Green Chemistry

PAPER

Critical assessment of the efficiency of chitosan biohydrogel beads as recyclable and heterogeneous organocatalyst for C–C bond formation†

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The effectiveness of neutral pH chitosan hydrogel beads (CSHB) as a green organocatalyst for a variety of C–C bond forming reactions (*i.e.* aldol reaction, Knoevenagel condensation, nitroaldol (Henry) reaction, Michael addition) has been comprehensively evaluated. Reaction rates, conversions and selectivities were studied as a function of a series of input variables including size, pH and reactive surface area of the beads, catalyst loading, temperature, molecular weight of the biopolymer, concentration, solvent system and molar ratio of reactants. Moreover, the catalytic biohydrogel beads were characterized by a variety of techniques including, among others, SEM, FT-IR, TGA and DSC.

Introduction

With growing concern for our environment and stringent environmental regulations by governments, the emphasis of science and technology is shifting more and more from petrochemical-based feedstocks towards the optimal use of environmentally friendly and sustainable resources and processes.[1] In this regard, direct utilization of products derived from naturally occurring materials has become a prevalent means for a number of high-tech applications.

Within this context, and during the past few decades, biopolymers have attracted increasing attention in both academic and industrial worlds owing to their unique properties, such as biodegradability, biocompatibility and antibacterial activity.[2] Among these biopolymers, cellulose and chitin are the most important biomass resources and the most abundant organic compounds on Earth.[3] Chitin, poly(β-(1 → 4)-*N*-acetyl-D-glucosamine), is the main component of the cell walls of fungi, the exoskeletons of arthropods such as crustaceans and insects, the radulas of molluscs and the beaks of cephalopods.[4] Depending on its source, chitin occurs as two allomorphs, namely the α and β forms, and it is usually extracted by acid treatment to dissolve calcium carbonate followed by alkaline treatment to solubilise proteins.[5] Chitosan, the most important derivative of chitin, can be obtained by extensive deacetylation under alkaline conditions (Fig. 1).[6] However, chitosan is rarely 100% deacetylated resulting actually in a hydrophilic random copolymer of β-(1–4)-linked glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). Their relative ratio defines the degree of deacetylation (DDA) that controls important properties of the polymer such as its basicity, viscosity and solubility, which are also influenced by the polymer's molecular weight.[7] Indeed, the intrinsic pK_a of chitosan depends on the DDA, the ionic strength and the charge neutralization of amine groups. In practice, it usually lies within 6.3–6.7 for completely neutralized amine functions when the deacetylation does not exceed 50%, which leads to protonation in aqueous acidic solution with a charge density of the resulting polyelectrolyte dependent on the exact pH and DDA values.[8,9]
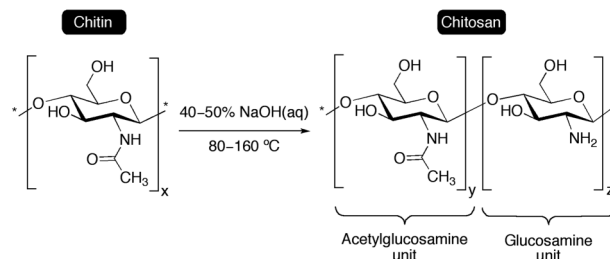

Fig. 1 General preparation of chitosan by deacetylation of chitin under alkaline conditions, which are chosen depending on the biopolymer source and the desired DDA.[10]

Perhaps one of the biggest advantages of chitosan as a raw material is that its dilute acidic solutions can be readily cast into films and fibers, or coagulated into well-defined spherical

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particles by spraying into alkaline solution. While chitosan has been widely used in agriculture, food, and biomedical applications,[4,11] such physical-chemical versatility and good processability has driven its use also in the field of heterogeneous catalysis, especially during the last decade.[12] The presence of both hydroxyl and amino groups in the chitosan make it useful as a chelating agent. Most studies in this area have focused on exploiting its complexation properties with metal ions and as a polymeric matrix for the synthesis of nanoparticles.[12,13,14] Although chitosan-supported organocatalysts have been recently reported,[15] the direct use of this amine-containing biopolymer, as a green chemistry approach,[16] in base catalysis has so far been scarcely investigated. In the other hand, it is known that the chitosan normally has a very low surface area (*ca.* 1.58 m^2 g^{-1}), their aerogels can display a surface area up to 350 m^2 g^{-1} with high content of accessible basic sites (up to 5.2 m^2 g^{-1} of –NH$_2$ groups).[17] This was exploited by Quignard and co-workers to prepare chitosan aerogel microspheres, obtained under supercritical CO$_2$ conditions, and used as a catalyst for the synthesis of monoglyceride by fatty acid addition to glycidol in toluene at 70 °C.[18] They have also reported very recently the use of chitosan aerogel as a recyclable, heterogeneous organocatalyst for the asymmetric direct aldol reaction in water.[19] Shukla and co-workers have described the use of powered chitosan, prepared through the hydrogel synthesis route, as a high-temperature catalyst for the synthesis of jasminaldehyde by the Claisen-Schmidt condensation of 1-heptanal and benzaldehyde under solvent-free conditions.[20] In 2006, some of us showed a preliminary study on the potential of chitosan hydrogel beads (CSHB) as a recyclable organocatalyst for both aldol and Knoevenagel reactions in DMSO.[21] In the field of low-molecular-weight (LMW) gels (*i.e.* gels made of proline-containing LMW gelators) as self-supported heterogeneous selective catalysts, the more recent seminal work from Miravet, Escuder and co-workers[22] should also be featured.

The preliminary studies carried out in our group dealing with the use of CSHB in organocatalysis provided some ambiguous results, which motivated us to investigate this material in more detail. Thus, we report here the results of a comprehensive study aimed to gain a better understanding of the exact role of chitosan hydrogel used directly as a green organocatalyst for C–C bond forming reactions (*i.e.* atom-economical reactions) – which are in the broad sense a prerequisite for all life on earth – and the variables that can impact its performance.

Results and discussion

Preparation and characterization of the catalyst[23]

Catalyst preparation. In order to evaluate the scope of CSHB as organocatalyst, uniform-size spherical hydrogel beads were prepared by adaptation and optimization of reported procedures based on the alkaline coagulation of an acidic viscous chitosan solution added using a dropping funnel (Fig. 2A). Thus, almost spherical shaped beads with narrow size distribution (average diameter = 4.0 ± 0.1 mm) were reproducibly obtained by adjusting the distance between the tip of the dropping funnel and the coagulating medium to 1.5 cm and a falling rate of drops controlled at approximately one drop per second (Fig. 2B–C).
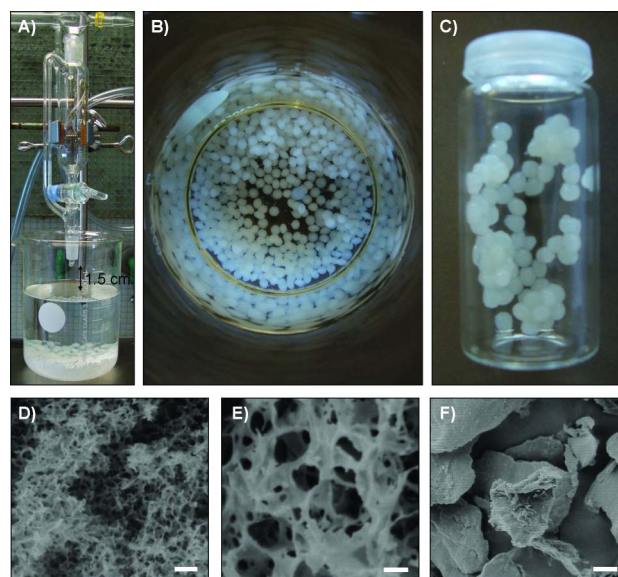

Fig. 2 A) Experimental setup used for the preparation of spherical CSHB with an average diameter of 4.0 ± 0.1 mm from commercially LMW chitosan. B) Aspect of CSHB during the maturing process in NaOH aqueous solution. C) Macroscopic view of milk-white colour CSHB after maturing. D) Representative SEM image of the freeze-dried cryogel beads made from the CSHB (scale bar 5 μm; magnification 2000×). E) Zoom in of picture D) (scale bar 1 μm; magnification 10000×). F) SEM image of commercially powdered chitosan (PCS) (scale bar 20 μm; magnification 500×).

CSHB with a mean diameter of 2.2 ± 0.2 mm were obtained using a syringe equipped with a 0.8 mm diameter needle. One of the most critical aspects during the evaluation of the CSHB catalyst is the meticulous washing protocol of the matured beads, thereby ensuring the removal of trapped hydroxyl ions (OH$^-$), which otherwise might influence the expected base catalysis by the free amino groups –NH$_2$ on the chitosan backbone, upon slow diffusion-controlled leaching of OH$^-$ during the reaction. In order to demonstrate this hypothesis, different batches of beads at different pH were prepared by tuning the wash procedure. The exact pH of the filtrate in each case was measured using a pre-calibrated pH meter. The use of previously reported pH indicators like phenolphthaleine,[21] proved to be unreliable for this study.[23] The general correlation of the pH of the filtrate with the internal pH of the beads was checked by extensive trituration-dissolution of the beads and measuring the pH of the resulting solution.

Morphology. Scanning electron microscopy (SEM) investigations of the corresponding freeze-dried CSHB showed the heterogeneous porous nature and well-developed networks of the beads with internal pores up to 2 μm in diameter (Fig. 2D–E), in contrast to the amorphous structure of commercially powdered chitosan (Fig. 2F). Such heterogeneous and layered structure of the CSHB surface can strongly favour the adsorption of small molecules and ions presented in the medium *via* electrostatic interactions (non-specific or physical adsorption), hydrogen bonding and/or van der Waals forces.[24]

The average porosity of the beads regardless of the diameter was estimated in 74 ± 2%, with a calculated moisture content of 94 ± 1%. The aqueous swelling of the chitosan was translated

in a much higher percentage of accessible –NH_2 groups (55–65%) in comparison to both powdered commercially chitosan and dried chitosan beads (2.5 and 1.7%, respectively),[21] which should enhance the potential base catalytic activity of the former.

