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Alkaline hydrolysis of vinclozolin: Effect of humic acids aggregates in water

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ABSTRACT

The influence of natural organic substances as humate colloidal aggregates in water solutions upon the chemical stability of vinclozolin has been investigated in basic media. A large inhibition (9 times-fold) has been observed and it has been rationalized in terms of a micellar pseudophase model. The observed behaviour could increase significantly the half-life of this fungicide. Moreover, these experimental results have been compared with the corresponding ones of other substances in these natural colloidal aggregates.

1. Introduction

Vinclozolin (V) is a dicarboxamide non-systemic pesticide (Scheme 1) used to control fungal diseases, blights, rots and moulds in vineyards (grapes), fruits (strawberries, raspberries and kiwi), vegetables (snap beans, lettuce and onions) or different types of ornamentals [1–5]. It may also be used on turf grass for golf courses [6]. Being highly effective against common fungi caused by *Botrytis* spp., *Sclerotinia* spp. or *Monilinia* spp. [7–9]. It is well known that this fungicide is moderately persistent in soil [10] and it is only partially broken down by soil microorganisms [11–13]. Vinclozolin may degrade [14] to 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide (see Scheme 1A) or 2-[(3,5-dichlorophenyl)-carbamoyl]oxy-2-methyl-3-butenoic acid (see Scheme 1B). Both reactions will continue to degrade to a third degradation product, 3,5-dichloroaniline (see Scheme 1C). In water, it can be degraded by photolysis and/or hydrolysis processes, which are favoured under neutral or basic conditions [15]. The acute median lethal dose (LD_{50}) for V is greater than 15,000 $mg\,kg^{-1}$ in rats [10].

Humic substances, and more specifically humate colloidal aggregates (HCAs), represent a large portion of organic matter in natural environments and soils [16,17]. In fact, these organic colloids include highly decomposed organic matter generally more known as humus. Their nature as colloids and their behaviour in solution is quite similar to the micellar aggregates [18–20]. Their ability to absorb or adsorb different types of pollutants, both organic and inorganic, made reconsider the use of these substances from a chemical point of view, since traditionally they were considered carriers. The adsorption properties and the respective compartmentalization of different substrates by humic substances cause changes in the chemical reactivity when a reaction occurs in the presence of these kind of colloidal aggregates [21]. Therefore, to understand the role that humate aggregates currently have in environmental processes, it requires the knowledge of their role from a chemical kinetic view [22] because they can act as solvents, and toxic reagents scavengers or natural catalysers. Furthermore, it has been considered that the primary route of elimination of organic xenobiotics such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), pesticides or other substances is related to the microorganism activity found in soil, neglecting the essential importance of humus. However, the properties of these humate aggregates and their catalytic capacity can be assumed the need to consider other means of disposal [23–29].

New insights in the V reactivity, which is widely used in agriculture, on the basis of a pseudophase model, were carried out because the major composition of the organic matter in soils is HCAs [30] and, moreover these compounds are also present in some conditions in the natural waters [31]. Thus, the aim of the present work

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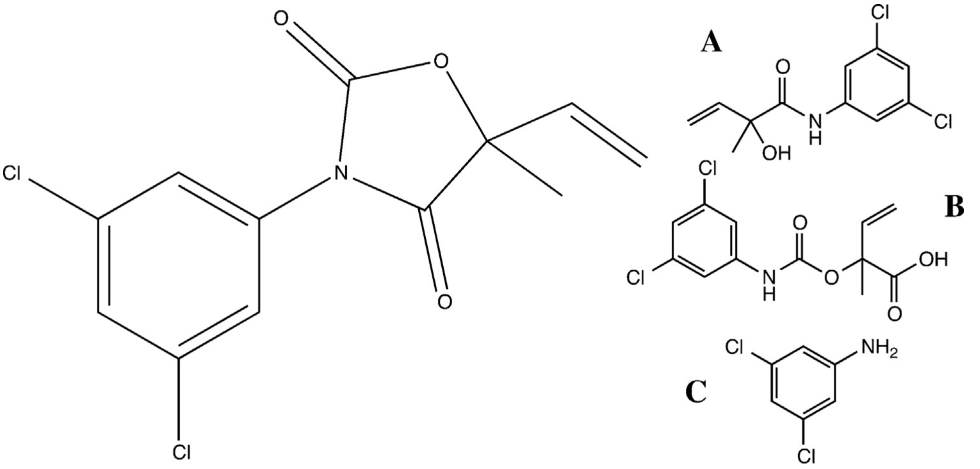


