SCHOOL OF SCIENCE

Department of Industrial Chemistry "Toso Montanari"

Second cycle degree in

Low Carbon Technologies and Sustainable Chemistry

Classe LM-71 - Scienze e Tecnologie della Chimica Industriale

Study of the activity and enantioselectivity of alginate-based catalysts in Michael addition reactions

Experimental degree thesis

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ABSTRACT

This thesis, resulting from an internship work performed at a research laboratory of the Department of Industrial Chemistry "Toso Montanari" of the University of Bologna, describes a study on the activity and enantioselectivity of various metal-alginate gel beads as heterogeneous catalysts for some representative reactions. Alginates are a class of biopolymers extracted from brown algae, which can be considered as a cheap and renewable biopolymer. For the preparation of the catalysts, we followed typical preparation protocols, delivering a small library of alginate gel beads featuring different cross-linking metals. These catalysts were tested in two Michael addition reactions, using three representative solvents. Promising results were obtained in the addition of diethyl malonate to nitrostyrene, where relatively higher rates were observed for alginate containing alkaline earth metals, compared to first-row transition metals or alginic acid. A possible explanation could be made considering the mechanism of the reaction, where the alkaline environment provided by the alkaline earth metal based material can promote the reaction.

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1. Introduction

1.1 Green chemistry

In the last few decades, the field of green chemistry has drawn a lot of attention due to the increasing need for balance the chemical innovation and the economic and environmental goals. Through the past few years, it was pointed out that the main steps on which scientists can work on and improve are the efficiencies of chemical reactions and the following downstream process including separation and purification. Nowadays, many highly inefficient atomic processes, polluting compounds, reagents, unrecovered and hardly reused catalysts, and large volume of volatile organic solvents are routinely employed. To solve these problems, one of the major efforts has been put towards reducing the final wastes of the reactions, especially when the separation is needed for recycling of catalysts and solvents.

Green chemistry is defined as "the design of chemical products and processes to reduce or eliminate the use and consumption of chemicals" by P.T Anastas and J.C Warner. ([1]) It can be achieved by careful planning of molecular design and relevant synthesis to lower the negative impacts on environment. Always thinking about environment and safety issues, currently, this method has been applied by many industrial sectors such as cosmetics, aerospace, electronics, agrochemicals, energy, household products and pharmaceuticals and so on. ([2])

Taking this into consideration, the search for an approximate optimal synthesis has been accomplished with the application of several technologies including novel catalysis, which will be the subject of this thesis.

1.2 Asymmetric catalysis and asymmetric catalysis with biopolymers

Indeed, the molecules generally adopted as chirality inducers, excluding enzymatic catalysis are low molecular weight compounds. They can be either natural molecules and their derivatives or synthetic molecules depends on the specific needs.

In the recent decades, organic synthesis aimed at enantiomerically pure compounds has gained significant attention. This importance may lie on the fact that, in most cases, molecules in biological systems have different features and properties depending on the enantiomeric form in which they are present, scientific advances in the field of organic synthesis have made it possible to control the spatial orientation of the newly formed bonds in many reactions, and new research is still undergoing to make these processes more efficient.

The control of spatial orientation to achieve stereoisomerically pure compound is commonly achieved through different strategies such as resolution of racemic mixtures, synthesis from chiral pool or asymmetric synthesis from prochiral compounds. ([3]) Tremendous research efforts have focus on asymmetric catalysis and stoichiometric asymmetric synthesis. The latter is based on stoichiometric amounts of chiral reagents and chiral auxiliaries and involves diastereoselective reactions. A chiral auxiliary is specific component that binds to the substrate through covalent bonds, allowing a stereoselective reaction, and it is subsequently removed from the product, leaving no trace.

Asymmetric catalysis is traditionally based on enantiopure chiral organic molecules with low molecular weight as enantioinducers. These include amino acids and their derivatives, alkaloids, synthetic molecules like BINOL or BINAP. These molecules can behave as ligands for metals (organometallic catalysis) or act as catalysts themselves (organo-catalysis). In the past few years, research on this subject has also been directed towards the use of chiral catalysts which are more environmentally friendly and those which are derived from renewable resources. And this effort rose successively with the above-mentioned concept "green chemistry", and by the guidance of this concept it has been focused specifically on functionalized molecules,

already present in nature with their own chirality, and possibly prone to be used as chiral catalyst without cumbersome manipulations. Besides, they are available in large quantities and do not compete with agricultural production. ([4])

Figure 1. The representation of BINAP based Rh asymmetric catalysis. ([5])

However, historically, one of the first examples of asymmetric catalysis outside the enzymatic realm employed biopolymers as a source of chirality was dated back to 1956, by Akabori and his co-workers. ([6]) Their approach consisted of the ability of a polypeptide, a biopolymer, to induce chirality. The reduction of a Pd (II) salt on the silk fibroin in the formation of a protein bound Pd (0) catalyst, led to hydrogenate some imine derivatives with modest but at the time revolutionary enantioselectivity. Anyway, because of the dissatisfactory outcomes obtained, this approach was not pursued deeply for many years, and asymmetric catalysis mostly focused on low molecular weight organic molecules as enantioinducing elements.

Nevertheless, some significant examples of using biopolymers as asymmetry inducing elements in catalysis have been reported. The use of the helical structure of ds DNA as scaffold for asymmetric catalysis, stands out for its elegance. ([7])

Roelfes and Feringa, ([8]) have shown that by considering the incorporation of a metal "cofactor" into the biopolymer structure, the potential of this polynucleotide molecule in asymmetric catalysis was very promising. This incorporation would also promote the activity expressed by the biopolymer beyond its natural function. Their study started with a Diels-Alder reaction catalyzed by copper (II) which was brought into contact with

the DNA double helix ([9]) via non-chiral ligand, a pyrimidine derivative. By the cause of the proximity of the metal catalyst, they were able to obtain high enantiomeric excesses and achieve good diastereoselectivity too. They were also able to demonstrate the accelerating effect of DNA on the reaction compared to the copper complex alone. ([10]) The consequent use of the DNA structure in many other types of reactions allowing the formation of C-C bonds, showed that also in many organic reactions can be performed. In this thesis, another organic biopolymer is used instead of DNA, which is the alginate. However, they both share a similarity in binding with metal and working as a catalyst.
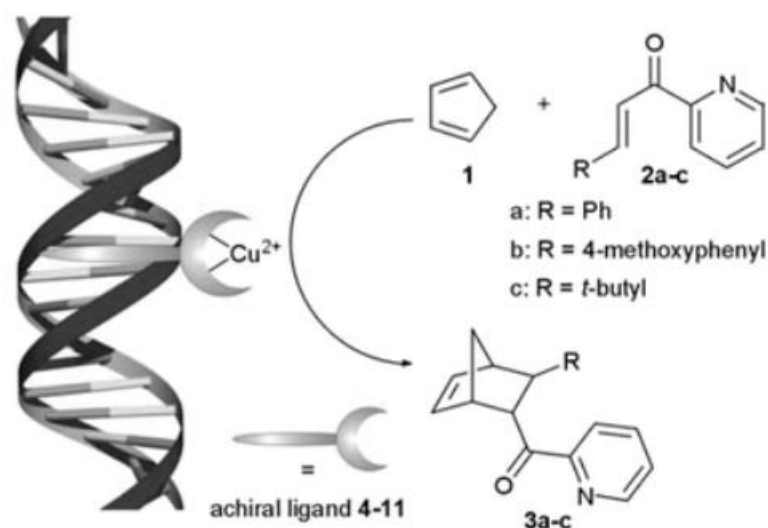

