Annie Y. Vargas, Hugo A. Rojas, Gustavo P. Romanelli and José J. Martínez*

Synthesis of 1,4-dihydropyrimidines with immobilized urease: effect of method immobilization on magnetic supports

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Abstract: The effect of the urease immobilization method was studied on magnetic supports for the Biginelli/Hantzsch reaction. For this purpose, Fe_3O_4/SiO_2 was modified with 3-aminopropyl-triethoxysilane and then activated with glutaraldehyde. A ratio of 500 mg of enzyme per gram of support at 4°C and 18 h were sufficient for the physical adsorption, while 24 h were required for covalent bonding. The Biginelli and Hantzsch reactions were used to evaluate urease application in multicomponent reactions (MCRs). The synthesis of 1,4-dihydropyrimidines was successfully performed using immobilized urease favoring the Hantzsch product. The magnetic properties of the supports allow easy separation, and the urease immobilized by both methods improved the enzymatic activity compared to that of free urease.

Keywords: Biginelli/Hantzsch reaction; magnetic supports; urease immobilized.

1 Introduction

Urease (E.C 3.5.1.5) is broadly used in the analysis of blood urea content, liquor treatment, and sensor development and it decomposes urea into ammonia and carbamate via hydrolysis reaction [1–4]; however, an interesting recent application of urease is its use as a catalyst in multicomponent reactions (MCRs) [5]. The MCRs involve the condensation of three or more reagents in one step. Also, they increase the reaction efficiency due to their high atom economy and are performed in several steps without isolation of intermediates or changing the reaction condition [6, 7]. The Biginelli and Hantzsch reactions are the most recognized MCRs for the synthesis of 1,4-dihydropyrimidines (DHPs) and dihydropyrimidin-2(1H)-ones (DHPMs) [8]. These compounds and their derivatives have biological activity with pharmacological applications including antiviral, antitumor, antibacterial, and anti-inflammatory activities [9]. In this way, Tamaddon and Ghazi [5] reported the use of free urease as catalyst in the Biginelli reaction, obtaining 1,4-dihydropyridine in water with 100% conversion. To the best of our knowledge, immobilized urease has not been used in organic synthesis.

Urease was immobilized on colloidal particles functionalized with poly 4-vinyl-N-ethyl pyridine bromide [10], starch [11], and ZnO nanowires [12]. However, the recovery of the enzyme immobilized on these particles is often limited [1, 2]. To address the issue of reuse, urease immobilized on magnetic solids was used [1, 13–15]. The paramagnetic properties of these solids allow easy separation of the enzymes using an external magnetic field, besides acting as contrast agents for neuro-magnetic resonance imaging [16, 17].

Generally, core-shell magnetic materials are employed as magnetic supports. For example, silica-coated magnetic nanoparticles provide many silanol groups on the surface. These reactive groups are used directly in the subsequent surface functionalization [14, 18]. Organosilanes are used to produce free $-NH_2$ groups that serve for further attachment of enzymes by physical adsorption [19]. Besides, a subsequent activation with glutaraldehyde as cross-linking agent favors enzymatic stability due to the multipoint covalent attachment [20, 21].

In particular, the use of magnetic solids for the immobilization of urease allows increasing storage time and thermal stability due to easy separation, high thermal stability, and low toxicity for biological systems [22]. Also, the activity of the immobilized urease is not affected by the change in broad ranges of pH [1]. Pogorilyi et al. [14] argued that the sorption capacity of urease in Fe_3O_4/SiO_2 increases when the surface is functionalized with amino

***Corresponding author: José J. Martínez,** Escuela de Ciencias Químicas, Facultad de Ciencias. Universidad Pedagógica y Tecnológica de Colombia UPTC, Avenida Central del Norte 39-115, Tunja, Boyacá, Colombia, e-mail: jose.martinez@uptc.edu.co. http://orcid.org/0000-0002-4906-7121
Annie Y. Vargas and Hugo A. Rojas: Escuela de Ciencias Químicas, Facultad de Ciencias, Universidad Pedagógica y Tecnológica de Colombia UPTC, Avenida Central del Norte 39-115, Tunja, Boyacá, Colombia
Gustavo P. Romanelli: Centro de Investigación y Desarrollo en Ciencias Aplicadas "Dr. J.J. Ronco" (CINDECA), Departamento de Química, Facultad de Ciencias Exactas, UNLP-CCT-CONICET. Calles 47 N° 257, B1900 AJK, La Plata, Argentina

or alkyl groups (methyl or *n*-propyl). The adsorbed urease was near 94%, and the residual activity was 73%.