Scheme 1. Structural formula of vinclozolin and its three principal degradation products (A–C).

is to analyse the effect of HCAs "like-micelles" aggregates upon the V stability in order to evaluate if the presence of large amount of organic matter could modify the efficiency of V hydrolysis decomposition pathway.

2. Materials and methods

All reagents used in the present investigation were of the maximum commercially available purity, and none required further purification. HCAs used in this study were isolated from soil using the method described elsewhere [32]. 3-(3,5-Dichlorophenyl)-5-methyl-5-vinyloxazolidine-2,4-dione, better known as vinclozolin, was obtained from Sigma–Aldrich (Steinheim, Germany). Sodium hydroxide and acetonitrile were Panreac reagent (Barcelona, Spain). All aqueous solutions were prepared by weight using double-distilled water.

The kinetic tests were conducted under pseudo first-order conditions ($[V] \ll [OH^-]$). Reactions were monitored through the first-order basic hydrolysis of vinclozolin using a Varian Cary 50 Bio spectrophotometer with the observation cell thermostated at $(25.0 \pm 0.1)\,^\circ$C. When it was necessary, a rapid mixing stopped-flow unit supplied by Applied Photophysics was used. These spectra were carried out between 200 and 800 nm, and because HCAs absorb in the UV–vis region, the spectrum of HCAs in absence of reaction was used as blank. In each instance, it was observed that the final spectrum of the products of the reaction coincided with one obtained in pure water, guaranteeing that the presence of HCAs micelles would not alter the product of the reaction studied.

The basic hydrolysis of vinclozolin was monitored spectrophotometrically measuring the disappearance of the absorbance at its maximum wavelength, more specifically at 242 nm. As an example, Fig. 1 shows the increase in absorption caused by the V decomposition along in time in HCAs. Finally, nonlinear regressions were carried out using a commercial package called pro Fit 6.2 supplied by QuantumSoft in order to obtain the kinetic coefficients. The rate equation to measure the V disappearance is the following:

$$-\frac{d[V]}{dt} = k_W[V_t][OH_t^-] = k_{obs}[V_t] \qquad (1)$$

where [V] is the concentration of vinclozolin pesticide and k_W and k_{obs} are the bimolecular rate and the pseudo-first rate constants, respectively, for the basic hydrolysis of vinclozolin.

Integrating Eq. (1) and expressing the concentration in terms of absorbance, Eq. (2) can be obtained being A_t, A_0 and A_∞ the absorbance at times zero, t, and infinity, respectively.

$$A_t = A_0 \exp(-k_{obs}t) + A_\infty(1 - \exp(-k_{obs}t)) \qquad (2)$$

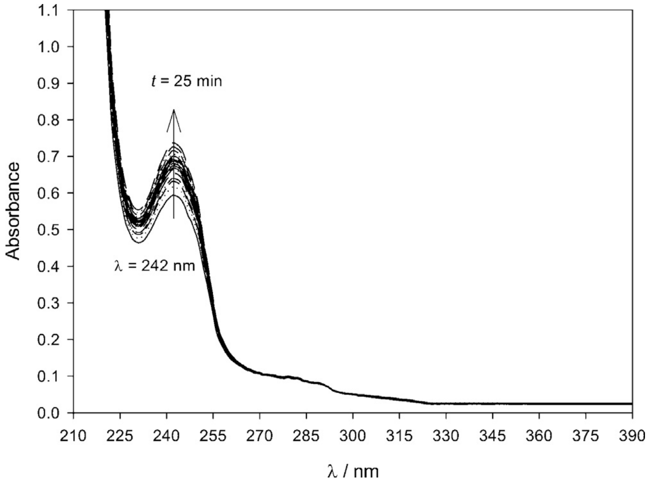


Fig. 1. Spectrograms showing the basic hydrolysis of vinclozolin in humate colloidal aggregates. $[HCAs] = 6.67\ mg\ L^{-1}$; $[V] = 5 \times 10^{-5}$ M; $[OH^-] = 2 \times 10^{-4}$ M; $T = 25\,^\circ$C; $\lambda = 242$ nm.

