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NANOESTRUTURAS DE QUITOSANA / HEXAMOLIBDATO PARA LIBERAÇÃO ORAL DE FÁRMACOS

CHITOSAN / HEXAMOLYBDATE NANOSTRUCTURES FOR ORAL DRUG DELIVERY

كيتوسان / هيكساموليبدات مركبات نانوية لتوصيل الأدوية عن طريق الفم

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RESUMO

Introdução: Nas últimas décadas, avanços substanciais foram alcançados no campo da entrega de medicamentos por meio da criação de formulações de liberação controlada, levando a avanços médicos significativos. O objetivo principal desses sistemas de liberação controlada é sustentar a concentração desejada do medicamento na corrente sanguínea ou em tecidos específicos por um período prolongado. Uma classe de nanoestruturas são os polioxometalatos (POMs), que são nanoclusters aniônicos carregados negativamente compostos por metal e átomos de oxigênio. Os POMs são notáveis por sua capacidade de serem controlados precisamente em tamanho e forma. Essas características, combinadas com sua carga negativa inerente, contribuem para sua estabilidade e os tornam estruturas altamente versáteis. **Objetivo**: Este estudo teve como objetivo examinar a auto-montagem de híbridos orgânicos-inorgânicos usando hexamolibdato de tipo Lindqvist de polioxometalato (POM) e quitosano para sintetizar novas nanoestruturas 3D. Essas nanoestruturas foram então investigadas quanto à sua adequação como nanocarreadores para o medicamento de quimioterapia Temozolomida (TMZ). A liberação de TMZ do nanocarreador foi estudada sob várias condições de pH. **Métodos**: Este estudo compreende duas partes práticas. A primeira parte foca na síntese de nanoestruturas hierárquicas projetadas para carregar medicamentos de TMZ. A quantidade de medicamento carregado na nanoestrutura preparada de POM-Quitosano foi determinada usando Cromatografia Líquida de Alta Eficiência (HPLC) em vários tempos de carga. A segunda parte do estudo investiga o processo de liberação do medicamento TMZ das nanoestruturas hierárquicas. Esse processo de liberação é examinado em duas soluções tampão com valores de pH distintos. **Resultados**: As características de superfície, tamanho, composição química e identidade das nanoestruturas foram analisadas usando técnicas como HPLC, FTIR, DRX, MEV e XPS. **Discussão**: Os nanocarreadores demonstraram um comportamento de liberação intrigante dependente do pH, sugerindo sua aplicação potencial em sistemas de entrega de medicamentos. **Conclusões**: Neste estudo, preparamos com sucesso novos nanocarreadores de quitosano e POM carregados com TMZ (21% de carga) por meio de auto-montagem para entrega oral de medicamentos. A forma de nanocarreador preparada de TMZ foi completamente caracterizada por DRX, FTIR, XPS e MEV. A forma oral preparada de TMZ mostrou uma excelente liberação dependente do pH do TMZ, onde a taxa de liberação em pH 7.4 foi consideravelmente mais rápida do que em pH 2.8.

Palavras-chave: *Chitosano, entrega de medicamentos, nanoestruturas hierárquicas, polioxometalatos (POMs), Temozolomida (TMZ).*

ABSTRACT

Background: In recent decades, substantial progress has been achieved in the field of drug delivery through the creation of controlled-release formulations, leading to significant medical advancements. The primary objective of these controlled release systems is to sustain the desired drug concentration in the bloodstream or specific tissues for an extended duration. One class of nanostructures is polyoxometalates (POMs), which are negatively charged anionic nanoclusters consisting of metal and oxygen atoms. POMs are notable for their ability to be precisely controlled in size and shape. These characteristics, combined with their inherent negative charge, contribute to

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their stability and make them highly versatile structures. **Aim:** This study aimed to examine the self-assembly of organic-inorganic hybrids using polyoxometalate (POM) Lindqvist-type hexamolybdate and chitosan to synthesize novel 3D nanostructures. These nanostructures were then investigated for their suitability as nanocarriers for the chemotherapy drug (Temozolomide TMZ). The release of TMZ from the nanocarrier was studied under various pH conditions. **Methods:** This study comprises two practical components. The first part focuses on synthesizing hierarchical nanostructures designed to load TMZ drugs. The amount of drug loaded onto the as-prepared nanostructure of POM-Chitosan was determined using High-Performance Liquid Chromatography (HPLC) at various loading times. The second part of the study investigates the release process of the TMZ drug from the hierarchical nanostructures. This release process is examined in two buffer solutions with distinct pH values. **Results:** The surface characteristics, size, chemical composition, and identity of the nanostructures were confirmed using techniques such as HPLC, FTIR, XRD, SEM, and XPS. The results confirm the successful complexation of chitosan with POM Lindqvist-type hexamolybdate. **Discussion**: The nanocarriers demonstrated an intriguing pH-dependent release behavior, suggesting their potential application in drug delivery systems. **Conclusions:** In this study, new nanocarriers of chitosan and POM were successfully prepared and loaded with TMZ (21% loading) via self-assembly for oral drug delivery. The as-prepared nanocarrier form of TMZ was fully characterized via XRD, FTIR, XPS, and SEM. The as-prepared oral form of TMZ showed an excellent pH-dependent release of TMZ, where the release rate at pH 7.4 was considerably faster than at pH 2.8.

Keywords: Chitosan, Drug delivery, Hierarchical nanostructures, polyoxometalates (POMs), Temozolomide (TMZ).