Figure 2. Schematic representation of DNA catalysis with copper ion and a Diels-Alder reaction with DNA catalyst from Roelfes and Feringa. ([11])

1.3 Polysaccharides used in asymmetric catalysis.

Surely, polysaccharides are a type of biopolymers that can be attractive for asymmetric catalysis. Unfortunately, as far as it is concerned, only a few numbers of examples have been reported. Most of them are related to the use of chitosan, a D-glucosamine polymer, ([12]) derived from chitin, a polysaccharide of mainly marine origin and identified by the presence of an amide functionality. Despite these limited cases, the examples

still demonstrate how these polysaccharides can have good potential and exploitability, and that the presence of a specific functional group, amine in the case of chitosan, may bring more opportunities than other common polysaccharides including for example, cellulose. It is important to underline how the most common chiral stationary phases applied in enantioselective chromatography are based on polysaccharide materials, mainly amylose, functionalized and supported on silica. However, till now, this class of biopolymers has received little attention as regards its use for asymmetric induction in catalytic processes.
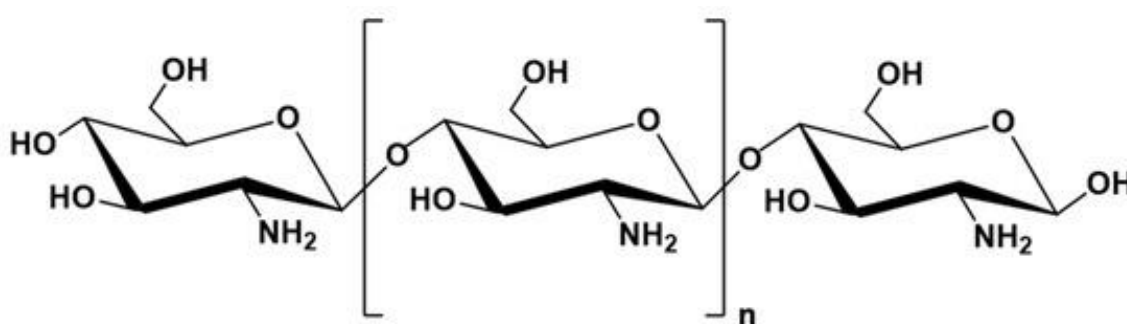

Figure 3. Natural structure of Chitosan. (-NH_2 as the proton acceptor when below it's pKa) ([13])

Polysaccharides obtained from marine sources have some interesting features that can be easily exploited successfully. They can be regarded as functionalized molecules, which is natural occurring the chirality and is not requiring for further treatments either for disposal or in experiments. As above mentioned, they are in large quantities and do not compete with agricultural production, which is a large advantage for application.

1.4 Alginate

From chitosan we can move to discuss about other natural polysaccharides. An important class of these molecules is alginates, which is implemented in this research. Alginates possess carboxylic groups, which allow the molecule to create coordination

structures with divalent metals through the carbonyl structures. This peculiarity establishes the possibility of applying them in the context of Lewis acid catalysis, for example. Besides, currently most catalytic systems within organometallic catalysis operate by transition metals, and they can provide this possibility to be used with alkaline earth metals. Alkaline earth metals are generally abundant on the earth crust, usually they cause less industrial pollution problems than transition metals, so they are in line with the basic concept of the "green chemistry" and they are also economically friendly with a quite lower price than that of the transition metals. On the other hand, alginates nowadays are widely applied in several fields that is not related to catalysis, such as biomedical, including immunoselection of cell transplants, slow-release systems, in vitro tissue engineering and 3D bioprinting. ([14]) ([15])

Alginates are natural-occurring polymers in brown macro-algae, present as an insoluble mixture of sodium, calcium, magnesium, strontium, and barium salts with alginic acid. ([16]) The structure of alginates can be divided into two sections, which are composed of mannuronic (M) and guluronic (G) acid linked by 1, 4 glycosidic bonds, as shown in the figure below.
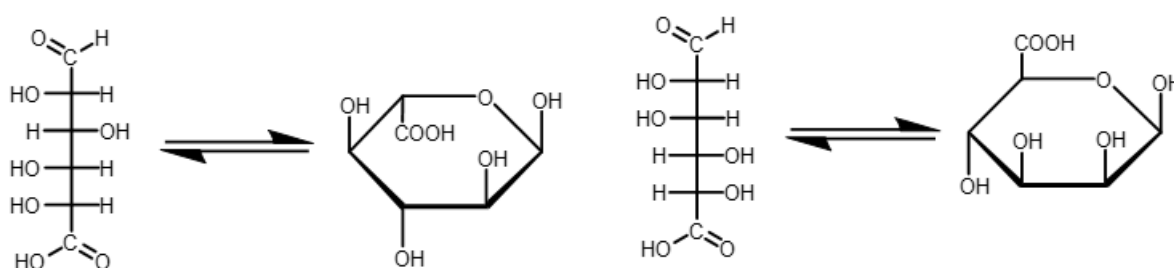

Figure 4. Linear and cyclic strucutres of α-L-guluronic acid (G) and β-D-mannuronic acid (M).

They are considered as the alter ego of cellulose in algae. However, considering the differences, alginates are formed by gulose and mannose derivatives instead of glucose, and instead of having only hydroxide groups, alginates are formed by uronic acids, which contains the carboxylic functional groups. The presence of the special functional groups allows an interaction and combination with metal cations providing the molecules special properties. And the ability to function as a ligand explains the

use of alginates as a carrier for enzyme immobilization as an example ([17]). As previous literature reported, when metals or clusters are incorporated into the polymers, specific metal/polymer interactions can lead to exceptional catalytic behaviors, especially Lewis acid metals ([18]). In the presence of those divalent metals such as calcium, barium, alginates form hydrogels by a mechanism of ionic cross-linking where the gel properties are influenced by the content and length of the G blocks. Typically, the structure of the alginates and consequently their characteristics depend on the way in which the monomers are connected. In the actual conditions, both the blocks have two different but defined chair conformations.
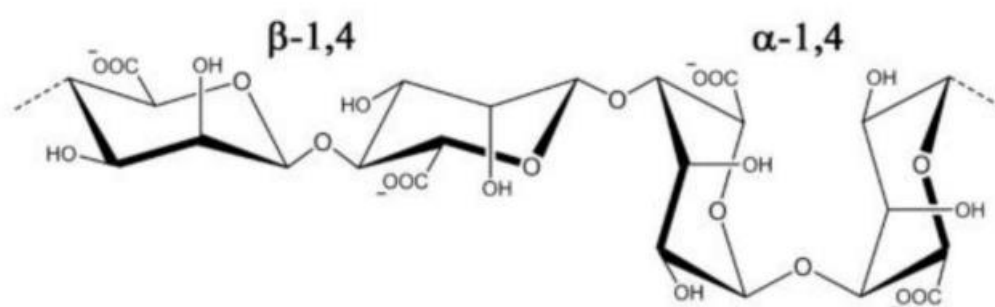

Figure 5. The schematic representation of possible linkage between the guluronate and mannuronate.

