Synthesis and properties of cyclodextrin-malic acid copolymers

Marcin Lukasiewicz, Magdalena Marciniak

Faculty of Food Technology; Agricultural University in Krakow
ul. Balicka 122; PL-30-149 Krakow, Poland
e-mail: rrlukasi@cyf-kr.edu.pl

Abstract

The presented work focuses on malic acid and β -cyclodextrin polycondensation processes. The cyclodextrin/malic acid co-polyesters were synthesized using β-cyclodextrin and DL-malic acid. Na_2HPO_4 was used as a catalyst and the reaction was conducted in solvent-less conditions. Poly reaction were studied as a function of reaction time and temperature. As a result of investigation a series of oligo- and polymeric materials with high water solubility was obtained. The material still possesses the complexation ability. The basic properties of obtained copolymers were investigated including its chemical composition that was studied using HPLC method. The amount of incorporated cyclodextrin as well as thermal transformation of malic acid was studied as well. According to result we get we can state that new route for low molecular malic acid cyclodextrin copolymers were developed. The product was obtained by means of green chemistry method in solvent less system with simple reaction set-up.

Keywords

Cyclodextrin, polester, malice acid, biodegradable polymer

Introduction

Cyclodextrins sometimes called cycloamyloses are non-reducing cyclic glucose oligosaccharides originated from the cyclomaltodextrin glucanotransferase (E.C. 2.4.1.19; CGTase) catalyzed degradation of starch. The described cyclic oligosaccharides, are composed of 5 or more α-D-glucopyranoside units which are linked together with a 1-4 glicosydic bond in the same manner as in amylose or amylopectine (main components of starch) [1]. The smallest known cyclodextrin is a 5-membered macrocycle while the largest one contains 32 1,4-anhydroglucopyranoside units. The most important from the scientific and industrial point of view cyclodextrins are constituted only by 6-8 glucopyranoside units and known as α-, β- and γ- cyclodextrins respectively. They can be topologically represented as toroids with the larger and the smaller openings of the toroid exposing to the solvent secondary and primary hydroxyl groups respectively. Because of this arrangement, the interior of the toroids is not hydrophobic, but considerably less hydrophilic than the aqueous environment and thus able to host other hydrophobic molecules. On the contrary the exterior is sufficiently hydrophilic to impart cyclodextrins (or their complexes) water solubility [2].

The formation of the so called inclusion compounds greatly modifies the physical and chemical properties of the host molecule, mostly in terms of water solubility. This is the main reason why cyclodextrins have attracted much interest in many fields. In most cases the mechanism of controlled degradation of such complexes is based on pH change of water solutions, leading to the cleavage of hydrogen or ionic bonds between the host and the guest molecules. Alternative means for the disruption of the complexes take advantage of heating or action of enzymes able to cleave α-1,4 linkages between glucose monomers.

Except cyclodextrins, nowadays there is planty of cyclodextrin derivatives known to science and industry [3–6]. Among them there is a strongly increasing attention paid to the cyclodextrin polymers [7], [8]. The conventional way for their preparation is the crosslinking process employing some bifunctional reagents as e.g. di-epoxides and diisocyanates, or linking the rings to a macromolecular support [9]. According to that method both soluble as well as insoluble polymers may be obtained. It is worth to emphasize those cyclodextrin polymers possess the same complexation properties as simple cyclodextrins do, so they may play a role as host macromolecules for several low molecular compounds. In case when only a few cyclodextrin rings are incorporated in the polymer structure the product is mostly soluble in water showing a much higher solubility than the parent cyclodextrin. The complexes of these polymers are also

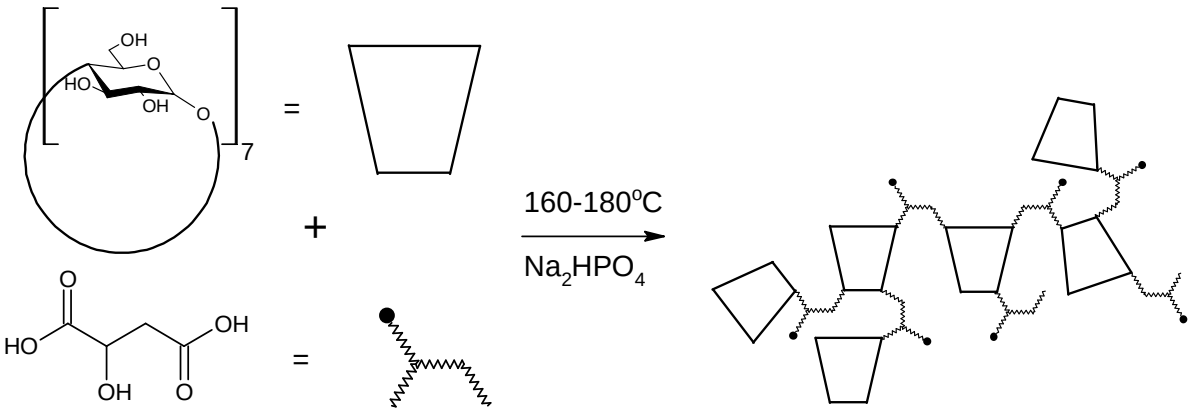
highly soluble; they do not precipitate from the solution and therefore these polymers are good solubilizers for the included guest compounds. Among plenty of linkers that may play as polymers structure building blocks only several are known to be safe for human body and may be used in pharmaceutical formulation or in food industry. Citric acid derivatives are one of the good example. There is some method for obtaining poly(β-cyclodextrin-co-citric acid). One of them was developed in our lab and includes simple solvent less polycondensation of cyclodextrin, citric acid and catalyst [10]. From the other hand, in food industry it is sometimes a strong need for avoiding high acidity of additives. In this case the citric acid may be easy replaced by malic acid, that is also a multifunctional cross linker but its lower acidity allow to use them in some special cases.

