Microwave assisted transesterification of β-cyclodextrin

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Abstract:

From several decades microwave assisted synthesis is known to speed up many reactions and processes. Better yield, shortening in the reaction time and easy set-up are also the one of the most important factors on this case. In opposite there are also a lot of processes known in which enzymes or whole microorganisms are inactivated by action of electromagnetic field with microwave frequencies. In some cases however it was shown that microwaves may also activate the enzymatic transformations influencing enzyme/reagent fitting or protein conformation. The phenomenon has also been observed in case of some carbohydrates. According to that in presented study enzymatic transesterification of β-cyclodextrin using vinyl esters of long chain carboxylic acids has been presented. All the reactions were performed at microwaves and in conventional conditions. Lipase from porcine pancreas was used as a biocatalyst. Reactions were carried out in DMSO or DMF as solvents using fatty acids vinyl esters as acyl donors (vinyl stearate and and vinyl laurate respectively). After the reaction the products were precipitated from the solution in several stages. Products were analyzed according to overall yield and degree of substitution. The structure of the obtained esters was investigated by means of FTIR spectroscopy. As a result the conclusion may be drowning out that microwaves induced processes by means of better yield and higher degree of substitution. The shortening in the reaction time was also observed and process looks promising as an alternative way for introducing acyl donors into carbohydrate molecules however further, detailed study on the transesterification should be done.

Keywords:

microwave, cyclodextrin, transesterification, lipase

Introduction:

Cyclodextrins (CD) are a group of cyclic oligosaccharides with specific properties. Due the distinctive design cyclodextrin can create a "guest - host" inclusion complexes that have been applied in many industries, including: pharmaceutical, cosmetic, food, in order to stabilize and protect sensitive substances against oxygen, moisture, or light[1]. Modification of cyclodextrins by introducing additional functional groups of various kinds can further extend the functionality of the same CD as well as their complexes. Up to now a long list of cyclodextrin modification protocols is known[2]. Most of them suffer from multi-stage procedure, low yield or low selectivity. According to that there is a strong need to find new more efficient procedures for incorporating of some side groups into the cyclooligosacharide structure.

On the other hand microwaves are known to speed up many reactions and processes[3]. Better yield, shortening in the reaction time and easy set-up are the one of the most important factors on this field. On the other hand even in industry several processes are known in which microorganisms or enzymes are inactivated in electromagnetic field with microwave frequencies[4]. Several researches that have already been done have shown however that microwaves may also promote the enzymatic transformations influencing the protein conformation and enzyme/reagent fitting. The phenomenon has also been observed in case of carbohydrates[5]. According to that in presented study enzymatic transesterification of β-cyclodextrin has been presented.

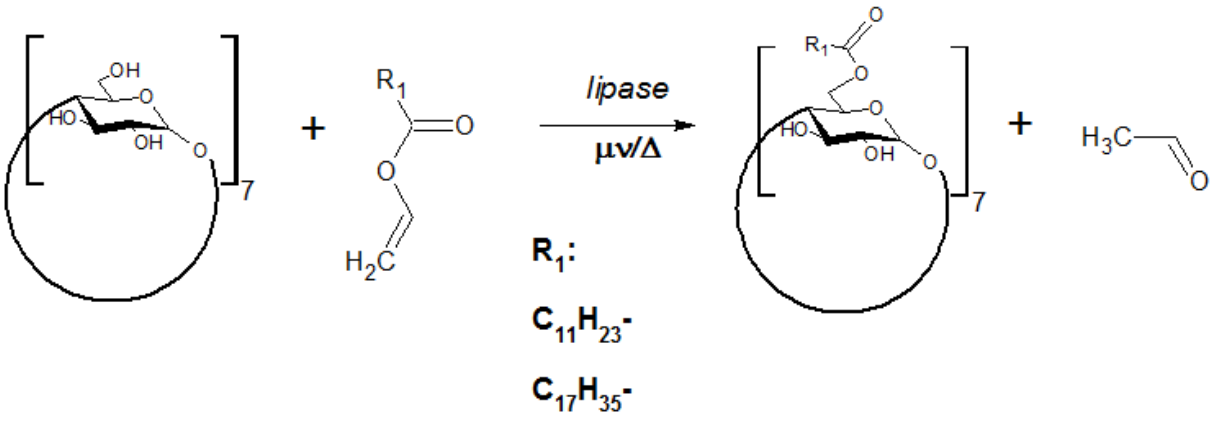
Experimental:

All reagents used in experiments were purchased from Sigma-Aldrich (Poland) except CD that was purchased from Roquette (France).