In the present article, we report the immobilization of urease in Fe_3O_4–SiO_2–NH_2, and Fe_3O_4–SiO_2–NH_2 activated with glutaraldehyde, and we study the effect of the immobilization method, and the application of immobilized urease on the Biginelli/Hantzsch reaction.

2 Materials and methods

2.1 Synthesis of supports

Fe_3O_4 nanoparticles (F) were synthesized by the co-precipitation method [23], using a molar ratio of Fe(II)/Fe(III) = 0.5 at pH = 11. F were encapsulated with SiO_2 using a ratio Fe_3O_4 : SiO_2 = 1 : 1 [24]. Then, 0.5 g of Fe_3O_4 particles was dispersed in a mixture of ethanol (200 ml), deionized water (100 ml), and concentrated ammonia aqueous solution (6 ml). The mixture was sonicated for 1 h. Subsequently, 1.75 ml of tetraethylorthosilicate (TEOS, 99%, Sigma Aldrich) was added dropwise and stirred for 3 h. The Fe_3O_4–SiO_2 solid was filtered, washed with deionized water, and dried under vacuum at 60°C. The ratio between the magnetic particles and the silica was 1 : 1.

The functionalization with 3-aminopropyl-triethoxysilane (APTES, 99%, Sigma Aldrich) using the method described by Díez et al. [25] was used to obtain Fe_3O_4–SiO_2–NH_2 (FSN). Briefly, Fe_3O_4–SiO_2 was sonicated in ethanol solution (75%), and then APTES, 99% was added and vigorously stirred for 7 h under inert atmosphere. Finally, the solids were filtered, washed with ethanol solution (50%), and dried under vacuum at 343 K.

To obtain activated Fe_3O_4–SiO_2–NH_2 with glutaraldehyde (FSN-A), the Fe_3O_4–SiO_2–NH_2 support was suspended in 10 ml of glutaraldehyde solution (9.25%, pH 9.3) [26]. Then, the suspension was stirred at 70°C for 20 min. Afterwards, the mixture was filtered and washed with water. The nanoparticles were suspended in 25 ml of polyethylenimine (5%, Sigma Aldrich), and the suspension was incubated at 25°C for 3 h. Then, it was filtered and washed with water. The support was again suspended in 25 ml glutaraldehyde solution (1%), incubated at 25°C for 30 min, washed with water, and dried under vacuum.

2.2 Support characterization

X-ray diffraction (XRD) measurements were carried out with a RigakuMiniflex II using Cu Kα radiation (λ = 1.54056 Å). X-ray photoelectron spectroscopy (XPS) analyses were performed in a Thermo Scientific Escalab 250 XI Photoelectron spectrometer with monochromatic Al K radiation (hυ = 1486.6 eV). Core-level peak positions were determined after background subtraction according to Shirley using Avantage software.

2.3 Immobilization of urease

In a general procedure, magnetic supports were added to 0.01 M sodium phosphate buffer pH 5.8 with stirring at 4°C. Afterwards, the solids were dispersed with a urease solution (Jack beam 5 U/ml, Sigma Aldrich) for 24 h. The supernatant was collected by centrifugation. The magnetic particles with immobilized enzyme were washed several times with 0.01 M solution of phosphate buffer and stored in 1 ml of phosphate buffer. The amount of immobilized urease was calculated from the initial urease amount minus the amount remaining in the supernatant using the Bradford method [27].

2.4 Urease activity

To optimize the immobilization process, free and immobilized urease activities were determined spectrophotometrically at 488 nm. The enzymatic activity was expressed as micromoles of ammonium produced per minute. The relative activity is the ratio between the enzymatic activity of the immobilized enzyme (Ai) and the enzymatic activity of the free enzyme (Ao) expressed as:

$$\%\ \text{Relative activity} = \frac{\text{Ai}}{\text{Ao}} \times 100 \tag{1}$$

The catalytic efficiency was used as an index for comparison of enzymes acting on the same substrate. It is also known as catalytic or constant yield potential [28], and it is defined as

$$\text{Catalytic efficiency} = \frac{V_{\max}}{k_m} \tag{2}$$

2.5 Optimization of urease immobilization

The immobilization of urease was optimized by modifying only one variable in each experiment and keeping all others constant. The effect of immobilization time was studied in the range 0–18 h. The temperature was varied in the range 4°C–30°C, and the support to urease ratio (mg/g support) varied from 300 to 5000. A pH value near 6.0 was used because as previously reported, this pH was better for urease activity. The stability of immobilized enzyme was evaluated at 4°C for 30 days, and the reuse was studied for 5 recycles using the same concentration of urea to maintain urease activity constant.