Experimental

Sodium phosphate dibasic dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), DL-malic acid, methyl orange, hydrochloric acid, sodium carbonate dodecahydrate ($Na_2CO_3 \cdot 12H_2O$), fumaric and malonic acids were delivered by POCh, Poland. β-Cyclodextrin was purchased from Roquette, France. All the chemicals were analytical grade and used as received.

Copolymerization

Copolymerisation of cyclodextrin and malic acid was done according to scheme 1. In details, in a solution of 3.82g malic acid (28 mmol) and 3.6g $Na_2HPO_4 \cdot 12H_2O$ (10 mmol) in 10ml of distilled water 6g of CD (4,75 mmol based on dry basis) were dissolved and the solution was dried for about 1h at 110°C. The powdered mixture was transferred into a Petri dish and heated in an laboratory dryer at temperature 160, 170 or 180°C for 60, 90 or 120 min respectively. A crude product was weighted and powdered. The specific amount of product was weighted (about 2g) and dispersed in 50ml of water. After centrifugation, the residue was dried and weighted in order to estimate the soluble and insoluble fraction as well as the reaction yield.



Hydrolysis of polyesters

In a capped vial mixture of 100mg of insoluble fraction was mixed with 3ml of 1M Na_2CO_3 and heated at 95oC for 1h. After heating was finished the mixture was left to finish the saponification for about one week. After hydrolysis, the mixture was neutralized using 1M HCl. Obtained samples were left for chromatographic analysis.

Chromatographic analysis of carboxylic acids in polyester fractions

HPLC analyses were carried out using system consisted of pump (Knauer), 20 μl injection valve and Smartline UV Detector 2500 (Knauer UV detector). A LiChrospher RP-18 column (Knauer) 250x4.0 mm i.d. with pore size of 100Å and particle size 5μm was used in all the analyses. Chromatographic conditions were as follows: UV detector wavelength 218 nm; mobile phase: 5 mM H_3PO_4 in 50 mM NaH_2PO_4; flow rate 0.8ml min-1; column temperature: 30°C. Quantities of the carboxylic acids were determined based on calibration curves made for the each acid separately.

