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Development of Active Polyvinyl Alcohol/β-Cyclodextrin Composites To Scavenge Undesirable Food Components

Carol López-de-Dicastillo,[†] María Jordá,[‡] Ramón Catalá,[†] Rafael Gavara,[*,†] and Pilar Hernández-Muñoz[†]

[†] Packaging Laboratory, Instituto de Agroquímica y tecnología de Alimentos, IATA-CSIC, Avenida Agustín Escardino 7, 46980 Paterna, Spain

[‡] ITENE, Parque Tecnológico, c/Albert Einstein 1, 46980 Paterna, Spain

ABSTRACT: Active food packaging systems based on the incorporation of agents into polymeric package walls are being designed to purposely release or retain compounds to maintain or even increase food quality. The objective of this work was to develop polyvinyl alcohol (PVOH)/β-cyclodextrin (βCD) composite films that can be applied to reduce undesirable component content such as cholesterol in foods through active retention of the compounds in the package walls during storage. Cyclodextrins were added to PVOH in a proportion of 1:1 and cross-linked with glyoxal under acidic media to reduce its water-soluble character. Three different cross-linking procedures were used: cross-linking of the polymer/polysaccharide mixture in solution and film casting, PVOH.βCD*; cross-linking of the polymer, addition of βCD, and casting of the mixture, PVOH*.CD; and casting of a PVOH film, addition of a βCD/glyoxal solution onto the film, and cross-linking during drying, PVOH.CD*. Characterization studies showed that the PVOH*.CD and PVOH.CD* films provided the best physical characteristics with the lowest release values and the highest barrier properties. As a potential application, materials were tested as potential cholesterol-scavenging films. There was a significant reduction in the cholesterol concentration in milk samples when they were exposed to the materials developed.

KEYWORDS: β-cyclodextrin, polyvinyl alcohol, active material, composites, cross-linking, cholesterol, scavenger

INTRODUCTION

The development of active materials, defined as those that are designed to produce a beneficial interaction with matter, are at the forefront of materials research in applications such as chromatographic fillers, membranes, and active packagings. This latter technology is presently focusing the attention of the food industry, because, in combination with other nonthermal technologies, it can improve the quality and safety of food products. Active packaging materials are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food.

β-Cyclodextrin (βCD) is a cyclic oligosaccharide composed of seven glucose units with a ring structure characterized by a hydrophilic external surface and a hydrophobic cavity. This unique structure enables CD molecules to form inclusion complexes, entrapping all or part of a "guest" molecule inside their cavities, presenting potential interest as agents to retain or release entrapped substances. Food ingredients, bioactive compounds, or flavor compounds can be complexed by CDs to protect them against oxidation, light-induced reactions, heat-promoted decomposition, or loss by volatility or sublimation or to reduce undesired tastes/odors.[1,2] Empty βCD is also being used in the food industry to entrap or remove undesirable compounds such as bitter components from coffee and tea,[3] although reducing cholesterol in food is probably the main commercial use of βCD in the food sector.[4]

Several applications have been described in which cyclodextrins or cyclodextrin derivatives have been immobilized in different polymeric supports as membranes for separation of mixed components.[5−8] Most of these membranes were prepared by solution/casting, and various cross-linking agents were added to immobilize the oligosaccharides.

In a previous study, βCD was immobilized in an ethylene−vinyl alcohol copolymer film by conventional extrusion.[9] The resulting materials presented scavenging activity of apolar substances. This activity was also studied for its potential use in the active packaging of food.[10]

Polyvinyl alcohol (PVOH) is a water-soluble, partially crystalline polymer, with technological potential as a biodegradable material.[11,12] This polymer has excellent film-forming, emulsifying, and adhesive properties. It is odorless and nontoxic in many applications.[13,14] It has high tensile strength and flexibility, as well as high oxygen and aroma barrier properties. Nevertheless, these properties are dependent on humidity. Water, which acts as a plasticizer, reduces its tensile strength, but increases its elongation and tear strength. PVOH has a high water permeation rate, and it is completely hydrosoluble unless it is cross-linked. Several multifunctional compounds capable of reacting with the hydroxyl group may be used as cross-linkers.[15]

The objective of this work was to develop materials based on polyvinyl alcohol containing cyclodextrins, which were resistant to water and applicable to the reduction of undesirable component content such as cholesterol in food through active retention of the compounds in the package walls during storage. To reduce the water-soluble character of PVOH, the CD/polymer composites were cross-linked with glyoxal under acidic media. The effect on the final properties of the order of addition of the different components and of the cross-linking process was analyzed. The final materials were characterized by the determination of

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CD immobilization, glyoxal migration, and thermal, barrier, and morphological properties. As an example of the capacities of the materials developed in active packaging, their application in the reduction of cholesterol content in food was also studied.

MATERIALS AND METHODS

Chemicals and Reagents. Gohsenol type AH-17 (saponification degree 97−98.5% mol and viscosity 25−30 mPa·s) polyvinyl alcohol (PVOH) was obtained from The Nippon Synthetic Chemical Co. (Osaka, Japan). βCD was purchased from Wacker Fine Chemicals, S.L. (Barcelona, Spain). Glyoxal 40% solution in water, hydrochloric acid 37%, and orthophenylenediamine (OPD) were obtained from Sigma (Madrid, Spain). Reagent grade hexane and methanol were from Merck (Barcelona, Spain). Cholesterol, 5α-cholestane, and potassium hydroxide were obtained from Fluka Biochemika (Sigma, Madrid, Spain). Water was obtained from a Milli-Q Plus purification system (Millipore, Molsheim, France). Whole UHT milk (Hacendado, Mercadona, Valencia, Spain) was acquired from a local supermarket.

