Revista de la Asociación Española de Materiales Compuestos

MATERIALES COMPUESTOS

http://revista.aemac.org

http://revista.aemac.org/
Vol 3, nº 1, pág. 60-63

ISSN: 2531-0739

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Efecto bacteriostático de films poliuretano-urea con extractos bioactivos incorporados por diferentes rutas en medio acuoso

RESUMEN

Historia del artículo:
Recibido 5 de Mayo 2017
En la versión revisada 5 de Mayo 2017
Aceptado 31 de Mayo 2017
Accesible online 21 de Junio 2017

Palabras clave:
Dispersiones de poliuretano-urea
Extractos de plantas
Rutas de incorporación
Films bioactivos

La conciencia medioambiental ha promovido el estudio de nuevos materiales enfocado a sistemas más respetuosos con el medio ambiente, promoviendo tanto procesos de síntesis verde como el origen renovable de los compuestos de partida. En este sentido, los poliuretano-urea dispersables en agua resultan una familia muy versátil dando lugar a una amplio rango de propiedades, así como una gran variedad de aplicaciones. Además, cabe destacar que su dispersabilidad en agua ofrece la posibilidad de adicionar aditivos solubles, como los extractos de plantas.

Por ello, en ese trabajo se ha empleado la planta *Melissa officinalis L.* para la obtención de extractos bioactivos, que se han incorporado a la dispersión de poliuretano-urea en diferentes porcentajes empleando tres rutas de incorporación. Las dispersiones y los films preparados a partir de éstas, se han caracterizadodesde el punto de vista fisicoquímico, térmico, y mecánico, entre otros. Por último, las propiedades antibacterianas de los films han sido estudiadas tras 1 y 4 días de incubación, donde se ha observado que el contenido y la ruta de incorporación del extracto influyen en el comportamiento de los films frente a patógenos comunes (*Staphylococcus aureus, Escherichia coli y Pseudomonas aeruginosa*).

Bacteriostatic effect of waterborne polyurethane-urea films containing bioactive plant extracts incorporated by different routes

ABSTRACT

Keywords:
Polyurethane-urea dispersions
Plant extracts
Incorporation routes
Bioactive films

The environmental awareness has promoted the development of new materials towards eco-friendly systems based on both, green synthesis processes as well as the renewable origin of the raw compounds. In this way, focusing on synthesis methods, the use of waterborne polyurethane-urea dispersions have gained attention due to their versatility leading to a wide variety of applications, broadening the range of applications. In addition, it is worth nothing that the dispersibility in water offers the possibility of incorporating soluble additives, such as plant extracts.

Therefore, in this work Melissa *officinalis* L. plant was selected in order to obtain bioactive plant extract, in order to be incorporated to a waterborne polyurethane-urea dispersion, varying their content as well as using three different incorporation routes. These dispersions were characterized and employed in the preparation of films which were analyzed from the viewpoint of physicochemical, thermal and mechanical properties, among others. Finally, the antibacterial properties of the films were analyzed after 1 and 4 days of incubation, where it was observed that the content and incorporation route of the extract influenced in the behavior of the films against common pathogens (*Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa*).

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1 Introduction

The environmental awareness has promoted the development of green synthesis routes and in this way, waterborne polyurethane (WBPU) and polyurethane-urea (WBPUU) dispersions have gained attention due to the reduction of volatile organic compounds generation [1]. Furthermore, their versatility and tailorable properties become them attractive for their use in different applications [2-4]. In addition, the waterborne character of the dispersions offers the opportunity of incorporating renewable water soluble additives, becoming an interesting approach towards the challenge of preparing new eco-friendly materials [5-6], providing improved or even additional properties, opening the range of applicability. In this context, among others, the use of plant extracts incorporated as biologically active agents can modulate the properties of the WBPUU films besides enhancing their antimicrobial properties.

Therefore, in this work, Melissa officinalis L. plant extract was selected for the incorporation to a WBPUU dispersion. In this way, three addition routes were designed for the incorporation of the extract in different weight contents (1, 3 and 5 wt%). WBPUU based dispersions were used for preparing bioactive films, which were characterized from the viewpoint of physicochemical, thermal and mechanical properties. Furthermore, a preliminary antibacterial test was performed against common pathogens (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*).