FTIR spectroscopy. The FT-IR spectrum of PCS showed the expected bands at 1645 cm^{-1} (amide I, C=O stretching), 1588 cm^{-1} (N–H angular deformation of amino groups), 1420 cm^{-1} (–CH_2 bending vibration), 1377 cm^{-1} (C–O stretching of primary alcoholic groups –polysaccharides conformation sensitive area–), 1321 cm^{-1} (amide III), as well as the bands corresponding to the symmetric stretching of C–O–C in the region 1010–1090 cm^{-1}. The broad band between 2990–3600 cm^{-1} corresponds to –OH and –NH stretching absorption, whereas the aliphatic C–H stretching can be observed between 2850–2950 cm^{-1} (Fig. 3). In the other hand, 4 mm diameter CSHB showed also the broad but more intense peak between 2990–3650 cm^{-1} related to the stretching vibrations of the –OH and –NH groups also involved in hydrogen bonding. The band at 2881 cm^{-1} is again attributed to C–H stretching, whereas amide II band, N–H bending, CO stretching of acetyl groups and free –NH_2 groups converge in the area between 1600–1645 cm^{-1}.
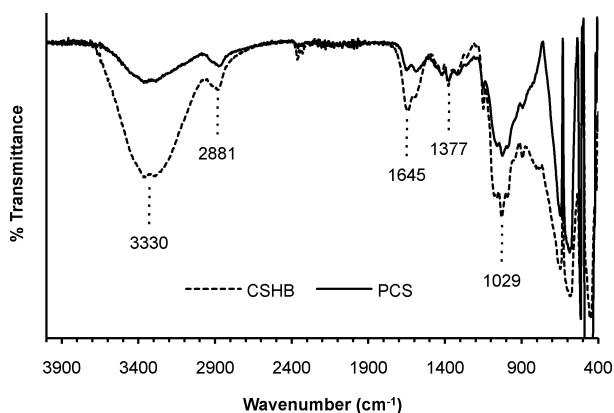

Fig. 3 FT-IR spectra of PCS and CSHB (4 mm diameter).

As for PCS, the zeta potential of CSHB[25] is also defined by the protonation/deprotonation features of the amine groups since it is positive in acidic solutions and negative in basic solutions, with a point of zero found at about pH 6.6, which is close to the pK_a values for the –NH_2.[26]

Thermal characterization. (1) Thermogravimetric analysis (TGA): TGA curve of CSHB showed expected weight loss at two stages (see ESI†). The first one (ca. 95% weight loss) was found in the region below 250 °C, which is attributed to the water content and in agreement with the estimated value (the weight loss in the same region for solid samples due to absorbed atmospheric water was ca. 1.8%). The second weight loss for CSHB (ca. 2.3%) was observed in the region between 250 and 450 °C, which is attributed to the decomposition of the polysaccharide chain by comparison with the TGA spectrum of the solid samples (ca. 50–60% weight loss in the same region). In general, the decomposition temperature of chitosan is molecular weight dependent (the lower the molecular weight, the lower the degradation temperature).[27] The lower decomposition pattern of the CSXG with respect to PCS is attributed to the higher packing

density of the former. (2) Differential scanning calorimetry (DSC): The DSC thermograms (see ESI†) of commercially PCS and CSHB were consistent with the above TGA and literature data.[28] PCS showed an expected exothermic peak centered at 294 °C, which corresponds to the degradation of the biopolymer backbone, whereas the CSHB showed also a broad endothermic peak centered at 115 °C, which is properly ascribed to the loss of water (the equivalent peak due to evaporation of the absorbed water in PCS was centered at 96 °C). The corresponding exothermic peak of the CSHB was centered at 289 °C.

Catalyst performance in the aldol reaction

Despite the low pK_a value of the amine group in the chitosan, there are a few aspects that should be taken into consideration when testing the catalytic potential of chitosan–based materials: 1) For monoamines, there is only a single pK_a value, but for polyamines the actual number of pK_as is related to the total number of amine groups in the polymer. Thus, the pK_as are used to calculate the overall concentration of conjugate base present for a given amine,[29] which ultimately is influenced by the polymer polydispersity index (PDI), polymer chain length and the length of the spacer between amines. 2) In general, amines are more basic in polar aprotic solvents (e.g. DMSO) than in water. In the context of gel systems, it is also worth considering the potential enhancement of basicity of the system upon gel formation,[30] which could take place on the aminated surface of CSHB. 3) The possibility of thermodynamic control in amine-catalyzed Aldol-type reactions involves several reversible steps and a modest exothermicity in reaction with aldehydes, which contribute to the success of the reaction even when weak bases are used to produce only low concentrations of the corresponding nucleophilic intermediates.

Astoundingly, and apparently in contrast with previous observations,[21] neutral pH CSHB with average diameters of 4.0 ± 0.1 mm showed very low activity towards direct aldol reactions between 4-nitrobenzaldehyde (**1a**) as acceptor and acetone (**2**) (model reaction I) or cyclohexanone (**5**) (model reaction II) as donor in DMSO. The reaction was initially run at RT using 17 mol% of catalyst[23] in agreement with previous report.[21] A molar ratio aldehyde:ketone 1 : 13.6 was employed to minimize self-condensation of the acceptor and favour cross-condensantion.[18,20,21] No product formation was detected in control experiments without catalyst, using dried gel beads instead of CSHB or using commercial PCS (see ESI,† Table S2).[31] Nevertheless, instead the expected quantitative conversion of the aldehyde,[21] only 4% conversion was achieved after 24 h.[32] No significant improvement was observed neither by increasing three-fold the catalyst loading nor at higher temperature.[23] The small differences were observed within the experimental error. In spite of the extremely low conversions, the ratio aldol product:dehydration product (99 : 1) was in agreement with that previously reported.[21,33]

With this set of data in our hands, and during the preparation of several CSBH batches, we realized that "just" a problem in controlling the washing step in the catalyst preparation could perhaps explain, at least to some extent, the observed enormous discrepancy with the previous report where

Table 1 Correlation between pH of CSHB and conversion to the β-hydroxycarbonyl aldol product[a]

Entry	ArCHO		pH[b]	Time (h)	Conversion (%)[c]
1	4-Nitrobenzaldehyde	(1a)	6.57	24	6
2	4-Nitrobenzaldehyde	(1a)	6.61	24	8
3	4-Nitrobenzaldehyde	(1a)	6.87	24	4
4	4-Chlorobenzaldehyde	(1b)	6.87	18	0
5	2-Naphthaldehyde	(1c)	6.87	18	0
6	4-Nitrobenzaldehyde	(1a)	7.00	24	12
7	4-Nitrobenzaldehyde	(1a)	7.34	24	39
8	4-Nitrobenzaldehyde	(1a)	7.34	30	59
9	4-Nitrobenzaldehyde	(1a)	7.62	24	30
10	4-Nitrobenzaldehyde	(1a)	7.87	24	45
11	4-Nitrobenzaldehyde	(1a)	7.87	24	31[d]
12	4-Nitrobenzaldehyde	(1a)	10.96	24	42
13	4-Nitrobenzaldehyde	(1a)	—[e]	24	0

[a] Reation conditions: 1a–c (1.0 mmol), 2 (1 mL, 13.6 mmol), beads number = 20 (corresponding to 17 mol% of free amino groups with respect to the aldehyde), DMSO (3 mL), RT. CSHB used in the following entries correspond to the same batch preparation: Entries 1, 8 and 9 (batch 1); entries 2, 6 and 12 (batch 2); entries 11 and 12 (batch 3). [b] Herein, the reported relative pH values correspond to the filtrate after washing. [c] Determined by ¹H NMR of the crude product based on the aldehyde proton (see ESI†). Estimated relative error = ± 2. [d] Result of the first recycling of entry 11. [e] Control experiment in which no catalyst was employed. Note: The selectivity 3:4 was estimated as 99 : 1 based on ¹H NMR analysis.

phenolphthalein-indicator was used to monitor the pH of the filtrates.[21] In order to demonstrate this hypothesis, several CSHB batches were prepared under different washing protocols to guarantee hydrogel beads with different basicity. Thus, pH dependent experiments could be performed as described in Table 1. Neutral CSHB afforded only 12.3% (Table 1, entry 6) conversion when 4-nitrobenzaldehyde was used as model substrate, which did not show conversion in the absence of the catalyst (Table 1, entry 13). In general, the use of slightly basic CSHB resulted in an expected conversion enhancement (Table 1, entries 7, 9, 10, 12), which could be further enhanced by longer reaction times (Table 1, entry 8 vs. 7). Such correlation is even reflected in those cases where further washings were done for the beads from the same batch, resulting in a drastic reduction in conversion (Table 1, entry 7 vs. 1, entry 9 vs. 6 or 2). Therefore, the low activity of the phenolphthalein-indicator at pH < 8.3 (colour change interval = 8.3–10, from colourless at pH < 8.3 to fuchsia at higher pH) should be taken into consideration in order to ensure the preparation of neutral CSHB, which should be cross-checked with the electrical measurement of the proton concentration inside the beads and/or conductivity measurements of the filtrates.[34] Hence, we anticipate that the earlier reported aldol conversions using CSHB could be determined under basic conditions rather than a neutral environment due to sufficient trapping of hydroxide ions,[21] which would indeed enhance the catalysis. This could also explain the drop of the conversion after a second run of the CSHB at pH 7.87 (Table 1, entry 10 vs. 11). In contrast, CSHB batches displaying pH values between 6.57 and 6.87 showed very little activity in the case of 4-nitrobenzaldehyde (Table 1, entries

1–2) and no activity whatsoever in less activated substrates like 4-chlorobenzaldehyde (Table 1, entry 4) or 2-napthaldehyde (Table 1, entry 5) under identical conditions to those previously reported,[21] indicating that simply the accessible free primary amino groups presented in these hydrogel beads of the native chitosan are not active enough to promote satisfactorily the formation of the required enamine intermediate[35] under the present hydrogel conditions. Despite the fact that CSHB would in principle fulfil the requirements to alter the selectivity of the reaction (in the case in which the beads act as a nanoreactor),[36] no induction of stereoselectivity due to the chiral backbone of the biopolymer was observed.