Film Preparation. Films of PVOH containing β-cyclodextrin were obtained by solution−extension−evaporation ("casting"). This technique has the advantage of reducing the thermal degradation of the composite with respect to conventional extrusion and can be compared to film-coating techniques.

PVOH solution (5% w/v) was prepared in Milli-Q water by heating it at 85−90 °C with constant stirring for 2 h. The solution was allowed to cool, and the film was cast on a flat PS surface. Films of ca. 50 μm were obtained.

PVOH.CD composite films (1:1 w/w) were obtained by adding a βCD solution (10% w/v) to the PVOH solution, stirring the mixture for 30 min, and film casting (PVOH.CD sample).

To improve the water resistance of the PVOH films and composites, the polymer was cross-linked with glyoxal[15] by various procedures. In this work, cross-linked materials are marked with an asterisk (*). Five percent (w/w) of glyoxal with respect to the weight of PVOH and HCl was added to the PVOH solution (to adjust the pH to 3.5), and it was heated to 75 °C for an additional 2 h. The cross-linked PVOH film (PVOH* sample) was obtained from this solution by casting.

In a first development, a βCD solution (10% w/v) was added to the previously cross-linked PVOH solution. This mixture was then stirred for 30 min and the film was cast (PVOH*.CD sample). In a second development, glyoxal and HCl were added to the aqueous solution containing PVOH and βCD and heated to 75 °C for 2 h. The cross-linked film was obtained by casting ((PVOH.CD)* sample). In a third development, a water solution containing the cyclodextrins (10% w/w), glyoxal, and HCl was added over a cast film of PVOH and allowed to dry. Cross-linking occurred during the drying process to obtain the PVOH.CD* sample. In all of the developments, the PVOH/βCD ratio was 1:1 (w/w) and casting was carried out in a chamber at 40 °C and 15% RH. The final films were ca. 65 μm thick.

Immobilization Studies. A study of βCD release from the films was carried out by determining the global migration from the polymer into water as aqueous food simulant following EU regulations (UNE-EN 1186-3).[16] A piece of each developed film was immersed in Milli-Q water, at a relation of 6 dm^2/L, for a period of 10 days at 40 °C, and the global migration values were determined on days 1 and 10 by calculating the weight loss according to the method prescribed in the applicable EC directives.[17−19] All of the samples were measured in triplicate.

Simultaneously, the amount of glyoxal released from the materials into water (as food simulant for aqueous food products) was also analyzed by HPLC after derivatization with OPD.[20,21] Three milliliters of simulant, or standard solution of glyoxal, was mixed with 1.8 mL of a 0.5% w/v solution of OPD in Milli-Q water. The mixture was kept at room temperature in the dark for 12 h. After membrane filtration (0.45 μm), samples were subjected to chromatographic analysis using an Agilent Technologies 1200 series HPLC equipped with an Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm particle size). The mobile phases were 0.15% acetic acid (solvent A) and 80% methanol containing 20% solvent A (solvent B). The gradient started with 20% solvent B over a period of 2 min, then it was changed linearly to 40% solvent B over a period of 20 min and to 100% solvent B over a period of 5 min, maintained for 5 min, and then changed to 20% solvent B for 5 min. The flow rate was 0.8 mL/min, and the column temperature was set at 30 °C. Peaks were detected by measurement of UV absorbance at 312 nm. A calibration using standard glyoxal solutions in the 0.5−10 mg/kg range was performed.

Swelling Assays. A cross-linked PVOH* blank and materials with cyclodextrin materials were accurately weighed and placed in a 100 mL glass vial with 70 mL of water. The samples were removed after 1 day, cleaned with soft paper to remove surface water, and weighed. Films were dried in a vacuum oven (Heraeus, Germany) at 50 °C for 2 days and weighed. The degree of swelling was calculated as the water uptake (difference between wet and dry weights) per 100 g of dry sample.

Fourier Transform Infrared Spectroscopy (FTIR). Fourier transform infrared spectroscopy (FTIR) was used to characterize the presence of specific chemical groups in the materials. Very thin (5−10 μm thick) PVOH films were obtained and analyzed by FTIR using transmittance mode. FTIR spectra were obtained in the range from 4000 to 500 cm^{-1}, with a resolution of 2 cm^{-1} and 64 scans (Bruker Tensor 27 equipment).

PVOH-βCD composites were characterized by attenuated total reflection FTIR (ATR-FTIR). Spectra were also obtained in the range from 4000 to 600 cm^{-1}, with a resolution of 4 cm^{-1} and 64 scans per test. Results were recorded in duplicate.

Thermal Analysis. Thermal properties were determined with a DSC model Q2000 from TA Instruments (New Castle, DE). Thermograms were obtained from 25 to 250 °C with a 10 °C/min heating ramp. The glass transition temperature, melting temperature, and melting enthalpy were calculated using TA Universal Analysis software.

Thermogravimetric analyses were also carried out using a Mettler Toledo TGA/SDTA/851 thermal analyzer (Columbus, OH). The samples were heated from room temperature to 600 °C under a nitrogen atmosphere to determine any evaporation of volatile compounds, as well as the degradation temperatures of the new materials.