2 Experimental

2.1 Synthesis of waterborne polyurethane-urea

For the synthesis of WBPUU, poly(ε-caprolactone) diol (PCL) (Mw = 2000 g mol^{-1}), purchased from BASF, ethylenediamine (EDA) supplied by Panreac, isophorone diisocyanate (IPDI) kindly provided by Covestro and 2,2-bis(hydroxymethyl) propionic acid (DMPA) purchased from Aldrich, were employed.
The synthesis of waterborne polyurethane-urea was carried out by two steps polymerization process and the reaction progress was followed by dybutilamine back titration method (ASTM D 2572-97), using an isocyanate/hydroxyl groups ratio of 1.67 in the prepolymer and 5 wt% of DMPA, resulting in a hard segment content around 32 wt%. In the first step PCL and DMPA (previously neutralized with TEA) were reacted with IPDI. The resultant prepolymer was cooled and the dispersion formation step was carried out by adding distilled water dropwise. In the second step, the chain extension was carried out with EDA. A WBPUU dispersion with a solid content about 35-40 wt% was obtained.

2.2 Plant extracts infusions

Plant extract was obtained from *Melissa officinalis L.* by infusion method. The plant was added to boiling distilled water. The mixture was filtered and the resultant solution was lyophilized.

2.3 Bioactive films preparation

Three alternative incorporation routes were designed for the preparation of the Melissa-based WBPUU at contents of 1, 3 and 5% (wt%, prepolymer-basis):

1. In the first method, **post-method**, the required amount of extract was dissolved in distilled water and incorporated dropwise to the synthesized WBPUU under mechanical stirring.

2. In the second method, **in-situ method**, the extract was dissolved in the amount of distilled water employed for the phase inversion step.

3. In the third method, **pre-method**, the extract was dissolved in a small amount of distilled water and incorporated to the reactor just before water addition. .

Melissa-based WBPUU films were prepared by solvent casting. The dispersion was poured into a Teflon mold and allowed to dry at room conditions during 1 week. The resultant films were stored in a desiccator before characterization. Samples were coded as MelissaXy, where “X” was referred to Melissa weight content in the polyurethane-urea and “y” specifies extract incorporation route “post”, “in-situ” or “pre” Furthermore, neat polyurethane-urea was coded as WBPUU.

2.4 Characterization

Particle size and distribution of base WBPUU and WBPUU dispersions containing Melissa extract were analyzed using a Mastersizer 3000 Hydro particle size analyzer of Marlvern by averaging 5 measurements of the diluted dispersions at 25 ºC.
Thermal behavior of bioactive films were analyzed by differential scanning calorimetry (DSC) using a DSC 204 F1 Phoenix equipment of Netzsch. Samples were subjected to a heating scan from -75 to 250 ºC at a scanning rate of 10 ºC min^{-1}.
Mechanical behavior of bioactive films was determined using a MTS Insight 10 testing machine provided with a 250 N load cell and pneumatic grips to hold samples. Five specimens were averaged for each system at room temperature. Films tensile modulus (E), yield stress (σ_y), stress at break (σ_b) and strain at break (ε_b) were determined from stress–strain curves obtained at a crosshead speed of 50 mm min^{-1}.
Antibacterial properties of films were analyzed by static tests. The assays were performed using Gram positive bacteria *Staphylococcus aureus* ATCC 19213 and Gram negative bacteria *Escherichia coli* ATCC 10536 and *Pseudomonas aeruginosa* ATCC 9027 as test microorganisms. The method was based on the Kirby-Bauer modified test [7]. Briefly, the bacteria inoculums were prepared by aseptically transferring 4 isolated colonies of each one, to separate test tubes containing nutrient broth, which were then incubated for 1 day at 37 °C. The inoculums were diluted to 0.5 McFarland turbidity standard (corresponding to a concentration of 1.5–3.0 x 108 CFU/mL) using sterilized Ringer solution. The concentration of the bacteria dilutions were also controlled by UV-visible spectrophotometry by measuring the absorbance at 625 nm. Then, the bacteria solutions were inoculated in Mueller Hinton Agar plates, using a sterilized swab. The inoculated plates were left to dry for a short period of time. After that, a piece of sample with 1.5 cm of diameter of the waterborne polyurethane-urea films containing plant was placed in the center of the plate. The plates were incubated at 37 °C for 24 h. After this period, the plates were analyzed to measure the diameter of the inhibition zone and the growth of the bacteria on the surface or behind the film. After, the incubation maintained for a further 4 days in order to evaluate the possible growth of the inhibition zone caused by the extract diffusion and the bacteria biofilm formation on the films surface