All the reactions were performed at microwaves and in conventional conditions according to Scheme 1. Lipase from porcine pancreas was used as a biocatalyst. Reactions were carried out according in DMSO or DMF as solvents using fatty acids as acyl donors (stearic and lauric acid respectively). After the reaction was finished the product was precipitated from the solution in several stages – Scheme 2. Product was analyzed according to overall yield and degree of substitution. The structure of the obtained esters was investigated by means of FTIR spectroscopy.

In a typical experiment 2.268 g (2mmol) of dried CD was dissolved in 50 mL of an appropriate solvent (DMSO or DMF respectively). After the carbohydrate was dissolved a biocatalyst was added to form a smooth suspension. The amount of catalyst was 0.01 of the CD sample (by means of mass balance). To the suspension and acyl donor was added as follows: 1.242 g

(4mmol) of vinyl stearate or 0.905 g (4mmol) of vinyl laurate. The reaction mixture was stirred for next 4hours in a water bath (conventional condition) at 50°C or irradiated for 30 min using RM-800 multimode microwave reactor (Plazmatronika, Poland). The power of the irradiation was set to 1.1W/g of the reaction mixture in DMSO and 1.3W/g for DMF mediated processes. Those levels allow keeping the reaction mixture at 50±5°C. In case of overheating above the level of 60oC the irradiation was cut off. During processing the temperature was controlled using a two-channel fiberoptic thermometer Reflex RFX-2 (Neoptix, Canada).



Scheme 1. Transesterification of CD by vinyl esters of fatty acids

After the reaction was finished obtained suspension was centrifuged (5000rpm, 20min) in order to remove the enzyme. Next steps of purification were shown on Scheme 2.

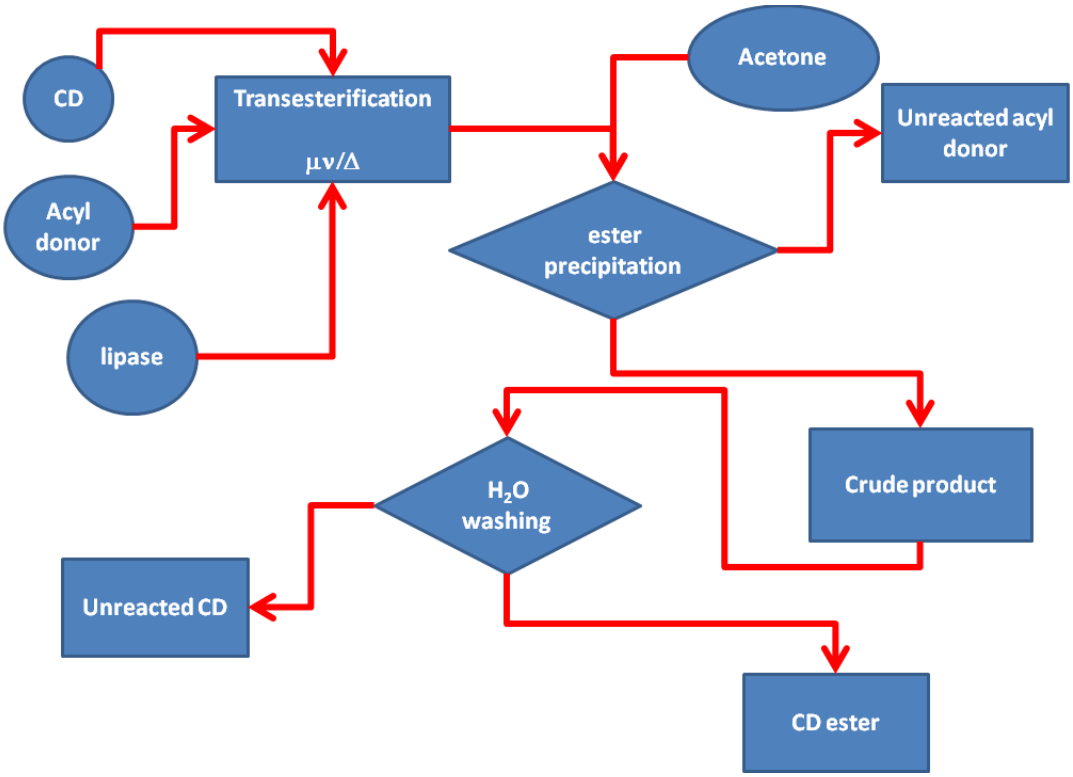
Analysis of products was performed using HPLC chromatography as follows:

Analysis of CD and CD esters[6]

CD and CD ester concentration were determined using a Knauer (Germany) chromatograph equipped with refractive index detection (Knauer, Germany). Measurement parameters were as follows: eluent AN/water 87:13 (v/v), flow rate 1 mL/min detection RI, column NP-NH2 Lichrosphere (250 × 4 mm, 5 μm particle size) (Knauer, Germany), sample volume 20 μL.

