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Irina Ioannou, Eduardo Barboza, Gaëlle Willig, Thomas Marié, Andreia Texeira, et al.. Implementation of an Enzyme Membrane Reactor to Intensify the  $\alpha$ -O-Glycosylation of Resveratrol Using Cyclodextrins. Nosheen Akhtar. Updates in pharmacology, Vide Leaf, 2021, 978-93-90014-03-3. hal-03523797

**HAL Id: hal-03523797**

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Submitted on 12 Jan 2022

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## Book Chapter

# Implementation of an Enzyme Membrane Reactor to Intensify the $\alpha$ -O-Glycosylation of Resveratrol Using Cyclodextrins

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Published **August 11, 2021**

This Book Chapter is a republication of an article published by Florent Allais, et al. at Pharmaceuticals in April 2021. (Ioannou, I.; Barboza, E.; Willig, G.; Marié, T.; Texeira, A.; Darme, P.; Renault, J.-H.; Allais, F. Implementation of an Enzyme Membrane Reactor to Intensify the  $\alpha$ -O-Glycosylation of Resveratrol Using Cyclodextrins. *Pharmaceuticals* 2021, 14, 319. <https://doi.org/10.3390/ph14040319>)

**How to cite this book chapter:** Irina Ioannou, Eduardo Barboza, Gaëlle Willig, Thomas Marié, Andreia Texeira, Pierre Darme, Jean-Hugues Renault, Florent Allais. Implementation of an Enzyme Membrane Reactor to Intensify the  $\alpha$ -O-Glycosylation of Resveratrol Using Cyclodextrins. In: Nosheen Akhtar, editor. Updates in Pharmacology. Hyderabad, India: Vide Leaf. 2021.

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**Fundings:** We are grateful to the Grand Est region, the Conseil Départemental de la Marne and Grand Reims for their financial support.

**Author Contributions:** All authors have given approval to the final version of the manuscript; Acquisition and analysis data: Eduardo Barboza, Thomas Marié, Gaëlle Willig, Pierre Darme; Supervision of the work: Andreia Teixeira, Jean-Hugues Renault, Florent Allais; Writing, critical analysis of the data, reviewing the paper: Irina Ioannou, Gaëlle Willig, Jean-Hugues Renault, Florent Allais

**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abstract

The *O*-glycosylation of resveratrol increases both its solubility in water and its bioavailability while preventing its oxidation, allowing a more efficient use of this molecule as bioactive ingredient in pharmaceutical and cosmetic applications. Resveratrol *O*-glycosides can be obtained by enzymatic reactions. Recent developments have made it possible to obtain selectively resveratrol  $\alpha$ -glycosides from the  $\beta$ -cyclodextrin-resveratrol complex in water with a yield of 35%. However, this yield is limited by the partial hydrolysis of the resveratrol

glycosides produced during the reaction. In this study, we propose to intensify this enzymatic reaction by coupling the enzymatic reactor to a membrane process. Firstly, membrane screening was carried out at the laboratory scale and led to the choice of a GE polymeric membrane with a cutoff of 1 kDa. This membrane allowed the retention of 65% of the  $\beta$ -cyclodextrin-resveratrol complex in the reaction medium and the passage of 70% of the resveratrol  $\alpha$ -*O*-glycosides in the permeate. In a second step, this membrane was used in an enzymatic membrane reactor and improved the yield of the enzymatic glycosylation of up to 50%.

## Keywords

Process Intensification; Enzyme Membrane Reactor; Enzymatic *O*-Glycosylation; Cyclodextrins; Resveratrol

## Introduction

Resveratrol is a polyphenol belonging to the stilbene family and is found in some fruits such as grapes, blackberries or peanuts. It has been shown that resveratrol consumption has beneficial effects on human health. For instance, anticancer effects on pancreatic cells have been demonstrated [1]. Moreover, resveratrol has the ability to quench free radicals believed to be the initiators of neuronal degeneration related to Alzheimer disease [2]. The prevention of diabetes, kidney failure or cardiovascular disease has also been demonstrated [3]. However, some limitations in the use of resveratrol in the pharmaceutical, food and cosmetic fields had been shown [4]. Indeed, the results of *in vitro* studies have not been confirmed by those obtained *in vivo*. This can be explained by a very low bioavailability of resveratrol, which leads to a rapid elimination of the molecule in the body, as well as its low solubility in water [5]. The concentrations measured in the plasma blood are thus lower than those required to have a significant effect [6]. Nature has already solved this issue as resveratrol is mainly found in vegetal matrix in its  $\beta$ -*O*-glycoside form, also known as piceid. *O*-Glycosylation not only allows an increase in resveratrol water solubility and bioavailability, but it also prevents oxidation [7,8]. Resveratrol

