Some infectious agents frequently act on human body. Multi-drug resistant microorganisms (MDR) develop the capacity to stabilize biofilms. The use of antimicrobials loaded nanoparticles can defeat antibiotic resistance mechanism. The major aim of this study was the synthesis and physico-chemical characterization of Colistin molecules intercalated nanoparticles in order to enhance antibiotic efficacy against multi-drug resistant microorganisms. Advanced characterization techniques were used to analyze new nanostructures containing antibiotics in order to improve antimicrobial efficacy of the free drug. Nano-encapsulated Colistin is presumed to be more efficient in the eradication of severe infections caused by MDR.

Keywords: nanoparticles, Colistin, infection, sepsis, pediatrics, epidemiology, neurosurgery, cardiology, pulmonology.

Colistin is an antibiotic possessing a bactericidal effect against Gram (-) bacteria being used since 1950s. Yet, plenty of side effects such as nephrotoxicity and neurotoxicity effect and the existence of safer alternatives limited the drug administration. Although the usage of this antibiotic was interdicted for many years due to its severe toxicities, it was reintroduced again due to occurrence of multi-drug resistant (MDR) microorganisms. These pathogens strength is expressed as microorganism resistance to at least three groups of antibiotic molecules with peculiar activity against Gram (-) bacteria. This stringent issue of antibiotic resistance and the low rate of new drugs development generated reconsideration of Colistin usage [1-6].

Nosocomial infections caused by MDR infectious agents increased remarkably representing at the moment a common global problem being a real problem for epidemiology field. There are incoming threats to current medicine from the appearance of MDR causing healthcare associated infections to people having immunity deficiencies of all ages from prematures, new born, pregnant, confinement after birth to young and old people respectively.

Sepsis is a worldwide healthcare matter and a big challenge that healthcare specialists deals with. In last few years, it became a major cause of death and incidence increased risk of developing antimicrobial resistance [7-21]. Antimicrobial treatment must be administrated when sepsis is suspected. Sites involvin sepsis are respiratory tract, genitourinary tract abdomen soft tissue infections, injuries, the central nervous system and the cardiovascular system concerning different specialists in pediatrics, neurosurgery, cardiology especially pulmonology areas. Even in some cases the source is unknown, most of pathogens depend on infection area [22-26].

Many countries use in clinical practice antibiotics such as penicillin, aminoglycoside, quinolone, cephalosporin and carbapenem for Gram (-) bacterial infection treatment. Increased prevalence regarding Gram (-) bacteria resistance against novel antibiotic including carbapenem was reported mostly in developed countries. The interest for old antibiotic polymixins was reopened as saving therapy in the management of infection caused by MDR pathogen agents including Pseudomonas, Klebsiella, Acinetobacter and Enterobacter species. Polymyxins represent a group of cationic polypeptide antibiotics with five different compounds namely polymyxin A-E, Colistin being denoted as pylymyxin E in figure 1. It is a multicomponent antibiotic mixture, the two important constituents of which are Colistin A and Colistin B with one carbon included in the fatty acyl chain distinguishing the two components.

![Fig. 1. Chemical structures of colistin](http://www.revistadechimie.ro)
Materials and methods
Preparation of Colistin loaded liposomes by dry film method

For liposomes preparation by dry film method, 500 mg of dioleoylphosphatidylcholine (DOPC) was dissolved in 1.5 mL of chloroform:methanol (in a volume ratio of 2:1) with cholesterol at a molar ratio of 2:1 DOPC : cholesterol. Then, the solvent was eliminated under vacuum using a rotary evaporation and the dry lipid film was washed using a continuous stream of nitrogen gas in order to remove trace solvent.

The obtained dry film was dispersed in 10 mL of Colistin solution and stirred until the lipid was entirely dispersed.

Empty liposomes were obtained by hydration of the lipid film with ultrapure water. Finally, liposomes were sonicated for 15 min on a cooled water bath.

Preparation of Colistin loaded liposomes by freeze-drying method

Freeze-drying method using t-butanol cosolvent system was used to prepare liposomes containing Colistin. 200 mg of DOPC were dissolved in 5 mL t-butanol to prepare phase A. Phase B was prepared by dissolution of Colistin in 15 mL water containing 100 mg/mL sucrose. Then, 10 mL of phase B was added to phase A and shaked until clear. The obtained product was iced at -80°C for 8 hours then loaded into a freeze drier with a shelf temperature of -40°C. This step was carried out for 48 hours succeeded by a drying step at 25°C for 24 h.

Results and discussions
Zeta potential of liposomes prepared with increasing concentrations of Colistin is presented in figure 2. The liposomes assumed the predicted charge from cationic Colistin, denoting direct association of the direct coalition of the amphiphilic lipopeptide with the liposome nanostructure.

Increasing Colistin concentration did not had as consequence a linear increase in surface charge.

Figure 4a shows the encapsulation efficiency for the antibiotic with increasing concentration of Colistin in the obtained formulation by using both methods of preparing liposomes. At concentration of 20 mg/mL, there was a bit higher encapsulation part for the freeze-dried preparation. Obtained formulation by using both methods of preparing liposomes exhibit synergistic effect against Gram (-) bacteria beyond the activity of antibiotic alone. Liposomes can be designed to intercalate drug and merge with bacterial cells delivering their payload directly to the targeted cells. These new nanostructures type Colistin-liposomes maximize the administration of antibiotic by exhibiting superior penetration into biofilm and by controlling the release of the drug [37-41].

By dry film method and freeze-drying of liposome preparation were obtained liposomes with similar z-average size and z-average distribution with diameters around 190 nm and 160 nm respectively, as presented in figure 3.
Figure 4b proves that there is a straight relationship between molar ratio of Colistin:phospholipid with increasing of drug concentrations for both preparation process.

Figure 5 demonstrates differences in encapsulation efficiencies between Colistin loaded into liposomes prepared with or without cholesterol, with rising antimicrobial agent concentration. A higher encapsulation was obtained by inclusion of cholesterol to DOPC in molar ratio of 2:1.

In vitro release of the antibiotic from liposomes is presented in figure 6. For release of Colistin from liposomes was essential to dilute the dispersion into a release medium with a significant concentration and observe the resulting antibiotic release. After 10 min, half of drug content was free in the release medium and throughout the 72 h the equilibration of antibiotic concentration upon dilution was produced.

**Conclusions**

In last few years, the administration of Colistin for serious infections treatment caused by multi-drug resistant microorganisms increased worldwide. Due to their activity and biocompatibility, lipid nanoparticles represent an encouraging choice to the currently available therapy.

The adverse effects of the active antibiotic molecules could be reduced due to the controlled drug release. Based on sustained release of the drug and the uniform distribution through the target area, the efficacy of the antibiotic intercalated nanoparticles could be enhanced.

The results of this work revealed that Colistin loaded nanoparticles are efficient in treatment of health care-associated infections caused by multi-drug resistant pathogens.

**References**

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Manuscript received: 03.09.2019