HYDROLYSIS KINETIC STUDY OF N-VINYLBENZYLIDENE-3-AMINOPYRIDINE AND ITS COPOLYMERS IN HOMOGENEOUS MEDIA

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(Submitted: 21 September 2014, accepted: 17 November 2014)

ABSTRACT: The present paper reports on the systematic hydrolysis kinetic studies of the monomer N-vinylbenzylidene-3-aminopyridine **Im** and its corresponding copolymers $\mathbf{Cp_a}$ and $\mathbf{Cp_b}$. These studies were established in homogeneous media with pH range from 4 to 10 using UV-Vis spectrophotometer for analysis. The experimental kinetic constants were calculated and a hydrolysis mechanism was proposed. The pH-rate diagram profile was also determined for the monomer and copolymers.

Key words: 3-aminopyridine, monomer, copolymer support, hydrolysis mechanism, kinetic, Schiff base.

RÉSUMÉ: Cet article est consacré à l'étude systématique des cinétiques d'hydrolyse du monomère N-vinylbenzylidene-3-aminopyridine **Im** et ses copolymères $\mathbf{Cp_a}$ et $\mathbf{Cp_b}$. Ces études ont été établies dans des milieux homogènes avec une plage de pH de 4 à 10 en utilisant un spectrophotomètre UV-Vis pour analyse. Dans cette gamme de pH, le mécanisme d'hydrolyse a été étudié et les constantes cinétiques expérimentales ont été calculées. Les courbes d'hydrolyse ont été tracées aussi pour le monomère et les copolymères synthétisés.

Mots clés: 3-aminopyridine, monomère, support de copolymère, mécanisme d'hydrolyse, cinétique, base de Schiff.

INTRODUCTION

A great number of polymers is used as supports of active compounds in the pharmaceutical field [1]. These supports permit the control of drug release over a defined period of time and represent a significant pathway for optimizing drug effects through dosage forms. Chemical grafting of the active agent on polymers or other molecules constitutes one of the most technologies of the active molecule formulation or modification [2, 3]. The linkage between the drug and the polymer can be obtained by various functions such as amide, carbonate, ester or imine groups [3-5].

In this domain, the styrenic and the acrylic polymers are widely used by researchers [6, 7]. Some parameters such as geometry, functional group and group spacer can modify the drug release. In the present research, the imine linkage has been chosen because it can be easily hydrolysable in all range of pH media and allows a grafting of amine-terminated drug precursors and the stability of the azomethine function has been largely discussed [8-10]. In addition, a great number of Schiff bases have been prepared and used as substrates for the preparation of industrially and biologically active compounds and their biological activities as for example antimicrobial, antifungal and antitumor agents have been demonstrated [11-13].

The present research is focused on the study of the stability of different formulations i.e monomer and copolymers based on the 3-aminopyridine as active ingredient. This intermediate

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active molecule constitutes the principal part in triapine (3-aminopyridin-2-carboxaldehyd-thiosemicarbazone) which has a broad anti-cancer activity [14].

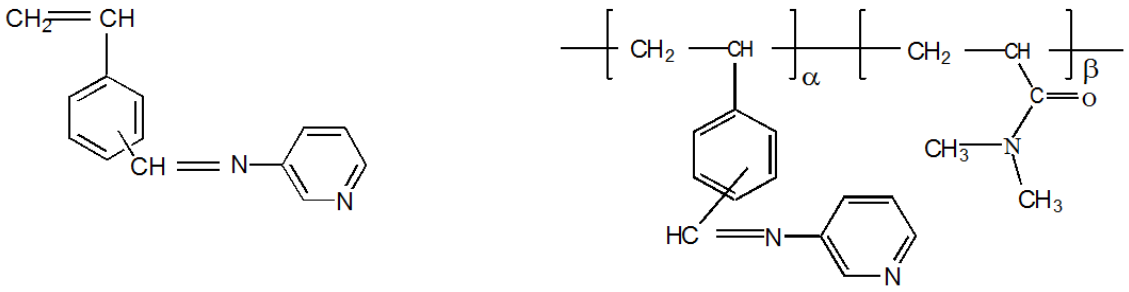
In our previous works [15], we have reported the synthesis of these new compounds; in fact, 3-aminopyridine was grafted on vinylbenzaldehyde (VBA) as a monomer support by means of an azomethine bond to acquire N-vinylbenzyliden-3-aminopyridine **Im**. The copolymerization of the monomer **Im** with N,N-dimethylacrylamide (DMA) leads to two hydrosoluble copolymers $\mathbf{Cp_a}$ and $\mathbf{Cp_b}$ with different masses. In the same paper [15], the hydrolysis kinetics of the synthetic formulations and so the 3-aminopyridine release has been investigated in heterogeneous media. However, in the present paper, we reported the complete and systematic hydrolysis study of these formulations in homogeneous (hydro-alcoholic) media in the pH range from 4 to 10.

