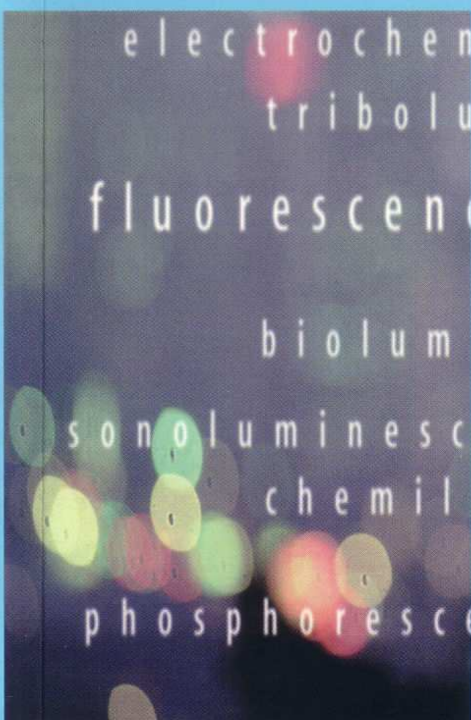
Volume 15 Number 2 March–April 2000

LUMINESCENCE

The Journal of Biological and Chemical Luminescence



Formerly the Journal of Bioluminescence and Chemiluminescence

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JBCHE7
15(2) 59 – 130 (2000)
ISSN 1522–7235

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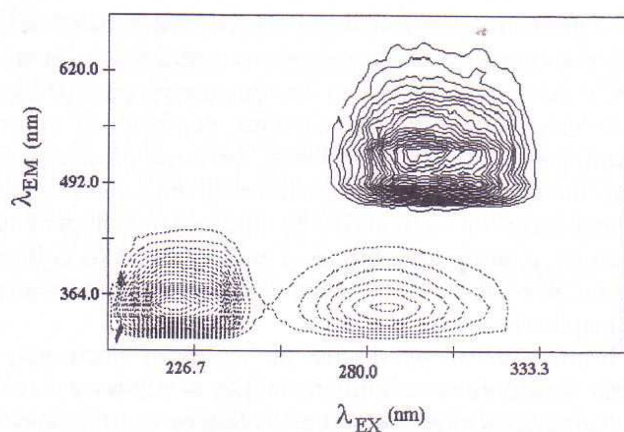

Figure 1.

used in the treatment of several diseases. It is well known that the fraudulent consumption of this type of substance is quite common these days as a β-adrenoceptor in sports with little physical activity.

Propranolol is rapidly and almost completely absorbed after oral administration, and undergoes extensive first-pass metabolism. In 48 h, less than 4% of a dose is excreted in the urine as unchanged drug and about 22% as NA. After intravenous doses, a greater proportion of NA is excreted in the urine. This metabolite is also the major one in plasma. NA presents native fluorescence and Fig. 1 shows the total fluorescence spectrum (broken line).

A selective and sensitive room-temperature phosphorimetric method for the direct determination of NA in biological fluids is described. The method is based on obtaining a phosphorescence signal from NA using $TlNO_3$ as a heavy atom perturber and Na_2SO_3 as a deoxygenator, without need of any special kind of organized medium. This technique is named 'non-protected room-temperature phosphorescence' (NP–RTP), which enables us to determine analytes in complex matrices without the need for tedious prior separation.

The determination was performed in thallium nitrate (0.065 mol/L) and sodium sulphite (0.015 mol/L). The pH value was 7.5, provided by adding sodium dihydrogen phosphate/sodium hydrogen phosphate (0.10 mol/L) at a measurement temperature of 20°C. In these conditions, the phosphorescence is rapidly developed. Fig. 1 shows the total phosphorescence spectrum (solid line). The linear calibration range was 20–1000 ng/mL when the phosphorescence intensity is measured at 527 nm with excitation at 295 nm.

A complete statistical analysis was applied to calibration graph: least squares regression, least median squares regression (to test the presence of outliers or leverages), residual analysis (to test the homocedasticity) and ANOVA analysis (to test the linearity of the regression line). The detection limit, calculated according to the Clayton criterion, was 15.8 ng/mL.

The proposed procedure was applied satisfactorily to the determination of NA in urine and serum samples. A dilution of urine of 1:200 was necessary to carry out the method. In the case of serum, the dilution was 1:10. The solutions were spiked with a amount of NA to test procedure. The recovery ratios, of known amount of NA added to the samples, were obtained by using standard additions methods. The assay results, expressed as a percentage of recovery, were 97.5% and 96.5% for urine and serum, respectively.

Simultaneous fluorimetric determination of amiloride and triamterene in urine using partial least-squares calibration

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Triamterene and amiloride are diuretics which have been included in the list of forbidden substances of the International Olympics Committee since 1990.

The fluorescence maxima characteristic of triamterene and amiloride are located in the UV region. Amiloride shows two broad peaks that are located at $\lambda_{ex1} = 285$ and $\lambda_{ex2} = 365$nm, with emission at $\lambda_{em} = 413$nm. Triamterene presents either two broad peaks, which are located at $\lambda_{ex1} = 268$ and $\lambda_{ex2} = 365$nm, with emission at $\lambda_{em} =$ 437nm. In this region, urine presents two bands that produce a background fluorescence, preventing the determination of triamterene and amiloride. The qualitative composition of the fluorescent metabolites of urine from healthy people of both sexes and different diets are practically constant, so that different samples of urine display the same type of fluorescence, with hardly any variation in the form of the spectrum and in the location of the fluorescence maxima, although it is possible to observe some variations in its intensity.

