference between salbutamol and excipients. Another advantage is the enhancement of the fluorescent signal of the ternary complex obtained and thus increased sensitivity.

The development of this highly sensitive fluorimetric method for the determination of salbutamol based on the sensitization of europium is an important contribution to its quality control in different pharmaceutical forms. The low detection limits obtained highlight the application of this methodology in biological fluids.

Acknowledgements

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Table 4 Salbutamol found on commercial samples using the method proposed

Sample, $n = 6$	Salbutamol found on commercial sample			
	Ventolin® (Glaxo Wellcome): 100 μg salbutamol per dose aerosol inhaler	Asmatol® (Roux Ocefa): 100 μg salbutamol per dose aerosol inhaler	Salbutol® (Altana Pharma S.A.): 100 μg salbutamol per dose aerosol inhaler	Ventoplus® (Lab. Phoenix): 100 μg salbutamol solutions for nebulizer
1	108.9	101.3	104.3	96.3
2	110.3	103.2	101.7	98.2
3	109.1	100.9	103.5	99.3
4	108.2	99.8	104.2	97.7
5	106.8	100.7	102.5	95.8
6	108	102.2	104.7	94.7
X ± SD	108.55 ± 1.18	101.35 ± 1.19	103.5 ± 1.16	97 ± 1.7

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