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Heterogenization of an organic catalyst by adsorption on alginic acid gels

Tesi di Laurea Sperimentale

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Abstract

A sustainable approach focused on the practice of green chemistry was used to develop a method which improved the performances of a catalyst system based on a natural and non-toxic substance. A benchmark Michael addition reaction was performed employing 9-amino(9-deoxy)*epi quinine*, adsorbed on alginic acid gels by hydrogen bonds, as catalyst. Compared to conventional heterogeneization of this organic catalyst, the present approach is more straightforward and employs as support a renewable biomaterial instead of oil-derived polymers. The optimization of the adsorption protocol was carried out to obtain an active and heterogeneous system able to work under different reaction temperatures. The Michael addition reaction rate, heterogeneity, enantiomeric excess and recyclability of the catalytic system were studied. The influence of temperature, additives and the presence of water were successfully investigated. The heterogeneity of the catalyst was perfectly preserved, therefore the catalyst could be easily recovered. Two optimal conditions were disclosed, differing in reaction temperature and catalyst pre-treatment. A scale-up is performed with good results for the first three reactions cycles (conversions: 100%, 87% and 55% respectively). The enantiomeric excess is determined as 98%. The results of this project demonstrated that a green catalytic system has a great potential to be competitive with more classic heterogeneous catalysts.

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1. Introduction

1.1. Organocatalysis

In the last two decades, the use of small organic molecules to catalyze organic transformations has become more and more frequent.[1] It is now widely accepted that organocatalysis is one of the main branches of enantioselective synthesis, the other being enzymatic and organometallic catalysis.

Before 1998, the state of the art in asymmetric catalysis involved basically metal-based chiral catalysts, which allowed a wealth of oxidations, reductions, σ-bond insertions, π-bond activations and Lewis-acid-catalyzed reactions. Although their impact on chemical synthesis cannot be understated, some organometallic systems can be expensive, toxic and/or sensitive to atmosphere conditions such as the presence of air and moisture. On the contrary, organic molecules are generally insensitive to oxygen and moisture in the atmosphere, so there is no need for special reaction vessels, or for ultra-dry reagents and solvents.

The development of organocatalysis, as a complementary mode of catalysis, showed the opportunity to save costs, time and energy, as well as to implement easier experimental procedures and reductions in chemical wastes. A wide variety of organic reagents are naturally available from biological sources as single enantiomers, which enables easy and cheap preparations of organic catalysts.

Despite organic systems are usually less reactive than organometallics, they are more biodegradable and most importantly do not contain transition metals, which may be harmful for humans and/or the environment. Small organic molecules are instead typically non-toxic and environmentally friendly, increasing the safety of catalysis in both biological and chemical research. Even if it might appear that low turnover numbers limit the potential uses of organocatalysis for industrial applications, the most salient considerations for large-scale-catalytic processes are costs and safety. In this sense, organocatalysts are often cheaper than metal-based systems and they do not require the removal of toxic catalyst-related impurities from the waste stream, which may have a large financial impact.

The catalyst **QNA** used in this project is a derivative of a cheap *Cinchona* alkaloid, *quinine* (**QN**), which has been exploited in the past as pharmaceutical for the treatment of diseases, mainly malaria. Among all organic catalysts that have been employed in recent years, the 9-amino-9-deoxy-*epi*-chincona derivatives have shown great ability to catalyze several transformations, including organocascade reactions proceeding either through iminium ion/enamine sequence or enamine/iminium ion formation.[2] The great versatility and the excellent levels of enantioselectivity shown by 9-amino-cinchona derivatives in organocatalyzed reactions has promoted the development of a modern approach to the synthesis of active pharmaceutical ingredients, fine chemicals and chiral intermediates.

From the natural product *quinine*, the catalyst **QNA** is synthetized through a Mitsunobu type reaction with an inversion of the stereochemistry, as shown in Figure 1.

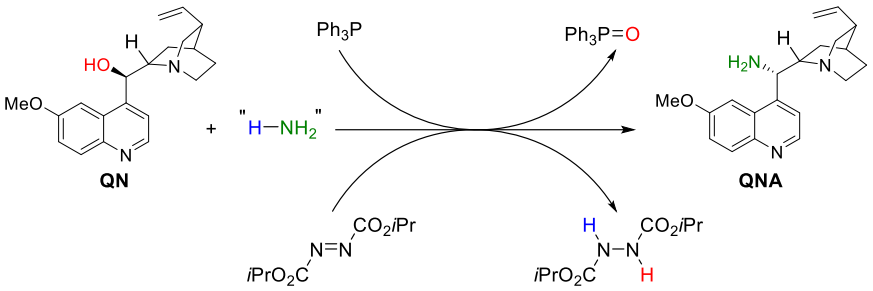


Figure 1

The quinine **QN** reacts with an ammonia surrogate to form the desired catalytic compound **QNA** ((*S*)-(6-methoxyquinolin-4-yl)((1*S*,2*R*,4*S*,5*R*)-5-vinylquinuclidin-2-yl)methanamine). The reaction proceeds in presence of a dialkyl azodicarboxylate and triphenylphosphine. At the end of the reaction two co-products (hydrazide and phosphine oxide) are also formed.

As **QNA** is most conveniently isolated and stored as a salt because of its higher stability, it needs to be converted to free **QNA** before being employed as a catalyst (Figure 2). The salt is neutralized with concentrated ammonium hydroxide and **QNA** is extracted with dichloromethane.

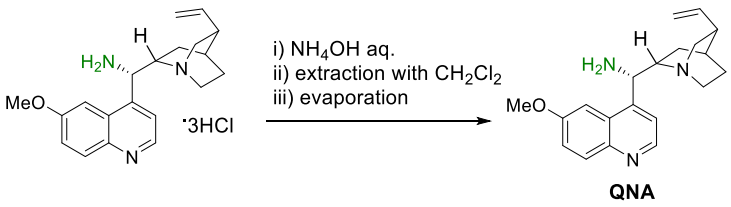


Figure 2

The catalyst is composed of three amine functions highlighted in Figure 3: the nitrogen contained in the bicycle is the most basic (red), while the amine function linked to the chiral carbon (green) is the active part of the catalyst. The remaining amine function (blue) does not have an obvious role in the reactions.

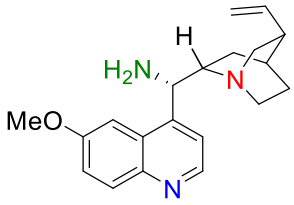
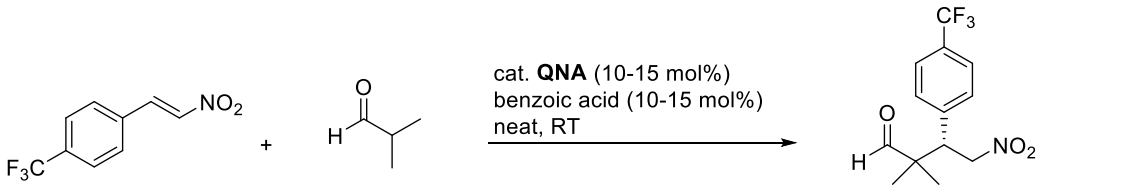


Figure 3

This simple alkaloid derivative, available via one-pot procedure from commercial starting materials, has proven to promote highly enantio- and diastereoselective Michael-type addition between enolizable carbonyl compounds and nitroalkenes.[3] The Michael reaction between nucleophiles and nitrolefins represents a particularly attractive target, due to the availability and reactivity of nitroalkenes, the possibility for suitably designed catalyst systems to create hydrogen bonds with the nitro functionality and obviously the high utility of the nitroalkane adducts.

In this thesis, to investigate the performances of the catalyst, the Michael addition reaction of *iso*butyraldehyde **2** to (*E*)-1-(2-nitrovinyl)-4-(trifluoromethyl)benzene **1**, forming the nitroalkane **3**, is employed as test reaction (Figure 4).

It is known that this reaction can be efficiently carried out under homogeneous conditions using a combination of **QNA** and an acidic co-catalyst, resulting in high enantioselectivity, favoring the (*S*) enantiomer of product.[3]



1: (*E*)-1-(2-nitrovinyl)-4-(trifluoromethyl)benzene

2: isobutyraldehyde

(*S*)-**3**: (*S*)-2,2-dimethyl-4-nitro-3-(4-(trifluoromethyl)phenyl)butanal

Figure 4

The catalytic cycle detailed in Figure 5 involves the formation of a key enamine intermediate, that can form only if the catalyst is present; otherwise no product is obtained. On the first step, the aldehyde carbonylic group is activated to form an imine by condensation, from which one water molecule is released. The subsequent tautomerism brings to the obtainment of the corresponding enamine intermediate. At this point, the nitrostyrene substrate **1** enters the cycle suffering the addition of the highly nucleophilic enamine species, approaching the enamine with its electron poor double bond. In the last step, the hydrolysis of the resulting iminium ion leads to the release of the product **3**. The catalyst **QNA** is now in its active form ready to initiate other cycles until its deactivation or limiting reagent exhaustion.