الخلاصة :

الخلفية: في العقود الأخيرة، تم تحقيق تقدم كبير في مجال توصيل الأدوية من خلال إنشاء مركبات إطلاق خاضعة للرقابة، مما أدى إلى تقدم طبي كبير. الهدف الأساسي لأنظمة الإطلاق الخاضعة للرقابة هذه هو الحفاظ على تركيز الدواء المطلوب في مجرى الدم أو أنسجة معينة لفترة طويلة. إحدى فئات الهياكل النانوية هي بولي أوكسوميتالات(POMs) ، وهي عبارة عن مجموعات نانوية أنيونية سالبة الشحنة تتكون من ذرات المعدن والأكسجين. تتميز POMs بقدرتها على التحكم بدقة من حيث الحجم والشكل. تساهم هذه الخصائص، جنبًا إلى جنب مع شحنتها السلبية المتأصلة، في استقرارها وتجعلها هياكل متعددة الاستخدامات للغاية. **الهدف:** تهدف هذه الدراسة إلى فحص التجميع الذاتي للهجين العضوية وغير العضوية باستخدام مادة البولي أوكسوميتالات (POM) وهيكساموليبدات من نوع ليندكفيست والكيتوسان لتجميع هياكل نانوية ثلاثية الأبعاد جديدة. تم بعد ذلك فحص هذه البنى النانوية للتأكد من ملائمتها كحاملات نانوية لعقار العلاج الكيميائي .TMZ تمت دراسة إطلاق TMZ من الناقل النانوي في ظل ظروف مختلفة للأس الهيدروجيني. **طريقة العمل** : تتكون هذه الدراسة من عنصرين عمليين. يركز الجزء الأول على تصنيع الهياكل النانوية الهرمية المصممة خصيصًا لتحميل دواء .TMZ تم تحديد كمية الدواء المحملة على البنية النانوية المحضرة لـ POM-Chitosan باستخدام تحليل كروماتوجرافي سائل عالي الأداء (HPLC) في أوقات تحميل مختلفة. يبحث الجزء الثاني من الدراسة في عملية إطلاق عقار TMZ من الهياكل النانوية الهرمية. يتم فحص عملية الإطلاق هذه في وجود محلولين عازلين بقيم pH مميزة. **النتائج:** تم تشخيص الخصائص السطحية والحجم والتركيب الكيميائي وهوية الهياكل النانوية بنجاح وذلك باستخدام تقنيات مثلHPLC ، FTIR، XRD، SEM, AFM, XPS. تثبت النتائج نجاح تكوين المعقد بين الهكساموليبيدات (POM) والكيتوسان. **المناقشة:** أظهرت الناقلات النانوية سلوك إطلاق مثير للاهتمام يعتمد على الرقم الهيدروجيني، مما يشير إلى إمكانية تطبيقها في أنظمة توصيل الدواء. **الاستنتاجات:** في هذه الدراسة، نجحنا في إعداد ناقلات نانوية جديدة من الكيتوسان و POMمحملة بـ TMZ(وكانت نسبة التحميل 21٪) عن طريق التجميع الذاتي كوسيلة لتوصيل الدواء عن طريق الفم. تم تمييز شكل الناقل النانوي المُجهز من TMZ بشكل كامل عبر XRD و AFM FTIRو XPSو .SEMأظهر الشكل المُجهز لـ TMZ إطلاقًا ممتازًا يعتمد على الرقم الهيدروجيني لـTMZ حيث كان معدل الإطلاق عند درجة الحموضة 7.4 أسرع بكثير من الرقم الهيدروجيني 2.8.

كلمات مفتاحية : كيتوسان , توصيل الادوية , هياكل نانوية هرمية , أوكسوميتالات , تيموزولومايد

1. INTRODUCTION

Temozolomide (TMZ) drug is (3,4-dihydro-3-methyl-4-oxoimidazo-[5,1-d]-as-tetrazine-8-carboxamide TZ) the TMZ is an oral chemotherapy drug that is primarily used in the treat certain types of tumors such as brain tumors, especially glioblastoma multiforme (GBM), which is the most common and aggressive form of brain cancer. It is also used in the treatment of anaplastic astrocytoma and mixed gliomas (Delello Di Filippo *et al*., 2021; Hotchkiss & Sampson, 2021). The Temozolomide drug belongs to a class of drugs, which called the factors alkylating agents. It works by damaging the DNA in cancer cells, which inhibits their ability to divide and grow and consequently helps to slow down or stop the growth of tumors. TMZ is commonly used as a primary medication for treating GBM (glioblastoma multiforme). However, high doses and frequent administration of TMZ are needed owing to its short half-life, and GBM cells often develop resistance to it. (Emamgholizadeh Minaei *et al*., 2019) Consequently, this leads to significant side effects and limits its effectiveness. Therefore, new strategies for the controlled release of TMZ are required to address these limitations and would aim to maintain therapeutic levels of the drug over extended periods. One promising approach is nanomedicine, where Nanocarriers, such as nanoparticles or liposomes, can be designed to capture TMZ and release it slowly and selectively

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at the tumor site. (Cui *et al*., 2016; Dang & Guan, 2020) The implementation of these nanocarriers can also provide many advantages. First, it can enhance the stability of a drug, prevent deterioration, and ensure its effectiveness throughout the delivery process. Second, it can improve the solubility of TMZ, availability, and biological compatibility, hence facilitating its distribution within the body. In addition, these nanocarriers could increase the possibility of delivering the drug to the targeted cancer cells, reducing exposure to healthy tissue and thus improving the safety profile of treatment. (Alshawwa *et al*., 2022; Din *et al*., 2017; Kamaly *et al*., 2016; Tewabe *et al*., 2021) By utilizing nanomedicine, researchers explore improving TMZ treatment efficiency while minimizing its side effects. This involves the development of new nanostructures with diverse and improved properties to serve as carriers for the drug.

Polyoxometalates (POM) are nanoclusters composed of negatively charged oxygen species and metals that can be controlled accurately in shape and size. They have received great interest in systems drug delivery. POM has illustrated the ability to improve the biocompatibility and modify the bioactivity and cytotoxicity of the drug. As inorganic building blocks, POMs offer promising prospects for the development of advanced drug delivery systems, facilitating the targeted and controlled release of therapeutic agents while enhancing their efficacy and reducing potential side effects (em Bioquímica, n.d.; Khalilpour *et al*., 2021; Zhang *et al*., 2019). Several studies have focused on synthesizing hierarchical nanostructures combining chitosan and POMs for oral drug delivery (Mikušová & Mikuš, 2021). However, most of these studies have primarily utilized Keggin, Anderson, and Dawson POMs in conjunction with bioactive components like chitosan to create multifunctional 3D nanostructures (Azmana *et al*., 2021; Shah *et al*., 2015; X. Wang *et al*., 2022). Bio-nanocomposites based on chitosan can specifically penetrate the membranes of cancerous cells and impart anti-tumor action through several enzymatic, antiangiogenic, immune-stimulating, and apoptotic mechanisms. The anticancer impact of chitosan-metal complexes is due to their ability to scavenge free radicals and interact with cellular DNA (Fang *et al*., 2020; W. Wang *et al*., 2020).