*O*-glycoside derivatives can be obtained through chemical synthesis or enzymatic reaction. While chemical synthesis leads to a large amount of chemical wastes due to the need of activating group at the anomeric position as well as protection/deprotection steps, most of the enzymatic reactions previously developed - involving phosphorylase, cyclodextrin-glucanotransférase (CGTase) or glycosidase - use hazardous organic solvents such as DMSO or acetone [9]. New developments in biocatalytic glycosylation have thus been attempted to overcome these drawbacks. Recently, resveratrol  $\alpha$ -*O*-glycosides were obtained selectively from the  $\beta$ -cyclodextrin-resveratrol complex in water with a yield of 35% [10]. This optimal yield was obtained by studying, with design of experiments, the effect of five parameters (CGTase and cyclodextrin concentrations, cyclodextrin/resveratrol ratio, pH and temperature). However, enzymatic processes can suffer from substrate and/or product inhibition or adverse equilibria. For CGTase, these disadvantages are due to its capacity to catalyze four types of reaction: cyclization, coupling, disproportionation and hydrolysis [11,12]. Moreover, the transglycosylation can release some type of sugars, which can be used by the enzyme and inhibit glycoside production [12]. Research efforts have been directed to these disadvantages to some extent. Different strategies from the literature on enzymatic process intensification had been reviewed [13]. Different techniques were listed: enzyme membrane reactors, membrane contactors, cascade reactions, and sorption. These technologies can be used to intensify enzymatic processes resulting in an increase in the product yield, process productivity, enzyme stability and/or process sustainability [14]. The main limitation of the CGTase-catalyzed  $\alpha$ -*O*-glycosylation reaction is the hydrolysis of the resveratrol  $\alpha$ -*O*-glycosides during the reaction. This hydrolysis is favored over time and decreases glycosylation yield [10].

Herein, to avoid this side-reaction, in-stream resveratrol  $\alpha$ -*O*-glycosides removal by using an enzyme membrane reactor has been investigated. This strategy of intensification would enable to increase the reaction yield and improve the sustainability of  $\alpha$ -*O*-glycosylation processes. A membrane screening according to their cut-off point was first performed on a model solution. Then,

the selected membranes were implemented with the aforementioned CGTase-catalyzed  $\alpha$ -*O*-glycosylation reaction as an enzyme membrane reactor.

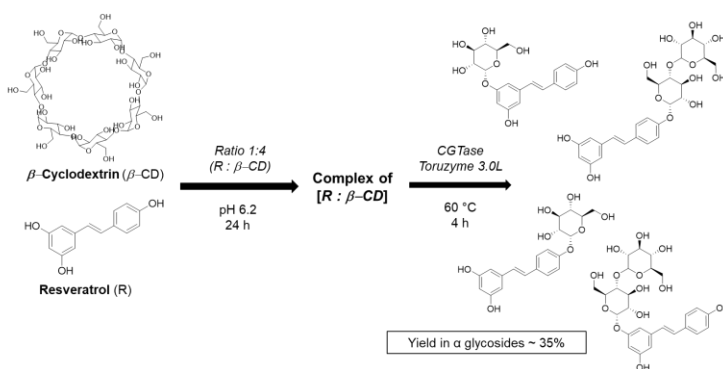
## Material and Methods

### Chemical Products

Resveratrol, piceid and  $\beta$ -cyclodextrin were purchased from Tokyo Chemical Industry Europe (TCI Europe). Salts for buffer solutions were acquired from AcrosOrganics. Methanol and acetonitrile with analytical grade were respectively purchased from Fischer Chemical and VWR. Toruzyms 3.0L was bought from Novozymes.

### Obtention of the Resveratrol $\alpha$ -glycosides from $\beta$ -cyclodextrin-Resveratrol Complex in Water

The enzymatic  $\alpha$ -*O*-glycosylation of resveratrol was carried out in MES (2-(*N*-morpholino) ethanesulfonic acid) buffer.  $\beta$ -cyclodextrin (70 mg) was mixed with 3.6 mg of resveratrol (4:1 molar ratio) in 4 mL of buffer at pH 6.2 and 80 °C with the addition of CGTase (Toruzyms 3.0L) at a concentration of 157 U/g of  $\beta$ -cyclodextrin. The yield of the enzymatic reaction obtained after 2 h is 35% (Figure 1).



**Figure 1:**  $\alpha$ -*O*-glycosylation reaction of resveratrol with  $\beta$ -cyclodextrin.

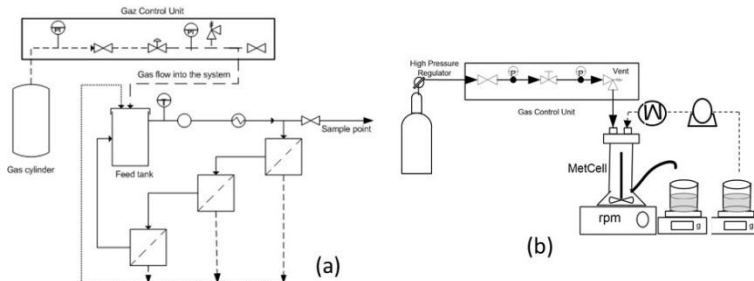
## Membrane Screening on Model Solution

### Composition of the Model Solution

The model solution to be implemented for the membrane selection is a mixture of  $\beta$ -cyclodextrin, resveratrol and piceid.  $\beta$ -cyclodextrin (20 g, 17.6 mmol), resveratrol (1 g, 4.3 mmol) and piceid (600 mg, 1.5 mmol) are dissolved in 400 mL of pH 6.2 phosphate buffer (0.05 mol/L). The solution is then heated for 24 h at 80 °C, in a flask covered with aluminum foil. After 24 h, the solution is cooled to 60 °C before being integrated into the membrane system. A 500  $\mu$ L aliquot is used for analysis by HPLC.