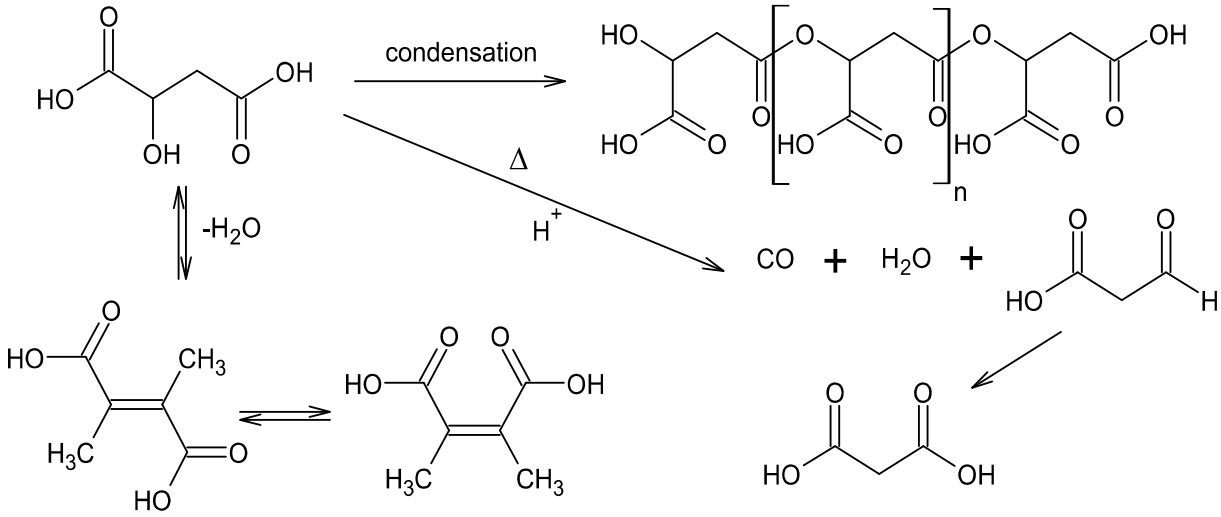
Complexation ability of the polymer

Cyclodextrin content in the polymer was determined using spectrophotometric method based on decolorization of methyl orange upon complexation by cyclodextrins [10]. In specific the mixtures of 0.023 mM methyl orange in 0.1 M HCl (absorbance of A_0=0.877 at 508nm) with different concentrations of cyclodextrin (concentration 0.2-10mM) were prepared and absorbance (A) at 508 nm were measured against 0.1M HCl as a blank. A calibration curve was plotted as a $1/(A_0-A)$ dependency of $1/(A_0-A)$ against 1/[CD].

A suspension of 50 mg of sample in 5 ml of 0.023 mM methyl orange in 0.1M HCl was prepared. In case of insoluble fraction, after 30 min the polymer was centrifuged and absorbance of the supernatant at 508 nm was measured against 0.1M HCl as a blank sample. Complexation ability was calculated based on the cyclodextrin content measured using anthron method.

Results and Discussion

As in the case of citric acid-cyclodextrin polymers the malic acid condensation process looks theoretically complicated. The desired reaction cours (see Scheme 1) may be interrupted by several side processes (see Figure 2). Among them self condensation of malic acid into poly(malic acid), decarboxylation to malonic acid or dehydratation to fumaric and/or maleic acids may take place. Some other rearrangements are also possible. According to obtained results we have excluded the decarboxylation process (no traces of malonic acid was detected using HPLC). On the other hand both maleic and fumaric acids are presented in product as both free or bounded acid. The amount of fumaric acids does not reach the level of 5% (w/w) and maleic acid 1%(w/w). HPLC analysis allows also to state that conversion of malic acid is quite high and reaches 50 to 95% depending on raction conditions (temperature 160-180°C, time 60-120min and the amount of catalyst 0-3,5g) - see Scheme 3-5. At presented work the molar ratio of malic acid, cyclodextrin and catalyst was set up as 6:1:2.



The obtained products may be divided into two separate fraction i.e. water soluble (WS) or water insoluble (WI). From practical point of view WS is more interesting due to its complexation of some hydrophobic compounds and making tham water soluble. The WS and WI fraction were obtained by solubilization of reaction product in water. The WI was separated by filtration and WS was than lyophilized. As can be seen at Figure 6 the amount of WI increases when higher

temperatures and longer reaction time is applied. It is probably the consequence of carbonization of both cyclodextrins or applied acid in the presence of phosphate ions. The detailed investigation of the WI composition will be done as a next step of investigation.

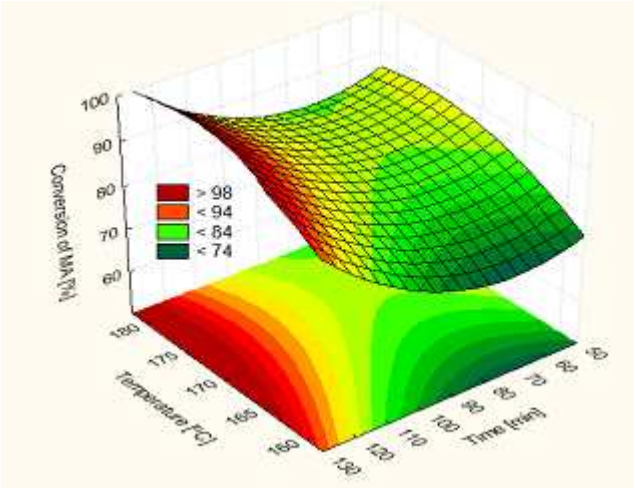


Figure 3. Conversion of Malic acid (MA) vs. reaction temperature and time at fixed catalyst amount (10mmol)