Barrier Properties. *Water Vapor Permeability (WVP).* WVP tests were carried out at 35, 50, 60, and 70% relative humidity (RH) and 23 °C using a PERMATRAN-W model 3/33 Mocon (Lippke, Neuwied, Germany). The film samples were conditioned in the cells for 10 hs, and then the transmission values were determined every 45 min.

Oxygen Permeability. The oxygen permeation rates of the materials were determined at 50, 75, and 90% RH and 23 °C using an OXTRAN model 2/21 ML Mocon (Lippke). The film samples were conditioned in the cells for 10 h, and then the transmission values were determined every 45 min.

Milk Sample Preparation. Taking into account results from migration tests, films were previously immersed in water for 24 h to eliminate the amount of CDs and glyoxal that would have been released into the food product and then dried with a paper tissue. Thus, the release of CD molecules into water and their involvement in cholesterol/CD complex formation cannot be considered to be responsible for the decrease of cholesterol concentration in milk.

To simulate the conditions of a conventional package for pasteurized milk, a piece of film was put in contact with real milk at a surface/volume ratio equivalent to that of a 1 L carton box. Approximately 12.8 cm^2 of each film was immersed in 20 mL of pasteurized milk and kept at 23 °C for 1 week in a closed vial covered by aluminum foil to avoid any potential light effect. The milk package, films, vials, and milk were handled under sterile conditions to prevent the risk of bacterial

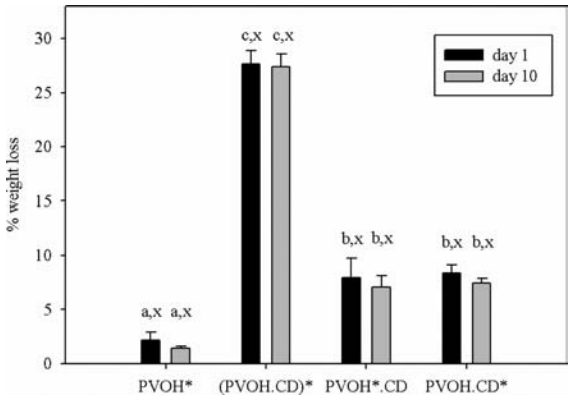

Figure 1. Global migration (expressed as weight loss) from cross-linked PVOH films to water after 1 and 10 days of exposure. Lower case letters a−c indicate significant differences in migration of the same day between samples; x and y indicate significant differences in migration of the same sample on different days.

contamination and milk spoilage. The experiments were carried out in quintuplicate.

Cholesterol Analysis. The determination of cholesterol in milk was carried out by a simple and rapid method based on direct saponification of the samples with methanolic KOH solution.[22] A 0.3 g sample of milk was accurately weighed into a sample preparation vial, and 5 mL of methanolic 0.5 M KOH solution was added, followed by 40 μL of a hexanolic solution of 5α-cholestane (1 mg/mL), as internal standard. The vial was tightly closed, and its contents were vortexed for 15 s. The vial was then immersed in a 75 °C bath and kept there for 25 min. Several vials with different samples could be handled conveniently by placing them in a wire basket. Following heating, the vials were cooled to room temperature, 1 mL of water and 5 mL of hexane were added, and the contents were vortexed vigorously for 1 min and then centrifuged for 3 min at 1500 rpm. An aliquot of the upper phase was injected in a gas chromatograph for analysis.

Total cholesterol was determined on a TRB-STEROL model fused capillary column (30 m × 0.22 mm × 0.22 μm) (Teknokroma S. Coop. C. Ltd.a., Barcelona, Spain) using an HP5890 gas chromatograph (Agilent Technologies, Barcelona, Spain) equipped with a flame ionization detector. The chromatographic conditions were as follows: He as the carrier gas, 4 μL sample injection volume, 1/20 split ratio, isothermal running at 285 °C for 15 min, injection port temperature and flame ionization detector temperature at 300 °C. Quantification of cholesterol was done through a five-point calibration curve with 5α-cholestane as internal standard. A linear peak area/concentration response (r = 0.998) was observed within the tested range (1−300 mg/L). The analysis was carried out in triplicate, and the determination of cholesterol in control/blank samples was done with each batch of test samples. Results are expressed as the average ± standard deviation concentration (w/v).

Statistical Analysis. One-way analyses of variance were carried out. The SPSS computer program (SPSS Inc., Chicago, IL) was used. Differences in pairs of mean values were evaluated by the Tukey b test for a confidence interval of 95%. Data are represented as the average ± standard deviation.

RESULTS AND DISCUSSION

Untreated PVOH and PVOH.CD films, cross-linked PVOH*, and cross-linked PVOH films containing βCDs, that is, PVOH*.CD, (PVOH.CD)*, and PVOH.CD*, were successfully obtained following the procedures described under Materials and Methods. All films were transparent and uncolored, without the visual presence of discontinuities or βCD aggregates, with a thickness of ca. 45 ± 5 μm (polymeric films) or 65 ± 5 μm (composite films).

Immobilization of βCDs in the Polymeric Film. The degree of immobilization of the oligosaccharides in the polymer matrix was determined by a migration study, which consisted of immersion of the film samples in water for 10 days at 40 °C followed by evaluation of global migration and specific glyoxal migration values. The first important observation was that all films manufactured from cross-linked materials had highly improved water resistance compared to untreated films of PVOH or PVOH with βCDs, which lost their integrity by exposure to water. This result was an indication of a successful cross-linking process.