The structure of the alginate therefore can be divided into 3 possible sequences, which are GG, MM, and GM or MG, (see the figure below) each with different characteristics, depending if they are based on a single type of monomer or alternation of both. ([19]) Different alginates have different G/M ratio, for example, in this project a commercial alginate named Protanal 200S is mainly used, extracted from a nature alga, (Laminaria digitata) which has a content of G/M approx. 70:30. It performed extremely well in enantioselectivity in the previous research than those containing a higher percentage of mannuronate.
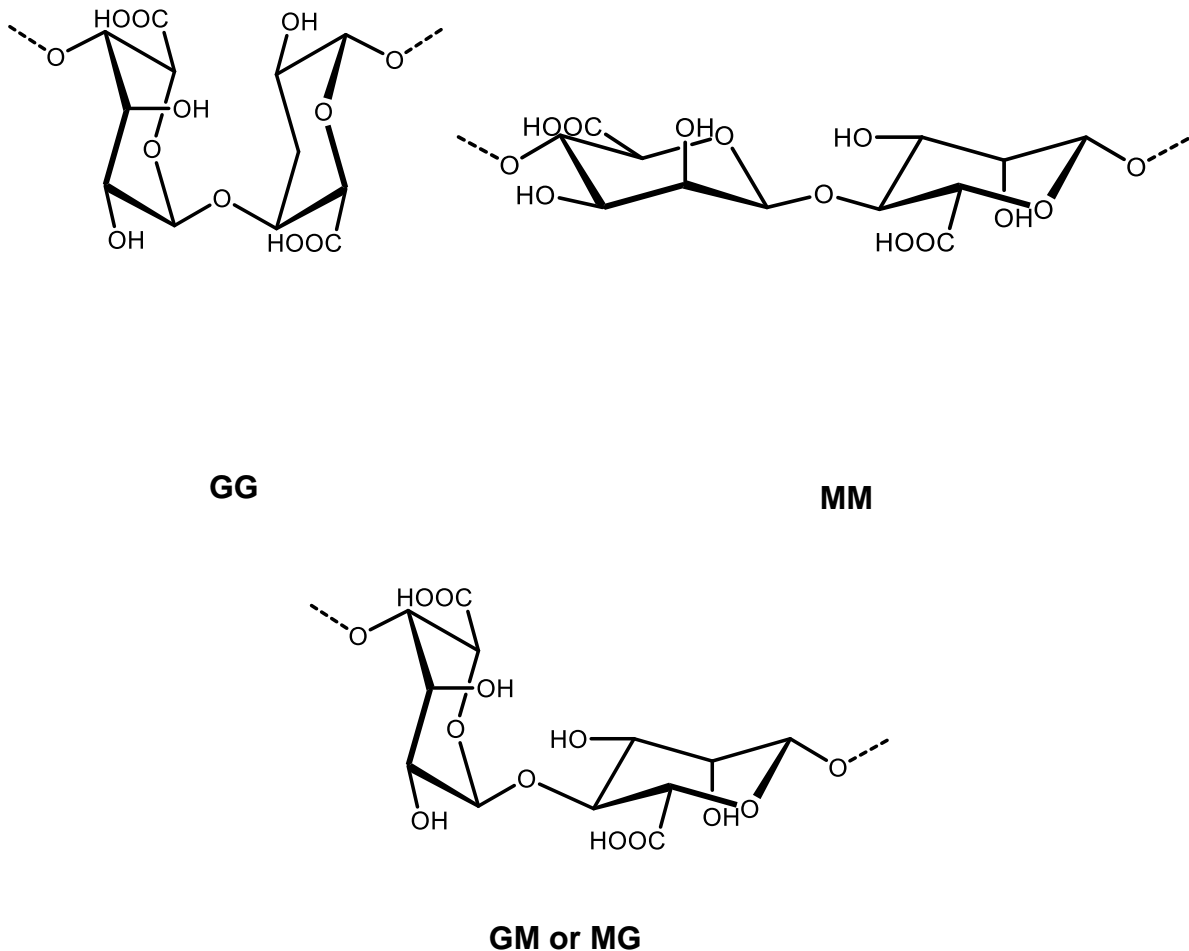
GG

MM

GM or MG

Figure 6. Structure of possible sequences of guluronate (GG), mannuronate (MM) and guluronate- mannuronate (GM or MG).

1.5 Alginate gels

After the alginate is obtained from industrial processes, the alginate gels can be then prepared. While sodium alginate is soluble in water, alginates tend to form spontaneously stable gels when they are exposed to dilute aqueous solution of divalent cations or to acidic solution. This capability is because of the predisposition of the carboxyl groups of the guluronate monomer to coordinate with these metals, as above mentioned, therefore, forming a stable structure, or by lowering the pH value, favoring therefore the production of alginic acid gels.

Alginate gels are classified according to two categories, the first one is the type of the counterion of the carboxylic group (Ionotropic gel or acidic alginic gel), the second is the type by of formulation through contacting (hydrogel, solvogel, aerogel, xerogel)

1.5.1 Ionotropic gel

As mentioned above, the alginates possess a high ability to bind divalent metals. Indeed, affinity increases following this order: Mn<Zn, Ni, Co< Fe< Ca < Sr < Ba < Cd < Cu < Pb ([20]). However, it is important to underline that even calcium is found in a low position, it is still the most studied and used ion. The mechanism of the interactions is a contact between oxygen atoms of the carboxyl groups, also because the M-block units corresponds to an almost absence of selectivity, a dimerization of two G residues occurs. This promotes the binding of two G chain of opposite sides, producing a diamond-shaped cavity and generating a junction zone named as "egg box". ([21])
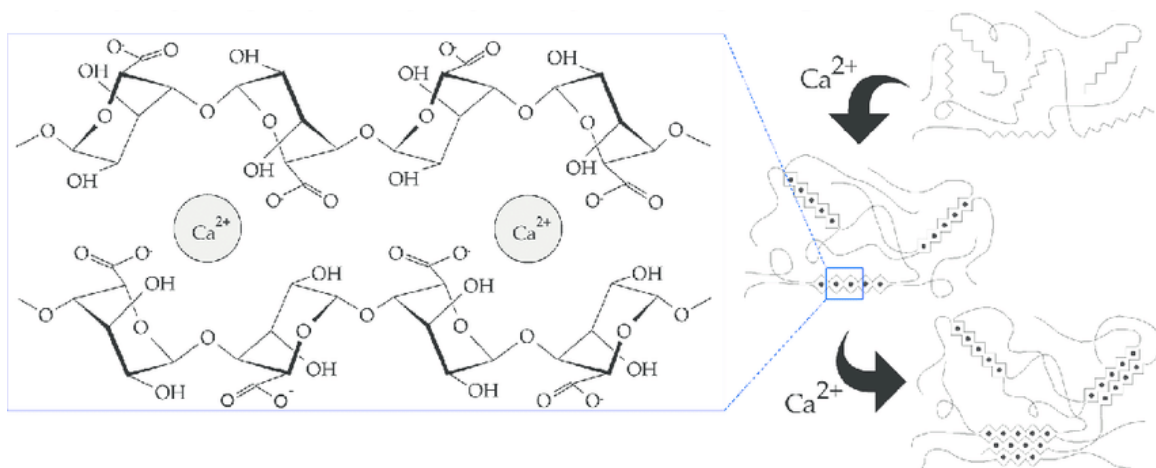

Figure 7. Schematic representation of the mechanism of "egg box" model in gelation of alginate with calcium.