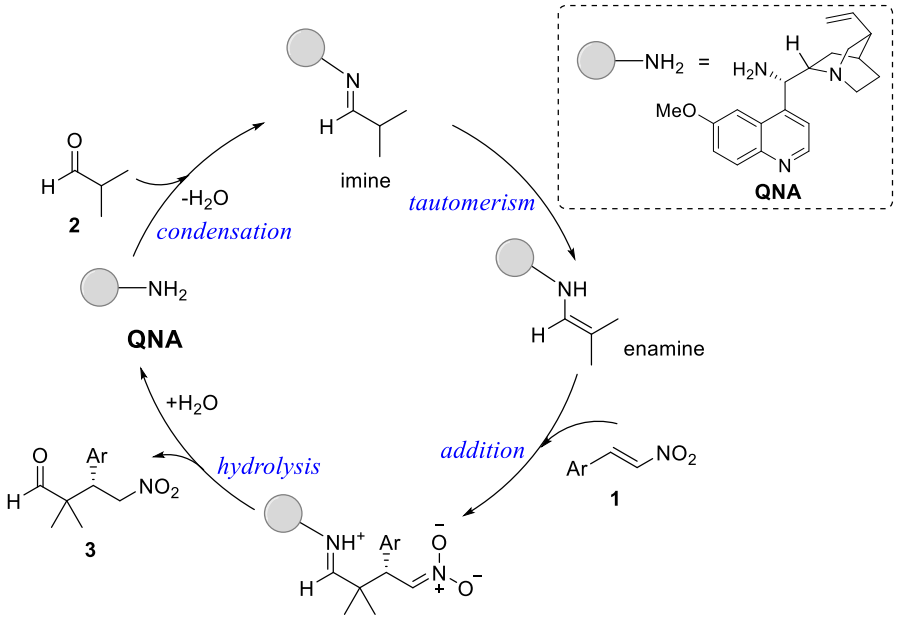


Figure 5

The acidic co-catalyst, presumably protonating the basic tertiary amine of the catalyst, can assist in all steps of the cycle. This type of reaction is highly enantioselective (*ee* > 95%). This feature is mainly determined during the enamine attack to the nitroalkene substrate.

1.2. Supported organic catalyst

One of the most explored approaches to overcome the limitations regarding organocatalytic systems such as high catalyst loading, difficult product separation and catalyst recycling is the immobilization of organocatalysts on solid supports.[4] Typically,

the immobilization is carried out *via* covalent attachment onto supports as polystyrene (PS), poly(ethylene glycol) (PEG), and inorganic solids. The general strategy to prepare polystyrene-supported 9-amino-cinchona derivatives involves several different steps (Figure 6). First of all there must be a linker suitable for polymerization on the quinuclidine ring: the double bond of the quinine is converted in a triple bond to allow the addition of a styrene moiety. The resulting alcohol is then converted into the amine employed in a radical copolymerization with divinylbenzene in the presence of azobis*iso*butyronitrile (AIBN) as radical initiator.[5]

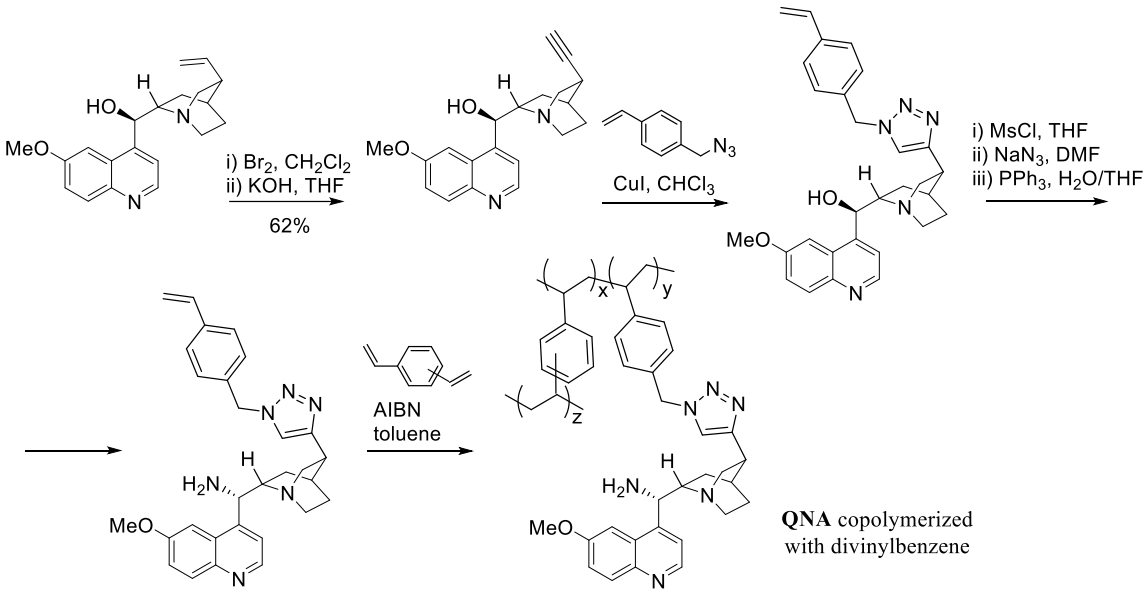


Figure 6

However, if compared to their homogeneous versions, most of these supported systems demonstrated reduced activity and selectivity, requiring higher catalyst loadings (from 10 to 20% mol) to attain reasonable yields. Moreover, these commonly used covalent immobilization methods usually involve multiple synthetic manipulations and the subsequent structural perturbations may lead to deterioration of their catalytic behaviors.

An alternative and conceptually appealing catalyst immobilization strategy can be achieved linking the catalyst to the support by non-covalent interactions.[4] Such approach requires minimal synthetic operations, as it is usually realized simply mixing catalyst and support in an appropriate medium. Furthermore, minimal structural perturbation enables in principle optimal activity and selectivity. However, leaching of the catalyst from the support is relatively more facile compared to the covalent approach.

Among all modes of non-covalent immobilizations (ion-pairs, hydrophobic interactions, as well as self-assembled gel-type organocatalysts), an acid-base interaction has been used in this project. Such type of interaction has already proven to be successful using e.g. sulfonic acid derivatized polystyrene as support and a secondary-tertiary amine as catalyst. The Figure 7 shows the ability of the PS-sulfonic acid supported catalyst (a proline derivative) to drive direct aldol reactions.

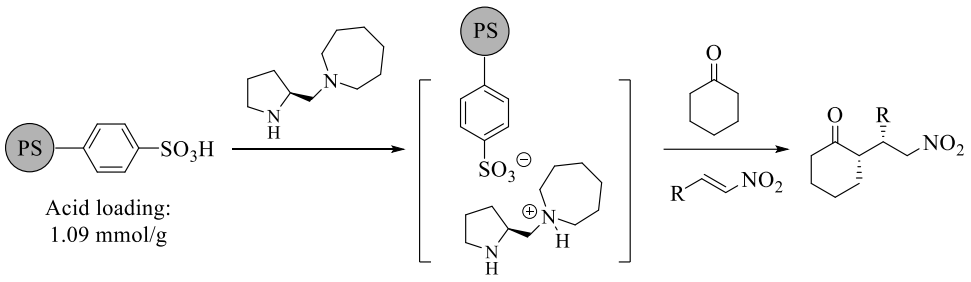


Figure 7

1.3. Alginic acid

Alginates are natural polysaccharides derived from seaweed which satisfy the appropriate requirements for heterogeneous catalysts and supports: they are stable in most organic solvents and can be manipulated to form gels presenting a high surface area and many accessible surface functionalities.[6]

This family of polysaccharides is mainly produced by brown algae. They are constituted of (1→4) linked β-D-mannuronic (M) and α-L-guluronic (G) residues (Figure 8), according to three types of sequences $(M)_m$, $(G)_n$ and $(M, G)_x$.

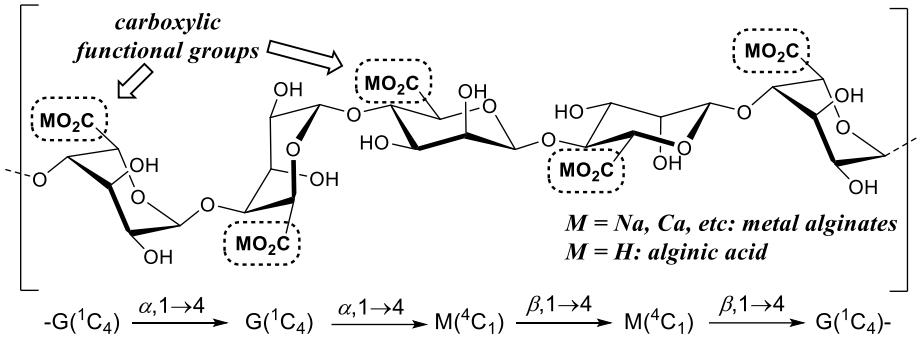


Figure 8

Their availability in nature is virtually unlimited and industrial processes for their extraction already run at the plant scale.

Most applications of alginates in drug release systems and as supports in catalysis are based on their ability to form strong gels with divalent or trivalent cations (Figure 9) or under acidic conditions.

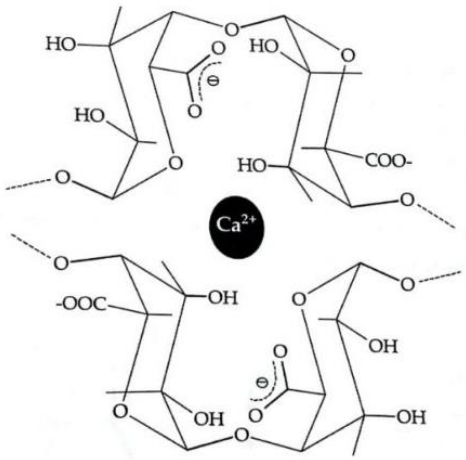


Figure 9

The possibility to exploit this polysaccharide as a solid support starts with its natural tendency to form, in an aqueous media and at low values of pH, highly dispersed alginic acid *hydrogel* spheres.[7] The high abundance of functional groups in alginate polymeric structure (i.e. 5.6 mmol/g carboxylate groups) and the dispersed 3D arrangement of the polymeric chains are responsible for the easy entrapment of catalytic species and for the interaction with the substrates involved in the given reaction. After solvent exchange from water to a chosen solvent, it is possible to obtain the corresponding *solvogel* spheres. For gels in which water has been replaced by an alcoholic solvent, the label *alcogel* can also be used. Solvent exchange is carried out either by soaking the beads directly in the new solvent (one-step) or by sequential soakings in different water-solvent mixtures with progressively increasing content of the new solvent (multi-step).

Depending on the rapidity of the exchange and the nature of the chosen solvent, a partial shrinkage of the hydrogel beads must be taken into account: the increase of organic solvent interferes with the release of water from the gel structure, leading to a reduction of surface tension in the gel pores. This decrease of the capillary pressure of the gel structure is responsible for the reduction in volume of the beads during solvent exchange.