The objective of this study is to estimate the hydrolysis constants of these new pyridines' Schiff bases and to evaluate the drug release profile in homogeneous media and finally to compare these results with the drug release in heterogeneous media.

EXPERIMENTAL SECTION

1. Preparation and characterization of monomer and copolymers

The synthesis of monomer **Im** and opolymers $\mathbf{Cp_a}$ and $\mathbf{Cp_b}$ and also their characterization are detailed in our previous paper [15]. We report here only the basic results of their characterization.



Im $\mathbf{Cp_a}$ and $\mathbf{Cp_b}$

Figure 1 : Structure of monomer **Im** and copolymers $\mathbf{Cp_a}$ and $\mathbf{Cp_b}$.

The monomer support **Im** was characterized by FTIR and ^{1}H NMR spectroscopy. IR (cm^{-1}): 1701 (C=O), 3033-3006 (C-H of Py), 3087 (C-H aromatic), 1624 (C=N), 1604 (C=C aromatic), 1575 and 1477 (C=C and C=N of Py), 914-989 (C-H vinylic), 709 (C-H of Py). ^{1}H NMR (ppm): 8.71-8.69 (m, 3H, meta and para of Py), 8.51-7.0 (m, CH of Ph and Py), 6.89-6.81 (2t, 3H, CH_2=CH-), 5.97-5.38 (2d, 3H, CH_2=CH-).

The principal results of the copolymers characterization ($\mathbf{Cp_a}$; obtained using 5% of initiator and $\mathbf{Cp_b}$; obtained using 5 ‰ of initiator) were:

$\mathbf{Cp_a}$: IR (cm^{-1}): 3020-2950 (C-H of CH and CH_2), 2920 (C-H of CH_3), 1660 (C=O amide), 1600 (C=C aromatic). ^{1}H NMR (ppm): 8.42 (m, 1H, -C<u>H</u>=N-), 8.03-7.1 (m, CH of Ph and Py), 4.25 (s, 1H, OC-C-<u>H</u>), 2.9 (m, 3H, C<u>H</u>$_3$), 2-0.8 (m, 3H, C<u>H</u>$_2$- and C<u>H</u>-). ^{13}C NMR (ppm): 175.08 (<u>C</u>=O), 37.47 (<u>C</u>H_3), 36.22 (<u>C</u>H), 14.5 (<u>C</u>H_2). Viscosimetric mass Mv=3120, incorporation ratios α=0.1394 and β= 0.8606. Glass transition temperature Tg=30 °C.

$\mathbf{Cp_b}$: IR (cm^{-1}): 3040-2970 (C-H of CH and CH_2), 2940 (C-H of CH_3), 1668 (C=O amide), 1600 (C=C aromatic). ^{1}H NMR (ppm): 8.46 (m, 1H, -C<u>H</u>=N-), 8.4-7.08 (m, CH of Ph and Py), 4.0 (s, 1H, OC-C-<u>H</u>), 3.0 (m, 3H, C<u>H</u>$_3$), 1.60-0.8 (m, 3H, C<u>H</u>$_2$- and C<u>H</u>-). ^{13}C NMR (ppm): 175.13 (<u>C</u>=O), (37.49) <u>C</u>H_3, 36.64 (<u>C</u>H), 36.21 (<u>C</u>H_2). Viscosimetric mass Mv=10260, incorporation ratios α=0.1059 and β= 0.8941. Glass transition temperature Tg=36 °C.

