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CYCLODEXTRINS CARBONATE ENTRAPPED IN POLYMERIC MEMBRANES: CATALYTIC BEHAVIOUR

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Flat sheet membranes made of modified polyetheretherketone known as PEEK-WC, charged with O-octyloxycarbonyl b-cyclodextrins (b-CD) were prepared by the phase inversion method and characterized. Their catalytic behaviour for the p-nitrophenylacetate (PNPA) hydrolysis to p-nitrophenol (PNP) was investigated. The b-CD acyclic carbonate derivative seems to have an effective catalytic action when incorporated in the PEEK-WC membrane. The membranes were tested at different temperatures and substrate concentrations and the value of activation energy for the reaction was estimated.

Օ օկտիլօքսիկարբոնիլ b-ցիկլոդեքստրինով (b-ՑԴ) մոդիֆիկացված պոլիեթերեթերկետոնի (PEEK-WC) հիման վրա ֆազաինվերսիոն մեթոդով սինթեզվել և բնութագրվել են հարթ մեմբրաններ: Ուսումնասիրվել է p-նիտրոֆենիլացետատի (PNPA) p-նիտրոֆենոլ (PNP) հիդրոլիզի նրանց կատալիտիկ ակտիվությունը: Ցույց է տրվել, որ PEEK-WC մեմբրանում ընդգրկված b-ՑԴ-ի կարբոնատային ածանցյալը դրսևորում է էֆեկտիվ կատալիտիկ ակտիվություն:

На основе полиэфирэфиркетона (PEEK-WC), модифицированного О-октилоксикарбонил b-циклодекстрином (b-ЦД), фазоинверсионным методом синтезированы и охарактеризованы плоские мембраны. Изучена их каталитическая активность гидролиза p-нитрофенилацетата (PNPA) в p-нитрофенол (PNP). Показано, что карбонатное производное b-ЦД, включенное в PEEK-WC мембрану, проявляет эффективную каталитическую активность.

Cyclodextrins are a series of cyclic oligosaccarides produced from starch by the action of cyclodextrin glucosyltransferase. Cyclodextrins were first discovered by Villiers in 1891 as degradation products of potato starch. Cyclodestrins are composed of D-glucopyranose residues bonded by a-(1-4) linkages. Three well characterized and commercially available members of this family are a-, b-, and g-cyclodextrins, made up of six, seven, and eight glucopiranose residuees, respectively.

Within a cyclodextrin molecule, each D-glucopyranose residue assumes the C1 (chair) conformation. The molecular geometry of a cyclodextrin can be described as a truncated cone, a doughnut, or even a lampshade, with one opening being wider than other, and the molecule possessing a hydrophilic outer surface and hydrophobic inner cavity. All secondary hydroxyl groups (C2-OH and C3-OH) are on the wider end of the cavity whereas all primary hydroxyls (C6-OH) are on the narrower end.

This non-inverting, somewhat rigid structure may flex or compose, but not

collapse, and it can efficiently form molecular inclusion complexes with various organic and inorganic "guest" compounds.

Molecular inclusion compound are formed when one molecule, commonly known as the "guest" inserts within the confines of the other, commonly known as the "host". A distinctive characteristic of the host molecule is that its binding sites are oriented along the same direction in space. The bond sites on the enclosed molecule, by contrast, diverge. Although no covalent bonds are formed between the host and the guest, lower energy forces hold the two molecules in a rigid crystalline structure which is easily disrupted to ensure timely release of the entrapped guest. In fact, the ability to reversibility form molecular inclusion complexes becomes the basis for almost all cyclodextrin applications.

Cyclodextrins are water soluble, although enhanced or dimished aqueous solubility can be achieved via chemical or structural modifications. b-cyclodextrins exhibit a remarkably lower solubility than do a- and g-cyclodextrins. This characteristic stems from a rigid intramolecular hydrogen bonding network between the secondary hydroxyl group belt found on the wider aperture of the b-cyclodextrin molecule.