Alginate gels in their wet state are advantageous for the high dispersion and accessibility of the hydrophilic polysaccharide chains. On the other hand, dried materials allow easier

handling, storage and transport; a drying method that helps maintaining the beneficial properties of the wet state in the dry state is highly desirable.

Among all drying techniques, CO_2 supercritical drying is an effective method to preserve dispersion, open porosity and superior textural properties of the wet state in a dry form, giving the resulting aerogel spheres. The near zero surface tension of the supercritical solvent and its mild critical points conditions (31 °C, 74 bar) prevent the collapse of the gels and any temperature-dependent change in the structure during drying.

Considering the low miscibility of water and liquid carbon dioxide, direct drying of the hydrogel is not possible and an intermediate exchange step has to be carried out. Ethanol has historically been the solvent of choice, since it is not able to dissolve or alter the gel network and present complete miscibility with both water and CO_2.[8] Once the alcogel is obtained (through multiple exchanges with alcoholic solutions), it is introduced in a pressure vessel and washed with liquid CO_2, in order to replace the ethanol in the gel. The resulting impregnated beads are then compressed and heated above the critical point to attain the supercritical state; release of pressure above the critical temperature allows to remove CO_2 without forming any liquid-vapor interface, leading to the resulting aerogel (Figure 10).

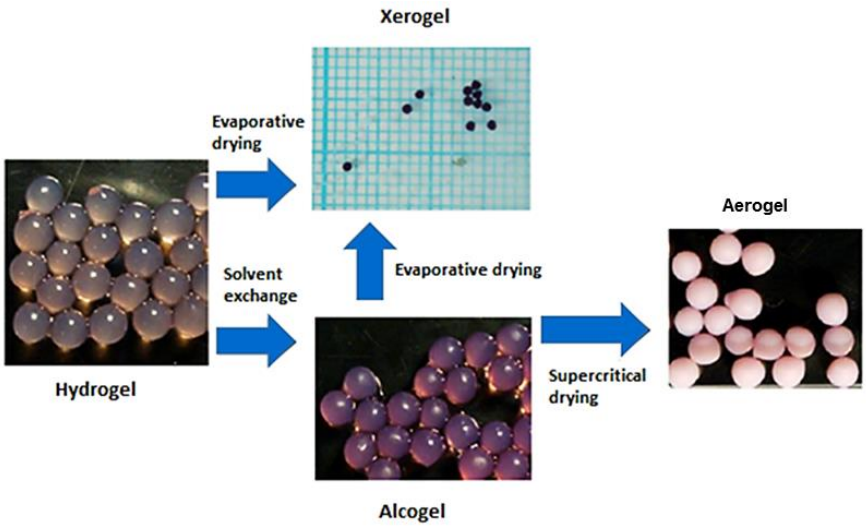


Figure 10

Direct evaporative drying, e.g. the removal of the gel solvent by evaporation, is undoubtedly the simplest and less expensive drying procedure. However the structure of the wet gel is not retained as the beads are almost completely shrank and a virtually non-porous material, called *xerogel*, is obtained.

2. Aim and objectives

The aim of this project was to develop a heterogeneous organocatalyst able to catalyze stereoselective reactions, ensuring both sustainability and recyclability in a catalytic process. The work has focused on the adsorption of **QNA** catalyst on alginic acid gels, and on testing its performances in a benchmark Michael addition reaction between nitroalkene **1** and isobutyraldehyde **2** (Figure 11).

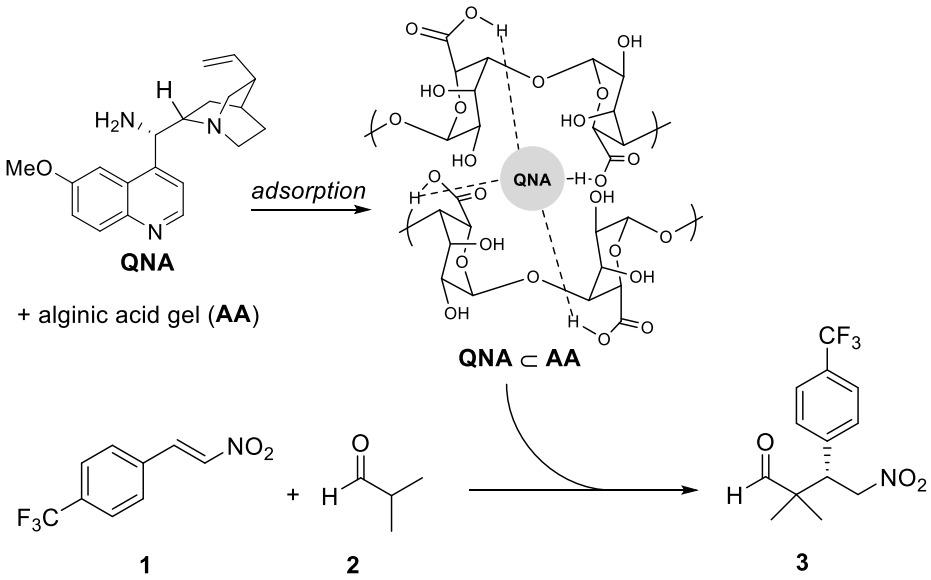


Figure 11

The catalyst performance was evaluated in terms of i) activity; ii) stereoselectivity; iii) heterogeneity; iv) recyclability. More in detail, the parameters that have been assessed are reported below.

a. The optimization of the adsorption protocol of **QNA** on the alginic acid support was intended to limit the catalyst leaching process in the reaction and to obtain a heterogeneous system able to work under different reaction temperatures. The main aspects that have been refined are:
    - Catalyst : Alginic acid ratio;
    - Amount of solvent;
    - Addition order of catalyst solution and alginic acid beads;
    - Water percentage in the adsorption solvent.

b. The research for optimal conditions to guarantee the best catalytic activity in the test reaction focused on:
    - Reaction temperature;
    - Purity of the aldehyde;
    - Choice of the reaction solvent with respect to the content of water.

This project was the result of a collaborative effort with the laboratories directed by Dr. Nathalie Tanchoux and Dr. Françoise Quignard (CNRS Montpellier, France), specialized in the manipulation and characterization of biopolymeric materials.

3. Experimental section

3.1. Materials and methods

3.1.1. NMR analysis

The characterization of the products, the determination of adsorbed catalyst percentage on the support and of reaction conversion were performed by NMR analysis.

Instrument:	NMR 300 MHz Varian Inova-300
Internal standard:	Bibenzyl
Solvent:	Deuterated chloroform or methanol

3.1.2. HPLC analysis

The enantiomeric excess of compound **3** was determined by HPLC analysis using a chiral column. The analytical conditions used were:

Instrument:	HPLC Varian ProStar 320
Column:	Chiralcel OD no. OD00CE-HK055
Detection:	UV-Vis λ=236 nm
Injected volume:	20 μL
Flow:	0.75 mL/*min*
Eluent:	*n*-Hexane/Isopropanol 80:20
Retention time:	$t_{r\_(R)\text{-}3}$ = 12.4 *min*, $t_{r\_(S)\text{-}3}$ = 21.3 *min*

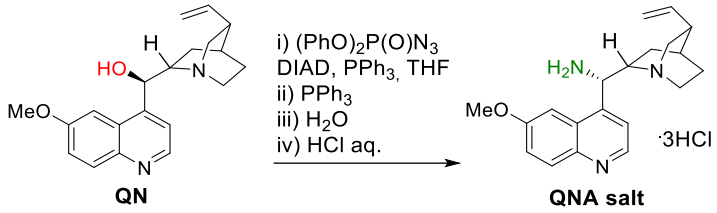
3.1.3 Equipment

All the catalytic reactions were performed in a 10 mL reaction tube with cap under gentle (<300 rpm) magnetic stirring. An oil bath equipped with a thermocouple thermometer was used to heat up the reaction mixture at given temperature and to keep its value constant throughout the reaction.

3.2. Catalyst synthesis [9]

The synthesis of the catalyst consists of two steps: i) a Mitsunobu reaction followed by a Staudiger reduction to perform the conversion of the *epi*-9-amino Chincona alkaloid; ii) neutralization of the trihydrochloride salt of the catalyst by basification with NH_3 solution. These synthetic steps are detailed below.

3.2.1. Synthesis of the trihydrochloride salt of the *epi*-9-amino *quinine* derivative



5.01 g (15.40 mmol) of *quinine*, 4.85 g (18.50 mmol) of triphenylphosphine and 60 mL of anhydrous THF are added under nitrogen atmosphere to a three neck flask equipped with a reflux condenser with a nitrogen pipe, a septum, glass stoppers and a magnetic bar. The mixture is stirred for 5 minutes at room temperature, then cooled at 0 °C in an ice/water bath. After 5 minutes of stirring at 0 °C, 3.70 mL of di-*iso*propyl azodicarboxylate DIAD (18.80 mmol) are added slowly, over 5-6 minutes, to the reacting mixture, which turns yellowish. 5 minutes after the DIAD addition is complete, 4.00 mL of diphenylphosphoryl azide (18.50 mmol) are added dropwise, over 15 minutes, to the reaction flask. The mixture is left stirring for 15 minutes at 0 °C; then the ice/water bath is removed and the stirring continued until the following day.

The next morning the mixture looks like a yellowish suspension. The flask is then heated up to 45 °C with an oil bath and the mixture is left stirring at this temperature for 2 hours, providing a homogeneous solution. At the end of this period, the complete exhaustion of the *quinine* is checked by TLC, using as eluting mixture ethyl acetate/methanol 10:1. A second equal amount of triphenylphosphine (4.85 g, 18.50 mmol) is added to the reaction mixture, which is followed by a moderate development of gas. The mixture is then kept stirring at 45 °C for 3 hours, long enough for the gas evolution to stop. At this point, 3.50 mL of distilled water are added to the flask, and the mixture is left stirring overnight at 45 °C.