Figure 1 presents the results from the migration tests. As can be seen, the migration of all the samples was independent of time. This kind of effect matches the case of a migration process restricted to the more external zones of the polymeric sample or a very fast migration process. This latter hypothesis is more plausible, because the film matrix is highly plasticized by water. Migration followed this order: (PVOH)* < PVOH.CD* ≈ PVOH*.CD < (PVOH.CD)*.

Control PVOH* samples presented migration values close to 2.1%, and part of this migration corresponded to unreacted glyoxal. The rest could be due to residues and additives from the original PVOH samples. Unfortunately, non-cross-linked PVOH film samples could not be tested because the films disintegrated in water.

With respect to migration from samples containing βCDs, the highest global migration values (>25%) were measured for the (PVOH.CD)* sample, that is, when the cross-linking reaction was done with PVOH and cyclodextrins simultaneously in the cross-linking solution. The presence of βCDs probably reduced the PVOH interchain cross-linking, resulting in a polymeric matrix with low resistance to water. As a consequence of the partial disintegration of the matrix, the release of PVOH molecules together with βCDs and unreacted glyoxal increased the migration values.

In the PVOH*.CD, the glyoxal reacted for 2 h with the −OH groups of the polymer, and then βCDs were added to the solution and films were cast. Efficient cross-linking of the polymer was obtained, resulting in a more stable film that reduced not only the water sensitivity of the film but also the release of oligosaccharides into the aqueous simulant.

The PVOH.CD* samples presented the lowest migration results (no significant differences with respect to PVOH*.CD). This unexpected result might be a consequence of partial dissolution of the polymeric film in the βCD solution and entrance of the cross-linking agent in the film and a cross-linking process that occurs simultaneously with the film drying. Apparently, the addition of the βCD + glyoxal solution to the previously cast PVOH film resulted in severe plasticization/partial dissolution of the PVOH film and transfer of both βCD and glyoxal to the film matrix. The reduction of film water content during the reaction may produce a very efficient cross-linking reaction. Otherwise, linkages would have been limited to the βCD molecules and the PVOH molecules in the contacting surface, and the bulk of the polymer and the other surface would have been untreated, which would have implied large migration values due to dissolution of PVOH in the liquid media.

Comparison of the migration values of these samples with results from a similar work but using EVOH as polymer matrix[9] revealed that PVOH-based materials had lower migration values than EVOH materials obtained by extrusion. In that work,

Table 1. Migration of Glyoxal from Cross-Linked Films to Water after 1 and 10 Days of Exposure and Swelling Results

	migration[a] (mg/dm^2)		
sample	day 1	day 10	swelling degree (%)
PVOH*	4.4 ± 0.3 b	4.1 ± 0.5 b	91.6 ± 4.8 x
(PVOH.CD)*	13.1 ± 1.5 a	12.7 ± 0.9 a	266.3 ± 24.0 z
PVOH*.CD	2.2 ± 0.1 c	2.4 ± 0.2 c	115.2 ± 5.9 xy
PVOH.CD*	2.0 ± 0.1 d	1.8 ± 0.1 d	139.5 ± 6.6 y

[a] Lower case letters a−d indicate significant differences in migration among the samples on the same day; x−z indicate differences in swelling degree among the samples.

βCDs were incorporated by melt mixing during extrusion; that is, immobilization was not achieved by chemical linkages. Because the content of cyclodextrins incorporated in the PVOH films was higher and PVOH sensitivity to water is greater, higher βCD release results were expected. A possible cross-linking of the βCDs to PVOH molecules might be the cause of this greater immobilization.

Among the potential migrants released from the films developed, glyoxal requires a specific migration study. This cross-linking agent is used as a strengthening substance in protein and starch coatings[23] and in the production of epoxy resins.[24] FDA Regulations about Food Contact Substances limit the use of glyoxal to a level not to exceed 6% by weight of the starch or protein fraction of the coating solids. The only migration limit detected in these regulations is the specific migration limit of 7.7 mg/dm^2 (0.5 mg/in^2).

Table 1 shows the results of the glyoxal-specific migration test, together with data from swelling tests, which will be discussed below. The sample of cross-linked polymer released nearly 4.4 mg/dm^2 of glyoxal, 15% of the glyoxal initially added. The PVOH*.CD and PVOH.CD* samples had the lowest values, ca. 2 mg/dm^2, <7% of the nominal glyoxal addition. This result appears to indicate that βCDs were also involved in the reactions of the cross-linking agent.

As can be seen, (PVOH.CD)* was the only sample that exceeded the migration limit. In fact, 40% of the glyoxal added was released from the film, indicating a very inefficient reaction. The lower concentration of polymer and glyoxal in the mixed solution might be the cause of this effect. For all of the materials, no effect of time on migration was observed, probably as a consequence of a very fast migration process, finished during the first 24 h.

Swelling Results. Absorption of large quantities of water leads to a reduction of interpolymer interactions, an increase in flexibility of the polymer chain, and, as a consequence of both, a film expansion that directly affects the diffusivity of low molecular weight substances in the matrix and, obviously, the retention capacity of oligosaccharide molecules. Chemical cross-linking of the polymer with glyoxal results in the generation of covalent bonds between PVOH molecules that maintain the integrity of the polymeric network and reduce polymer chain flexibility and mobility.

