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Inhancment of meropenem potency by methotrexate nanoparticles

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Abstract---The MTX was converted to MTX nanoparticles by the modified method based on changing the pH gradually . For the first time MTX NPs+Meropenem complex were prepared and evaluated as a potential tool to overcome antimicrobial resistance and to improve pharmacokinetics of the drug, the results showed that the antibacterial activity of complex (MTX NPs plus MEM) has increased (from 1(µg/ml) to <0.5(µg/ml) for p1 , from 2(µg/ml) to 1(µg/ml) for p10 and from 8(µg/ml) to 4(µg/ml) for p48).

Keywords---MTX, MTX NPs, UV-Vis spectra analysis, AFM, SEM, FTIR, MEM.

Introduction

Meropenem, under the brand name Merrem , is an intravenous β-lactam antibiotic used to treat a variety of bacterial infections, It is in the carbapenem family of medications[1]. Meropenem usually results in bacterial death through blocking their ability to make a cell wall[2]. The spectrum of action includes many Gram-positive and Gram negative bacteria (including Pseudomonas) and anaerobic bacteria. The overall spectrum is similar to that of imipenem, although meropenem is more active against Enterobacteriaceae and less active against Gram-positive bacteria[3].Meropenem exerts its bactericidal action by binding to penicillin-binding proteins (PBPs) in the bacterial cell wall and inhibiting peptidoglycan cross-linking associated with cell wall synthesis, which ultimately leads to cell death[4,5].

Multidrug resistant (MDR) bacteria have become an extremely serious public health concern, Bacteria can develop resistance through various mechanisms[6,7]. However, various types of NPs are still considered to be non-toxic and are used to reduce the toxicity hazards of other therapeutic agents[8].

Antibiotic resistance has become one of the biggest threats to public health worldwide due to the misuse and abuse of antibiotics. Combined use of nanoparticles (NPs) with conventional antibiotics constitutes a promising alternative for fighting antibiotic resistance as NPs could restore the activity of the existing antibiotics[9,10]. Interactions of antibiotics with nanoparticles is the most common among studies dedicated to the testing of combined action of nanoparticles with antibiotics and moreover some studies have found that the efficacy of antimicrobial agents can be enhanced by combining them with nanoparticles against different pathogens, including *Staphylococcus* aureus, *Pseudomonas aeruginosa* and *Escherichia* coli [11,12].

Methods

Methotrexate (MTX) nanoparticles preparation and characterization

Synthesis of methotrexate nanoparticles from methotrexate done in nanotechnology lab, Department of Biotechnology, College of Science, University of Baghdad by [13].The characterization of prepared nanoparticles were measured by Atomic force microscopy (AFM), Fourier-transform infrared spectroscopy (FTIR), Ultraviolet (UV) spectrophotometer and Scanning electron microscopy (SEM).

MTX nanoparticles plus Meropenem (MTX Nps-MEM) complex

In the current study, MTX Nps- MEM complex was prepared, which is a component of MTX nanoparticles plus Meropenem in a 1: 1 ratio, according to the following method:

- The mixture of 5 ml of MTX nanoparticles (MIC) concentration was prepared previously and 5 ml of Meropenem (16,8,4,2,1 and 0.5 µg/ml).
- The mixture was put in the ultrasonic path at 4500 kh for 25 min.
- The characterization of prepared complex nanoparticles was measured by Fourier-transform infrared spectroscopy (FTIR).

Determination of Minimal Inhibitory Concentration of MTX Nps- MEM complex against *Pseudomonas aerujinosa* by micr titer plate method

Micro-titer plate's method [14] with some modifications was used as the follows:

- *Pseudomonas aeruginosa* isolates were inoculated on nutrient broth and incubate for 24 hrs at 37°C.
- Mueller Hinton broth was prepared.
- Micro-titer plates of 96 flat well were used in this method.
- One hundred µL of prepared Mueller Hinton broth was placed in all wells of the plate (in the first horizontal line) except the last well (well number 12).
- Meropenem dilutions were prepared between: 0.5 -16µg/ml
- Meropenem plus Methotrexate nanoparticles concentrations were prepared by adding (MIC) of MTX NPs for dilutions of meropenem (16,8,4,2,1,0.5 µg/ml).

- One hundredμL of every dilution of Meropenem and Meropenem plus methotrexate nanoparticles complex was putted in the wells that market for each one and mixed very well
- Inoculums were prepared, by transferring 3-5 colonies into a tube of 5ml of normal saline to obtain culture with 1.5 × 10^8CFU/ml, and adjusting to turbidity standard of McFarland 0.5, suspensions were used within 30 min. of preparation.
- Ten μL of bacterial suspension was added to the wells .
- The positive and negative controls were prepared in Last line of plate .
- SixteenμL of resazurin pigment was added to all the wells.
- Plate was incubated at 30˚C for 18 – 24 hours.
- The lowest concentration of antimicrobial agent that completely inhibits the growth was considered the minimal inhibitory concentration (MIC).