To ensure that partial volatilization of acetone (b.p. = 50.5 °C at 760 mm Hg) was not decreasing the reaction rate, we also tested the reaction between 1a and the non-volatile cyclohexanone (5) (b.p. = 155.7 °C at 760 mm Hg), which has similar basicity in DMSO (pK_a = 26.4 for 2 and 26.5 for 5) and represents a well-studied substrate for comparison in the same reaction.[15a,19] Taking into consideration the latest results reported on the use of chitosan aerogels as catalyst for the asymmetric aldol reaction in water,[19] both DMSO and water were used to evaluate the performance of CSHB. No conversion was observed when the reaction was carried out in absence of any chitosan-based material (Table 2, entries 1 and 3). Similarly, no conversion was observed after 48 h when commercially LMW PCS was used in DMSO (Table 2, entry 2), and only

Table 2 Aldol model reaction II between 4-nitrobenzaldehyde (1a) and cyclohexanone (5) under different conditions[a]

Entry	Solvent[b]	Catalyst	Conversion (%)[c]	dr (anti/syn)[c]
1	DMSO	—	0	—
2	DMSO	PCS[d]	0	—
3	H₂O	—	0	—
4	H₂O	PCS[d]	8 (46[e])	69 : 31 (69 : 31[e])
5	H₂O:DMSO (1 : 1)	PCS[d]	2	61 : 39
6	H₂O	CSHB	13 (77[e])	68 : 32 (70 : 30[e])
7	DMSO	CSHB	3	56 : 44
8	H₂O:DMSO (1 : 1)	CSHB	6	70 : 30
9	H₂O:toluene (4 : 1)	CSHB	9	66 : 34
10[f]	H₂O	CSHB	75[h]	68 : 32
11[g]	H₂O	CSAB	85[h]	70 : 30

[a] Reaction conditions: 1a (1.0 mmol), 5 (13.6 mmol), pH = 6.80, beads number = 20 (corresponding to 17 mol% of free amino groups with respect to the aldehyde), solvent (3 mL), 48 h, RT. [b] The amount of water held by the CSHB (20 beads) was estimated in ca. 0.5 mL, which is not included in the total volume of solvent described in the reaction conditions. [c] Determined by ¹H NMR spectroscopy of the crude product. Batch-to-batch estimated relative error = ±0.5%. Relative configurations were assigned by comparison with reported literature data. [d] Powdered chitosan: 28 mg (corresponding to 17 mol% free amino units with respect to the aldehyde). [e] Result obtained under the following conditions: 22 mol% free amino groups with respect to the aldehyde, 0.1 mmol 1a, 2.0 mmol 5, 0.5 mL H₂O, 48 h, RT. [f] Data reported in ref. 19 using different CSHB under the conditions specified in footnote e. No specific details regarding the preparation and characterization of those CSHB used were given, which would be necessary for a precise comparison with our beads. [g] Data reported in ref. 19 using chitosan aerogel beads (CSAB) as catalyst under the conditions described in footnote e. [h] Isolated yield of the β-hydroxycarbonyl compound 6a.

8% when the reaction was carried out in H_2O (Table 2, entry 4). In the latter case, a moderate *anti*:*syn* diastereoselectivity (68 : 32) was achieved. As expected from these results, the use of PCS in H_2O:DMSO 1 : 1 (v/v) afforded almost no conversion whatsoever (Table 2, entry 5). Disappointedly, when we tested our CSHB as catalyst, the reaction conversion was only slightly increased to 13% in H_2O (Table 2, entry 6) with almost no detriment in the diastereoselectivity (69 : 31), 6% in H_2O:DMSO 1 : 1 (v/v) (Table 2, entry 8), and 3% in DMSO (Table 3, entry 7). In the latter case, the *anti*:*syn* ratio dropped by *ca.* 60% in comparison to the reaction in pure water. Addition of toluene as a co-solvent to improve the solubility of the reactants in the aqueous medium provoked slightly deterioration of both conversion and selectivity (Table 2, entry 9 *vs.* 6). These findings would be in agreement with the key role of solvent, and especially the beneficial effect of water, in base-catalyzed aldol-type reactions.[21,37]

Unfortunately, our CSHB were found to be *ca.* 5 times less effective (in terms of yield but not in terms of selectivity) than the chitosan aerogel, PCS and CSHB formulations reported previously by Quignard and co-workers under analogous conditions (Table 2, entry 6 *vs.* 10–11).[19] At this point, and in agreement with previous observations,[19] we checked that the use of slightly more diluted conditions (0.2 M *vs.* 0.3 M), a higher excess of ketone (20 equiv *vs.* 13.6 equiv) and 5 mol% increased catalyst loading, provided a remarkable enhancement of the conversion in both PCS and CSHB-catalyzed processes with almost no variation in the diastereoselectivity (Table 2, entries 4 and 6). Hence, we decided to evaluate which of the different parameters represent a significant contribution to the conversion (Fig. 4). The results of the experimental design clearly showed that only the reaction scale caused a major impact (*e.g.* 75% conversion for **F** *vs.* 7% for **E**).
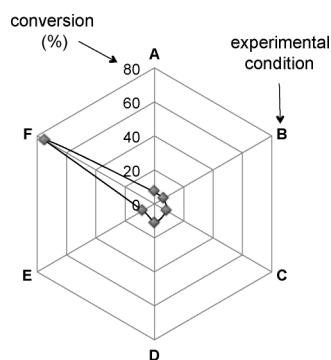

Fig. 4 Radial diagram showing the effect of catalyst loading (**B** *vs.* **C**), molar ratio (**A** *vs.* **B**; **C** *vs.* **D**), concentration (**A** *vs.* **E**) and reaction scale (**E** *vs.* **F**) on the conversion of the aldol reaction between **1a** and **5** catalyzed by 4 mm diameter CSHB. Experimental conditions: **A** = 22 mol% catalyst, 1 mmol **1a**, 20 mmol **5**, 0.3 M in **1a**; **B** = 22 mol% catalyst, 1 mmol **1a**, 13.6 mmol **5**, 0.3 M in **1a**; **C** = 17 mol% catalyst, 1 mmol **1a**, 20 mmol **5**, 0.3 M in **1a**; **D** = 17 mol% catalyst, 1 mmol **1a**, 13.6 mmol **5**, 0.3 M in **1a**; **E** = 22 mol% catalyst, 1 mmol **1a**, 20 mmol **5**, 0.2 M in **1a**; **F** = 22 mol% catalyst, 0.1 mmol **1a**, 2 mmol **5**, 0.2 M in **1a**. Constant conditions: 48 h, RT. Estimated error = ± 0.5%.

In the other hand, the effect of the molecular weight of the chitosan was found to be statistically insignificant in this reaction in terms of conversion and selectivity. For example, the use of

Table 3 Knoevenagel condensation reaction between different aldehydes **1a–k** and malonitrile (**7**) catalyzed by CSHB in DMSO at RT[a]

Entry	Aldehyde **1a–k**	Product **8a–k**	Time (min)	Conversion (%)[b]
1	**1a**	**8a**	5	99
2	**1b**	**8b**	5	100 (92[c])
3	**1c**	**8c**	5	83
4	**1d**	**8d**	5	62
5	**1e**	**8e**	5	100 (93, 80)[d]
6	**1f**	**8f**	5	100
7	**1g**	**8g**	60[e]	100
8	**1h**	**8h**	5	93
9	**1i**	**8i**	5	55[f]
10	**1j**	**8j**	5	84[f]
11	**1k**	**8k**	30	100 (84[c])

[a] Reaction conditions: **1a–k** (1.0 mmol), **7** (1.1 mmol), DMSO (3 mL), mean pH = 6.9, beads number = 20 (corresponding to 17 mol% of free amine groups with respect to the aldehyde), RT. [b] Determined by ^{1}H NMR spectroscopy of the crude product based on the aldehyde proton. Batch-to-batch estimated error = ± 0.5%. [c] Yield previously reported in the literature for the uncatalyzed process in water reaction. Reaction time = 3 min for entry 2 and 30 min for entry 11. [d] Experiments using CSHB from different batches with slighly different pH values: 6.7 for 93% conversion and 6.5 for 80% conversion. [e] Reaction time was not optimized. [f] Conversion calculated respect to malononitrile instead of the aldehyde due to the lower boiling point of the latter.

medium (MMW) or high molecular weight (HMW) PCS lead to 51% conversion (*anti/syn* = 71 : 29) or 78% conversion (*anti/syn* = 68 : 32), respectively. These values are in the same range than those obtained under the same conditions using LMW PCS (46% conversion, *anti/syn* = 69 : 31 (Table 2, entry 4). A similar behaviour was observed with the hydrogel beads, which lead to 13% conversion (*anti/syn* = 70 : 30) in the case of MMW chitosan or 7% conversion (*anti/syn* = 72 : 28) in the case of HMW chitosan (for comparison, see Table 2, entry 6).[38]

With these results in hand, we decided to explore also some other important variables that could greatly influence the catalyst performance in the case of the hydrogel beads. The foregoing findings and a meticulous study of the experimental details provided for the preparation of similar CSHB,[39] motivated us to evaluate first the foreseeable effect of the surface area to volume ratio (SA:V) of the CSHB in the aldol reaction rate. For a given shape, high SA:V decreases linearly with increasing size and provides a strong driving force to speed up chemical reactions upon minimization of thermodynamic free energy. In this context, smaller spherical CSHB (2.2 ± 0.2 mm in diameter) were also tested in the aldol model reactions. However, no significant difference was observed compared to 4-mm CSHB in terms of conversion and selectivity. The result most likely suggests, at least within the studied size range, a basicity mismatch effect rather than a SA:V effect. Moreover, in contrast to the aerogels,[19] the addition of 20 mol% of 2,4-dinitrophenol as a catalyst for the formation of the enamine intermediate did not provide better results in the case of CSHB (17 mol%) for the model aldol reaction I (conditions: 18 h, RT).