Cyclodextrins are modified chemically in order to alter their solubility behaviour and complexation properties, and to induce groups with certain specific functions. Derivatives that have a greater aqueous solubility than their parental cyclodextrins are desired in many applications. Cyclodextrins can also be chemically modified to produce derivatives that are much less water soluble than their parents. A reduced aqueous solubility is necessary, for example, in applications where a low residue of cyclodextrin is required.

In this work a O-octyoxycarbonyl b-CD derivative was used. The effect of the immobilised CD derivative in polymeric membrane on the rate of the hydrolysis reaction of PNPA to PNP was analysed. In aqueous basic solutions cyclodextrins cleave phenyl acetates by acyl transfer from the ester to an ionised hydroxyl group of the cyclodextrin. Catalytic activity of b-CD carbonate is a function of DS and of the strength of the alkaline medium. In homogeneous system the catalytic action of CD derivatives was found to decrease with increasing of the substitution degree [3]. Moreover the b-CD acyclic carbonate derivatives are not stable because of the hydroplysis of carbonate at basic pH, whereas the rate constant for the CD-catalysed reaction is maximal at a pH of 12 to 13 [1].

The most important characteristic of cyclodextrins is their ability to form inclusion complexes with a wide variety of guest compounds without formation of covalent bonds. Since the cavity diameter of a cyclodextrin varies with the number of glucose units in the ring, selecting an appropriately sized cyclodextrin to fit the geometric parameters of a guest compound is of primary importance in forming an inclusion complex.

It is important to keep in mind that complexation reaction between a cyclodextrin and a guest molecule is a rapid and reversible process in solution. Complexation reactions usually occur in presence of a solvent, typically water. Water favors the formation of a complex on the basis of hydrophobic interaction.

The breadth of industries which can benefit from cyclodextrin technology range

from agricultural and pharmaceutical, to chemical process industries and analytical and diagnostic, to foods, flavors, and cosmetics, essentially any industry which consumes organic based materials can benefit from cyclodextrins.

Membrane preparation and characterization was the first aim of the work, in order to evaluate the possibility to combine catalytic action of b-CD immobilised in polymeric matrix with membrane technology.

Membrane technology offer a wide range of applications, but also a great number of advantages with respect to other traditional techniques.

It is well known, however, that the properties of the membranes in the applications where they separate molecules which have very close molecular dimensions are widely influenced by the choice of the polymer. In this work a particular kind of PEEK, named PEEK-WC (poly(oxa-p-phenylene-3,3-phthalido-p-phenylene-oxy-phenylene), was used. The polymer is characterised by the presence of the cumbersome lattonic group that reduces the degree of cristallinity thus making it more soluble in some chlorohydrocarbon solvents and also in DMF and DMSO and, as a consequence, it is then possible to obtain PEEK-WC membranes that are now studied for many applications.

The b-CD acyclic carbonate derivative seems to be an efficient nucleophilic catalyst when incorporated in the PEEK-WC membrane [2]. It seems to posses a high nucleophilicity towards the reagent PNPA with exceptional lability of the intermediate on the reaction pathway leading to the formation of the product by a large rate acceleration. In any case, when the same reaction was carried out in a membrane reactor, even if without cyclodextrins, the reaction rate results higher with respect to the batch reaction.

The PNPA hydrolysis takes place with an inclusion complex in which the phenyl group of the ester stays in the hydrophobic cavity of the CD [1]. The efficiency of the ester cleavage is enhanced in the presence of immobilised membrane cyclodextrin since the PNPA orients its phenyl group into the cavity in geometries that are suitable for the acyl transfer [2].

In previous studies the effect of b-CD derivatives with different DS was investigated and the procedure of membrane preparation was optimised; by using b-CD carbonate with DS equal to 7 and in concentration of 7.5 wt% a conversion degree of 100% was reached [4].