2.6 Kinetic parameters of immobilized urease

The method of Lineweaver-Burk was used to determine the The Michaelis constant (k_m) value. The enzymatic activity was tested using 50 ul immobilized urease and modifying the substrate concentration from 0.02 to 0.1 mol/l. The reaction conditions were 10 min, pH 5.8, and 30°C.

2.7 Application of immobilized urease for the synthesis of 1,4-DHPs using the Biginelli synthesis

Free and immobilized urease was added to a mixture of 3 mol of benzaldehyde (99%, Sigma Aldrich), 1 mol of ethyl acetate (EtOAc) (99.5%, Sigma Aldrich), and 2 mol of urea (98%, Sigma Aldrich). The mixture was stirred at 70°C in water until the product precipitated. The reaction was monitored by thin layer chromatography using hexane:EtOAc (70 : 30). The reaction product was washed with cold

water and filtered to give the pure 1,4-DHP product, and then the yield was calculated.

3 Results and discussion

3.1 Characterization of magnetic supports

The crystalline structure of FSN and FSN-A particles was investigated by XRD. As shown in Figure 1, the diffraction peaks (2θ) at 18.2°, 30.3°, 35.6°, 43.3°, 53.8°, 57.3°, and 62.8° are ascribed to the (111), (220), (311), (400), (422), (511) and (440) planes of Fe_3O_4 (JCPDS no. 19-0629). The signal near $2\theta = 22°$ is assigned to the amorphous silica. This signal is broader in FSN-A.

X-ray photoelectron spectra were obtained to compare the surface modification performance of FSN-A in the cross-linking process with glutaraldehyde and polyethylenimine. The XPS spectra collected in the region of N 1s are shown in Figure 2. The N 1s peak was deconvoluted into two spectral bands at 399.1 and 401.1 eV, which correspond to protonated and unprotonated species present in APTES [29]. By considering the relative proportions of these two species, it is concluded that unprotonated species increase in the cross-linking process.

Figure 3 displays the XPS in the Fe 2 $p_{3/2}$ and $p_{1/2}$ regions. The absence of signals associated with Fe 2 $p_{3/2}$ and $p_{1/2}$ suggests that the Fe_3O_4 particles were totally covered in the FSN-A solid.

3.2 Urease activity

The optimization of urease immobilization by physical adsorption was performed in FSN, while covalent

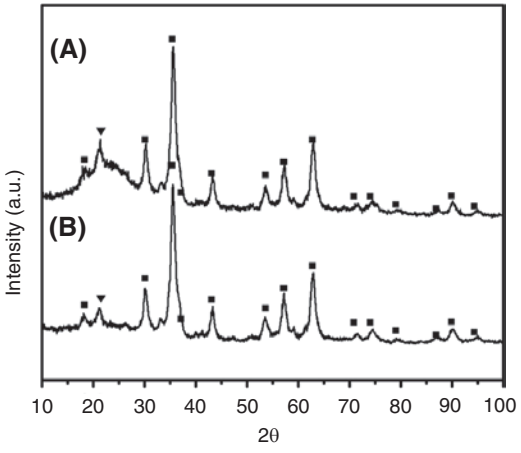

Figure 1: X-ray diffraction patterns for: (A) FSN and (B) FSN-A. (■) Fe_3O_4, (▼) SiO_2.

