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# Fluorimetric Determination of Histamine in Fish Using Micellar Media and Fluorescamine as Labelling Reagent[1]

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An analytical method based on the use of fluorescamine to produce a fluorescent derivative with histamine and combined with micellar-enhanced fluorescence detection of the formed complex is developed for the sensitive and rapid determination of histamine in fishes. The fluorescence properties of the obtained complex in water and micellar solutions of sodium dodecyl sulfate (SDS), cetyltrimethylammonium chloride (CTAC) and brij-700 are reported. Physicochemical variables influencing the sensitivity of the method (pH, micellar, fluorescamine and NaCl relative concentrations) have been optimized. The stability of the formed complex, as shown by kinetic study, depends on the pH of the solution. Linear calibration curves allowing an effective histamine determination were established with large linear dynamic range (LDR), and low limits of detection (LOD) between 0.5 and 33 ng $mL^{-1}$, according to the solvent. Application to the analysis of fish samples (sardines) yielded satisfactory results. The method seems to be suitable for environmental fish quality control.

**KEY WORDS:** Histamine; fluorescamine; fluorescence; micellar media effect; fish analysis.

## INTRODUCTION

The halieutic and derived products are the principal sources of proteins of our populations. The fish, the molluscs and the shellfish, during postmortem period, produced some biogene amines (tyramine, putrescine, histamine, etc.) by microbial action. These metabolites which were very toxic are used like indicators of decomposition (loss of quality) and scombroïde poisoning [1,2]. Indeed, a study of the Australian agency for food safety showed that 3.5% of fish and 14.3% of their derived finished products contain histamine coming from the amino acid histidine by enzymatic decarboxylation. A product is unsuitable for consumption when the histamine content exceeds 100 $\mu$g/g of product [2,3]. Above this concentration level, histamine can cause very serious allergies including respiratory disorders, abdominal cramps, diarrhoea, vomiting, etc. In particular cases, these health problems can lead to death [1]. In consequence, an effective analytical method of monitoring the histamine content in halieutics products is necessary to avoid collective food poisoning.

Since its discovery in 1916, a variety of techniques and detectors have already been used for determining histamine in various matrices. Biological (enzymatic or radio enzymatic) [4–6] as well as chromatographic methods (TLC, GC, and HPLC) and spectroscopic methods (UV-Visible absorption, infra red, and fluorimetry) [7–13] have been reported for their analytical interests. However, some of them suffer from interferences due to spectral bands overlapping effect and poor resolution. For example, HPLC method associated with the UV-visible detector is less powerful in the short UV wavelengths where histamine as many other amines absorb in general. Moreover, the retention times of histamine and many other amines

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are often similar both in GC and HPLC. For these reasons, the fluorimetric methods were proposed these last years and were widely used for the analysis of histamine because of their great sensitivity and selectivity. However, direct fluorimetry is not applicable because histamine has no specific fluorophore ring allowing its analysis by fluorescence. This difficulty is partly overcome by the use of derivatization reactions allowing to transform histamine by the mean of a fluorogenic molecule into a fluorescent derivative [8,10,12]. The fluorogenic labelling reagents which were generally used are molecules including orthophthalaldehyde (OPA), dansyl chloride, NBD chloride and the fluorescamine. From the different studies [13], it has been observed that the detection of very low amount of analyte requires selectivity and specificity for the labelling reagent, and high sensitivity for the method; if not, one needs to research the conditions of medium allowing achieving these objectives. Thus, a good pre-treatment prior to detection will make possible to remove most of the interfering molecules such as amines, amino acids, alcohols, and even other biogenes amines [11].

Nowadays, the most widely employed fluorimetric method for the analysis of histamine is the one leading to a fluorescent derivative histamine-OPA, which was originally described by Lerke and Bell [12]. In the later method, the reaction between histamine and OPA proceeds efficiently at room temperature in alkaline medium and then, the pH of the obtained complex histamine–OPA solution is shifted back in acid zone before the measurement of the fluorescence signal. Lerke and Bell justified this return in acid medium by the fact that, the complex histamine–OPA was not stable under the alkaline derivatization conditions. Moreover, even after the acidification process, the stability of the excited fluorophore is too poor since it is necessary to wait 25 min before measuring the fluorescence signal; which appears to be a time-consuming procedure for routine analysis of real samples.

In our laboratory, the first method based on the measure of the fluorescence signal of the complex histamine–OPA in alkaline medium, after optimization of the analytical variables (pH, concentrations of histamine and OPA, reaction time, etc.) was developed [8]. The ultimate goal of the later work was to propose an alternative to the method until now employed (i.e., Lerke and Bell method). Although a good calibration line is obtained, the question of the stability of the formed fluorophore was not completely solved and moreover, the formation of the complex is not instantaneously done in all the various prospected solvents [8]. It is for this reason that we propose in this work to develop another method of histamine condensation reaction using fluorescamine as a fluorogenic reagent [14–16].