2. UV–Vis Analysis and Hydrolysis Kinetics

The hydrolysis of monomer **Im** and copolymers $\mathbf{Cp_a}$ and $\mathbf{Cp_b}$ was studied at a temperature of 37 ± 0.1 °C using an UV-vis spectrophotometer (Shimadzu UV-2401 PC; Tokyo, Japan) equipped with a

thermostatically controlled cell holder in homogeneous media, i.e., 20% V/V ethanol buffer mixtures where the ionic strength is maintained at 0.01.

To follow the hydrolysis kinetics, 2.70 mL of buffer solution and 0.06 mL of distilled water were poured into the UV–vis cell and shaken and then 0.24 mL of monomer (10^{-3} M) or copolymer (10^{-2} M) ethanolic solution were added and shaken too. Complete UV spectra of solution were registered over time intervals consistent with the advancement of the reaction state at T=37 ± 0.5 °C. For the rapid kinetics especially at an acidic pH, only the change in UV absorption with time at the selected wavelength was recorded.

We must indicate that the hydrolysis media were prepared using the buffered aqueous solution/ethanol according to the methods of Perrin [16] and Michaelis- Mizutani [17]. A Tacussel digital pH meter was used for the pH measurements in cells.

RESULTS AND DISCUSSION

1. Hydrolysis study of Schiff bases (monomer and copolymers)

In general, complete UV spectra of the solutions were registered for slow kinetics, examples of UV-Vis spectra of monomer and copolymers hydrolysis are given in figures 2 and 3. Additional example reproducing a rapid kinetic is given in figure 4.

Some researchers demonstrated that the hydrolysis reaction of the imine functional group passes through the carbinolamine group [18] and the presence of isobestic "i" points as mentioned in figure 3 indicates that there was no accumulation of any intermediate, especially the α-amino alcohol, during the hydrolysis process of copolymers **Cp_a** and **Cp_b**.

From the results, we remarked that the hydrolysis kinetics of these Schiff bases in ethanol buffer mixtures are considered as a first-order reaction since it is indicated by the linearity of plot of log (D_c^t) versus time (equation 1):

$$\log D_c^t = \log D_c^0 - \frac{k_{exp}}{2{,}3} t \qquad \text{eq. 1}$$

where $D_{t,c}$ is the corrected optical density at time t, $D^t_c = D_t - D_\infty$; D_t is the optical density at time t; and D_∞ is the optical density at the end of hydrolysis (infinite time).

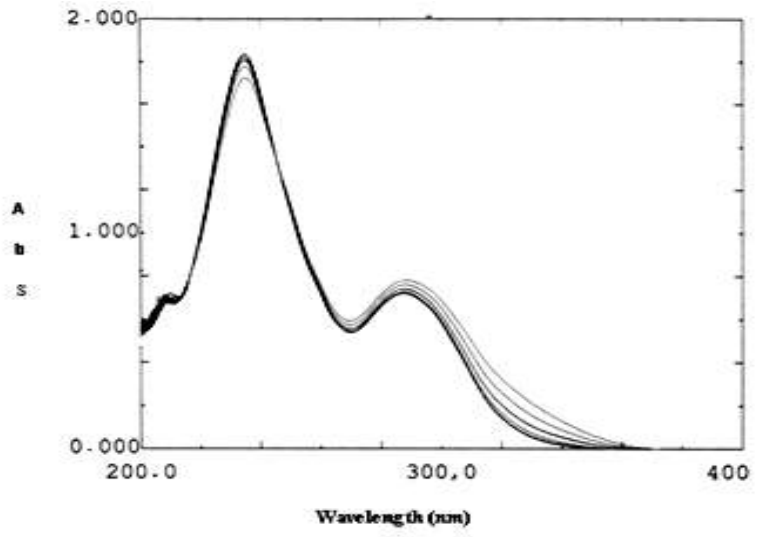


Figure 2: UV Kinetic curves of **Im** hydrolysis at pH = 6.18.