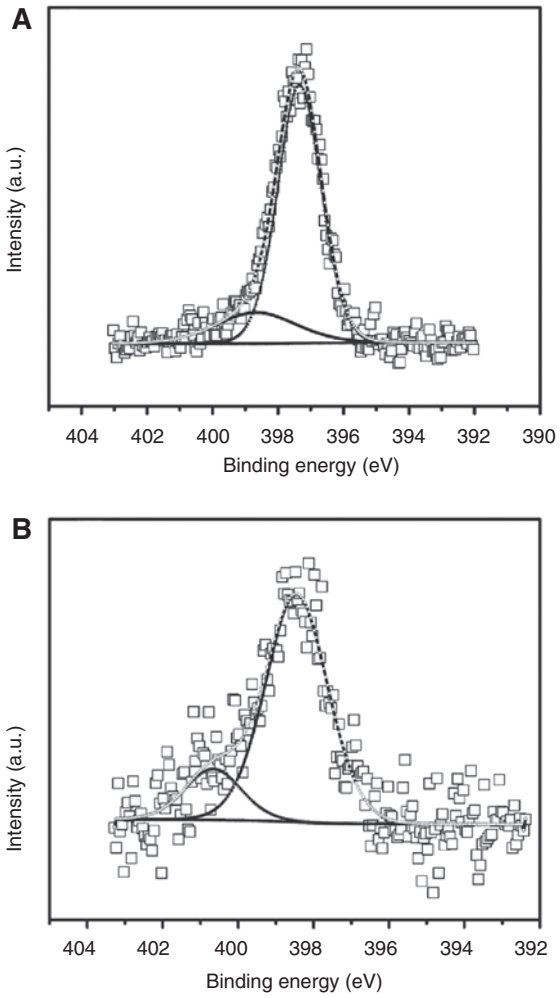

Figure 2: X-ray photoelectron spectra in the region of N 1s (A) FSN-A and (B) FSN.

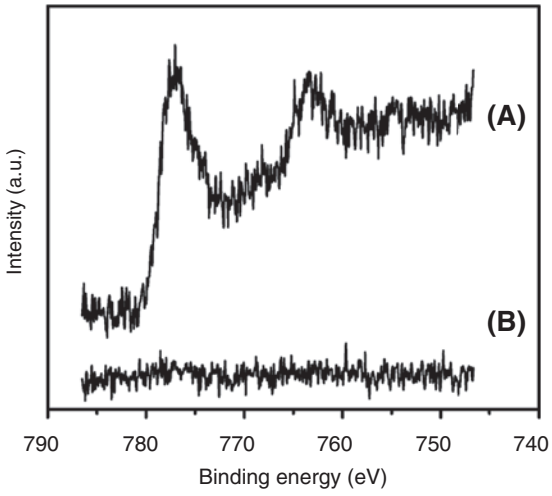

Figure 3: X-ray photoelectron spectra in the region Fe 2p. (A) FSN and (B) FSN-A.

Table 1: Relative activity and adsorbed urease at different concentrations of urease in the process of immobilization (physical and covalent immobilization).

Urease concentration ($mg/g_{support}$)	Physical adsorption FSN[a]		Covalent bonding FSN-A[b]	
	Relative activity (%)	Adsorbed urease (%)	Relative activity (%)	Adsorbed urease (%)
300	80	54	80	87
500	75	41	85	90
1000	71	39	47	90
5000	69	38	47	90

[a]FSN, Fe_3O_4–SiO_2–NH_2; [b]FSN-A, activated Fe_3O_4–SiO_2–NH_2.

immobilization was studied with the FSN-A support. Table 1 lists the results of adsorbed urease and enzymatic activity expressed as relative activity at different concentrations of urease. In both methods, the amount of coupled urease and relative activity decreased with an increase in urease concentration. This is due to the competition of enzyme molecules by surface of chemical groups [11] and possible blocking pores of the support by proteins neighborhood [30, 31]. Higher protein concentrations did not yield better immobilization.

Similar results by physical adsorption were reported by Pogorilyi et al. [14] using a poly (3-aminopropyl) siloxane matrix; they indicated 94% adsorbed urease and 73% residual activity. However, in the covalent adsorption, the values of relative activity are lower than other previously reported ones. Krishna et al. [2] found a relative urease activity near 90% on chitosan beads activated with 3% glutaraldehyde. In this work, the same result was obtained with 0.1 mg/ml and 1% glutaraldehyde concentration in activation time.

The coupling time for attachment of urease on the surface of solids was also optimized by varying it from 4 to 24 h (Figure 4). The immobilization time should be optimized due to conformational changes in the tertiary structure of the enzyme that occur during this process. Regarding physical adsorption, the highest percentage of immobilization and relative activity was found at 20 h (Figure 4A). Pogorilyi et al. [14], and Ayhan et al. [32] reported a higher percentage of enzyme adsorption and relative activity between 94% and 100%, with shorter immobilization time (1–4 h). This difference with our results is due to the immobilization temperature used by these authors (25°C).