The advantage of the fluorescamine is that the formation of the fluorophore is almost instantaneous in neutral and alkaline solutions [14]; moreover fluorescamine is a specific derivatizer for the amine function. Like histamine, fluorescamine is non-fluorescent, and its hydrolysis products are too non-fluorescent [17]. In order to increase the stability of the fluorophore signal and the sensitivity of the method, we have employed micellar solutions of brij-700, cetyltrimethylammonium chloride (CTAC) and sodium dodecyl sulfate (SDS). Indeed, micelles are microcapsules which are able to encapsulate and protect the excited singlet state of many fluorophores thus inducing in some cases, an enhancement of fluorescence signal [18–21].

Therefore, in this study, we established a spectrofluorimetric procedure for histamine determination in different media using fluorescamine as a labelling reagent. After an extensive investigation of the effect of various physicochemical parameters, including solvent systems, pH, micellar concentration and the complex signal stability, the applicability of the method to fish samples (sardines) was studied. The performance of the fluorescamine method in terms of simplicity, sensitivity, and rapidity are compared to literature data obtained with other techniques of histamine determination [7–9,22,23].

## EXPERIMENTAL

### Reagents

Histamine (96%, m/m), fluorescamine (98%, m/m), and sodium hydroxide (97%, m/m), analytical-reagent grade were purchased from Sigma-Aldrich (Taufkirchen, Germany). Hydrochloric acid (36%, m/m), sodium chloride (99.9%, m/m) and trichloroacetic acid (TCA, 99%, m/m) were obtained from Labosi (Oulchy-Le-Chateau, France).

### Solvents

Sodium dodecyl sulfate (SDS, 98%, m/m), cetyltrimethylammonium chloride (CTAC, 25%, wt. solution in water) and brij-700 were from Sigma-Aldrich. They were used with a monomer concentration at least 3–5 times over the critical micellar concentration (cmc). Spectroscopic-grade solvents including methanol and acetonitrile (Merck, Darmstadt, Germany) were utilized for preparing the stock solutions of histamine and fluorescamine. Distilled water was used for preparing aqueous solutions of the surfactants.

## Apparatus

All fluorescence measurements were performed at room temperature with Kontron spectrofluorimeter, Model SFM-25 (Zurich, Switzerland) connected to a micro-computer and controlled by software Lcwin. A standard Hellma, (Mulheim, Germany), quartz fluorescence cuvette (1-cm pathlength) and a micropipette pipetman of 10–100 $\mu$L (Gilson, France) were used. All analytical measurements were carried out under the same conditions of voltage (high voltage = 400) and sensitivity (factor = 1.5).

## Procedures

### *Solutions Preparation*

Stock solutions of fluorescamine ($10^{-3}$ M) were freshly prepared by dissolving the compound in acetonitrile to avoid its hydrolysis. Histamine is solubilized in 10 mL of water, to which is added solid NaCl (in order to match the real conditions of the purified and neutralized histamine extracts from halieutic products), then adjusting to 50 mL with acetonitrile to obtain a final histamine stock solutions ($10^{-3}$ M) in NaCl (0.2 M). Serial dilutions in water or acetonitrile were performed to obtain working standard solutions. All solutions were protected against light with aluminum foil and stored in a refrigerator.

Stock solutions of CTAC (0.1 M), SDS (0.1 M), brij-700 ($10^{-3}$ M), NaOH (1 M), and HCl (0.1 M) were prepared in 50 mL of distilled water and used for serial dilutions.

The histamine–fluorescamine complex condensation reaction takes place into a 5-mL volumetric flask by transferring 10–100 $\mu$L aliquots of both fluorescamine and histamine working standard solutions and then, 1 mL of water. After gently shaking, one add the required micellar solution volume, NaOH or HCl solutions (when needed) and distilled water. The solutions were again shaken before analytical measurements.

### *Analytical Measurements*

An aliquot of the obtained histamine–fluorescamine complex solution was placed in the quartz cuvette and the uncorrected fluorescence emission spectra were recorded between 400 and 550 nm at a scanning speed of 480 nm/min. Fluorescence intensity was monitored at fixed analytical excitation ($\lambda_{ex}$) and emission ($\lambda_{em}$) maximum wavelengths of the complex by measuring the spectra height signal. Linear calibration curves were obtained at these $\lambda_{ex}$ and $\lambda_{em}$ values. All fluorescence measurements were corrected for the solvent (background) signal with the appropriate blanks. All fluorescence signal measurements were carried out in triplicate and the results were expressed as mean values. Microcal Origin, version 6.00, application software was used for the statistical treatment of the data.

### *Extraction Procedures*

Among the different extraction procedures usually employed (methanol, water–methanol 25:75, HCl 0.1 N and TCA 5%), the TCA method appears to be the most efficient [22]. The later method is therefore adopted in this study.

A total of 10 g of crushed flesh sample is mixed with TCA (10%, m/v) solution. After vigorous shaking, TCA precipitated all soluble proteins and amino acids. After filtration, the aqueous phase sample suspected to contain histamine (and eventual others remaining interfering amines) was transferred into a chromatographic column filled with a cation exchange resin. When the column tap is opened, it was several times washed with an acetate tampon pH = 4.62. Indeed, we exploit the selectivity due to the medium acidity for a selective separation of the remaining amines with histamine on resin. Thus, after several washing the others amines are involved out of the column while histamine ($pk_a$ = 5.0 and 9.7) remains fixed on the resin. It is then possible to recover histamine using hydrochloric acid solution (HCl 0.2 N).

