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RESEARCH ARTICLE

Characterization of Fipronil in Micellar Medium with Brij 35

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ABSTRACT

Fipronil is a phenyl pyrazole used as an insecticide to combat termites, moths, and locusts. Its low solubility in water represents a problem with its commercial formulations. In this work, an aqueous micellar medium was formed with Brij 35, calculating the critical micellar concentration at 0.01% (w/v) by UV-visible spectrophotometry and conductimetry. In this medium, the apparent pka was estimated at 12.6 from spectrophotometric data by the graphical method and Partial Least Squares Regression. Finally, its degradation kinetics in this medium at pH 12 was studied, finding that it conforms to a first-order model, with a half-life of 3.3 h, higher than the 2.4 h reported by other authors for an aqueous medium. In conclusion, the micellar medium favored the solubility of the compound in aqueous medium, allowing the estimation of its pka and retarding its chemical degradation.

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1. INTRODUCTION

Fipronil (FP) is an insecticide first registered in the United States of America in 1996. Since it presents effective biological activity at low doses. FP is currently used to control lepidopteran (moths), orthoptera (locusts, grasshoppers), and isoptera (termite) pests in extensive crops such as rice, corn, potatoes, and grains, as well as horticulture. It is also used to control other insects, such as cockroaches, ants, lice, and mites, for domestic or veterinary purposes. Its use is not recommended for animals intended for human consumption due to its accumulation in fatty tissue and viscera [1].

FP belongs to the group of phenyl pyrazoles (Fig. 1). Its solubility in water is low (2 mg L^{-1} at 20 °C), while it is high in organic solvents such as acetone, dichloromethane, and toluene (greater than 1 g L^{-1}). Consequently, its partition constant is high ($\log K_{o/w} = 4$), showing an affinity for hydrophobic matrices such as proteins and lipids [2]. It is stable under acidic conditions but hydrolyzes rapidly under alkaline conditions following pseudo-first-order kinetics. In soil, its half-life varies depending on the soil conditions, the microorganisms present in the medium, and the presence of oxygen [3], [4]. Its mode of action is selective towards insects and non-mammals, and it affects their nervous system by contact or ingestion, causing everything from excitement and convulsions to death due to disruption of the central nervous system [5]. On the other hand, the development of resistance to this pesticide in the target organisms has not been documented.

Due to the lipophilic nature and persistence in the environment of the original compound and its primary metabolites, its bioaccumulation and toxic effects are also recognized in secondary organisms. In particular, oxidative stress is relevant to FP neurotoxicity in animals such as fish, tadpoles, bees, rats, birds, and livestock. Furthermore, FP induces sperm toxicity, nephrotoxicity, growth retardation, and thyroid effects. Regarding its hepatotoxicity, it has been proposed that the mitochondria are mainly affected during FP metabolism, significantly altering the formation of reactive oxygen species or oxidative stress. On the other hand, the primary metabolites of FP, such as FP sulfide, desulfinyl, and sulfone, are also highly toxic to insects and secondary animals [6], [7]. Notably, including FP in micelles has been found to reduce their toxicity in aquatic organisms [8].

One of the main problems faced while preparing their commercial formulations is their low solubility in water, which is why suspensions are formed with polar organic solvents, such as alcohol. The

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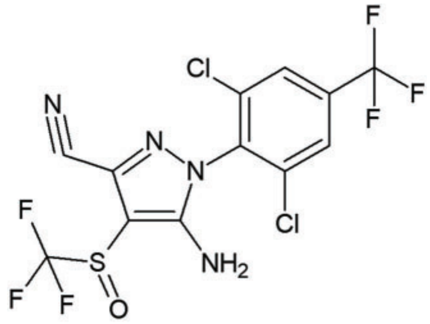

Fig. 1. Chemical structure of FP.

use of surfactants to form micelles is one of the most used strategies to increase the solubility of organic compounds in aqueous medium [9]. Furthermore, in recent years, the influence of the micellar medium on reaction rates has been explored with great interest, given that it constitutes an organized medium that provides numerous compounds with excellent stability [10], [11]. On the other hand, physicochemical parameters such as pKa have different values in aqueous media compared to micellar media due to the interactions between the compound of interest and the hydrophobic or hydrophilic part of the micelles, which influences their acidity or basicity. Therefore, they are recognized as apparent constants [12], [13].