The percentage of immobilization in covalent adsorption is reached in 6 h, and the highest relative activity in 24 h (Figure 4B). However, the activity of the covalently bound urease was significantly lower than that of the adsorbed urease, in agreement with the results of Pogorilyi et al. [33]. Covalent grafting is a method that can change the enzymatic structure.

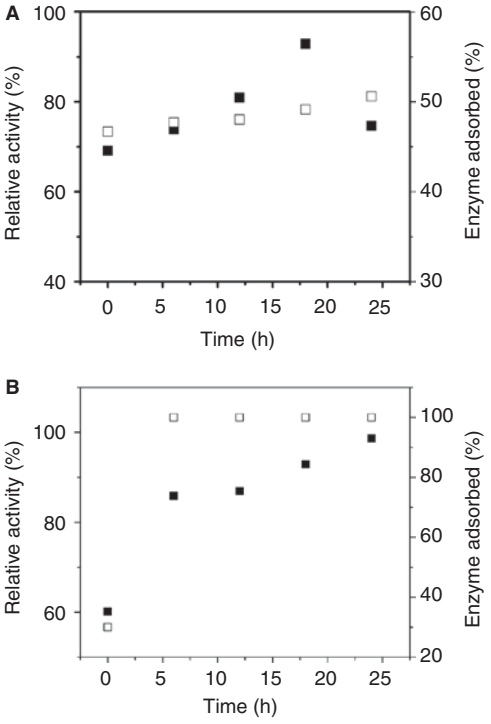

Figure 4: Effect of time immobilization of urease by physical immobilization and covalent bonding.
Relative activity (■) and adsorbed urease (□).

The effect of immobilization temperature on enzymatic activity is shown in Table 2. A temperature of 4°C for both immobilization methods was chosen to improve urease stability. Enzymatic deactivation occurs at room temperature [2, 34, 35] and increases in adsorbed urease by physical adsorption.

3.3 Stability, reuse, and kinetic parameters

The stability of immobilized urease is shown in Figure 5. The enzymatic activity was studied for 30 days. Using both methods of immobilization, the relative enzymatic activity

Table 2: Relative activity (Rel. act) and adsorbed urease (Ads. Enz.) at different temperatures of immobilization for both methods.

Temperature (°C)	Physical adsorption FSN[a]		Covalent bonding FSN-A[b]	
	Rel. act. (%)	Ads. Enz. (%)	Rel. act. (%)	Ads. Enz. (%)
4	71	52	89	90
19	66	43	70	78
25	50	35	53	28

[a]FSN, Fe_3O_4–SiO_2–NH_2; [b]FSN-A, activated Fe_3O_4–SiO_2–NH_2.

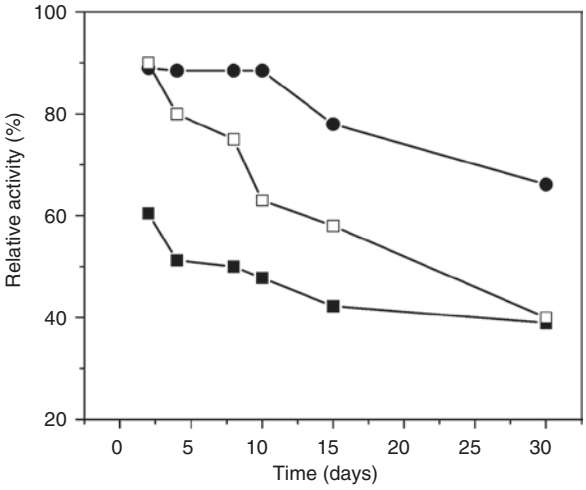

Figure 5: Stability study in terms of relative activity (%) free urease (●) urease immobilized by physical adsorption (■) and by covalent bonding (□).

is near 40% after 30 days, while the relative activity of free urease is 70% at the same time. This behavior is explained by the desorption or denaturation of the enzyme [34].

The enzyme reuse was studied in 10 cycles (Figure 6), obtaining a relative activity near 20% in 8 cycles. Other authors such as Ispirli Doğaç et al. [34] and Krishna et al. [2] reported the enzyme reuse for 11–14 cycles, respectively; this is because they did not wash out the excess dye.