In order to improve the catalysis the O-octyoxycarbonyl b-CD derivative was dispersed in the casting dope. In this way the CD carbonate catalytic activity in the membrane can be reached by the substrate with a better efficiency.

In this study the effect of some parameters on the catalytic activity of the membranes functionalised with b-CD has been examined. The membrane have been characterised and tested at different temperatures and substrate concentrations. The value of activation energy was estimated.

The reaction has been carried out at same temperatures and substrate concentrations, in the same membrane reactor on a membrane made only of PEEK-WC.

By using immobilised membrane b-CD, a significant improvement of reaction rate in comparison with the PEEK-WC membrane was observed.

Materials and Methods. PEEK-WC, poli (oxa-*p*-phenylene-3,3-phtalido-*p*-phenylenxoxa-*p*-phenylene-nexoxi-*p*-phenylene) was supplied from Chanchung Institute of Applied Chemistry, Academia Sinica. The polymer powder was washed with methanol at room temperature and then dried in a vacuum oven before membrane preparation.

b-cyclodextrin was supplied from Roquette Italia (Cassano, Spinola, Italy). Its *O*-octyloxycarbonyl b-CD derivative was synthesised according to the literature and the average degree substitution (DS 7) was determined via quantitative FT-IR analysis [5].

p-nitrophenylacetate and *p*-nitrophenol were purchased from commercial sources (Fluka Chemicals) and were used without further purification.

The membranes were prepared following the traditional phase inversion process [6] which permits the production of membranes with an asymmetric pore structure. The solvent was N,N-Dimethylformammide (DMF) and non solvent water.

PEEK-WC membranes. The purified polymer (15 wt%) was dissolved in DMF by magnetically stirring overnight to allow complete solution at room temperature. The solution was cast knife on a glass plate. The knife was supplied from Braive Instruments. The knife high was set at 250 mm and the time of initial evaporation in air, at room temperature, was 45 s. the cast film was immersed in a coagulation bath containing distilled water; the cast film were kept in water for 10 min and then transferred to fresh distilled water for 2 hours.

PEEK-WC/O-octyloxycarbonyl b-CD membranes. This membrane was prepared as above, using 7,5 wt% solution of CD derivative in the polymer solution. b-CD derivative was added after complete dissolution of the polymer and the solution was dissolved by magnetically stirring for a day or more to allow complete solution at room temperature.

O-octyloxycarbonyl b-CD derivative, insoluble in water until 60 °C about, was used instead of b-CD to prevent loss of CD during the membrane formation process.

To confirm the absence of b-CD derivative in the coagulation bath a thin layer chromatography (TLC) test was carried out with a solution with b-CD derivative in EtOH and a part of first coagulation bath solution. Ferric "orcinolo" was put on the layer of TLC and it is clear the presence of b-CD in the first solution and the absence in the second one.

In the casting solution the ratio PEEK-WC: b-CD derivative was 2:1. It was been supposed that after phase inversion process the ratio is the same and, after drying, all solvent is gone out of the membrane. A part of the utilised membrane of area 11,9 cm^2 has been dried and weighted (0,121 g). So the b-CD weight was 0,0403 g. the utilised membrane was of 133 cm^2 area, so the total b-CD weight was 0,45 g. The molar concentration of b-CD derivative immobilised in membrane in the active area has been determined and it was 0,152 M.

All membranes were stored in water.

Reactor equipment. The membrane reactor configuration is described in Figure 1.

A flat cell was used. The solution of PNPA in phosphate buffer, pH 8 permeated through the membrane with constant flow rate having a pressure difference as driving force, DP = 0,01 ± 0,002 bar. The membrane area was 133 cm^2. The reactor calibration has been done at room temperature and with distilled water. Various tests at different temperatures have been carried out by using a thermostatic bath.