On the other hand, Lindqvist-type hexamolybdate, a specific type of polyoxometalate, has attracted growing interest due to its potential applications in electronic and photonic devices (Anyushin *et al*., 2020; Cameron *et al*., 2018). Interestingly, the combination of chitosan and Lindqvist-type hexamolybdate in the fabrication of 3D nanostructures has not been previously reported. This study aims to illustrate the formation of nanostructures using POMs, specifically Lindqvist-type hexamolybdate, with chitosan as a nanocarrier for pH-controlled oral drug delivery of TMZ. The prepared nanostructures were thorough.

Figure 1. Structure of the POMs used in this study.

2. MATERIALS AND METHODS:

2.1. Materials

Chitosan was purchased from Central Drug House (CDH) Scientific. Acetic acid and phosphate-buffered saline (PBS) were purchased from HiMedia (India). TMZ was obtained from Sigma-Aldrich. Glycine was purchased from Thomas Baker. The pH meter from OAICTON-2100, Singapore. Centrifuge from Hettich-Universal II- Germany.

2.2. Instrumentation

The analysis involved various instruments, including the High-Performance Liquid Chromatography, UFLC-20A, Shimadzu, Japan. Fourier-transform infrared spectroscopy, FT-IR-8400S, Shimadzu, Japan. X-Ray Diffraction X-Ray, Siemens model D500. Scanning Electron Microscopy (SEM), ZEISS model, UK. Atomic Force Microscopy (AFM), DME Denmark model and X-Ray Photoelectron Spectra (XPS), Germany Bes Tek device model.

2.3. Methods

2.3.1 Synthesis of 3D nanostructures

The nanostructures were created using POM-chitosan in a 1:1 ratio. In a typical procedure,

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separate mg of chitosan and 1 mg of POM (hexamolybdate) 1 mg were prepared in an acetic acid solution 0.5 mL. The POM solution was immediately combined with the chitosan solution. Within 30 seconds of this combination, the solution's color changed to a pale yellow. The mixture was then left to age for half an hour, followed by centrifugation at 5000 rpm for 5 min, followed by washing three times with deionized water to obtain the final product. Several analytical techniques were employed to characterize the nanostructures. FTIR provided information about functional groups and chemical bonds present in the nanostructures. XRD was utilized to examine the crystallographic properties, while SEM allowed for the visualization of surface morphologies and topographies. XPS provided information about the elemental composition and chemical states of the nanostructures. AFM also provides information about nanocomposite size, shape, and surface roughness.

2.3.2. Loading of as-prepared nanostructures with TMZ

Temozolomide (TMZ) was selected as the model drug for this study. An aqueous TMZ solution of 0.5 mg/mL, 5 ml, was submerged in a nanopowder of POM-chitosan. After shaking the mixture for 12 hours at 25°C, the result was washed three times with deionized water. After that, the product was centrifuged for five minutes at 5000 rpm to remove any unbound medication. The amount of unloaded TMZ that remained in the supernatant was measured by HPLC under the chromatographic conditions listed in Table 1 to ascertain the percentage of TMZ loaded onto the nano-POM-chitosan.

2.3.3. Release (in vitro) of loaded TMZ

TMZ release was achieved by immersing a sample of nanostructures loaded with TMZ 10 mg under agitation on a shaker plate 500 rpm by using two pH mediums, and the pH was measured by a digital PH meter and control by using a certain buffer solution: an acidic medium (pH 2.8, glycine-HCl buffer solution), and in neutral medium (pH 7.4, phosphate-buffered saline (PBS) solution) over a period of 24 h. The amount of TMZ released in each case was determined by taking 0.5 ml aliquots of the supernatant at timed intervals. Finally, the amount of TMZ released was measured by applying HPLC analysis using the UFLC system of a Shimadzu 20A with a UV detector. The chromatographic conditions that were optimized were illustrated in Table 1.

3. RESULTS AND DISCUSSION:

3.1. Results

3.1.1. Fourier-transform infrared (FT-IR)

The Fourier-transform infrared spectra of POM-Chitosan-TMZ nanocomposites were individually compared with those of TMZ (Temozolomide) and POM (polyoxometalate). The comparison revealed complexation between POM and the nanocomposite, as shown in Figure 2**.**

3.1.2. X-ray diffraction analysis (XRD)

Figures 3 A, B, C, and D demonstrated the XRD data for POM hexamolybedate; chitosan; the as-prepared nanostructure before loading with TMZ, and the as-prepared nanostructure after loading with TMZ.

3.1.3.Scanning Electron Microscopy (SEM) analysis

The scanning electron microscope has long been the preferred technology for determining the surface shape and fundamental physical characteristics of materials. This technique determines the particle shape and size distribution of the as-prepared nano structure before and after being loaded with TMZ, shown in figure 4 and 5.

3.1.4. X-Ray Photoelectron Spectra (XPS)

X-ray photoelectron spectroscopy was performed to identify the elemental composition of the as-prepared nano structure prior to and after being loaded with TMZ, as explained in Figure 6.

3.1.5. Atomic Force Microscopy (AFM)

Atomic Force Microscopy was used to determine the roughness of the prepared nanostructure before and after it was loaded with TMZ, as demonstrated in Figures 7 and 8.

3.1.6. Release behavior of TMZ loaded onto as-prepared nanostructures

After studying the loading of TMZ onto an as-prepared nanostructure, the drug release profiles of TMZ -loaded to an as-prepared nanostructure in [pH 2.8] glycine-HCl buffer solution and [pH 7.4] phosphate-buffered saline

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(PBS) solution was shown in Figure 9.

3.2. Discussion

3.2.1. Fourier-transform infrared (FT-IR)

The Fourier-transform infrared (FT-IR) spectra of POM-Chitosan-TMZ nanocomposites were individually compared with those of TMZ and POM (polyoxometalate). The comparison revealed the most characteristic bands of each component, POM and TMZ, with slight shifts observed at 794 cm-1, 960 cm-1, and 1446 cm-1, specifically for POM. These shifts indicate complexation between POM and the nanocomposite, as depicted in Figure 1. Based on the given information, the shifts in the characteristic bands of POM in the nanocomposite provide evidence of complex formation between POM and TMZ. These shifts may be attributed to changes in the molecular environment or interactions between the components, indicating the successful incorporation of POM into the Chitosan-TMZ nanocomposite.