### The Membrane Process

Experiments at laboratory scale were performed with a METCell filtration system in dead-end and cross flow configurations with a gas control unit (Evonik Industries, Essen, Germany) (Figure 2).



**Figure 2:** METCell filtration system (a) in cross-flow and (b) in dead-end configuration.

Membranes are supported by a porous stainless-steel disc. This system allows the study of three planar membranes (for an effective area per membrane of 13 cm<sup>2</sup> and a total volume treated of 600 mL) in cross-flow configuration or with the use of a single membrane in dead-end configuration (active surface of 51.4 cm<sup>2</sup> and a treated volume of 250 mL). The liquid circulates in the system through a recirculation pump GC-M23.JF5S.6 (Micropump INC, Vancouver, WA USA), which promotes the

agitation in cross-flow configuration. In dead-end configuration the agitation is made possible thanks to a cross-head magnetic bar. This system makes it possible to implement from microfiltration to reverse osmosis. The HPLC pump, Model 306 (Gilson, Middleton, USA) allows working in diafiltration mode and not only in concentration mode or total recirculation. In all cases, the pressure is controlled by a cylinder of inert gas (N<sub>2</sub>) and can be ranged from 1 to 65 bar. The temperature is controlled by a hot plate RCT basic (IKA, Baden-Württemberg, Germany) and a cooler system Minichiller300 (Peter Huber Kältemaschinenbau AG, Offenburg, Germany). The amount of permeate is measured gravimetrically, by acquisition (precision 0.1 g) with a balance ME4001 (Mettler Toledo, Greifensee, Switzerland).

### **Screening of the Membranes**

The membrane choice was guided by both temperature resistance and cut-off thresholds. The membranes must withstand a temperature of 60 °C corresponding to the temperature of the glycosylation reaction. Membranes used in the membrane process are listed in Table 1.

The membrane selection was made considering three parameters: the retention rate of resveratrol and glycosides and/or the molar yield in glycosides.

The retention rate assesses the membrane separation efficiency for the target compound. The METcell system is used in total recirculation mode. After compacting the membranes in water at 60 °C, at maximum pressure (15 bar) and until constant permeate flux (usually 30 min), membrane permeability is assessed. Membrane water permeability is determined with ultrapure water at 5 pressures (15, 12, 10, 8 and 6 bars). Tests start with the highest pressure up to the lowest pressure, with a stabilization time of 10 min between each pressure. Membrane water permeability is evaluated before and after the filtration with the solution in order to detect fouling. Each test is realized with a new membrane coupon.



**Table 1:** Characteristics of the used membranes.

<i>Supplier</i>	<i>Type</i>	<i>Membrane</i>	<i>Material</i>	<i>Cut_off threshold (Da)</i>	<i>Maximum temperature and pressure</i>	<i>pH</i>
General Electrics	UF	<i>GE</i>	Proprietary thin film	900	70 °C / 40 b	1-11
		<i>GH</i>		1400	70 °C / 27 b	
		<i>PT</i>	Polyethersulfone / polysulfone	5000	70 °C / 10 b	
Microdyn Nadir	NF	NP030	PES	400	95 °C / 40 b	0-14
Hydranautics - Nitto	NF	HYDRACoRe 70 pHT	Sulfonated polyethersulfone	720	60 °C / 41 b	2-11

After compacting and evaluating water permeability, the water is removed from the tank and the model solution containing resveratrol,  $\beta$ -cyclodextrin and piceid is placed in the system at 60 °C. The pressure is then adjusted to the maximum pressure used in the membrane test. The system is then tested at 5 pressures; a stabilization of 10 minutes being performed between each pressure. At each pressure, the permeate flow rate is measured to determine the permeability of the solution. An HPLC sample of 1 mL is taken in the retentate and in the permeate to measure the concentration of resveratrol and piceid, and thus to determine the retention rates as in equation 1.

$$R = 1 - \frac{C_p}{C_a} \quad (1)$$

Where R is the retention rate,  $C_p$  the concentration of the compound in the permeate and  $C_a$  the concentration of the compound in the retentate.

The molar yield is calculated when only the permeate can be monitored over time (dead-end configuration). The molar yield of  $\alpha$ -*O*-glycosides (Y) is given by equation 2.

$$Y = \frac{N_{(glycosides)t}}{N_{(resveratrol)_0}} \times 100 \quad (2)$$

Where  $N_{(glycosides)t}$  represents the quantity of  $\alpha$ -*O*-glycosides (mol) at a t time of the reaction and  $N_{(resveratrol)_0}$  the initial quantity of resveratrol (mol).

