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Immunological Study in Vivo of Synthesis Nanoparticules used in Rheumatoid Arthritis

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Abstract— *The synthesis of a nanoparticules used in drug delivery plays an important role in determining its targeting specificity and efficacy in vivo. A conventional approach relies on the surface conjugation of a nano-sized particle with two functionally distinct types of molecules, one as a targeting ligand, and the other as a therapeutic agent to be delivered to the diseased cell. However, an alternative simplified approach can be used, in which a single type of molecule displaying dual function as both a targeting ligand and therapeutic agent is conjugated to the nanoparticle. In this paper, we evaluate the validity of this new strategy by using methotrexate(MTX) and xerogel- methotrexate, (xerogel-MTX), Naproxine(NAP.), xerogel-Naproxine (xerogel-NAP.) the aim in this paper to define the procedures of sample and the applicability of FTIR and AFM and UV-Visb. techniques towards the characterization of the surface details with sub-nanometer resolution in nanoparticles (NPs) modified by MTX. And NAP ligands. To reach this aim, we prepared and analysed xerogel, xerogel-NAP. Xerogel-MTX NPs functionalized on the surface with ligands having different chemical nature and composition and capable to provide to the NPs physical chemical properties required for specific application. We tested the resulted NPs in vivo, using the whit rats animals, engineered with direct against arteries Rheumatology inflammation, proceed the evolution of some immunity parameters during the period of treatments.*

Keyword— xerogel, FTIR, AFM, immunology , nanoparticules

I. INTRODUCTION

In many applications of biomaterials, the formulation of the biomaterial is specifically designed such that it is presented to the human body as an entity with dimensions at the nanoscale[1]. It is important that a material has distinctly different properties from the bulk material as a consequence of its occurrence as discrete entities for it to be considered as a nanomaterial. With this in mind, we can see that many of the developments in drug and, especially, gene delivery, during recent years have focused on the potentially far greater efficiency[2], in both functionality and targeting, that can be achieved at the nanoscale. In a similar manner, some of the biotechnological aspects of biomaterials, including agents for enhanced imaging and separation applications, will rely heavily on nanoscale properties. Nanoparticle-based targeted drug delivery carries several advantages over conventional chemotherapy[3], including specificity, solubility, increased retention time, and enhanced target drug concentration.[4] Despite the recent explosion in the production ofanti inflammatory a nano therapeutics, the application of most of these approaches in clinical studies has suffered significant. One of the reasons for this is the difficulty in producing consistent, large-scale batches of homogeneous multifunctional molecules, a problem that is exacerbated when multiple functionalities (e.g., a targeting molecule and a drug) are conjugated onto the surface of the nanoparticle[5]. for the delivery of chemotherapeutic drugs such as the anti folate methotrexate (MTX)[6]. Importantly, our prior studies have shown that the arthritis inflamation targeting of a conjugate Xerogel-MTX, Xerogel NAP. resulted in improved chemotherapeutic index as compared to free MTX and free NAP. [7-12] Xerogel. An alternative, which allows elaborating by using a sol-gel method as an intermediate step, with much lower temperatures than the usually needed by traditional methods, plus it is particularly efficient in produce transparent jel at relatively low cost [13]. Several efforts have been conducted in order to obtain sol-gel derived-(Xerogel) xerogel films, starting with the use of zinc nitrate and silver nitrate as metal precursor. A possible alternative is the incorporation of MTX. And NAP. As conjugated polymer into the silver- zinc-sol reduced by ethylene glycole [14,15], which not only could stabilize the sol, but also could reduce the xerogel during synthesis procedures, and, as also increments the viscosity of the sol, In this work, antibacterial and Ag/ZnO resulted (Xerogel) NPs, characterization of the Xerogel, Xerogel-MTX and Xerogel-NAP. By Infrared spectra to indicate the functional group on the surface of xerogel and the Xerogel conjugated. Previously we identified the romatology factor as main target organs for various nanomaterials after mouth up take administration [16-22].

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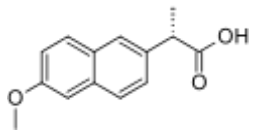
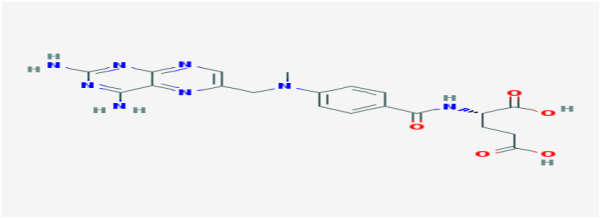
the behaviour to surface states in nanomaterials where the surfaces are becomes prime importance as the size decreases. Synthesized xerogel and then conjugated it with the solution of Methotrexate, N-_4-[(2,4-diaminopteridin-6-yl-methyl)methylamino]benzoyl_-L-glutamic acid, MTX (500mg) and naproxen(500mg) 50ml H_2O to obtain xerogeland - MTX) and (xerogel-NAP).



Naproxen



Methotrexate

II. MATERIALS AND METHODS

Preparation of nanoparticles

All NPs analysed were obtained according to the oxidation reductions of cations(Ag and Zn) via sol-gel processes procedure. The functionalization can be inserted during NPs formulation by using EG. as reducer and stabilizer agent in 70% ethanol after the NPs formation by activating the functional groups onto NPs surfaces and subsequent conjugation with ligands (MTX) and NAP. On the surface of the resulted Xerogel.

Chemicals

Silver nitrate(41mg/ml) and Zinc anitrate hexahydrate (70mg/ml), 25ml Ethanol 95% and 5ml Ethylene Glycol(from BDH)(England), Sodium carbonate(from merk germany). methotrexate (MTX) from BDH.