Analysis of unreacted acyl donors[7]

Unreacted vinyl esters were determined using a Knauer (Germany) chromatograph equipped with refractic index detection (Knauer, Germany). Measurement parameters were as follows: eluent AN/MeOH/AcOH 89:9:2 (v/v), flow rate 1 mL/min detection UVVIS; λ=208nm, column RP-C18 Lichrosphere (250 × 4 mm, 5 μm particle size) (Knauer, Germany), sample volume 20 μL.



Scheme 2. Process set-up for enzymatic transesterification of CD

FTIR analysis

FTIR spectra were collected to confirm the ester formation. All the spectra were recorded on FTIR BIORAD FTS-165 Fourier transform infrared spectrophotometer as KBr disks. Analysis was done with resolving capability of 4 cm−1 and scanning range 4000–500 cm−1.

Degree of substitution (degree of esterification)[8]

Degree of esterification was expressed as degree of substitution (DS) and was established using method evaluated by Miladinov and Hanna 32. The method consists in ester saponification with 1 M NaOH and then back titration with 0.5 M HCl to the original pH measured before NaOH addition. A sample (approximately 2 g) was accurately weighted using analytical balance, placed in a 250 mL conical flask, and mixed with 25 mL of distilled water. The mixture was conditioned in water bath with stirring for about 1 h at 30°C. After conditioning was finished the pH of the mixture was measured. Next 10 mL of 1 M NaOH was added. The sample was then conditioned for another 48 h at 50°C to fulfill the saponification of ester. In the last stage the sample was titrated with 0.5 M HCl to the original pH.

Results and discussion:

As a result of investigation a series of the CD ester has been obtained. The formation of ester bond between CD and an acyl donor has been confirmed using FTIR spectroscopy (see supplemental data). The overall reaction progress may be characterized as an example of low efficient reaction. These observations does not depend on reaction condition. On the other hand there are some easy to seen differences when one compare the conventional and microwave assisted processes. As can be seen in Table 1 and 2 microwave assisted processes results in higher yield and higher degree of substitution.

Table 1. Efficiency of CD tranesterification

Acyl donor (R_1)	Condition	Solvent	Product yield[1], %	Product purity[2], %	Donor conversion[3], %
$C_{17}H_{35}$	Δ	DMSO	9	85	17
	μν		11	80	29
	Δ	DMF	15	92	34
	μν		14	94	41
$C_{11}H_{23}$	Δ	DMSO	14	80	37
	μν		18	95	45
	Δ	DMF	23	71	21
	μν		31	92	44

[1] – *separated product, determined gravimetrically*
[2] – *by means of HPLC*
[3] – *determined (HPLC) after washing out by acetone*

But the differences are also observed in case of microwave assisted reaction only. In fact it might be the result of heat distribution in reaction mixture containing different types of solvent i.e. DMSO and DMF respectively. In Table 3 the initial heating rate is presented. Those heating rates were calculated as a first derivative of heating curves in both types of processes. It is easy to see that heating rate in microwaves is about three times higher than those observed in conventional experiments. The higher heating rate at μν condition results in faster achieving of final temperature level what may influence on yield and degree of substitution.

On the other hand the differences between DMSO and DMF systems may be the result of its physicochemical properties. In Table 4 some important parameters of both solvents has been collected. The dipole moments of both solvent are similar and almost twice as μ for water however dielectric permittivity of water is twice a dielectric permittivity of DMSO or DMF.

Table 2. degree of substitution for obtained esters

Acyl donor (R_1)	CD derivative	Solvent	Degree of substitution[1], %
$C_{17}H_{35}$	Δ	DMSO	0,11
	μν		0,16
	Δ	DMF	0,08
	μν		0,29
$C_{11}H_{23}$	Δ	DMSO	0,14
	μν		0,23
	Δ	DMF	0,27
	μν		0,33

[1] – *determined by saponification method*

There are also the significant differences between dielectric permittivity of DMSO and DMF. As the result a faster heating (heating rate) of DMSO mixtures may be observed. Participation of an acyl donor type may be negligible in this case due to its relative low polarity when compared to the solvent. Additionally the solvent molecules are the majority in the solution, so the solution dielectric permittivity is almost the solvent dielectric permittivity. The energy dissipation is mainly due to solvent dipolar losses.