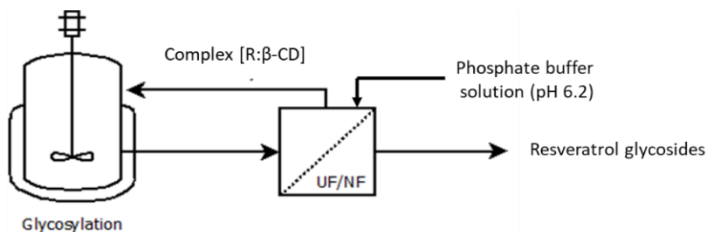
Membrane permeability ( $L_p$ ) was defined according to equation 3.

$$J_p = L_p \times TMP \quad (3)$$

Where  $J_p$  ( $L \cdot h^{-1} \cdot m^{-2}$ ) and TMP (bar) represent the permeate flux and the transmembrane pressure, respectively. The permeate flux, corresponding to the permeate flow rate per unit area of the membrane; is then determined for 5 pressures. A straight line is obtained by plotting  $J_p$  according to the transmembrane pressure.  $L_p$  corresponds to the slope of this straight line.

## Enzyme Membrane Reactor

The enzyme membrane reactor (Figure 3) used to intensify the  $\alpha$ -*O*-glycosylation of the resveratrol consists in a stirred tank in which enzymatic  $\alpha$ -*O*-glycosylation is coupled to a membrane process allowing the passage of small molecules (resveratrol  $\alpha$ -*O*-glycosides).



**Figure 3:** Enzyme membrane reactor used to intensify resveratrol glycosylation.

In this case, the system is configured in a recirculation mode as the objective is to remove the glycosides from the reaction medium containing the  $\beta$ -cyclodextrin-resveratrol complex in order to intensify the enzymatic  $\alpha$ -*O*-glycosylation. The permeate is isolated and a phosphate buffer solution (pH 6.2), placed in a diafiltration tank, is added to the reaction medium. A level probe coupled to a regulation loop makes it possible to maintain a constant filtration volume. The membranes tested are those chosen in the screening part. The membrane process is the METCell system used in crossflow configuration at 6 bar. Regular samples of 1 mL were taken in the permeate stream and in the retentate to determine the retention rates and the process yield.

## HPLC Analysis

The contents of resveratrol and  $\alpha$ -*O*-glycosides of resveratrol were determined by HPLC on a Dionex Ultimate 3000 system (Dionex Corporation, Sunnyvale, USA). The HPLC system and the method have already been well described [10].

## Complexation Simulations

The coordinates for the  $\beta$ -cyclodextrin were obtained from the X-ray crystal structure of a  $\beta$ -amylase/ $\beta$ -cyclodextrin complex (Protein DataBank code: 1BFN, resolution of 2.07 Å). Optimized  $\beta$ -cyclodextrin, resveratrol and piceid structures were obtained after conformational optimization with the open-source molecular builder and visualization tool Avogadro 1.2.0 (<http://avogadro.cc/>). The MGL Tools 1.5.6 with AutoGrid 4 and AutoDock 4.2 were used to set up and perform docking calculations between resveratrol and piceid as flexible guests, while  $\beta$ -cyclodextrin was used as rigid host. Initially, hydrogen atoms were added into the structure. Then, the partial atomic charges for both resveratrol and piceid as well as for the  $\beta$ -cyclodextrin were calculated using the Gasteiger-Marsili and Kollman methods, respectively. A grid box of the size of the  $\beta$ -cyclodextrin was generated using AutoGrid 4 and the ligands were then docked into the  $\beta$ -cyclodextrin cavity by AutoDock 4.2 with the following parameters: run number = 100 and search parameters = lamarkian genetic algorithm. All the other parameters were the default values. The docking analysis was performed using AutoDockTools by combing the docking pose populations and the corresponding binding energies.

## Results

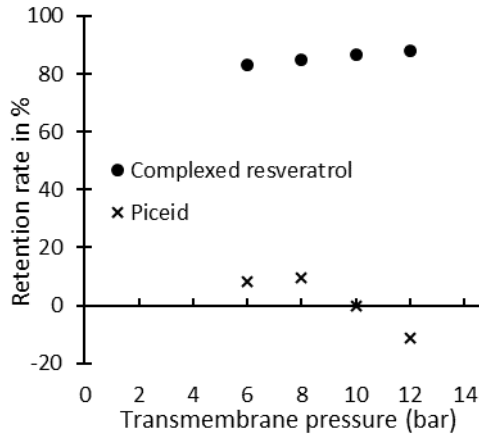
The intensification of the enzymatic reaction consists in two steps: (i) the choice of the membrane allowing the best separation between the reaction medium and the  $\alpha$ -*O*-glycosides, and (ii) the implementation of the enzyme membrane reactor. The first step was conducted on a model solution containing commercially available natural 3-*O*- $\beta$ -D glycoside of resveratrol (i.e., piceid) whereas the second one used a real medium.

