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Preparation of Polymeric Membranes Entrapping β-Cyclodextrins and Their Molecular Recognition of Naringin

E. FONTANANOVA*, A. BASILE, A. CASSANO and E. DRIOLI
Institute on Membrane Technology, ITM-CNR, c/o University of Calabria, Via P. Bucci, cubo 17/C, 87030 Rende (CS), Italy

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Abstract

An amorphous polyetheretherketone known as PEEK-WC and a β-cyclodextrin derivative (O-octyloxycarbonyl-β-CD), insoluble in water, were used to prepare flat sheet membranes by the diffusion induced phase separation. It was found that the β-CD derivative entrapped in membranes is able to form a complex with naringin, a bitter component present in grapefruits. The recognition properties of the β-CD derivative immobilized in the PEEK-WC membranes towards naringin have been investigated as a function of the amount of β-CD derivative present in the casting solution. A significant improvement of the efficiency of the O-octyloxycarbonyl-β-CD was observed when an inclusion complex with naringin in the membrane was formed compared to when the β-CD derivative is dispersed in an aqueous solution of naringin. Washing with an alkaline solution allows an easy regeneration of the membranes and permits their reuse. No recognition properties were found for PEEK-WC membranes made without the β-CD derivative.

Introduction

Excessive bitter taste reduces the quality of commercial fruit juices. For this reason the development of new methods to remove the bitter components at the lowest possible cost, while maintaining organoleptic quality and stability of the finished product, is an important industrial goal.

Limonin (limonoic acid 3,19:16,17 dilactone) and naringin (4′,5,7-trihydroxyflavanone-7-rhamnoglucoside) are bitter constituents of citrus fruits. In particular, limonin is the main bitter component of the orange juice [1], while naringin is dominant in grapefruits [2].

Several attempts have been made to decrease the concentration of bitter compounds in citrus juice including treatment of the juice by bacteria [3], enzymatic and chemical methods [4, 5], molecular imprinted polymeric membranes [6] and β-cyclodextrin polymers [7, 8].

β-cyclodextrins (β-CDs) are cyclic oligomers composed of seven α-1,4-linked-D-glucopyranose units (Figure 1). The main feature of β-cyclodextrin is a hydrophobic cavity (0.62–0.78 nm), which enables these molecules to form inclusion complexes with some organic compounds containing hydrophobic groups able to fit their cavity [9].

Previous studies have shown that a β-cyclodextrin polymer, synthesized using epichlorohydrin as cross-linking agent, is effective to remove limonin and naringin in orange and grapefruit juice [7, 8].

In this work we have studied the recognition properties of O-octyloxycarbonyl-β-CD derivative immobilised in polymeric membranes towards naringin (Figure 2).

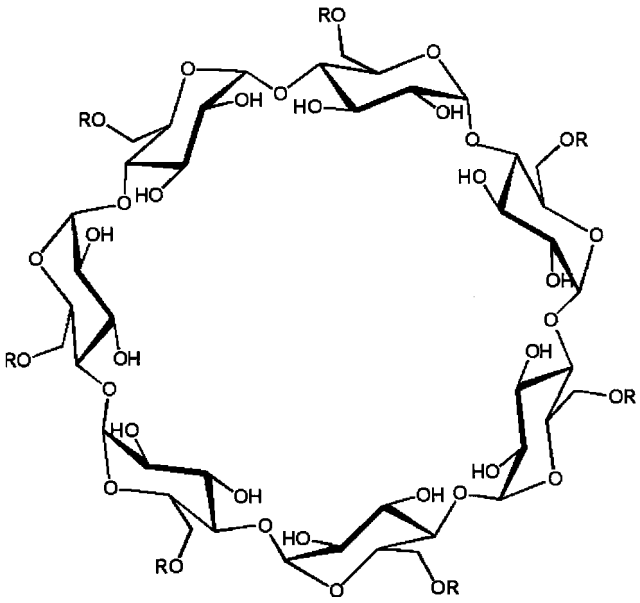

Figure 1. Structure of the O-octyloxycarbonyl-β-cyclodextrin, R = $COO(CH_2)_7\ CH_3$.