Brij 35, Brij L23, or polyoxyethylene (23) lauryl ether- is a non-ionic surfactant with a density of 1.05 g mL^{-1} [14]. Currently, there is a wide variety of applications for Brij 35, including its use as a support for chemical and enzymatic reactions [15], as well as a vehicle in pharmaceutical preparations and agricultural formulations [16]. Regarding its analytical applications, Brij 35 has been used to form micellar media in the separation of drugs by capillary electrophoresis [17], as well as the mobile phase in liquid chromatography [18]. Also, some authors used it in sample treatment with techniques such as liquid-liquid microextraction [19].

In this work, the critical micellar concentration (CMC) of Brij 35 in the presence of FP was estimated using UV-Vis spectrophotometry and conductimetry techniques. From this, the apparent pKa of FP in a micellar medium with the non-ionic surfactant was determined. Finally, the degradation kinetics of FP in the micellar medium were evaluated since, in basic conditions of the aqueous medium, a low half-life time was reported due to hydrolysis.

2. Materials and Methods

2.1. *Instrumentation*

An Agilent Technologies UV-visible spectrophotometer (model Cary 300), a Fisher Sci potentiometer (model Acumet Basic AB15) with a Thermo Scientific combined pH electrode, and an Ohaus conductimeter (model ST3100C) were used. Data analysis was done with Pirouette v 4.5 software from Infometrix.

2.2. *Reagents*

All reagents were at least analytical grade. The FP was 99.8% pure, and the Brij 35 was an analytical grade from Sigma-Aldrich. Methanol (MeOH) was HPLC grade from Tedia, and water was tridistilled. A standard solution of FP of 200 $\mu g\ mL^{-1}$ dissolved in MeOH was prepared and stored at 4 °C protected from light. Solutions of NaOH and HCl at different concentrations were prepared for pH adjustment.

2.3. *Procedure*

2.3.1. *Estimation of the CMC of Brij 35 in the presence of FP and KCl*

Solutions containing FP at 20 $\mu g\ mL^{-1}$ and 0.1 mol L^{-1} of potassium chloride (KCl) were put together, in which the concentration of Brij 35 was varied in the range of 0.002% to 0.04% (w/v). For each one, the absorption spectrum was recorded against a reactive blank in the wavelength range of 200 to 350 nm, with a resolution of 1.5 nm. In the case of conductimetric measurements, the equipment was previously calibrated with a standard KCl 1 400 $\mu S\ cm^{-1}$; conductance readings were recorded for the same solutions.

2.3.2. Determination of the Apparent pKa of FP in Micellar Medium

A solution of 20 μg mL^{-1} of FP, 0.02% (w/v) of Brij 35, and 0.1 mol L^{-1} of KCl was composed, and its pH was varied in the range from 5 to 14 units. The pH was adjusted for each sample with HCl or NaOH solutions, and its absorption spectrum was recorded. All tests were carried out at 25 °C. The pKa was determined by the graphical procedure (from corrected absorbances) and by Partial Least Squares Regression (PLS). In both cases, the same spectrophotometric data was used. The behavior of the acid species was considered since, for these, there were no spectral interferences from other chemical species.

2.3.3. Kinetics of FP Degradation in Micellar Medium

A solution of 20 μg mL^{-1} of FP and 0.01% (w/v) of Brij 35 was prepared, and the pH was adjusted to 12 with NaOH. The absorbance signal was recorded at 278 nm (absorption maximum) at different time intervals from 30 s to 150 min. The zero-to-second-order models were evaluated using spectrophotometric data to identify the order of the reaction.

