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Synthesis and characterization of methotrexate nanoparticles

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Abstract--The MTX was converted to MTX nanoparticles by the modified method based on changing the pH gradually with exposure to ultrasound and shaking, changing the pH with exposure to ultrasound plays an significant role in the formation of nanoparticles, and this is shown in some previous studies. As the change in pH affects the nature of bonding between molecules, as well as the strength of bonding that depends on the change of electrical charges. The exposure to ultrasound waves will greatly affect the breakdown of large particles into small particles that reach the level of nanoparticles. The MTX NPs formation was characterized by UV-Vis spectra analysis, Atomic force microscopy (AFM) analysis, Scanning electron microscope (SEM) and Fourier-transform infrared spectroscopy (FTIR), which indicate that MTX NPs formation was in Nano sized (57.11 nm) with spiny or needle shape.

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

Introduction

An anionic anti-cancer medication known as methotrexate (2,4-diamino-N10-methyl propylglutamic acid, MTX)[1] is widely known for the success it has had treatment a number of cancers, including head and neck cancer, lymphoma, leukemia, breast, osteosarcoma, and lung cancer [2,3]. Additionally, it is used to treat autoimmune and inflammatory conditions such Crohn's disease, rheumatoid arthritis, psoriasis, and multiple sclerosis [4]. Among hydrophobic medications, methotrexate (MTX) is now used to treat a variety of illnesses[5]. A synthetic counterpart of folic acid called MTX is an antimetabolite that suppresses the immune system [6]. Multiple sclerosis, psoriasis, and rheumatoid arthritis are inflammatory disorders that are treated with MTX due to its

immunomodulation and anti-inflammatory properties[7]. A chronic inflammatory skin disease known psoriasis is rather common and is characterized by an inflammatory invasion and hyperproliferative keratinocytes[8]. Additionally, MTX demonstrated epidermal proliferation inhibitory properties and is the go-to treatment for many cutaneous disorders. MTX is a routinely given medication for moderate to severe psoriasis, despite the possibility that using it could result in unfavorable side effects include hepatotoxicity and myelosuppression[9]. The topical application of MTX, particularly in people with mild psoriasis, may be able to reduce these side effects.

Numerous trials have tried different topical MTX formulations, with encouraging but not totally satisfying outcomes. MTX's high molecular weight, poor solubility in ointment formulations, ionized nature at physiological pH, and a reduction in the passive diffusion through layers of skin, especially in psoriatic plaques, which are distinguished by increased epidermal thickness, were the main causes of its insufficient percutaneous penetration [5]. [10] evaluated the therapeutic uses of MTX administration via several Nano vehicles, noting the potential to lessen side effects such toxicity. Its topical distribution has been improved by the use of several formulations. The methods used ranged from iontophoresis, liposomes, nanogels, and lipid carriers to penetration enhancer sticky tapes as an occlusive covering as well as the use of adhesive tapes as occlusive coverings and penetration enhancers[5].

Although MTX is often used as an efficient medication to treat a variety of cancers, autoimmune disorders, and inflammatory illnesses, there are certain restrictions on its usage. Due to its weak water solubility and limited permeability, MTX has a low bioavailability[11]. Additionally, Due to the medication's quick and thorough renal clearance, MTX has a short half-life and low drug concentration in target tissues. [12]. The immune response and cancer metastasis are both significantly influenced by the lymphatic system[13]. MTX is an anti-cancer and immune-related agent, as was already noted. The immune system and the transport of MTX to the lymphatic system are tightly connected. Through diffusion through the intercellular space of porous lymphatic walls, nano-sized medicines can be delivered to the lymphatic system[14]. Reduced MTX dosage requirements and increased therapeutic benefit from nanoscale drug carriers can minimize adverse effects by improving target delivery of MTX.

Polymer-based NP has been researched in many different methods. Numerous investigations have shown that the cytotoxic effects of drug polymeric NPs are superior to those of free drugs[15]. There is a limit to how consistently you can get outcomes in vivo. As far as we are aware, there have been few research on the pharmacokinetics (PKs) and distribution of prepared formulations to the lymphatic system or target/normal tissues. In order to increase MTX's bioavailability, selectivity, and lower its toxicity when administered orally and intravenously (IV), the goal of this work was to create MTX-loaded NPs.