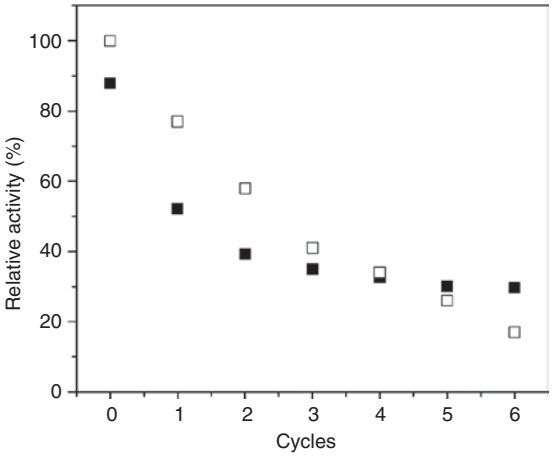

Figure 6: Immobilized urease recycles. Physical adsorption (■) covalent bonding (□).

The kinetic parameters of immobilized urease were compared to those of free urease. The V_{max} values and the k_m values are presented in Table 3. It can be observed that the maximum velocity (V_{max}) value decreases, but the k_m value considerably increases. The lowest k_m values obtained mean a loss of enzyme affinity by substrate when the enzyme is immobilized. This is because many active sites of the enzyme are buried or blocked inside the support surface. This phenomenon occurs preferentially in the covalent bonding method. However, both methods show similar catalytic efficiency.

3.4 Syntheses of 1,4-DHPs

Syntheses of 1,4-DHPs were carried out with benzaldehyde, ethyl acetoacetate, and urea catalyzed by immobilized

Table 3: Comparison of kinetic parameters of urease immobilized by physical adsorption and covalent bonding.

Support	k_m (mM·ml·$^{-1}$)	V_{max} (μmol/l·min^{-1})	Catalytic efficiency (%)
Free urease	0.0015	77.51	51
Urease-FSN[a]	0.0038	54.64	15
Urease-FSN-A[b]	0.0042	71.42	17

[a]FSN, Fe_3O_4–SiO_2–NH_2; [b]FSN-A, activated Fe_3O_4–SiO_2–NH_2.

Table 4: Yield (%) of urease-catalyzed synthesis of 1,4-DHPs.

Support	Yield to Hantzsch product (%)	Yield to Biginelli product (%)
Free urease	27.29	11.60
Urease-FSN[a]	90.57	10
Urease-FSN-A[b]	78.27	5.3
FSN[a]	NR	NR

[a]FSN, Fe_3O_4–SiO_2–NH_2; [b]FSN-A, activated Fe_3O_4–SiO_2–NH_2.

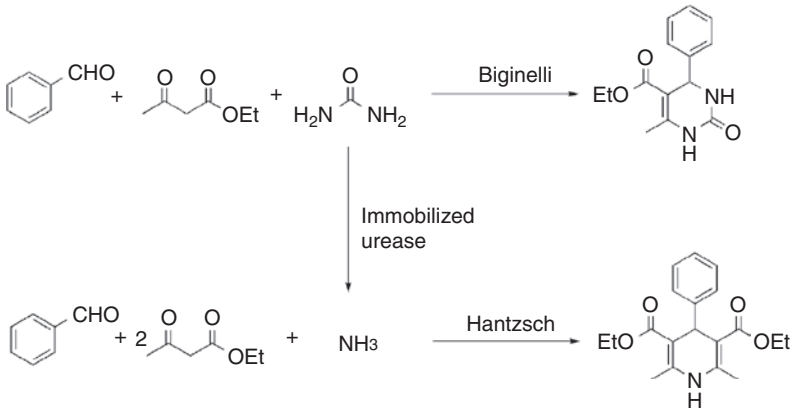

Figure 7: Routes in the formation of Biginelli and Hanztch products.

and free urease. The products of the Biginelli/Hantzsch reactions were obtained by isolating them via dilution of the crude product with cold water. The results of yields are listed in Table 4. Product A was separated by distillation of ethanol, and the remaining solid B was isolated by filtering. The experimental control using only the supports without urease under similar conditions did not give the product even after 24 h, indicating that the presence of urease is necessary for urea dissociation.