Experimental animal

Thirty-five male healthy young Wistar Albino rat used in this study, whose weights ranged from 250-370 g and their ages (10-12 weeks), which were brought from the Animal House Laboratory in the College of Veterinary / University of Al-Qadisiyahand the Animal House Laboratory of the College of Pharmacy of /University Karbala and the Department of Control and Monitiring of Drugs / Ministry of Health / Baghdad, these animals were placed in special plastic cages dimensions (25 × 40 × 15) cm covered with metal grates in the animal house of the College of Pharmacy University of Kerbala. So from time to time sponsors change,the animals were left for two weeks to acclimatize in the laboratory and natural lighting was adopted 12 hours a day anddarking 12 hours at night. To ensure that they were free from various diseases, oral dosage was 0.5 ml of Sodium sulfadimidine in 1 liter of water and 0.5 mg of Ampicillin WSP (20%)in 1 liter of water for 5 consecutive days.

Induced induction of rheumatoid arthriti The infection was developed by injecting 0.1 ml of Complete Freund Adjuvant(CFA) containing *Mycobacterium tuberculosis* in the right foot of the Achmad area of the rat. After weighing the foot size with the caplierverneir before the injection, Foot size after 14 days of injection and after six weeks of treatment [23] . The introduction of the CFA model is now being developed. This results in chronic inflammation, which is characterized by the similarity of these characteristics in the case of rheumatoid arthritis affecting humans [24]. The males of the white rat are randomly assigned to each group and are fed orally according to the weight of the rat body for the first six weeks (full dosage period) and the second three weeks (half the dosage period) for each study group respectively.

Design

1. The first group (G1): Is dosage daily with a physiological saline solution and consider as a negative control group.
2. The second group(G2): Is injecting by CFA and is a positive control group.
3. Group 3: G3 arthritis Is developed and dosage orally 14 days after the development of50.3 *mg*/ Xerogel nanoparticule in 0.5ml.
4. Group 4: G4 arthritis Is developed and dosage orally 14 days after the development arthritis of methotrexate free (MTX) 0.5mlof 0.125 mg / 250 g of animal weight by two doses per week.
5. Group 5 (G5): arthritis Is developed and dosage orally 14 days after the development of free nabroxen(NAP)0.5mlof 12.8mg / 250 g of animal weight by two doses per week.
6. Group 6 (G6): arthritis Is developed and dosage orally 14 days after the development of Xerogel-MTX.
7. Group 7 (G7): arthritis Is developed and dosage orally 14 days after the development of Xerogel-NAP.

Release kinetics of MTX from microspheres.

In vitro

Release of MTX from xerogel

For experiments were performed with release of MTX and NAP. Concentrations using UV-Vis. Techniques at the maximum adsorptions of MAT and NAP. The accumulation released quantity of MTX was greatest during the first hour, realization a plateau in the 180min, the initial amount of released MTX was generally slow.

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Processing the data with kinetic modules equations of, found that the pseudo second order was the most adapted modul Fig.(1), the terminal half-lives, and 10 mg/g of liquid MTX. Figure presents the cumulative release of the drug. The amount of MTX released during 180 min incubation ranged from 1.1–98% of MTX . In contrast, direct incubation of the prepared with original MTX solution without a prior washing procedure yielded raised amounts of released MTX, which was higher**.** In this study, we have described in detail the kinetics of MTX release from xerogel. The release of MTX and of NAP. Fig.(2 ABCD) from xerogel as a potential local therapy applied. Subsequent detailed in vitro works evaluated elution kinetics of MTX from the xerogel. All authors reported the greatest elution during the first hours, with rapid decrease during the following hours, and stabilization to a constant rate of release after two hours of incubation. In our study, using the UV-Vis method and advanced software data processing, we showed similar results. Moreover, the calculated half-life for elution of around ten hours means that this phase of MTX and NAP. release lasted approximately 4 hours . This finding was supported by absolute amounts of methotrexate, which was released from xerogel showed that the mean amount of MTX eluted from xerogel over a four hours period ranged from 1.1% to 98%. Thus, most of the dose was available for slow release from this porous material. Accordingly, we selected the MTX dose below this limit. The next question was the MTX formulation. Therefore, we intend to compare potential changes in release from xerogel prepared with liquid MTX. Our data demonstrated for the first time that xerogel prepared with liquid MTX releases MTX more rapidly, reaching a higher concentration at 180min, but the release half-life is shorter. In conclusion, our results demonstrated differences for the first time in MTX and NAP. elution kinetics from xerogel.