### Screening of Membranes on Model Solution

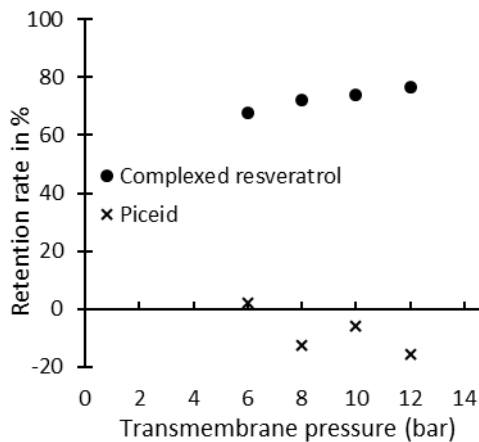
#### Proof-of-Concept of the Selective Filtration

The proof-of-concept of the selective filtration of a  $\beta$ -cyclodextrin-resveratrol inclusive complex (named *complexed*

*resveratrol*) and piceid solutions was demonstrated by using two membranes GE and GH whose cut-offs are 0.9 and 1.4 kDa, respectively. The retention rates as a function of the transmembrane pressure are shown in Figure 4.



(a)



(b)

**Figure 4:** Retention rates according to the transmembrane pressure for (a) GE and (b) GH membranes.

As expected, due to the molar mass difference (390 g/mol for the piceid and 1328 g/mol for the complexed resveratrol), low retention rates (8.6% for GE and 2.2% for GH, at 6 bar transmembrane pressure) were obtained for the piceid solution while high retention levels (83.2% for GE and 67.6% for GH, at 6 bar transmembrane pressure) were obtained for the complexed resveratrol. The negative values obtained can be explained by the rapid elimination of piceid in the permeate. Negative values have already been observed in the literature [15,16].

### Membrane Screening on Model Solution

Membrane screening was then performed on a *model solution* composed of  $\beta$ -cyclodextrin (20 g, 17.6 mmol), resveratrol (1 g, 4.3 mmol), and piceid (0.6 g, 1.5 mmol) dissolved in 400 mL of phosphate buffer. A 4/1  $\beta$ -cyclodextrin/resveratrol ratio was chosen as it was the ratio used for the reaction in the batch mode in Marié et al.'s procedure [10]. Cut-off thresholds ranging from 0.4 to 5 kDa were tested. Table 2 indicates the retention rates for the piceid for the different tested membranes.

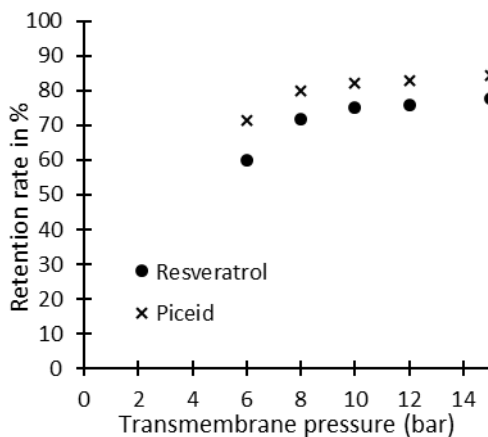
**Table 2:** Piceid retention rates according to the cut-off thresholds of the membranes.

Supplier	Microdyn Nadir	Hydranautics - Nitto	GE	GH	PT
Membrane cut-off threshold (kDa)	0.4	0.72	0.9	1.4	5
Piceid retention rates (%)	> 90	> 90	71.4 (6 bar)	89.1 (6 bar)	56.4 (6 bar)

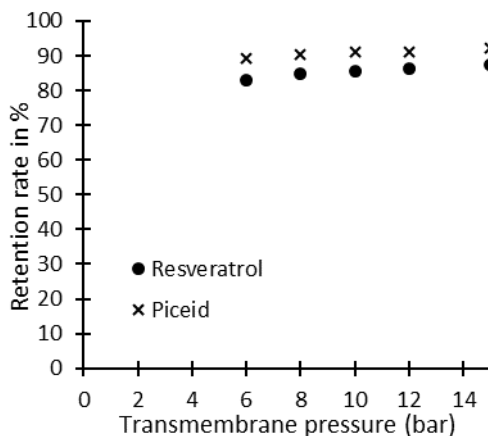
In these conditions -  $\beta$ -cyclodextrin in excess, 6 bar transmembrane pressure - the retention rates obtained for the piceid are high whatever the membrane cut-off threshold. With a GE membrane, a retention rate of the piceid with no free cyclodextrin in the solution (*vide supra*) was equal to 8.6% whereas a value of 71.4% was obtained with this model solution when  $\beta$ -cyclodextrin is available for piceid complexation. At this stage, one can conclude that two different inclusion complexes

can be formed: cyclodextrin-resveratrol and cyclodextrin-piceid. For the next steps of our study, the membranes with the lowest piceid retention rates (*i.e.* the highest cut-off threshold) are chosen (GE, GH and PT) as they could provide a highest selectivity between the  $\beta$ -cyclodextrin-resveratrol inclusive complex and the free piceid.