This is a stable three-dimensional structure, and with this a hydrogel can be observed, in which each cation binds to four monomers of guluronate by multi coordination, exploiting the oxygen atoms of the carboxylic groups and of the hydroxyl groups. This

stability, as mentioned before, is preferred in the corresponding asymmetric catalysis with higher percentage of this G monomer. Besides, the length is another important factor, usually 8-20 continuously G monomers are necessary to from a stable structure. ([22])

1.5.2 Alginic acid gel

This gel is formed when an alginate solution is added to another solution with lower pH than the value of acidic dissociation constants of the two monomers: guluronate has a pKa of 3.65, while mannuronate has a pKa of 3.38. This is due to if the value if lower than the dissociation constants, the acidic form of the two monomers will be favored and an ion exchange will happen between the metal coordinated with carboxylic groups and the protons present in solution. The formation of this acidic gel only happens when an acidic solution is added slowly into alginate, drop by drop, since the formed gel has to be stabilized by intermolecular hydrogen bonds, which means an intense decrease in pH will provoke the precipitation of insoluble alginic acid. However, the mechanism of formation of the alginic acid gel is not fully comprehend yet. The reason why it relies on its relatively limited industrial applications is still under study.

Above two types are classified according to their type of cross-linkage and medium.

1.5.3 Hydrogel

Generally, hydrogels are hydrophilic macromolecules capable of absorbing a large amount of water, resulting an increased volume. ([23]) These types of gels can also be obtained from alginates as introduced above. There is a copolymeric network stabilized by hydrogen bond (alginic acid) or cross-linking ions (metal ion alginates). The formation of the gel particles can occur by external (diffusion method) or internal gelation, differing on how the cross-linking ions are introduced into the alginate polymer.

However, the diffusion one is the most popular method, which has been used in this work and is detailed in the experimental part.

In short, a sodium alginate solution is added dropwise to the corresponding metal chloride solution or acidic solution. The formation of hydrogel spheres is immediately apparent, associated to the rapid jellification of the outer layer of the drop, allowing a rapid shaping and control size of the gel.
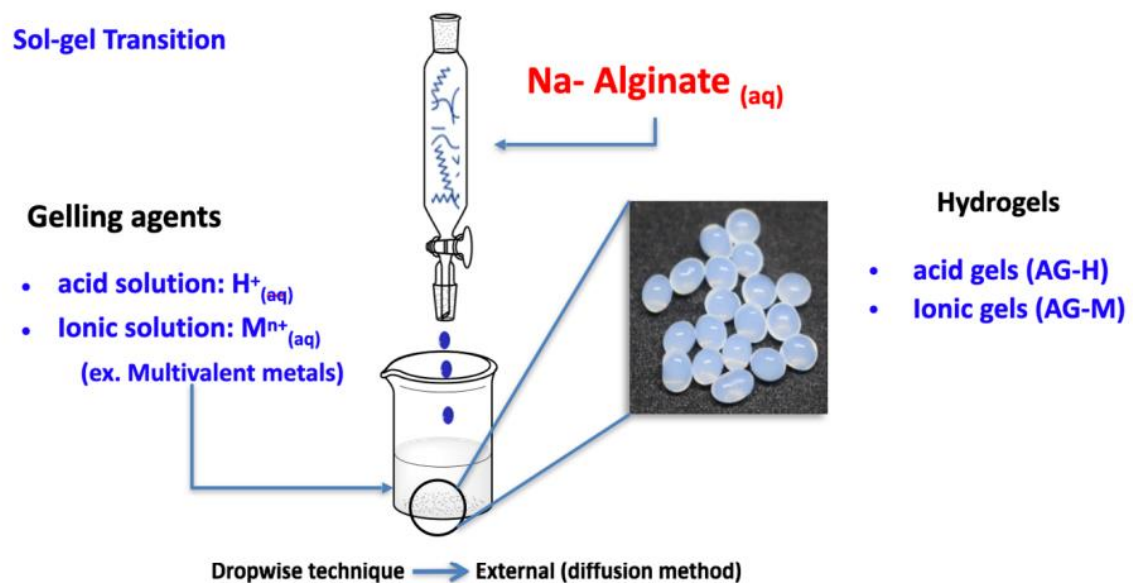

Figure 8. The common method of formation of various types of alginate gel beads

The key point of this diffusion method lies in the fast migration of cations from their aqueous solution into the alginate solution. There are various parameters that can influence the properties and morphology of the final gel spheres. (shape and size mainly) For example, a high viscosity of the alginate solution can change the final shape of the sphere; also, the time a drop will take to descend from the funnel and the stirring rate will influence the shape.

As for the internal method, it consists in mixing the alginate solution with an insoluble source of divalent cations, and a gelation inducer agent. The required low solubility of divalent metal ion compound in pure water allows its uniform distribution in alginate solution before gelation occurs. However, the simplicity and faster gelling kinetics of

diffusion method still makes it one of the most widespread strategies to produce gel beads at lab scale.

1.5.4 Solvogel

The synthesis of hydrogels is the previous step to obtain solvogels, which is then applied in catalysis. These gels, instead of water, contain organic solvents. In this thesis, the water of the hydrogel was replaced with ethanol, therefore in this case the solvogel can be also called alcogel since the role of the organic solvent is exerted by an alcohol. Ethanol is indeed one of the most used solvents because of the ideal characteristics that the solvent which replaces water molecules must have, first, it does not break the structure of the gel and will not cause shrinkage of the structure, besides, it is miscible with water, and finally it is compatible with the final applications of the solvogel. In fact, a lot of attention needs to pay to the phenomena of shrinkage since the release of water involves a reduction in the surface tension of the gel pores, resulting in a reduction in capillary pressure with consequent unfavorable reduction in the volume. ([24]) That is why a multistep process is needed and consisting in the gradual soaking of the gel with an increasing concentration of the solvent in each step using new solvent/water mixture. With this a solvent exchange can be achieved to remove water fully out of the gel beads, mitigating the shrinkage at the same time. Consequently, this protocol is often used in obtain solvogels, instead of soaking the hydrogel directly in the new solvent.

1.5.5 Aerogel

Aerogels are "dry gels" obtained from alcogels by replacing the organic solvent with a gas, thus generating a light porous structure. Supercritical drying of solvogels with supercritical CO_2 is the most used method. The reason why they are not obtained from hydrogels but from solvogels is that in general alcohols are more soluble in carbon

dioxide than water. This method allows to preserve the high porosity and the same properties that the starting hydrogel possesses, because the reduced temperature used in this process minimizes the changes that could occur at the molecular level, thus maintaining the conformation and non-covalent interactions between each polymer molecular chains. ([25]) The advantage of using a supercritical fluid is that there are no intermediate situations in which a liquid-vapor transition is present, thus avoiding the collapse of the three-dimensional structure ([26]). A less efficient alternative method is to use freezing-drying processes from hydrogels, which, however, generally lead to a slightly shrinkage of the structure compared to the first approach.

1.5.6 Xerogel

The last type of gels is the xerogels. They are always obtained by eliminating the solvent from the alginate structure, however, in this case, by direct evaporation, which leads to a collapse of the porous network, leading to a product with a lower volume due to the shrinkage of the material. Usually, an evaporation is needed to obtain xerogel. There will be the formation of menisci inside the pores due to the simultaneous presence of a liquid and a gas phase. And when the solvents are removed, the surface tension of the liquid contained in the pores creates an intense capillary pressure which is able to collapse them. However, for this type of gel, the application in catalysis is limited because of its low surface area.

1.6 Alginates gels in catalysis

In conclusion, the high surface area, the abundance of functional groups in the polymeric structure of the alginate (5.6 mmol/g of carboxylate groups), the stability in most organic solvents and the porous structure are some appealing characteristics which make these polysaccharide gels as promising materials for catalysis ([27]).