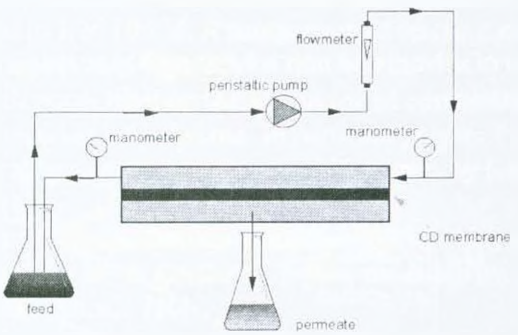

Figure 1. Membrane Reactor scheme.

Reactor operation and analytical method. A standard solution of PNP 0,02 M in acetonitrile was prepared. In the typical experiment, 1.26 10^{-4} M, 9.58 10^{-5} M, 7.76 10^{-5} M, 5.55 10^{-5} M, 4.05 10^{-5} M and 2.74 10^{-5} M PMPA solutions in phosphate buffer were prepared. The solution was placed in the membrane reactor and the permeate analysis has been done about every 5 minutes.

Initial PNPA and PNP concentrations were determined spectrophotometrically at 299 nm and 401 nm, respectively, using a 1 cm quartz cell and a Shimadzu UV-160A UV-VIS recording spectrophotometer, at room temperature. The reference compartment contained a phosphate buffer. A calibration curve for PNPA and PNP in the phosphate buffer was made and the curves were approximated with a linear function, Figure 3.

To verify the effective catalysis of b-CD derivative, the same experimental tests have been carried out by using a PEEK-WC membrane in the membrane reactor.

Because hydrolysis of esters occurs spontaneously in alkaline solutions, also in the absence of CD an increase of PNP is observed.

The reaction rate has been determined by working at different temperatures (15, 20, 30, 40, and 55°C).

Results and discussion. *Membranes.* The different structures of phase inversion membranes are related to the polymer and CD concentrations in the casting solution [7]. The actual membrane formation process is usually a diffusion induced phase separation (DIPS) process.

The membrane forming system is composed of PEEK-WC, *O*-octyloxycarbonyl b-CD derivative, DMF as solvent and water as non solvent. All components are only miscible in a concentration range between 15 wt% PEEK-WC/2,5 wt% *O*-octyloxycarbonyl b-CD derivative and 15 wt% PEEK-WC/7,5 wt% *O*-octyloxycarbonyl b-CD derivative [4].

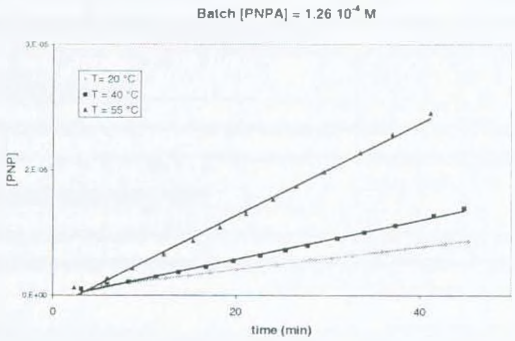

Figure 2. Reaction course in the bulk at different temperatures.

The different membrane structure, porosity and permeability properties, mainly depend upon diffusivities and the ratio of solvent and non solvent exchange that are influenced by components concentration.

Both membranes show a linear dependence of water flux on the applied pressure gradient, at constant temperature.

Hydrolysis of PNPA in the bulk. Noted above, during the alkaline hydrolysis of PNPA an increase of PNP concentration was observed. In Figure 2 the reaction course in the bulk at different temperatures is shown.

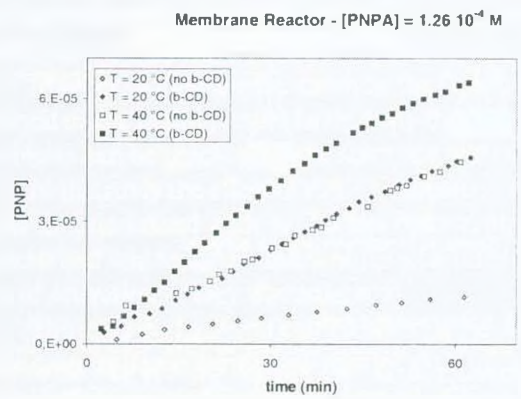

Figure 3. Reaction coures in the b-CD carbonate membrane reactor and in the same without b-CD derivative at 20 and 55 °C, for [PNPA] equal to 1.26 10^{-4} M.