3.2.2. X-ray diffraction analysis (XRD)

XRD measurements were used to investigate the as-prepared nanostructures. Fig. 3-C presents the XRD patterns of the as-prepared POM-chitosan compared to POM (Figure 3-A) and Chitosan (Figure 3-B), where several characteristic Bragg reflections of Lindqvist-type hexamolybdate POMs ($2\theta = 16.5^{o}$), as well as other peaks at $2\theta = 20^{o}$, 26.5^{o} and 29.5^{o} for Chitosan, could clearly be observed, indicating the successful complexation of Chitosan with POM Lindqvist-type hexamolybdates through strong interactions. Compared to its POM and Chitosan precursors, the POM-Chitosan structure showed broader peaks, which can be attributed to the smaller crystalline particle size, confirming the nano-character of the resultant POM-Chitosan composite. These results indicated the formation of the nanostructures, also supported by the SEM analysis. After loading TMZ into the POM-Chitosan nanocomposite, the XRD patterns (Fig.3-D) showed minimal change compared to the POM-Chitosan composite, though border peaks were observed.

3.2.3. Scanning Electron Microscopy (SEM) analysis

The morphology of the as-prepared nanostructures of POM-chitosan before and after loading with TMZ was investigated via SEM. As can be shown in Figure 3, which demonstrates the SEM micrographs of the POM-chitosan nanostructure, the nanostructure itself consists of hierarchical microsphere structures with a size of about 1 µm constructed from a multitude of nanoplates of thicknesses ranging from 20-113 nm and widths of 200-500 nm. 3D hierarchical structures were formed from the central connection of these nanoplates. The loading of TMZ onto the hierarchical microsphere structures resulted in totally different morphological (shape and size) structures compared to POM-Chitosan. As can be seen from Fig. 4, nanosphere particles with diameters ranging from 13-42 nm are formed upon loading with TMZ. These considerable changes in both morphology and size can be attributed to the TMZ loaded onto the surface of the POM-Chitosan nanostructure. These findings are consistent with the XRD results, which showed a smaller particle size change upon TMZ loading compared to the naked POM-Chitosan nanostructures.

3.2.4. X-Ray Photoelectron Spectra (XPS)

X-ray photoelectron spectroscopy (XPS) was employed to determine the elemental composition of the nanocomposites. The findings are depicted in Figure 3. The XPS analysis verified the presence of carbon, nitrogen, and molybdenum, as evidenced by the respective peaks observed. The C1s peak was measured at a binding energy (BE) of 290 eV, indicating the presence of carbon in Chitosan (and TMZ in (b)). The N1s peak appeared at a BE of 404 eV, representing the amido groups in Chitosan. Moreover, the Mo3d peak at a BE of 237 eV confirmed the existence of POM Lindqvist-type hexamolybdate. Notably, the N1s band exhibited two distinct peaks: the first one, observed at 404 eV, was associated with alkylamines, while the second peak at higher binding energy indicated the protonation of the amine groups in chitosan. This finding further supported the presence of electrostatic interactions between POM and chitosan.. XPS results again confirm the successful complexation of chitosan with POM Lindqvist-type hexamolybdate.

3.2.5. Atomic Force Microscopy (AFM)

Gaining useful information from AFM images is best achieved through their display, as the images provide a 3D representation of the sample's surface. Typically, AFM images are viewed in either 2D or 3D, allowing for the

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visualization of height variations. Adjusting the color scale and contrast of the images can enhance the presentation of height changes, making them more visually impactful. The images were captured from different positions on the sample. To address the unevenness of the sample stage, line-by-line leveling was applied during image processing to normalize the Z range. The data extracted from these images was examined to gather information about roughness, feature height, and grain analysis. The 3D images have been deliberately exaggerated in the Z range to highlight the sample's features. The color variation in the images represents the height of the sample within the Z range, with the dark brown indicating the lowest point (zero) and the bright yellow representing the highest point.

3.2.6. Release behavior of TMZ loaded onto as-prepared nanostructures

The potential of the nanostructures to act as carriers in oral drug delivery was explored by studying the loading and release behavior of TMZ in media of different pHs. Upon loading TMZ onto the nanostructures, a color change from pale yellow to green was seen, indicating the successful loading of TMZ. The amount of TMZ loaded onto the POM-chitosan nanostructures was determined via HPLC and was found to be about 21% (by weight), representing a high loading efficiency. This could be attributed to the large relative surface area (smaller particle size compared to POM-chitosan before loading) of the nanostructures. The employment of pH changes within the gastrointestinal (GI) tract is one of the most interesting methods in the design of oral delivery protocols for drugs in which two pH changes are chosen, the first mimicking the acidic conditions of the stomach (pH 1–3), and the second the more basic conditions of the intestines (pH 5–8). Fig. 8 shows the release profile of TMZ at pH 2.8 and pH 7.4 over 24 h, from which it can clearly be seen that release was pH-dependent. As can be seen from Fig. 6, at pH 7.4 the release of TMZ reaches 97% in 24 h, where in the first ten hours, the amount released shows a dramatic increase, at up to 90%, which is then followed by a more sustained release pattern over the following 14 hours. On the other hand, at pH 2.8 the total amount of TMZ released over 24 h was only 73%. An ‘eruption’ release of up to 45% was seen in the first 60 minutes at pH 7.4 and 2.8, which can be attributed to the physisorption of TMZ onto the dispersed POM-chitosan nanostructure powder. Compared to the pH 7.4 release profile, the amount of TMZ released at pH 2.8 in the first four hours was relatively smaller, at up to 50%, indicating that the absorption of TMZ is favored at lower pH. Thus, it can clearly be concluded that the release behavior of TMZ from POM-Chitosan nanostructures was pH-responsive. The above results clearly demonstrate the potential use of POM-Chitosan nanostructures as nanocarriers for the oral delivery of TMZ in the therapy for certain cancers.