Knoevenagel reaction

With the lessons learned from the case of the aldol reaction, we further re-evaluated the neutral CSHB as organocatalyst for the Knoevenagel condensation reaction at RT in DMSO. This modification of the aldol condensation was tested for an expanded variety of aromatic, heteroaromatic and aliphatic aldehydes in combination with activated methylene compounds as donors including malononitrile ($pK_{a(water)}$ = 11.1; $pK_{a(DMSO)}$ = 11.10), ethylcyanonacetate ($pK_{a(DMSO)}$ = 13.10), barbituric acid ($pK_{a(water)}$ = 4.01) and Meldrum's acid ($pK_{a(DMSO)}$ = 7.33).

Good to excellent conversions (*i.e.* 55–100%) to the desired condensation product were quickly achieved in the reaction between malononitrile and a variety of aromatic, heteroaromatic and aliphatic aldehydes (Table 3). Thereby, aryl compounds with electron withdrawing groups (*e.g.* Table 3, entries 1–2) afforded approximately 1.6-fold higher conversion[40] rate than those with electron donating groups (*e.g.* Table 3, entries 4–5). The desired condensation product was also obtained in good yield with more hydrophobic substrates such as 2-naphthaldehyde (Table 3, entry 3). The reaction rates in the case of aliphatic aldehydes (Table 3, entries 9–10) were comparable to those observed for activated aromatic aldehydes. Double condensation in the case of terephthalaldehyde was also achieved in very good yield without difficulties (Table 3, entry 8). In agreement with previous observations,[41] heteroaromatic substrates like 2-furaldehyde were found to react more slowly than other aromatic aldehydes (Table 3, entry 11). It was previously reported that the reaction rates in Knoevenagel condensations are slowed when

bulky reagents were used.[42] However, the use of 2-substituted isomers (Table 3, entries 6–7) provided the same results as the 4-substitued isomers (Table 3, entries 1–2), which indicates a negligible effect of the steric effect of these 2-substitued isomers in our system, albeit other substituents with higher *A*-values were not evaluated (*A*-value$_{(Cl)}$ = 0.43 kcal mol^{-1}; *A*-value$_{(NO2)}$ = 1.1 kcal mol^{-1}).

In contrast to some reports in the literature,[43,23] at least in our hands and using the conditions depicted in Table 3, we could not observe conversion of aldehydes **1a–1b** within 5 min in pure water and in the absence of any catalyst. In any event, the use of efficient green base-catalysts in aprotic solvents like DMSO would overcome the limitations of working with unstable aldehydes (*e.g.* aldehydes containing hydrolyzable silanes, aldehydes containing water-sensitive functional groups, aliphatic or water-insoluble aldehydes like **1a–1b** with slow kinetic in/on pure water). As a proof-of-concept, highly reactive *tert*-butyl chloride was quantitatively hydrolyzed to *tert*-butanol in 0.3 M water after 1.5 h at RT, whereas it remained stable in DMSO and in the presence of 20 hydrogel beads (17 mol% of free –NH$_2$ groups; estimated amount of trapped water = 0.5 mL).

As expected, the reactions with ethylcyanoacetate were in general slower than with more acidic malonitrile (Table 4), albeit no difference in terms of catalyst stability was observed in any case. Moreover, only one geometric isomer (*E*-isomer) was obtained in all cases. Amongst the tested aryl aldehydes, only 4-nitrobenzaldehyde reacted quantitatively in 5 min (Table 4, entry 1). Less activated aldehydes needed 1 h to react completely (Table 4, entries 2, 8), which was not possible with more electron rich aryl substrates even after 5 h (Table 4, entries 4–5). Herein, low reactive substrates like 2-naphthaldehyde produced only modest conversion after 1 h (Table 4, entry 3). Moreover, the steric effects showed relatively high influence in comparison to the reaction with malonitrile, which can be also overcome with longer reaction times (Table 4, entries 6–7). In general, kinetic rates for activated aromatic aldehydes were found to be higher than for their aliphatic or heteroaromatic partners (Table 4, entries 9–11). Control experiments carried out in the absence of CSHB or in the presence of PCS (Table 4, entry 2) confirmed the utility of the CSHB as heterogeneous catalyst for the Knoevenagel condensation reaction. It is also worth mentioning that in none of the cases, side reactions like self-condensation or Cannizaro products were observed. Although the studied beads also showed activity under solvent-free conditions, the use of solvent clearly facilitates the molecular collisions and the access to the CSHB –NH$_2$ groups (Table 4, entry 11).

Kinetic studies of a model Knoevenagel reaction between **1f** and **9** catalyzed by CSHB in DMSO (Table 4, entry 6) led to a first-order rate constant = 4.9 ± 0.1 × 10^{-2} min^{-1}, which is in the same range as that reported in the literature for comparable processes.[23] Moreover, a much lower reaction rate was observed in the case of other activated methylenes containing heterocyclic compounds such as barbituric acid or Meldrum's salt (see ESI,† Table S3), most likely due to steric effects.

With the "ideal synthesis/catalysts" concept[44] in mind, we further evaluated the recovery and reusability of the catalyst in the model condensation between **1a** and **7** (Table 5). After the work-up of the reaction, no visible physical changes were observed on the CSHB catalyst surface. The catalyst was found

Table 4 Knoevenagel condensation reaction between different aldehydes **1a–k** and ethylcyanoacetate (**9**) catalyzed by CSHB in DMSO at RT[a]

Entry	1a–k	Product 10a–k	Time (min)	Conversion (%)[b]	E/Z ratio[c]
1	1a	10a	5	100	100/0
2	1b	10b	5	31 (100[d], 3[e], 0[f])	100/0
3	1c	10c	5	9 (35[d])	100/0
4	1d	10d	5	28 (29[d], 75[g])	100/0
5	1e	10e	5	29 (35[d], 83[g])	100/0
6	1f	10f	20	97	100/0
7	1g	10g	60	92	100/0
8	1h	10h	40	100	100/0
9	1i	10i	60	55[h]	100/0
10	1j	10j	60	12[h]	100/0
11	1k	10k	60	100 (45[i])	100/0

[a] Reaction conditions: **1a–k** (1.0 mmol), **9** (1.1 mmol), DMSO (3 mL), mean pH = 6.9, beads number = 20 (corresponding to 17 mol% of free amine groups with respect to the aldehyde), RT. [b] Determined by ^{1}H NMR spectroscopy of the crude product based on the aldehyde proton. Batch-to-batch estimated error = ± 0.5%. [c] Determined by ^{1}H NMR of the crude product. [d] Conversion after 60 min. [e] Control experiment using PCS (50 mg), reaction time = 5 min. [f] Control experiment without catalyst, reaction time = 5 min. [g] Conversion after 5 h. [h] Conversion calculated with respect to ethylcyanoacetate instead of the aldehyde due to the lower boiling point of the latter. [i] Conversion obtained under solvent-free conditions (60 min, RT).

to be easily recovered and retain full activity for at least 4 runs with high TON (> 5800) and TOF (> 1100 min^{-1}), which indicate both efficient catalyst recovery and good catalyst lifetime.[44]

The general greater effectiveness of the Knoevenagel condensation in comparison to the previous aldol reaction disguises the expected pH dependence activity in the former. In spite of this, a similar trend of pH-triggered conversion could also be perceived for model Knoevenagel condensations between **1b** and **9** or **1e** and **7** (Table 6).
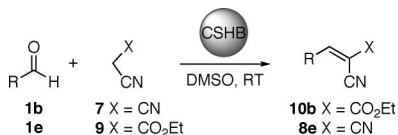
The concrete participation of the primary –NH$_2$ groups of the chitosan in the aldol-like reactions was further demonstrated by submitting the CSHB to an imine cross-link process

with glutaraldehyde to form CS–N=CH(CH$_2$)$_3$CH=N–CS.[45] This heterogeneous cross-linking reductive amination slightly reduced the positive ζ-potential at pH < 6.7 and enhanced the mechanical stability of the beads. However, blocking the primary –NH$_2$ groups should cause an erosion of the CSHB for catalysis by decreasing the accessibility of substrate molecules to active basic sites. Indeed, when the model reaction between aldehyde **1f** and ethylcyanoacetate (**9**) (Table 4, entry 6; conversion = 97%) was carried out in the presence of the cross-linked CSHB, the conversion dropped drastically to *ca.* 36%. Some conversion is still observed most probably due to an incomplete imine cross-linking process caused by partial polymerization and/or

Table 5 Recycling experiments in the Knoevenagel condensation reaction between **1a** and **7** catalyzed by CSHB in DMSO at RT[a]

Entry	Catalytic cycle	Time (min)	Conversion (%)[b]	TON[c] (± 59)	TOF (min^{-1})[d] (± 12)
1	Fresh catalyst	5	99	5823	1164
2	First cycle	5	99	5823	1164
3	Second cycle	5	99	5823	1164
4	Third cycle	5	99	5823	1164

[a] Reaction conditions: **1a** (1.0 mmol), **7** (1.1 mmol), DMSO (3 mL), mean pH = 6.9, beads number = 20 (corresponding to 17 mol% free amine units with respect to the aldehyde), RT. [b] Determined by ^{1}H NMR spectroscopy of the crude product based on the aldehyde proton. Batch-to-batch estimated error = ± 0.5%. [c] Turnover number defined as the molar ratio of converted substrate to catalyst loading. [d] Turnover frequency defined as the molar ratio of converted substrate to catalyst loading per unit of time.