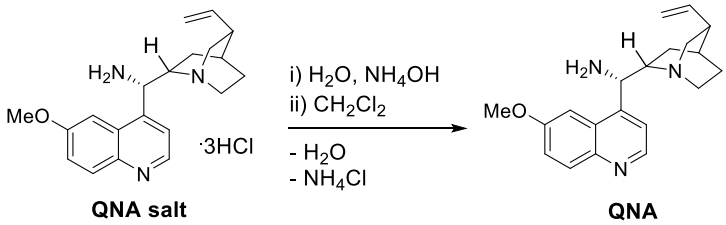
Once the reaction is complete, the work-up is carried out. The reaction mixture is cooled at room temperature and transferred in a 500 mL flask, using 15 mL of CH_2Cl_2 to rinse the reaction flask. The solvents are removed under vacuum; CH_2Cl_2 and a magnetic bar are

added at the resulting oil. At the homogeneous solution obtained by magnetical stirring, 80 mL of 2M HCl_{aq} are slowly added. After 10 minutes of vigorous stirring, the biphasic mixture is transferred in a 250 mL separating funnel, using two 5 mL portion of 2M HCl_{aq} and one 5 mL portion of CH_2Cl_2 to rinse the flask. The phases are separated, and the aqueous phase is transferred into a flask using 10 mL of methanol to complete the transfer. The solvents are removed first at the rotary evaporator, and then at the high-vacuum pump: a bright yellow solid is obtained and allowed to dry completely for several hours at the vacuum pump.

The trihydrochloride **QNA** salt is then purified through crystallization from methanol/ethyl acetate. A pale yellow solid is obtained, again dried at the high-vacuum pump with an overall yield of 92%. The final product is analyzed by ^{1}H-NMR in CD_3OD.

^{1}H-NMR (300 MHz, CD_3OD): δ 9.19 (d, J=5.6 Hz, 1H), 8.48 (d, J=6.2 Hz, 1H), 8.32 (d, J=9.3 Hz, 1H), 8.08 (d, J=2.1 Hz, 1H), 7.97 (dd, J_1=2.5 Hz, J_2=9.3 Hz, 1H), 6.11-5.94 (m, 1H), 5.90 (d, J=10.5 Hz, 1H), 5.38 (d, J=17.5, 1H), 5.27 (dt, J=10.3, 1H), 4.84-4.68 (m, 1H), 4.23 (s, 3H), 3.95-3.80 (m, 1H), 3.76-3.47 (m, 2H), 3.38 (s, 1H), 2.95 (s, 1H), 2.19-2.03 (m, 2H), 2.01-1.85 (m, 1H), 1.32 (s, 1H), 1.24-1.10 (m, 1H) ppm.

3.2.2. Salt neutralization and free amine QNA production



QNA salt **QNA**

0.5 g (1.155 mmol) of salt are weighted in a vial and dissolved in water. The water solution is basified using a concentrated ammonium hydroxide solution and the pH is checked with pH-indicator paper (highly basic solution). The solution is transferred in a separatory funnel and extracted four times with CH_2Cl_2 with a ratio of ca. 1:3 H_2O/ CH_2Cl_2 phases. All the organic solutions are collected in a beaker and dried on magnesium sulfate. The solution is then filtered over a filter paper and transferred in a round bottom flask to remove solvents at the rotatory evaporator. The obtained oil is dissolved in 5 mL of dichloromethane, transferred in a vial, dried over a nitrogen flux and then with a high vacuum pomp. The catalyst, a viscous yellowish oil, is obtained in quantitative yield and stored at 4 °C.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.75 (d, J=4.8 Hz, 1H), 8.04 (d, J=9.2 Hz, 1H), 7.66 (bs, 1H), 7.46 (d, J=5.1 Hz, 1H), 7.39 (dd, J_1= 3.2 Hz, J_2= 9.6 Hz, 1H), 5.89-5.73 (m, 1H), 5.06-4.93 (m, 2H), 4.60 (d, J=10.1 Hz, 1H), 3.98 (s, 3H), 3.35-2.99 (m, 3H), 2.88-2.74 (m, 2H), 2.29 (bs, 1H), 1.68-1.50 (m, 3H), 1.44 (bt, 1H), 0.85-0.72 (m, 1H) ppm.

3.2.3. Alginic acid solvogel preparation[6]

1g of alginate Protanal 200S is dissolved in 50 mL of distilled water to obtain a 1% m/V viscous and colorless sodium alginate solution, which is left under magnetic stirring overnight.

The resulting sodium alginate solution is added drop by drop with a 10 mL syringe without needle to a HCl 1M solution under magnetic stirring. As no pressure is applied, it is the gravity force causing the fall of the drops and permitting to obtain beads with a homogeneous mass. The beads are left under stirring overnight to mature.

The thus obtained beads are rinsed with water on a Büchner with filter paper until the pH of the collected washings in the Erlenmayer flask results within values 6-7. To get the alcogels from the hydrogels, the beads are soaked in EtOH/H_2O solutions. The percentage of EtOH in the washing mixtures is increased every time from 10% to 100%. The alcogel is stored at 4 °C in EtOH.

The obtained alcogel beads look whitish and slightly transparent (**Figure 12**, left).

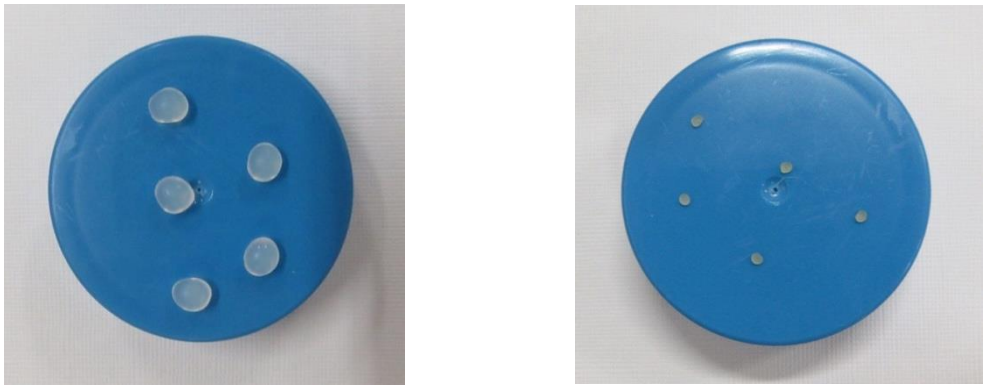


Figure 12

To determine the exact weight and thereby the exact number of functional groups available in each support beads, two vials are weighted without cap. In each vial are added 10 beads: the vials are connected to high vacuum pomp overnight to completely dry the solvogel (giving a xerogel) and then weighted. Each bead has a mass of ca 0.7 mg (Figure 12, right). Each gram of support corresponds to 5.7 mmol of carboxylic groups. Therefore, in each

bead there are 3.99 μmol of carboxylic acid available to interact with amine group of the catalyst.

3.2.4. Catalyst immobilization on alginic acid solvogel

To obtain the optimal ratio between the functional groups of the catalyst and the ones in the support (1:2.5), 146 alginic acid solvogel beads (corresponding to 0.58 mmol of carboxylic units) are added to a flask and covered with a solution of 9:1 ethanol/water. 75 mg (0.232 mmol) of **QNA** are weighted in a vial and dissolved with 5 mL of the ethanol/water solution. The mixture is transferred in a dropping funnel and the vial washed with 2 portions of 10 mL of the same solution to complete the transfer. The mixture is added dropwise to the flask containing the beads under slow magnetic stirring.

As the addition is completed, the funnel is also rinsed with 20 mL of the ethanol/water solution and the washing solution added to the beads. The adsorption reaction proceeds overnight under magnetic stirring at room temperature.

After 24*h* the adsorption is completed. The solvents are removed and collected in a round bottom flask. The solvogel beads are rinsed three times with absolute ethanol to remove water; a solvent exchange from ethanol to toluene (reaction solvent) is then performed with 5 washes of the beads with pure toluene. During the solvent exchange, the beads went from whitish to almost transparent.

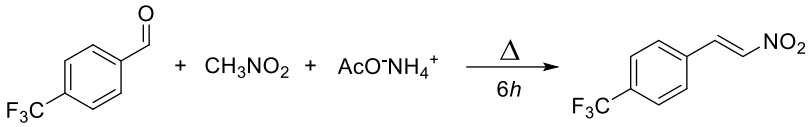
All washings are collected in the same round bottom flask and its content evaporated at rotatory evaporator. Residues are then dissolved in $CDCl_3$ and 31.7 mg (0.174 mmol) of bibenzyl are added as internal standard.

^{1}H-NMR analysis permitted to determine the exact amount of adsorbed catalyst, by comparing the integration of the signal related to the methoxy group of the catalyst at 3.97 ppm with the signal related to benzylic protons of the bibenzyl at 2.90 ppm (Equation 1).

$$Adsorption\ [\%] = \left(1 - \frac{mol\ bibenzyl\ STD}{mol\ QNA} \cdot \frac{4}{3} \int QNA\_MeO\_peak\right) \cdot 100 \qquad \text{(Eq. 1)}$$

Each bead contains ca. 0.00156 mmol of catalyst (80% of catalyst adsorbed). The beads have been stored in toluene at 4 °C.

3.2.5. Nitrostyrene synthesis



Into a one neck flask equipped with a reflux condenser and a magnetic bar are placed 10 mL of 4-(trifluoromethyl)benzaldehyde (73.5 mmol), 120 mL of nitromethane (2.205 mol) and 1.42 g of ammonium acetate (18.4 mmol). The resulting mixture is vigorously stirred at 100 °C. After 6*h*, the mixture is allowed to cool to room temperature, and a work up is performed in a separatory funnel with H_2O and CH_2Cl_2 for the separation of the two phases: the organic phase is extracted, dried over $MgSO_4$ and filtered through filter paper. The product is dried at the rotatory evaporator and purified by chromatographic column with petroleum ether/ethyl ether 9:1 as eluting mixture. The elution is followed by TLC analysis and two main fractions are collected separately: the first eluate fraction is composed of pure product, whereas in the second fraction a 10% of unconverted reagent is present in addition to the product. Both fractions are dried at the rotatory evaporator for the quantitative determination of the yield.

The product is obtained as a yellow-brown solid with an overall yield of 64%.