Table 3. Basic physicochemical properties of solvents used

Solvent	**ε_r'**	**μ [D]**
DMSO	47,2	3,96
DMF	38,3	3,82
Water	80,4	1,85

Salmoria, G.V., Dall'Oglio, E., Zucco, C.; Tetrahedron Letters ; 1998, 39, 17, 2471-2474

The overall product yield of the transesterification process does not reach the 35% in all cases however for conventional condition is about 10% lower (see Table 1). The yield for DMF mediated reaction is in each case a little bit higher than those observed for DMSO.

Similar trends may be also observed in case of product purity or donor conversion. In case of purity some amount of undefined products with high molecular mass has been observed in DMF. The both origin of the phenomenon as well as its structure needs further investigation. For now a hypothesis may be drawn that some agglomerates of CD vinyl ester and enzyme may be arise or the radical polymerization and grafting on CD chain may take place.

Table 4. Initial rate of heating

Reaction condition	Acyl donor	DMSO	DMF
		V_h [deg/min]	
Δ	Lauric	0,86	0,79
μν		3,14	2,84
Δ	Stearic	0,84	0,81
μν		3,03	2,92

The most important factor for described reaction system is a degree of transesterification (see Table 2). In all the investigated reaction those factors are below 0,35% what means that efficiency of the process is low. The explanation of phenomenon may be the enzyme substrate configuration. Although the hydroxyl groups of CD are located on external wall of the molecule the cyclic composition of the carbohydrate may be the key factor for steric hindrance and lack of fit. The second reason may be found when focus on acyl donor molecules. Those molecules are rather nonpolar (except the some polarity of ester group). According to that the inclusion complexes between CD and vinyl ester may takes place. The phenomenon may also inhibit the transesterification processes. The higher degree of substitution observed in case of microwave enhanced processes might be explained using better fitting of substrate and enzyme by means of reorientation of molecules in high-frequency EM field. Additionally higher collision frequency and reaction mechanism may influence on the process efficiency as well. The direct energy transfer obtained using microwaves may also facilitate the crossing of energy barrier for the reation [4].

Conclusion:

As a result it might be stated that microwave induced processes show better yield and higher degree of substitution. The shortening in the reaction time was also observed and process looks promising as an alternative way for introducing acyl donors into carbohydrate molecules.

(1) Research conducted allow stating that microwave assisted enzymic transesterification of cyclodextrin can be an interesting alternative to conventional conditions. The structure of the product is heating method independent what was proved by means of FTIR spectroscopy.

(2) In all case the similar yield and substitution was observed however the shortening of the

reaction time was about 2.5 times detected in microwave assisted processes

(3) The results suggest that the novel method developed enzymatic modification of cyclodextrins will be a handy tool to functionalize these compounds

(4) In all the microwave experiments a low level of microwave irradiation was applied (90W). These allow to omitt the problem of enzyme inactivation1 that is commonly observed in case of enzymatic processes performed at μν.

Supplemental data:

Table 5. FTIR bands for obtained esters

ID	FTIR bands
CD laurate	FT-IR (cm^{-1}): 3445 (w), 2954. 2927 (s) -C-H stretching, 2864 (s) -C-H stretching), 1744 (s) -C=O stretching, 1467 (m) -CH_2 scissoring, 1378 (w) -C-H scissoring, 1352 (m) -C-H scissoring, 1252, 1248 (m), -C-O stretching, 1171 (m) -C-O stretching, 1114 (w) -C-O stretching, 1018(w) -C-O stretching, 880, 720 (w) -C-O stretching.
CD stearate	FT-IR (cm^{-1}): 3439 (w), 2927 (s) -C-H stretching, 2855 (s) -C-H stretching), 1742 (s) -C=O stretching, 1467, 1462 (m) -CH_2 scissoring, 1436 (w) -CH_2 scissoring, 1378 (w) -C-H scissoring, 1352 (m) -C-H scissoring, 1254, 1247 (m), -C-O stretching, 1191 (w) -C-O stretching, 1170 (m) -C-O stretching. 1117 (w) -C-O stretching, 1017(w) -C-O stretching, 876, 871 (w) -C-O stretching.

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