Among the properties, the most important is the three-dimensional structure. This will allow accessibility of active functions, fundamental for the possible entrapment of catalytic species and interaction with substrates in the reaction. Before, there were already some studies on the use of alginates as supports for organic molecules used in organometallic catalysis, as supports for metal nanoparticles, heterogenous Bronsted acid catalysis, and like this project, as catalysts creating interactions between the polymer and a metal ion. These studies make it possible not to just limit the use as supports as enzymes for bio-catalysis. One example is the Cu^{2+} coordinated alginate, that is a regioselective catalyst in the 1,3-cycloadditions of azides and alkynes ([28]).

Therefore, given the excellent properties of these eco-friendly polymers extracted from biomass, the use of alginates in catalysis is the main subject of ongoing studies.

2. Goals

This work follows previous research some time ago from the colleagues in the same laboratory, which shares some similarities in the catalyst aspect. In the past years, a project had been developed and finished using barium alginate solvogels as catalysts to carry out an enantioselective reaction. For that project, the Friedel-Crafts addition of indoles to nitroalkenes was developed.
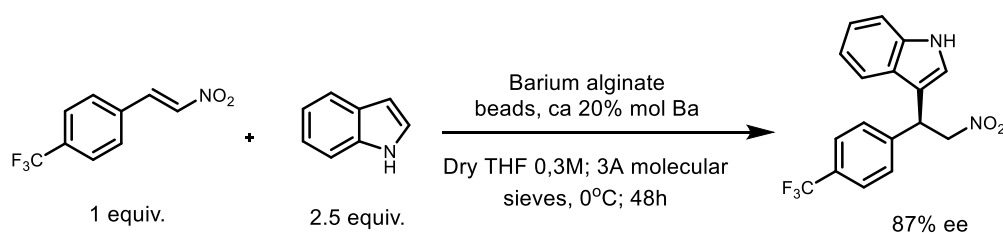

Figure 9. The scheme of Friedel-Crafts reaction.

This has been the first demonstration that alginate gels can be used as chiral enantioselective catalysts for an asymmetric reaction. This reaction likely proceeds via activation of the nitroalkene by barium, which acts as a Lewis acid. Possibly, one of the carboxylate groups can assist the reaction by coordinating the indole N-H and favoring the formation of a highly ordered transition state.
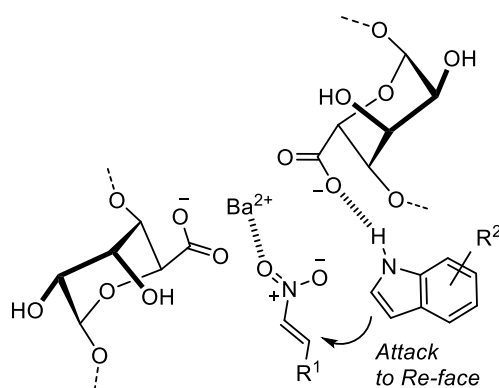

Figure 10. The representation of catalysis of barium in Friedel-Crafts addition of nitroalkene to indole.

The goal of my thesis is to investigate preliminarily if alginates are affective as chiral catalysts in mechanistically distinct reactions. In particular, the process of Friedel-Crafts does not involve genuinely charged intermediates, that is, no deprotonation or protonation is happening. Thus, it was decided to investigate a completely different process, which is soft enolization. The difference lies in a soft enolization is usually coupled with a deprotonation process, which leads to the formation of an enolate. Metal catalysts (like alginates) can in principle promote the formation of enolates. The metal is Lewis acidic and coordinates the oxygen of a carboxylic group. As a result, the electrons are attracted by the metal and moving away from the protons, which makes them more acidic and more likely to be removed, even by a relatively weak base like the carboxylate. This process is therefore called "soft enolization" ([29]). As result of deprotonation a metal enolate is formed. For testing the performance of alginate, two "Michael additions" reactions were chosen, which play an important role in obtaining new molecules and is an important reaction in fields like pharmaceutical, industrial chemistry etc. ([30])
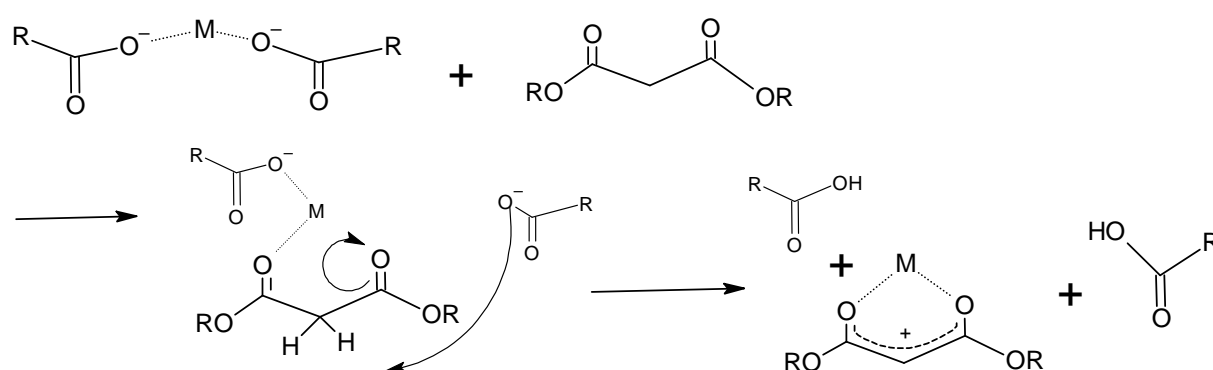

Figure 11. The representation of mechanism of soft enolization with metal catalysts.

More in detail, the specific goals are summarized into the following three points:

1) Preparation of a small library of alginate gel beads using different divalent metals.
2) Study two specific reactions (addition of diethyl malonate with nitrostyrene, and of nitromethane to chalcone). The reactions will be primarily carried out without the metal-alginate catalyst to get the racemate products as the reference compounds for HPLC analysis.

3) Carrying out the reactions using the other synthesized metal-alginate beads. Compare the activity using H-NMR analysis and enantiomeric excess (%) between different catalysts using HPLC equipment.

3. Results and Discussion

3.1 Preparation of the metal-alginate gel beads

The preparation of gel beads started from a Protonal 200S alginate powder, apart from one case where Protonal 200DL was used. During the preparation, the sodium inside the powder is replaced by several kinds of divalent metals. To do that, 1g of powder is weighted and dissolved in 50ml distilled water in an Erlenmeyer flask. Solution is stirred until a transparent and viscous liquid is formed. After that, 0.1M of metal chloride powder is dissolved in 100ml distilled water in a beaker, for the metal, Ba, Ca, Sr, Cu, Co, Ni, Cr, Zn are chosen, and for proton a 1 M HCl is used. The dissolved sodium alginate is added to the solution dropwise from a dropping funnel. And in the preparation of barium beads, a syringe is used for dropping the dissolved alginates to compare the properties of the final beads. The spheres can be observed as soon as the drop contact the solution, however, they are usually very transparent in the beginning, meaning that they are not mature and a continuously magnetically stirring is required under room temperature and pressure, which usually lasts overnight.

Picture 1. The picture of preparation of Cu-alginate beads from dropping of sodium alginate.

The gel beads are filtered and washed with water for 5 times per 10 minutes under magnetic stirring for removing the ions in solution after maturation. To better preserve and use the gel beads, these hydrogels are switched to a 100% alcogel using an increasing gradient concentration of ethanol and water mixture (10%, 30%, 50%, 70%,

90%, 100%). Each substitution takes 15 minutes under magnetic stirring, after that the solvogel is obtained. After this, some 3Å molecular sieves wrapped by filter paper are placed inside the liquid and the liquid is kept in fridge for 3 days to remove water completely outside the system. The molecular sieves were preheated under vacuum and then kept in a nitrogen gas atmosphere for a better absorption of water.