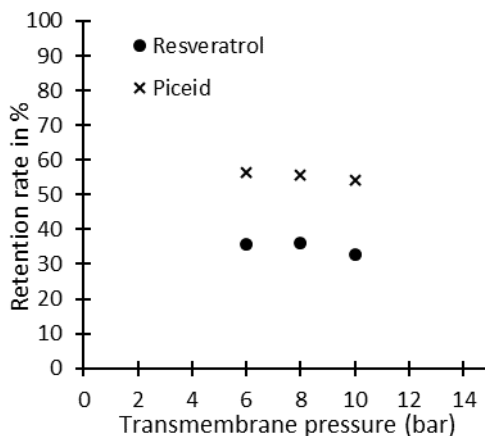
Figure 5 shows the evolution of the retention rates of resveratrol and piceid as a function of the transmembrane pressure.



(a)



(b)



(c)

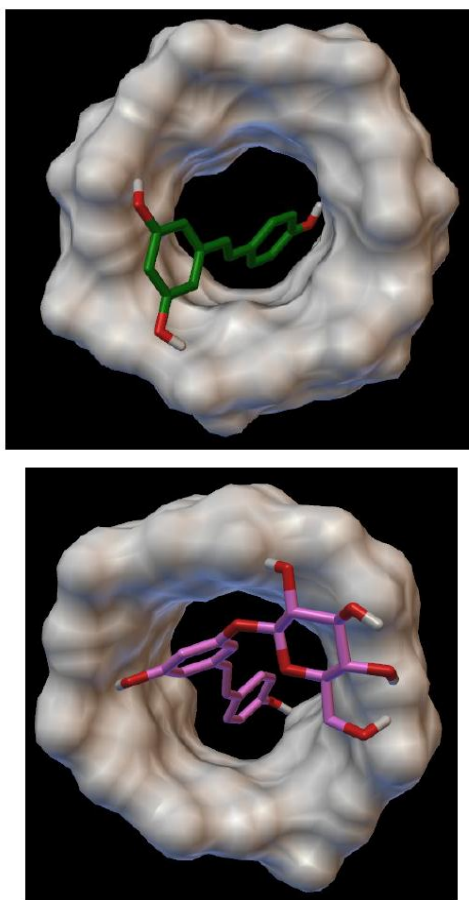
**Figure 5:** Retention rates for complexed resveratrol and piceid with regards to the transmembrane pressure for (a) GE, (b) GH and (c) PT membranes.

Piceid retention rates are higher than those of resveratrol – which is under its inclusion complex form in these conditions - whatever the membrane and the selectivity, confirming that piceid was also under an inclusion complex form. Modeling experiments by using the Autodock4 docking program have confirmed that both resveratrol and piceid can be complexed into the  $\beta$ -cyclodextrin cavity through their monophenolic aromatic cycle. Figure 6 shows that the glucose moiety of piceid remained outside the  $\beta$ -cyclodextrin cavity, thus increasing the size of the inclusion complex.

Two information can be deduced from these preliminary experiments. Firstly, the model solution led to the formation of an inclusion complex between piceid and  $\beta$ -cyclodextrin, thus preventing the selective retention of resveratrol and the transfer of its *O*-glycosylated form (i.e., piceid) in the permeate. Thus, to eliminate this problem in the case of an enzymatic  $\alpha$ -*O*-glycosylation carried out under real conditions, the  $\alpha$ -*O*-glycosides of resveratrol synthesized must be continuously removed from the permeate during the reaction. Secondly, the PT membrane has a too high cut-off threshold and did not



provide enough retention of the  $\beta$ -cyclodextrin-resveratrol inclusion complex. This membrane will therefore not be implemented in the enzyme membrane reactor.



**Figure 6:** Optimal complex geometries in the case of resveratrol (left, binding energy =  $-5.32 \text{ kcal.mol}^{-1}$ ) and piceid (right, binding energy =  $-5.24 \text{ kcal.mol}^{-1}$ ).

### Determination of the Membrane Permeabilities

The effect of the filtration of the model solution on membrane water permeabilities was then studied for the GE and GH membranes. Table 3 shows the values of  $L_p$  calculated.

**Table 3:** Membrane water permeability ( $L_p$ ), at 60°C, before and after filtration.

$L_p$ ( $L.h^{-1}.m^{-2}.bar^{-1}$ )	Before filtration	After filtration	Evolution
GE	7.34	5.65	- 23%
GH	6.73	4.64	- 30 %

The water permeability of the GE membrane obtained before filtration is higher than the manufacturer's specification ( $1.21 L.h^{-1}.m^{-2}.bar^{-1}$ ) and the data in the literature (at 25 °C,  $1.88 \pm 0.088 L.h^{-1}.m^{-2}.bar^{-1}$ ) [15], especially because of the temperature used in this study. An increase in temperature leads to a decrease in the dynamic viscosity of water and therefore an increase in permeability, according to Darcy's law. The temperature can also cause the pores to deform.

The same reflection can be applied to the GH membrane. The manufacturer's specification for the water permeability of the GH membrane is  $3.30 L.h^{-1}.m^{-2}.bar^{-1}$  and the data developed in the scientific literature are at 25 °C,  $3.22 \pm 0.17 L.h^{-1}.m^{-2}.bar^{-1}$ ; at 23 °C,  $2.7 \pm 0.5 L.h^{-1}.m^{-2}.bar^{-1}$ ; at 30 °C,  $3.8 L.h^{-1}.m^{-2}.bar^{-1}$  [17-19]. According to the manufacturer's specifications, the permeability of the GE membrane should be lower than that of GH, which is contrary to the data obtained. The composition of each of these membranes is not specified by the manufacturer, so temperature can affect these two membranes differently.