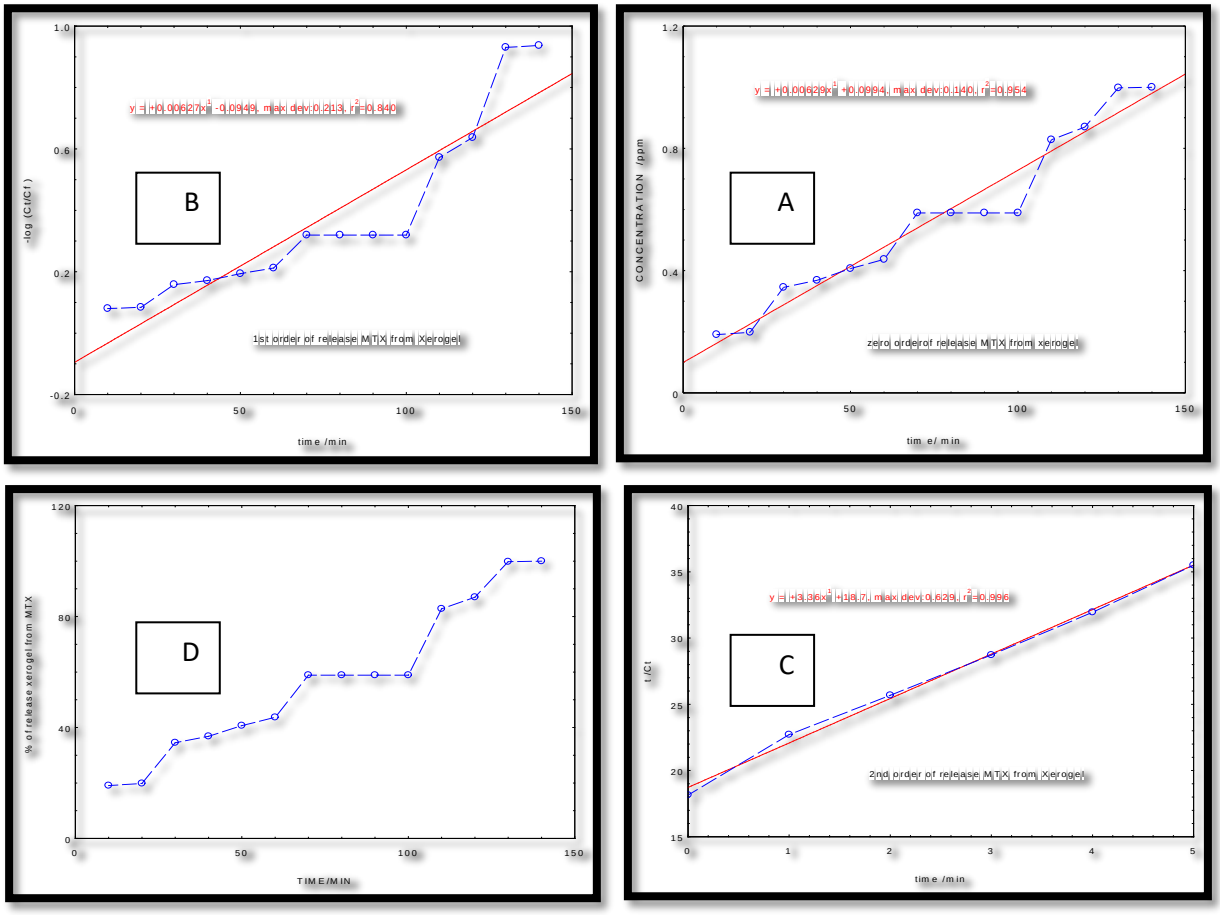

Fig.1: Fitting the release of MTX from the xerogel in to 0.5M sodium carbonate ,A-Zero order ,B-1st pseudo order, C-2nd pseudo order ,D - The percentage of release mtx from xerogel

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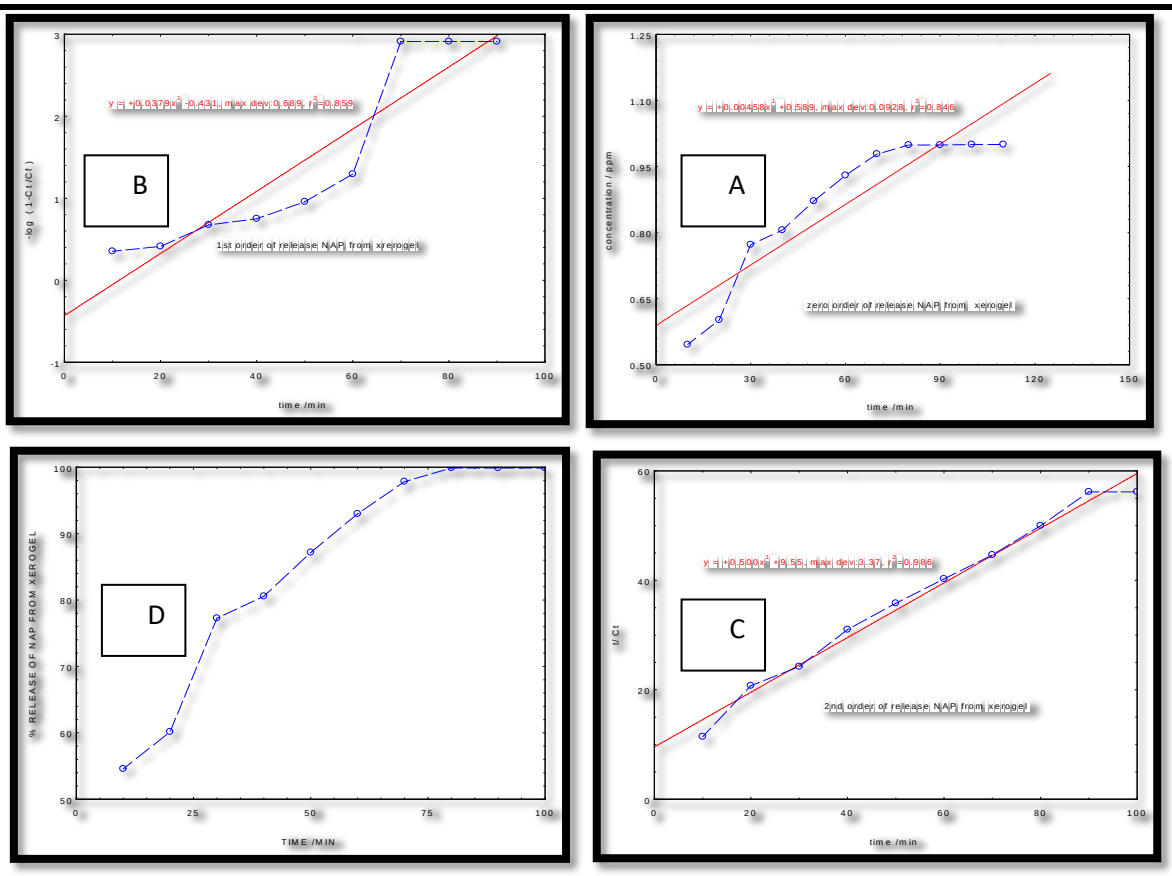