3. RESULTS AND DISCUSSION

3.1. Determination of the CMC of Brij 35 in the Presence of FP and KCl

Previous studies have determined that the CMC changes depending on the solutes present in the solution, in addition to the surfactant [20]–[22]. Therefore, the CMC of Brij 35 was determined in the medium used to determine the pKa since this can vary depending on a) the concentration of FP as a hydrophobic organic species, b) the alcohol added as a dissolution vehicle of the analyte, and c) the electrolytes added to fix the ionic strength of the medium.

CMC can be measured through the change in the physicochemical properties of the micellar solution, such as osmotic pressure, turbidity, solubilization, surface tension, or conductivity. Fig. 2A shows the variation in conductivity of FP at 20 μg mL^{-1} with different concentrations of Brij 35. As can be seen, there is a change in the trend of this signal at 0.01% (w/v), which corresponds to the CMC under these conditions. Also, Fig. 2B shows the absorbance at 278 nm (where an absorption maximum occurs in the fipronil spectrum) as a function of the concentration of the surfactant, and a change in the tendency can be recognized with the concentration of Brij 35 similar to the conductimetry tests. The change in the trend of the analytical signals is due to the organization of the monomers in the working medium into spheres to form micelles. Therefore, the CMC of Brij 35 under working conditions was 0.01% (w/v).

3.2. Determination of pKa of FP in Micellar Medium

The pKa of a substance can be defined as the pH at which 50% of the substance is in its acidic form (HA) and the rest in its conjugate base form (A$^-$) or vice versa. It is essential to know this value since it allows the prediction of the molecule's behavior in environmental compartments and biological systems [23].

The solubilization of compounds in micellar medium allows parameters such as pKa to be determined, which is of particular interest in the case of drugs and other compounds with biological activity. Thus, the pKa of several benzodiazepines, which are poorly soluble in water, has been determined with ionic surfactants, concluding that there are electrostatic interactions between the protonated forms of

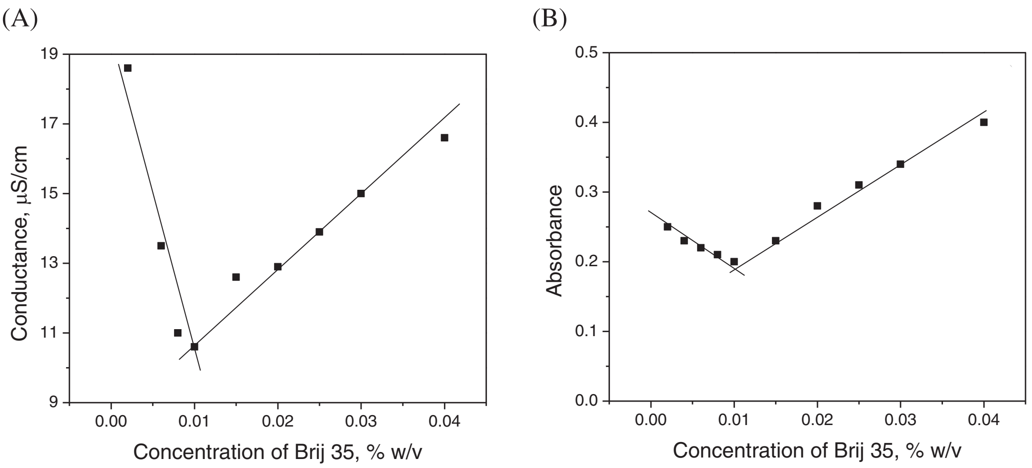

Fig. 2. CMC of Brij 35 in FP by conductimetry (A) and UV-vis spectrophotometry (B).

the drugs and the surfactants used in the study [24]. Likewise, the study of the acid-base balances of rupatadine fumarate has been reported in the presence of anionic, cationic, and non-ionic surfactants, finding variations between the apparent pka estimated in the different media concerning that reported in aqueous media [25].