Hydrolysis of PNPA in the membrane reactor. PNPA hydrolysis reaction is an ideal model reaction for its simple

mechanism, widely investigated, to obtain information on the catalytic and selectivity properties of CDs [8,9,10].

Reaction course in the b-CD carbonate membrane reactor and in the same without b-CD derivative at 20 and 55°C for [PNPA] = 1.26 10^{-4} M are reported in Figure 3. It is clear that the reaction rate was significantly higher when the reaction was carried out in presence of b-CD.

The first step of the reaction mechanism of the CD catalysed hydrolysis of ester is the substrate inclusion into the CD cavity [11]. The second step is the nucleophilic attack of a secondary CD hydroxyl into the carboxyl group of the substrate and CD acylation. Following steps are the dissociation of the inclusion com-

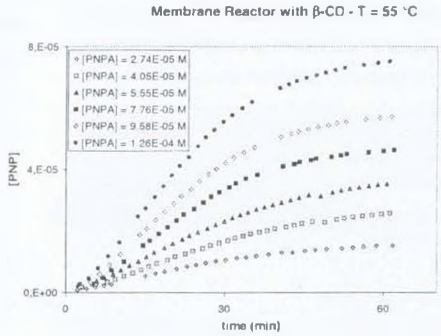

Figure 4. Reaction courses at 15, 20, 30, 40 and 55 °C for intial [PNPA] equal to 1.26 10^{-4} M.

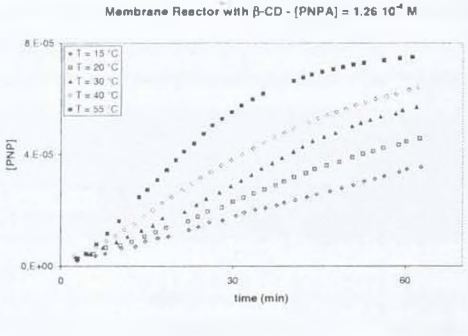

Figure 5. Comparison of reaction rate at 55 °C for intial [PNPA] equal to 2.74 10^{-5}, 4.05 10^{-5}, 5.55 10^{-5},7.76 10^{-5}, 9.58 10^{-5} and 1.26 10^{-4} M.

plex and hydrolysis of the acylated CD. CD deprotonated secondary hydroxyl groups are the active species, while the primary ones are not involved.

In Figure 4 are reported the reaction courses at different temperatures for PNPA initial concentrations equal to 1.26 10^{-4} M. A comparison of reaction rate at the same temperature (55°C) are reported in Figure 5.

The rare of the hydrolysis depends both on the PNPA initial concentration and the temperature at which the reaction is carried out. In particular, higher PNPA concentration and higher temperatures produce a higher reaction rate, by following a pseudo-first order kinetic [10].

Kinetic of reaction. b-CD carbonate derivatives catalysed the hydrolysis reaction acting as synthetic enzymes with lower costs and higher productivity. The reaction follows a pseudo-first order kinetic [10].

Initial rate, V_0, values for the reaction carried out in b-CD carbonate reactor were determined.

The reaction has been schematised as follow:

$$C + S \qquad CS \qquad P + C$$

here C, S, CS and P symbolise the catalyst (*O*-octyloxycarbonyl b-CD), substrate (PNPA), inclusion complex and product (PNP), respectively.

Two parameters define this king of kinetic, The Michaelis constant, K_M, and the maximal velocity of the reaction, Vmax, that occurs at high substrate concentrations when the CD is satured.

