

Spectrofluorimetric and cyclodextrin-enhanced spectrofluorimetric methods for the determination of naphazoline in nasal and eye drops

Jamshid L Manzoori* & Mohammad Amjadi

Department of Analytical Chemistry, Faculty of Chemistry, Tabriz University, Tabriz, Iran
Email: manzoori@tabrizu.ac.ir

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A spectrofluorimetric method has been developed for the determination of naphazoline based on its fluorescence activity. This method allows the determination of 0.1-3.0 $\mu\text{g ml}^{-1}$ naphazoline in aqueous solution with excitation and emission wavelengths at 281 and 330 nm respectively. The complexation between naphazoline and β -cyclodextrin gives a 1:1 complex. A cyclodextrin-enhanced spectrofluorimetric method has also been developed which has better sensitivity and limit of detection (15 ng ml^{-1}) as compared to that in the absence of cyclodextrin. Both methods have been applied satisfactorily to the determination of naphazoline in pharmaceutical preparations.

Naphazoline, 2-(1-naphthylmethyl) imidazoline (Structure I) is a sympathomimetic drug having direct α -agonistic activity. It has a marked vasoconstrictor action on α -receptors of vascular smooth muscle. It is used as an important ingredient of various pharmaceutical preparations especially nasal and ophthalmic solutions. Several reports on naphazoline determination have appeared in literature. Gas chromatography^{2,3} high performance liquid chromatography⁴, capillary electrophoresis⁵, atomic absorption and emission spectrometry⁶, spectrophotometry^{7,8} first and second-derivative spectrophotometry^{9,10} and room-temperature phosphorescence^{11,12} have been used for the determination of naphazoline in different pharmaceutical preparations, alone or with other active ingredients.

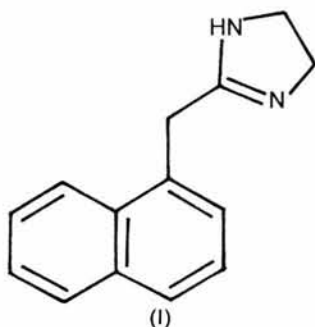
Since spectrofluorimetry has the advantages of both sensitivity and simplicity, it has found extensive use in the determination of inorganic, organic, and biological materials. Survey of literature revealed that

there is no spectrofluorimetric method for the determination of naphazoline. Cyclodextrins (CDs) are naturally occurring cyclic oligosaccharides that have a hydrophobic internal cavity and are able to form inclusion complexes with various guests of correct dimensions. The resulting modifications in absorption and fluorescence properties of guest molecules, have extensively been used for analytical applications^{13,14}, especially for improving performance of spectrofluorimetric methods^{15,20}.

In the present study, the fluorescence characteristics of naphazoline and naphazoline- β -CD complex were investigated. Based on the obtained results, sensitive spectrofluorimetric and cyclodextrin-enhanced spectrofluorimetric methods have been developed for the determination of this drug. The methods have been applied to the determination of naphazoline in nasal and eye drops.

Materials and Methods

Fluorescence spectra and intensity measurements were made on a Shimadzu RF-540 spectrofluorimeter equipped with a 150W xenon lamp, using 1.0 cm quartz cell, excitation and fluorescence emission wavelengths of 281 and 330 nm, respectively. Slit widths of both monochromator were set at 5 nm. All measurements were performed at $25 \pm 0.1^\circ\text{C}$ by use of a thermostated cell holder and a thermostatically controlled water bath. Absorption spectra were recorded using a Shimadzu UV-265FW spectrophotometer. A Metrohm (model 654) pH meter was used for pH measurements.



All reagents used were of AR grade. Triply distilled water was used throughout. Naphazoline hydrochloride was obtained from Sina Darou Laboratory (Tehran, Iran). A $200\text{ }\mu\text{g ml}^{-1}$ stock standard solution of naphazoline was prepared by dissolving the appropriate amount of naphazoline HCl in water. The α - and β -cyclodextrins (α - and β -CD) were purchased from Merck and $1.3\text{ (}10^{-2}\text{ mol l}^{-1}\text{)}$ stock solutions were prepared in water. β -CD was recrystallized once from boiling water.

Influence of pH

The change in fluorescence of both naphazoline and naphazoline- β -CD complex solutions as a function of pH was studied by the following procedure. To a stirred hydrochloric acid solution of naphazoline or naphazoline- β -CD, NaOH solution ($0.1\text{--}2\text{ mol l}^{-1}$) was added in small increments. For each pH point an aliquot of solution was extracted and fluorescence was measured. The initial concentrations of naphazoline and β -CD in the reaction vessel were 4.75×10^{-6} and 0.01 mol l^{-1} respectively. The ionic strength of solution was maintained at 0.1 mol l^{-1} using NaCl.