To check the effectiveness of this extraction procedure, three tests were carried out: (i) Before the extraction procedure, three standard histamine solutions were submitted to similar extraction procedure as the real sardine sample. The measurement of their fluorescence signals gave an average recovery rate close to 98% after comparison with the same histamine concentration which was not passed through the column. (ii) After the extraction procedure of histamine from real sardine sample, 20 mL of acetate was transferred into the column; the tap is completely opened. The obtained solution, mixed with fluorescamine does not present an emission signal of fluorescence at 460 nm. Similarly, no fluorescence emission is observed when the column is again washed with a new HCl 0.2 N solution. This shows that all the histamine presents in the column was recuperated during the first treatment. (iii) The precipitate of fish flesh where histamine was extracted (during the first treatment) is rewashed then filtrated. The obtained solution follows a second same procedure of extraction as described above. The emission spectrum presents a very weak signal after addition of fluorescamine showing that histamine is totally recuperated from the fish.

## RESULTS AND DISCUSSION

In this study, we have to investigate, in the first time, the physicochemical properties of the obtained histamine–fluorescamine complex in several solvents system (water and micellar solutions of brij-700, CTAC and SDS) and afterwards, develop a valid quantitative analytical procedure for histamine determination. Our preliminary experiments showed that the condensation reaction between histamine and fluorescamine is instantaneous in neutral or alkaline medium.

### Preliminary Studies

*Fluorescence Spectra of the Complex Histamine–Fluorescamine*

Histamine and fluorescamine were found to be naturally non-fluorescent in all solvents or media under study, whereas an intense fluorescence band appeared upon mixing the two compounds in neutral or alkaline medium. The fluorescence excitation and emission spectra were recorded (Fig. 1) after adding into the fluorescamine solution (placed in the 5-mL volumetric flask), a known amount of histamine. The results showed that the excitation and emission spectra of the formed histamine–fluorescamine complex have similar shape in all the solvents system under study, i.e., water, brij-700, CTAC, and SDS. The excitation spectra present two peaks, located at 272 nm for the weakest and 380 nm for the strongest, while the emission spectra gave a single peak located at 460 nm.

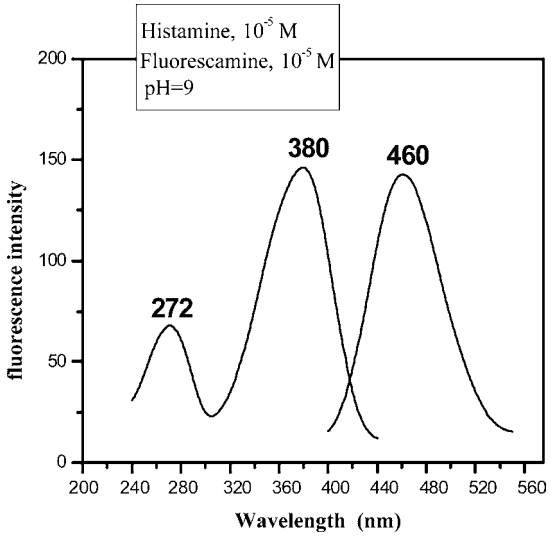


**Fig. 1.** Fluorescence excitation and emission spectra of the formed complex histamine–fluorescamine in water at pH = 9.

**Table I.** Optimization of the Analytical Conditions for the Determination of the Complex Histamine–Fluorescamine

| Solvent[a] | $L_{ex}/L_{em}$ (nm)[b] | pH | Complex stability |
|---|---|---|---|
| Aqueous media | | | |
| $H_2O$ | 380/460 | 7 | Until 15 min |
| Brij-700 | 380/460 | 10 | Yes |
| SDS | | 10 | Yes |
| | 380/460 | 1.44 | Poor |
| CTAC | | 10 | Poor |
| | 387/458 | 1.13 | Medium |
| $CH_3CN$ media | | | |
| CTAC | | Neutral | Yes |
| | 387/458 | Acid | Poor |

[a] Micellar concentrations: [Brij-700] $=10^{-4}$ M, [SDS] = 0.05 M, [CTAC] = 0.4 M.

[b] Analytical excitation ($\lambda_{ex}$) and emission ($\lambda_{em}$) maximum wavelengths of the spectra.

No significant wavelengths shift of these bands occurred on changing the solvent, whereas a red-shift (∼7 nm) of the excitation band and a blue-shift (∼2 nm) of the emission band took place when CTAC is used instead of other solvents. The selected analytical optimum wavelengths are summarized in Table I.