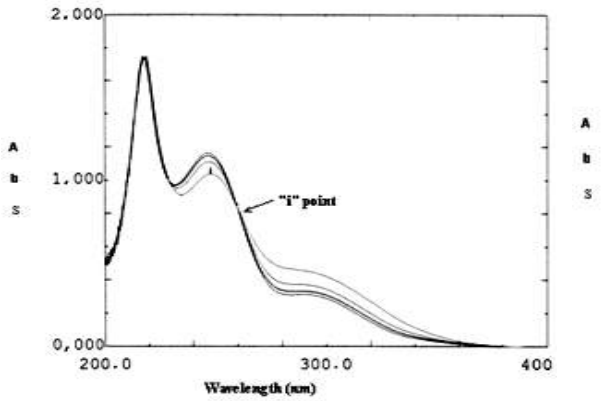


Figure 3: UV Kinetic curves of **Cp$_a$** hydrolysis at pH= 6.18.

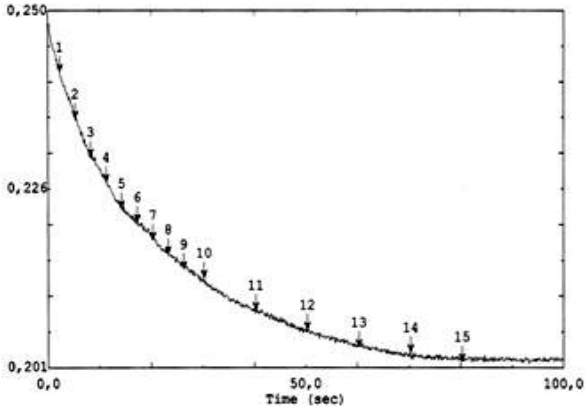


Figure 4: UV Kinetic curves of Cp$_a$ hydrolysis at pH= 4.62.

Examples of straight lines of the hydrolysis data of monomer and copolymers are given in figure 5.

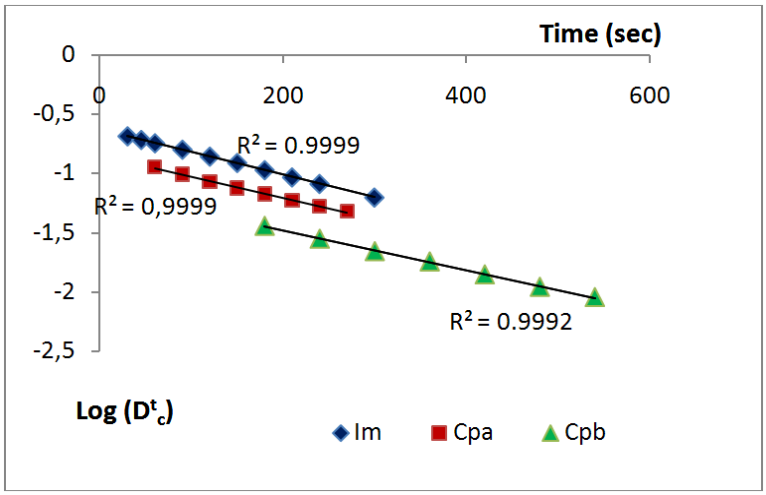


Figure 5: Plots of log (D^t_c) as a function of time for monomer and copolymers hydrolysis at pH = 6.18.

The experimental kinetic constants (k_{exp}) were then calculated from the slope of the log(D) = f(t) equation and the initial corrected optical density (D^0_c) was determined by extrapolation at t = 0.

We specify that the analytical wavelength λ_a was selected from the monomer **Im** and copolymers **Cp$_a$** and **Cp$_b$** UV-bands at each pH; at λ_a the UV-band disappears completely at infinite time, suggesting that the reaction is complete in these conditions. So, λ_a was 334 nm for **Im**, 320 nm for **Cp$_a$** and 300 nm for **Cp$_b$**.