Result

MTX nanoparticles preparation and characterization

The MTX was converted to Methotrexate nanoparticles by the modified method based on changing the pH gradually .The characteristics of MTX nanoparticles were done by using UV-Vis Spectroscopy, atomic force microscope (AFM), scanning electron microscope (SEM) and fourier-transform infrared spectroscopy (FTIR) analysis.UV-Vis spectra analysis showed absorption peak at 405nm for MTX NPs. The results of AFM showed the existence of a number of nanoparticles with different diameters, but the average of these diameters was 57.11 nanometres. SEM showed the prepared MTX nanoparticles were typically nanoparticles (57.11nm diameter) with spiny or needle shape ,FTIR showed the possible functional groups of biomolecules involved in MTX NPs .The above analysis proved strongly that the particles that prepared from MTX were typically nanoparticles[13].

Preparation and characterization of MTX NPS-MEM complex

MTX NPs-MEM complex were prepared by using the minimum inhibitory concentration (MIC) of MTX NPs (62.5 μg/ml) that added to serial concentration of Meropenem (MEM) (16,8,4,2,1 and 0.5 μg/ml) in 1:1 ratio of volume and Formation of complex confirmed by FTIR. The FTIR spectrum of meropenem (MEM) showed that absorption peaks located at about 3438.84-3409.91, 3001.03-2935.46, 1575.73, and 1423.37 cm -1 in the region 400-4000 cm-1 . The peaks at 3438.84-3409.91cm -1 assigned to O-H stretching and N-H stretching, The band at 3001.03-2935.46cm -1 associated with C-H stretching , The band at 1575.73cm -1 corresponds to the N-O stretching ,The peak at 1423.37 assigned to O-H bending as shown in figure (1).

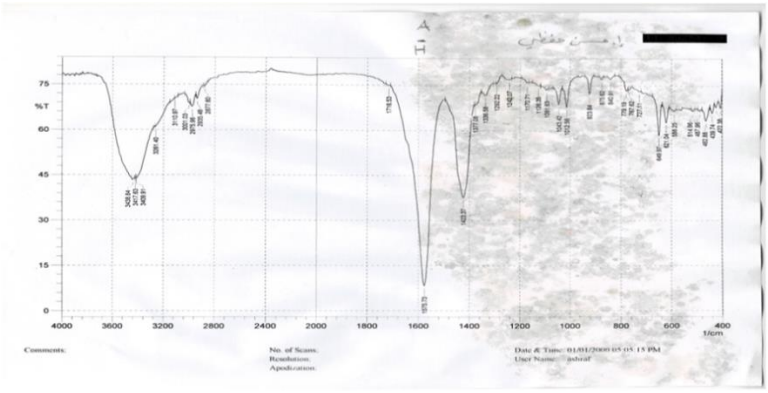

Figure 1. FTIR analysis of (meropenem)

The FT-IR spectrum of MTX-NPs + meropenem complex showed that absorption peaks located at about3427.27, 2360.71-2329.85, 1639.38 , 1556.45 , 1413.72 , and 642.25-524.60 cm -1 in the region 400-4000 cm-1 . The peaks at 3427.27 cm -1 assigned to O-H stretching and N-H stretching, The band at 2360.71-2329.85 cm -1 associated with the O=C =O, The band at 1639.38 cm -1 corresponds to the C=C stretching ,The peak at 1556.45 assigned to N-O stretching, The peaks at 1413.72 cm -1 assigned to O-H bending , and the peaks at 642.25-524.60 cm -1 assigned to metal oxygen , as shown in figure (2).

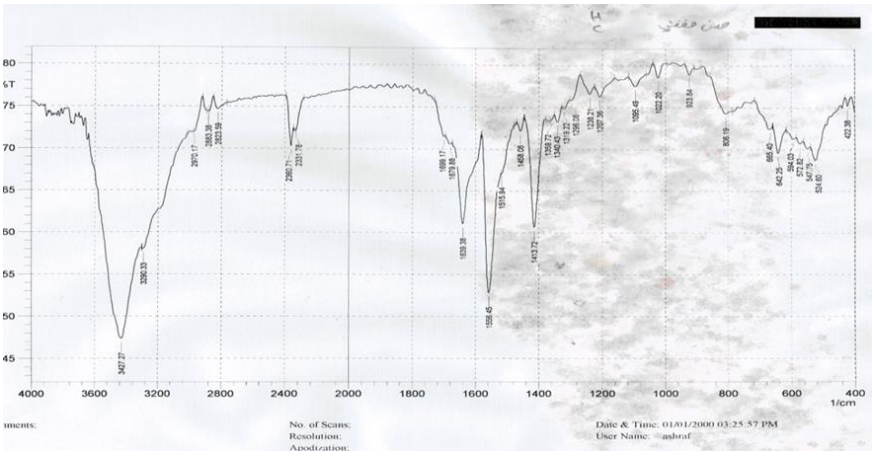

Figure 1.2. FTIR analysis of MTX NPs + meropenem complex

Table 1
Frequency of Absorption, Bonds and Functional group of MEM and MEM-MTX NPs complex