*Characterization of the Complex Histamine–Fluorescamine Formation*

To evaluate the fluorophore formation, we investigated the evolution of fluorescence intensity with histamine concentration for fixed amount of fluorescamine. In a $10^{-5}$ M solution of fluorescamine prepared in acetonitrile, when increased amounts of histamine were added, a progressive signal increase is observed (Fig. 2), resulting from chemical reaction between histamine and fluorescamine. The curve displaying the variation of integrated intensity *versus* histamine concentration (shown in Fig. 2 inset) gives the same correlation coefficient (0.9932) as the curve based on the peak intensity of the same data. Moreover, the sensitivity of the method (slope value of the regression equation) was slightly better for the peak intensity procedure. Hence, for further measurement, the peak intensity method was used.

The usually indicated fluorophore based on the known reactivity of fluorescamine with primary amines [14,15,17,18] was the pyrrolinone derivative (molar mass 371 g/mol). After gas chromatography–mass spectrometry investigation, combined with IR and UV-visible studies, we have found that the derivative histamine–fluorescamine molar mass is 353 g/mol and its structure is as shown in Fig. 3. Indeed, Weigele and Udenfriend have

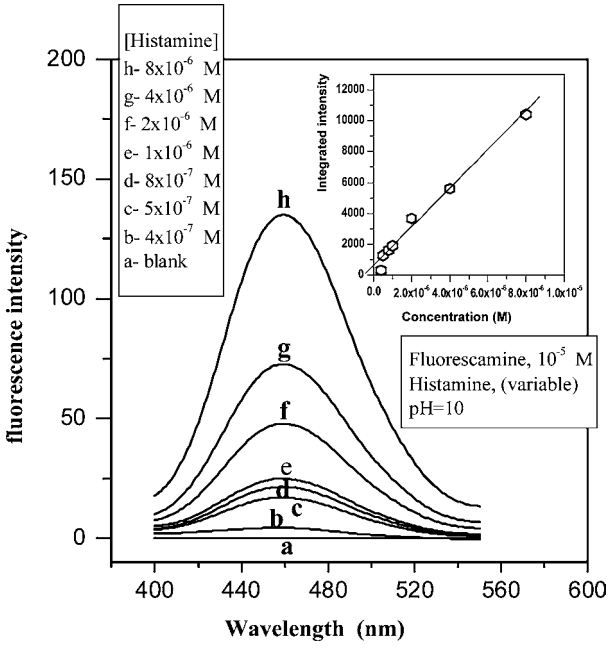


**Fig. 2.** Evolution of fluorescence intensity of the obtained fluorophore in function of histamine concentration, for fixed amount of fluorescamine.

shown that the pyrrolinone derivative according to the pH of the media or the nature of the amine may evolve to another compound [14,17].

*Order of Addition of the Reagents in the Volumetric Flask*

The condensation reaction between histamine and fluorescamine was firstly done by mixing aliquots of the two compounds in the volumetric flask, thereafter one adds micelles solution, NaOH or HCl when needed, before adjusting to 5 mL with distilled water.

Because of the problems due to the effect of cosolubilization of multiple solutes in micelles [21], the order of addition in the flask the three reagents (i.e., histamine, fluorescamine and micelle) has a noticeable effect on measurements to carry out. Hence, we have tested three cases of procedures for the preparation of the final solution before analytical measurements.

i. In the first procedure (HMF), the three reagents are introduced into the flask in following order: histamine (H), micelle (M) and fluorescamine (F), before adjusting the flask with distilled water.
ii. For the second procedure (FMH), the reagents are introduced into the flask according in the following sequence: fluorescamine (F), micelle (M) and finally histamine (H) before distilled water.
iii. During the third procedure, (HFM or FHM), the following sequence is used: histamine (H) or fluorescamine (F), and micelle (M).

For fixed amounts of the reagents (histamine $5 \times 10^{-6}$ M and fluorescamine $10^{-5}$ M) in the 3 cases, the relative intensities of measured fluorescence signal were 3, 5 and 135, respectively, for HMF, FMH and HFM or FHM. The emission spectra of the complex histamine–fluorescamine in SDS (Fig. 4) and in the other micellar media give similar effect.

These results show that procedure (iii) is more indicated for the analysis of histamine in aqueous micellar solutions. In the first two procedures, the micelle prevents