The determination consists of a combination of different fluorimetric techniques (non-variable angle synchronous, and matrix isopotential synchronous) and PLS regression. The determination was performed in an ethanol–water (1/1, v/v) medium at apparent pH of 6.3, adjusted by using sodium citrate–citric acid (0.1 mol/L) as buffer solution and at a temperature of 20°C. The best range of concentrations for the relationship between fluorescence intensity and concentration was found to be up to 100 and 320 ng/mL for triamterene and amiloride, respectively. These ranges were selected also because, at these concentrations, triamterene and amiloride show similar fluorescence intensities. The urine was considered

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as a third component and its dilution was varied from 1:40 to 1:60.

In the design of the calibration matrix, three components were considered: triamterene, amiloride and urine. The calibration matrix consists of a combination of a factorial design with two levels for each factor and a central composite design. In order to reach a satisfactory result, the calibration matrix was implemented with three samples, with only one of the components considered.

To perform the appropriate selection of the number of principal components in the PLS-1 algorithm, cross-validation, leaving out one sample at a time, was used. The predicted concentrations were compared with the known concentrations of the compounds in each calibration sample, and the predicted error sum of squares (PRESS) was calculated. The PRESS was calculated in the same manner each time a new factor was added to the PLS-1 model. To select the optimum number of factors, the criterion proposed by Haaland and Thomas was used. The significance level was attributed by using an F-test ($\alpha = 0.25$).

Known concentrations of all tested samples, included in the calibration matrix, were compared with the predicted concentrations, by cross-validation, for the spectral data sets employed. The values of the root mean square difference, which is an estimate of the absolute error of prediction by cross-validation for each component in the calibration matrix, were used. The squared correlation coefficients obtained for the predicted vs. actual concentrations were also used.

In addition, to evaluate the predictive ability of the method for each compound, PLS-1 was applied to the spectral data sets for a series of problem mixtures, the concentration of which were taken from a random numbers falling within the concentration ranges of the calibration matrix. To quantify the predictive performance, the standard and relative errors of prediction were calculated.

Interactions of a lipopeptide derived from VP3 capside protein of hepatitis A virus with biomembrane models

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Hepatitis A virus (HAV) is a non-enveloped hepatotropic that belongs to the family Picornaviridae. The viron capsid is composed of the structural proteins VP1, VP2, VP3 and possibly VP4. Although hepatitis A is not considered a severe disease, it is still a source of mortality in both developed and developing countries, mainly because there is no specific treatment other than supportive care. Therefore the best therapy is prevention, by stopping transmission of the virus and/or rendering susceptible individuals resistant to infection by active immunization. However, the poor yields of HAV in cell cultures (1) cause high production costs and pose serious difficulties to the use of this vaccine in developing areas. As an alternative, the use of new strategies based on synthetic peptides that can elict an efficient immune response offers the advantage of high purity, a well-defined structure and safety (2). The continuous epitope VP3 (110–121) [FWRGDLVFDFQV] is highly immunogenic even in the absence of adjuvants (3). In order to potentiate the immune response, a lipopeptide derivative,

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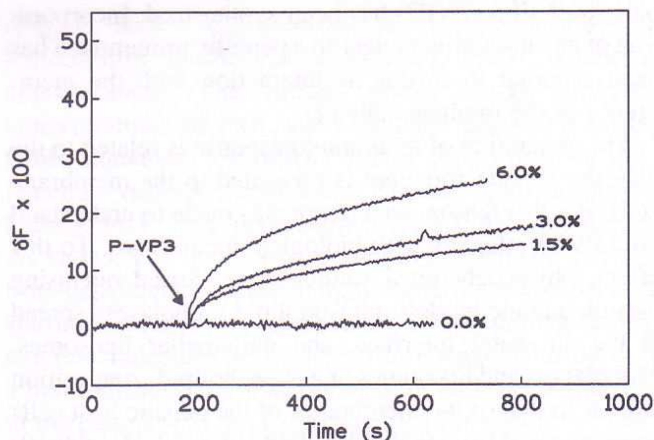

Figure 1. Lipid mixing induced by PVP3 (mol% as indicated) measured by resonance energy transfer. Vesicles are POPC/SM/DPPE/DOTAP (40:33:12:15) containing 0.6% NBD-PE or Rh-PE (1:1 mixture, lipid concentration 133 μmol/L, excitation 460 nm, emission 530 nm).

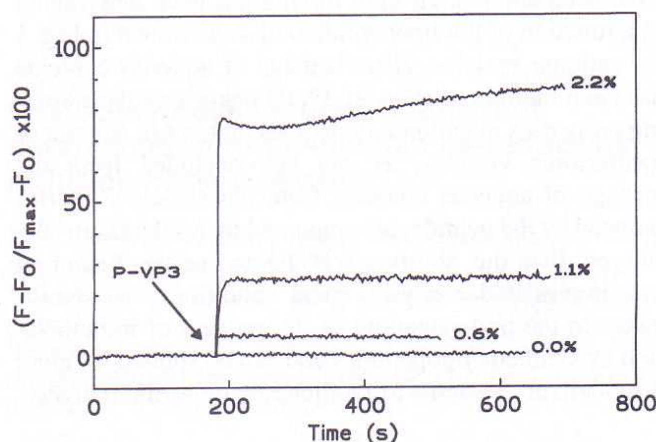

Figure 2. Effect of palmitoyl VP3(110–121) concentration on leakage of ANTS/DPX co-encapsulated in POPC/SM/DPPE/DOTAP (40:33:12:15) vesicles (lipid concentration 240 μmol/L, excitation 360 nm, emission 530 nm).

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