

EVALUATION OF ANTIMICROBIAL ACTIVITY OF SOME TYPES OF INCLUSION COMPLEXES OF ERYTHROMYCIN WITH β -CYCLODEXTRIN ON *STAPHYLOCOCCUS AUREUS*

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Abstract

Erythromycin may be bacteriostatic or bactericidal depending on the organism and drug concentration. The purpose of this study was to evaluate the antimicrobial activity of the inclusion complexes of erythromycin with β -cyclodextrin synthesized by three methods: kneading, co-precipitation and freeze-drying.

The inclusion compounds were synthesized using a molar ratio of erythromycin: β -cyclodextrin of 1:1.

To determine the antimicrobial activity of erythromycin and its inclusion complexes, we used the disk diffusion method on Muller-Hinton medium and *Staphylococcus aureus* ATCC 25923 was used as quality control organisms.

Our determinations showed that the tested complexes erythromycin- β - cyclodextrin had a strong antimicrobial activity.

Rezumat

Eritromicina poate fi bacteriostatică sau bacterică, în funcție de organism și de concentrația ei. Scopul acestei lucrări a fost acela de a evalua activitatea antimicrobiană a unor complecși de incluziune ai eritromicinei cu β -ciclodextrina obținuți prin trei metode: frământare, coprecipitare și liofilizare.

Compușii de incluziune au fost sintetizați utilizând un raport molar eritromicină: β -ciclodextrină de 1:1.

Pentru determinarea activității antimicrobiene a eritromicinei și complexelor s-a utilizat metoda difuzimetrică pe mediu Müller-Hinton și ca microorganism- test s-a folosit *Staphylococcus aureus* ATCC 25923.

Rezultatele obținute au evidențiat o sensibilitate a microorganismelor la complexii eritromicină- β - ciclodextrină.

Keywords: erythromycin, β -cyclodextrin, antimicrobial activity, *Staphylococcus aureus*

Introduction

Erythromycin (Figure 1) is a macrolide antibiotic produced by *Streptomyces erythreus*. It inhibits the bacterial protein synthesis by binding

to bacterial 50S ribosomal subunits; binding inhibits peptidyl transferase activity and interferes with the translocation of amino acids during translation and assembly of proteins. Erythromycin may be bacteriostatic or bactericidal depending on the organism and drug concentration [5,11,15,17].

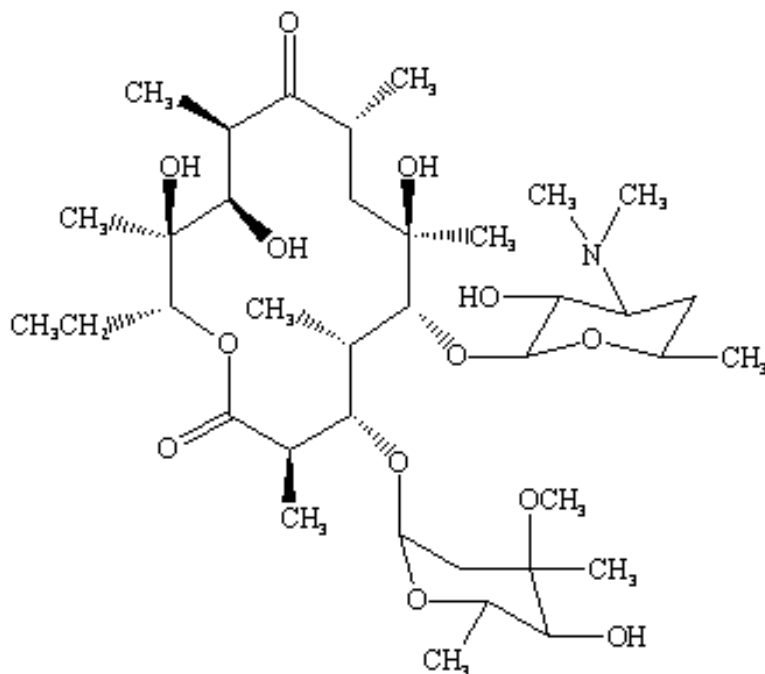


Figure 1
Chemical structure of erythromycin

The chemical name of erythromycin is (3*R*, 4*S*, 5*S*, 6*R*, 7*R*, 9*R*, 11*R*, 12*R*, 13*S*, 14*R*) -4-[(2, 6 - dideoxy-3- *C*-methyl - 3 - *O* - methyl - α - L - *ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[(3, 4, 6 - trideoxy - 3 - dimethylamino - β - D - *xylo* - hexopyranosyl)-oxy]oxacyclotetradecane-2,10-dione.