4. CONCLUSIONS

In this research, the nanocarriers from POM and Chitosan were effectively synthesized and loaded with TMZ (loading efficiency of 21% using the self-assembly method). Oral drug delivery is the primary goal for developing and innovating these nanocarriers. The as-prepared nanocarrier form of TMZ was fully characterized via XRD, FTIR, XPS, and SEM. The XRD analysis of nanocomposite gives insightful information about crystal structure and arrangement and aids to understanding their general composition. The FT-IR Analysis contributed to determining and confirming the chemical structure of the nanocomposite by identifying the functional group present in the sample. The XPS analysis was used primarily to provide information about the nanocomposite composition by identifying the type of elements within these nanocomposites. The SEM analysis was used to determine the structural features and surface morphology of the nanocomposite by providing a better understanding of their physical properties. The oral form of TMZ loaded within the nanocomposite was evaluated, and release behavior was shown to depend on the pH. The comparison of the release rate of TMZ from the nanocomposite in PH 7.4 and pH 2.8 found that the amount of release in pH 7.4 was faster than in pH 2.8. The ability of these nanocomposites to effectively control the TMZ release through their pH-dependent release behavior makes them a promising method for oral drug delivery. Generally, the successful production thorough characterization of the POM-Chitosan nanocomposite loaded with TMZ and their pH-dependent release behavior demonstrated the potential of these nanocomposites to be used as effective carriers in oral drug delivery. These results provide new possibilities for enhancing the delivery of TMZ, maximizing the therapeutic efficiency, and eventually minimizing the side effects.

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5. DECLARATIONS

5.1. Study Limitations

1. The study only focuses on synthesizing and characterizing the POM-Chitosan nanocomposite loaded with TMZ. No *in vitro* or *in vivo* studies were conducted to evaluate the anticancer efficacy, cytotoxicity, or biocompatibility of the developed nanocarrier system.

2. The release observations were performed only under simulated gastrointestinal pH conditions (pH 2.8 and 7.4). The behavior of the nanocarrier system in other physiological environments, such as blood or tumor microenvironments, was not investigated.

3. The potential for targeting the nanocarrier system to specific cancer cells or tissues was not explored, which could enhance the therapeutic efficacy and reduce side effects.

4. No long-term stability studies were conducted to determine the shelf-life and storage conditions of the developed nanocarrier system.

5. It did not investigate the potential toxicity or environmental impact of the POM-Chitosan nanocomposite, which is essential for future clinical translation and commercialization.

6.

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5.3. Funding source

The research was founded by the researcher.

5.4. Competing Interests

The authors declare no conflicts of interest.

5.5 Open Access

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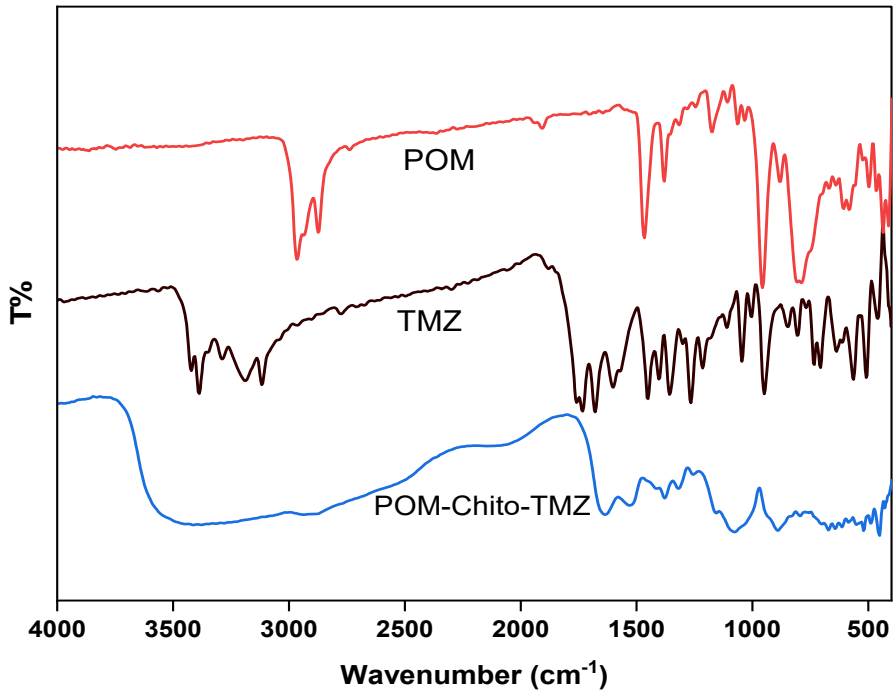

Figure 2. *FTIR spectra of the POM-Chitosan nanocomposite loaded with TMZ(blue), POM Lindqvist-type (red), and TMZ(black) .*

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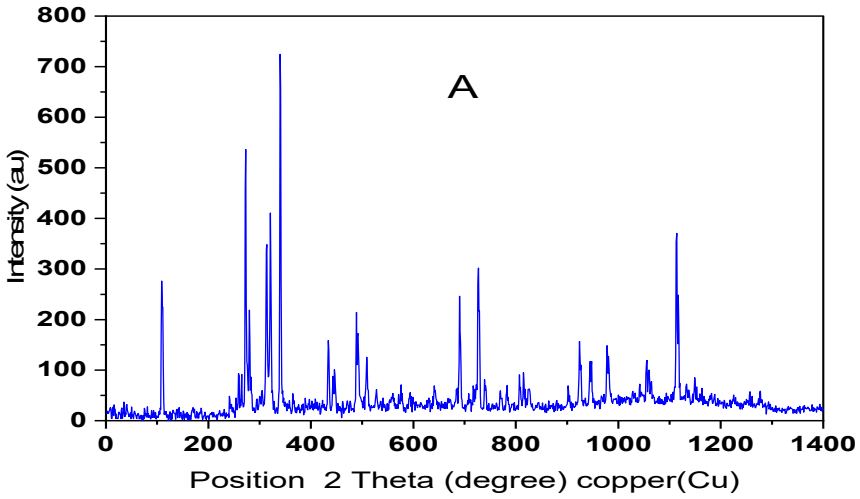
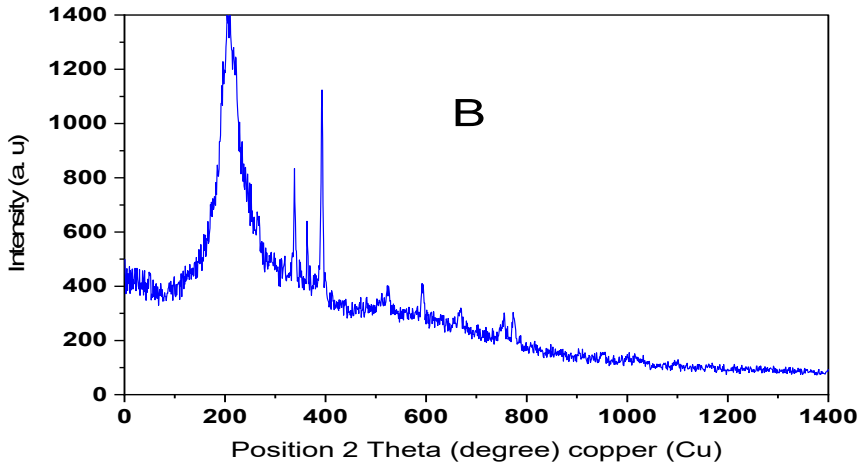

