PHYSICAL CHEMISTRY 2014

12th International Conference
on Fundamental and Applied Aspects of
Physical Chemistry

The Conference is dedicated to the
25. Anniversary of the Society of Physical Chemists of Serbia

---

September 22-26, 2014
Belgrade, Serbia

PHYSICAL CHEMISTRY 2014

12th International Conference
on Fundamental and Applied Aspects of
Physical Chemistry

Proceedings
Volume I

The Conference is dedicated to the
25. Anniversary of the Society of Physical Chemists of Serbia

---

September 22-26, 2014
Belgrade, Serbia

ISBN 978-86-82475-30-9

Title: PHYSICAL CHEMISTRY 2014 (Proceedings)
Editors: Ž. Čupić and S. Anić
Published by: Society of Physical Chemists of Serbia, Studenski trg 12-16, 11158, Belgrade, Serbia
Publisher: Society of Physical Chemists of Serbia
For Publisher: S. Anić, President of Society of Physical Chemists of Serbia
Printed by: "Jovan" Priting and Publishing Company; 200 Copies;
Number of pages: 6+ 441; **Format**: B5; Printing finished in September 2014.

Text an Layout: "Jovan"

200 - *Coppy priting*

SYNTHESIS AND CHARACTERIZATION OF LEVAN-AMPHOTERICIN B CONJUGATE

B. Kekez[1], G. Dj. Gojgić-Cvijović[2], D. M. Jakovljević[2]
V. P. Beškoski[1,2], M. M. Vrvić[1,2]

[1]Faculty of Chemistry, University of Belgrade, 11158 Belgrade, Studentski trg 16, POB 51, Serbia; [2]Institute of Chemistry, Technology and Metallurgy, University of Belgrade, 11158, Belgrade, Njegoševa 12, POB 473, Serbia (djakovlj@chem.bg.ac.rs)

ABSTRACT

In this work, microbial polysaccharide levan was functionalized by introducing aldehyde groups in the glycan molecule and coupled with polyene antibiotic amphotericin B. Resulting conjugate was characterized by spectroscopic data (UV-Vis and FT-IR) and by results of elemental analysis.

INTRODUCTION

Levan is nonlinear polyfructan with (2,6)-linked β-fructosyl units and (2,1)-branches (Fig.1). Due to the high molecular mass and solubility in water this polysaccharide has many potential uses in different industries [1]. The selection of levan in the synthesis of drug derivatives is justified on the basis of its blood biocompatibility, solubility and biodegradability. Amphotericin B is a polyene antifungal antibiotic (Fig. 2), macrocyclic lactone, produced by *Streptomyces nodosus*. It is used in medicine intravenously, orally and parenterally, however its insolubility in water leads to undesirable side effects [2].

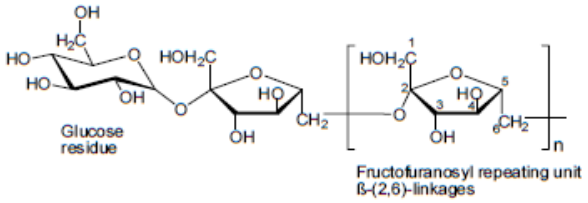

Figure 1. Levan

The aim of the present work was synthesis and spectroscopic characterization of amphotericin B conjugate with activated levan obtained by introducing aldehyde groups in the glycan chain in order to obtain drug with improved solubility in water which would potentially expanded the use of this antibiotic without harmful side effects.

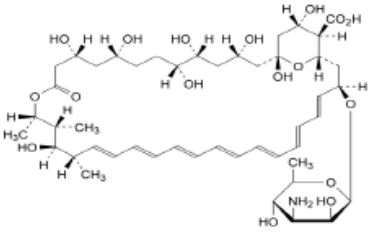

Figure 2. Amhotericin B

EXPERIMENTAL

Levan used in this work was produced by *Bacillus licheniformis* NS032 [3]. Polyaldehyde derivative of this fructan was obtained by reaction with periodate salts in distilled water. Coupling reaction between oxidized levan and amphotericin B was performed in 0.1 M borate buffer (pH 11.0) in the dark, at 40°C, with stirring, during 48 h. Resulting conjugate was purified from unbounded molecules by dialysis (dialysis tubing MWCO 8,000 obtained from Sigma Aldrich Co) against distilled water until negative probe on antibiotic in the surrounding water. Conjugate then was lyophilized (Christ Alpha 2-4 LD plus lyophilizer). Characterization of synthesized product was performed by UV-Vis (GBC Cintra 40 spectrophotometer) and FT-IR (Thermo-Nicolet instrument, Model 6700 equipped with Smart Orbit Diamond ATR accessory) spectral techniques and by the results of elemental analysis, too.