After 3 days, the molecular sieves are removed. To find out the amount of the catalyst beads need to be applied in the catalytic reaction, the amount of metal-alginate complex must be measured. In the following step, 10 beads are collected in a vial for getting the total weight. Then the vial was connected to a vacuum pump for evaporating the solvent (ethanol) out, the final constant number of weights can be obtained after some time. Then the weight per bead was obtained. Next, the content of metal inside the catalytic system was calculated, expressing the content of metal as mole per bead and using the following equation:

$$mmol\ (M) = \frac{weight\ of\ 1\ bead\ in\ mg}{[350.232 + atomic\ weight\ of\ M](\frac{mg}{mmol})}$$

For every metal and the proton, it follows the same procedures except for Ba, another type of alginate 200DL is also applied. Furthermore, for barium, the use of a syringe instead of a dropping funnel was also tested. Since the syringe has a narrower nozzle, it was thought to give smaller drops and thus smaller beads. However, this was not the case. The result of each type of gel beads are shown in the following table.

M	Alginate	M/G	Solvent	W(mg)/bead	Micromole (M)/bead
Cu	200S	3/7	EtOH	1.4	3.098
Ni	200S	3/7	EtOH	1.42	3.177
Ba	200S	3/7	EtOH	1.47	2.793
Ba (from syringe)	200S	3/7	EtOH	1.7	2.44
Ba	200DL	1/1	EtOH	1.7	3.23
Co	200S	3/7	EtOH	1.24	2.77
Ca	200S	3/7	EtOH	1.89	4.41
Sr	200S	3/7	EtOH	1.4	2.94
Zn	200S	3/7	EtOH	1.16	2.55
H	200S	3/7	EtOH	1.15	5.89

Table 1. The type of alginate, the solvent, the weight, and the content of metal and proton of every type of gel bead.

3.2 Preparation of the racemate reference compounds.

All the investigations started off from preparation of racemic reference compounds samples, which is necessary for devise suitable conditions for the separation of the two enantiomers by HPLC. In the beginning, 0.1 mmol of nitrostyrene and 2 equivalents of diethyl malonate are put together while for the second reaction 10 mmol of nitromethane and 0.01 mmol are mixed, nitromethane is in large excess for making the reaction happening more easily. Both reactions applied triethylamine as the catalyst. The appearance of a light-yellow color from the colorless liquid can be observed after around 3 hours and the product is confirmed by TLC test in a mixture of 20% of ethyl acetate and 80% of hexane as the eluent for the TLC, the result can be observed under a UV light, the proof of the reaction happened is the disappearance of the trace of limiting agent on the TLC silica plate. To guarantee a high purity in the later HPLC analysis, the compound was purified by a column chromatography using toluene to dissolve the products and hexane/EtOAc mixtures as eluent. After that, it was further checked by a $CDCl_3$ ^{1}H-NMR of the purity, and the mixture is stored in a small and labeled vial in a container for later analysis. In the analysis of HPLC, an analytical ADH column was used, with a flux of 1 mL/min with 10% of isopropanol and 90% of hexane mixture. The two enantiomers were detected by a UV detector. Since it is the reference compound, two peaks presenting the same area can be seen in the result, meaning a successful separation of the enantiomers.
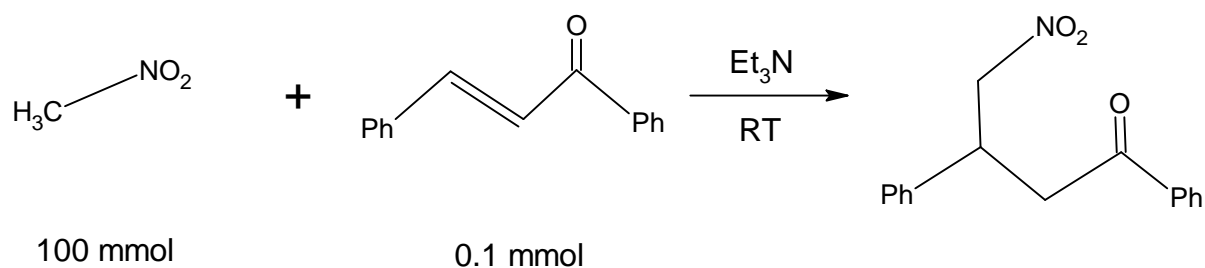
100 mmol   0.1 mmol
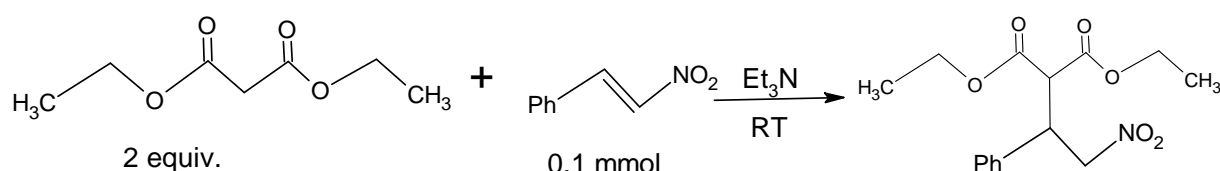
Figure 12. Preparation of the racemic reference compounds.

