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Development study of new topical formulations with erythromycin

Lacramioara OCHIUZ[1], Andreea CRETEANU[1], Manuela HORTOLOMEI[2], Catalina Anisoara PEPTU[3], Iulian STOLERIU[4], Marcel POPA[5], Cristian PEPTU[6]

[1]*Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania*
[2]*Ester Farm LTD, Iasi Romania*
[3]*Faculty of Chemical Engineering and Protection of the Environment, "Gheorghe Asachi" Technical University, Iasi, Romania*
[4]*Faculty of Mathematics, "Alexandru Ioan Cuza" University, Iasi, Romania*
[5]*The Academy of Romanian Scientists, Bucharest, Romania*
[6]*"Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania*

Abstract

Erythromycin is a macrolide antibiotic prescribed for the topical treatment of acne. Pharmacologically, erythromycin has the disadvantage of being poorly soluble in water. This leads to formulation challenges in semisolid dosage forms. In recent years, many published studies have shown the ability of cyclodextrins to form complexes with drugs. These new complexes are characterized by much improved solubility and permeation compared to the „parent" molecule. The aim of this study was to synthesize an inclusion complex of erythromycin and lactide-β-cyclodextrin for the formulation of semisolid bases and the development of innovative topical preparations with erythromycin. The erythromycin-lactide-β-cyclodextrin complex was characterized by scanning electron microscopy and Fourier-transform infrared spectroscopy. Semisolid formulations were pharmacologically evaluated by in vitro dissolution test and kinetic analysis of drug release by fitting to representative mathematical models. The results obtained showed a prolonged release of erythromycin from erythromycin-lactide-β-cyclodextrin formulations and a higher permeability coefficient of these formulations compared to the erythromycin-based release.

Keywords: erythromycin, cyclodextrins, semisolid dosage forms

INTRODUCTION

Local antibiotherapy is one of the most effective acne therapies. Erythromycin (ER) is a macrolide antibiotic administered topically for over 30 years in the form of gels, ointments or hydroalcoholic solutions in acne therapy. The use of ER as a base for topical dosage forms raises some technological challenges due to the physicochemical properties of this substance [1-3]. The main disadvantage of ER is its poor water solubility (2 mg/ml) that limits both formulation using hydrophilic bases and skin permeability [4-5]. Cyclodextrins (CDs) are biocompatible cyclic oligomers of glucose, with a hydrophobic core and a hydrophilic exterior. CDs are used to improve

Corresponding author:
Assist. Prof. Andreea Creteanu, MD, PhD
E-mail: acreteanu@gmail.com

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the bioavailability of drugs by increasing their solubility and/or their dissolution rate after including the poorly water-soluble substances (such as ER) in the hydrophobic cavity of CDs. Adding CDs leads to improved solubility and stability of the drug substance, increased permeability of low aqueous solubility, decreased toxicity and even to reduced active dose as a result of increased bioavailability. CDs increase skin tolerability by reducing the irritant effect of certain substances [6]. We have included ER to lactide modified β-cyclodextrin, in order to improve the therapeutic effect of topically administered ER. The aims of the present study were: to synthesize and describe a new complex with prolonged release of ER with lactide modified β-cyclodextrin (CD-LA_E); to investigate the CD-LA_E complex by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR); to analyze the effect of the semisolid base on the *in vitro* release characteristics of ER in the CD-LA_E complex by assessing the permeability coefficient and the release kinetics by fitting on mathematical models.