Molecule characterization:

^{19}F-NMR:	(300 MHz, $CDCl_3$) CF_3 peak: - 63.18 ppm
^{1}H-NMR:	(300 MHz, $CDCl_3$): δ 8.03 (d, J=13.5 Hz, 1H), 7.74-7.65 (m, J=8.8 Hz, 4H), 7.62 (d, J=13.5 Hz, 1H) ppm.

The ^{19}F-NMR signal will be taken as a reference for the peaks integration in the analysis of reaction conversions, as it is constant for each solvent.

3.3. Michael addition

3.3.1. Basic method

14 beads, which correspond to 0.0218 mmol of catalyst **QNA**, are added to a reaction tube. 450 μL (4.25 mmol) of toluene are added to the beads, followed by 22.8 mg (0.105 mmol) of nitrostyrene **1** and 48 μL (0.525 mmol) of isobutyraldehyde **2**. Reaction is performed at 40 °C under gentle magnetic stirring.

In order to study the heterogeneity of the catalyst, two reactions are carried out in each experiment. As the conversion reaches 20-40%, the catalyst beads are removed from one of the two reaction tubes; in case of high heterogeneity, the reaction stops right after beads removal. Conversion is followed by ^{19}F-NMR analysis.

Molecule characterization:

^{19}F-NMR: (300 MHz, $CDCl_3$) CF_3 peak: - 62.75 ppm

3.3.2. Scale-up of the optimized conditions and product purification

A small reaction scale-up is necessary in order to obtain enough product for purification and characterization. 18 beads, which correspond to 0.028 mmol of catalyst **QNA**, are added to a reaction tube and pre-treated at 60 °C in 600 μL of water saturated toluene as solvent for 24*h*. As the pre-treatment is completed, 30.4 mg (0.14 mmol) of nitrostyrene **1** and 64 μL (0.70 mmol) of isobutyraldehyde **2** are added in the reaction tube, cooled to room temperature. Reaction is then performed at 40 °C under gentle magnetic stirring. After 24*h*, when conversion is ca 93%, the reaction mixture is removed and collected in a vial, while the catalyst beads remain in the reaction tube. Catalyst beads are washed four times with 1 mL of toluene; all the washings are joined to the isolated reaction mixture.

The beads, carefully washed with toluene, are now ready to be employed in new reaction cycles to determine catalyst recyclability.

Conversion is checked by ^{19}F-NMR analysis, carefully sampling a minimal amount to not alter the yield.

The product is isolated by chromatography column conditioned with Silica gel for gravity chromatography and 7:3 petroleum ether/diethyl ether as eluting mix. The reaction mixture is deposited directly as plate on the top of the column.

The elution of the products is checked by TLC analysis. TLC is performed with TLC Silica gel 60 F_{254} and *n*-hexane/diethyl ether 8:2 as eluting mixture. As reference the nitroalkene reagent **1** is dissolved in diethyl ether and seeded. The product **3** does not have conjugation, therefore difficult to see with an UV lamp. For this reason, potassium permanganate stain is used to visualize the TLC, making the spots well visible.

The product is dried at the rotatory evaporator. A ^{1}H-NNR analysis is performed to determine the purity of the product, dissolved with deuterated chloroform.

To determine the enantiomeric excess, a HPLC analysis is performed how described in chapter 3.1.

Molecule characterization:

HPLC: $t_{r\_(R)\text{-}\mathbf{3}}$ = 12.4 *min*, $t_{r\_(S)\text{-}\mathbf{3}}$ = 21.3 *min*

^{19}F-NMR: (300 MHz, $CDCl_3$) CF_3 peak: - 62.75 ppm

^{1}H-NMR: (400 MHz, $CDCl_3$): δ: 9.50 (s, 1H), 7.95 - 7.46 (m, 2H), 7.35 (dd, J_1= 8.1 Hz, J_2=.7 Hz, 2H), 4.89 (dd, J_1= 13.3 Hz, J_2= 11.4 Hz, 1H), 4.74 (dd, J_1= 13.3 Hz, J_2= 4.1 Hz, 1H), 3.80 (dd, J_1= 11.4 Hz, J_2=4.1 Hz, 1H), 1.14 (s, 3H), 1.03 (s, 3H) ppm.

4. Results and discussion

4.1. Preliminary tests: catalyst adsorption on alginic acid

The adsorption protocol of **QNA** on alginic acid supports had already been preliminary explored in previous projects. The aim of this part of the research was to improve the reproducibility of the existing method and, at the same time, to optimize adsorption parameters.

The first step was to determine which solvent could maximise the ability of alginic acid gels to adsorb the catalyst from solution. To monitor the catalyst adsorption process, its residual content in the adsorption solvents after 20-24*h* at room temperature was quantified by ^{1}H-NMR analysis using bibenzyl as internal standard. These tests were all performed using 0.014 mmol of **QNA**, 10 AA aerogel beads (which correspond to 0.028 mmol of carboxylic units) and ca. 0.5 mL of solvent (Figure 13).

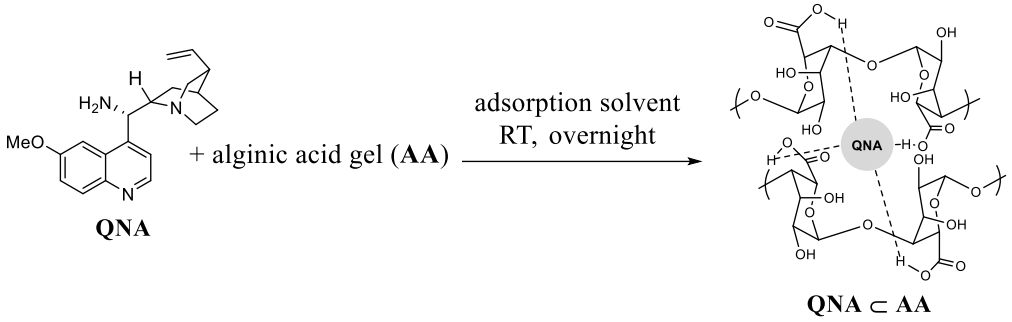


Figure 13

A solvent screening (Table 1) showed that adsorption efficiency is very similar and unsatisfactory with solvents of moderate polarity (tests 1 to 6). The use of a lipophilic alcohol like *iso*propanol gave results in line with those of aprotic solvents (test 7). On the contrary, the adsorption was much more efficient when carried out in a protic polar solvent, such as ethanol (test 8).

As can be seen, when **QNA** is adsorbed in ethanol, adsorption occurs with considerable efficiency. Given the excellent result obtained with this polar protic solvent, the influence of water as co-solvent was also investigated. It was found that a water percentage in the solvent ethanol as adsorption mixture had a positive effect, probably due to the inherent ability of polysaccharides to swell in aqueous media, leading to a more dispersed and

accessible structure for the catalyst and therefore a more efficient adsorption. However, increasing amounts of water during the adsorption to >10% values led the beads to break jellifying the whole solvent.

Table 1

Test	Solvent	Residual QNA (%)
1	Toluene	55
2	CH_2Cl_2	50
3	$CHCl_3$	62
4	EtAc	68
5	CH_3CN	66
6	THF	69
7	*i*-PrOH	61
8	EtOH	14
9	EtOH/H_2O 90/10	10
10	EtOH/H_2O 92/8 (inverse addition)	10
11	EtOH/H_2O 80/20 (inverse addition)	n.d.[(a)]

[a] The residual amount of catalyst in the adsorption mixture could not be determined as the beads broke.

Furthermore, the first adsorption protocol involved adding the beads one by one to the catalyst solution. Nevertheless, the large amount of base experienced by the first added beads could break the H-bonds responsible for their structure, as observed in some experiments. Therefore, an inverse order of addition was tested, performing better results in terms of beads structure and percentage of residual catalyst in the adsorption mixture (test 10). Being EtOH as the major component of the adsorption mixture, the possibility of using more readily available alcogel beads instead of aerogel was also tested. This experiment gave results fully comparable to the use of aerogel.

Thus, according to the existing optimized procedure, the adsorption should take place on solvogel beads using ca EtOH/H_2O 90:10 as adsorption mixture (initial concentration of **QNA** 0.25 M), 2.5 equivalents of carboxylic acid functions per **QNA** catalyst unit and by adding the catalyst solution to the beads, soaked in the adsorption solvent, at room temperature. Since the catalytic reaction has to be performed in toluene, the EtOH solvent was replaced by toluene, rendering toluene solvogel beads to be tested in the reaction as heterogeneous catalyst.

4.2. Preliminary tests: feasibility of the test reaction

In order to study the activity and heterogeneity of the adsorbed catalysts, two reactions (I and II) were set up at the same time and conditions for each experiment. Solvogel beads were removed from reaction II as the conversion, followed by ^{19}F-NMR analysis on the mixture, reached approximately the value of 30%.

To prove the feasibility of this approach, a catalyst obtained by adsorbing **QNA** in EtOH/H_2O 92:8 mixture was applied in the test reaction at 60 °C (Figure 14).

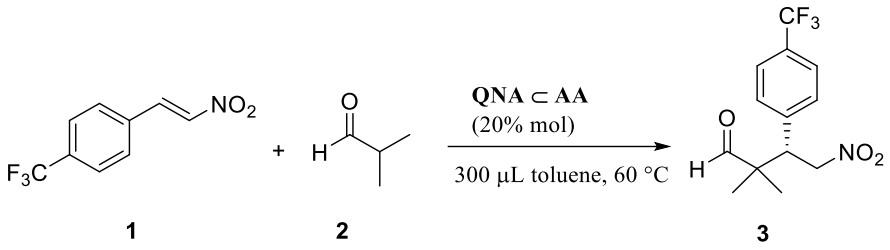


Figure 14

As can be seen in Figure 15, the catalyst is active (yield > 90% after 24 *h*) and fully heterogeneous, as the reaction II stops right after beads removal. Also the enantioselectivity is very good: an enantiomeric excess of ca 97% was determined by chiral stationary phase HPLC on the crude mixture.