Table 6 Correlation between pH of CSHB and conversion towards the Knoevenagel product in DMSO at RT[a]

Entry	Aldehyde	Donor	Product	pH	Conversion (%)[b]	TON[c] (± 117)
1	**1b**	**9**	**10b**	6.49	15	882
2	**1b**	**9**	**10b**	6.99	31	1824
3	**1b**	**9**	**10b**	10.48	72	4235
4	**1e**	**7**	**8e**	6.49	80	4706
5	**1e**	**7**	**8e**	6.99	87	5118
6	**1e**	**7**	**8e**	8.44	100	5823

[a] Reaction conditions: **1b** or **1e** (1.0 mmol), **7** or **9** (1.1 mmol), DMSO (3 mL), mean pH = 6.9, beads number = 20 (corresponding to 17 mol% free amine units with respect to the aldehyde), RT, reaction time = 5 min. [b] Determined by ^{1}H NMR spectroscopy of the crude product based on the aldehyde proton. Batch-to-batch estimated error = ± 0.5%. [c] Turnover number defined as the molar ratio of converted substrate to catalyst loading.

irreversible entrapment of glutaraldehyde within the cross-linked beads.[45] However, the foregoing results point out another significant factor that may influence the –NH$_2$ catalysis of CSHB. The ability of chitosan to readily form imines in the presence of aldehydes under mild conditions could support also a potential change from amine to imine catalysis, at least to a certain extent, with a consequent modification of the surroundings of the active site. Hence, a possible combination of both amine and imine base catalysis (apparently favourable to the former) should be considered in the mechanism of CSHB-catalyzed reactions like Knoevenagel condensations, where imines are usually the key intermediates. In this sense, imine grafted silicas have been already described as mild and effective base catalysts for Knoevenagel and Michael reactions.[46]

At the core of our research perspective, the particular case of biohydrogel materials in catalysis is framed within a scientific challenge devoted towards altering the selectivity of chemical transformations by arranging the potential reactants in organized and confining media.[36] In combination with the inherent chirality of the chitosan, the high active surface area of CSHB and its 3D porous network might amplify the potential stereoselection in the aldol-like reactions. However, the fact that almost negligible enantiomeric excess (< 1%) was observed in the aldol reaction may denote a role of the biohydrogel as an immobilized base catalyst instead of a chiral nanoreactor, which is likely to be

the case for aerogel microspheres. Indeed, no apparent change in light-scattering was observed for CSHB in water between 0 °C and 60 °C, which suggest a permanent close packed structure within that range of temperature. In order to further verify or falsify the above hypothesis we run some model reactions using hemispherical CSHB obtained by cutting in half the original spherical beads. For the same number of beads, the total reactive surface area (SA) will be higher for the hemispherical CSHB (Fig. 5, *top*). Thus, the comparison between the ratio of the surface areas [SA$_{2(hemispherical\ CSHB)}$/SA$_{1(spherical\ CSHB)}$] with the ratio of the specific reaction rates [$K_{2(hemispherical\ CSHB)}$/$K_{1(spherical\ CSHB)}$/] should provide insight about the reaction pathway. No major difference between these ratios would be expected if most of the substrate molecules were transformed on the CSHB surface (Fig. 5, *top*, path b), whereas a much higher kinetic ratio would underline primary reactivity inside the beads (Fig. 5, *top*, path a). In our case, a fairly good linear correlation between surface area of the CSHB and the ratio of the reaction rates was observed (Fig. 5, *bottom*). Thus, the studied CSHB seems to behave most likely as a base-supported catalyst, which is also consistent with the fact that no trace of reaction product could be detected at any reaction time inside the beads. Hence, the modest differences observed between conversions and isolated yields[40] should be attributed to partial adsorption/absorption of the starting materials onto/inside the beads and/or minor
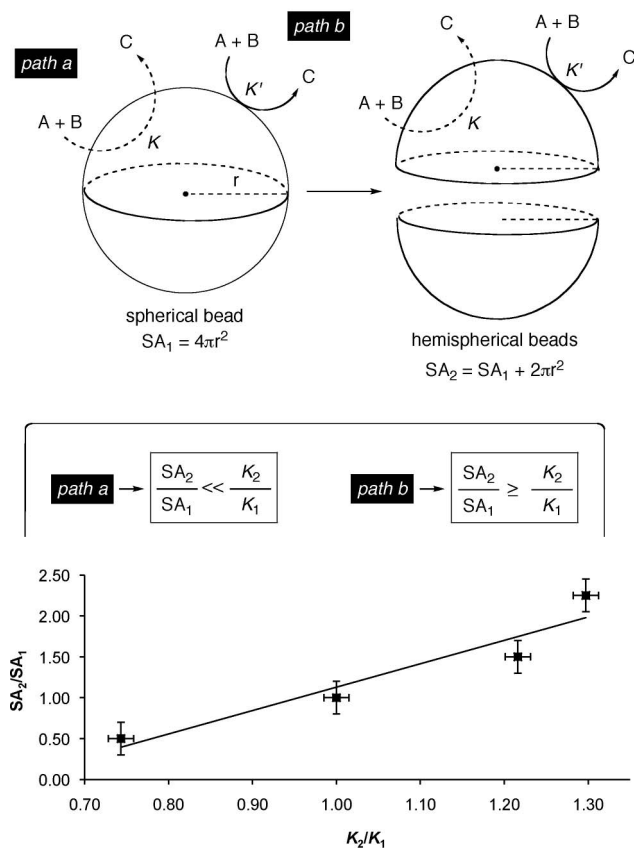
Table 7 CSHB-catalyzed model nitroaldol (Henry) reaction in different solvents[a]

Entry	Solvent	Catalyst	$T/°C$	Time (h)	Conversion (%)[b]
1	MeOH	CSHB	20	24	33
2	MeOH	CSHB	30	24	99 (98[c], 80[d])
3	MeOH	CSHB	30	24	97[e], 92[f], 78[g], 27[h]
4	EtOH	CSHB	30	24	99
5	H_2O	CSHB	25	12	98 (15[i], 5[j])
6	DMSO	CSHB	25	12	100 (74[i], 33[j])
7	H_2O	—	25	16	2%
8	DMSO	—	25	24	< 1%
9	MeOH	—	30	24	0
10	MeOH	ADCSHB	30	24	0[k]
11	MeOH	PCS	30	24	37[l]
12	MeOH	PCS	30	24	99[m]

[a] Reaction conditions: **1a** (1.0 mmol), **11** (10.0 mmol), solvent (3 mL), mean pH = 6.9, catalyst beads number = 20 (corresponding to 17 mol% of free amine groups with respect to the aldehyde). [b] Determined by ^1H NMR spectroscopy of the crude product based on the aldehyde proton. Batch-to-batch estimated error = ± 0.5%. [c] Conversion using 15 CSHB units. [d] Conversion using 10 CSHB units. [e] Conversion using 7 equiv of **11** with respect to **1a**. [f] Conversion using 5 equiv of **11** with respect to **1a**. [g] Conversion using 3 equiv of **11** with respect to **1a**. [h] Conversion using 1 equiv of **11** with respect to **1a**. [i] Recycling experiment: Conversion after second cycle. [j] Recycling experiment: Conversion after third cycle. [k] ADCSHB = Air dried chitosan hydrogel beads ($m_{(20\ units)}$ = 28 mg). [l] Control experiment performed using 28 mg of PCS as catalyst. [m] 28 mg of PCS was used as catalyst and 0.54 mL of H_2O was added.

Fig. 5 *Top*: Relationships between reactive surface areas (SA_1, SA_2) and reaction rate (K_1, K_2) for two potential modes of action of CSHB with diameter r: (1) as a nanoreactor (path a); and (2) as a supported base. Theoretical considerations were made on the basis of perfect spherical beads. *Bottom*: Surface area to reaction rate plot for the Knoevenagel condensation reaction catalyzed by CSHB.

lost during product isolation, mainly due to volatility issues and/or deficient washing steps. In the other hand, the possibility of hydrogen bonding at the surface of the beads between the aldehyde and the water molecules of the hydrogel network could assist the condensation reaction at the water/organic solvent interface.

In this regard, UV-vis experiments showed for example a significant adsorption of 2-nitrobenzaldehyde onto CSHB (*ca.* 20 mol% after 1 h at RT).[23] Diffusion studies of other small molecules in CSHB have confirmed that the solute transport through the gel beads occurs faster at higher temperatures and primarily by a Fickian diffusion mechanism with general activation energies in the range of 20–30 kJ mol^{-1}.[47] At neutral pH, the negative zeta potential of the CSHB would indeed favour the adsorption of electron-deficient aldehydes. Experiments with metal-doped CSHB have also showed that the catalytic reaction is more likely located on the external layers of catalyst particles. In this case, the strong decrease of kinetic rates with increasing size of catalytic beads confirms a higher contribution of the resistance to intraparticle diffusion in the control of reaction kinetics.[48]

Nitroaldol (Henry) reaction

Encouraged by the latter results we decided to evaluate also the CSHB as a heterogeneous catalyst for the classical and valuable nitroaldol (Henry) reaction, which involves the reaction of nitroalkanes with carbonyl compounds (*i.e.* aldehydes, ketones) in the presence of an ionic or non-ionic base-catalyst to form β-nitroalcohols under a wide range of experimental conditions.[49] One of the main drawbacks of this powerful atom-economical reaction is the formation of several by-products that complicate the isolation of the desired compounds. These by-products include mainly polymerizable nitroalkenes (formed upon dehydration of the β-nitroalcohols, especially in the case of aryl aldehydes), self-condensed products in the case of sterically hindered substrates (*i.e.* Cannizzaro reaction epimerized β-nitroalcohols and products derived from the Nef reaction.[49a] In order to optimize the formation of β-nitroalcohols, a careful control of the basicity of the reaction medium and long reaction times are usually required. In the case of aromatic aldehydes, the selectivity of the Henry reaction is strongly dictated by the electronic nature of the substituents and their ability to favour either the imine or ion-pair mechanism.[49]