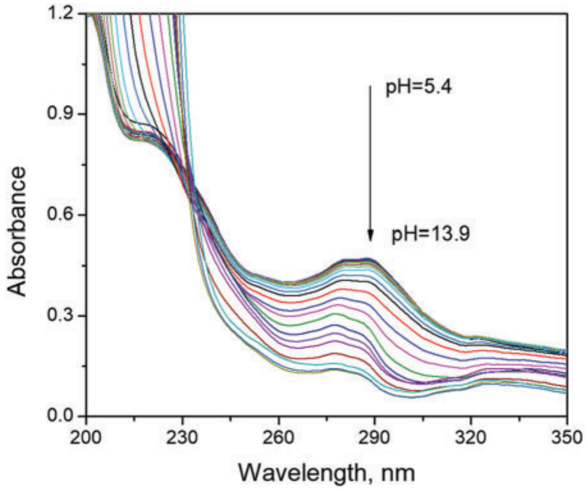

Fig. 3. Absorption spectra of FP in the micellar medium at distinct pH values.

On the other hand, it is essential to determine the pKa of FP since there are no reports of this value in the literature. On the other hand, at alkaline pH, FP degrades quickly, reaching a half-life close to 2 h at a pH of 12 [26]. Fig. 3 shows the absorption spectra recorded for FP at 20 $\mu g\ mL^{-1}$ in a micellar medium at different pH conditions. A hypochromic effect is observed in the absorption band, with a maximum at 278 nm as the pH increases. An isosbestic point can be recognized at 229 nm, close to the FP band between 210 and 223, which is also distorted due to the contribution of the hydroxyl ions under alkaline conditions.

According to Fig. 4A, the pKa can be determined through the absorbance signal at 278 nm. At this wavelength, there is a 100% concentration of the acid species at pH below 10, while at pH above 13.5, it remains constant at its minimum value, corresponding to a concentration of 0%. Half of the difference in analytical signals corresponds to the conditions in which this species reaches a concentration of 50%, where the pH is equal to the apparent pKa, which was 12.6.

Furthermore, given the spectral interferences in alkaline conditions, the PLS chemometric technique was used to estimate the concentrations of the HA species in the pH range studied. A matrix of five samples was constructed, with three presenting a concentration of 0 HA while two had HA at 100 [27]. The absorption spectra were considered in the range of 210 to 310 nm, and mean centering was considered data pretreatment.

Table I presents the data reported for choosing the optimal number of Principal Components (PCs). From the cross-validation (SEV) and calibration (SEC) standard errors, there is the uncertainty of choosing between one and two PCs; according to SEV, it is desirable to choose one, while the SEC shows that it is preferable to choose two PCs. From the analysis of the residual and reconstructed spectra with one and two PCs, it was concluded that the differences lie in the modeling of the signals

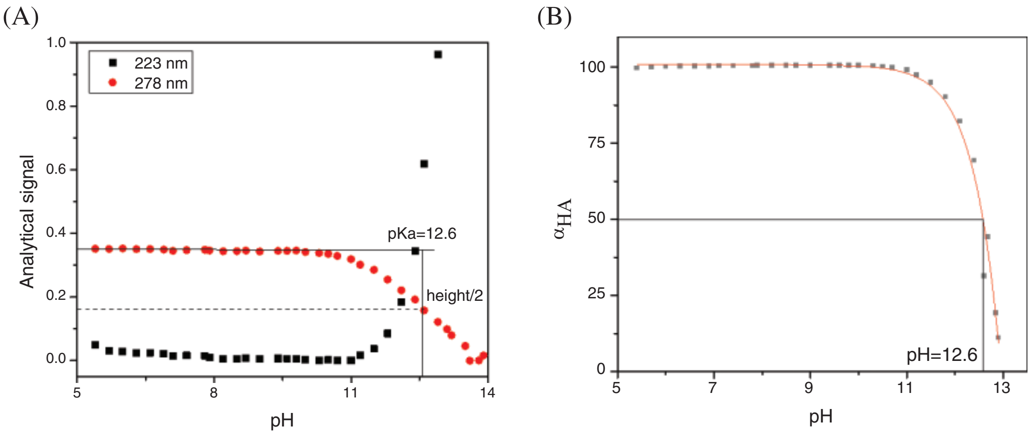

Fig. 4. Determination of pKa by graphical method with corrected absorbances (A) and a mathematical method by PLS (B).