Methods

Methotrexate (MTX) nanoparticles preparation

The standard method of [16] was followed with novel modification in nanotechnology lab, Department of Biotechnology, College of Science, University of Baghdad. Vial of Methotrexate (5ml) was added to 50 ml of deionized distilled water and put in ultrasonic (4500 kh for 25 second) for mixing and then pH was adjusted to 10 by adding NaoH (1 N) and left an hour and put in ultrasonic path . The pH was readjusted to 4 by HCl (1N) and left for an hour. The pH of mixture was readjusted to 7 by NaoH (1 N) for an hour. 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 NPs characterization

1UV-Vis spectra analysis

By determining the wavelength of the MTX solution in the UV-VIS spectrum of a spectrophotometer with a resolution of 1 nm in a 2 ml with quartz cuvette a 1 cm path length, it was determined that the reaction mixture included MTX NPs. By utilizing a blank reference to adjust the spectrophotometer, The samples were scanned between 300 and 900 nm at a scan rate of 500 nm/min. All of the samples' UV-Vis absorption spectra were captured, and the numerical data were plotted[17]. This test was administered at the chemistry department's labs at the University of Baghdad's College of Sciences.

Atomic force microscopy (AFM) analysis

MTX nanoparticles was examined using atomic force microscopy. A silica glass plate was treated with a few drops of the synthesized MTX nanoparticles to form a thin layer, which was then left to dry in the dark at room temperature. After that, the AFM was used to scan the deposited film glass plate[18]. This examination was conducted in the laboratories of the Department of chemistry /College of Sciences, University of Baghdad.

Scanning electron microscope (SEM)

Investigating the typical particle size and form of nanoparticles a scanning electron microscopy approach. After being sonicated with distilled water, the dried sample of the MTX NPs solution was dropped onto a glass slide and let to dry. The samples were then coated with a thin layer of platinum to make them conductors [19].

Fourier-transform infrared spectroscopy (FTIR)

It is a technique for acquiring the infrared spectrum of an object's absorption, emission, or reflection., and photoconductivity. It is employed to find several functional groupings in MTX nanoparticles. FTIR spectrum is recorded between 4000 and 400 cm^{-1} . For FTIR analysis, the polymer was dissolved in chloroform

and layered on a NaCl crystal and after evaporation of chloroform, the polymer film was subjected to FTIR[20].

Result

MTX nanomaterial preparation

The MTX was converted to MTX nanoparticles by the modified method based on changing the pH gradually with exposure to ultrasound and shaking [16](figure 1), changing the pH with exposure to ultrasound plays an significant role in the formation of nanoparticles, and this is shown in some previous studies. As the change in pH affects the nature of bonding between molecules, as well as the strength of bonding that depends on the change of electrical charges The exposure to ultrasound waves will greatly affect the breakdown of large particles into small particles that reach the level of nanoparticles[21].

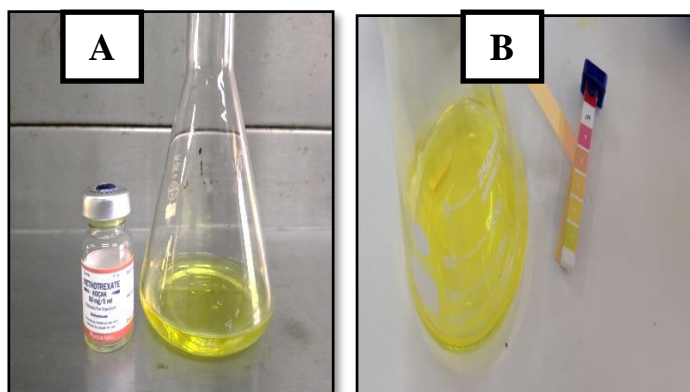


Figure 1. A: Methotrexate solution at Ph 9 B: Methotrexate solution at Ph 4

MTX NPs characterization

UV-Vis spectra analysis

The corresponding UV-Vis absorption spectra are showed in figure (2).The control solution (Supernatant of MTX nanoparticles solution) showed no evidence of absorption in the range 300 to 900 nm. The samples were exposed to the MTX NPs solution showed absorption peak around (405) nm. The wide resonance's presence indicates the aggregation of the MTX NPs nanoparticles in the solution. [22] observed that absorption peak emerged at (393.56 nm) for Ag MTX NPs and (389.43 nm) for PEG-Ag MTX NPs.

