"GREEN" IMPREGNATION OF BIOPOLYMERS FOR APPLICATION IN FOOD PACKAGING

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Abstract

The possibility of impregnating biopolymer films with active substances, using "clean" supercritical technology was the main purpose of this study. Known anti-oxidants, ascorbic acid and co-enzyme Q10, were impregnated in several biopolymer films (pectin, alginate, carrageenan, PHB/V and their mixtures). A "clean" supercritical fluid impregnation process was used to prepare these biopolymer/drug systems. Experiments were carried out at different operating conditions in order to evaluate the impregnation process. These systems were characterized using scanning electron microscopy (SEM) analysis.

Introduction

Nowadays, one of the most important global concerns is the waste produced and accumulated from chemical processes, and packaging for consumer items, especially food. Therefore, natural biodegradable materials and cleaner technologies are an area of growing interest worldwide. In the polymer industry, biopolymers present a valuable and interesting solution for the food packaging waste problem especially in combination with a clean technology, such as the use of supercritical fluids. The food packaging industry has special interest in these biopolymers since they degrade in a short period of time when exposed to a biologically active environment. [1] They are naturally recycled by biodegradation, in a manner analogous to the natural biogeochemical cycles in nature. [2]

Most foods deteriorate in quality and safety during transport, processing, and storage. Oxidative reactions have the greatest impact in limiting the shelf life of perishable foods. Several impregnation techniques are used in the food packaging industry to try and solve this major problem. [3], [4] However, most of these impregnation methods use organic solvents, which are not regarded as "green".

Supercritical fluid impregnation, being a "green" process, presents an alternative technology to replace organic solvents by carbon dioxide. In this technique, the carbon dioxide is used to both dissolve the drug, and swell the polymer, enabling rapid impregnation.[5]

In the present study, different biopolymers were impregnated using known anti-oxidants, of synthetic and natural origin (ascorbic acid and co-enzyme Q10). The biopolymers used were alginate, carrageenan, pectin and different blends of these natural polymers. The impregnations were carried out at different operational conditions and the systems prepared were characterized through SEM.

Materials and Methods

Materials

Pectin, (CAS 9000-69-5), Carrageenan, Type I (CAS 9000-07-1), Alginic acid sodium salt from brown algae, (CAS 9005-38-3), Copolymer of (R)-3-Hydroxybutyric acid and (R)-3-Hydroxyvaleric acid (~3:1), (CAS 92267-82-8), were purchased from Sigma. Ascorbic acid, (CAS 50-81-7) and Co-Enzyme Q_{10}, (CAS [303-98-0], pureza de 98%) purchased from Sigma. Trichloromethane stabilized with ethanol (99% purity), (CAS 200-663-8) , Ethanol (99,5% purity) (CAS 200-578-6), both purchased from Panreac Quimica SA. Carbon dioxide, (99.998 mol %) was supplied by Air Liquide. All chemicals were used with no further purification.

Film Preparation

Alginate, carrageenan, pectin and their mixtures (0.6 g) were dispersed in 1–2 ml of ethanol to avoid lumping during solution preparation. Water (30 ml) was then added with vigorous stirring. The suspension was heated at 60 ± 5 ºC with constant stirring until a homogeneous solution was obtained (approximately 20 min). The clear solution was then poured into a polystyrene petri dish, taking care that all air bubbles escape from the viscous medium. The cast solution was then allowed to dry in air for 2–3 days to form a film.

The PHB/V films were made by mixing 0.5 g of Copolymer (R)-3-Hydroxybutyric acid and (R)-3-Hydroxyvaleric acid (~3:1) with 6 ml of trichloromethane. The resulting solution was then poured into a glass dish and left to dry in air for 1-2 days.

Methods - *Supercritical impregnation process*

The impregnation was performed in a batch or semi-continuous apparatus presented schematically in figures 1a) and 1b), respectively:

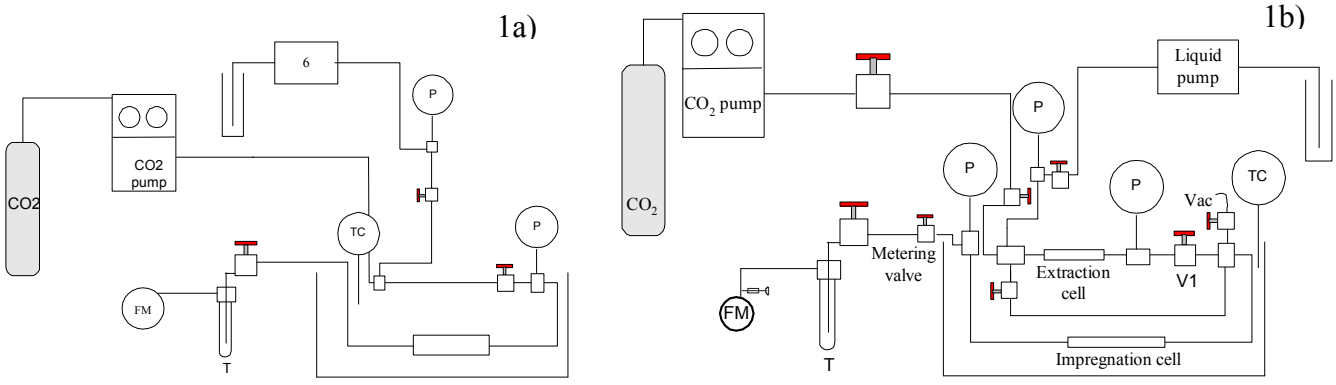

Figure 1: Schematic representation of the impregnation apparatus a) batch mode b) semi-continuous mode

When the batch impregnation is performed, the high pressure vessel is loaded with the active compound and the polymeric matrix. The cell is immersed in a thermostatized water bath heated by means of a controller (Ero Electronic LMS-491-13), that maintains temperature within ± 0.1ºC (TC), stirred with a magnetic stirrer. Carbon dioxide is pumped into the cells

using high pressure piston pump (Haskel model MCPV-71) until the operational pressure is attained. The pressure inside the cells is measured with a pressure transducer (P) (LEO2 0..300 bar). The contact time was 3 hours and afterwards the system was depressurized to atmospheric pressure.
The semi-continuous process has been described elsewhere by Duarte *et al.* 2005 [6]. Briefly, a tubular batch extractor is initially loaded with the anti-oxidant and packed between a sequence of a filter paper, cotton and net metallic discs that work as filters of small particles. The polymer is loaded, in the same manner, in the impregnation cell. The cells are then immersed in a thermostatized water bath heated by means of a controller that maintains temperature within ± 0.1ºC (TC) (Ero Electronic LMS-491-13). Carbon dioxide is pumped into the cells using high pressure piston pump (Haskel model MCPV-71) until the operational pressure is attained. In the intervening time the cells are not connected to each other, however they are at the exact same pressure. The pressure inside the cells is measured with a pressure transducer (P) (LEO2 0..300 bar). The solubilization of the drug in the supercritical fluid and the swelling of the polymer due to the presence of carbon dioxide take place during one hour, a typical equilibrium time. Opening valve V1 the cells are placed in contact and the continuous process of impregnation starts. A saturated stream of the active compound in carbon dioxide passes through the polymeric matrix, for a predetermined period of time and at a very slow rate so that the impregnation can occur. The outflow is regulated by a micrometric valve (Hoke 1315G4Y), in order to maintain a constant pressure in the system and a slow carbon dioxide flow, which is measured with a flowmeter (FM) (Alexander Wright DM3C). The solid that might be solubilized in the gas is collected in a small glass trap (T). After an impregnation experiment the system is depressurized to atmospheric pressure.

Film Characterization - Scanning electron microscopy (SEM)
The morphology of polymer samples was analysed and imaged by scanning electron microscopy (SEM, Leica 5440) after carbon sputter coating.

Results and Discussion

Impregnation experiments were performed either in a batch or semi-continuous mode in order to evaluate the performance of these two techniques.
The impregnation experiments that were performed in batch mode are summarized below:

# experiment	Biopolymer (mass %)	Drug	Pressure (bar)	Temperature (K)	Impregnation Time (h)
1	Alginate:Pectin (50:50)	Ascorbic Acid	180	313	3
2	PHB/V	Ascorbic Acid	180	313	3
3	Alginate:Carrageenan (50:50)	Ascorbic Acid	180	313	3

The experiments performed in a semi-continuous process were carried out at pressures ranging from 100bar to 180bar and temperatures from 308 K to 323 K. Also, different contact times during the impregnation process were studied.
A summary of the experiments performed is listed in the following table:

# experiment	Biopolymer (mass %)	Drug	Pressure (bar)	Temperature (K)	Impregnation Time (h)
1	Alginate:Carrageenan (75:25)	Ascorbic Acid	180	313	3
2	Alginate:Carrageenan (50:50)	Ascorbic Acid	180	313	3
3	Alginate:Pectin (75:25)	Ascorbic Acid	180	313	3
4	Alginate:Pectin (50:50)	Ascorbic Acid	180	313	3
5	Alginate (100)	Ascorbic Acid	180	313	3
6	Pectin (100)	Ascorbic Acid	180	313	3
7	Alginate:Carrageenan (50:50)	Co-Enzyme Q_{10}	100	313	2
8	Alginate:Carrageenan (50:50)	Co-Enzyme Q_{10}	125	313	2
9	Alginate:Carrageenan (50:50)	Co-Enzyme Q_{10}	150	323	2
10	Alginate:Carrageenan (50:50)	Co-Enzyme Q10	150	308	2
11	Alginate:Carrageenan (50:50)	Co-Enzyme Q10	150	313	2
12	Alginate:Carrageenan (50:50)	Co-Enzyme Q10	150	313	3

The resulting impregnated polymer films were cut into sections and characterized using SEM. From the SEM images of the films impregnated in batch mode, figures 2 and 3, it is possible to observe ascorbic acid on the surface of the polymeric matrixes.
Nevertheless particles of the active compound can also be seen in the cross-section of the film, assuring the success of the impregnation (figure 3).

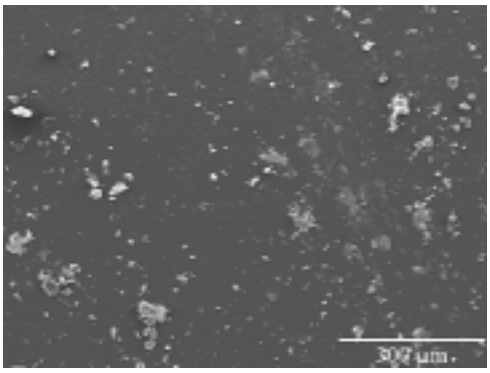

Figure 2: SEM picture of the surface of PHB/V matrix impregnated with ascorbic acid in batch mode.

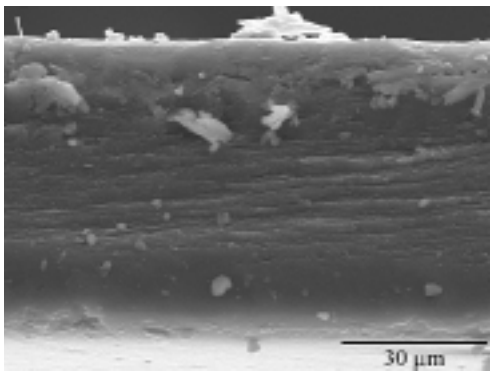

Figure 3: SEM picture of a cross-section of alginate:pectin (50:50) matrix impregnated with ascorbic acid at 180 bar and 313K, in batch mode.

In figure 4 it is possible to see co-enzyme Q_{10} in the cross-section of a film of alginate:carrageenan (50:50), that was impregnated in a semi-continuous mode.

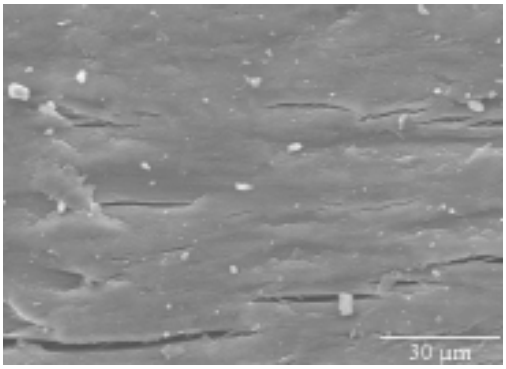

Figure 4: SEM picture of a section cut of alginate:carrageenan (50:50) matrix impregnated with co-enzyme Q_{10} at 150 bar and 313K, in semi-continuous-mode.

When semi-continuous impregnation was performed, only small amounts (much less than in the batch mode) of the antioxidant were found on the surface of the films, corresponding to the active compound dissolved in the carbon dioxide stream that precipitates, when the depressurization takes place.
Therefore, the rate of depressurization is an operational factor that has to be taken into account when impregnation experiments are carried out.

Conclusions

This work is part of an on going project which aim is the impregnation, using a "clean" technology, of biopolymer matrixes with active compounds, such as antioxidant or antimicrobial agents, with applications in the food packaging industry.[7]
The preliminary work presented here proves the success of the supercritical fluid impregnation process, using CO_2 as a solvent for the drug and a swelling agent for the polymer. SEM analysis of the biopolymer films impregnated, using batch and semi-continuous modes, showed that in both cases the bioactive compound is found inside the polymer.
On-going studies include the determination of yields of impregnation and kinetic studies related with the release of the antioxidant from the polymer matrixes.

Aknowlegements

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