Fig.2: Fitting the release of NAP from the xerogel in to 0.5M sodium carbonate ,A-Zero order ,B-1st pseudo order, C-2nd pseudo order ,D - The percentage of release NAP from xerogel

FTIR characterization of MTX

Fourier transform infrared spectrometry *(FT-IR).* – The FT-IR spectra of methotrexate and the resulted xerogel were obtained on an FT-IR spectrometer, Mode spectrum Bruker (UK) over the range 400–4000 cm^{-1}.

By special liquid cell.

Spectrum of pure methotrexate fig.2B active substance (MTX) shows characteristic absorptions band as a broad signal at 3450 cm-1 (O–H) stretching from carboxyl groups superposed with the O-H stretching from crystallization water), at 3080 cm^{-1} (primary amine N–H stretching), at 1670-1600 cm^{-1} assigned to C=O stretching (–C=O stretching from carboxylic group) and C=O stretching from amidic group, so the C=O band is splited into a doublet in the MTX sample). The bands corresponding to N-H bending from amidic group appear in the 1550–1500 cm^{-1} spectral range, partly overlapping with the aromatic –C=C stretching. Another prominent bands, such as 1400-1200 cm^{-1} correspond to –C–O stretching from carboxylic group, 930 cm^{-1} to O–H bending out of plane and 820 cm^{-1} to C–H - 2-adjacent hydrogens on an aromatic ring, *para* substitution. All the bands identified in the FTIR spectrum are in good agreement with the molecular structure of MTX and confirm its purity.

FTIR analysis of the tablet (MTX-xerogel) fig.2C, is not offering relevant spectroscopic information due to the fact that the pharmaceutical formulation contains a low quantity of MTX comparing to the ones of excipients. Mainly, the FTIR spectrum consists of superposing of characteristic bands of used excipients. Due to superposing and to the fact that excipients are in a higher concentration than MTX , the characteristic bands of the active substance are significantly decreased, making them almost indistinguishable in the spectrum. Although a band around 3500 cm^{-1} can be assigned to O–H stretching of carboxyl groups and two weak bands are still appearing in the spectrum of MTX, namely the ones corresponding to C=O stretching(36).

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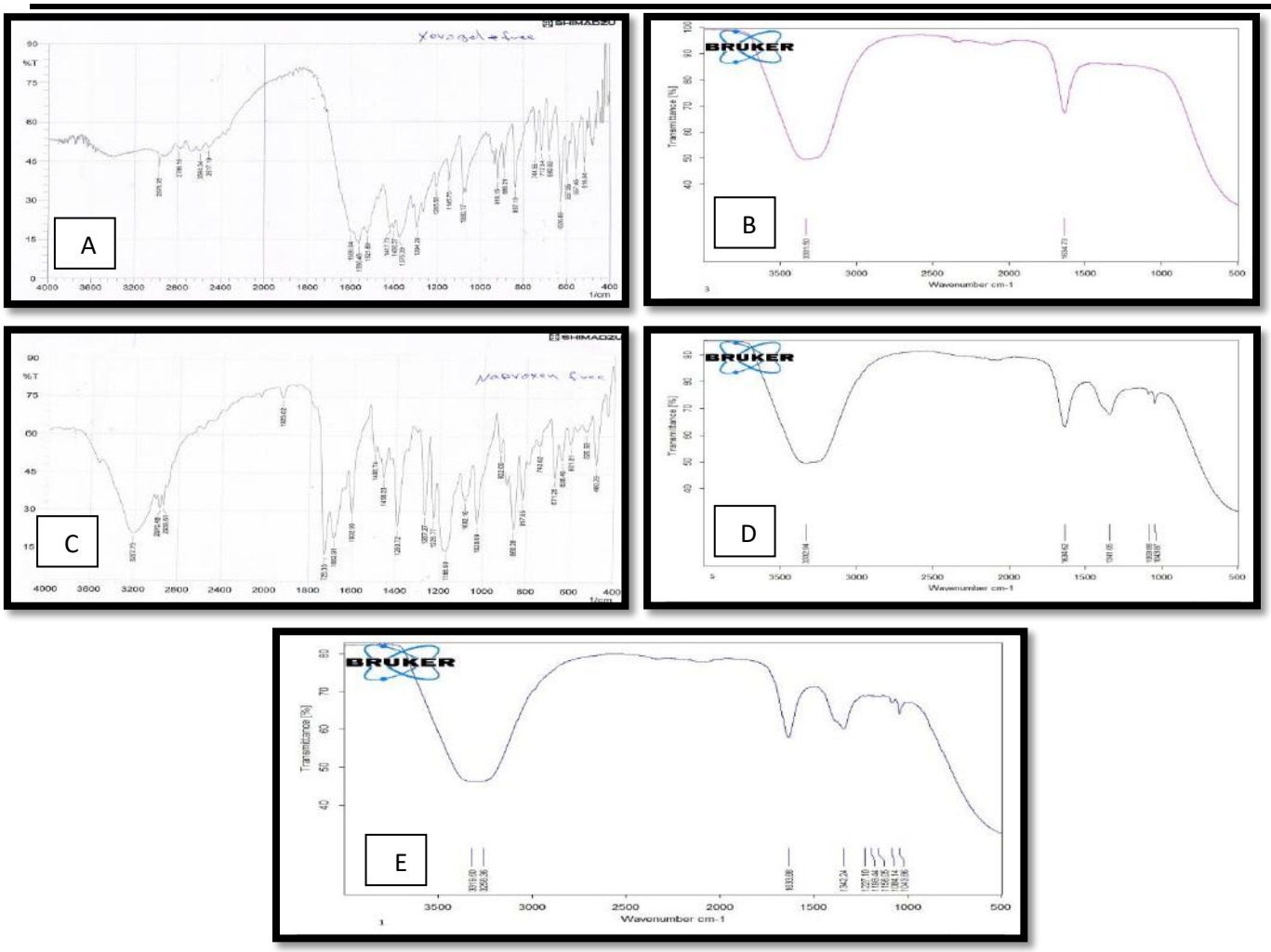