The value of the apparent kinetic constants of Schiff bases (monomer and copolymers) at each pH are regrouped in table I. The kinetic results demonstrated that for all compounds, the hydrolysis constant k_{exp} decreases with increasing pH; the hydrolysis mechanism in these media is discussed bellow. Regarding the type of drug support (**Im**, **Cp$_a$** and **Cp$_b$**), we noted that in acidic pH equal to 4.68, the hydrolysis kinetic of monomer was more rapid than the copolymer ones (*Table I, entry 1*). In pH=6.18, the hydrolysis speed was practically the same for monomer and copolymers (*entry 2*). However when the pH exceeded 7 (7.71 and 9.91), the hydrolysis of imine bond became more slow for the copolymer **Cp$_b$** (*entries 3,4*).

Table I: Hydrolysis kinetic constants of the monomer **Im** and the copolymers supports **Cp$_a$** and **Cp$_a$** based of 3-aminopyridine.

Entry	pH	Im	Cp$_a$		Cp$_b$	
		k_{exp} x10^4 (sec^{-1})	k_{exp} x10^4(sec^{-1})	$\frac{k_{exp}^{Im}}{k_{exp}^{Cpa}}$	k_{exp} x10^4(sec^{-1})	$\frac{k_{exp}^{Im}}{k_{exp}^{Cpb}}$
1	4.68	580	480	1.20	460	1.26
2	6.18	44	41	1.07	39	1.13
3	7.71	5.8	5.2	1.12	3.6	1.6
4	9.91	1.8	2.5	0.72	0.8	2.25

The results can be discussed on the base of both the azomethine bond stability and the molecular weight of the support (monomer and copolymers). In fact, in acidic media, the imine bond is rapidly disrupted due to the nitrogen protonation and since the mass of monomer **Im** is lower than those of copolymers **Cp$_a$** and **Cp$_b$**, the hydrolysis is then rapid and facile for the monomer. However, when the pH increases more than 6, the diminution of the hydrolysis speed for the copolymers can be caused by the high molecular mass of polymer chain which can obstruct the water penetration; in this case, the hydrolysis of copolymers **Cp$_a$** and **Cp$_b$** which possesses the higher molecular weight became the lower.

2. Morphology of the hydrolysis curves

The experimental kinetic constants of the hydrolysis reaction of all products **Im**, **Cp$_a$** and **Cp$_b$** in the pH range from 4 to 10 were calculated; the pH-rate profiles i.e. log k_{exp} = f(pH) are plotted in figure 6.

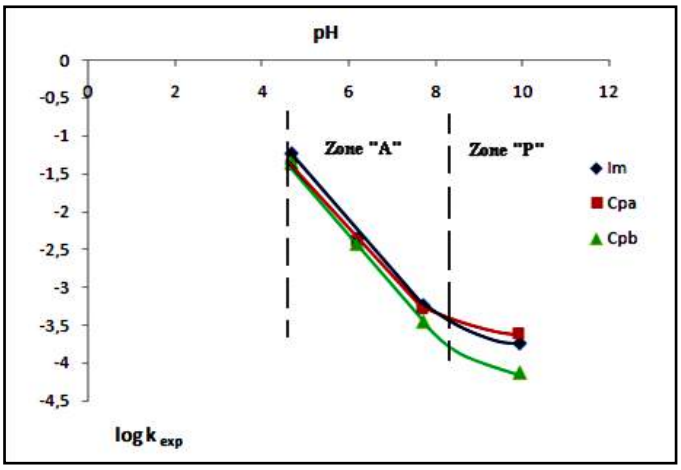


Figure 6: Hydrolysis curves of compounds **Im**, **Cp$_a$** and **Cp$_b$**.

As reported in previous researches [19-24]**,** the hydrolysis curves of Schiff bases derived from aromatic and aliphatic amines can be composed from a number of domains. For the present study and in the pH range 4-10, only two zones appeared; a zone "A" where the kinetic constant decreased when pH increased, and the beginning of the plateau "P" (zone P) where the kinetic constant became independent from the pH medium.

The scheme 1 recovers the general proposed mechanism of Schiff base (S) hydrolysis in pH range from 0 to 14 [25] where the general observed kinetic constant was expressed by the following equation:

$$k_{obs} = \frac{k_1 k_5 [H^+] + K_e k_2 k_5}{([H^+] + K_{SH+})(k_{-1}[H^+] + k_{-2} + k_5)} \qquad \text{eq. 2}$$

where K_e is the autoprotolysis constant of water.