This paper is a contribution to the development of inclusion chemistry type nanoconjugates cyclodextrin - drug with the role of obtaining controlled-release antimicrobial agents (the field of nanomedicine), a priority area of the European Knowledge Area [3,10,14,16].

A first phase of the study was the synthesis and characterization of host-guest nanoconjugates type of cyclodextrins and erythromycin to increase the bioavailability and efficiency of drug substance molecules [12].

The purpose of this study was to evaluate the antimicrobial activity of the inclusion complexes of erythromycin with β -cyclodextrin synthesized by three methods: kneading, co-precipitation and freeze-drying.

Materials and Methods

All materials were reagent grade and used without further purification. Erythromycin and β -cyclodextrin were obtained from Sigma Aldrich GmbH, Germany.

To determinate the antimicrobial activity of erythromycin and its inclusion complexes we used the disk diffusion method on culture Müller-Hinton medium, agar poured uniformly on a thin layer of 4 mm, pH 7.4. As test microorganisms it was used *Staphylococcus aureus* ATCC 25923. *Inocula* were prepared in nutritive broth at a density adjusted to a 0.5 Mc Farland turbidity standard for disc diffusion. The inoculated plates were then incubated at 37°C, 24h. The turbidity was determined with a nefelometer - DEN 1 Biosan. The active substance content of a standard tablet of erythromycin was 15 μ g and the same concentration was established for inclusion complexes. Filter paper discs (4 mm in diameter) were impregnated with solutions of the complexes erythromycin - β -cyclodextrin and placed on seeded plates [1]. As a control it was used a blank disk impregnated with methanol, used as solvent. The activity was determined after a 24 hours incubation at 37°C [7, 9,13].

Results and Discussion

We examined the Petri dishes after 24-48 hours and recorded the diameter of the inhibition zones for the test-microorganism (mm). The results are illustrated in figure 2.

In accordance with the requirements of quality control, we used standard M100-S16, 2006, *Staphylococcus aureus* ATCC 25923, with the 15 μ g erythromycin disk which should be within the diameter of inhibition between 22 - 30mm [2, 4, 6, 8, 15, 17].

The sensitivity of *Staphylococcus aureus* ATCC 25923 was higher than the tested complexes, compared with the standard antibiotic erythromycin. Each sample was used in triplicate in order to determine the antibacterial activity.

The results are presented in table I and represent the antibacterial activity of the three-cyclodextrin erythromycin nanoscale systems (noted P1, P2, P3) obtained by different methods.