Fig.2: FTIR spectrum show the characteristics vibrations of the different functional groups ,A-Xerogel free, B-MTX free C-NAP free , D-Xerogel-MTX , E- Xerogel-NAP

Atomic force microscopy (AFM): Atomic force microscopy (AFM) was used to better clarify the morphology and the surface structure of the samples. The atomic force microscopical observations were performed with an Atomic Force Microscope (AFM model, AA3000, Advanced Angstrom Inc- USA) at about 20°C operating in air and in *Non- Contact* (NC) mode using a commercial silicon tip-cantilever (high resolution noncontact "GOLDEN" Silicon Cantilevers NSG-11, NTMDT, tip diameter=5–10 nm; with stiffness about 40 Nm^{-1} and a resonance frequency around 170 kHz. After the purification, the sample dispersed in distilled water were applied on a freshly cleaved mica disk (1 cm×1 cm); 30 min after the deposition, the water excess was evaporated. The AFM images were obtained with a scan rate 1 Hz and processed using a ProScan Data Acquisition software. Two kinds of images are obtained: the first one is a topographical image and the second one is indicated as "error signal". This error signal is obtained by comparing two signals: the first one, direct, representing the amplitude of the vibrations of the cantilever, and the other one being the amplitude of a reference point. The images obtained by this method show small superficial variations of the samples. Images were flattened using second-order fitting background curvature and slope from the images.

Figure 3: surface alterations after conjugation with MTX prepared evident, without any preliminary preparative treatment of the samples. Structurally, (Xerogel/MTX NPs) were characterized by larger aggregates having irregular shapes. The analysis of the topographical images of the samples Figure 4 showed that, in accordance with the size measured by PCS analysis, the diameters of Xerogel/ MTX (127nm) fig.3 were in a wide range (100 nm). The NPs were characteri1zed by regular shape and fragmented contours. Additionally, the surface appeared characterized by the presence of grains any more, as already observed for similar systems [24,25]. The "Error signal" image and 3D reconstruction Figures 2 well detailed the surface of NPs where spherical structures with diameters of about nm are present. This structure

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could be recognized as Ab molecules, as the images strongly agree with AFM images showing trimeric structures with comparable size of XEROGEL [26]. Based on these results, AFM imaging appears particularly attractive to characterize particulate matter based on organic materials with high spatial resolution, such as Ab anchored on NPs surface, without any concern about scattering cross sections and sample treatment procedures, through AFM scanning, once the suspension was deposed on a mica