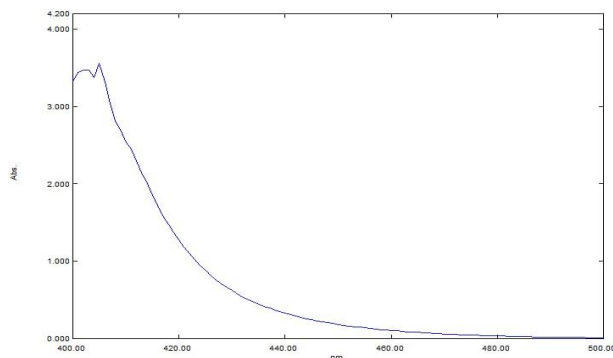


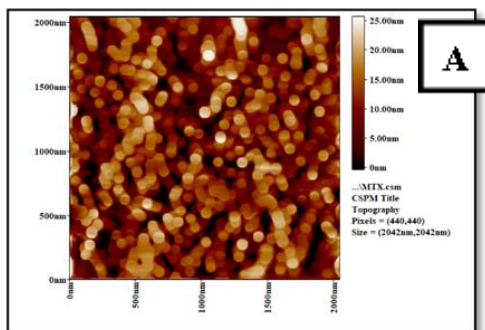
Figure 2. UV-Vis spectrophotometry of Methotrexate nanoparticles

Atomic force microscope (AFM)

The reaction mixture characterized by AFM. table (1) and figure (3) indicated that MTX NPs formed by Methotrexate solution was in a nano size (57.11 nm). Demonstrated that MTX NPs exhibit different shapes . On the other hand result of [23] showed that the HA-MTX NPs were well distributed with size of about 100 nm.

Table 1
The Cumulation size of Methotrexate Nanoparticles synthesis measured by AFM technique

Avg. Diameter:57.11 nm			<=10% Diameter:35.00 nm			<=50% Diameter:55.00 nm			<=90% Diameter:70.00 nm		
Diameter (nm)<	Volum e(%)	Cumulat ion(%)	Diameter (nm)<	Volum e(%)	Cumulat ion(%)	Diameter (nm)<	Volum e(%)	Cumulat ion(%)	Diameter (nm)<	Volum e(%)	Cumulat ion(%)
15.00	0.32	0.32	40.00	5.47	12.54	65.00	10.29	63.99			
20.00	0.64	0.96	45.00	8.36	20.90	70.00	12.22	76.21			
25.00	2.25	3.22	50.00	9.97	30.87	75.00	14.15	90.35			
30.00	0.96	4.18	55.00	10.61	41.48	80.00	9.65	100.00			
35.00	2.89	7.07	60.00	12.22	53.70						



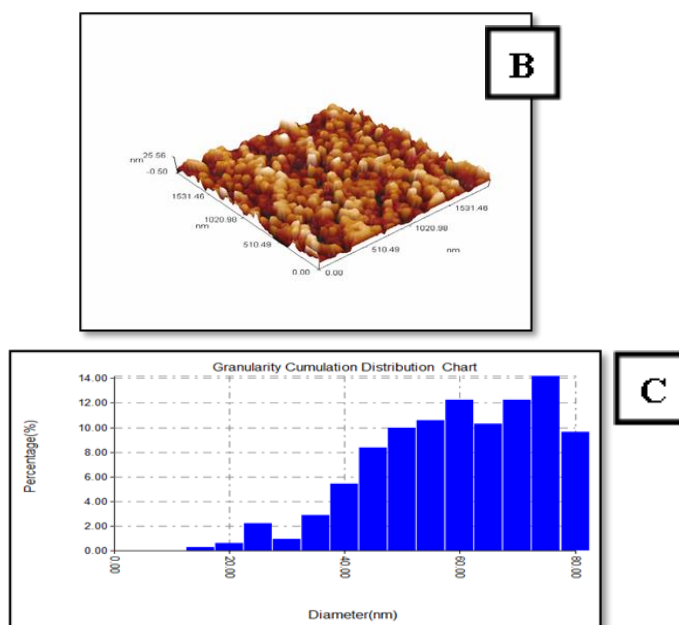
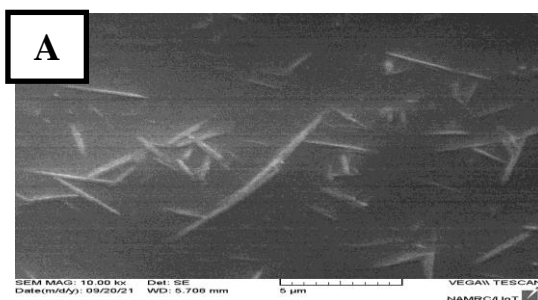