Table 1 includes the results of the swelling study. Unfortunately, the swelling caused in samples not treated with glyoxal could not be measured owing to the disintegration of the samples in liquid water. Samples containing cyclodextrins presented significantly higher swelling values than cross-linked PVOH, probably as a consequence of the large portion of the film constituted by oligosaccharides (50%) and the fact that they are hydrophilic and include large cavities, which become filled with water molecules when immersed in water. A comparison between the materials containing βCDs shows that the (PVOH.CD)* film samples had the highest swelling values ($p < 0.05$), double the swelling observed for the other two materials, in agreement with the migration results. No significant differences were observed between PVOH*.CD and PVOH.CD*, indicating a similar degree of cross-linking of the composite matrix.

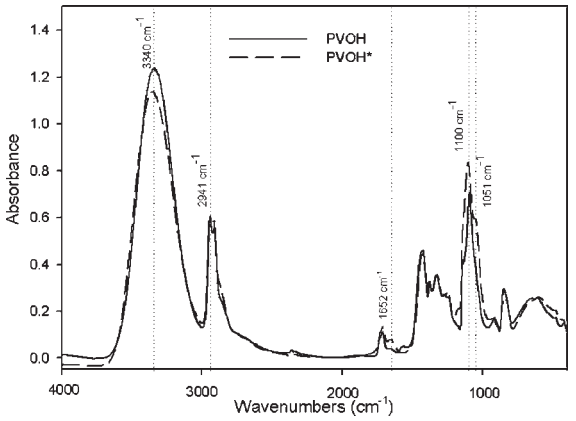

Figure 2. Transmission FTIR spectra from untreated (PVOH) and treated (PVOH*) polymeric films.

Fourier Transform Infrared Spectroscopy (FTIR). Infrared spectrophotometry was used to observe the cross-linking effect of glyoxal on the PVOH-based materials. Figure 2 shows the transmission FTIR spectra recorded for the untreated PVOH and cross-linked PVOH* films (5 μm thick films were especially prepared). The most relevant features of the PVOH spectrum are the large, wide O—H stretching band (3000−3600 cm^{-1}), the asymmetrical (2941 cm^{-1}) and symmetrical (2907 cm^{-1}) C—H stretching bands, the C═O (1735 cm^{-1}) and C—O (1708 cm^{-1}) stretching bands related to unhydrolyzed acetate groups, the secondary O—H in-plane bending band (1426 cm^{-1}), the C—H wagging vibrations band (1330 cm^{-1}), the C—C—C stretching band (1144 cm^{-1}), and the C—O stretching band (1094 cm^{-1}).

As can be seen, important changes appear with the cross-linking process. As a bifunctional cross-linker, one aldehyde group of the glyoxal molecule reacts with hydroxyl groups of the PVOH polymer chain by forming a hemiacetal structure. Cross-linking occurs by forming acetal bridges between the pendant carbonyl group of the glyoxal molecule and the hydroxyl groups of another PVOH chain. Therefore, efficient cross-linking implies a reduction of available hydroxyl groups, which was clearly evidenced by the reduction of the relative intensity of the O−H stretching band (3000−3600 cm^{-1}). The formation of acetal bridges was confirmed by the presence of new features at 1053, 1104, 1180, and 1244 cm^{-1} related to the presence of ether groups. The presence of a shoulder at ca. 2850 cm^{-1} related to the stretching of C−H in the neighborhood of carbonyl substituents and the new band at 1652 cm^{-1} corresponding to the carbonyl of glyoxal and hemiacetal groups indicated the presence of unreacted glyoxal.[25] The absence of the peak at 1141 cm^{-1} indicated the reduction of crystallinity with the cross-linking process.[26]

Figure 3 shows the ATR-FTIR spectra of PVOH*, βCD, and various composites. The difficulty of obtaining very thin films of

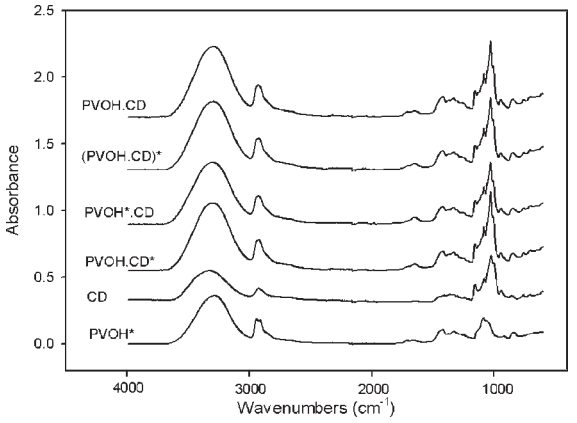

Figure 3. ATR-FTIR spectra of PVOH−βCD composites and pure components.