MATERIALS AND METHODS

β-cyclodextrin (Sigma Aldrich, Germany), *erythromycin,* purity 99.85% (Zhejlang Sanmen Hengkang Pharmaceutical Co. Ltd. China) *lactic acid, alcohol, methanol* – pharmaceutical purity – were purchased from MedChim Company, Bucharest. *Hydroxypropylcellulose (HPC)* 150 - 4000 cP was received as a sample from Nisso Chemical Europe GmbH, Germany. *Carbopol 940 (C 940), propylene glycol, triethanolamine, cetyl alcohol, petroleum jelly, glycerol monostearate* and *isopropyl-myristate* - pharmaceutical purity - were purchased from MedChim Company, Bucharest. *Soya lecithin, Lutrol F 127 (127 L)* and *Lutrol F 68 (L 68)* were donated by BASF Germany. The preparation of gels used pure water and double distilled water (Millipore conductivity – 0.01 Ms/cm). All reagents used met the quality requirements of RP X. *In vitro* dissolution tests were conducted on Nylon synthetic membrane with a 50 mm pore diameter and pores of Ø = 45µm (Millipore, Merck Germany).

Preparation of the erythromycin β-cyclodextrin-lactide complex – (CD-LA_E)

The polarity of the β-CD molecule has been adjusted by preparing lactide modified cyclodextrin (CD-LA). In order to obtain CD-LA, β-CD was functionalized with an average of 3 units of lactide. The β-CD derivative modified with oligoester lactide (LA) was prepared by LA ring opening exclusively with β-CD by Shen method [7]. The complex erythromycin - lactide modified β-cyclodextrin (CD-LA_E) was prepared following the method described by Song [6]. The work technique consists in preparing a CD-LA aqueous solution, concentration 0.125 g/ml, and an ER alcoholic solution of 0.25 g/ml. The two solutions were mixed in a molar ratio of 1:1. The ER solution was added dropwise under continuous stirring for 30 min. The complex obtained was washed three times with alcohol to remove uncomplexed ER. In the last stage, the complex was lyophilized at - 30 ° C after being frozen for 12 hours at - 20 ° C, in order to remove the solvent completely.

Physical-chemical characterization of the CD-LA_E complex

Scanning electron microscopy (SEM) was performed using a scanning electron microscope Shimadzu SSX 550 by visualizing a constant sample quantity.
Mass spectrometry with electrospray ionization (ESI-MS) is known as a simple and quick method applied to the characterization of CD derivatives. This method was applied for the characterization of the CD derivatives obtained by ring-opening and oligomerization with β-butyrolactone and [8-9], D, L – lactide [7] and other cyclic esters [10]. In our studies we used an *AGILENT 6520 QTOF spectrometer* equipped with electrospray ionization. The analysis of ESI-MS was performed under the following conditions: VCAP = 4000 V, fragmentor voltage = 175 V, temperature of drying gas = 325 °C, gas flow = 5 L/min, nebulization pressure = 35 psig, injection flow = 0.5 ml/min.
Fourier transform infrared absorption spectroscopy (FT-IR) – carried out with a Vertex 70 FT-IR spectrometer (Bruker, Germany), using the encapsulation technique in KBr. The KBr pellets were prepared by mixing 3 mg of sample with 500 mg of KBr of appropriate purity. 128 records were made for each sample, 2 cm^{-1} resolution in absorbance and transmittance. The spectra obtained were compared with those presented in the literature [11].

Preparation of dermal preparations with CD-LA_E

CD-LA_E was dispersed in the base, so that the gels have a final concentration of 2% free erythromycin. We used 6 different bases abbreviated in Table 1.

TABLE 1. *Abbreviation of gel formulations with CD-LA_E*

Gel Formulati	Abbreviati
HPC gel, 3,5%	B1-CE 2%
C 940 gel, 2,5%	B2-CE 2%
L/H emulsion	B3-CE 2%
L 127 gel, 15% and L 68 gel, 3%	B4-CE 2%
L 127 gel, 13%	B5-CE 2%
Organogel	B6-CE 2%