A modified polyetheretherketone, known as PEEK-WC (Figure 3), was used for preparing the membranes. This polymer has a high thermal, chemical and mechanical stability. Moreover, unlike PEEK, it is amorphous and soluble in various organic solvents and is suitable for preparing membranes by the phase inversion technique [10]. Previous studies of molecular dynamics simulation performed on the β-CD derivative included in PEEK-WC membranes have shown that the β-CD derivative is slightly distorted but this

* Author for correspondence. E-mail: efontananova@itm.cnr.it

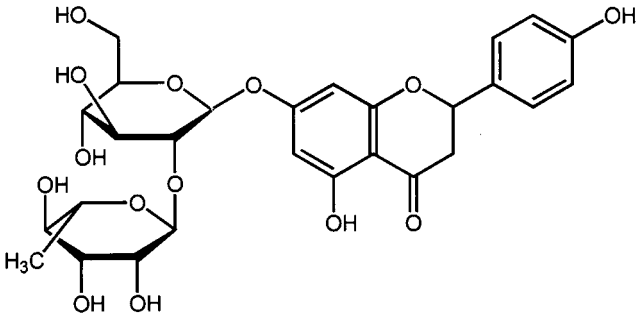

Figure 2. Structure of the naringin.

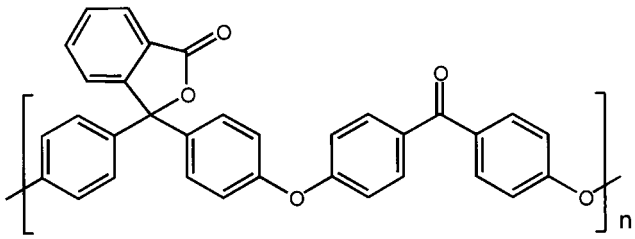

Figure 3. Structure of the repeat unit of the PEEK-WC.

effect does not modify the possibility of the inclusion of guest molecules. More important seems to be the disposition of the O-octyloxycarbonyl groups that are immersed into the polymer matrix randomly, by non bonding interactions with the polymer. In some cases the groups can be inserted into hydrophobic cavity of β-CDs obstructing the guest inclusion [11].

Experimental

All the solvents and compounds used for preparing both β-cyclodextrin derivative and membranes were from Sigma-Aldrich and were used without further purification. UV-VIS spectra were recorded on a Perkin Elmer Lambda EZ201 spectrophotometer. Scanning electron microscopy (SEM) images were obtained using a Cambridge Instruments Stereoscan 360. The casting knife was supplied by Braive Instruments.

PEEK-WC was supplied by the Chanchung Institute of Applied Chemistry, Academia Sinica. The polymer was Soxhlet extracted with methanol and then dried in a vacuum oven before its use.

O-octyloxycarbonyl-β-cyclodextrin (average degree of substitution = 7) was synthesized according to the method reported in literature [12] with minor modifications.

The flat sheet membranes were prepared following the diffusion induced phase separation technique [13]. The membranes were prepared from PEEK-WC using N,N-dimethylformamide (DMF) as solvent and distillated water as non solvent. A solution (15 wt%) of PEEK-WC was prepared dissolving the polymer in DMF by magnetic stirring overnight at room temperature to allow complete solubilization. The solution was knife cast on a glass plate by setting the knife gap at 250 μm. The cast film was then immersed in a coagulation bath containing distilled water at room temperature. The membranes containing O-octyloxycarbonyl-β-cyclodextrin were prepared following the same methodology by adding the β-CD derivative to a homogeneous solution of PEEK-WC in DMF (Table 1). The O-octyloxycarbonyl-β-CD derivative, insoluble in water, was used instead of β-CD to avoid β-CD loss during the membrane formation process [14]. Before and after their use, all membranes were stored in distilled water.