A
Intensity (a.u)
Position 2 Theta (degree) copper(Cu)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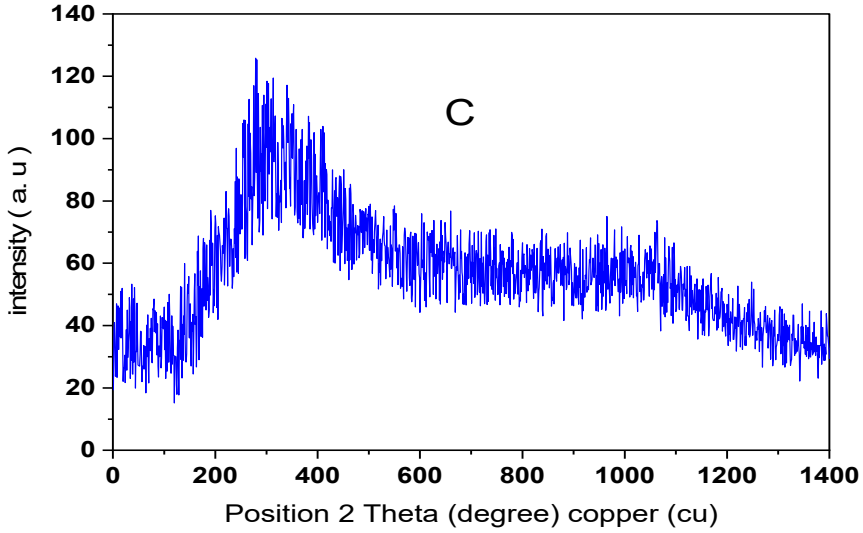
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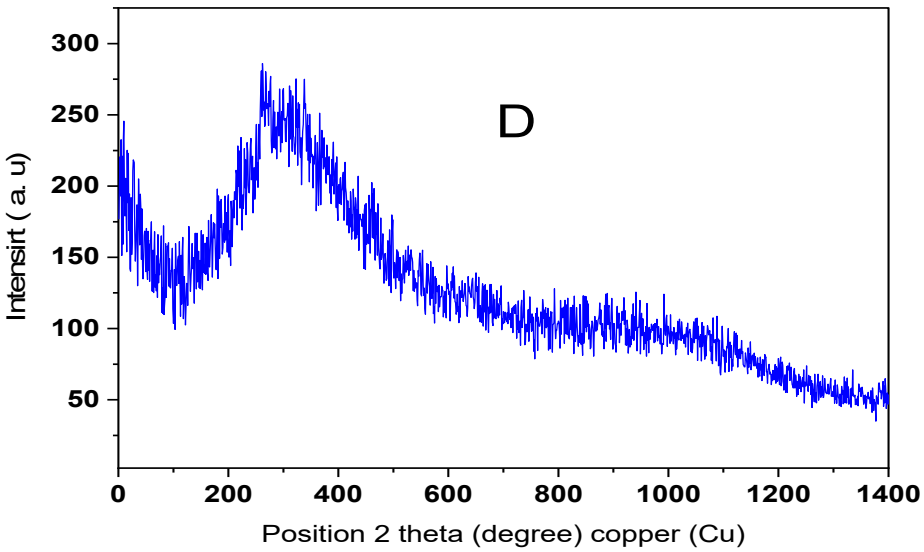

Figure 3. *: XRD patterns of (A) POM hexamolybedate; (B) chitosan ; (C) the as-prepared nanostructure prior to loading with TMZ; and (D) the as-prepared nanostructure after loading with TMZ.*

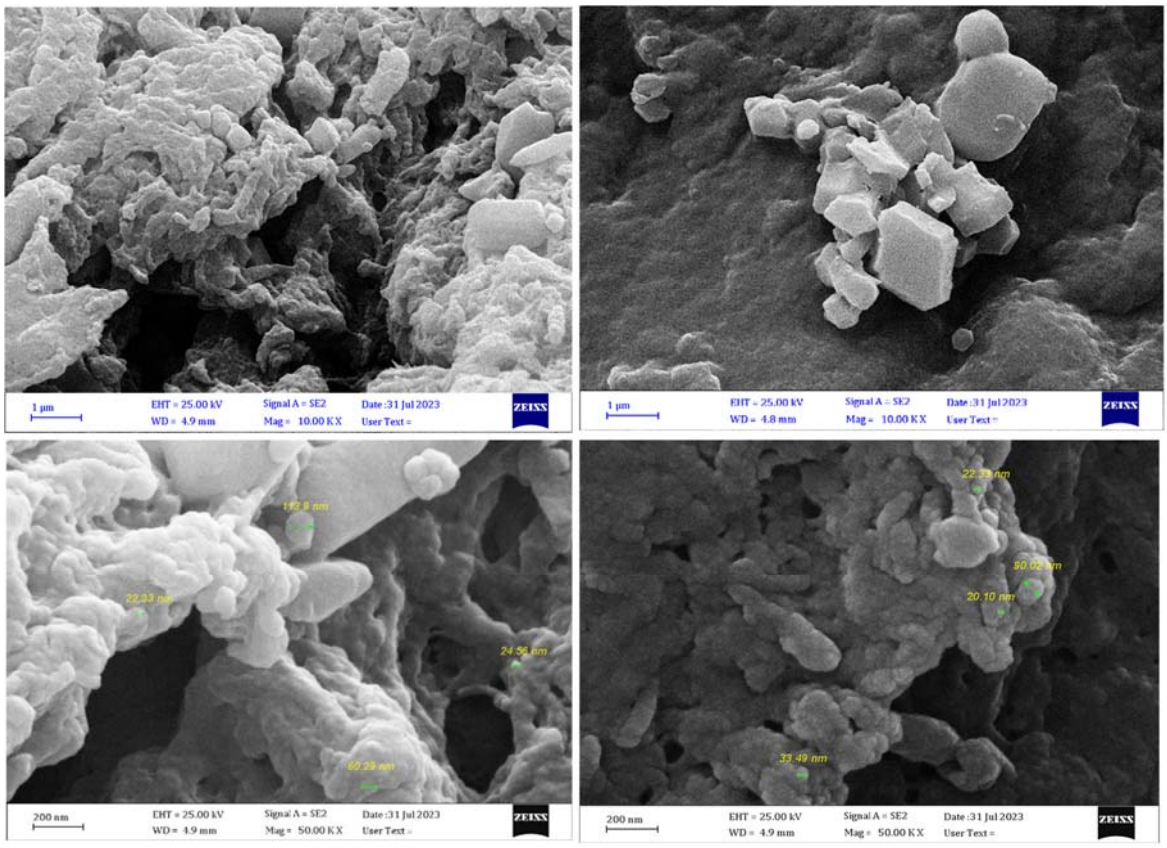

Figure 4. *SEM images of the as-prepared nanostructures prior to loading with TMZ.*