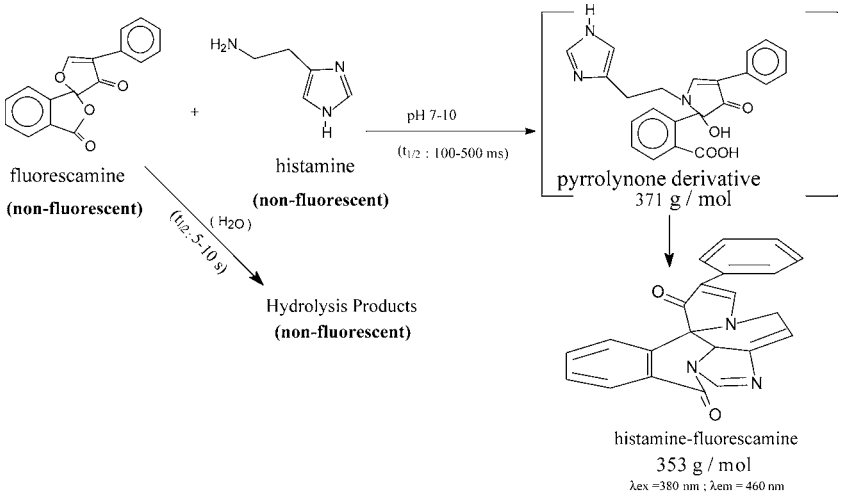


**Fig. 3.** Reaction scheme of the condensation of histamine with fluorescamine in neutral or alkaline aqueous media.

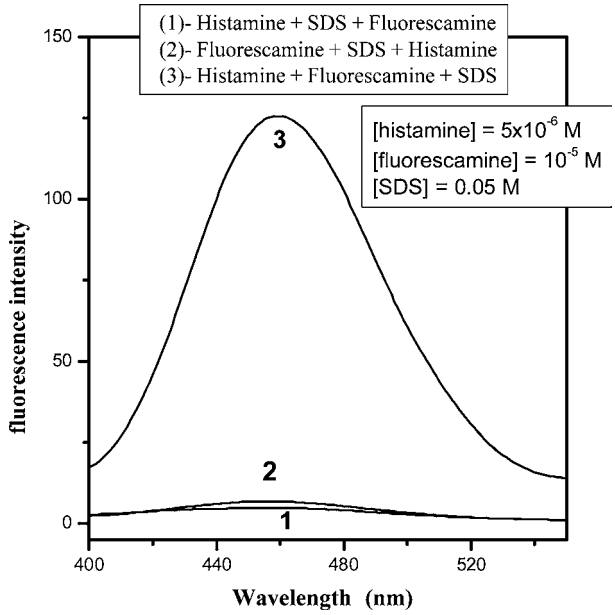


**Fig. 4.** Variation of fluorescence intensity according to the order of addition of the different reagents.

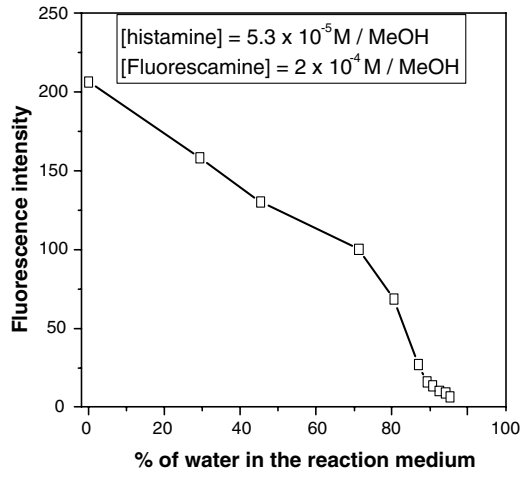


**Fig. 5.** Influence of water percentage on the condensation reaction.

the formation of the complex between histamine and fluorescamine due to an encapsulation of one of the reagents, and the resulting intermolecular interactions that compete with the reaction process diminish fluorescence signal.

*Influence of Water on the Condensation Reaction*

The influence of the amount of water on the condensation reaction was checked in the case of the histamine–fluorescamine complex formation. In a 5-mL volumetric flask containing histamine methanolic solution ($5.3 \times 10^{-5}$ M), when increased amount of water is introduced in the condensation medium before adding fluorescamine ($2 \times 10^{-4}$ M), the fluorescence intensity of the formed complex decreases with increasing water percentage (Fig. 5). The decrease of fluorescence intensity is probably due to the competition of water molecules with histamine for the reactivity-site of the fluorescamine. This is in agreement with the fast hydrolysis reaction ($t_{1/2}$ = 5–10 s) noted for the fluorescamine in aqueous medium [17]. Consequently, the allowed maximum percentage of any water during the condensation reaction was chosen to be as possible, low and constant, for all measurements to carry out. That is why in our histamine extraction procedure in real sardines' sample (Lerk and Bell extraction method), we have introduced into the column a large volume of fish filtrate (8 mL). After purification, the solution suspected to contain histamine is diluted with an organic solvent (methanol or acetonitrile) in order to reduce water proportion before condensation reaction.

## Optimization and Selection of the Analytical Parameters

To develop a valuable method of histamine determination, the influence of several parameters on the fluorescence signal of the complex histamine–fluorescamine were examined, and the optimum values based on the result obtained by triplicate determinations were selected.

*Fluorescamine Concentration Effect*

*Determination of Histamine–Fluorescamine Complex Stoichiometry.* Before studying the effect of the amount of fluorescamine on the complex signal measurements, we have for the first time, checked the histamine–fluorescamine complex stoichiometry using organic solvent. In fact, the later study could not be conducted in aqueous media where immediate hydrolysis reactions of fluorescamine ($t_{1/2}$ = 5–10 s) may compete with the reaction of complex formation. Hence in a methanolic solution of both compounds, when increased amount of histamine (ranged from $0.4 \times 10^{-6}$ to $3.2 \times 10^{-6}$ M) is added to a fixed concentration of fluorescamine ($2 \times 10^{-6}$ M), a progressive signal increase is observed. Plots of the relative fluorescence intensity of the complex versus the histamine concentration give two straight lines with correlation coefficient values larger than 0.9 (Fig. 6). The first line characterizes the progressive formation of the complex while the second represents the addition of excess amount of histamine in the medium. The intersection point of the