Influence of β -CD concentration

The influence of β -CD on the fluorescence of naphazoline was studied in neutral media ($pH=7.0$). The naphazoline concentration was held constant in $4.75\times 10^{-6}\text{ mol l}^{-1}$, while the β -CD concentration was varied from 0 to $1.1\times 10^{-2}\text{ mol l}^{-1}$. Individual samples were prepared according to the following procedure; A $100\text{ }\mu\text{l}$ aliquot of $2.37\times 10^{-4}\text{ mol l}^{-1}$ naphazoline solution (prepared by dilution of stock solution) was transferred into a 5 ml volumetric flask and 0.5 ml of 0.2 mol l^{-1} phosphate buffer ($pH=7.0$) was added. Then an appropriate amount of β -CD solution (0.013 mol ml^{-1}) was added and the flask was filled to the mark with water. The resulting solutions were shaken and allowed to equilibrate and their fluorescence intensities were read within 1 h. All measurements were made at least in duplicate.

For each 5.0 ml calibrated flask, after addition of a suitable amount of naphazoline standard solution, 0.5 ml of phosphate buffer ($pH=7.0$) was also added and diluted up to the mark with water. The solutions were thermostated at $25\pm 0.1^{\circ}\text{C}$ and their fluorescence was measured at 330 nm using an excitation wavelength of 281 nm .

In the case of cyclodextrin-enhanced fluorescence method, two stock standard solutions were prepared: $1.0\times 10^{-2}\text{ mol l}^{-1}$ solution of β -CD at $pH\ 7.0$ (phosphate buffer) and solution containing $20.0\text{ }\mu\text{g ml}^{-1}$ naphazoline and $1.0\times 10^{-2}\text{ mol l}^{-1}$ of β -CD. Small volumes of the latter were transferred into 5.0 ml calibrated flask and diluted up to the mark with the former solution. Thus, the concentration of naphazoline was varied from $0\text{--}2\text{ }\mu\text{g ml}^{-1}$ while the β -CD concentration was kept constant.

Pharmaceutical preparations

Three pharmaceutical products commercialized in Iran were analyzed: Naphazoline (nasal drop) with a nominal content of 0.5 mg ml^{-1} of naphazoline hydrochloride, Naphazoline (eye drop) with a nominal content of 1 mg ml^{-1} of naphazoline hydrochloride and Naphazoline+Antazoline (eye drop) with a nominal content of 0.5 mg ml^{-1} of naphazoline hydrochloride and 5 mg ml^{-1} of antazoline phosphate (all products were manufactured by Sina Darou, Tehran, Iran).

For the analysis, 1.0 ml of each product was diluted to 50.0 ml in a calibrated flask and a suitable portion of this solution was tested for the determination of naphazoline by using both the above methods.

Results and Discussion

Fluorescence characteristics of naphazoline in aqueous solution

Naphazoline exhibits the analytically useful, native fluorescence in aqueous solution derived from the naphthalene fluorophore. Figure 1 shows the fluorescence excitation and emission spectra of this drug in neutral media. As can be seen naphazoline presents two peaks at excitation wavelengths of 227 and 281 nm . This is explained by the light absorption promoting an electron from the ground electronic state to the second and first excited singlet states, respectively. The fluorescence emission spectrum gives a band with a characteristic wavelength of 330 nm , corresponding to the transition from the first excited singlet state to the ground state.

The influence of pH in the range of $2\text{--}12$ on the fluorescence of naphazoline was studied using NaOH and HCl. The results indicate that the spectral characteristics are almost independent of pH while the fluorescence intensity markedly decreases at basic solution ($pH>9$). Phosphate buffer solution of $pH=7.0$ was used for pH adjustments. Concentration of this

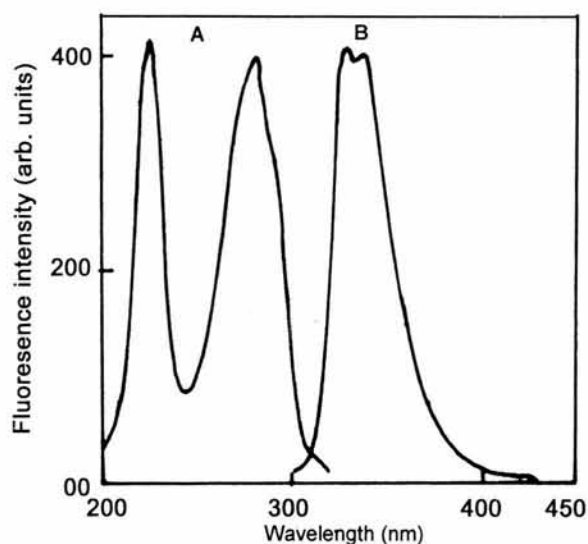


Fig. 1—Fluorescence excitation (A) (λ_{ex} =227 and 281 nm) and emission (B) (λ_{em} =330 nm) spectra of naphazoline ($1 \mu\text{g ml}^{-1}$) in aqueous solution.

buffer did not affect the fluorescence spectrum up to 0.1 mol l^{-1} . The optimum pH range for the determination of naphazoline was considered as pH 5-9.