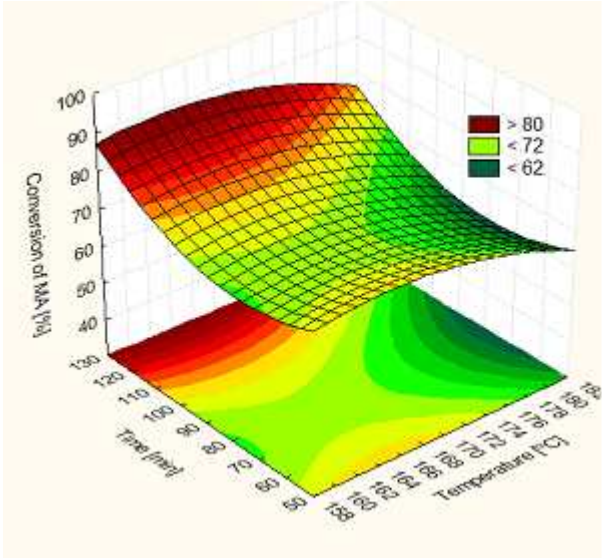


Figure 4. Conversion of Malic acid (MA) vs. reaction temperature and time at fixed catalyst amount (5mmol)

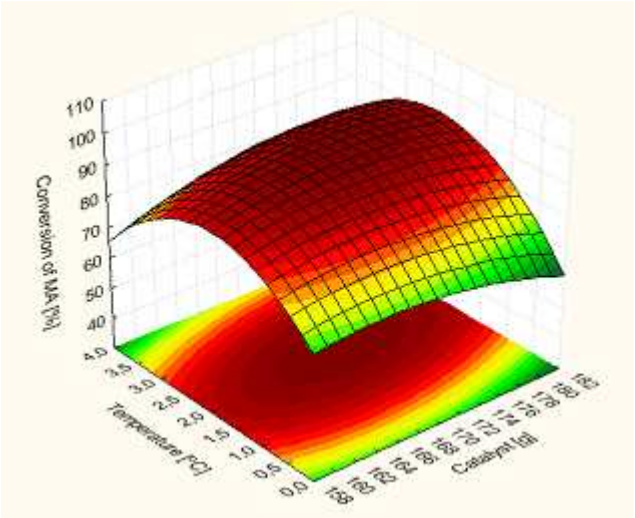


Figure 5. Conversion of Malic acid (MA) vs. reaction temperature and time noncatalytic process

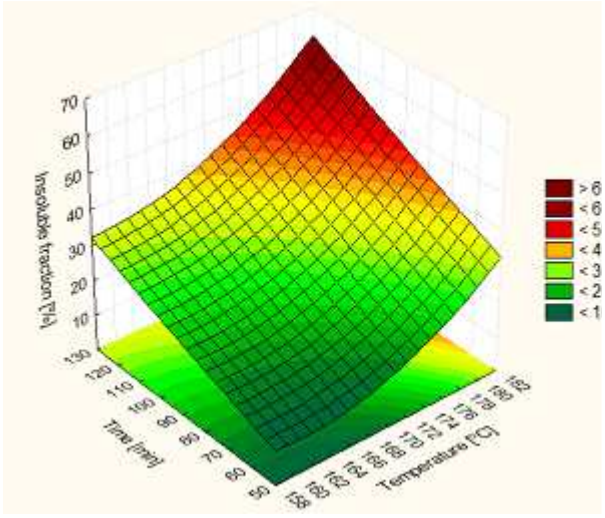


Figure 6. The changes in WI fraction vs. temperature and reaction time at fixed catalyst amount (10mmol)

The most important factor in case of cyclodextrin polymer is the ability to make some inclusion, host-guest complexes. The properties is strictly linked with cyclodextrin molecules presented in

the sample. The sugar content of WS was done using spectrometric, anthron method. The results are presented at Figure 7. It is easy to detect the strong variation of cyclodextrin content in the sample. At low temperature and longer reaction time the amount of cyclodextrin is the highest. Because we don't know if it is the bounded or free cyclodextrin we can only state that at these condition the amount of sugar in WI fraction must decrease. The phenomenon needs furthe examination including complexation ability itself.

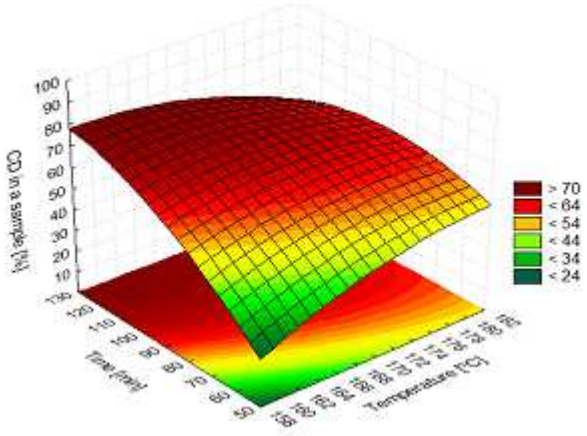


Figure 7. The changes in cyclodextrin presened in WS fraction vs. temperature and reaction time at fixed catalyst amount (10mmol)

Conclusions

According to result we get we can state that new route for low molecular malic acid cyclodextrin copolymers were developed. The product was obtained by means of green chemistry method in solvent less system with simple reaction set-up.

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