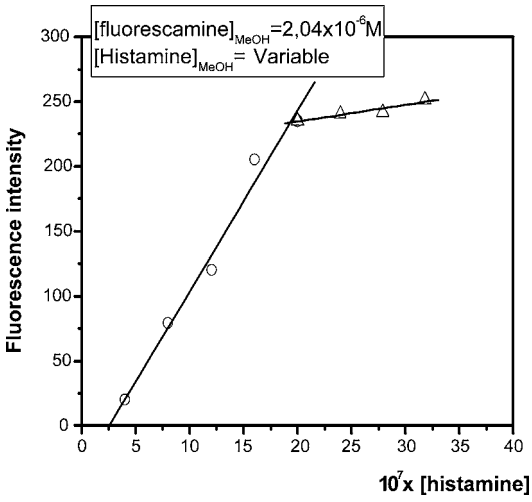


**Fig. 6.** Stoichiometry of the condensation reaction between histamine and fluorescamine in methanol.

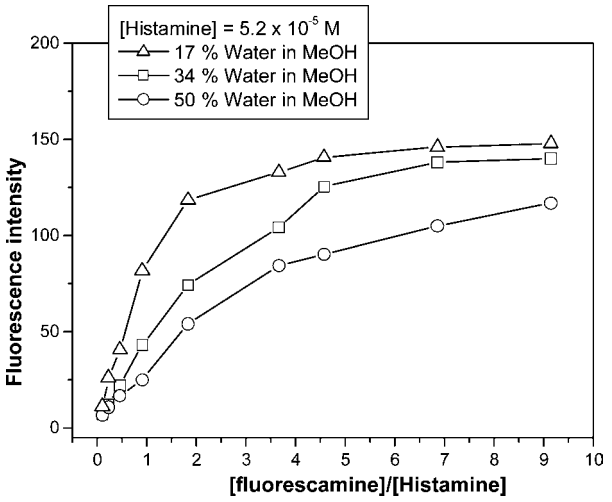


**Fig. 7.** Effect of fluorescamine concentration on the condensation reaction in the presence of (△) 17%, (□) 34%, and (○) 50% (v(v) of water in methanol.

two lines indicates clearly the end of the reaction. This feature suggests that the stoichiometry of the complex is 1:1.

*Optimization of the Fluorescamine Concentration.* According to the above study, non-aqueous media appear to be the more convenient media for the condensation reaction of histamine with fluorescamine. Unfortunately, the extraction methods of histamine from real samples are generally accomplished in a whole or a semi-aqueous medium. For instance, the present AOAC official method, for determining histamine, cadaverine and putrescine rates used the mixture water–methanol (25:75, v/v) [10]. The Lerk and Bell method [12] used aqueous hydrochloric acid. Consequently, in order to match the real conditions of histamine samples preparation, the effect of fluorescamine concentration on the formation of the complex is studied in the presence of various amount of water added in a methanolic solution of both compounds (Fig. 7). This study is destined to predict the amount of the fluorescamine concentration that can be used, in function of the proportion of water present in the reaction medium.

Plots of the complex fluorescence intensity *versus* the concentration ratio fluorescamine/histamine (ranged from 0 to 10, with a constant histamine concentration) showed that the maximum of fluorescence, attained at the end of the condensation reaction, is similar for different water percentage. In fact, this maximum obviously corresponded to the emission of the same fluorophore concentration. However, the concentration ratios fluorescamine/histamine needed to reach these maxima are 5 and 7 in the presence of, respectively, 17 and 34% (v/v) of water but is greater than 10 for water percentage over 50%; while it would be plainly 1 in a non-aqueous solvent. This difference in fluorescamine concentration is due by the fact that few part of the amount of the fluorescamine is destroyed through hydrolysis reaction.

We can conclude that for histamine determination in a semi-aqueous solvent system, an excess amount of fluorescamine at least 10-times greater relative to that of histamine should be used in order to avoid an under estimation of histamine concentration in real samples.

### *pH Effect*

The pH is a factor affecting both fluorescence intensity and the micelles-analyte binding abilities. It is therefore important to know its impact on the fluorescence emission of the complex under study in the various solvents prospected, i.e.; water and the micellar solutions of CTAC, SDS and brij-700.

The results obtained in the different media (Fig. 8) show that the curves of fluorescence signal versus the pH present different patterns according to the solvent used. Indeed, neutral solvents (water and brij-700) present high fluorescence intensities for a large pH scale ranging approximately between 4 and 11, whereas for the ionic surfactant (SDS and CTAC), the variation of fluorescence intensities in function of pH was characterize by a well-defined maximum obtained at pH values of 1.44 and 1.13 respectively for SDS and CTAC.