With the aim of studying the influence of ionic strength, aqueous solutions of naphazoline at various concentrations of NaCl were prepared. The results showed no fluorescence intensity variations for concentrations between 0 and 0.1 mol l^{-1} NaCl; higher concentrations led to a decrease in fluorescence signal.

Another factor that affects the fluorescence intensity is temperature; the fluorescence intensity of naphazoline decreases when the temperature increases from 15 to 45°C . This decrease is nearly linear with temperature coefficient of $1.5 \pm 0.3\% ^\circ\text{C}^{-1}$. Since this value is greater than 1, it can be inferred that internal conversion is not the main non-radiant deactivation process for naphazoline²¹. A temperature of 25°C , close to room temperature, was selected for spectrofluorimetric determination.

Inclusion complex of naphazoline with β -CD

Although α - and β -CD were investigated, only β -CD produced considerable changes in the absorbance and fluorescence spectra of naphazoline. It can be inferred that the cavity dimension of β -CD is convenient for the complex formation with naphazoline. Absorption and fluorescence spectra of naphazoline at different concentrations of β -CD were

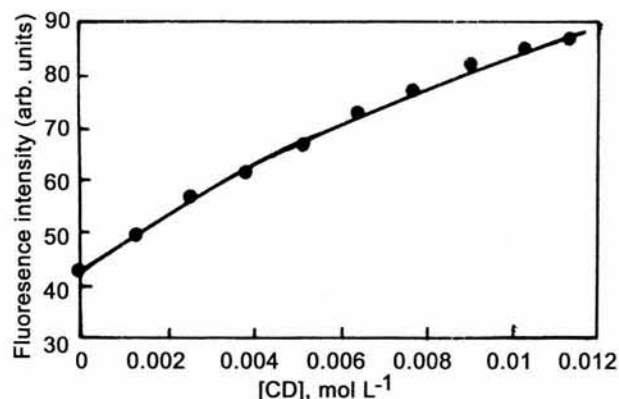
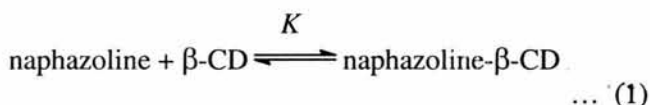


Fig. 2—Influence of β -CD concentration on the fluorescence signal of naphazoline at pH=7.0. The curve is the non-linear fit of the data assuming the formation of a 1:1 complex.

studied. Spectra shows that while the presence of β -CD produces a small change in absorption spectra, the changes in fluorescence signals are significant. Upon inclusion of a fluorophore, CD offers a more protective microenvironment and generally enhances the fluorescence of the guest molecule by shielding the excited state from non-radiative decay processes that normally occur readily in bulk aqueous solution. Assuming that β -CD forms a 1:1 inclusion complex with naphazoline, the following expression can be written:



The formation constant of the complex (K) is given by:

$$K = [\text{naphazoline-}\beta\text{-CD}] / [\text{naphazoline}][\beta\text{-CD}] \quad \dots (2)$$

where $[\text{naphazoline-}\beta\text{-CD}]$, $[\text{naphazoline}]$ and $[\beta\text{-CD}]$ are equilibrium concentrations. The value of formation constant can be calculated from the changes in the fluorescence spectra taking into account that (i) the β -CD is in large excess with respect to naphazoline and therefore its free and analytical concentrations are similar, (ii) the variations in the fluorescence signals are proportional to complex concentration and (iii) at high β -CD concentration essentially all the naphazoline molecules are complexed. Thus the following equation is obtained:

$$F - F_0 = (F_\alpha - F_0) K[\text{CD}]_0 / (1 + K[\text{CD}]_0) \quad \dots (3)$$

where F is the fluorescence intensity of naphazoline solution at each β -CD concentration tested; F_0 and F_α are the signals in the absence of β -CD and when all naphazoline molecules are complexed, respectively. Figure 2 shows the fluorescence emission at 330 nm of naphazoline as a function of the β -CD concentration. The formation constant was calculated by a non-linear regression analysis using the Eq. (3). The obtained value for K is $44 \pm 9 M^{-1}$.