Evaluation of in vitro release of erythromycin

The *in vitro* dissolution test was performed on an Enhancer cell with a diameter of 2.5 cm, employing an SR 8 Plus Series device (AB & L Jasco), according to the following protocol: *dissolution medium:* phosphate buffer pH 7.4, 100 ml; *mass of sample:* each formulation studied used 0.5 g *Nylon synthetic membrane* with a pore diameter Ø = 45µm (Millipore, Merck Germany); *temperature:* 37 °C ± 0.2 °C; *harvest interval:* the test was carried out over a 12-hour interval; every 60 min, we harvested a sample volume of 1 ml, which was replaced with fresh medium; *rate:* 100 rpm.
The synthetic membrane was placed in the dissolution medium for 24 hours, prior to performing the *in vitro* test, so as to obtain optimal hydration and expansion of the pores. The harvested samples were filtered through a 0.45 µm filter, and then chromatographed by High Pressure Liquid Chromatography method [12].

Determination of the permeability coefficient of erythromycin

The permeability coefficient was calculated by the following equation [13]:

$$\mathbf{Kp = J/C \times A} \quad (1)$$

in which:
Kp - permeability coefficient (cm/h);
J - rate of permeation of drug substance or flow in stationary phase (µg/h);
C - concentration in the donor compartment (µg/ml)
A - contact surface area (cm^2)

Evaluation of release kinetics of erythromycin

Experimental data obtained from studies of ER release through biological or synthetic membrane were analyzed by fitting on four mathematical models (zero-order model, first-order model, Higuchi model and Korsmeyer-Peppas model), according to the equations presented by Kierstan et al. and Lucero et al. [14, 15].
Data fitting was performed by linear and nonlinear regression using Matlab 7.1. Data were presented as a mean ± the standard deviation and were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

The amorphous morphology of the powders of CD-LA and CD-LA_E complex was observed by SEM (Fig. 1.a and Fig. 1.c, respectively.). This morphology is consistent with the data reported in the literature [16]. Pure free ER shows a semicrystalline structure, as shown in Figure 1.b. Thus, the mixing process implemented in the CD preparation step facilitates the interaction of CD with ER, with the alteration of the original crystalline structures of these compounds and the formation of an amorphous structure during criodessication, as shown in Fig. 1.c.
β-CD was functionalized with a variable number of lactide units from 1 (m/z = 1296) to 6 (m/z = 2016), with an average of 3 units, as shown in Fig. 2. For example, the peak at m/z = 1728 corresponds to a CD molecule carrying 4 lactide units, according to the following stoichiometric calculation: 1728 = 1134(CD)+4x144(LA)+18(NH_4^+). In the structure of the CD-LA_E complex, the molar ratio between CDLA and ER is 2.12 to 1.
ER characteristic spectrum shown in Fig. 3.a presents the following specific absorption bands: 3522 cm^{-1} corresponding to the – OH groups, 2974 cm^{-1} – CH_2 , 1715 cm^{-1} – C=O, 1639 cm^{-1} – OH groups, 1464 cm^{-1} – $N(CH_3)_2$, 1379 cm^{-1} – OH, 1271 cm^{-1} – OH, 1194 cm^{-1} – C-O-C, 1096 cm^{-1} – C-O/C-C, 1009 cm^{-1} – C-N, 903 cm^{-1} – N-CH_3.
The CD-LA spectrum, shown in Fig. 3.b manifests the following specific absorption bands: 3400 cm^{-1} – OH groups, 2932 cm^{-1} – CH_2, 1747 cm^{-1} – C=O, 1639 cm^{-1} – OH groups, 1456 and 1402 cm^{-1} – CH_3 groups from lactide, 1155 cm^{-1} – C-O-C, 1080 cm^{-1} – OH groups, 1030 cm^{-1} – C-O/C-C.
The spectrum obtained for the CD – LA_E complex shown in Fig. 3.c allows the observation of distinct absorption bands resulting from the contribution of the mixture components, erythromycin and CD-LA, together with modified peaks due to inclusion of ER in CD-LA. Thus, the bands corresponding to the carbonyl group of ER at 1715 cm^{-1} were displaced as a result of the inclusion process at 1657 cm^{-1}, the bands observed 1740 cm^{-1} corresponding to the carbonyl groups of the CD-LA. At the same time, the bands corresponding to the – OH groups in the ER structure at 1639 cm^{-1} were displaced by complexation to 1611 cm^{-1}.
The results of the *in vitro* dissolution test showed that ER solubility was greatly enhanced by complexation, the amount of ER released from the gels with CD-LA_E being in the range of 76.23 to 89.01%. (Fig. 4).