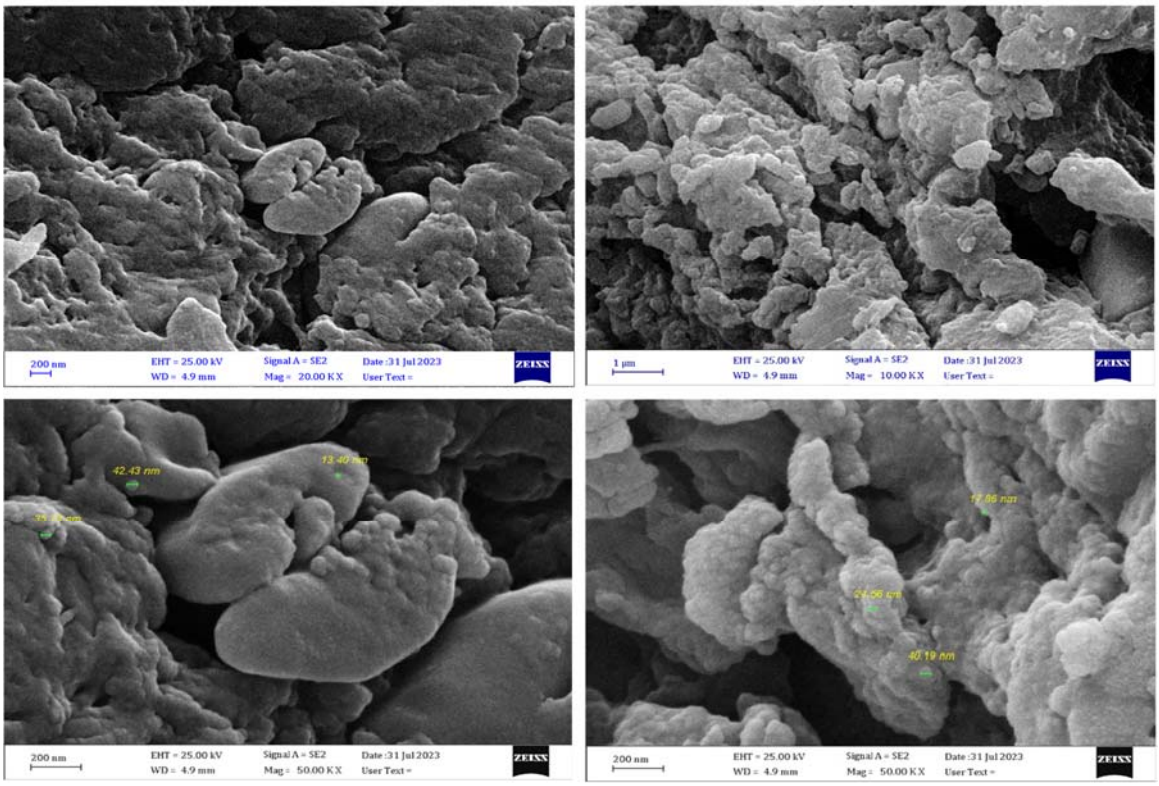

Figure 5. *SEM images of the as-prepared nanostructures after loading with TMZ.*

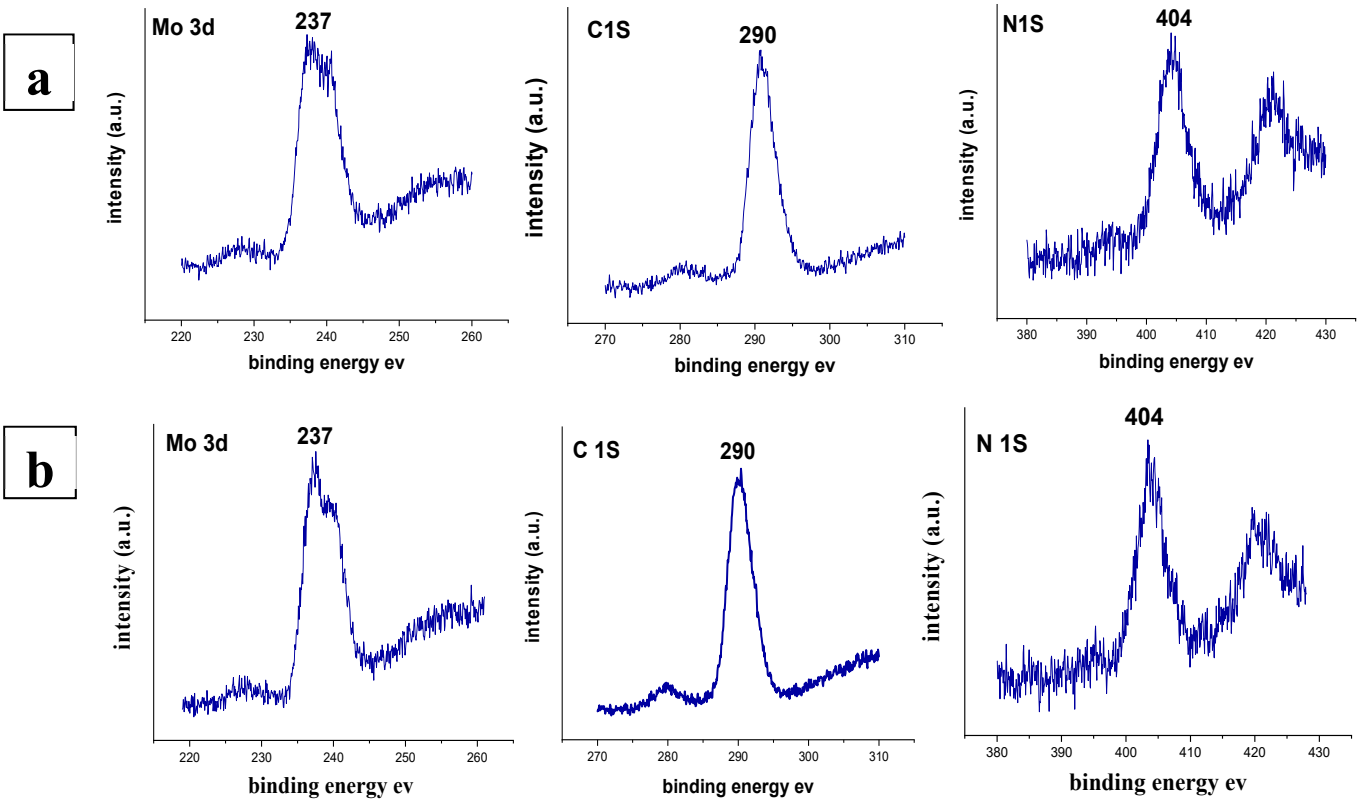

Figure 6. ***:*** *X-Ray photoelectron spectra of: (a) the as-prepared POM-Chitosan nanostructure prior to loading with TMZ; and (b) the as-prepared POM-Chitosan nanostructure after loading with TMZ.*

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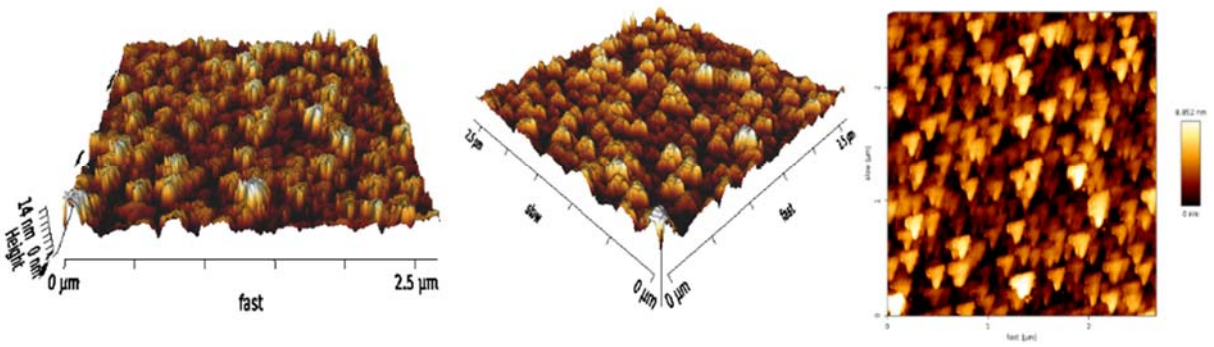

Figure 7. *AFM images of the as-prepared nanostructures prior to loading with TMZ*