RESULTS AND DISCUSSION

Synthesis of levan-amphotericin B conjugate was done by coupling reaction between aldehyde groups of oxidized polysaccharide and amine groups of antibiotic (Fig. 3).

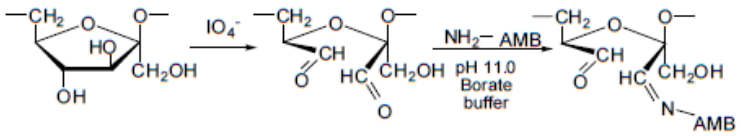

Figure 3. Synthesis of levan-amphotericin B conjugate (AMB-amphotericin B)

In UV-Vis spectrum of oxidized levan no absorption bands were observed (Fig. 4), however, spectrum of synthesized conjugate (Fig. 4) was significantly different, with the typical absorption peaks in the region 340 nm - 430 nm, that characteristic for amphotericin B (Fig. 5). On the basis of

the spectroscopic data it can be concluded that polyaldehyde polymer of levan was successfully coupled with polyene antibiotic amphotericin B.

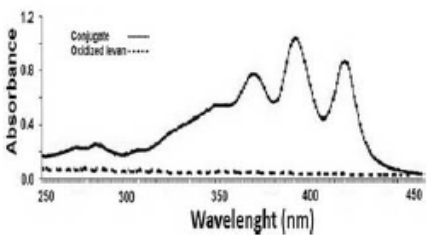

Figure 4.
UV-Vis spectrum of of conjugate and oxidized levan

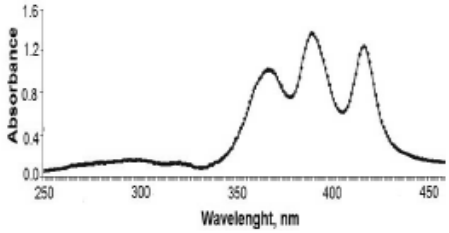

Figure 5.
UV-Vis spectrum of amphotericin B

Elemental analysis showed the content of nitrogen in the synthesized product, unlike the starting aldehyde-functionalized fructan that did not contain nitrogen. Results of elemental analysis were shown in Table 1.

Table 1. Results of elemental analysis

	% N	% C	% H
Polyaldehyde levan	/	44.40	6.04
Conjugate levan-amphotericin B	3.46	49.12	6.55

The synthesized levan-amphotericin B conjugate was further characterized by FT-IR data (Fig. 6). The FT-IR spectrum of product showed signals specific for polyaldehyde fructan and antibiotic. In the FT-IR spectrum of conjugate among the signals characteristic for levan [4] and band at 1730 cm^{-1} specific for carbonyl group in polyaldehyde glycan, peaks at 1449 cm^{-1}, 1401 cm^{-1}, and 1010 cm^{-1} can be observed relating to $-CH_3$-CH_2, $-COO^-$ and -C-C-H for chromophore, respectively, characteristic for the peaks of amphotericin B [5]. The strong absorbtion at 1604 cm^{-1}, relating to imine stretching vibration confirmed that amphotericin B has been covalently coupled to oxidized levan.