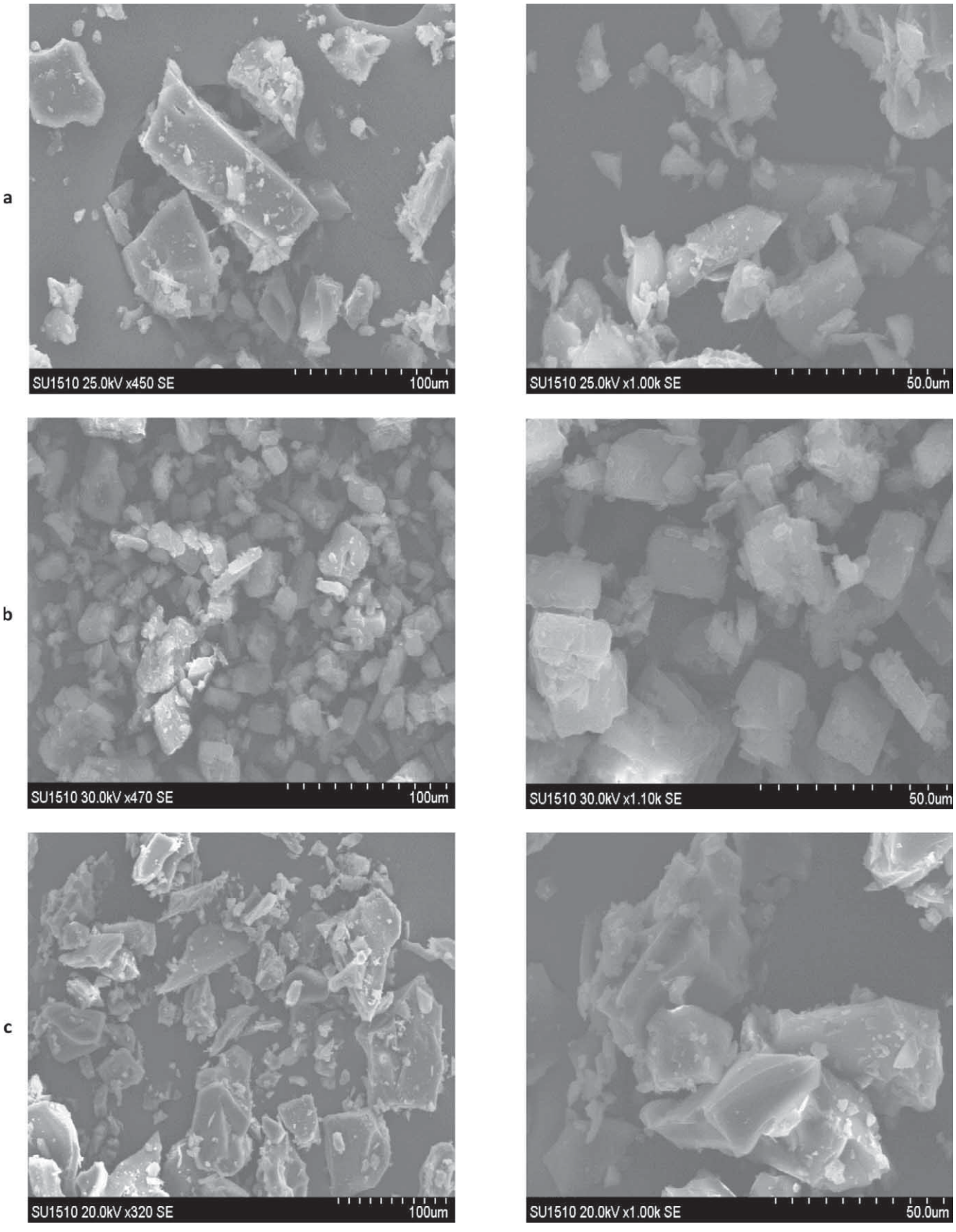

FIGURE 1. *SEM image of CD-LA (a), ER (b) and CD-LA_E (c)*

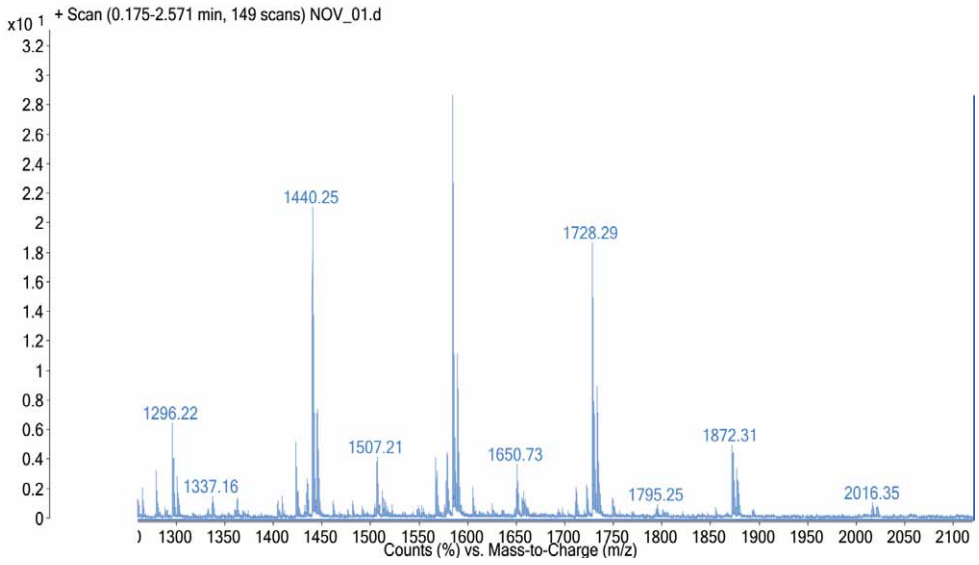

FIGURE 2. *ESI MS spectra of the CD-LA derivative*

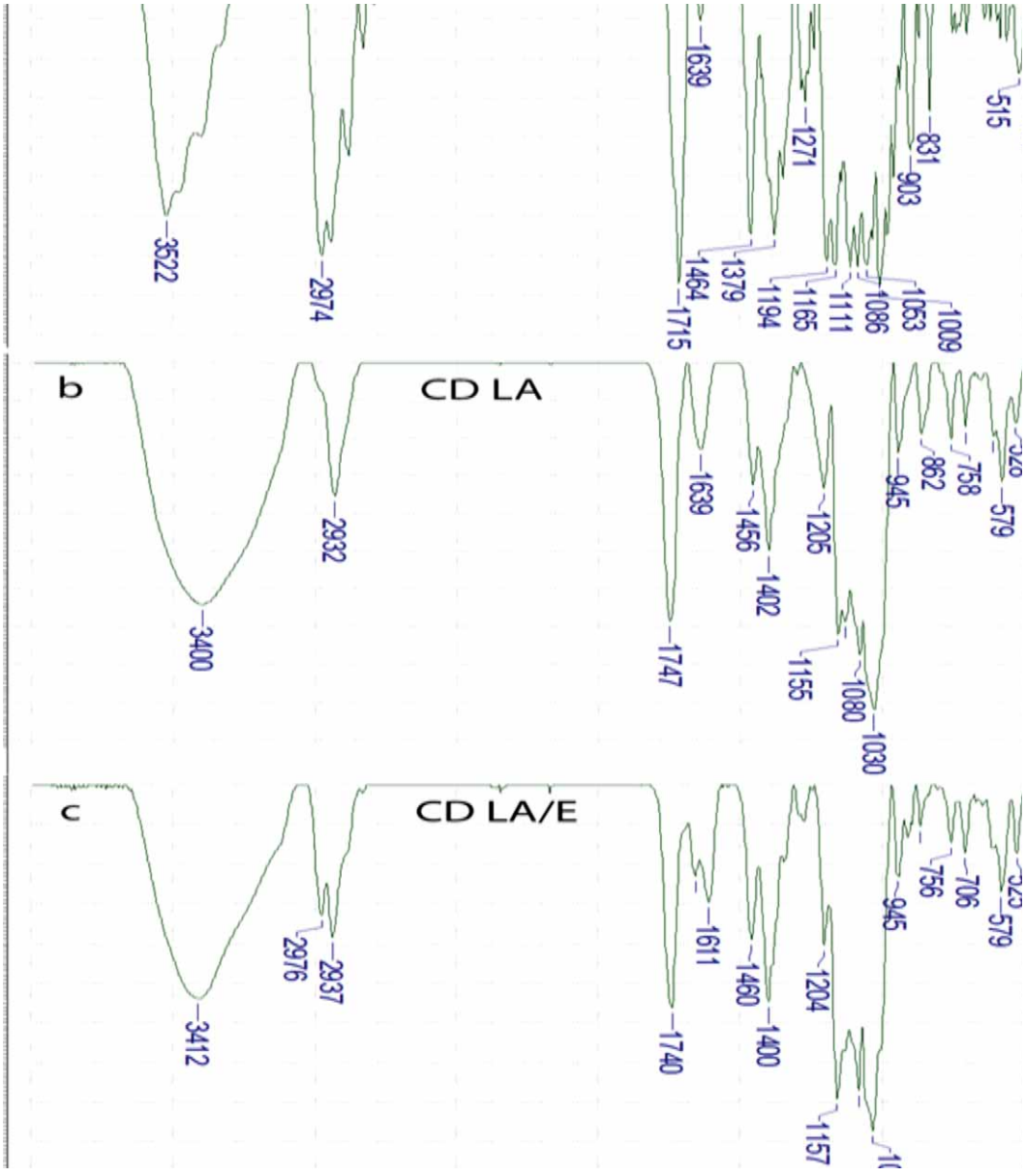

FIGURE 3. *FTIR spectrum: ER (a), CD-LA (b), CD-LA_E (c)*

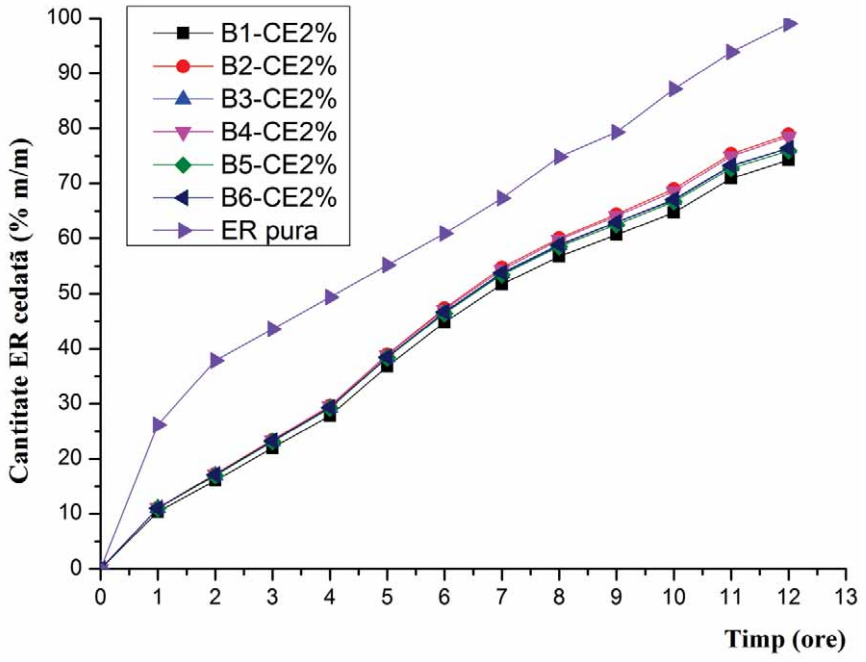

FIGURE 4. *Cumulative profile of the* ***in vitro*** *release of ER from gels with CD-LA_E*

As shown in Table 2, the permeation coefficient of ER in the CD-LA_E complex is greater from the Carbopol-based gel and Lutrol ER 127-based gel. The release kinetics of complexed ER is carried out by diffusion, according to the results obtained by fitting the data from the *in vitro* dissolution test (table III).