In order to evaluate the performance of the CSHB as an organocatalyst for the Henry reaction, we first investigated the model reaction between 4-nitrobenzaldehyde (**1a**) and excess nitromethane (**11**) in the presence of nearly neutral CSHB in both protic and aprotic polar solvents that are frequently used in this transformation (Table 7). As expected, the solvent used did not show a very large influence on the outcome of the

reaction.[50] The reaction was driven to full conversion in 12 h at RT when either water or DMSO were used as solvent (Table 7, entries 5–6),[51] whereas in the case of MeOH or EtOH 24 h and 30 °C were needed to achieve similar results (Table 7, entries 2, 4). A temperature increment of only 10 °C was enough to increase the conversion to the desired nitroaldol product **12a** from 33% to 99% (Table 7, entry 1 *vs.* 2). Regarding the aldehyde:nitromethane molar ratio, 1 : 10 was found to be optimum for a catalyst loading of 17 mol% (Table 7, entries 2–3). In contrast, equimolar amounts of reactants in MeOH afforded only 27% conversion after 24 h at 30 °C (Table 7, entry 3). Nearly no conversion was observed when the reaction was run either in H_2O, DMSO or MeOH in the absence of CSHB (Table 7, entries 7–9). Interestingly, the use of air dried CSHB was also unsuccessful (Table 7, entry 10), whereas the commercialy PCS afforded a modest 37% conversion (Table 7, entry 11). The latter could be driven to 99% conversion by adding to the reaction mixture approximately the amount of H_2O estimated in 20 hydrogel beads (*ca.* 30 equiv of H_2O with respect to the aldehyde) (Table 7, entry 12). As pointed out by Quignard and co-workers, these results could be explained by the dramatic effect that the method used for drying these biomaterials could have on the accessibility of the surface catalytic groups.[19] In agreement with previous observations for the aldol reaction, almost no enantiomeric excess (< 1%) was detected in MeOH, H_2O or DMSO either for the nitroaldol version.

In contrast to the Knoevenagel condensation reaction, the recycling model experiments carried out showed a remarkable catalyst deactivation right after the first virtually quantitative cycle (Table 7, entries 5–6), being even more severe in water than in DMSO. Although the exact deactivation mechanism in the case of hydrogel beads remains unclear, blocking of the basic catalytic sites by chemical poisoning of the surface of the beads seems to play a major role. In this sense, factors like the chemical evolution of intermediate imines, large excess of nitromethane, slow reaction kinetics and the presence of a protic solvent could contribute to the formation of an inactive coat blocking the active surface of the CSHB. In order to support this hypothesis the model nitroaldol reaction between **1a** and **11** (Table 7, entry 6) was carried out using beads previously matured under three different conditions: (A) beads matured in a solution of **1a** (1 mmol) in DMSO (3 mL) for 12 h at RT; (B) beads matured in a solution of **11** (10 mmol) in DMSO (3 mL) for 12 h at RT; (C) beads matured in a solution of **1a** (1 mmol) in DMSO (3 mL) for 5 min at RT. The conversion values obtained in each case were 23% (A), 95% (B) and 95% (C). The results point out that the time in which the reactants are in contact with the beads can be crucial for performance of the catalyst. In agreement to these results, the recycled beads in the case of the Knoevenagel condensation (reaction time = 5 min) did not show a major detriment of the catalytic activity. In contrast, the longer reaction time observed in nitroaldol facilitates the blocking of catalytic amine groups. The results of the experiments (A) and (B) point out that the chemical evolution of intermediate imines on the bead surface, by reduction Cannizzro-like processes or formation of cross-linked aminals, play a main role in the catalyst deactivation (Fig. 6, a–b). To a lesser extent, nitroalkanes could be also partially associated with this process since they can interact with the free amine groups by formation of molecular

(or charge-transfer) complexes, or with the hydroxyl groups of polysaccharides forming nitroalkyl ethers,[52] which would be in tautomeric equilibrium with the corresponding nitronic acid species (*aci*-form, $R^1R^2C{=}NOOH$). Moreover, the formation of the *aci*-form is also known to be catalyzed by water[53] or amines[54] through two stabilizing hydrogen bonds, which could also support the faster catalyst deactivation observed in pure water. Moreover, remarkable stable dimers of the *aci*-form could be established like in the case of carboxylic acids.[55] In any event, the overall effect would be a hindered access to the free amino groups on the surface of the hydrogel beads causing a detriment in the catalytic activity (Fig. 6, c). Moreover, SEM images of the catalytic beads after the nitroaldol reaction suggested the presence of both layered structures and new agglomerated moieties on the surface (Fig. 7). Such clustered structure could be also observed in the case of less active glutaraldehyde cross-linked beads.
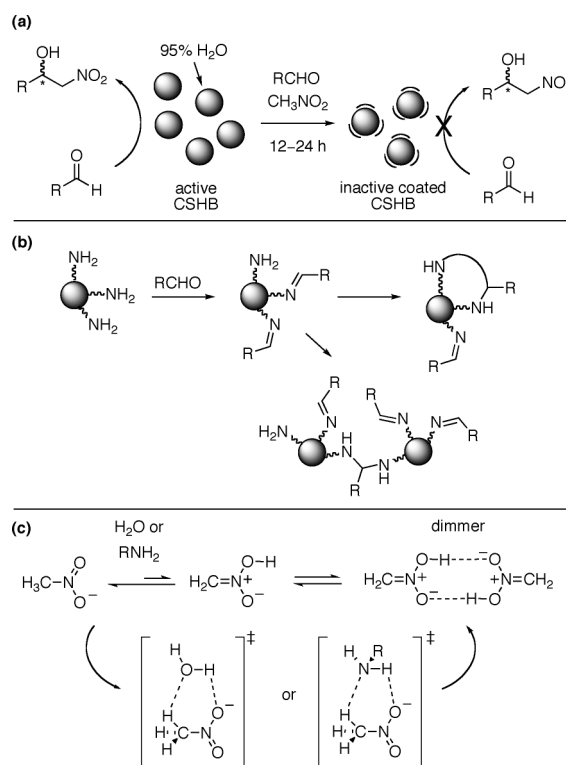

Fig. 6 Plausible contributions to the catalyst deactivation in the nitroaldol reaction (a): formation of lineal or cyclic aminals from intermediate imines (b); formation and H-bonding stabilization of *aci*-nitromethane catalyzed by hydroxyl or amine groups (c).

Due to the high toxicity of methanol and the best results obtained in pure water, the latter was used as the green solvent[56] to study the performance of the biohydrogel catalyst in the Henry reaction with different aldehydes (Table 8). As expected, aldehydes bearing strong electron-withdrawing groups (*i.e.* **1a**, **1f**) were converted to the desired nitroaldol product **12** much faster and high TON than less electrophilic aldehydes (*i.e.* **1l**, **1b**, **1g**) (Table 8, entries 2–3 *vs.* 1, 4–5). For 2-substituted isomers **1f** and **1g** no major steric effect was observed during the reaction (Table 8, entries 3, 5 *vs.* 2, 4, respectively). In the case of the less reactive chloro-substituted aldehydes like **1b**,
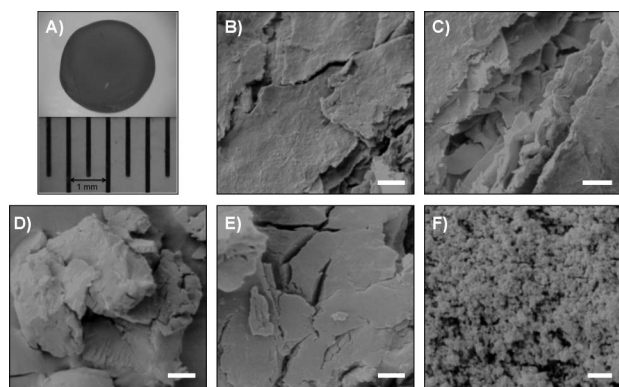
Fig. 7 A) Digital photograph of a CSHB under an optical microscopy (magnification 10×). B–C) Representative SEM images showing the layered surface structure of the CSHB (B: scale bar 20 µm; magnification 500×; C: scale bar 10 µm; magnification 1000×). E–F) Representative SEM images showing the clustered surface structure of the CSHB after the 3rd cycle in the nitroaldol reaction in water (E: scale bar 50 µm; magnification 200×; F: scale bar 10 µm; magnification 1000×). F) SEM image of the freeze-dried cryogel beads made from the glutaraldehyde cross-linked CSHB (scale bar 2 µm; magnification 5000×).

the conversion could be enhanced to *ca.* 70% after 4 days at RT or up to 85% after 24 h at 40 °C. As is shown, *β*-hydroxy nitroalkanes were always obtained as the major product, except for 4-methoxybenzaldehyde and furfuraldehyde, which afforded a mixture of nitroalkane and nitroalkene (dehydrated product) in a ratio 2 : 1 (Table 8, entries 6–7). With aliphatic aldehydes such as isovaleraldehyde (Table 8, entry 8), almost only dehydrated product was detected.