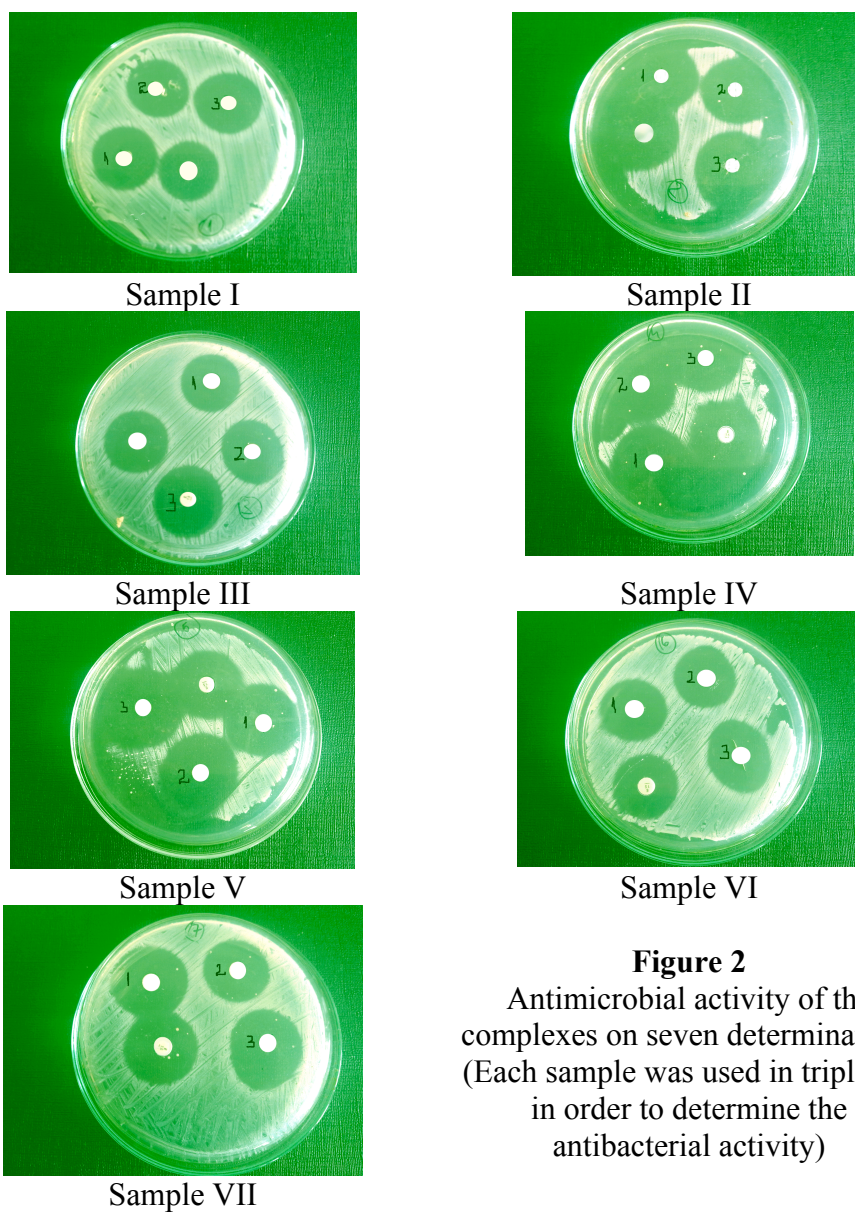


Figure 2
Antimicrobial activity of the
complexes on seven determinations
(Each sample was used in triplicate
in order to determine the
antibacterial activity)

Our determinations showed that there is an inhibitory activity on the tested bacteria in the presence of the studied nanoscale systems.

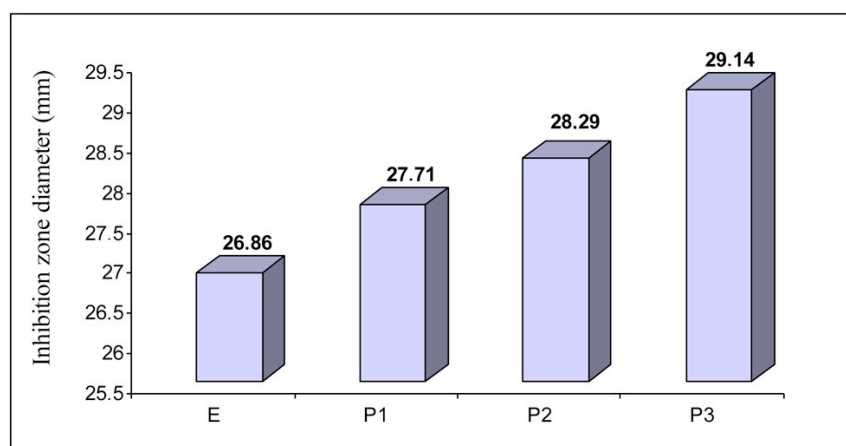
The highest antimicrobial activity was registered by the complex P3, obtained by co-precipitation, with the inhibition zone diameter 29.14 ± 1.86 mm, followed by sample P2 (freeze-drying), the inhibition zone diameter being 28.29 ± 2.28 mm (Table I).