Evidence for the existence of 1:1 complex can be obtained by using the double reciprocal plot (Benesi-Hidebrand's method) given by:

$$1/(F-F_0) = 1/(F_\alpha-F_0)K[CD]_0 + 1/(F_\alpha-F_0) \quad \dots (4)$$

A linear plot of $1/(F-F_0)$ vs. $1/[CD]_0$ was obtained, indicating a 1:1 stoichiometry for the complex. On the other hand, if a plot of $1/(F-F_0)$ vs. $1/[CD]_0^2$ was constructed, a downward concave curvature was obtained, confirming that the stoichiometry of naphazoline- β -CD is not 1:2.

For both spectrofluorimetric and cyclodextrin-enhanced spectrofluorimetric methods, a series of standard solutions (three replicate for each) of naphazoline were measured by the above procedure. The equation for calibration graphs in all cases is $F = h + mC$, where F is the fluorescence intensity (in arbitrary units) and C is the concentration of naphazoline expressed in $\mu g\ ml^{-1}$. The calibration graphs were found to be linear in the range of 0.1-3 and 0.05-2 $\mu g\ ml^{-1}$ in the absence and presence of β -CD, respectively. The detection limit (calculated as $3S_b/m$, where S_b is the standard deviation of the blank) was found to be 25 and 15 ng/ml in the absence and presence of β -CD respectively, while quantification limit ($=10 S_b/m$) was found to be 80 and 50 respectively. As can be seen, the analytical parameters, specially the sensitivity, significantly improve in the presence of β -CD.

In order to study the precision of the spectrofluorimetric and cyclodextrin-enhanced spectrofluorimetric methods, a series of nine solutions of 1 $\mu g\ ml^{-1}$ naphazoline were measured on the same day. By applying the IUPAC definition, the relative errors were 0.5% and 0.6%, and the relative standard deviations for nine replicate analyses were 1.8% and 2.8%, respectively.

Application

Naphazoline was satisfactorily determined in three pharmaceutical products commercialized in Iran by

Table 1—Results obtained by the application of proposed methods to pharmaceutical preparations

Sample	Spectro-fluorimetric method ^a	CD-enhanced spectro-fluorimetric method ^a	Spectrophotometric method ^a
NaphazolineHCl 0.5 mg ml ⁻¹	0.49 $\pm$ 0.01 (99 $\pm$ 2)	0.48 $\pm$ 0.01 (96 $\pm$ 2)	0.50 $\pm$ 0.02 (100 $\pm$ 4)
NaphazolineHCl 1 mgml ⁻¹	0.98 $\pm$ 0.04 (98 $\pm$ 4)	0.97 $\pm$ 0.02 (97 $\pm$ 4)	0.96 $\pm$ 0.01 (96 $\pm$ 1)
Naphazoline HCl 0.5 mg ml ⁻¹ + Antazoline HCl 5 mgml ⁻¹	0.49 $\pm$ 0.02 (98 $\pm$ 3)	0.49 $\pm$ 0.03 (98 $\pm$ 5)	

Average of three determinations $\pm$ SD. Recoveries were calculated based on amounts reported by the manufacturing laboratory. All values are given in mg ml⁻¹.

using both spectrofluorimetric and cyclodextrin-enhanced spectrofluorimetric methods. According to spectral characteristics of fluorescence spectra obtained for these preparations there is no interference from the excipients. Table 1 shows the results for three replicate analyses. The results were compared with those obtained using an independent method (spectrophotometric method based on reaction of naphazoline with alkaline nitroprusside²³). As can be observed in Table 1, there is good agreement between the methods; application of Student's *t*-test and F-test indicate that both methods are accurate²⁴. It should be noted that due to the lack of selectivity, the spectrophotometric method could not be applied to the determination of naphazoline in the presence of antazoline.

Conclusion

Direct spectrofluorimetry has been shown to be a sensitive, accurate and precise procedure for the determination of naphazoline. The complex formation between naphazoline and β -CD was studied. Both the stoichiometry and equilibrium constant for the inclusion complex were evaluated. The results indicate that the inclusion of naphazoline in the β -CD cavity produce fluorescence signals which are larger than those obtained in the absence of β -CD, leading to a corresponding improvement in the analytical method. Both methods were applied successfully to the determination of active constituent of commercial pharmaceuticals. In comparison with other methods such as spectrophotometry^{7,23}, HPLC⁴ and room-

temperature phosphorescence¹¹, the present method is more sensitive, simple and less time consuming.

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