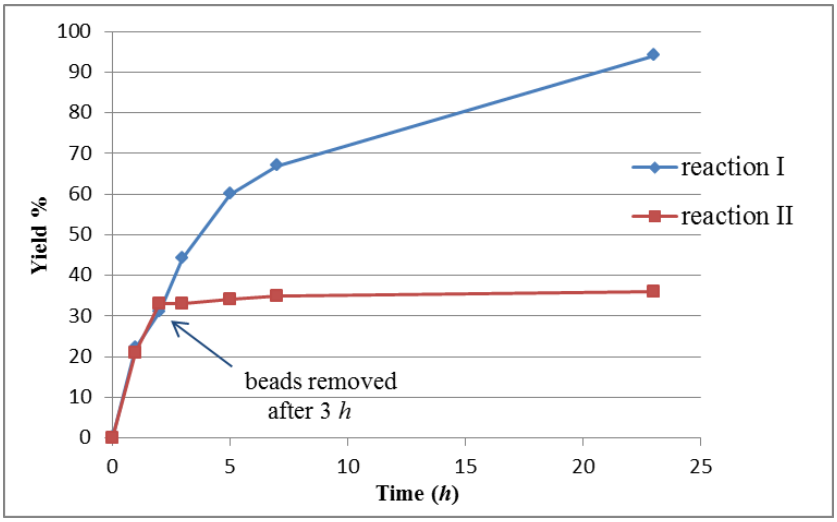


Figure 15

The results achieved with this catalyst as solvogel in toluene were the best obtained up to this point, a considerable improvement compared to previous attempts performed before this work.

4.3. Development of a new optimized protocol

To determine the final optimized conditions to adsorb the catalyst, the main aspect to clarify seemed to be the exact amount of water to add in the adsorption mixture.

The existing protocol included the use of solvogel beads and of a relatively small amount of solvents (initial concentration of **QNA** in solution ca. 0.25 M) to perform the adsorption, resulting in a "concentrated" catalyst solution. However, as the solvogel beads are soaked with ethanol, the quantification of the exact EtOH/H_2O ratio in the adsorption mixture proved to be difficult. Furthermore, some reproducibility problems were encountered, as in some cases gel breaking or inactive catalysts were obtained even by applying the very same adsorption protocol that gave the results reported in the previous paragraph.

To overcome these issues, our collaborators in Montpellier developed an alternative adsorption protocol, designed to allow a precise control of the ratio between ethanol and water and a better reproducibility. The strategy was to increase the amount of solvent used during the adsorption (approximately 10 times greater than the previous protocol, initial concentration of **QNA** ca. 0.025 M), resulting in a more "diluted" catalyst solution, and to use aerogel beads retaining no extra solvent inside the gel structure, instead of solvogel beads. Even if the amount of catalyst adsorbed was slightly inferior to the previous protocol (in this case 15-20% of **QNA** remains in solution), the adsorption process under these conditions appeared to be less sensitive to small changes in the percentage of water present in the mixture, providing a higher heterogeneity of the encapsulated catalyst and a better reproducibility of the method.

However, solvogel materials are much easier to obtain, considering the extra step of CO_2 supercritical drying needed to achieve the corresponding aerogel. In order to distinguish between aerogels and solvogels in terms of performances in the test reaction, two catalysts were prepared by adsorption (with the new diluted protocol) on aerogel and solvogel beads under the same conditions (EtOH/H_2O 90:10 as adsorption mixture, 2.5 equivalents of carboxylic acid functions per **QNA** catalyst unit and by adding the catalyst solution to the beads at room temperature).

Two reactions were run in parallel at 60 °C for each catalyst, with 20% of catalytic loading and toluene as reaction solvent.

As can be seen in Figure 16, the two catalysts showed almost identical behavior, with reference to both activity and heterogeneity.

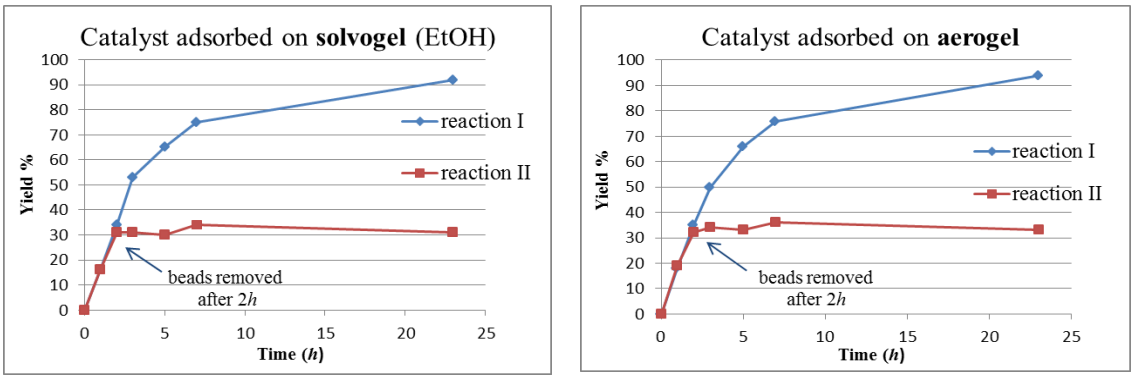


Figure 16

Given these results, it was possible to conclude that solvogel and aerogel beads can be indiscriminately employed as support for the catalyst adsorption.

At this point, it was attempted to vary the water/ethanol ratio in the adsorption mixture to study the influence of this parameter on the method and to assess the different catalytic behaviors in the test reaction at 60 °C (results shown in Figure 17).

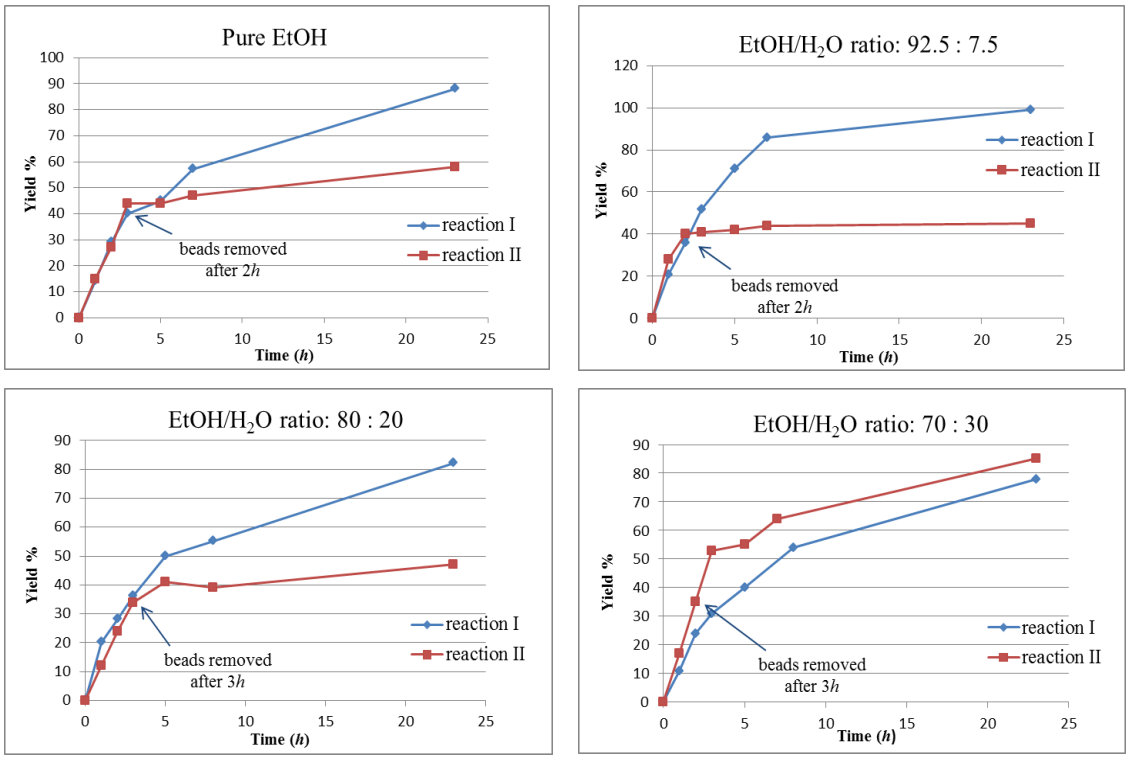


Figure 17

When the catalyst is adsorbed in pure EtOH (Figure 17: top, left), both activity and heterogeneity are worsened if compared to the excellent results obtained by adding a 7.5%

of water as co-solvent in the adsorption mixture (Figure 17: top, right). This catalyst was the most active (full conversion after 24 *h*) and heterogeneous, showing similar behavior to the one adsorbed by adding a 10% of water (see Figure 16).

Under the new diluted condition, the beads were less sensitive to higher percentages of water in the adsorption mixture, so it was possible to exceed the limit of 10% value without breaking the beads or jellifying the solvents during catalyst adsorption.

However, as shown in Figure 17, raising the amount of water up to 20% (bottom, left) resulted in moderate yield (88% after 24 *h*) and heterogeneity, while an increase of 30% (bottom, right) led to solvent jellification and breaking of the beads during the reaction, giving poor heterogeneity.

According to the existing optimized method, after the adsorption process is completed and the beads are washed with pure EtOH to get rid of the excess water, a solvent exchange to toluene (reaction solvent) must be performed for the use of the beads in the reaction.

So far, to prepare the toluene solvogel beads, several washings were carried out at increasing amounts of toluene in EtOH (10, 30, 50. 70. 90, 100%). Since a direct switch from EtOH to pure toluene would simplify the solvent exchange, it was attempted to prepare the solvogel beads by performing three direct washings with pure toluene. The beads obtained with this method showed no evident changes in the structure or in the catalytic behavior, proving the potential of the direct switch.

Finally, a new catalyst was prepared using an increased ratio between the alginic acid functions and the catalyst (AA/**QNA**=5, instead of the usual 2.5), in order to see if a greater number of free functional groups could increase catalyst adsorption. However, it was found that the large amount of free protons of AA quenched the active amino group on the **QNA**, preventing its interaction with the substrate (i.e. the catalyst was not active).