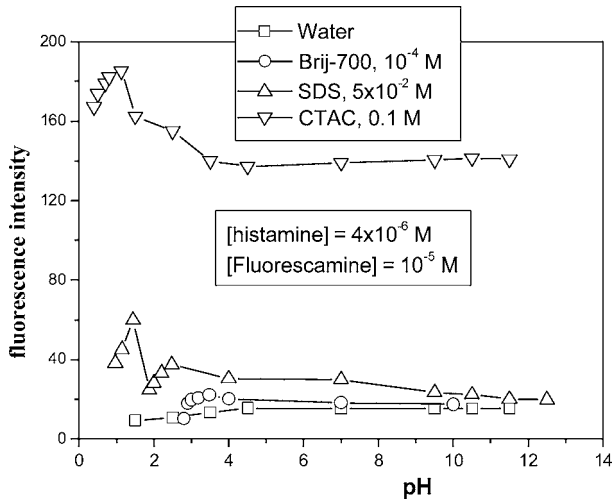


**Fig. 8.** Effect of pH on the fluorescence intensity of the complex histamine–fluorescamine in the different studied media.

However, for all solvent systems, the above studies do not reveal the major information about the relative stability of the formed complex, since these curves were recorded in a relatively short time, at the maximum emission for each pH values. To obtain more information on the stability of the formed complex, kinetic studies were performed. The results (Fig. 9a) show that the obtained complex is relatively stable in water and aqueous micellar solutions of brij-700 and SDS at pH = 10, whereas, a continuous signal decrease with time is observed for the aqueous cationic surfactant CTAC. However, in the same CTAC micelles, the complex signal became more stable when using acetonitrile like solvent of the predominant phase, instead of water (Fig. 9b). Even the hydrochloric acid medium ($2 \times 10^{-3}$ M) enhance strongly the emission signal, the complex stability is very poor (Fig. 9b). This means that the later medium could not be the best choice for analytical purpose; but it would always be possible to conduct a reliable analytical study with the remaining other media (water, SDS, brij-700, CTAC in acetonitrile, CTAC in acidic aqueous media). The final optimum conditions were summarized in Table I.

*Surfactant Concentration Effect*

For the tree micelles used, only CTAC produced a significant signal change, which was characterized by an increase of fluorescence intensity with increasing the CTAC concentration, close to the critical micellar concentration (cmc) value until saturation is reached. The final optimum micellar concentrations selected were $10^{-4}$, $5 \times 10^{-2}$ and 0.4 M, respectively for brij-700, SDS and CTAC.

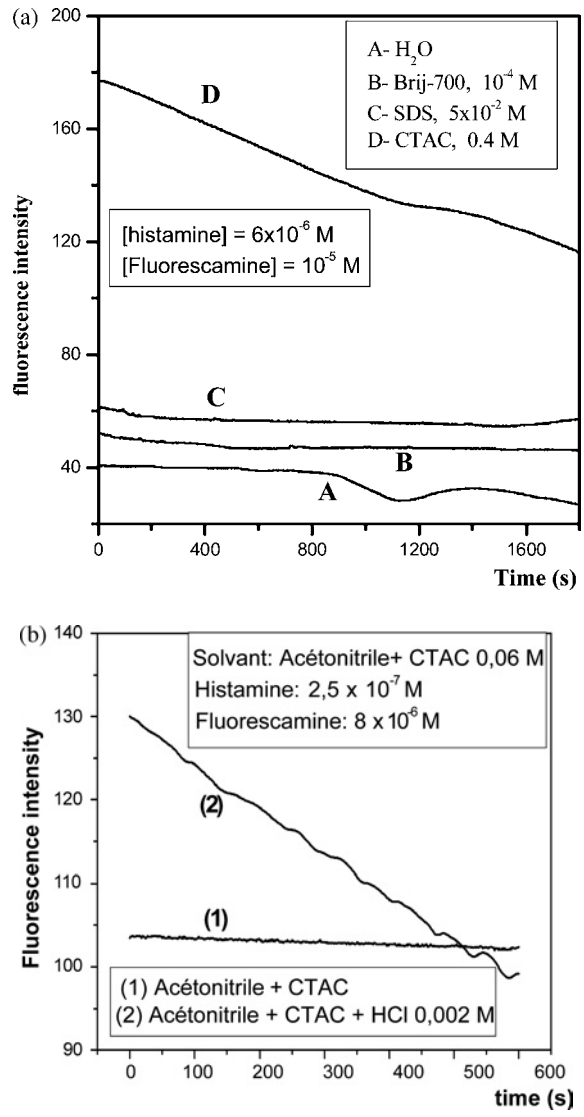


**Fig. 9.** Evolution of fluorescence intensity of the formed complex with time: (a) in the different studied media at pH 10, and (b) in the CTAC-acetonitrile medium.

*Saline NaCl Concentration Effect*

This study is justified by the fact that histamine is generally extracted from real samples with hydrochloric acid (HCl 0.2 M). Since the condensation reaction between histamine and the fluorescamine takes place in neutral or basic medium, a progressive addition of NaOH on the hydrochloric acid extract allows entire neutralization of the acid and, further addition of an excess amount of fluorescamine permit to determine the histamine content in real samples.