CONCLUSIONS

The present study synthesized and characterized an ER complex obtained by inclusion in lactide-modified β-CD. Changing β-CD structure aimed at increasing the polarity of the CD molecule and improving ER binding capacity. Scanning electron spectroscopy showed that by complexation ER alters its crystalline structure and becomes amorphous. The study also showed that specific bands for certain groups in the structure of erythromycin were displaced during the inclusion process. In the structure of the CD-LA_E complex, the molar ratio of CD-LA and ER is 2.12 to 1. CD-LA_E manifests a good compatibility with a wide range of bases for semi-solid preparations. By complexation, both solubility and permeability of ER were improved. According to the results of *the in vitro* test, all the formulations studied showed a release of above 75% over 12 hours. Of the bases studied, the gels based on Carbopol 940 and polyoxyethylene-copolymers, B2-CE2 % and B4-CE2%, respectively, exhibited the best characteristics of active principle release.

TABLE 2. *Parameters specific of ER permeation from CD-LA_E gels (n = 6)*

Formula	Permeation parameters	
	J_{ss} (µg/cm²/h)	K_p x 10^{-6} (cm/h)
	Synthetic membrane *(in vitro)*	
B1-CE 2%	30.4559 ± 7.2197	7115.86
B2-CE 2%	30.3371 ± 7.5029	7561.58
B3-CE 2%	25.0658 ± 7.7158	5424.43
B4-CE 2%	30.3625 ± 7.7720	7882.27
B5-CE 2%	28.2829 ± 7.7888	6926.99
B6-CE 2%	26.9587 ± 7.4907	6156.37

Conflict of interest: none declared
Financial support: none declared

TABLE 3. Parameters of the analysis of the release kinetics of ER from CD-LA_E gels

Formula	zero order		firstorder		Higuchi		orsmeyer-Peppas		
	K_0 g	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-0.5}$)	R^2	K_P h	n	R^2
Synthetic membrane									
B1-CE 2%	7.5498	0.9890	0.1357	0.9514	21.5391	0.9434	10.4687	0.85	0.9962
B2-CE 2%	7.8527	0.9843	0.1473	0.9495	22.4513	0.9490	10.8949	0.85	0.9959
B3-CE 2%	6.9357	0.9759	0.1125	0.9837	19.8826	0.9548	10.7219	0.80	0.9947
B4-CE 2%	8.0226	0.9822	0.1547	0.9464	22.9561	0.9515	12.3940	0.80	0.9958
B5-CE 2%	7.6682	0.9788	0.1381	0.9656	21.9656	0.9537	11.8511	0.80	0.9954
B6-CE 2%	7.2880	0.9783	0.1240	0.9757	20.8812	0.9538	11.2642	0.80	0.9954

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