In conclusion, the optimized adsorption protocol of **QNA** on alginic acid gels, which enabled the obtaining of highly active and heterogeneous supported catalysts, involved the use of: solvogel alginic acid beads, 2.5 equivalents of carboxylic acid functions per **QNA** catalyst unit, EtOH/H_2O 90:10 as adsorption mixture, an initial concentration of **QNA** ca. 0.025 M, and the direct solvent switch from EtOH to toluene. This protocol enabled the adsorption of 82-85% of the initially added **QNA**.

4.4. Optimization of reaction conditions

In this part of the research, experiments were carried out to determine the best conditions for the Michael addition reaction with reference to catalyst activity, heterogeneity and recyclability.

As usual, two reactions (I and II) were carried out in parallel for each experiment: variations from the usual reaction conditions are reported in each kinetic graph, specifying reactions temperatures, whether the catalyst or the reagents received special treatments and the solvent used. Kinetic curves were determined by ^{19}F-NMR analysis, carefully sampling a minimal amount to not alter the catalyst/substrate ratio. To perform recyclability tests, all the beads employed in a previous reaction were washed several times with pure toluene to get rid of residual startings.

Given the excellent results obtained with the catalyst prepared under the optimized adsorption conditions during the first reaction cycle at 60 °C, a second cycle was attempted to determine catalyst recyclability (Figure 18).

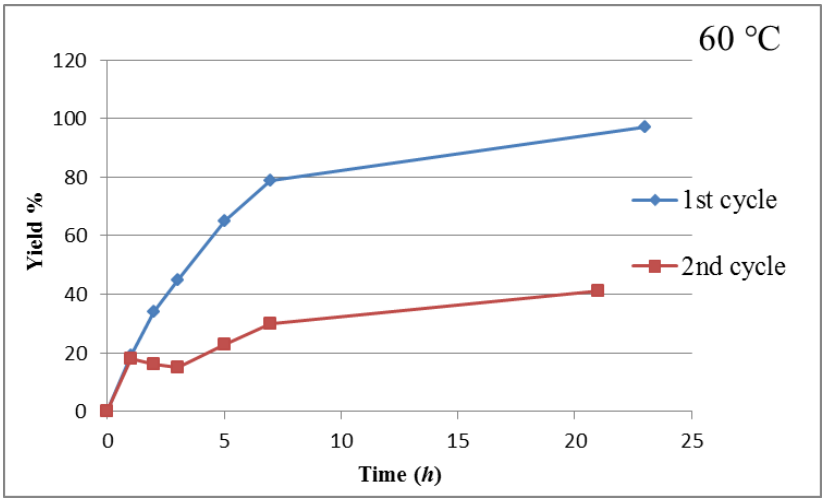


Figure 18

As shown, the catalyst gave poor yield when employed in the second reaction cycle. It was hypothesized that the high temperature of the reaction affected the longevity of the beads leading to faster deactivation and lowering the overall yields.

Therefore tests were carried out to determine how the catalytic behavior is affected at different temperatures, knowing that the catalyst was active and heterogeneous in the reaction at 60 °C, but not recyclable.

The first reaction, performed at room temperature (25 °C), was slow and the catalyst showed homogeneous behavior, since the reaction proceeded after beads removal (Figure 19, left). The second reaction was instead performed at 40 °C (Figure 19, right): while the reaction was slower, as expected, the catalyst resulted less heterogeneous if compared to the results achieved in the reaction at 60 °C.

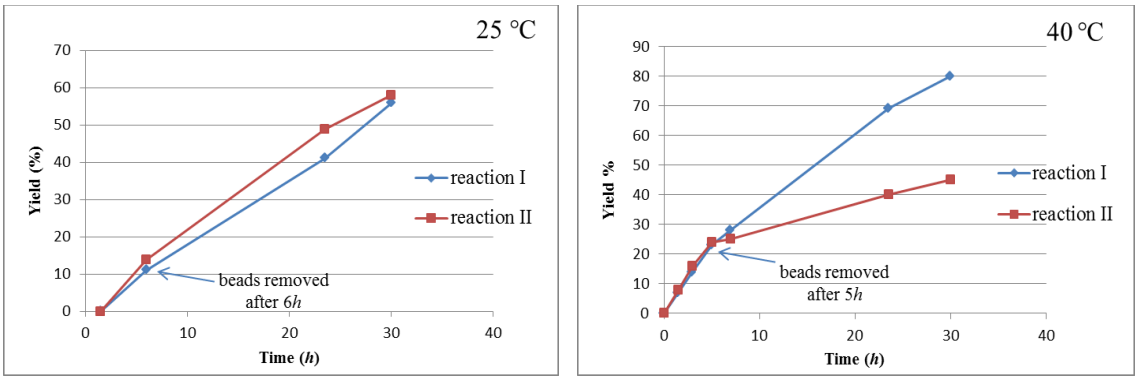


Figure 19

A second cycle was carried out using the beads already employed in reaction I performed at 40 °C. This recycle gave unexpected results: the conversion in the second cycle (Figure 20: ca. 45% after 24 *h*) was higher than the one obtained in the recycle of the beads employed in the test reaction at 60 °C (Figure 18: ca. 40% after 24 *h*), despite there was a much higher heterogeneity during the first cycle.

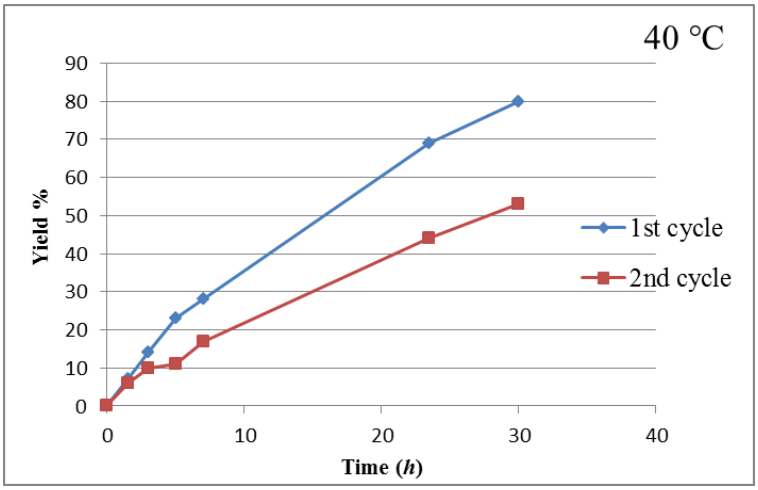


Figure 20

A possible explanation for the non-heterogeneous behavior of the catalyst in the reaction at 40 °C could be due to the presence of *iso*butyric acid, formed by air oxidation of the aldehyde. It was supposed that this acid could extract the catalyst from the support, leading to poor heterogeneity in the first reaction cycle and therefore low activity in the second

one. Although freshly distilled aldehyde was generally used, aldehyde oxidation could occur even upon short storage, or simply during the reaction.

To understand the aldehyde purity influence, the previous experiment performed at 40 °C using freshly distilled *iso*butyraldehyde was compared with an experiment where *iso*butyric acid was added in equimolar quantity with the catalyst (Figure 21, left and right respectively).

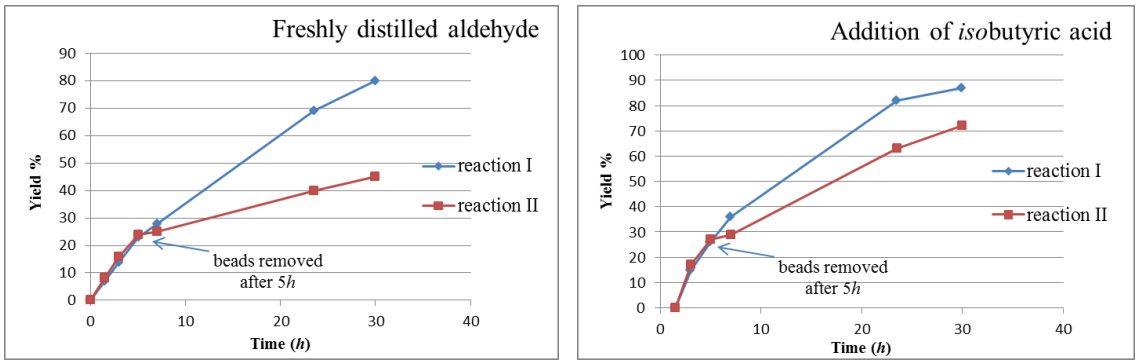


Figure 21

None of the two experiments showed fully heterogeneous behavior. Nevertheless, with the addition of *iso*butyric acid to the system, the heterogeneity of the catalyst was worsened even more. It was therefore demonstrated that the *iso*butyric acid impurity is able to interact with the catalyst, extracting it from the support. For this reason, *iso*butyraldehyde reagent must be distilled every week and stored under nitrogen atmosphere to avoid its oxidation as far as possible.

An additional reaction was performed under inert atmosphere (nitrogen) to determine whether the presence of oxygen, which might oxidize the aldehyde *in situ*, influences the reaction or not. No changes were observed from experiments carried out in presence of oxygen (under air): therefore, all subsequent experiments were performed without any precaution to exclude air.

As regards catalyst deactivation observed during the attempted recycling, it was hypothesized that nitrostyrene reagent itself might have interacted with the catalyst during the reaction, resulting in low conversion in the second cycle. This hypothesis was disproved through an experiment in which, after catalyst beads were left stirring for 24 *h* in a reaction tube with the nitrostyrene in toluene, the aldehyde was added and the reaction started. A ^{19}F-NMR analysis prior to the reaction showed no decomposition of the reagent, and the catalyst displayed similar activity to a fresh one. This result proved that the

nitrostyrene reagent alone is not capable to interact with (or deactivate) the catalyst in absence of *iso*butyraldehyde.

Considering that temperature could influence the structure of alginic acid and its capacity to trap the **QNA**, a pre-treatment of the catalyst beads was performed at 60 °C during 24 *h*, in toluene solution. After 24 *h*, ^{1}H-NMR analysis showed that there were no catalyst traces in the solvent, ensuring that leaching did not occur. These beads, when employed in the test reaction at 40 °C (Figure 22), showed a higher heterogeneity than the one obtained in the reaction performed at the same temperature, but without pre-treatment of the beads (Figure 19, right).