As shown in Table 4, the most interesting result is obtained when the enzyme is immobilized onto the support Fe_3O_4–SiO_2–NH_2 due to the highest yield to product A. Tamaddon and Ghazi [5] reported selectivity to product A of 60% and 80% conversion, carrying out the reaction under the same conditions using free urease.

The experimental control using only the supports without urease under similar conditions did not give the product even after 24 h, indicating that the presence of urease is necessary for urea dissociation. The mechanism of Hantzch and Biginelli has been well described by Kappe [36]. The urease acts hydrolyzing the urea and improving the NH_3 disponibility. As the urea is hydrolyzed by the enzyme the Hantzch product is favored. The Biginelli product is formed by the reaction of aldehyde with urea through acyl imine intermediate. The urease favors preferentially the Hanztch product while the Biginelli product is formed without the presence of the enzyme. Figure 7 shows schematically the summary of the products obtained.

Considering that some dihydropyrimidines are inhibitors of certain ureases [4] the products formed possibly cause the deactivation of the free enzyme, phenomena that does not occur when the enzyme is immobilized. It is noteworthy that urease immobilization by any of the immobilization methods enabled obtaining the product of the Hantzsch reaction. Although, the urease immobilized has a lower affinity of the enzyme towards urea (k_m values of immobilized urease is approximately 2.5 times higher than the free enzyme), the disponibility of NH_3 is sufficient for favoring the Hanztch product, and the confinement of enzyme in the support prevents the inhibition with products formed. However, this study will require a major reaction optimization

4 Conclusions

Fe_3O_4/SiO_2 was modified with APTES and then activated with glutaraldehyde allowing a total coverage of Fe_3O_4 particles. The solids can be easily separated from the reaction medium by applying a strong magnetic field. A ratio of 500 mg of enzyme per gram of support at 4°C and 18 h was enough for the physical adsorption, while 24 h were required for covalent bonding. Using both methods of immobilization, the relative enzymatic activity is near 40% after 30 days. The enzyme reuse was studied in 10 cycles obtaining a relative activity near 20% in 8 cycles. The Biginelli and Hantzsch reactions were used to evaluate urease application in an MCR. It is noteworthy that urease immobilization by any of the immobilization methods enabled obtaining products of the Hantzsch reaction.

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References

[1] Sahoo B, Sahu SK, Pramanik P. *J. Mol. Catal. B: Enzym.* 2011, 69, 95–102.