For the fluorescence properties of the obtained complex in saline medium, the curve giving the variation of

**Table II.** Analytical Figures of Merit for the Determination of Histamine in the Different Solvents Under Study

| Solvent | $\lambda_{ex}/\lambda_{em}$ (nm) | pH | Concentration range (ng/mL) | $r^a$ | LOD$^b$ (ng/mL) | LOQ$^b$ (ng/mL) | ALOD$^c$ (ng) |
|---|---|---|---|---|---|---|---|
| $H_2O$ | 380/460 | 7 | 500–1600 | 0.999 | 33 | 107 | 83 |
| Brij-700 | 380/460 | 10 | 500–1600 | 0.996 | 3 | 26 | 8 |
| SDS | 380/460 | 10 | 500–1600 | 0.995 | 6 | 17 | 15 |
| CTAC | 387/458 | 1.13 | 200–800 | 0.999 | 2 | 7 | 6 |
| CTAC in $CH_3CN$ | 385/458 | — | 200–800 | 0.996 | 0.5 | 2 | 1 |

[a] Correlation coefficient of the calibration curves.
[b] Limits of detection (LOD) and limits of quantification (LOQ), defined as the amount of analyte giving a signal-to-noise ratio of 3 and 10, respectively.
[c] Absolute limits of detection (ALOD), calculated using 2.5 mL sample volume.

fluorescence intensity of the complex versus NaCl concentration showed that the signal of the complex remains practically unchanged. A kinetic study revealed that the presence of NaCl does not affect the stability of the complex histamine–fluorescamine.

## Analytical Figures of Merit

To evaluate the analytical interest of the fluorescamine approach, we have established calibration graphs under optimum analytical conditions for the complex formed in all the studied media. Linear calibration plots were obtained and we determined the analytical figures of merit. The statistical treatment of the data, including the linear dynamic range (LDR), correlation coefficient ($r$), limits of detection (LODs) and quantification (LOQs) are summarized in Table II. The LODs were relatively low ranging between 0.5 and 33 ng/mL depending to the solvents. The absolute LODs are within 1–83 ng. These results indicate that the method is more suitable for determining histamine with micellar solutions because measurements are 5 to 13 times in micellar media and 66 times in mixture acetonitrile-CTAC more sensitive than in water. In most cases, the lowest histamine contents measured in aqueous media by our method (7 ppb) and the time for a test (25 min) were significantly lower than those given by several available commercial tests: (sensitivity within 0.5–5 ppm, duration of the test ranging from 35 to 60 min) [9,22,23].

## Analytical Application

Our regression equations were used for the analysis of sardine freshly purchased in a local market. The results of our analyses are summarized in Tables III and IV and they demonstrate the presence of a small amount of histamine in the studied fresh fish samples. Table III shows the results of three replicates in optimized CTAC media, while in Table IV, we present the results of histamine rate evolution in function of different storage conditions. The difference between the two sardine sample (A and B) resides in the day of their collection. The average value in fresh sardine sample (about 22 $\mu$g/g of flesh) is relatively below the estimated rate of toxicity of histamine in fishes (100 $\mu$g/g of flesh), but urges the local fishmongers to ameliorate their storage conditions.

## Analytical Advantages of the Fluorescamine in Micellar Media Method

Relative to other techniques of histamine analysis described in the literature, our micellar-enhanced fluorescamine method presents several analytical advantages:

- Instantaneous reaction of condensation relative to OPA or other labellers.
- Relative stability of the complex obtained in the different media under study.
- The sensitivity is about 6–66-times superior in, respectively, SDS and CTAC relative to water.
- The obtained LOQs in aqueous media (7 ppb) were lower than those reported previously for several available analytical and commercial methods of histamine determination [9].
- After extraction procedures, the duration of the test is about 25 min.

**Table III.** Rate of Histamine ($\mu$/g/g of Flesh) Found in Fresh Sardine's (Sample A)

| | Sample A1 | Sample A2 | Sample A3 | Average rate |
|---|---|---|---|---|
| CTAC aqueous acidic medium | 22.31 | 22.26 | 22.31 | $22.29 \pm 0.03$ |
| CTAC acetonitrile medium | 25.41 | 26.36 | 25.91 | $25.89 \pm 0.48$ |

**Table IV.** Rate of Histamine ($\mu$/g/g of Flesh) Found in Sardine's (Sample B) in Function of Different Conditions of Storage

| Solvent | 24 hr at room temperature[a] | 54 hr at room temperature | 54 hr at 10 °C |
|---|---|---|---|
| Water | 15.0 | 300.0 | 34.4 |
| Brij-700 | 17.6 | 272.1 | 33.2 |
| SDS | 18.8 | 262.1 | 35.7 |
| CTAC | 20.7 | 265.1 | 41.4 |
| Average rate | $18.0 \pm 2.4$ | $274.8 \pm 17.3$ | $36.2 \pm 3.6$ |

[a]Time, after fish capture.

## CONCLUSION

We have shown the usefulness of the effect of micelles for improving the sensitivity, stability, and simplicity of the fluorimetric method for the determination of histamine in fish samples. Our results demonstrate that the method should be a suitable alternative for fish quality control. Moreover, its application has revealed that nanogramme levels of histamine can be detected under better conditions using micellar media rather than aqueous solutions. The combination of the proposed method with flow injection analysis is currently being evaluated in our laboratory for the rapid determination of histamine.

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