Table 1. Composition of the casting solutions of the PEEK-WC membranes prepared with and without O-oct.-β-CD derivative

Membranes	PEEK-WC (wt%)	O-oct.-β-cyclodextrin (wt%)	DMF (wt%)
M-PWC	15.0	–	85.0
M-BCD-1	15.0	2.5	82.5
M-BCD-2	15.0	5.0	80.0
M-BCD-3	15.0	7.5	77.5

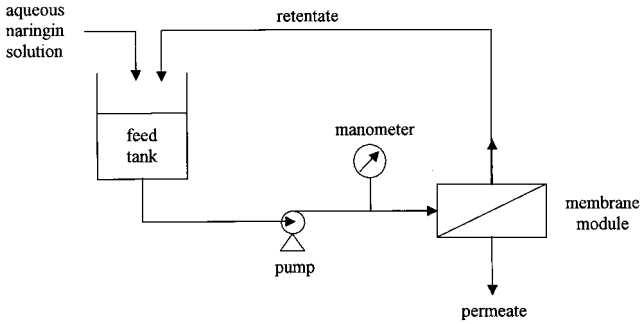

Figure 4. Flow sheet of the membrane separation system.

Membrane testing was performed using a laboratory equipment (Figure 4) constituted by a feed tank with a capacity of 2 liters, a centrifugal pump, a manometer for measuring the inlet pressure and a flat-sheet membrane cell realised in our laboratory. An aqueous solution of naringin (7.98 μmol/l) passed through the flat membrane disks having a surface area of 41.6 cm^2 at a transmembrane pressure (TMP) of 150 mmHg, at 25 °C, in a continuous process. The naringin content of permeate and feed samples, collected at regular intervals, was determined by UV absorbance at 280 nm (λ_{max}).

Results and discussion

The surfaces and the cross-sections of the membranes prepared by the phase inversion technique were examined by scanning electron microscopy (SEM). The SEM images showed an asymmetric structure for all membranes.

The PEEK-WC membrane (M-PWC, see Table 1) has an asymmetric structure (Figure 5) characterised by a thin dense skin layer (thickness = 1 μm) supported by a thicker (thickness = 48 μm) porous sponge-like substructure (Figure 6). The membranes containing the β-CD derivative are also asymmetric. In particular, the PEEK-WC membrane prepared from the casting solution containing 5.0 wt% of O-oct.- β-CD (M-BCD-2) is composed of a porous skin layer (average pore radius = 0.1 μm) and a porous substruc-

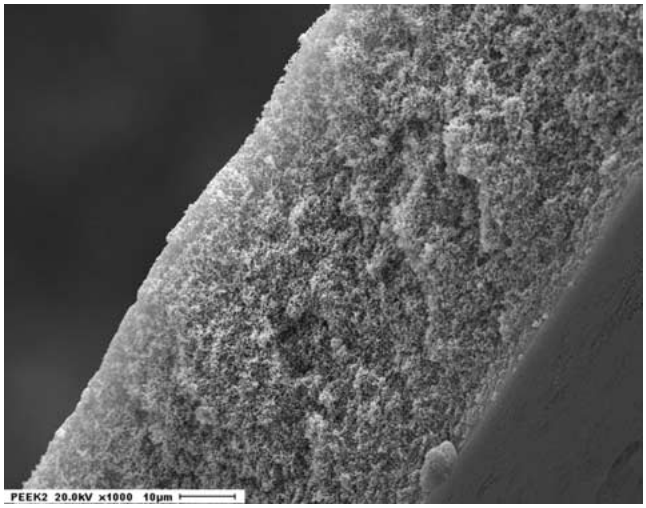

Figure 5. Cross-section (1000×) of the PEEK-WC membrane (M-PWC).