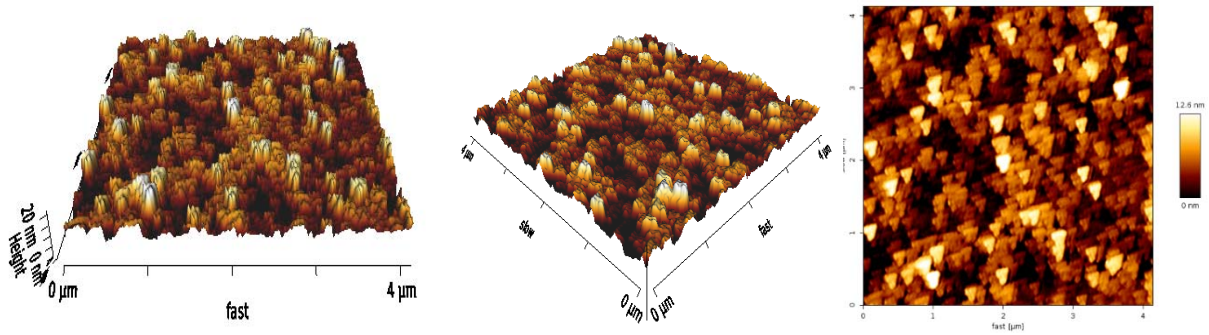

Figure 8. *AFM images of the as-prepared nanostructures after loading with TMZ.*

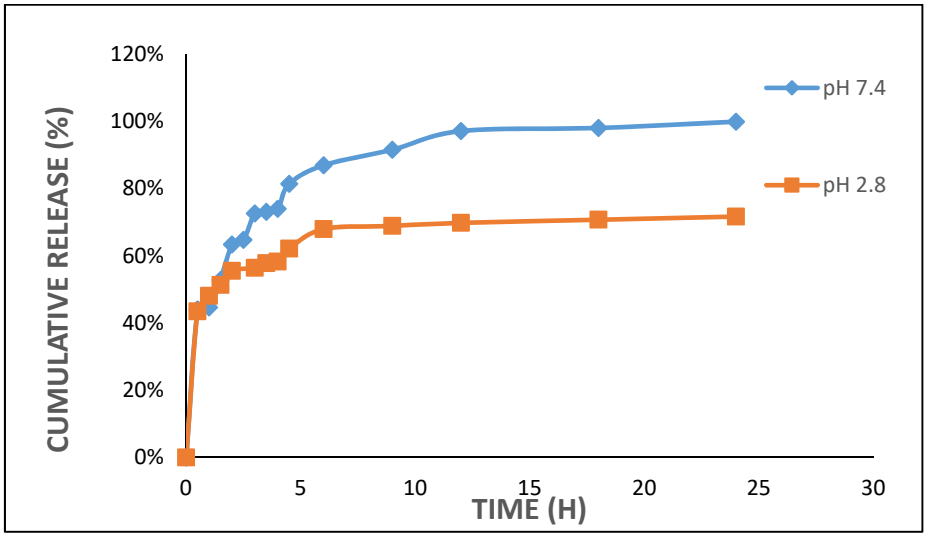

Figure 9. *Release behavior of TMZ loaded onto nanostructures at pH 7.4 (blue) and pH 2.8 (orange).*

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Table 1. *Chromatographic conditions*

Column	**C18**
Mobile phase	Acetonitrile 60:40 Water HPLC
Detector (Wavelength)	328 nm
Flow rate	1.0
Column temperature	37°C
Injection volume	20 µL
Run time	10 min

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