Table I

The values of inhibition zones of erythromycin- β -cyclodextrin complexes

No.sample	E(mm)	P1(mm)	P2(mm)	P3(mm)
I	25	25	27	27
II	30	30	30	30
III	26	25	27	27
IV	30	31	34	30
V	28	30	30	34
VI	23	25	25	26
VII	26	28	25	30
	26.86$\pm$1.85	27.71$\pm$2.03	28.29$\pm$2.28	29.14$\pm$1.86

E-standard, P1-kneading, P2-freeze-drying, P3-co-precipitation

**Figure 3**

Graphical representation of the inhibition zones diameter of the three complexes compared to the standard

E-standard, P1-kneading, P2-freeze-drying, P3-co-precipitation

In comparison with the standard reference - erythromycin 15 μ g/disc, the formed complexes showed an increased antibacterial activity at the same concentration/disc.

The antibacterial activity of the structural analogues of erythromycin has been reported by many authors [5].

In our paper we found that the complexes preserved the antibacterial activity of erythromycin.

Data are presented as the mean of seven measurements of both the tested products and the standard group.

There was a significant difference between the value for the kneading complex (P1) (27.71 $\pm$ 2.03mm) and standard erythromycin (26.86 $\pm$ 1.85 mm) ($p = 0.042$). When we compared the values for sample P2

(freeze-drying) ($28.29 \pm 2.28 \text{ mm}$) to the reference ($26.86 \pm 1.85 \text{ mm}$) important differences were recorded ($p = 0.020$). In comparison with the standard drug - erythromycin the mean value for co-precipitation complex (P3) ($29.14 \pm 1.86 \text{ mm}$) was significantly increased ($p < 0.001$) (figure 3).

By performing the statistical calculation of antimicrobial activity it was concluded that the samples comply with the quality control requirements (Figure 4).

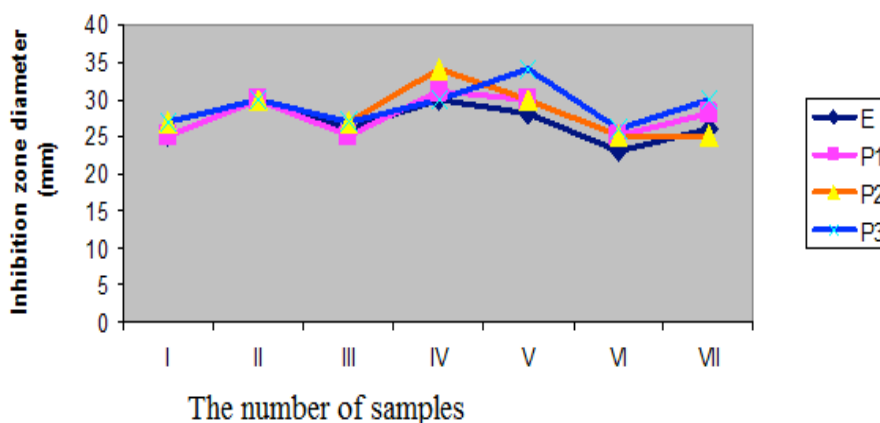


Figure 4

The values of inhibition zone diameter for the seven samples (I-VII)
E-standard, P1-kneading, P2-freeze-drying, P3-co-precipitation

Processing of experimental data allowed us to calculate the activity coefficient (r) of each sample representing the three complexes erythromycin - β - cyclodextrin obtained (Table II).

From the point of view of the antimicrobial activity, the studied samples were identical concerning quality and closely quantitatively to the standard.

Table II

Activity coefficient (r) of the antimicrobial activity of the complexes erythromycin - β - cyclodextrin

Samples	P1	P2	P3
Activity coefficient (r)	0.5696	0.5770	0.5742

P1-kneading, P2-freeze-drying, P3-co-precipitation

The obtained results allowed us the assertion that the antimicrobial action of inclusion complexes is reproducible in the condition that the

complexes were synthesized using the three methods: kneading, co-precipitation and freeze-drying.