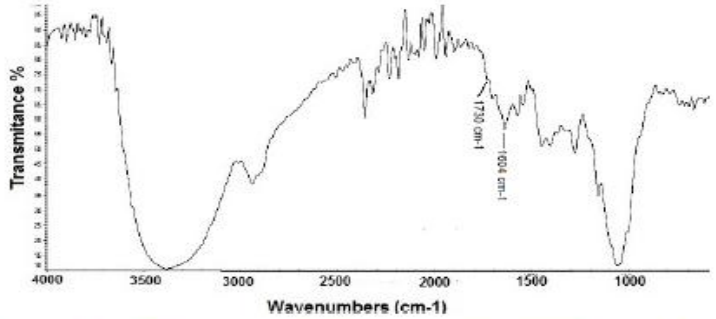

Figure 6. FT-IR spectrum of levan - amphotericin B conjugate

CONCLUSION

In this work a conjugate of aldehyde-functionalized fructan and polyene antibiotic amphotericin B was synthesized by reaction of covalent coupling. Resulting product was characterized by spectroscopic data (UV-Vis and FT-IR) and by the results of elemental analysis.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Education and Science of the Republic of Serbia through Project III 43004.

REFERENCES

[1] F. Freitas, V. D. Alves, M. A. M. Reis, Trends Biotechnol., 2011, 29, 388-398.
[2] R. Laniado-Laborín, M.N. Cabrales-Vargas, Rev. Iberoam. Micol., 2009, 223–227.
[3] G. D. Gojgić-Cvijović, J. S. Milić, T. M. Šolević, V. P. Beškoski, M. V. Ilić, L. S. Djokić, T. M. Narančić, M. M. Vrvić, Biodegradation, 2012, 23, 1-14.
[4] N. L. A. Kee, C. L Frost, B. I. Pletschke, Bioresources, 2012, 7, 4976-4993.
[5] M. Gagoś, M. Arczewska, Biochim. Biophys. Acta, 1798 (2010) 2124–2130.

SYNTHESIS AND CHARACTERIZATION OF LEVAN-AMPHOTERICIN B CONJUGATE

B. D. Kekez[1], G. Dj. Gojgić-Cvijović[2], D. M. Jakovljević[2A], V. P. Beškoski[1,2], M. M. Vrvić[1,2]

[1]*Faculty of Chemistry, University of Belgrade, 11158 Belgrade, Studentski trg 16, P.O. Box 51, Serbia;*
[2]*Institute of Chemistry, Technology and Metallurgy, University of Belgrade, 11158 Belgrade, P.O. Box 473, Njegoševa 12, Serbia;*

[a]djakovlj@chem.bg.ac.rs

Introduction

Levan is nonlinear polyfructan with (2,6)-linked β-fructosyl units and (2,1)-branches (Fig.1). Due to the high molecular mass and solubility in water this polysaccharide has many potential uses in different industries [1]. The selection of levan in the synthesis of drug derivatives is justified on the basis of its blood biocompatibility, solubility and biodegradability.

Amphotericin B is a polyene antifungal antibiotic (Fig. 2), macrocyclic lactone, produced by *Streptomyces nodosus*. It is used in medicine intravenously, orally and parenterally, however its insolubility in water leads to undesirable side effects [2].

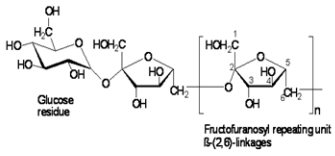

Figure 1. Levan

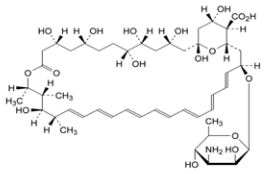

Figure 2. Amphotericin B

Aim

The aim of the present work was synthesis and spectroscopic characterization of amphotericin B conjugate with activated levan obtained by introducing aldehyde groups in the glycan chain in order to obtain drug with improved solubility in water which would potentially expanded the use of this antibiotic without harmful side effects.

Experimental

Levan used in this work was produced by *Bacillus licheniformis* NS032 [3]. Polyaldehyde derivative of this fructan was obtained by reaction with periodate salts in distilled water. Coupling reaction between oxidized levan and amphotericin B was performed in 0.1 M borate buffer (pH 11.0) in the dark, at 40°C, with stirring, during 48 h. Resulting conjugate was purified from unbounded molecules by dialysis (dialysis tubing MWCO 8,000 obtained from Sigma Aldrich Co) against distilled water until negative probe on antibiotic in the surrounding water. Conjugate then was lyophilized (Christ Alpha 2-4 LD plus lyophilizer). Characterization of synthesized product was performed by UV-Vis (GBC Cintra 40 spectrophotometer) and FT-IR (Thermo-Nicolet instrument, Model 6700 equipped with Smart Orbit Diamond ATR accessory) spectral techniques and by the results of elemental analysis, too.