3.3 Application of the metal-alginate catalysts to the reactions

For the catalytic reactions, the process follows a similar procedure to the one used for the racemic compounds. The catalyst loading was set at 20 mol% based on the amount of metal. It was decided to test three different solvents, respectively ethanol, tetrahydrofurfural (THF) and dichloromethane (DCM), for each metal catalyst, for each reaction.
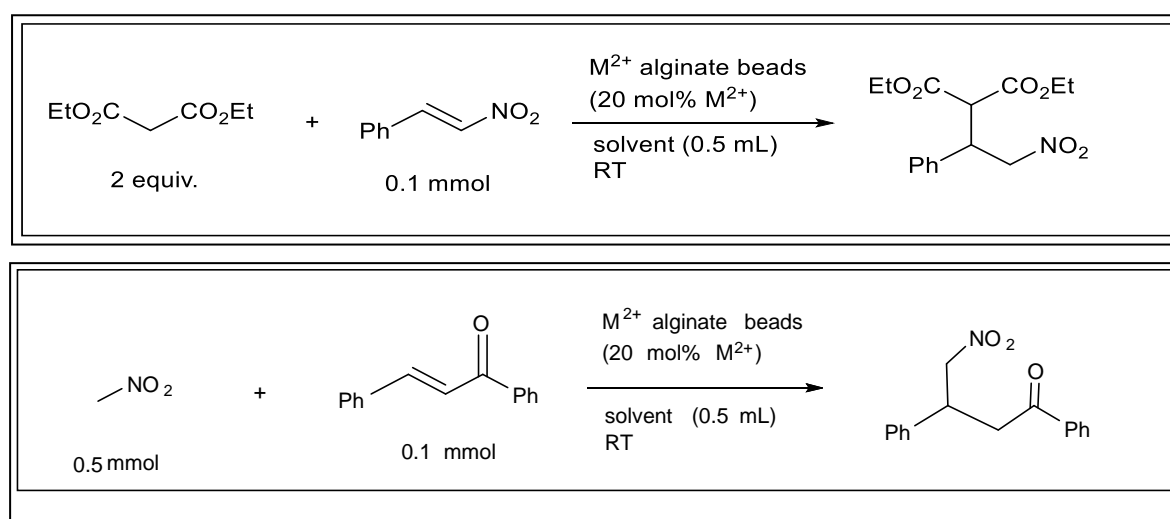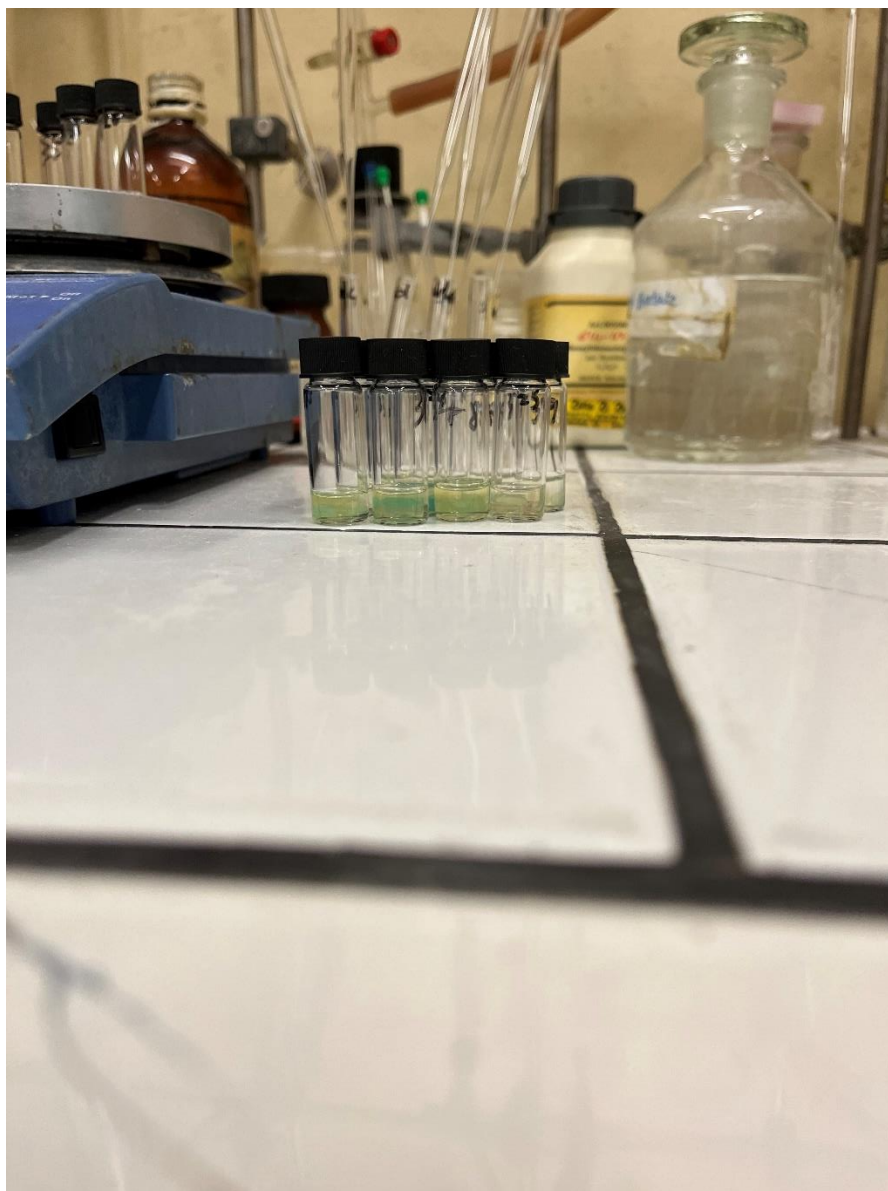

Figure 13. The scheme of catalytic reactions in the experiment. (reaction 1, reaction 2)

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Picture 2. The catalytic reactions in small vials

The reactants are weighed and transferred into small reaction vials without mechanical stirring. Usually, the amount of catalyst is 20 mol% based on metal, with respect to the limiting reagent, therefore based on Table 1, the number of each type of beads to be added could be calculated. After the addition of the gel beads, the reaction progress is checked by thin layer chromatography (TLC) every 24 hours, to see if there are some product in the reactions using a mixture of 80% of hexane and 20% of ethyl acetate as eluent. In case of visible product formation, the mixture is filtered on a small silica plug, and checked by ^{1}H-NMR spectroscopy. The enantioselectivity of the reaction is

determined by HPLC and is expressed in enantiomeric excess (ee%). The results of the reactions are shown in the following tables.

Reaction	Solvent	Metal	Observations and TLC analysis	ee% (254nm)
GW-008	DCM	Ca	6d: a trace of product	
GW-014	THF	Ca	24h: Product can be seen, 72h: lots of product	17%
GW-020	EtOH	Ca	24h:got turbid, 48h: some product, 72h: some product	0.16%
GW-011	DCM	Cu	After 6d: No product	
GW-017	THF	Cu	72h: trace of product	
GW-022	EtOH	Cu	48h: No product	
GW-024	DCM	Sr	24h: Product can be seen,48h: Some product,72h: Lots of product	8.02%
GW-030	THF	Sr	24h: reactions seems complete(No trace of limiting reagent)	6.78%
GW-036	EtOH	Sr	24h: some product, 48h: some product, 5d: some product	12.10%
GW-027	DCM	Ni	24h: no product, 48h: trace of product, 5d: some products	
GW-033	THF	Ni	24h: no product, 48h: some product, 5d: lots of product.	4.36%
GW-038	EtOH	Ni	24h: no product, 48h: a trace of product.	
GW-040	DCM	Ba(syringe)	24h: lots of products(little trace of limiting reagent)	4.10%
GW-041	THF	Ba(syringe)	24h: no trace of limiting reagent, reaction seems complete	12.80%
GW-042	EtOH	Ba(syringe)	24h: no reaction 48h: get turbid,no reaction, 72h: a trace of product	4.06%
GW-043	DCM	Ba(normal dropping)	24h: some product, 48h: some product, 72h: lots of product	2.43%
GW-044	THF	Ba(normal dropping)	24h: no trace of limiting reagent, reaction seems complete	16.19%
GW-045	EtOH	Ba(normal	24h: get turbid, no reaction, 48h: no reaction	

			dropping)	
GW-046	DCM	Co	24h: no reaction, 48h: no reaction, 72h: no reaction	
GW-047	THF	Co	24h: a little amount of product, 48h: a trace, 72h: a trace of product	0.45%
GW-048	EtOH	Co	24h: no reaction, 48h: no reaction, 72h: no reaction	
GW-049	DCM	Zn	24h: a trace of product, 48h: some product,72h: some product	0.50%
GW-050	THF	Zn	24h: no reaction, 48h: a trace of product, 72h: a trace of product	1.00%
GW-051	EtOH	Zn	24h: no reaction, 48h: a trace of product, 72h: a trace of product	
GW-052	DCM	Proton	24h: no reaction	
GW-053	THF	Proton	24h: no reaction	
GW-054	EtOH	Proton	24h: no reaction	
GW-055	DCM	Ba(200DL)	24h: lots of products, 48h: lots of products	0.24%
GW-056	THF	Ba(200DL)	24h: no trace of limiting reagent, reaction seems complete	12.00%
GW-057	EtOH	Ba(200DL)	24h: get turbid, a trace of product, 48h: some product	

Table 2. The activities, TLC and HPLC results of metal-alginate beads catalysts in reaction 1.