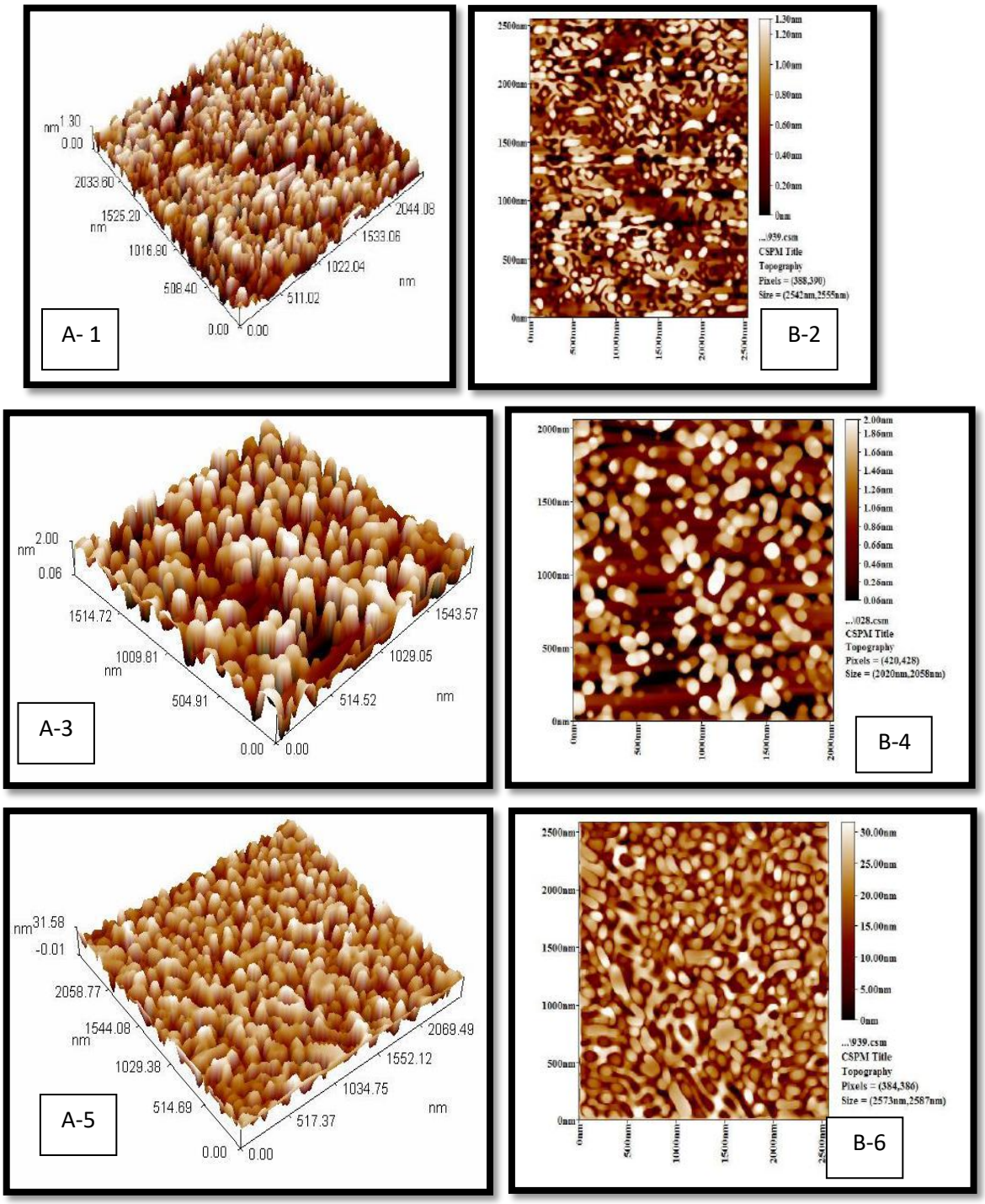

Fig.3: Atomic Forcing Microscope analysis of (xerogel NP free) and -NAP microphotographs;A-3D "Height'' image , " B-2D reconstruction" 1,2- xerogel NP free 3,4- Xerogel/MTX and 5,6- xerogel/ NAP

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Table [1] shows the Xerogel and the Xerogel conjugated with MTX and NAP. parameters evolutions predicted from AFM image.

Xerogel NPs	Avg. Diameter/ nm	Roughness Average(Sa)/ nm	Root mean square (Sq) /nm	Peak-Peak(Sy)/nm	Ten point height(Sz)/nm	Core Roughness depth (Sk)/nm
Xerogel NPs	**127.95**	**0.327**	**0.377**	**1.3**	**1.3**	**1.13**
Xerogel- MTX	**100.38**	**0.488**	**0.565**	**1.94**	**1.71**	**1.75**
Xerogel - NAP	**94.14**	**5.45**	**6.49**	**31.6**	**31.5**	**18.37**

This study demonstrates that AFM are really representing good options to be able to discriminate amongst the MTX and naproxen ligands conjugated on the Xerogel-MTX and Xerogel-NAP surface. These technologies would be really useful especially in the view of the upcoming of surface engineered nanomedicines. In fact, there is need for a precise, clear and accurate characterization of active surface of these new smart carriers. Advance surface-analysis technologies should be chosen in function of the ligands/molecules used for surface engineered of nanomedicines. In this view, in this paper, we showed that AFM can work, giving at the same time different information of different nature, converging to draw a more and precise "picture" of the novel nanomedicine.

The in vivo experimental results :

The results of Table(2) show that the development of rheumatoid arthritis in male rats by CFA injection in the positive control group G2 resulted in a significant increase in the IL-1β concentration at (86.99, 72.28)pg/ml for six weeks and for three weeks of infection respectively compared with a IL-1β concentration rate in the negative control group (G1) (6.85, 7.79) pg/ml for a full duration (six weeks) and for half duration (three weeks), respectively not injected with CFA. The results of this table also indicate a significant decrease in the concentration of IL-1β in the Xerogel treatment group and in the full treatment period compared with the G2 control group with a mean concentration of 69.34 Pg/ml, while its concentration for half the treatment period was approximately equal to its concentration level G2 group, three weeks after injury, reaching (75. 79) pg/ml .Treatment of male rats induced of rheumatoid arthritis by CFA with MTX (G4) , NAP (G5) significantly reduced ($P < 0.05$) at the IL-1β concentration level compared with the G2 positive control group for the full treatment period , The treatment results were MTX, NAP (33.71, 50.13) Pg/ml respectively for the 3-week treatment period the treatment results of MTX resulted in a significant decrease in IL- 1β and NAP treatment resulted in aobvious reduction in the IL-1β concentration at (42.70, 59.85) Pg/ml respectively compared to G2 positive group for half of the period (72.28) Pg/ml . As for G6 (Xerogel-MTX) nano particles with an IL-1β concentration (14.18) pg/ml indicate a significant decrease ($P < 0.05$) when compared with G2 positive control group for a complete treatment period, there is a significant reduction ($P < 0.05$) when compared to G3 treated with free Xerogel for the same period of treatment, as well as when compared with the G4 group shows a clear reduction in the concentration level ,While the G6 results for half the treatment period (38.52) pg / ml were compared to with (G6) treated with free MTX for a complete treatment period (33 .71)Pg/ml there was no significant difference between them, suggesting equal efficiency treatment for both treatments after calculating the T value.

Results shown in Table (2) for (G7) group Xerogel - NAP treatment indicating IL-1β concentration level significantly decreased (22.51) pg/ml compared to G2 positive control group and G5 group treated with free NAP as well as the G3 treatment group of free compound Xerogel NP for the full therapeutic period of those groups.When comparing the level of IL-1β concentration in the G7 group for half the period of treatment (25.49)Pg/ml , there was a significant decrease in the mean of T calculated compared to the G5 group treated with free NAP for a full period, indicating a reduction in time and dose Large up to 50%.