In zone "A" of figure 6, the kinetic constant increased when pH decreased subsequently we can confirm than that the hydrolysis reaction is catalyzed by acidity. If we considered as the protonated imine (SH^+) is attacked only by water molecules according to the reaction in step b, the theoretical equation 2 can be simplified to equation 3:

$$k_{obs} = \frac{k_1 [H^+]}{(K_{SH+})} \qquad \text{eq. 3}$$

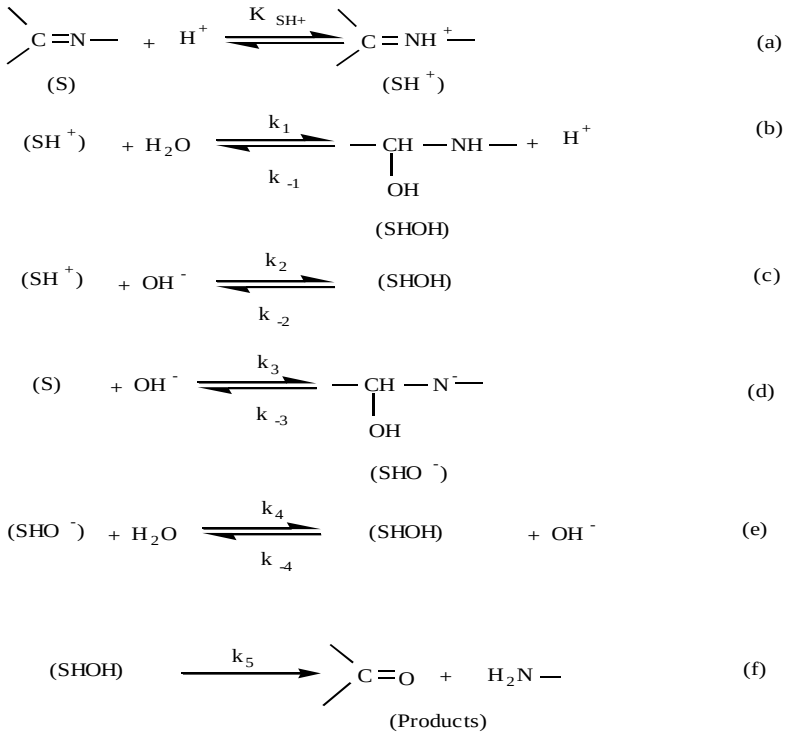
where the slope of the plot $\log(k_{obs})=f(pH)$ is equal to 1.

But, for the studied Schiff bases, the calculated slopes of the experimental linear plots of $\log k_{exp} = f(pH)$ were inferior than 1 and varied from 0.63 to 0.69 (in the pH range from 4 to 8). So, we can conclude that this equation is not sufficient to describe the hydrolysis behavior of these compounds. Consequently, if we considered that the hydrolysis reaction is simultaneously specific acid and base-catalyzed (scheme 1, step b and c), the following equation (eq. 4) became more convenient to describe the hydrolysis mechanism.

$$k_{obs} = \frac{k_1 [H^+] + K_e k_2}{([H^+] + K_{SH+})} \qquad \text{eq. 4}$$

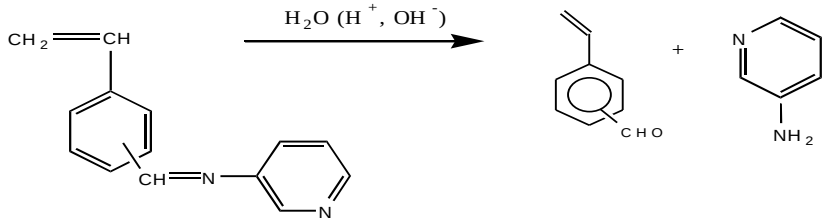
In alkaline media, when the pH=9.9, we remarked that a basic plateau "P" began. It showed that the kinetic constant is independent from pH. In this domain it has been demonstrated that the rate-limiting step corresponded to the nucleophile attack of hydroxide anion on the protonated imine (scheme 1, step c) [26]. The hydrolysis constant was then given by equation 5 where the pH of the hydrolysis media didn't affect it.