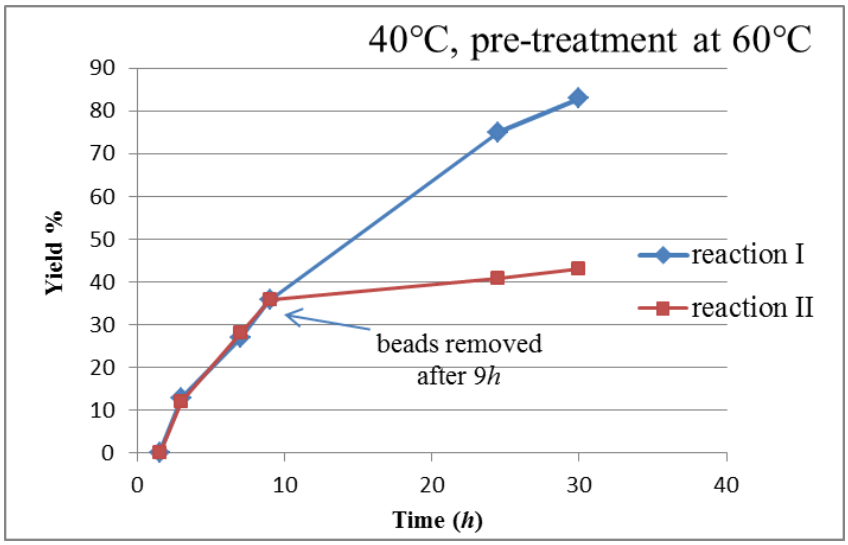


Figure 22

However, the recyclability (similar to the one without pre-treatment, Figure 20) was limited also in this case.

At this point, in the knowledge that water might influence the structure of the polysaccharide support, experiments were carried out focusing the attention on the understanding of the influence of water percentage in the reaction mixture.

Instead of pure toluene, a water saturated toluene solution (0.52 mg/mL) was employed (Figure 23, left) in the test reaction at 40 °C. The reaction showed to be faster as it reached full conversion after only 24 *h*. However, water did not have any influence on the heterogeneity of the system, which remained moderate.

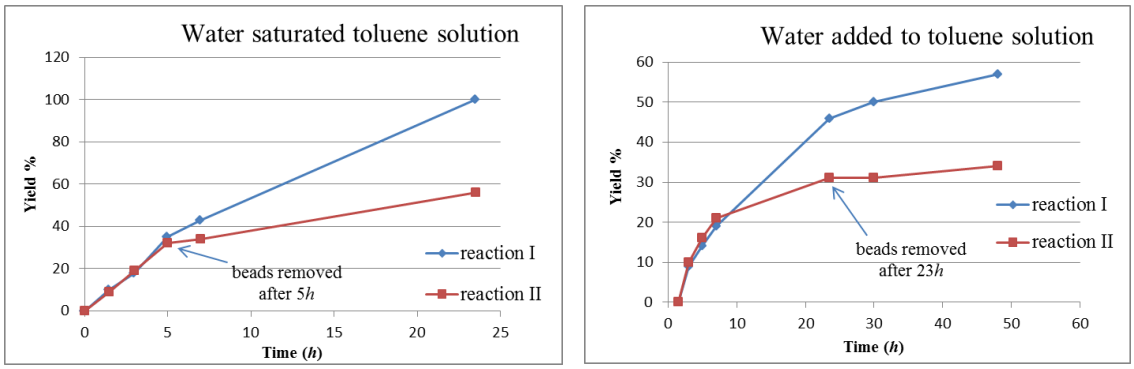


Figure 23

A second experiment with a larger amount of water was performed. Water was added to the reaction mixture in a concentration of 6.67 mg/mL, considering that the polysaccharide can adsorb water in larger amounts than the saturation limit of toluene. The reaction was very slow and it reached a conversion of ca 55% after 48 *h* (Figure 23, right). Hence, high amounts of water are not favorable to promote the catalyst activity.

The next experiment was performed combining the two conditions which had given the best results during previous experiments: the pre-treatment at 60 °C of the beads to improve heterogeneity and the addition of a small portion of water (using water saturated toluene as reaction solvent) to speed up the reaction (Figure 24). This experiment (and the accompanying recycling ones) was performed on a larger scale, to enable product isolation by chromatography on silica gel, its characterization by NMR and the precise determination of the enantiomeric excess of the reaction by chiral stationary phase HPLC (98% ee).

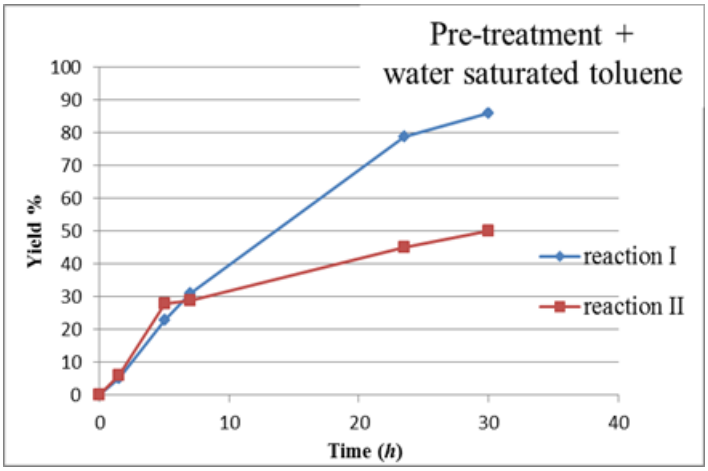


Figure 24

The conversion reached after 30 *h* is about 85%, lower than the one obtained in the reaction with water saturated toluene at 40 °C without pre-treatment of the beads (Figure 23, left).

A comparison between the results of heterogeneization experiments revealed that the catalyst pre-treated at 60 °C and employed in the test reaction at 40 °C with water saturated toluene as solvent was slightly less heterogeneous than the one pre-treated and used in the test reaction at 40 °C with pure toluene as solvent.

Despite the small decrease in heterogeneity, a second reaction cycle at 40 °C in water saturated toluene was attempted to determine the pre-treated catalyst recyclability. A conversion of ca 60% after 22 *h* was achieved, resulting in the best activity observed so far for the second reaction cycle (Figure 25).

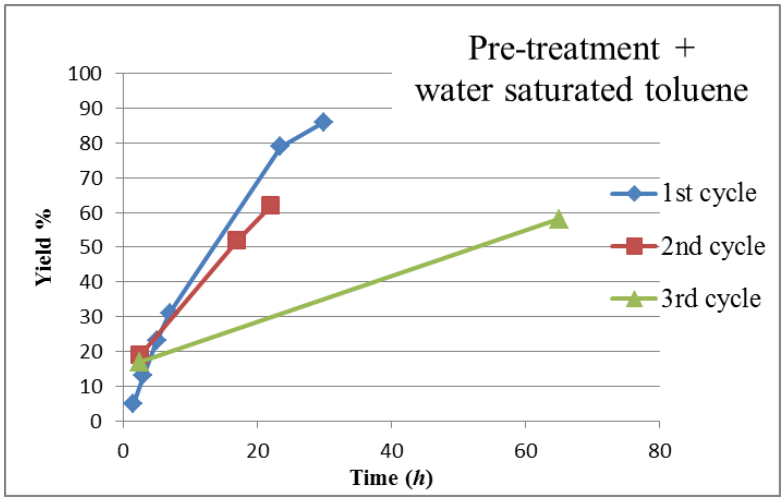


Figure 25

A third reaction cycle was also performed: the catalyst showed still moderate activity, since the conversion was ca 60% after 65 *h*.

Even though the combination of the two factors which seemed to improve the performances of the system (pre-treatment and water saturated toluene) resulted in moderately lower activity and heterogeneity, the recycling was much more improved from all previous tests, as even three cycles were possible.

Therefore, it was ultimately established that employing water saturated toluene as solvent helps to overcome the difficulty to recycle the catalyst in the reaction at 60 °C. The pre-treatment of the beads allows milder reaction conditions (40 °C), but more time is required

to achieve acceptable yields. If the reagents are not susceptible to high temperatures, the reaction can be directly performed at 60 °C.

This method is now regarded as the best one to exploit adsorbed **QNA** in the Michael addition reaction.

5. Conclusion and perspectives

This project demonstrated the possibility to develop a heterogeneous catalytic system based on the adsorption of **QNA** on alginic acid gel. The performances of the catalyst were investigated in a benchmark Michael addition reaction, with respect to activity, heterogeneity, stereoselectivity and recyclability.

Regarding the adsorption protocol, a new method was successfully developed, resulting in highly stereoselective and reasonably active and heterogeneous adsorbed catalyst. Parameters that were optimized include the ratio between the catalyst and the alginic acid, the correct addition order of catalyst solution and alginic acid beads, the amount of solvents to use and the proper percentage of water in the adsorption solvent.

With the aim of finding the optimal conditions to guarantee the best catalytic activity in the test reaction, the influence on the system of temperature, additives and presence of water was also determined. Considering the overall investigation, the best conditions to carry out this type of Michael addition were: *i)* a catalyst beads pre-treatment at 60 °C during 24 *h* and a reaction temperature of 40 °C; *ii)* a reaction temperature of 60 °C without pre-treatment. Good heterogeneities were achieved with both methods, which included water saturated toluene as solvent. As a matter of fact, the presence of water is a critical aspect towards recycling the catalyst as well as obtaining high conversions.

After the first reaction cycle, the catalyst could be easily recovered and reused, as the heterogeneity of the solutions was largely preserved. Moreover, given the durability of catalyst activity achieved under optimized conditions, it was possible to accomplish at least two reaction cycles with high conversions and excellent enantiomeric excesses.

This work proves the possibility of using alginic acid, a cheap and renewable bio-polymer, instead of oil-based materials for supporting/heterogenizing organic catalysts.

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