Table [2] Shows the effect of free MTX, NAP and xerogel NP and loaded with MTX, NAP treatment at the cytokine IL-1β pg / ml concentration levels in rat with rheumatoid arthritis

Treatment	Duration	Mean	standard error	LSD
Negative control(G1)	Full duration	6.85	2.50	**31.91**
	Half-term	7.97	0.32	**32.88**
	Total	**7.41**	1.67	**22.91**
Positive control (G2)	Full duration	86.99	20.96	

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Treatment	Duration	Mean	standard error	LSD
	Half-term	72.28	19.44	
	Total	**79.64**	19.04	
Xerogel(G3)	Full duration	69.34	0.00	
	Half-term	75.79	14.76	
	Total	**72.57**	15.07	**T Calculated**
MTX(G4)	Full duration	33.71	11.09	
	Half-term	42.7	4.90	
	Total	**38.21**	8.22	
NAP(G5)	Full duration	50.13	5.79	
	Half-term	59.85	1.00	
	Total	**54.99**	4.49	
XERO+ MTX (G6)	Full duration	14.18	5.07	
	Half-term	38.52	7.79	**-0.9**
	Total	26.35	8.42	
XERO+NAP (G7)	Full duration	22.51	6.20	
	Half-term	25.49	3.99	**8.87**
	Total	**24**	4.88	
Total	Full duration	40.44	15.37	
	Half-term	53.01	15.87	
	Total	**46.73**	15.80	

The results of Table 3 show that the induction of rheumatoid arthritis by CFA causes a significant increase in the concentration of TNF-α in the serum of males of white rats with a mean level(460.17, 449.61) Pg/ ml In the positive control groups (G2) for a complete treatment period and for half the period of treatment respectively compared with the negative control group G1 with a level of (72.53,73.39) Pg/ml for the two treatment periods respectively.The table also shows an increase in the concentration of TNF-α in the G3 group treated with the Xerogel nanoparticle for the total rate of full and half-term treatment (496.5) Pg /ml compared with an average in the positive control group (G2) as it reached (454.89 pg/ml).When treated with MTX/Xerogel (G6) for a complete treatment period there was a significant decrease of (P <0.05) in the concentration of TNF-α in this group with a concentration of (255.32) Pg/ml compared to Positive Control (G2) and G3 treated with free Xerogol and G4 (free MTX) , The concentration rate in these groups was (460.17, 481.77, 324.91) Pg/ml respectively and for a full treatment period, When comparing the results of the same group G6), for half the treatment period, the TNF-α concentration was significantly reduced by 267.54 Pg/ml compared to the G4 group (free MTX treatment), where TNF-α concentration was324. 91Pg/ml and a full treatment period.When comparing the results of the same group (G6) for half the treatment period, the TNF-α concentration was reduced (267.54) Pg / ml compared to the G4 group (free MTX treatment) full treatment period. The results of Table (3) for Group G7 treatment with xerogel / NAP nanoparticles show a significant decrease (P <0.05) at the TNF-α concentration level,the concentration level in the full treatment period for this group was 213.61 pg / ml compared to the positive control group G2 and the free Xerogel treatment group (G3) and free NAP treatment (G5) 314.69pg/ml each of them and respectively for a full treatment period.When comparing the low level of TNF-α concentration in the above-mentioned nanoparticles treated with NAP treatment for half the treatment period, the concentration level was reduced to approximately equal levels(307.39) pg/ml, compared to the free NAP treatment group (G5) for a complete treatment period,

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*Table [3] Shows the effect of free MTX, NAP and xerogel NP and loaded with MTX, NAP treatment at the **TNF. pg/ml** concentration levels in rat with rheumatoid arthritis*

Treatment	Duration	Mean	standard error	LSD
Negative control(G1)	Full duration	72.53	4.23	**158.41**
	Half-term	73.39	4.30	**186**
	Total	72.96	3.95	**122.16**
Positive control (G2)	Full duration	460.17	76.53	
	Half-term	449.61	67.71	
	Total	454.89	66.94	
Xerogel(G3)	Full duration	481.77	75.20	
	Half-term	511.22	97.87	
	Total	496.5	81.11	**T Calculated**
MTX(G4)	Full duration	324.91	6.69	
	Half-term	363.55	70.75	
	Total	344.23	47.43	
NAP(G5)	Full duration	314.69	10.03	
	Half-term	481.62	87.45	
	Total	398.15	70.09	
XERO+ MTX (G6)	Full duration	255.32	40.43	
	Half-term	267.54	29.48	4.8
	Total	261.43	32.89	
XERO+NAP (G7)	Full duration	213.61	39.69	
	Half-term	307.39		1.09
	Total	260.5	35.47	
Total	Full duration	312.12	77.34	
	Half-term	380.92	85.53	
	Total	346.52	82.60	