From Table 2, reporting the results obtained in reaction 1, the reaction proceeded well with alkaline earth metal catalysts, especially in the less polar DCM and THF solvents. As far as the enantioselectivity is concerned, calcium alginate in THF (GW-014) gives the highest ee% up to around 17% in the HPLC analysis while its activity may not be the highest (GW-014). Similar results can be found for barium and strontium in THF (GW-030, GW-041, GW-044). For DCM solvent (GW-024, GW-040, GW-055), it can be seen quite a lot of products, and a better ee% than that of transition metals, even it is not very high. While the worst results were with ethanol, the activity of the reactions was quite low and they didn't give a good ee% except from strontium (GW-036), where a 12% ee% is seen. Besides, there is not so many differences between barium beads

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prepared using a dropping funnel or a syringe, they both worked well in THF, and DCM, while in ethanol the activity was much lower (GW-040 to GW-045, GW-055 to GW-057). At the same it also gave a high enantiomeric excess from 12% up to 16%. Since this process is a base-promoted deprotonation, therefore based on the results that alkaline earth metals have high activity, we hypothesize that the alkalinity of different metals is the deciding factor of this process, since the hydroxides of alkaline earth metals have higher alkalinity which means that they can easily deprotonate the malonate, resulting in a better catalysis in the process.

For reaction 2, no relevant amount of product was observed in the first reactions performed with calcium, copper, strontium and nickel alginates in the three solvents. Thus, we hypothesized that the reaction probably would not happen in the later tests too, therefore investigations on this reaction stopped after 4 metal catalysts were tested.

3.4 Mechanism of Michael addition reactions

Michael addition reactions are a series of reactions that happens between Michael donors and Michael acceptors. The acceptors are usually olefins carrying electron withdrawing groups (EWG), such as acyl, cyano, nitro or sulfone groups. While for Michael donors they are usually a nucleophile such as malonates or ketones. In the Michael addition reactions, a deprotonation first happens, if the compound is in a basic environment, the process can be facilitated. As a result, an intermediate enolate is formed. After this, the nucleophilic group will attack carbon β to Michael acceptors, this conjugation pushes the electrons of carbon double bond onto carbonyl oxygen, forming a new enolate. Since the oxygen has two more electrons, it becomes possible for the whole system to be protonated. After the tautomerization of hydrogen from oxygen to α carbon, the product is finally formed. During the reaction, two sp2 hybridized carbons are both converted to sp3 hybridization, therefore there is a possibility to create a chiral carbon. As there are different nucleophiles, there are several types of additions for example, carbon-sulfur addition, carbon-nitrogen addition, carbon-carbon addition. In this research, carbon-carbon addition is carried out in the reactions.[31]
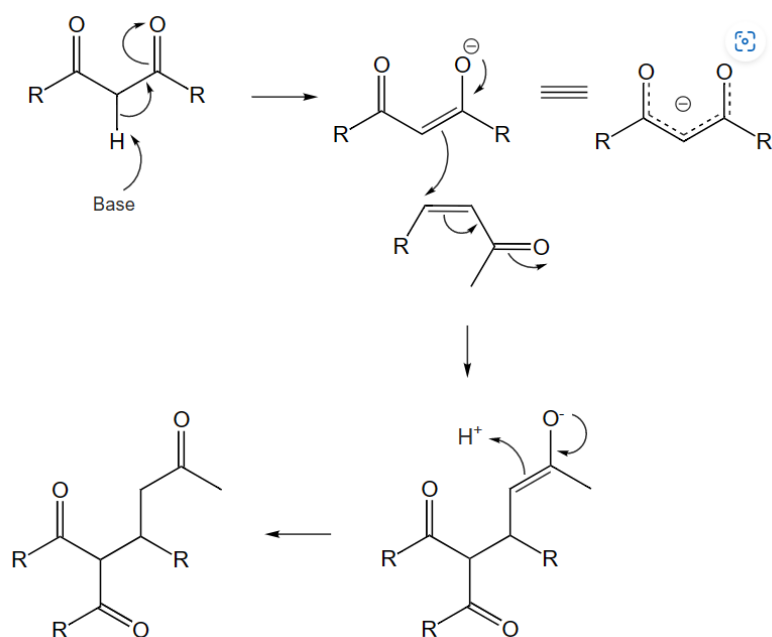
Figure 14. The mechanism of Michael C-C addition reactions ([32])

4. Conclusion

In this project, it is confirmed that several divalent metals and proton form gel beads with alginate successfully. Soft enolization was tested using these gel beads as a catalyst in two representative Michael addition reactions.

Firstly, the racemic reference compounds were synthesized using triethylamine as catalyst. The reactions went well with the product confirmed by TLC and H-NMR, and later successfully separated in HPLC analysis.

And for the late tests of the catalysis of activity and enantioselectivity, in general, alkaline earth metals show a higher activity and enantioselectivity (expressed by ee%), between them, barium showed a better activity and enantiomeric excess at the same time. Other metals could give a good enantiomeric excess but relatively lower activity. The reactions did not occur with transition metals and alginic acid except for nickel in THF, which might be ascribed to different basicity of the materials.

In conclusion, we found that barium alginates catalysts can be employed in a reaction based on soft enolization, providing a promising starting point for future investigation.

5. Experiment part

5.1 Materials and methods

Reagents and solvents in commercial use or available in the laboratory were used. To verify the purity of the products obtained and the progress of the reactions, ^{1}H-NMR spectra were performed using Varian Mercury 400 spectrometers. The enantiomeric excess of products was determined by chiral stationary phase HPLC Daicel Chiralpak AS-H columns, using an UV detector operating at 254 nm. To get the reference compound, the two enantiomers were obtained using triethylamine as a catalyst. And in HPLC analysis the retention times of the two enantiomers was established. And it was performed under the following conditions: AS-H, 1 mL/min, 90:10=Hexane: isopropanol, λ=254 nm, t_{min}= 11.8 min, t_{max}= 13.5 min. ([33])

5.2 General procedure for the catalytic reaction

Nitrostyrene (0.1 mmol) is added to a small vial followed by the solvent (0.5 mL) and malonate (0.2 mmol) and the catalyst beads corresponding to a 20 mol% loading based on metal amount. The vial is kept standing at RT without stirring and the reaction progress is followed by TLC (*n*-hexane/EtOAc 80:20). After the appropriate time, the mixture is filtered on a short plug of silica, the beads extracted with EtOAc, all extracts filtered on the same plug and the solvents evaporated with a rotavapor. The residue is analyzed by ^{1}H-NMR to determine the conversion, and by HPLC to determine the enantiomeric excess.
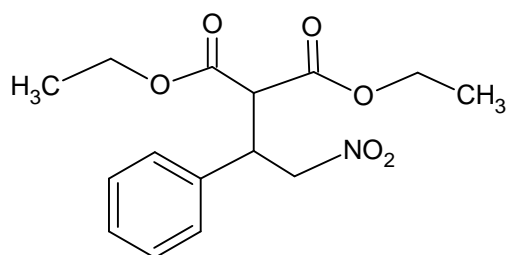
Diethyl 2-(2-nitro-1-phenylethyl) malonate:

^{1}H NMR (400 MHz, CDCl$_3$) δ = 7.35-7.21 (m, 5H), 4.92 (dd, J = 13.9, 5.0 Hz, 1H), 4.86 (dd, J = 13.1, 9.0 Hz, 1H), 4.27-4.20 (m, 3H), 4.01 (q, J = 7.2 Hz, 2H), 3.82 (d, J = 9.3 Hz, 1H), 1.26 (t, J = 7.1 Hz, 3H), 1.05 (t, J = 7.1 Hz, 3H).

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