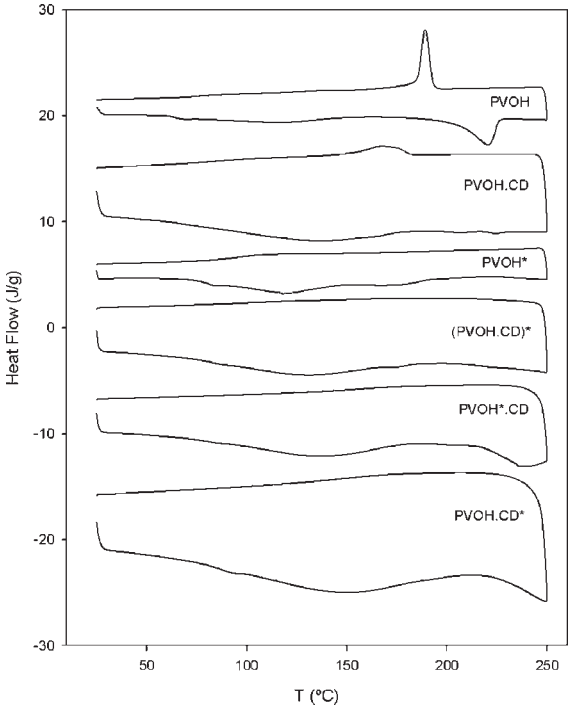

Figure 4. DSC thermograms of PVOH films during the first heating.

PVOH containing βCDs impeded assays in the transmission mode due to superabsorbance. The spectra are displaced on the absorbance axis to facilitate comparison.

As can be seen, the spectra of the various composites were very similar and practically corresponded to the addition of the PVOH* and βCD spectra. The characteristic bands of βCD appeared in all spectra at 995, 1003, 1025, 1078, and 1155 cm^{-1}, without any displacement. Moreover, these features within the same wavelength as that of the acetal groups hindered any effect on the PVOH cross-linking process. The O−H stretching band of the composites appeared at intermediate wavenumbers between those of the cross-linked PVOH and the βCD.

After the immobilization analysis, the films were dried and analyzed by ATR-FTIR. The spectra obtained (not shown) did not exhibit relevant differences with respect to those corresponding to nonmigrated films, indicating that the cross-linked films resisted the exposure to water and that the βCDs were successfully immobilized.

Thermal Properties. The materials developed in this study were analyzed by DSC and TGA. Figure 4 shows representative DSC thermograms of the various film samples. Untreated PVOH showed a glass transition, T_g, at 65.14 °C. This polymer exhibited a clear endotherm between 200 and 230 °C, with a minimum at 220.3 °C (melting temperature, T_m) that reveals the semicrystalline nature of the polymer. The cooling process (shown in Figure 4) exhibited a crystallization exotherm with a maximum at 189 °C. The other thermograms shown in Figure 4 correspond to cross-linked samples, with and without cyclodextrins, and a non-cross-linked composite film. The clearest effect of cross-linking is the absence of the melting endotherm. The chemical bonding produced by the reaction of glyoxal with the polymer (and possibly with βCDs) impeded the chain rearrangement needed to create crystalline structures. In the cooling process, no signs of crystallization processes were observed. Similar results were observed by Mansur et al. in a PVOH hydrogel cross-linked with glutaraldehyde.[26] The inclusion of the βCDs also affected the crystallization ability of the polymer in the non-cross-linked sample as observed in the first heating process, although the PVOH crystallized in the cooling process and presented a melting endotherm in the second heating process.

Table 2 shows the glass transition temperatures of the various samples. The addition of βCDs yielded greater stiffness, increasing the T_g value by about 5 °C. The cross-linking process also resulted in an increase in T_g because the generation of interchain covalent bonds reduced the flexibility of the polymeric molecules. Similar results have been observed in cross-linked proteins.[27,28] Incorporation of cyclodextrins produced further increased rigidity of the composite cross-linked materials.

The TGA thermograms showing the weight loss and its derivative with temperature for all of the materials and components are plotted in Figure 5. As can be seen, they show that there were several degradation processes. To determine their individual contributions to the mass loss, the wide bands presented in the derivative curve have been deconvoluted using Origin software with Gaussian or Lorentzian fitting. In all cases, a good fit was observed with $R^2 > 0.99$. The deconvolution of the PVOH* sample is included as an inset in Figure 5. The results of the various degradation contributions to mass loss are included in Table 2.

All samples showed a weight loss at temperatures slightly above 100 °C that is related to the evaporation of water. The degradation of the βCDs appeared in a single narrow feature at 330 °C. Glyoxal also degraded in practically a single process at 223 °C. On the other hand, PVOH underwent three degradation processes, the two more important ones occurring at 294 and 347 °C. According to Ballistreri et al. and Holland and Hay, the degradation of PVOH starts with the loss of water molecules by elimination of hydroxyl side groups and acetaldehyde, acetone, ethanol, unsaturated aldehydes, and ketones.[29,30] At higher temperatures, degradation continues with the breakdown of the polymer backbone and the release of aromatic substances (benzene, naphthalene, and derivatives). A non-cross-linked film of PVOH containing βCDs was prepared and included in this study. This particular sample showed the original TG features of the two components.