III. DISCUSSION

This came in line with the findings of the studies [27, 28] showed that the induction of rheumatoid arthritis with CFA caused an increase in the number of white blood cells in the blood of male white rats, This is also consistent with the results of the study [29], which attributed this increase to the fact that white blood cells are a major component involved in the inflammatory immune response as an indicator for measuring the condition of the disease and can be attributed to the increase of interleukin-1 (IL-1) released by phagocytic cells, as this article is working to increase the nomination of white blood cells from the bone joints into the bloodstream and consequently increase their accumulation and numbers . Pro-inflammatory cytokines are used as criteria for predicting the immunological effects of nanoparticles and their potential for cytotoxicity[30]. The therapeutic results of nanoparticles can be changed by knowing how to manage nanoparticles (Xerogel), it is possible to polarize the TH1and TH2 balance and produce cytokines towards a single specific pathway, for example. The treatment of poly-hydroxylated metallofullerenol leads to polarization of cytokine towards TH1 cytokines by reducing the production of TH2 and producing cytokines (IL-4, IL-5 and IL-6) and increasing TNF-α & IFN- γ cytokines through TH1 production in rat serum treated with this compound [45] ,due to the increased production of large amounts of cytokines (TNF-α, IL1-β, IL-12 and IFN- γ) pro-inflammatory cytokines to transcription the DNA of the production of those cytokines in the rat serum as a result of treatment of these nanoparticles modified compounds [31]. The results of the study [32] were consistent with the results of the current study. The above study suggested developing the DDS delivery system for localized MTX treatment used in the treatment of RA through the formation of small pellets with nanoscale size And work to change the therapeutic properties in vitro in

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vitro including control of emancipation, pH stability and length of storage period. The increased penetration of skin treatment three to four times with oleic acid with ufasomes compared to the free localized MTX or carbopol xerogel. At the end of the experiment, more than 50% of the dermal dose was found to be available.

The study [33] asserts that clinical application is very important to know the common bio-compatibility between the treatment of naproxen and DDSs. The use of Xerogel in the treatment of rheumatoid arthritis is widespread and is biocompatible with DDSs. Acidic pH can be reduced in the surrounding environment, so the DDSs delivery system should be completely biodegradable as the residue of the nanoparticles carrying can be a cause of progression of arthritis by enhancing the inflammatory media IL-1β and TNF-α, The results of the current study proved to be indicative of that method The design and size of the DDSs affect the properties of the treatment which becomes susceptible to surrounding environmental surroundings.

The findings of the current study agree with what [34] have found that loading the MTX treatment on the theranostic gold (Au) half-shell NPs or Polysialic acid (PSA) -trimethyl chitosan (TMC) NPs (Xerogel) Antibodies to the specific receptor of CD46 cells, and that loading MTX therapy is much more effective than free therapy in inhibiting the production of TNF-α tumor necrosis and significantly eliminating the progression of arthritis in experimental animals.

The results of the present study agree with the findings of [35] which confirmed that the effectiveness of hydrogel nanoparticles and considered naproxin therapeutic treatments to target biological sites due to its absorption and high control of the release of treatment in inflammatory areas, which protects the joint from the action of inflammatory agents and TNF - α The reason for this is several of them, high compatibility because of the containment of large amounts of water helps to accept the natural tissues and this reduces immune response trend, easily broken by the body, which makes them vulnerable to the devices and You reduce toxicity, as well as their ability to carry treatments and maintain until it reaches the target tissue, and also its ability to escape from the reticulo-endothelial system and access to the target tissue and regulate the liberalization of treatment. For patients starting treatment with a TNF inhibitor, concomitant use of MTX is also declining [36] . The exact mechanism behind the protective effect of antirheumatic treatment on Endothelial Dysfunction is not known[37]. Another explanation might be that the examined drugs might have a direct beneficial effect on the vessel walls, including the endothelium. It has been shown that MTX and anti-TNF treatments are associated with improvements in reverse cholesterol transport by various mechanisms [38-39].

Tumor necrosis factor), which play a major role in RA and other inflammatory diseases [40] . naproxen by its inhibition of IL-6, but was also a more potent modulator of IL-1 beta and TNF-alpha in RA synovial explants. The significance of these findings lies in the possible therapeutic benefit of pro inflammatory cytokine suppression in joint disease [41]. Anti-inflammatory drug naproxen suppresses the hypothalamic-pituitary-adrenal HPA-axis in the first weeks of treatment [42]. Nanofibre forming peptide amphiphiles were conjugated to naproxen through an esterase-sensitive linker. The amount of naproxen released, in the presence of enzymes, was influenced by the linker conjugating the drug to the supramolecular assembly. The acid functionality of naproxen is un altered upon enzymatic action and its bioactivity is preserved as shown by the COX-2 inhibitory assay. The biocompatible nature of the supramolecular assemblies make them ideal platforms for drug delivery applications [43]. This study indicates that concomitant naproxen does not abruptly alter the disposition of low-dose methotrexate in patients with rhetunatoid arthritis who have normal renal fonction [44] For example, MTX increases high-density lipoprotein (HDL) capacity to promote cholesterol efflux from cells [45]. MTX inhibits production of inflammatory mediators suchas metalloproteinases and IL-6, and expression of these proteins is known to require activation of NF-kB[56]. The results indicate that there isa role for MTX co-medication with TNF in PsA, and that this might be more important in patients receiving monoclonal antibodies [46] . MTX up regulates in the monocyte cell line U937 the productionof the pro inflammatory cytokines IL-1, IL-6 andTNF alpha. The folate pathway is implicated in this response,while the adenosine signaling pathway is probably not involved. These results may have implications for explaining mechanisms of some off-target actions of MTXsuch as mucositis and pneumonitis as well as decreased bone density in oncology patients. Identification of patientsin whom this response is significant might be useful in predicting the need for combination therapy with anticytokineagents [47] . The mechanism of action ofMTX in RA might be more anti inflammatory than immunosuppressive. This is supported by the rapid clinical response to drug treatmentand by data from in vitro and animal studies. The inhibition of interleukin-1 (IL-l) activity or other inflammatory cytokines by MTX may play an important role in the anti inflammatory effect of MTX. MTX effects in RA are not fully understood and further studies are needed to clarify its mechanism of action. MTX has crucial effects on the

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cascade of events initiated by some cytokines (IL-l, IL-6,].

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