$$k_{obs} = \frac{K_e k_2}{(K_{SH+})} \qquad \text{eq. 5}$$

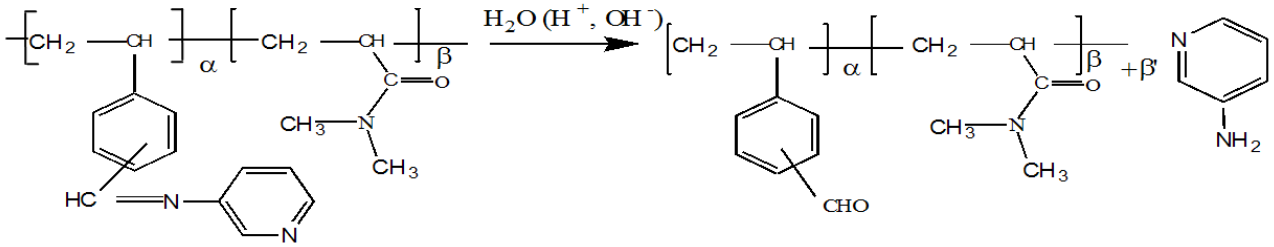


Scheme 1: A General hydrolyisis mechanism of Schiff bases

Finally, we confirmed that both for monomer and copolymers, the hydrolysis mechanism is simultaneously specific acid and base-catalyzed in the pH range from 4 to 8. However, in pH=9.9 the hydrolysis reaction is limited by the nucleophile attack of hydroxide anion on the protonated imine. Then, the hydrolysis reaction of the monomer N-vinylbenzyliden-3-aminopyridine **Im** and copolymers **Cp$_a$** and **Cp$_b$** can be written as following:



Scheme 2: Monomer hydrolysis reaction.



Scheme 3: Copolymers hydrolysis reaction.

3. Hydrolysis constants in homogeneous and heterogeneous media

As mentioned in introduction, the hydrolysis kinetics were studied previously in heterogeneous media and as demonstrated in our paper [15], the 3-aminopyridine released seems that be governed by diffusion. For the comparative study, we have selected basic media where pH was next to 8 because in acidic media the homogeneous hydrolysis was too fast and the hydrolysis rate cannot be calculated. In these conditions, the release and the kinetic constants are resumed in table II.

Table II: Values of the release and the kinetic constant's in heterogeneous and homogeneous media.

Formulation	**homogeneous media** ***K_{exp}** x10^4 (sec^{-1})	**heterogeneous media** ****K** ($min^{-1/2}$)
Im (monomer)	5.8	12.85
Cp$_a$ (copolymer)	5.2	12.45
Cp$_b$ (copolymer)	3.6	24.49

* $\log D_{t,c} = \log D_{0,c} - K_{exp}.t/2.3$, ** $\%P.a._{released} = Kt^{1/2} + b$

The results showed that the Schiff base hydrolysis is more rapid in homogeneous media and these remarks are hopeful since the drug efficacy can be improved where the hydrolysis is established in homogeneous media.

CONCLUSION

The present comparative study of hydrolysis kinetics of the monomer **Im** and the copolymers **Cp$_a$** and **Cp$_b$** derivatives of 3-aminopyridine in homogeneous media showed that the hydrolysis reaction is pseudo first order and the apparent kinetic constants were calculated and investigated in

the pH range from 4 to 10. The morphology of the hydrolysis curves log K_{obs} (s^{-1}) = *f*(pH) showed that the imine function complies the hydrolysis mechanism for this function as reported in literature independently of the support structure i.e. of monomer **Im** or copolymers $\mathbf{Cp_a}$ and $\mathbf{Cp_b}$. As well, the results demonstrated that the monomer hydrolysis is rapid than the copolymers whatever the pH of media. Finally, the results demonstrated that the imine function hydrolysis and so the 3-amino-pyridine release was rapid in homogeneous media. These reports must be important depending on the required application and the drug efficacy rate.