Conclusions

Our determinations showed that the three complexes erythromycin- β – cyclodextrin had strong antimicrobial activities.

There was a significant difference between the complex erythromycin- β -cyclodextrin obtained by co-precipitation method compared to the complexes obtained by freeze-drying or by kneading.

The research points out that the antimicrobial activity of erythromycin and of the erythromycin- β –cyclodextrin complexes is therapeutically appropriated.

References

1. *** Farmacopeea Română, Ed. a X-a, Editura Medicala, Bucuresti, 2008
2. *** European Pharmacopoea 7th edition, 2011
3. Akujobi C.N., Ofodeme C.N., Enweani C.A., Determination of antibacterial activity of *Carica Papaya* (Paw-Paw) extracts, *Nigerian Journal of Clinical Practice*, 13(1), 2010, 55-57.
4. Akiyama H., Fujii K., Yamasaki O., Oono T., Iwatsuki K., Antibacterial Action of Several Tannins Against *Staphylococcus aureus*, *J Antimicrob Chemother.* 2001, 48(4), 487-91.
5. Blondeau J.M., DeCarolis E, Metzler K.L., Hansen G.T., The macrolides. *Expert Opin Investig Drugs*, 2002(11), 189–215.
6. Carson, C.F., K.A. Hammer and T.V. Riley, 1995, Broth micro-dilution method for determination of susceptibility of *Escherichia coli* and *Staphylococcus aureus* to the essential oil of *Malaleuca alterifolia* (Tea tree oil). *Microbios*, 82, 181-185.
7. Codiță I., Ghid național de aplicare a procedurii de testare a sensibilității la antibiotice conform standardului CLSI/NCCLS, versiunea 1-2006, Editura Universitara „Carol Davila”, Bucuresti, 2007.
8. Çolak S.M., Yapici B.M., Yapici A.N., Determination of antimicrobial activity of tannic acid in pickling process, *Romanian Biotechnological Letters* Vol. 15(3), 2010, 5325-5330.
9. Clinical and Laboratory Standard Institute, *Performance Standards for Antimicrobial Susceptibility Testing*, M 100 S 19, 2009, 29(3), 40-52.
10. Hasan M. F., Das R., Khan A., Hossain M.S., Rahman M., The Determination of Antibacterial and Antifungal Activities of *Polygonum hydropiper* (L.) Root Extract, *Advances in Biological Research* 3 (1-2)2009, 53-56.
11. Lakis Z., D. Mihele, I. Nicorescu, V. Vulturescu, D.I. Udeanu, The antimicrobial activity of *Thymus Vulgaris* and *Origanum Syriacum* essential oils on *Staphylococcus Aureus*, *Streptococcus Pneumoniae* and *Candida Albicans*, *Farmacia*, 2012, 60(6), 857–865.
12. Marian E., Jurca T., Vicas L. Kacso I., Miclaus M., Bratu I., Inclusion compounds of erythromycin with β -cyclodextrin, *Rev. Chim. (Bucharest)*, 62(11), 2011, 1065-1068.
13. NCCLS: Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved Standard NCCLS Publication M2-A5, Villanova, PA, USA, 1993.
14. Raicu A.D., Mihele D.E., Ionita C., Nistorica V., Manea S. - Antimicrobial activity of *Ribes nigrum*, *Rosmarinus offinalis*, *Betula pubescens*, *Salix alba*, *Vaccinium myrtillus* gemoderivates, *Farmacia*, 2010, 58(6), 735–748.

15. Subedi S, Brahmadathan K.N., Antimicrobial susceptibility patterns of clinical isolates of *Staphylococcus aureus* in Nepal. *Eur Soc Clin Microbiol and Infect Dis* 2005(11), 235-237.
16. Şuta L-M, Vlaia L., Vlaia V., Olariu I., Hădărugă D. I., Mircioiu C., Study of the complexation behavior of tenoxicam with cyclodextrins, *Farmacia*, 2012, 60(4), 475-483.
17. Yilmaz G, Aydin K, Iskender S, Caylan R, Koksall I., Detection and prevalence of inducible resistance in staphylococci. *J Med Microbiol*, 2007(56), 342-345.

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