As an example, in Supplementary information, Fig. S1 shows a typical kinetic run for the basic hydrolysis of vinclozolin as well as the fitting of the experimental data to Eq. (2). Thus, k_{obs} values can be calculated. Fig. 2 shows the influence of $[OH^-]$ on k_{obs} in pure water and in HCAs, respectively. As can be observed a linear dependence between the pseudo-first order rate constant and $[OH^-]$ was obtained.

The influence of HCAs upon the basic hydrolysis of vinclozolin has been analysed in the present manuscript. Pseudo-first order conditions (see Supplementary information: Figs. S2 and S3) were kept in all experiments where the V concentration was kept in all of experiments and equal to 5.0×10^{-5} M. This concentration was chosen to optimize the change in absorbance with time during the kinetic process. Sodium hydroxide concentrations were chosen to obtain a suitable half-life time to monitor the reaction. HCAs concentration was varied between 0 and 0.08 g L^{-1}.

3. Results

Recent studies in our research group [33–36] showed that the presence of micellar aggregates could significantly modify the kinetic processes of some pesticide degradations used in agriculture.

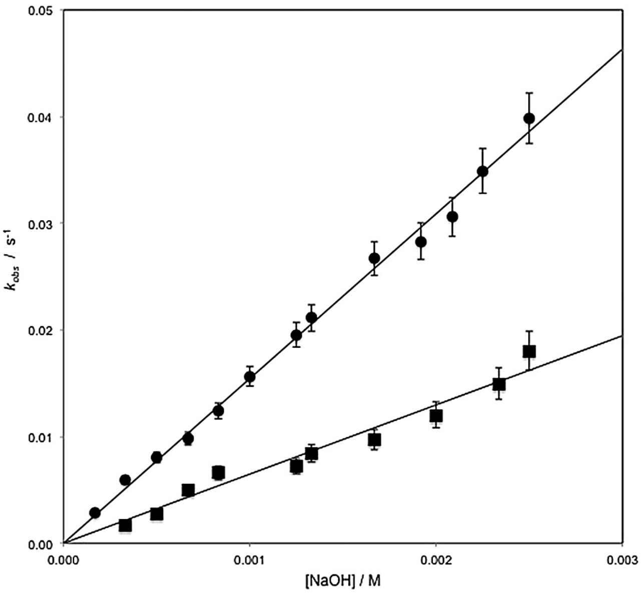


Fig. 2. Influence of the pseudo-first order rate constant, k_{obs}, on the basic hydrolysis of vinclozolin in pure water (●) and in the presence of humate colloidal aggregates (■). [HCAs] = 13.3 mg L^{-1}; [V] = 5 × 10^{-5} M; T = 25 °C; λ = 242 nm.

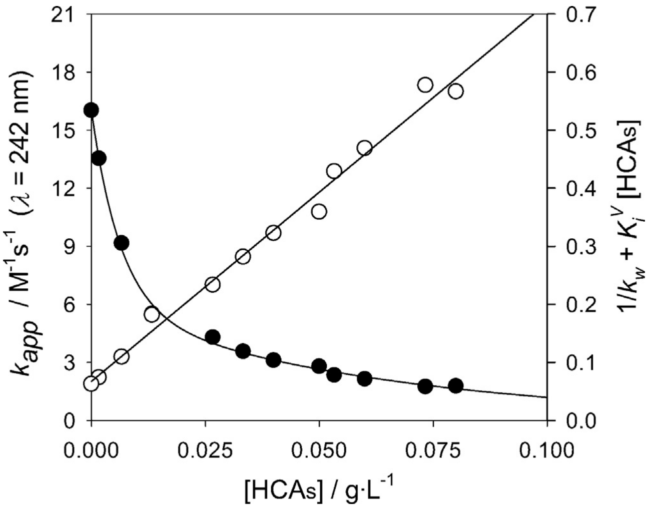


Fig. 3. Influence of [HCAs] upon the apparent bimolecular rate constant, k_{app}, for the V basic hydrolysis in the presence of humate colloidal aggregates. [V] = 5 × 10^{-5} M; [OH$^-$] = 1.67 × 10^{-3} M; T = 25 °C; λ = 242 nm.