Figure 3. MTX NPs under AFM (a) 2D image of Methotrexate Nanoparticles synthesis (b) 3D image of Methotrexate Nanoparticles synthesis (c) Granularity Distribution Chart of Methotrexate nanoparticles synthesis

Scanning electron microscope (SEM)

The MTX NPs were analyzed under the SEM to determine their predicted size and shape after being described by UV-vis spectroscopy and AFM examinations; the results supported the development of MTX NPs. The SEM image analysis revealed the production of MTX nanoparticles. The morphology of the nanoparticles was spiny or needle shape and within the size range of (57.11) nm figure (4). On the other hand result of [24] showed that the form of MTX NPs under SEM was spherical with slight aggregation. Another study by [25] observed that the MTX-loaded PLGA Under SEM, NPs had a rather limited distribution and were spherical in shape.



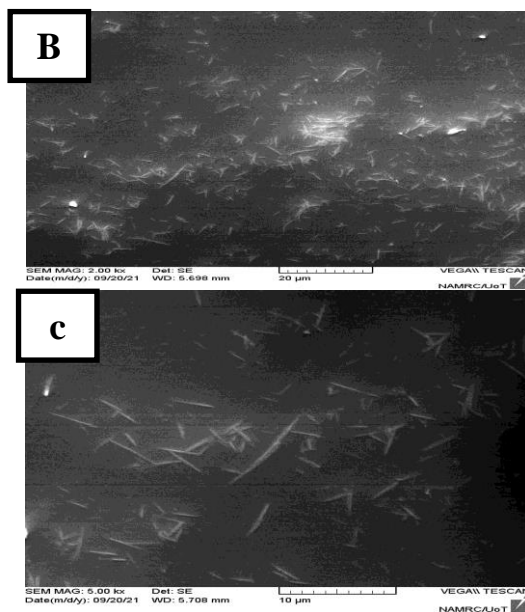


Figure 4. MTX NPs shape a: X5 ,b:X10 and c: X20 by SEM

Fourier-transform infrared spectroscopy (FTIR) analysis

To ascertain the potential functional groups of the biomolecules involved in MTX NPs . The FT-IR spectrum of methotrexate Nanoparticles demonstrated that they have absorption maxima at around 3433.06, 2360.71-2333.71, 1639.38 , 1560.30 , 1413.72 and 644.18-520.74 cm^{-1} in the region 400-4000 cm^{-1} . The peaks at 3433.06 cm^{-1} assigned to O-H stretching and N-H stretching, The band at 2360.71-2333.71 cm^{-1} associated with the O=O=O, The band at 1639.38 cm^{-1} corresponds to the C=C stretching ,The peak at 1560.30 assigned to N-O stretching, The peaks at 1413.72 cm^{-1} assigned to C-H bending , The peak at 1560.30 assigned to N-O stretching and the peaks at 644.18-520.74 cm^{-1} assigned to metal oxygen as shown in figure (5).

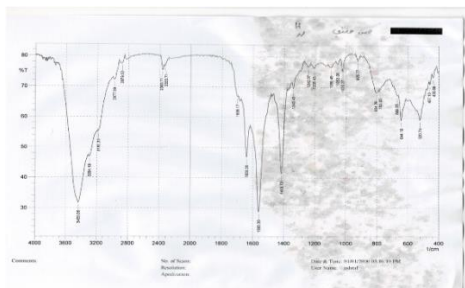


Figure 5. FTIR analysis of MTX nanoparticles

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