As can be seen in Figure 5, the cross-linked samples presented a different thermal behavior. The first degradation process observed from the cross-linked PVOH sample (PVOH*) took

Table 2. Thermal Properties of the Materials Developed and Their Components: Glass Transition Temperatures, Dehydration, and Degradation Peaks Expressed as Temperature of the Minimum at the Derivative and Attributed Percentage of Weight Loss

		dehydration		degradation					
material	T_g (°C)	T (°C)	loss (%)	T (°C)	loss (%)	T (°C)	loss (%)	T (°C)	loss (%)
glyoxal		125.9	53	152.2	10.2	223.4	36.8		
βCD		89.3	13	330.5	87				
PVOH	65.14	113.3	1.1	293.7	40.7	346.6	42.9	433.1	15.4
PVOH.CD	70.4	131.7	2.6	325.1	28.1	351.1	54.9	437.6	14.3
PVOH*	80.12	118.5	3.1	359.6	47.4	366.0	31.1	442.0	18.4
(PVOH.CD)*	84.56	127.5	3.4	326.6	45.3	380.2	51.3		
PVOH*.CD	82.90	132.0	3.7	309.0	35.7	369.0	55.6	435.8	5.0
PVOH.CD*	88.01	145.4	6.0	304.2	40.0	380.4	49.3	433.9	4.8

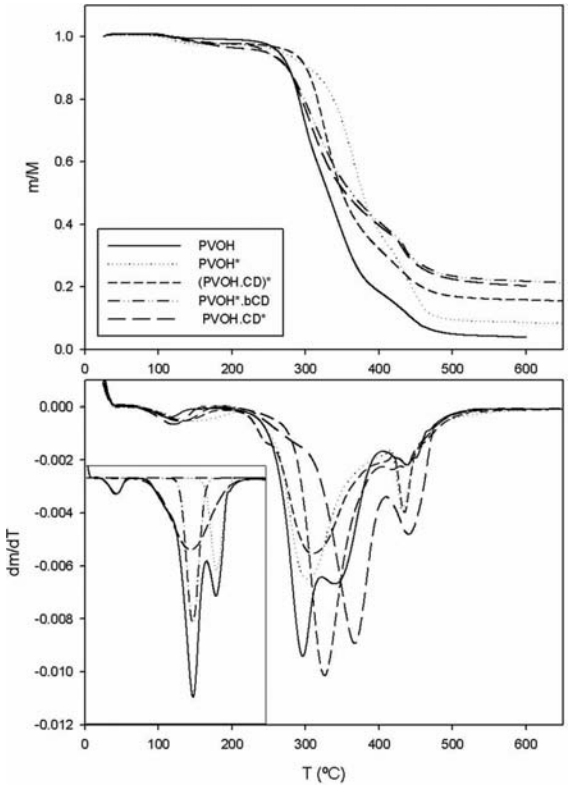

Figure 5. TGA and derivative curves of PVOH and composite films developed. (Inset) Deconvolution of the TGA derivative curve of PVOH* material, using a Gaussian algorithm (dotted and dashed curves represent the diverse contributions; solid line is the experimental curve).

place at 360 °C, well above that of the untreated PVOH. The cross-linking process, which makes use of the −OH groups, reduces the initiation of the degradation process by dehydration, causing this change. Variations in the temperature of the first and second degradation processes were also observed in all composites. The absence of the degradation band of the pure βCD (except in the (PVOH.CD)* sample) indicates that the oligosaccharide was involved in the cross-linking process.

Barrier Property Results. *Water Vapor Permeability.* The water vapor barrier of a polymer is a very important datum in packaging design. Owing to the hydrophilic character of PVOH, the analysis of the WVP of the PVOH samples was carried out at various relative humidity conditions, and the results are included in Table 3. As expected, all of the materials showed a strong dependence on the relative humidity. The sorption of large amounts of water caused an increase in polymer chain mobility and, consequently, in water diffusivity. Moreover, it is well-known that the water sorption isotherm for PVOH deviates from linearity toward higher values.[31] The PVOH* sample had significantly higher values ($p > 0.05$) than PVOH at all humidities tested. These differences were greater at the lowest humidities tested. The reduction in crystallinity induced by cross-linking as indicated by DSC and ATR analysis is one of the causes of this

Table 3. Water Vapor Permeability Values of PVOH-Based Materials

	$10^3 \times$ WVP[a] [g·m/(m²·day·atm)]			
	35% RH	50% RH	60% RH	70% RH
PVOH	5.25 ± 0.5 b,x	4.6 ± 0.5 c,x	13.5 ± 0.5 c,y	116.0 ± 2.5 c,z
PVOH*	8.5 ± 0.5 c,x	7.5 ± 0.5 d,x	40.0 ± 2.5 d,y	125.0 ± 5.0 d,z
(PVOH.CD)*	2.5 ± 1.0 a,x	3.0 ± 0.5 b,x	7.5 ± 0.5 a,y	87.5 ± 2.5 b,z
PVOH*.CD	2.0 ± 0.3 a,x	1.5 ± 0.3 a,x	6.0 ± 0.5 a,y	37.5 ± 2.5 a,z
PVOH.CD*	7.5 ± 0.5 cx	6.8 ± 0.5 d,x	11.3 ± 0.3 b,y	115.0 ± 2.5 c,z

[a] Lower case letters a−d indicate significant differences among the values of permeability at the same RH; x−z indicate significant differences among the values of permeability of a sample at different values of RH.