[2] Krishna BL, Singh AN, Patra S, Dubey VK. *Process Biochem.* 2011, 46, 1486–1491.
[3] Tibhe JD. *Green Process. Synth.* 2012, 1, 557–558.
[4] Khan A, Hashim J, Arshad N, Khan I, Siddiqui N, Wadood A, Ali M, Arshad F, Khan KM, Choudhary MI. *Bioorg. Chem.* 2016, 64, 85–96.
[5] Tamaddon F, Ghazi S. *Catal. Commun.* 2015, 72, 63–67.
[6] Puripat M, Ramozzi R, Hatanaka M, Parasuk W, Parasuk V, Morokuma K. *J. Org. Chem.* 2015, 80, 6959–6967.
[7] Silva GCO, Correa JR, Rodrigues MO, Alvim HGO, Guido BC, Gatto CC, Wanderley KA, Fioramonte M, Gozzo FC, de Souza ROMA, Neto BAD, *RSC Adv.* 2015, 5, 48506–48515.
[8] Tamaddon F, Moradi S. *J. Mol. Catal. A Chem.* 2013, 370, 117–122.
[9] Alvim HGO, da Silva Junior EN, Neto BAD. *RSC Adv.* 2014, 4, 54282–54299.
[10] Zhou J, Cao J, Huang W, Huang L, Wang Y, Zhang S, Yuan Y, Hua D. *Chem. Biochem. Eng. Q.* 2013, 27, 431–437.
[11] Luo Z, Fu X. *Starch – Stärke* 2010, 62, 652–657.
[12] Ali SMU, Ibupoto ZH, Salman S, Nur O, Willander M, Danielsson B. *Sensors Actuators B Chem.* 2011, 160, 637–643.
[13] Ashtari K, Khajeh K, Fasihi J, Ashtari P, Ramazani A, Vali H. *Int. J. Biol. Macromol.* 2012, 50, 1063–1069.
[14] Pogorilyi R, Melnyk I, Zub Y, Seisenbaeva G, Kessler V, Shcherbatyik M, Košak A, Lobnik A. *J. Sol-Gel Sci. Technol.* 2013, 68, 447–454.
[15] Ansari SA, Husain Q. *Biotechnol. Adv.* 2012, 30, 512–523.
[16] Das S, Carnicer-Lombarte A, Fawcett JW, Bora U. *Progr. Neurobiol.* 2016, 142, 1–22.
[17] Jaber J, Mohsen E. *Colloids Surf. B Biointerf.* 2013, 102, 265–272.
[18] Pogorilyi RP, Melnyk IV, Zub YL, Seisenbaeva GA, Kessler VG. *J. Mater. Chem. B* 2014, 2, 2694–2702.
[19] Kumar S, Jana AK, Dhamija I, Singla Y, Maiti M. *Eur. J. Pharm. Biopharm.* 2013, 85, 413–426.
[20] Alftrén J, Hobley TJ. *Biomass Bioenerg.* 2014, 65, 72–78.
[21] Tian Z, Wu K, Liu W, Shen L, Li G. *Spectrochim Acta Part A Mol. Biomol. Spectrosc.* 2015, 140, 356–363.
[22] Moghaddam FM, Ayati SE. *RSC Adv.* 2015, 5, 3894–3902.
[23] Wu W, He Q, Jiang C. *Nanoscale Res. Lett.* 2008, 3, 397–415.
[24] Luo B, Song XJ, Zhang F, Xia A, Yang WL, Hu JH, Wang CC. *Langmuir* 2010, 26, 1674–1679.
[25] Díez P, Villalonga R, Villalonga ML, Pingarrón JM. *J. Colloid Interf. Sci.* 2012, 386, 181–188.
[26] Bezbradica DI, Mateo C, Guisan JM. *J. Mol. Catal. B: Enzym.* 2014, 102, 218–224.
[27] Wenrich BR, Trumbo TA. *Anal. Biochem.* 2012, 428, 93–95.
[28] Eisenthal R, Danson MJ, Hough DW. *Trends Biotechnol.* 2007, 25, 247–249.
[29] Scaffaro R, Botta L, Re GL, Bertani R, Milani R, Sassi A. *J. Mater. Chem.* 2011, 21, 3849–3857.
[30] Valerio SG, Alves JS, Klein MP, Rodrigues RC, Hertz PF. *Carbohydrate Polym.* 2013, 92, 462–468.
[31] Amaya-Delgado L, Hidalgo-Lara ME, Montes-Horcasitas MC. *Food Chem.* 2006, 99, 99–304.
[32] Ayhan F, Ayhan H, Pişkin E, Tanyolaç A. *Bioresour. Technol.* 2002, 81, 131–140.
[33] Pogorilyi RP, Goncharik VP, Kozhara LI, Zub YL. *Appl. Biochem. Microbiol.* 2008, 44, 561–565.
[34] Ispirli Doğaç Y, Deveci İ, Teke M, Mercimek B. *Mater. Sci. Eng. C* 2014, 42, 429–435.
[35] Kutcherlapati SNR, Yeole N, Jana T. *J. Colloid Interf. Sci.* 2016, 463, 164–172.
[36] Kappe CO. *J. Org. Chem.* 1997, 62, 7201–7204.

Bionotes

Annie Y. Vargas

Annie Y. Vargas received her MS degree from the Pedagogical and Technological University of Colombia (2015). Her research interests focus on enzyme immobilization for the application in biomass transformations.

Hugo A. Rojas

Hugo A. Rojas received his PhD degree in chemistry from Conception University of Chile (2010). Currently, he is a titular professor at the Pedagogical and Technological University of Colombia. His research interests include the development of novel methodologies in organic synthesis, principally reduction and oxidation reactions. He published 44 scientific papers in international journals.

Gustavo P. Romanelli

Gustavo P. Romanelli is a titular professor at the National University of the Plata (UNLP-CCT-CONICET), Argentina. His research program focuses on the development of new recoverable catalysts for multicomponent reactions. He obtained his PhD from UNLP (1994). He published 250 internationally refereed journal papers on catalysis and organic chemistry.

José J. Martínez

José J. Martínez received his PhD degree from the National University of Colombia (2013). Currently, he is an Associated Professor at the Pedagogical and Technological University of Colombia. His current research interests include biomass transformation using heterogeneous catalysts and biocatalysts. He published 22 scientific papers on catalysis and green chemistry.