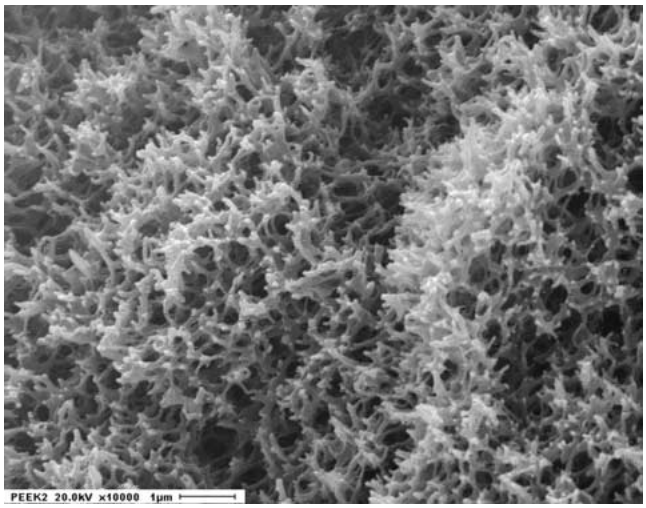

Figure 6. Particular of the cross-section (10000×) of the PEEK-WC membrane (M-PWC).

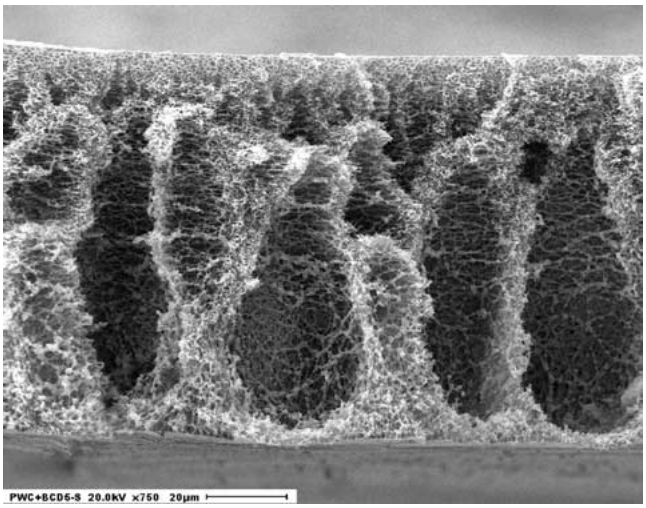

Figure 7. Cross-section (750×) of the PEEK-WC membrane prepared from the casting solution containing 5.0 wt% of O-oct.- β -CD (M-BCD-2).

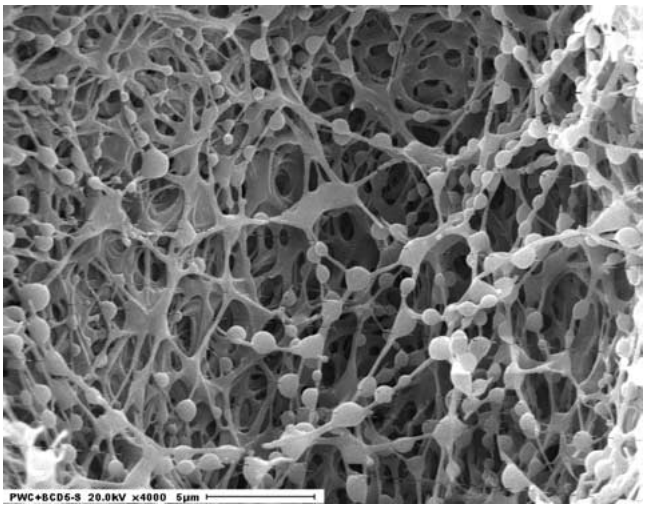

Figure 8. Particular of the cross-section (4000×) of the PEEK-WC membrane prepared from the casting solution containing 5.0 wt% of O-oct.-β-CD (M-BCD-2).

ture, characterized by a drop shaped and finger-like structure (Figures 7, 8).

A correlation between the membrane structure and the precipitation rate has been found. Systems with rapid precipitation rate tend to form a finger-like structure whereas systems with slow precipitation rate result in a sponge type-structure. The phase separation process has to be accelerated by addition of the hydrophobic β-CD derivative that encourages the polymer precipitation during the membrane formation. These results are in agreement with the phase inversion process analysis of Strathmann *et al.* [15].