A significant decrease in permeability is noticed for the two membranes (30% and 23% for the GH and GE membranes, respectively), suggesting a phenomenon of membrane fouling non-investigated in the framework of this study. Nevertheless, the permeate flow remained above 70% of its initial value, which is acceptable to continue the study.

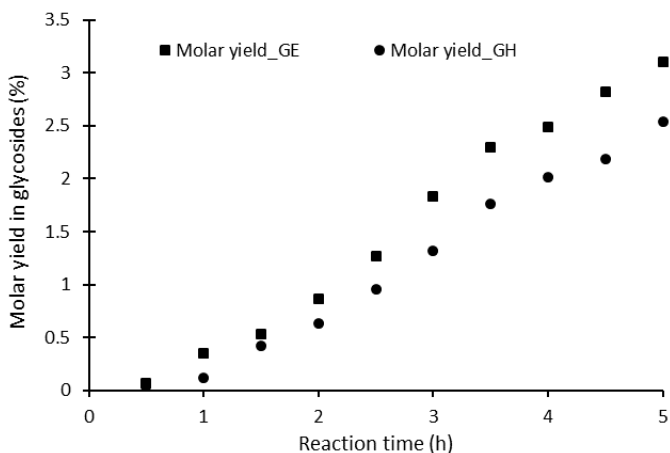
### Implementation of the Enzyme Membrane Reactor for the Resveratrol Glycosylation

The problem of selectivity between resveratrol and its  $\alpha$ -O-glycosylated forms could be avoided if the complexation kinetics

are sufficiently slow to promote the transfer of free  $\alpha$ -*O*-glycosylated forms across the membrane. The hyphenation between the unit operations of enzymatic *O*-glycosylation and membrane filtration has therefore been implemented. The dead-end and cross-flow configurations were both tested.

### Membrane Separation in Dead-End Configuration

The molar yield of the total glycoside forms in the permeate was monitored by quantitative HPLC during the course of the  $\alpha$ -*O*-glycosylation reaction (Figure 7).



**Figure 7:** Molar yield of the  $\alpha$ -*O*-glycoside of resveratrol in the permeate during the  $\alpha$ -*O*-glycosylation reaction with the GE and GH membranes.

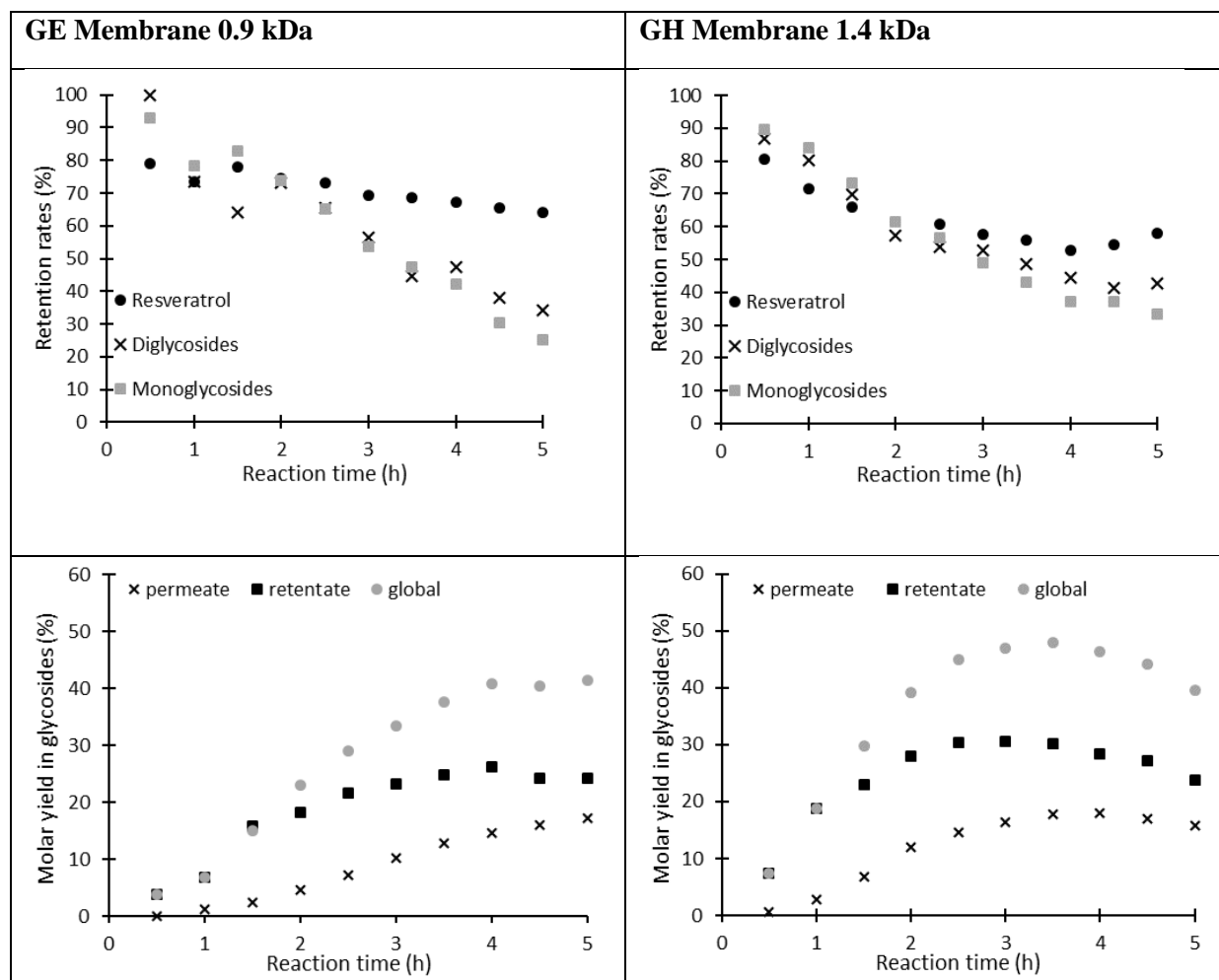
Low glycoside yields were obtained in the permeate, respectively 3.1% for the GE membrane and 2.5% for the GH using the dead-end configuration for the membrane filtration system. In addition, by measuring the  $\alpha$ -*O*-glycosides of resveratrol produced in the retentate, the overall yield of the  $\alpha$ -*O*-glycosylation reaction was also measured. After a reaction time of 5 hours, the overall  $\alpha$ -*O*-glycosides yields were 19.4% and 16.9% for the GE and GH membranes, respectively, whereas a global yield of 35% was previously reached in batch [10]. In conclusion, the dead-end configuration allowed the coupling between the two technologies but did not make it possible to