Table 4. Oxygen Permeability Values of PVOH-Based Materials

	$10^4 \times$ oxygen permeability[a] [cm³·m/(m²·day·atm)]		
	50 % RH	75% RH	90% RH
PVOH	0.32 ± 0.11 a,x	2.49 ± 0.41 b,y	184.86 ± 8.88 d,z
PVOH.CD	1.73 ± 0.21 c,x	34.03 ± 1.32 d,y	706.20 ± 14.22 e,z
PVOH*	0.48 ± 0.09 a,x	1.34 ± 0.05 a,y	52.14 ± 2.11 a,z
(PVOH.CD)*	0.84 ± 0.09 b,x	3.35 ± 0.22 bc,y	156.88 ± 0.45 c,z
PVOH*.CD	0.77 ± 0.01 b,x	3.56 ± 0.06 c,y	133.21 ± 7.79 b,z
PVOH.CD*	0.81 ± 0.11 b,x	3.28 ± 0.30 bc,y	145.37 ± 3.87 bc,z

[a] Lower case letters a−e indicate significant differences among the values of permeability at the same RH; x−z indicate significant differences among the values of permeability of the same sample at different values of RH.

Table 5. Cholesterol Concentration and Reduction of Cholesterol in Milk Exposed to Cross-Linked PVOH Materials at Days 2 and 7 at 23 °C[a]

	milk	PVOH*	(PVOH.CD)*	PVOH*.CD	PVOH.CD*
			Day 2		
cholesterol (mg/L)	151.3 ± 7.2 b,x	148.43 ± 1.06 b,x	141.94 ± 1.77 a,x	141.47 ± 0.61 a,y	142.16 ± 1.4 a,y
% reduction		1.90 ± 0.7	6.19 ± 1.17	6.50 ± 0.40	6.04 ± 0.92
			Day 7		
cholesterol (mg/L)	151.3 ± 7.2 c,x	147.76 ± 1.65 c,x	138.26 ± 3.12 b,x	136.15 ± 2.48 ab,x	130.69 ± 2.1 a,x
% reduction		2.34 ± 1.09	8.62 ± 2.06	10.01 ± 1.64	13.62 ± 1.39

[a] Lower case letters a−c indicate significant differences among the values of the same day between samples; x and y indicate significant differences among the values of the same sample at different days.

effect. At 70% RH, the permeability is still higher but much closer to the original PVOH permeability. The intercatenary bonds reduced chain mobility and swelling of the material, counterbalancing the observed effect of the more amorphous structure.

Among the composites, the PVOH.CD* sample presented a permeability profile very similar to that of PVOH. The manufacturing process of this particular sample (casting of PVOH film and addition of the glyoxal/βCD aqueous solution) might be responsible for this result. The other composites presented improved water barrier properties at all humidities. The incorporation of a substance with a less hydrophilic character than the polymer reduces water sorption and swelling of the composite. This effect, combined with the previously mentioned cross-linking action, is responsible for these improvements.

Oxygen Permeability. Gas permeability, especially oxygen, is also a key parameter for a packaging material. Permeation values were therefore determined for all samples at three humidities, owing to the RH effect on the barrier properties of this hydrophilic polymer. The results are included in Table 4. As expected, the WVP increases with RH for all samples as a consequence of the previously mentioned plasticization effect. This result is in agreement with permeation values of pure PVOH films.[32] Water molecules adsorbed by the polymer interact with the hydroxyl groups of the polymer and deteriorate intra- and intermolecular hydrogen bonds, facilitating the mobility of the polymer chains and diffusion of the molecules of oxygen. The generation of intermolecular chemical bonds in PVOH reduced molecular mobility and, subsequently, oxygen diffusivity. For this reason, PVOH* presented better oxygen barrier characteristics than untreated PVOH. Table 4 also shows that the incorporation of cyclodextrins increased the permeability to oxygen of the composite films. The presence of the cavity of the molecules of cyclodextrins can facilitate diffusion of oxygen molecules in the polymer matrix. Nevertheless, the cross-linked composite films are more efficient barriers than the untreated composite one.

Cholesterol Scavenger Results. As mentioned in the Introduction, one of the main applications of βCDs in food and pharmaceutical applications is the formation of inclusion complexes for (a) the protection of a sensitive ingredient or (b) the extraction of an undesired component. In this work, the potential use of the composites developed in the reduction of the cholesterol content of foods was explored, specifically, in milk. Table 5 shows the results obtained for the various materials. Untreated material films could not be analyzed because they lose their integrity as a result of exposure to milk. PVOH* films, used as controls, did not present any significant cholesterol retention during storage time. The low chemical compatibility between the film and the fatty molecule is probably responsible for this result. On the other hand, the immersion of βCD-containing PVOH films in whole milk reduced the cholesterol content by the formation of inclusion complexes. At day 2, (PVOH.CD)* reduced the cholesterol concentration by 6.2%. After 7 days of storage, the amount retained by the film increased slightly (6.5%), although the difference is not significant. PVOH*.CD and PVOH.CD* samples presented a similar reduction at day 2, but at day 7 the reduction was significantly higher, between 10 and 15%. (PVOH.CD)* presented severe swelling, increasing the film thickness by 300%. This large water retention produced a strong plasticization of the polymeric structure, allowing fast diffusion of the cholesterol molecules in the structure. Also, βCD molecules presented higher mobility, making possible the formation of cholesterol/βCD 1/2 and 1/3 inclusion complexes. In the other two samples the lesser degree of swelling produced a lesser plasticization of the polymer and, as a consequence, a slower diffusion of the cholesterol molecules in the polymer matrices. Also, a lesser number of 1/2 and 1/3 inclusion complexes increases the number of available βCD molecules, increasing the final cholesterol reduction.

AUTHOR INFORMATION

Corresponding Author

*Phone: +34 963900022. Fax: +34 963636301. E-mail: rgavara@iata.csic.es.

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