A large inhibition (9 times-fold) has been observed for the V basic hydrolysis (Fig. 3). This result is consistent with the corresponding one observed for hydrolysis of other xenobiotics. In this way, 3-hydroxy-carbofuran presents a 1.7 times-fold inhibition and 3-keto-carbofuran yields 1.5 times-fold inhibition [37]. In the case of iprodione basic hydrolysis, it was a 2 times-fold inhibition [38]. It should be noticed that this inhibition represents a significantly increase of V half-life time (from 25.96 s to 235.52 s) in presence of these HCAs. In Supplementary information, Fig. S4 shows the influence of [NaOH] upon the pseudo-first order rate constant, k_{obs}, of basic hydrolysis of vinclozolin at different HCAs concentrations. A slightly decrease in the reactivity of vinclozolin at higher [HCAs] was observed.

4. Discussion

To model these kinetic results, we must take into account that HCAs in water solution are micelle-like aggregates. Hence, these kinetic results can be rationalized in terms of a micellar pseudophase model [39–41].

Scheme 2 shows the mechanism of the V basic hydrolysis reactions in terms of the two-pseudophase model formed by HCAs and water. K_i^{V} and K_i^{OH} are the inclusion constants between micellar and water phases for V and hydroxyl ions, respectively, and k_{m} and k_{w} the basic hydrolysis rate constants in the micellar phase and water phase. Due to electrostatic considerations the hydroxyl ions concentration in the micellar pseudophase must be very low owing to the anionic character of the micellar head group and therefore the reaction rate contribution of micellar reaction pathway is negligible.

Pseudophase model implies that total reagent concentrations can be written as the sum of reagent concentrations in each pseudophase (Eqs. (3) and (4)).

$$[\mathrm{V}_t] = [\mathrm{V_w}] + [\mathrm{V_m}] \tag{3}$$

$$[\mathrm{OH}_t^-] = [\mathrm{OH_w^-}] + [\mathrm{OH_m^-}] \approx [\mathrm{OH_w^-}] \tag{4}$$

where subscripts m and w represents the concentrations in the micellar phase and water phase, respectively. As quote above, the V partition coefficient between water pseuophase and micellar pseudophase can be written as Eq. (5).

$$K_i^{\mathrm{V}} = \frac{[\mathrm{V_m}]}{[\mathrm{V_w}]\,[\mathrm{HCAs}]} \tag{5}$$

Hence, taking into account all these considerations and the previous Scheme, reaction rate can be obtained as the sum of the reaction rates in both pseudophases (Eqs. (6–8)):

$$-\frac{d[\mathrm{V}]}{dt} = \mathrm{V_w} + \mathrm{V_m} \tag{6}$$

$$\mathrm{V_w} = k_{\mathrm{w}}\,[\mathrm{V_w}]\left[\mathrm{OH_w^-}\right] \tag{7}$$

$$\mathrm{V_m} = k_{\mathrm{m}}\,[\mathrm{V_m}]\left[\mathrm{OH_m^-}\right] \tag{8}$$

Considering previous equations, Eq. (9) can be demonstrated:

$$-\frac{d[\mathrm{V}]}{dt} = \frac{k_{\mathrm{w}}[\mathrm{OH}_t^-]}{1 + K_i^{\mathrm{V}}[\mathrm{HCAs}]}[\mathrm{V}_t] \tag{9}$$

Comparing Eq. (9) with Eq. (1) k_{obs} can be deduced as (Eq. (10)):