We should indicate that our observations made with commercial PCS in the Henry reaction are in reasonable agreement with the data recently provided by Cui and co-workers.[15b] Nevertheless, the absence of data regarding the properties of the used chitosan makes a reliable comparison very difficult. For instance, aldehyde **1f** was nearly fully converted into the corresponding nitroalcohol product in 24 h (Table 8, entry 3), which was previously obtained in 47% (isolated yield) in 16 h.[15b] Under the assumption that the same solid chitosan material was used in both cases (*e.g.* same molecular weight and DDA), the observed deviations might be justified considering the difference in reaction times (*e.g.* 24 h *vs.* 16 h) and solvent concentrations (*e.g.* 0.3 M *vs.* 0.1 M in aldehyde), as well as possible loss of product during isolation/purification, to which the use of higher amounts of nitroalkane could also contribute (*e.g.* the ratio aldehyde:nitromethane 1 : 10 *vs.* 1 : 23).[15b]
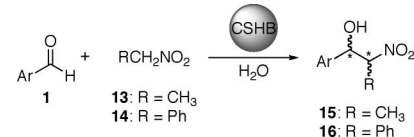
We finally confirmed that the size of the nucleophilic carbanion might play a more critical role than the pK_a of the donor in the mechanism of the CSHB-catalyzed nitroaldol reaction, in a similar way that has been demonstrated for some enzyme-catalyzed examples.[57] We found that the reaction rates and conversions in the presence of CSHB dropped significantly in the case of nitroethane (**13**) in comparison to nitromethane (**11**) (pK_a values:[57] **11** = 10.2; **13** = 8.6) (Table 9). In the other hand, several products were detected by TLC when phenylnitromethane was used as donor, but the NMR analysis of the reaction crude did not allow the unequivocal identification of the signals for the expected product. In general, CSHB performed comparatively

Table 8 CSHB-catalyzed nitroaldol (Henry) reaction between different aldehydes **1** and nitromethane (**11**) in water at RT[a]

$$\text{Ar}\overset{O}{\underset{1}{\text{CHO}}} + \underset{11}{CH_3NO_2} \xrightarrow[\text{H}_2\text{O}]{\text{CSHB}} \underset{12}{\text{Ar}\overset{OH}{\underset{*}{\text{CH}}}NO_2}$$

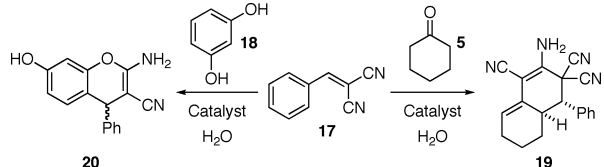| Entry	ArCHO	Product	Conv. (%)[b]	TON[c] (± 117)	TOF[d] (h^{-1}) (± 5)
1	benzaldehyde **1l**	**12l**	91 (99[e])	5353	223
2	4-nitrobenzaldehyde **1a**	**12a**[f]	99[g] (99[h])	5824	243
3	2-nitrobenzaldehyde **1f**	**12f**	96[g] (99[h])	5647	235
4	4-chlorobenzaldehyde **1b**	**12b**	13 (12[e])	765	32
5	2-chlorobenzaldehyde **1g**	**12g**	10 (10[e])	588	24
6	4-methoxybenzaldehyde **1d**	**12d**	23[i]	1353	25
7	furfuraldehyde **1k**	**12k**	16[i]	941	39
8	isovaleraldehyde **1j**	**12j**	76[k]	4471	186

[a] Reaction conditions: Aldehyde (1.0 mmol), **11** (10.0 mmol), mean pH = 6.9, catalyst beads number = 20 (corresponding to 17 mol% of free amine groups with respect to the aldehyde), solvent (3 mL), 24 h, RT. [b] Conversion was determined by ^{1}H NMR spectroscopy based on the aldehyde proton. Batch-to-batch estimated relative error = ± 0.5%. [c] Turnover number defined as the molar ratio of converted substrate to catalyst loading. [d] Turnover frequency defined as the molar ratio of converted substrate to catalyst loading per unit of time. [e] Conversion obtained using 28 mg of PCS as catalyst after 24 h. [f] The enantiomeric excess was determined by chiral-phase HPLC for this model reaction run in: a) DMSO (ee = 0.62%), b) MeOH (ee = 0.34%), c) H$_2$O (ee = 0.05%). Conditions: eluent = *n*-Heptane/i-propanol 70/30, flow rate = 0.5 mL min^{-1}, wavelength = 215 nm, run time = 30 min; retention time (RT): RT$_1$ = 14.34 min, RT$_2$ = 16.86 min. [g] Conversion obtained after 12 h using CSHB as catalyst. [h] Conversion obtained using 28 mg of PCS as catalyst after 12 h. [i] Molar ratio nitroalkane:nitroalkene product = 2 : 1, reaction time = 54 h. [j] Molar ratio nitroalkane:nitroalkene product = 2 : 1. [k] Only dehydrated product with traces of nitroalkane was observed.

better than PCS (Table 9, entry 2). Moreover, the experiments with nitroethane afforded *anti/syn* ratios 41 : 59, 45 : 55 and 45 : 55 for aldehydes **1l**, **1a** and **1b**, respectively, with negligible enantiomeric excess.[58] Again, a dramatic inactivation of the catalyst was observed during the recycling experiments (Table 9, entry 3).

Table 9 CSHB-catalyzed nitroaldol (Henry) reaction between aldehydes **1** and nitroalkanes **13–14** in water at RT[a]

Entry	ArCHO	Product	Time (h)	Conv. (%)[b]	dr[c] (anti/syn)	TON[d] (± 117)	TOF[e] (h^{-1}) (± 5)
1	**1l**	**15l**	26	43	41/59	2529	97
2	**1a**	**15a**	24	85 (62[f], 2[g])	41/59 (45/55[f])	4470	186
3	**1a**	**15a**	24	36[h] 12[i]	45/55 47/53	2118 706	88 29
4	**1b**	**15b**	26	26	45/55	823	32

[a] Reaction conditions: Aldehyde (1.0 mmol), **13** or **14** (10.0 mmol), mean pH = 6.9, catalyst beads number = 20 (corresponding to 17 mol% of free amine groups with respect to the aldehyde), H$_2$O (3 mL), RT. [b] Conversion was determined by ^{1}H NMR spectroscopy based on the aldehyde proton. [c] Determined by ^{1}H NMR spectroscopy of the crude product. Batch-to-batch estimated relative error = ± 0.5%. Relative configurations were assigned by comparison with reported literature data. [d] Turnover number defined as the molar ratio of converted substrate to catalyst loading. [e] Turnover frequency defined as the molar ratio of converted substrate to catalyst loading per unit of time. [f] Result of a control experiment performed with 28 mg of PCS as catalyst after 24 h. [g] Result of a control experiment performed without catalyst after 24 h. [h] Recycling experiment: Conversion after first cycle. [i] Recycling experiment: Conversion after second cycle.

Table 10 Performance of CSHB as organocatalyst in Michael additions in water in comparison with the uncatalyzed and PCS-catalyzed processes[a]

Entry	Catal.	Yield **19** (%)[b]	Yield **20** (%)[b]	TON[c] **19**	TON[c] **20**	TOF **19** (h^{-1})[d]	TOF **20** (h^{-1})[d]
1	None	12 (20[e])	8 (15[e])	—	—	—	—
2	PCS	26 (36[e])	19 (24[e])	1182 ± 91	864 ± 91	394 ± 30	288 ± 30
3	CSHB	27	23	1227 ± 91	1045 ± 91	409 ± 30	348 ± 30

[a] Reaction conditions: **17** (1.0 mmol), **5** or **18** (1.0 mmol), mean pH = 6.9, catalyst = Catat.: hydrogel beads number = 26, amount of PCS = 36 mg (corresponding to *ca.* 22 mol% of free amine groups with respect to **17**), H$_2$O (5 mL), reflux, 3 h. [b] Isolated yield. Batch-to-batch estimated relative error = ± 0.5%. [c] Turnover number defined as the molar ratio of converted substrate to catalyst loading. [d] Turnover frequency defined as the molar ratio of converted substrate to catalyst loading per unit of time. [e] Values as reported in ref. 59 The obtained lower yields in comparison with the reported values could be attributed to product loss during filtration/recrystallization (entry 1) or to possible critical differences between the properties of the specific chitosan used for the experiments – no specific technical data regarding the DDA of the biopolymer were previously reported – (entry 2).

Michael addition

Finally, the performance of neutral CSHB was also preliminarily evaluated towards two model Michael-like additions involving the reaction between benzylidenemalonitrile (**17**) and either (a) cyclohexanone (**5**) or (b) resorcinol (**18**) in water. The choice of these reactions was made on the basis of the catalytic effect of PCS previously observed in these transformations.[59] As shown in Table 10 (entry 1), the addition of either **5** or **18** to **17** in refluxing water and in the absence of catalyst yielded the corresponding racemic products aminopyrancarbonitrile **19** or chromene **20** albeit in much lower yields. However, the use of both PCS and CSHB under the same conditions afforded the desired products in higher yields (Table 10, entries 2–3). In terms of TON and TOF, no significant differences were observed between CSHB and PCS, suggesting a similar reactive surface of both catalysts for the Michael reaction under prescribed conditions.

Evaluation/optimization of Michael-like addition reactions catalyzed by CSHB, as well as further explorations of the scope

and limitations of the direct use of this and related biohydrogels in organocatalysis for biomedical purposes are in progress in our laboratory.

Conclusions

In conclusion, we have demonstrated that neutral LMW chitosan hydrogel beads with diameters ranging from 2 to 4 mm, is very limited in catalyzing the aldol reaction in both DMSO and aqueous conditions at a catalyst loading of 17 mol% (mol% of free amine groups with respect to the aldehyde). A meticulous washing protocol of the matured hydrogel beads was found to be critical to ensure the removal of trapped OH$^-$ ions, which otherwise would become the actual catalytic species upon their slow diffusion-controlled leaching during the reaction. The detailed study on the impact of the washing protocol on the catalytic activity of the hydrogel beads aims to provide a better standardization of production process to be able to prepare reproducible CSHB batches.

In contrast, neutral pH chitosan hydrogel beads displayed high catalytic activity and selectivity in both the Knoevenagel and nitroaldol (Henry) reactions with a variety of acceptors and donors. TONs up to *ca.* 5823 were achieved in both cases, whereas the highest TOFs ranged between *ca.* 1164 min⁻¹ for the Knoevenagel and *ca.* 243 h⁻¹ for the nitroaldol (Henry) reaction. In general, higher TONs and TOFs were routinely found for CSHB in comparison to PCS. Experiments with different nitroalkanes revealed that the carbanions with bigger size provided the lower reaction rates and conversions despite the higher acidity of the donor. A similar effect has been also observed in some enzymatic catalysis of this reaction. Nevertheless, the observed negligible enantioselectivites and the reactive surface-reactivity relationship studies confirmed that the biohydrogel beads act as an immobilized base catalyst rather than as a bionanoreactor. In general, no significant correlation between reaction time and selectivity was observed, and neither the SA:V ratio nor the molecular weight of the biopolymer were found to have a major influence on the catalytic performance of the hydrogel beads. Interestingly, CSHB were also found to be catalytically active towards model Michael additions, albeit with modest TON/TOF comparable to PCS. Optimization of the experimental variables leads to the conclusion that the reaction scale plays a major role in the performance of the biohydrogel catalyst.