Figure 9 shows the water flux as a function of the transmembrane pressure. Membranes obtained from casting solutions with greater amount of β-CD derivatives exhibit higher water fluxes. These results are in agreement with the SEM observation concerning a more open structure of the membranes when the β-CD derivative is present in the casting solution (Figures 6, 8).

The β-CD derivative, entrapped in polymeric membrane, maintains the recognition properties of β-CD and it is able to form an inclusion complex with naringin. When an aqueous solution of naringin is passed through the membranes entrapping O-octyloxycarbonyl-β-CD, a lower naringin concentration in permeate samples with respect to the initial feed, is observed.

The retained amount of naringin can be expressed by the following equation [16]:

$$Q = \sum_i (C_0 - C_i) V_i / W,$$

where Q is the amount of naringin retained into the membrane (μmol/g_{membrane}), C_0 is the initial naringin concentration in the feed (μmol/l), C_i is the concentration in the permeate (μmol/l), i is the i-th permeate sample ($i = 1, \ldots, 60$), V_i is the volume of the permeate i (l) and W is the weight of the dry membrane used (g). The β-CD derivative entrapped in PEEK-WC membranes (M-β-CD) interacts with naringin (N) and forms an inclusion complex (M-β-CDN). The inclusion reaction can be expressed as:

$$\text{M-}\beta\text{-CD} + \text{N} \rightleftarrows \text{M-}\beta\text{-CDN}$$

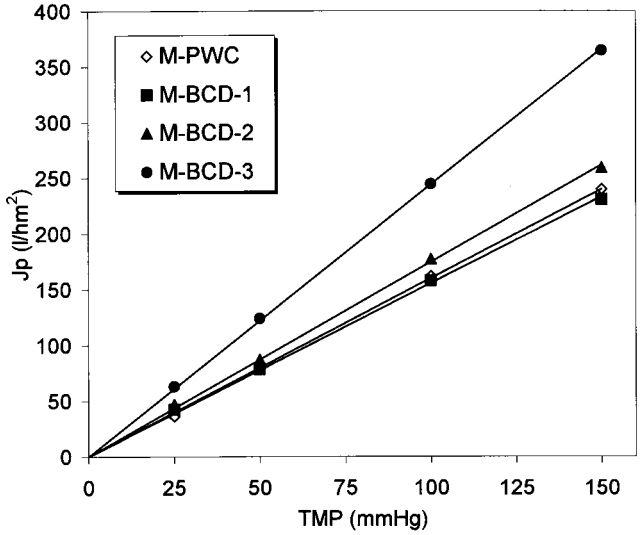

Figure 9. Water flux (Jp) versus the transmembrane pressure (TMP) at 25 °C of the PEEK-WC membranes containing different amount of O-oct.-β -CD (M-BCD-1; M-BCD-2; M-BCD-3) and of the PEEK-WC membrane made without β-CD derivative (M-PWC).

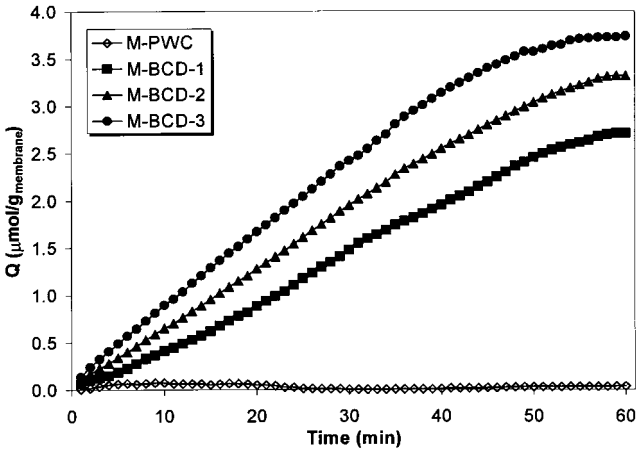

Figure 10. Retained amount of naringin (Q) as a function of time into the PEEK-WC membranes containing different amount of O-oct.- β -CD (M-BCD-1; M-BCD-2; M-BCD-3) and into the PEEK-WC membrane made without β-CD derivative (M-PWC).