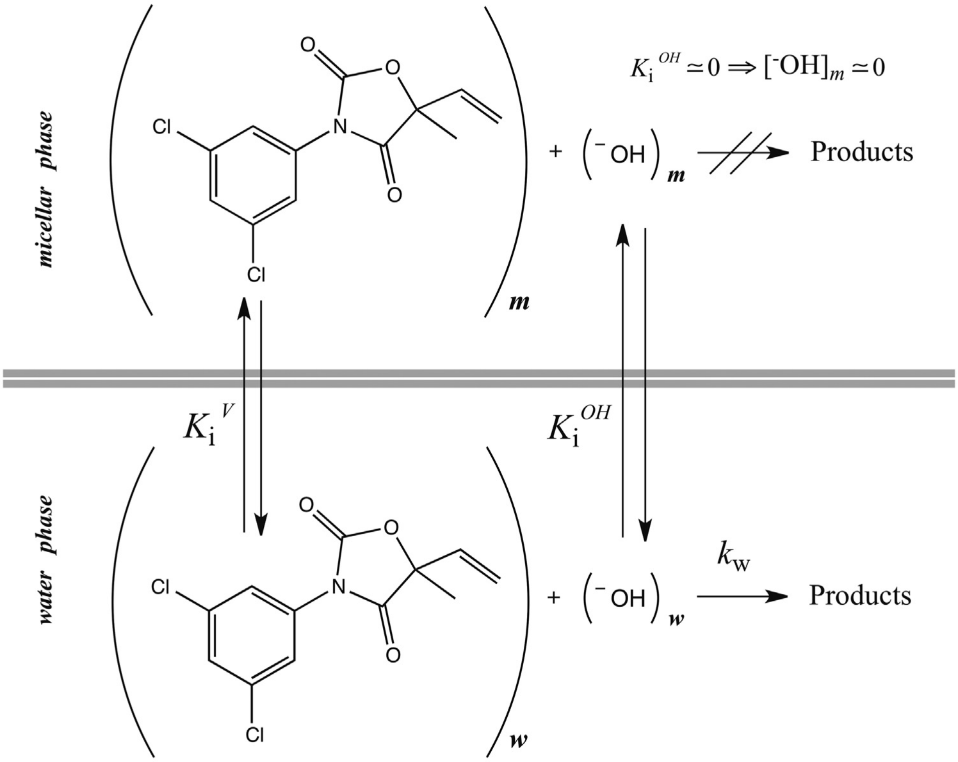
$$k_{\mathrm{obs}} = \frac{k_{\mathrm{w}}[\mathrm{OH}_t^-]}{1 + K_i^{\mathrm{V}}[\mathrm{HCAs}]} = k_{\mathrm{app}}[\mathrm{OH}_t^-] \tag{10}$$

where k_{app} is the apparent rate constant.

In order to confirm the pseudophase model in these microheterogeneous media, the V apparent rate constants were theoretically calculated. Fig. 4 shows the good correlation between the experimental results with those calculated from Eq. (10). These results help us to understand the role of organic matter in soils upon the stability and persistence of vinclozolin investigated in this work. As quote above, these kinetic results imply a significantly increase of V half-life time (Eq. (11)). The rate constant results for the basic hydrolysis of V, in the presence of these natural organic aggregates, is 9-fold lower than those in pure water. As we can see, pseudophase model predicts satisfactorily the kinetic behaviour of this fungicide in humate aggregates. This model represents a useful tool for a quantitative analysis of these kinetic measurements.

$$t_{1/2} = \frac{\ln 2}{k} \tag{11}$$

Finally this behaviour has been compared with the corresponding one in the same natural colloidal aggregates in the presence of other substances (*N*-methyl-*p*-toluene sulfonamide, 3-hydroxy-carbofuran, 3-keto-carbofuran, carbofuran and iprodione) with different behaviour [37,38,42] according electrostatic and



Scheme 2. Pseudophase model upon the basic hydrolysis of vinclozolin in HCAs.

hydrophilic/hydrophobic considerations. The value of the inclusion constant as well as the rate constants in pure water (k_2) were obtained by a non-linear fitting of the previous Eq. (10) to the experimental results and are shown in Table S1. As quote above, this binding constant calculated for vinclozolin in HCAs is relatively higher ($97 \pm 1\,L\,g^{-1}$) being this behaviour due to their differences in hydrophobicity and, hence, differences in their penetrability inside HCAs core (Supplementary information: Table S2). This implies that the micellar-like humate aggregate “protect” V of OH^- nucleophilic attack. The compartmentalisation of reactives justifies the large inhibition observed.