optimize the  $\alpha$ -*O*-glycosylation yield within a reasonable reaction time.

### **Membrane Separation in a Cross-Flow Configuration**

The cross-flow configuration was then evaluated. Figure 8 presents both the retention rates of the resveratrol and its different  $\alpha$ -*O*-glycosides and the molar yield of the total  $\alpha$ -*O*-glycoside forms in the permeate for the GE and GH membranes (0.9 and 1.4 kDa).

As highlighted in Figure 8, no selectivity between the resveratrol and its  $\alpha$ -*O*-glycosides was observed until a reaction time of two hours, this being due to the formation of inclusion complexes (vide supra) when the cyclodextrin was in a large excess compared to the  $\alpha$ -*O*-glycosides. After approximately 2.5 hours, the resveratrol retention rate stabilizes at 65% with the GE membrane and 55% with the GH one, while the  $\alpha$ -*O*-glycoside retention rates continued to decrease reaching 30% and 40% for the GE and the GH membrane, respectively, after 5 hours. Moreover, as expected, resveratrol glycoside removal as soon as they are produced by using membrane filtration led to an increase in the reaction yield by limiting the hydrolysis phenomenon after 4 h of reaction time [10]. Compared to the 35% yield obtained for the  $\alpha$ -*O*-glycosylation reaction without hyphenation with the membrane filtration unit operation, cross-flow configuration with the GE membrane resulted in a 40% yield after 4 h while a 50% yield was achieved after 3.5 h with the GH membrane. To summarize, intensification of the CGTase-mediated enzymatic  $\alpha$ -*O*-glycosylation of resveratrol through its coupling to the membrane process in cross-flow mode allows an increase of 15 units of the global yield of the reaction (from 35% to 50%).



**Figure 8:** Molar yields in glycosides and retention rates obtained during the coupling of the extractive reaction with GE and GH membranes.

## Conclusion

The main objective of the project was to improve the performances of the enzymatic  $\alpha$ -*O*-glycosylation of resveratrol by coupling the biocatalytic reaction with a membrane process in order to isolate the  $\alpha$ -*O*-glycosides of resveratrol and prevent their hydrolysis. After having conducted a membrane screening, two polymeric membranes were chosen (0.9 (GE) and 1.4 kDa (GH)) and then tested in both dead-end and cross-flow ultrafiltration configurations. Compared to the classic batch  $\alpha$ -*O*-glycosylation of resveratrol in presence of  $\beta$ -cyclodextrin and CGTase in water (35% yield), the coupling of the reaction to an ultrafiltration process enabled the increase of the reaction yield between 5 and 15%, depending on the membrane used, with a reaction time inferior to 5 h. The membrane of 0.9 kDa (GE) led to a stable yield of 40% after 4h of reaction whereas the membrane of 1.4 kDa (GH) resulted in a maximum yield of 50% at 3.5 h of reaction. The proof-of-concept of selective removal of  $\alpha$ -*O*-glycosides of resveratrol to prevent subsequent hydrolysis reported in this study provides interesting improvements of the  $\alpha$ -*O*-glycosylation of resveratrol as yield increases while reaction time decreases. An optimization step of the operating conditions would allow to further improve this integrated process.

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