Materials	Frequency of Absorption (cm^{-1})	Bonds	Compound class of Functional Groups
MTX NPs +Mem complex	808.12	C-H bending	Alkene
	642.25-524.60	Metal Oxygen	-
	3427.27	O–H stretching N–H stretching	Alcohol, phenols, Amine

	2360.71-2331.78	O=C=O stretching	Carbon dioxide
	1639.38	C=C stretching	Alkene
	1556.45	N–O stretching	Nitro compound
Meropenem	1413.72	O-H bending	Alcohol
	642.25-524.60	Metal Oxygen	-
	3438.84-3409.91	O–H stretching N–H stretching	Alcohol, phenols, Amine
	3001.03-2935.46	C-H stretching	Alkane
	1575.73	N–O stretching	Nitro compound
	1423.37	O-H bending	Alcohol

Antibacterial effect of MTX NPs-MEM complex by micro titer plate

The minimum inhibition concentrations (MICs) of MTX NPs-MEM complex were evaluated against four *P. aeruginosa* isolates(three isolate p1,p10 and p48 were MDR and one isolate p29 was sensitive to antibiotics) by Microtiter plate , as shown in figure (3).

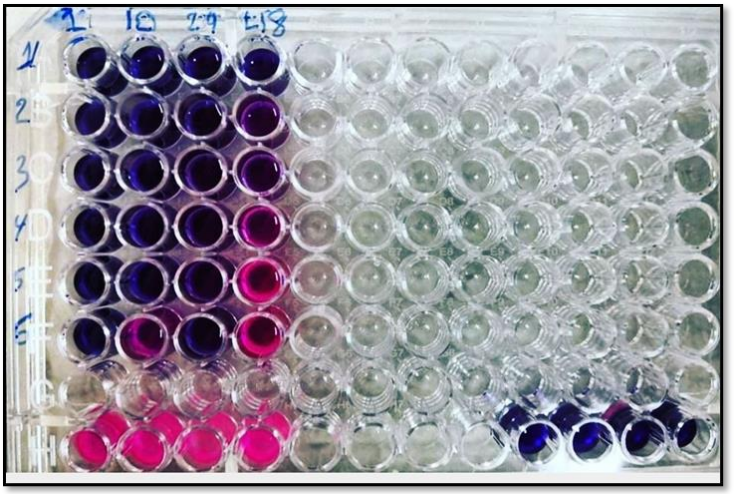

Figure 3. Antibacterial effect of MTX NPs-MEM complex by micro titer plate

The results of MICs for MTX Nps-MEM complex showed in table(2).

Table 2
The minimum inhibition concentration of MTX and MTX NPs

Isolate	MEM MIC (µg/ml)	Complex (MEM+MTX NPs) Conc (µg/ml)	Complex MIC (µg/ml)
p1	1	125+(16,8,4,2,1,0.5)	125+<0.5
p10	2	31.25+(16,8,4,2,1,0.5)	31.25+1
p29	<0.5	62.5+(16,8,4,2,1,0.5)	62.5+<0.5
p48	8	62.5+(16,8,4,2,1,0.5)	62.5+4

For the first time MTX NPs+Meropenem complex were prepared and evaluated as a potential tool to overcome antimicrobial resistance and to improve pharmacokinetics of the drug, the results showed that the antibacterial activity of complex (MTX NPs plus MEM) has increased (from 1(µg/ml) to <0.5(µg/ml) for p1 , from 2(µg/ml) to 1(µg/ml) for p10 and from 8(µg/ml) to 4(µg/ml) for p48) as shown in the table(3.5).[11] observed that meropenem and ZnO NPs showed indifference effect on *P. aeruginosa* . The result by [15] that showed a high efficiency Meropenem silver nanoparticles (mer-AgNPs) that increased antibacterial activity against both Gram-positive and Gram–negative bacteria and [16] showed a high loading efficiency of Meropenem into mesoporous silica nanoparticles (MSN–NH_2–MEP) against multidrug-resistance (MRD) *A. baumannii*. In other hand [17] the enhanced activity of antibiotics combined with silver NPs, especially meropenem, was weaker against non-resistant bacteria than against resistant bacteria and [18] showed a higher antibacterial activities of the drug-loaded nanoparticles (meropenem-loaded chitosan nanoparticles) were observed against methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*, as compared to the free drug.[19] was found that VAuNPs in combination with meropenem better potentiation effects against *P. aeruginosa* than that of vanillin alone . This study observed the effect of MTX NPs on the potency of meropenem against *P.aeruginosa* and indicate the enhancement of meropenem potency when used as complex(meropenem+MTX NPs).

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