References

[1] F. Freitas, V. D. Alves, M. A. M. Reis, Trends Biotechnol., 2011, 29, 388-398.
[2] R. Laniado-Laborín, M.N. Cabrales-Vargas, Rev. Iberoam. Micol., 2009, 223–227.
[3] G. D. Gojgić-Cvijović, J. S. Milić, T. M. Šolević, V. P. Beškoski, M. V. Ilić, L. S. Djokić, T. M. Narančić, M. M. Vrvić, Biodegradation, 2012, 23, 1-14.
[4] N. L. A. Kee, C. L Frost, B. I. Pletschke, Bioresources, 2012, 7, 4976-4993.
[5] M. Gagoś, M. Arczewska, Biochim. Biophys. Acta, 1798 (2010) 2124–2130.

Acknowledgement

This work was supported by the Ministry of Education and Science of the Republic of Serbia through Projects III 43004.

Results and discussion

Synthesis of levan-amphotericin B conjugate was done by coupling reaction between aldehyde groups of oxidized polysaccharide and amine groups of antibiotic (Fig. 3).

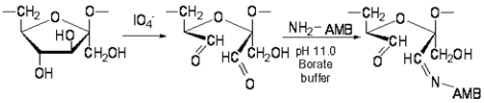

Figure 3. Synthesis of levan-amphotericin B conjugate (AMB-amphotericin B)

In UV-Vis spectrum of oxidized levan no absorption bands were observed (Fig. 4), however, spectrum of synthesized conjugate (Fig. 4) was significantly different, with the typical absorption peaks in the region 340 nm - 430 nm, that characteristic for amphotericin B (Fig. 5). On the basis of the spectroscopic data it can be concluded that polyaldehyde polymer of levan was successfully coupled with polyene antibiotic amphotericin B.

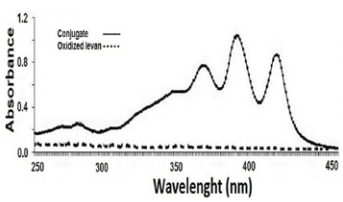

Figure 4. UV-Vis spectrum of conjugate and oxidized levan

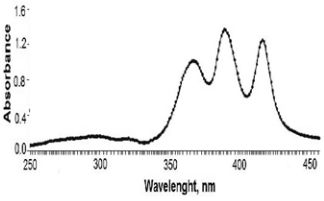

Figure 5. UV-Vis spectrum of of amphotericin B

Elemental analysis showed the content of nitrogen in the synthesized product, unlike the starting aldehyde-functionalized fructan that did not contain nitrogen. Results of elemental analysis were shown in Table 1.

Table 1. Results of elemental analysis

	% N	% C	% H
Polyaldehyde levan	/	44.40	6.04
Conjugate levan-amphotericin B	3.46	49.12	6.55

The synthesized levan-amphotericin B conjugate was further characterized by FT-IR data (Fig. 6). The FT-IR spectrum of product showed signals specific for polyaldehyde fructan and antibiotic. In the FT-IR spectrum of conjugate among the signals characteristic for levan [4] and band at 1730 cm^{-1} specific for carbonyl group in polyaldehyde glycan, peaks at 1449 cm^{-1}, 1401 cm^{-1}, and 1010 cm^{-1} can be observed relating to $-CH_3$-CH_2, $-COO^-$ and -C-C-H for chromophore, respectively, characteristic for the peaks of amphotericin B [5]. The strong absorbtion at 1604 cm^{-1}, relating to imine stretching vibration confirmed that amphotericin B has been covalently coupled to oxidized levan.

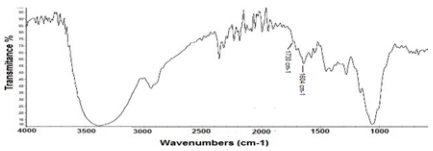

Figure 6. FT-IR spectrum of levan - amphotericin B conjugate

Conclusion

In this work a conjugate of aldehyde-functionalized fructan and polyene antibiotic amphotericin B was synthesized by reaction of covalent coupling. Resulting product was characterized by spectroscopic data (UV-Vis and FT-IR) and by the results of elemental analysis.