On the other hand, the main driving force for V association to micelar aggregate has a hydrophilic/hydrophobic nature, which is definitely probed (Fig. 5) attending the linear relationship observed between log K_s and their hydrophobicity measured in terms of log P value.

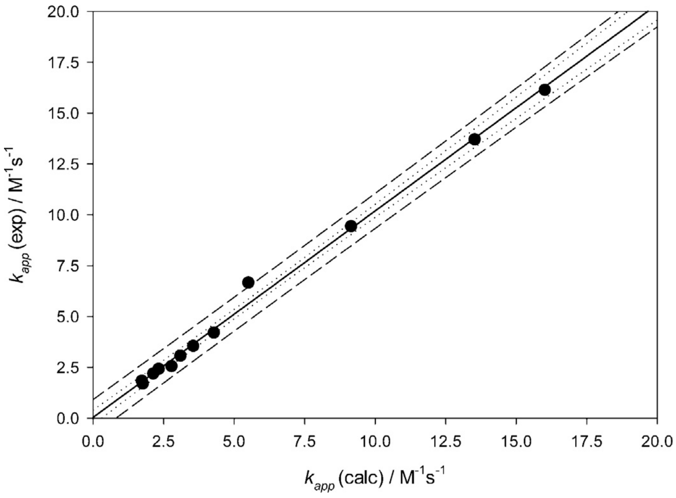


Fig. 4. Experimental rate constant, k_{app} (exp), and calculated rate constant, k_{app} (calc), for the V basic hydrolysis in HCAs. Dotted and short dashed lines represent the 95% confidence band and the 95% prediction band, respectively.

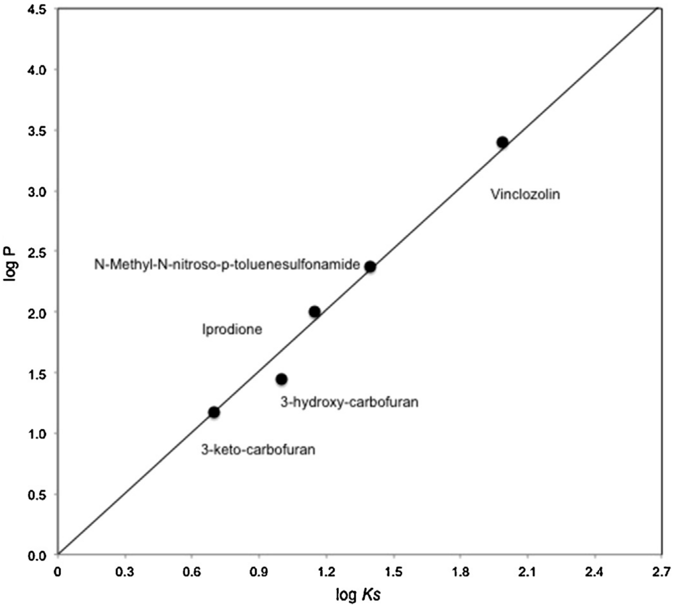


Fig. 5. log K_s for different reactives in HCAs as a function of log P coefficient.

5. Conclusions

To sum up, the results of this research allow us to guarantee the use of natural colloidal aggregates as new reaction media, taking advantages of their peculiar chemical properties [43], but especially the physical properties arising from their compartmentalization in different microenvironments and from the strong interactions of solvation. Similarities in the behaviour of HCAs with other colloidal aggregates provide us to assume that the organic material in natural water solution could be a chemical nanoreactor, which is able to concentrate and compartmentalize reagents. They can play an important role in various chemical processes, which occur in the environment and provide a natural route of xenobiotic elimination. In addition, these results show a high applicability in Green Chemistry due to the increased survival of vinclozolin in the environment. The presence of these HCAs involves, on the one hand the need for a minor "dosage" to achieve similar results, and on the other hand a continued impact over time upon the ecosystem where it is applied.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molcata.2015.02.017

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