REFERENCES

[1] V. Pillay, A. Seedat, Y. E. Choonara, L. C. du Toit, P. Kumar, V. M. K. Ndesendo, *AAPS Pharm. Sci. Tech*., **2013,** *14 (2)*, 692-711.
[2] N. Chafi, A. Benghalem, A. Mesli, *Eur. polym. J.*, **2003,** *39 (5)*, 1063-1070.
[3] S. Davaran, J. Hanaee, A. Klosravi, *J. controlled Release*, **1999,** *58(3)*, 279-287.
[4] A. Bichara, J-P. Montheard, J-L. Taverdet, *Die Ang. Mak. Chem*., **1998,** *254*, 47-54.
[5] R. G. Schoenmakers, P. Van de Wetering, D. L. Elbert, J. A. Hubbell, *J. Controlled Release*, **2004**, *95*, 291-300.
[6] H. Kamogawa, T. Sakai, H. Sohma, *J. Polym. Sci. Polym. Chem. Ed*., **1988**, *26*, 1335-1341.
[7] J. P. Monthéard, J. M. Vergnaud, M. Kolli, *Polymer. Bull*., **1990**, *23*, 331-334.
[8] F. Benachenhou, N. Mimouni, Y. Mederbel, R. Kaïd Slimane, *Arab. J. Chem*., **2012**, *5(2)*, 245- 250.
[9] A. S. Kirdant, V. A. Shelke, S. G. Shankarwar, S. G. A. G. Shankarwar, T. K. Chondhekar, *J. Chem. Pharm. Res*., **2011**, *3(4)*, 790-796.
[10] Z. Bengharez, Z. El Bahri, A. Mesli, *Int. J. Chem. Kinet*., **2013**, *45*, 404-414.
[11] S. Samadhiya, A. Halve, *Orient. J. Chem.*, **2001**, *17*, 119-122.
[12] M. A. Baseer, V. D. Jadhav, R. M. Phule, Y. V. Archana, Y. B. Vibhute, *Orient. J. Chem*., **2000**, *16*, 553-556.
[13] F. D. Karia, P. H. Parsania, *Asian J. Chem.*, **1999**, *11*, 991-995.
[14] A. R. Finch, M. C. Liu, S. P. Grill, W. C. Rose, R. Loomis, K. M. Vasquez, Y-C. Cheng, A. C. Sartorelli, *Biochem. Pharm*., **2000**, *59 (8)*, 983-991.
[15] K. Diaf, Z. El Bahri, Z. Bengharez, N. Chafi, A. Mesli, *Med. J. Chem.*, **2011**, *1(4)*, 154-162.
[16] D. D. Perrin, *Aust. J. Chem*., **1963**, *16*, 572-578.
[17] L. Michaelis, M. Mizutani, *J. Phys. Chem.*, **1925**, *116*, 146-160.
[18] C. K. Ingold, *Structure and Mechanism in Organic Chemistry*, 2nd Ed., Cornell University Press, Ithaca, New York, **1969**.
[19] A. Mesli, J. Tirouflet, *Bull. Soc. Chim. Fr.*, **1975**, *7-8*, 1753-1762.
[20] A. V. Willi, *Helv. Chim. Acta*, **1956**, *39*, 1193-1203.
[21] E. H. Cordes, W. P. Jencks, *J. Amer. Chem. Soc*., **1962**, *84*, 832-837.
[22] R. L. Reeves, *J. Amer. Chem. Soc.*, **1962**, *84*, 3332-3337.
[23] R. L. Reeves, W. P. Smith, *J. Amer. Chem. Soc*., **1963**, *85*, 724-727.
[24] A. Mesli, *Bull. Soc. Chim. Fr*., **1974**, *9-10*, 2129-2134.
[25] A. Ali-Bey, H. F. Loukil, G. Gosselin, A. Mathieu, A. Mesli, *J. Heterocycl. Chem*., **1982**, *19*, 617-624.
[26] R. L. Reeves, *J. Org. Chem*., **1965**, *30*, 3129-3135.