3 Results and discussion

The particle size distribution of the base WBPUU and Melissa-based WBPUU dispersions prepared by *in-situ* and by the pre-methods and distribution profiles are shown in **Figure 1a**. It was observed that, in general, and in comparison with the base WBPUU, the addition of extract to the WBPUU dispersion contributed to the broadening of the particle size distribution towards smaller particle sizes, acting as natural surfactants [8-10], favoring the dispersion formation and thus contributing to the achievement of smaller particles. However, the effect varied attending to the incorporation route due to the intercalation of the extract in among WBPUU nanoparticles, as can be seen in **Figure 1b**. In the case of *in-situ* method a progressive decrease in particle size was observed with increasing extract content, whereas by pre-method, the reduction in the particle size remained similar without relying on the extract content.

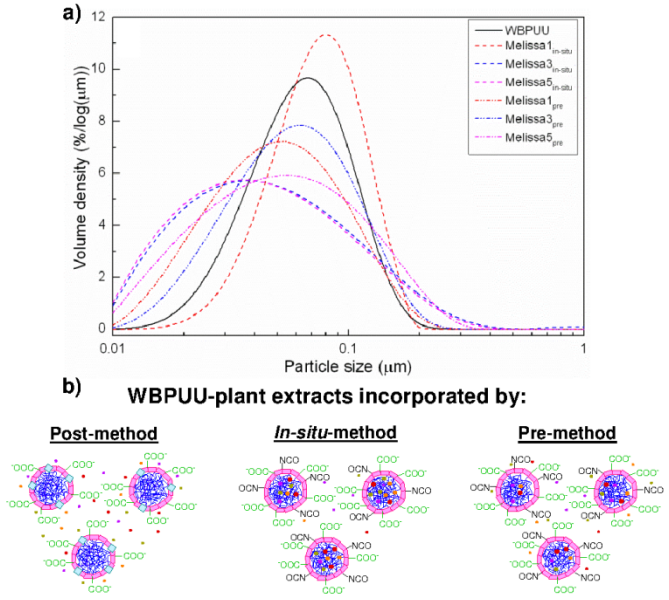

Figure 1. a) Particle size distribution of base WBPUU and Melissa-based WBPUU, b) Plant extract incorporation mechanisms

The thermal behavior of base WBPUU and WBPUU containing Melissa extract bioactive films was analyzed by DSC, and thermograms are shown in **Figure 2a**. Analyzing the base WBPUU film, it was appreciated that the polyurethane-urea film presented a T_{gSS} around -50 °C, which remained similar in the bioactive films series. Furthermore, a broad transition related with the long range order of hard segment domains [11] was observed. In general, it was observed that Melissa extract incorporation favored the ordering of hard segment domains, resulting in a progressive increase of both, T_{mHS} and ΔH_{mHS} values, except in Melissa3$_{in\text{-}situ}$ sample. It is thought that in this case different extract intercalation mechanisms were developed.

Mechanical behavior of base WBPUU and Melissa-based WBPUU bioactive films is shown by stress-strain curves in **Figure 2b**. It was observed that in general, Melissa extract conferred stiffness to the bioactive films presenting slightly higher E values and lower σ_b and ε_b values, except in the case of the use of 3 wt% content in *in-situ* and pre-methods. It is thought that at this percentage, greater extract quantity would result embedded inside nanoparticles, conferring flexibility to the system.