TABLE I: PARAMETERS REPORTED FOR SELECTING THE OPTIMAL NUMBER OF PCS

PC	Variance	Cumulative variance	SEV	SEC
1	357.14	92.01	7.79	3.63
2	31.03	99.99	35.34	0.26
3	6.3×10^{-4}	100	34.28	0.01

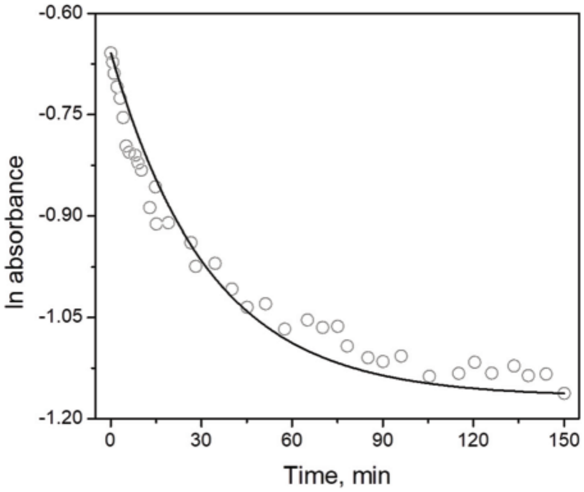

Fig. 5. Kinetics of FP degradation in the micellar medium at pH 12 (278 nm).

located between 210 and 235 nm, in addition to the fact that it is more convenient to consider the SEC as a parameter since it describes the prediction capacity of the calibration model. Therefore, it was decided to work with two PCs. According to the residual spectra of the calibration samples (spectra obtained experimentally – spectra constructed with two PCs using PLS), spectral noise is recognized between 210 and 235 nm because it is the saturation zone of the detector in the presence of high concentrations of the hydroxyl species.

Moreover, Fig. 4B shows the estimated concentrations for the HA species on the scale from zero to 100 (αHA) through the PLS calibration model with two PCs. This data series was subjected to mathematical analysis by Lagrange interpolation, resulting in a third-degree polynomial, whose function is: $y = 342.03x^3 - 12718.62x^2 + 157445.31x - 648761.45$. Its clearance allows us to deduce that for an αHA value of 50, the $\mathrm{pH} = \mathrm{pKa} = 12.6$, the same as that estimated by the traditional method.

3.3. *Kinetics of FP Degradation in Micellar Medium*

Bobé *et al*. [26] described the degradation kinetics of FP by hydrolysis under alkaline conditions, finding that the average lifetime at pH 9 is 32 days. However, when the pH is 12, the average lifetime is reduced to 2.4 h. In contrast, the micellar medium as an external environment protects FP, so the behavior described above is modified [28].

Fig. 5 shows the degradation kinetics of FP in a micellar medium with Brij 35 at pH 12. The observed trend of data corresponded to a first-order model. The graphical method determined the reaction order; according to the slope value, the rate constant (k) was −0.0035, and the estimated half-life under such conditions was 3.3 h.

As observed in other compounds, FP is more stable in a micellar medium compared to the aqueous medium at alkaline conditions [29]. According to other authors, the reactivity of some organic compounds decreases against oxidation or hydrolysis reactions in a micellar medium [30].

4. CONCLUSIONS

The CMC of Brij 35 in the presence of FP and KCl was estimated to be 0.01% (w/v). The micellar medium was an excellent alternative to solubilize the compound and determine its apparent pKa, whose value was 12.6. The micelles formed with Brij 35 generated a more stable medium for FP since its half-life increased compared to its value in water to 3.3 h. Thus, by solubilizing FP and stabilizing it against a hydrolysis reaction, Brij 35 in the form of micelles in an aqueous medium is a viable candidate from a chemical point of view as a vehicle for new formulations.

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CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

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