Catalytic CSHB are readily available, inexpensive, nontoxic nonhazardous, and requires no initiation step. In terms of recyclability, the CSHB catalyst could be efficiently recovered after the Knoevenagel condensation reaction by a simple filtration/washing protocol and reused several times without noticeable loss in activity. However, poisoning of the catalyst in the presence of aldehydes and nitroalkanes during extended periods of time was observed, thus seriously hindering its reusability in the nitroaldol (Henry) reaction. Moreover, the direct use of CSHB as organocatalyst could complement the use of other forms of the catalyst (*e.g.* PCS, CSAB) by (1) being compatible with biodegradable and low toxic organic solvents like DMSO and, therefore, with hydrolysable aldehydes or low reactive aldehydes in aqueous medium, (2) avoiding undesired alterations on the functionalized surface of the biopolymer due to imprecise drying protocols, and (3) enhancing the mechanical stability of the catalyst due to its elastic properties, without risking the accessibility of the functional groups.

Experimental section

See ESI† for detailed general procedures.

Characterization of new products

Reaction products **6a**,[60] **8f**,[61] **10e**,[62] **10l**,[63] **10m**,[64] **12d**,[65] **12j**,[65,66] **12k**,[65] **19**[59] and **20**[59] are known compounds and showed spectroscopic data identical with the literature.

2-(Naphthalen-2-ylmethylene)malononitrile **(8c).** Yellow solid, m.p.: 131–132 °C; R_f (30% EtOAc/n-hexane): 0.50; ¹H NMR (300 MHz, CDCl₃) δ (ppm) = 8.27 (d, J = 1.5 Hz, 1H), 8.07 (dd, J = 8.8, 1.9 Hz, 1H), 8.00–7.84 (m, 4H), 7.65 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ (ppm) = 159.8, 135.9, 134.5, 132.6, 130.0, 129.7 (2C), 128.6, 128.1, 127.8, 124.2, 114.0, 112.9,

82.2; FT-IR ν_{max} (cm⁻¹): 2922, 2225, 1733, 1583, 1464, 1583, 1464, 1375, 1270, 1185, 965, 868, 810, 745. Anal. Calcd. for $C_{14}H_8N_2$. ·1/5 H_2O: C, 80.91; H, 4.07; N: 13.48. Found: C, 81.02; H, 4.21; N: 12.73.

2-(2-Methylpropylidene)malononitrile (8i). Yellow liquid; R_f (20% EtOAc/n-hexane): 0.47; ¹H NMR (300 MHz, CDCl₃) δ (ppm) = 7.14 (d, J = 10.6 Hz, 1H), 3.02 (ddt, J = 13.3, 10.6, 6.6 Hz, 1H), 1.18 (d, J = 6.6 Hz, 6H); ¹³C NMR (75 MHz, CDCl₃) δ (ppm) = 175.0, 112.1, 110.4, 87.9, 32.8, 21.0; FT-IR ν_{max} (cm⁻¹): 2973, 2239, 1734, 1607, 1469, 1367, 1094, 955, 620.

2-(3-Methylbutylidene)malononitrile (8j). Yellowish liquid; ¹H NMR (300 MHz, CDCl₃) δ (ppm) = 7.35 (t, J = 8.1 Hz, 1H), 2.50 (dd, J = 8.0, 6.8 Hz, 2H), 1.94 (tt, J = 13.4, 6.7 Hz, 1H), 1.01 (d, J = 6.7 Hz, 6H).

(E)-Ethyl 2-cyano-3-(naphthalen-2-yl)acrylate (10c). Bright yellow solid, m.p.: 111–112 °C; R_f (30 EtOAc/hexanes): 0.50; ¹H NMR (300 MHz, CDCl₃) δ (ppm) = 8.37 (d, J = 6.7 Hz, 2H), 8.18 (dd, J = 8.7, 1.8 Hz, 1H), 7.98–7.82 (m, 3H), 7.68–7.51 (m, 2H), 4.41 (q, J = 7.1 Hz, 2H), 1.42 (t, J = 7.1 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ (ppm) = 162.7, 155.0, 135.4, 134.2, 132.8, 129.4, 129.2, 129.1, 129.1, 127.9, 127.2, 125.3, 115.8, 102.7, 62.8, 14.2; FT-IR ν_{max} (cm⁻¹): 2989, 2220, 1723, 1598, 1364, 1245, 1161, 1093, 1021, 815, 752. Anal. Calcd. for $C_{16}H_{13}NO_2$.: C, 76.48; H, 5.21; N: 5.57. Found: C, 76.66; H, 5.20; N: 5.48.

(E)-Ethyl 2-cyano-3-(2-nitrophenyl)acrylate (10f). Off-white solid, m.p.: 99–100 °C; R_f (30% EtOAc/n-hexane): 0.32; ¹H NMR (300 MHz, CDCl₃) δ (ppm) = 8.72 (s, 1H), 8.28 (dd, J = 8.2, 1.1 Hz, 1H), 7.91–7.66 (m, 3H), 4.42 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ (ppm) = 161.1, 153.2, 147.4, 134.5, 132.2, 130.6, 128.2, 125.5, 113.9, 108.7, 63.2, 14.1; FT-IR ν_{max} (cm⁻¹): 2999, 1721, 1604, 1570, 1519, 1342, 1259, 1200, 1090, 1007, 930, 886, 857, 798. Anal. Calcd. for $C_{12}H_{10}N_2O_4$.: C, 58.54; H, 4.09; N: 11.38. Found: C, 58.89; H, 4.17; N: 11.35.

(E)-Ethyl 3-(2-chlorophenyl)-2-cyanoacrylate (10g). Beige solid, m.p.: 52–53 °C; R_f (30% EtOAc/n-hexane): 0.57; ¹H NMR (300 MHz, CDCl₃) δ (ppm) = 8.69 (s, 1H), 8.29–8.17 (m, 1H), 7.55–7.35 (m, 3H), 4.40 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ (ppm) = 161.8, 151.2, 136.5, 133.7, 130.4, 129.9, 127.5, 114.9, 106.2, 63.0, 14.2; FT-IR ν_{max} (cm⁻¹): 2998, 2224, 1728, 1609, 1468, 1431, 1254, 1199, 1125, 1090, 1019, 893, 758.

(E)-Ethyl 2-cyano-4-methylpent-2-enoate (10i). Colourless liquid; R_f (20% EtOAc/n-hexane): 0.52; ¹H NMR (300 MHz, CDCl₃) δ (ppm) = 7.45 (d, J = 10.6 Hz, 1H), 4.30 (q, J = 7.1 Hz, 2H), 2.99 (ddt, J = 13.3, 10.6, 6.6 Hz, 1H), 1.34 (t, J = 7.1 Hz, 3H), 1.15 (d, J = 6.6 Hz, 6H); ¹³C NMR (75 MHz, CDCl₃) δ (ppm) = 169.2, 161.5, 113.6, 107.5, 62.5, 31.6, 21.3, 14.1; FT-IR ν_{max} (cm⁻¹): 2970, 1729, 1626, 1467, 1369, 1307, 1253, 1157, 1069, 1016, 956, 763.

(E)-Ethyl 2-cyano-5-methylhex-2-enoate (10j). Colourless liquid; R_f (20% EtOAc/n-hexane): 0.55; ¹H NMR (300 MHz, CDCl₃) δ (ppm) = 7.66 (t, J = 8.0 Hz, 1H), 4.30 (d, J = 7.1 Hz, 2H), 2.45 (dd, J = 8.0, 6.8 Hz, 2H), 1.90 (dp, J = 13.4, 6.7 Hz, 1H), 1.34 (t, J = 7.1 Hz, 3H), 0.98 (d, J = 6.7 Hz, 6H); ¹³C NMR

(75 MHz, CDCl$_3$) δ (ppm) = 162.8, 161.3, 113.8, 110.4, 62.4, 40.7, 28.2, 22.4, 14.1; FT-IR ν_{max} (cm^{-1}): 2969, 2876, 2231, 1730, 1627, 1466, 1370, 1281, 1256, 1169, 1072, 1048, 836, 758. Anal. Calcd. for C$_{10}$H$_{15}$NO$_2$. ·1/5 H$_2$O: C, 64.98; H, 8.40; N: 7.58. Found: C, 65.21; H, 8.33; N: 7.32.

Acknowledgements

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33 In general, aldol reactions may be catalyzed either by a base or an acid, where the nucleophile is an enolate anion or enol, respectively. In the latter case, catalytic protonation of the carbonyl oxygen increases the electrophilicity of the carbonyl carbon just enough so that it can be attacked by the enol, and promote the dehydration of the aldol product, as water is a good leaving group. Nevertheless, it is well known that aldol reactions proceed more efficiently under basic conditions where dehydration rarely takes place during the reaction since hydroxide is a poor leaving group.
34 Under the critical assumption that the content of Na$^+$ ions inside the beads reflect the actual hydroxide catalyst content, atomic absorption spectroscopy (AAS) was used to calculate these values for a series of beads that displayed different pH values of the filtrate after the washing protocol (see Experimental section). Unfortunately, AAS on these materials provided an average value of 9.96 ± 0.16 ppm of Na$^+$ ions for several batches of beads with pH values ranging from 6.49 to 8.44. Therefore, any attempt to correlate these AAS values with the actual concentration of hydroxide ions inside the beads would not be completely reliable. The same conclusion was reached from ICP measurements of CSHB, from which the theoretical concentration of hydroxide ions (related to the concentration of Na$^+$ ions) would be well below the minimum catalytic loading necessary for the reaction. Although it was not pursued, a more direct method of analysis could involve the treatment of a Ag(I) solution in the presence of Mn(II) and finely triturated CSHB. The existence of hydroxide ions in the gel matrix could promote the redox formation of MnO$_2$ and Ag(0), which could be further quantified by AAS.
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