The fraction of saturation of the β-CD derivative is defined as:

$$Y\% = \frac{N_{M\text{-}\beta\text{-}CDN}}{N_{M\text{-}\beta\text{-}CDN} + N_{M\text{-}\beta\text{-}CD}} * 100,$$

where $N_{M\text{-}\beta\text{-}CDN}$ are the moles of the host/guest complex and $N_{M\text{-}\beta\text{-}CD}$ are the moles of free β-CD in the membrane in condition of saturation of the membrane itself.

Figure 10 and Table 2 show that naringin is effectively removed from the aqueous solution by flowing the solution through membranes containing the β-CD derivative. Moreover, a greater amount of β-CD derivative entrapped in polymeric membrane leads to an increase in the retained amount of naringin.

The saturation of the membranes occurs, in our experimental conditions, after about 60 minutes. Saturated membranes can be restored, without loss of binding capacity, by washing them with a 0.01 N aqueous NaOH solution until no naringin is detected by UV analysis in the permeate samples. In Figure 10 it is also possible to observe that no recognition properties were detected for PEEK-WC membranes made without the β-CD derivative.

As shown in Table 2, under the reported operating conditions, only 2.4% of the β-CD sites show effective molecular recognition properties in M-BCD-1; 1.7% in M-BCD-2 and 1.4% in M-BCD-3. This behaviour can be explained assuming that most of the β-CD derivative is present in the bulk of the membranes, and increasing the amount of the β-CD derivative entrapped in the membrane, increases the number of the binding sites that are inaccessible or not correctly oriented. Nevertheless, the results obtained are very competitive with respect to other systems such as molecular imprinted polymeric membranes [6] where only 0.127 μmol of naringin for gram of membrane are retained, and only 0.03% of binding sites show effective molecular recognition properties.

Table 2. Retained amount naringin (Q) and fraction of saturation (Y%) of the membranes

Membrane	Q (μmol/g membrane)	Y%
M-PWC	0	–
M-BCD-1	2.71	2.4
M-BCD-2	3.32	1.7
M-BCD-3	3.74	1.4

An improvement of the process of naringin removal by using tubular membranes containing β-CD derivative instead of flat sheet membranes can be predicted, considering an easy membrane cleaning and a high packing density for these systems [17].

Experiments were also carried out in order to determine the capacity of the O-octyloxycarbonyl-β-CD derivative dispersed in an aqueous solution to form inclusion complexes with naringin. O-octyloxycarbonyl-β-CD derivative was dispersed in an aqueous solution of naringin under magnetic stirring at 25 °C for 5 hours. The solution was then centrifuged and the concentration of naringin in the solution was determinated by UV analysis. In these conditions only 0.01% molecules of β-CD derivative form an inclusion complex with naringin. This low capacity of the β-CD derivative to form a host/guest complex with naringin is probably due to the preferential arrangement of the hydrophobic O-octyloxycarbonyl groups into the hydrophobic cavity of the β-CD in aqueous solution. This process prevents the naringin inclusion in the cavity. On the other hand, the entrapment of the β-CD derivative in the polymeric membranes optimises the interaction with naringin and increases the stability of the host/guest complex.

Conclusions

In this work we have shown that the O-octyloxycarbonyl-β-CD derivative entrapped in polymeric membrane both recognizes naringin and retains it. It was also found that a greater amount of β-CD immobilised in the membrane leads to an increase in the retained amount of naringin. Entrapment of the β-cyclodextrin in membrane optimises the interaction with naringin, increases the stability of the host/guest complex β-CD/naringin and allows an easy regeneration of the system.

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