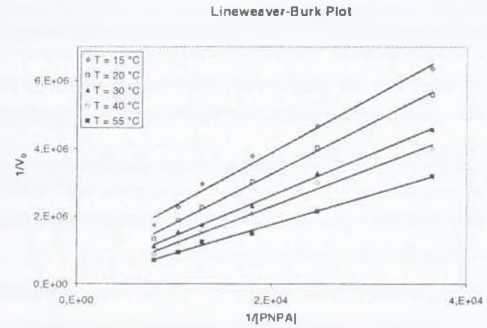

Figure 6. Lineweaver-Burk plot for the hydrolysis reaction carried out in the b-CD carbonate membrane reactor.

At very high values of [S], the initial velocity V_0 asymptotically approaches V_{max}.

To determine the values of Vmax and K_M, the Lineweaver-Burk or double reciprocal plot is used.

The Lineweaver-Burk plot for the hydrolysis reaction carried out in the b-CD carbonate membrane reactor is reported in Figure 6. The slope of the line is K_M/V_{max}, the $1/V_0$ intercept is $1/V_{max}$, and the extrapolated 1/[S] intercept is $-1/K_M$.

The kinetic constant has been obtained from this equation:

$$k_2 = \frac{V_{max}}{[C]_T}.$$

In literature the approximation of the b-CD determined kinetic to a first order kinetic is reported [12].

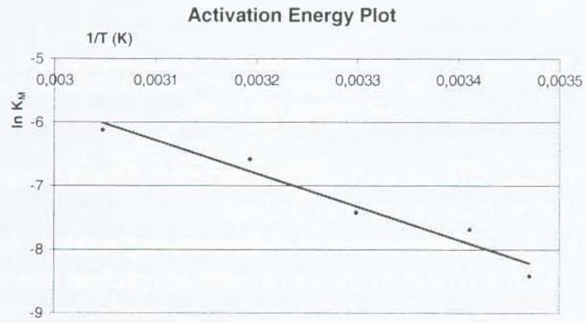

Figure 7. Estimation of the value of activation energy with Arrhenius equation.

To estimate the value of activation energy, the Arrhenius equation has been applied (see Figure 7):

$$\ln k = \ln k_0 + \left(-\frac{E_{att}}{R}\right)\cdot\frac{1}{T}.$$

Even if CD aren't enzymes and don't follow exactly a Michaelis-Menten kinetic, we can use this kind of relation with good approximation.

Conclusions. In previous studies [4,11] the influence of pH, DS of CD and CD concentration on the trend of hydrolysis reaction of PNPA to PNP carried out in a b-CD carbonate membrane reactor has been examined.

In this study we worked in optimal conditions determined previously [4,11], the effect of temperature has been examined and the value of activation energy has been estimated.

When hydrolysis reaction was carried out in the b-CD derivative membrane reactor, we found a value of activation energy of 32,6 kJ/mol, significantly higher respect the value listed in literature [?]. The values of activation energy for the batch hydrolysis of PNPA with and without b-CD reported in literature results, respectively, in 36.4 and 45.2 kJ/mol.

In the membrane, the CD carbonate derivative shows to have more stability

because of their chemical resistance to alkaline attack. The entrapment of CD in the polymeric membrane optimises the interaction with the substrate, increases the chemical stability of the catalyst, allows the reuse of the catalytic membrane, in fact the membranes may be easily washed and reused without any lost of activity.

In the membrane the acylic chains form bonds with the PEEK polymer so that the hydrophobic cavity is free and oriented to be more able for carrying out the catalytic action.

In conclusion, the use of a polymeric membrane functionalised with O-octyloxycarbonyl b-CD derivative to carry out the hydrolysis reaction of PNPA to PNP in phosphate buffer enhances the reaction rate with an enzyme-like behaviour, but improving productivity and stability and decreasing costs.

This study shows the performance of a novel design of catalytic membrane reactor, in which the specific properties of a non conventional catalyst immobilised in a polymeric membrane can promote and extend new applications of these systems.

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