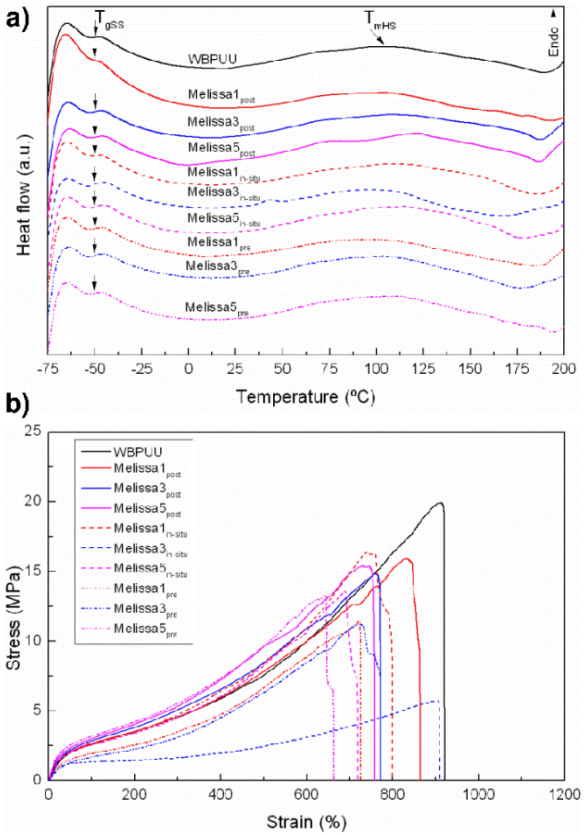

Figure 2. a) DSC thermograms and b) stress-strain curves of base WBPUU and Melissa-based WBPUU bioactive films

The antibacterial properties of the base WBPUU and Melissa-based WBPUU films were analyzed against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* microorganisms. It was observed that after 1 day of incubation, the base WBPUU and Melissa-based WBPUU film presented bacteriostatic properties against the three bacteria, hindering their growth on the film. Nevertheless, none of the samples showed an inhibition zone. However, it was observed that after 4 days of incubation the base WBPUU sample did not show inhibitory power against none of the assayed bacteria, whereas the addition of Melissa extract, as well as the incorporation route, resulted in different behaviors, which could be related with the bioactive components constituting the extracts and the different intercalation mechanisms [12-13]. The obtained results are summarized in **Table 1**. In the case of *Staphylococcus aureus* bacteria, Melissa-based films maintained the bacteriostatic effect in all analyzed samples, attributable to the composition of the extract [14]. Instead, in the case of *Escherichia coli* and *Pseudomonas aeruginosa* bacteria, at 1 and 3 wt% of extract incorporated by pre-method was not enough in order to maintain the bacteriostatic effect of the film. It could be related with the intercalation of the extract among and inside the WBPUU nanoparticles, as well as in 1 wt% of extract against *Pseudomonas aeruginosa* bacteria, taking into consideration that these bacteria is a more resistant pathogen.

Table 1. Antibacterial properties of base WBPUU and WBPUU containing Melissa extracts after 4 days of incubation

Sample	Melissa		
	S. aureus	*E. coli*	*P. aeruginosa*
1 post	V	V	X
3 post	V	V	V
5 post	V	V	V
1 *in-situ*	V	V	X
3 *in-situ*	V	V	V
5 *in-situ*	V	V	V
1 pre	V	X	X
3 pre	V	X	X
5 pre	V	V	X

V: There was not bacteria growth on the surface or behind the film
X: There was bacteria growth on the surface or behind the film

4 Conclusions

Different contents (1, 3 and 5 wt%) of Melissa officinalis L. extract were incorporated into the polyurethane-urea dispersions by using three different incorporation routes: post-method, *in-situ* method and pre-method, in order to prepare films with antibacterial properties. It was observed that the extract content, as well as the incorporation route, influenced the final properties of the prepared films. Regarding dispersions particle size, results revealed that the WBPUU particle size distributions broaden to lower values, which was related to the surfactant effect attributed to the used extract. Thermal properties of the films showed that extract promoted the ordering ability of hard domains. This fact influenced the mechanical properties of the films, where it was observed a stiffening effect in Melissa-based WBPUU films. In the case of *in-situ* and the pre-method (with 3 wt% of extract), films became more flexible, which was related with the intercalation mechanism of the extract within the polyurethane-urea nanoparticles. Antibacterial tests revealed that after 1 day of incubation, all base WBPUU and Melissa-based WBPUU series, showed bacteriostatic effect against the analyzed *S. aureus*, *E. coli* and *P. aeruginosa* bacteria. After 4 days of incubation, only some samples presented bacteriostatic effect, being the magnitude of the effect dependent on the extract content and incorporation route.

Acknowledgements

Financial support from the Basque Government (IT-776-13), the Spanish Ministry of Economy and Competitiveness (MINECO) (MAT2013-43076-R), (MAT2016-76294-R), POCI–01–0145–FEDER-006984 (LA LSRE-LCM) funded by ERDF through POCI-COMPETE2020 and FCT and NORTE-01-0145-FEDER-000006, funded by NORTE 2020, under PT2020 through ERDF is gratefully acknowledged. We also wish to acknowledge the "Macrobehaviour- Mesostructure-Nanotechnology" SGIker units from the University of the Basque Country, for their technical support. A.S-E thanks the University of the Basque Country for Ph.D. grant (PIF/UPV/12/201).

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