



Development and Characterization of TMZ Loaded HPMC-Montmorillonite Composites

*Makale Bilgisi / Article Info

Alındı/Received: 23.01.2025

Kabul/Accepted: 01.05.2025

Yayımlandı/Published: 01.10.2025

TMZ Yüklü HPMC-Montmorillonit Kompozitlerinin Geliştirilmesi ve Karakterizasyonu

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Abstract

In particular, using natural and large surface area natural inorganic materials such as clay minerals as carrier systems or adsorbents is important in terms of low cost. This study investigated the utility of montmorillonite (Mt) clay in both its raw form and when modified with hydroxypropyl methylcellulose (HPMC) for the encapsulation and release of temozolomide (TMZ) active ingredient. For this purpose, the raw Mt clay was first purified using the sedimentation method. Then, six different formulations were prepared using Mt, HPMC, and TMZ in different composition ratios. The release of TMZ was evaluated under simulated gastric fluid conditions at varying time intervals. As a result of the release tests, it was determined that the optimum formulation (F-6) showed the best controlled release of TMZ from HPMC-modified Mt. The F-6 formulation exhibited a rapid TMZ release of 50% in the first 45 min. In the later period, it was determined that the diffusion occurred more slowly for 360 min and reached approximately 90%. Korsmeyer-Peppas and Hixson's kinetics clearly explained the TMZ release of the generated formulations. Nonetheless, the presence of regulated drug diffusion (Fickian mechanism) and ion exchange mechanism is linked to n coefficient values in the Korsmeyer-Peppas model being smaller than 0.5.

Öz

Özellikle kil mineralleri gibi doğal ve yüksek yüzey alanlı doğal inorganik malzemelerin taşıyıcı sistem veya adsorban olarak kullanılması düşük maliyet açısından önemlidir. Bu çalışmada montmorillonit (Mt) kilinin hem ham haliyle hem de hidroksipropil metilselüloz (HPMC) ile modifiye edildiğinde temozolomit (TMZ) etken maddesinin kapsüllenmesi ve salınması için kullanılabilirliğini araştırıldı. Bu amaçla, ham Mt kili ilk önce sedimantasyon (çöktürme) yöntemi kullanılarak saflaştırıldı. Daha sonra, farklı bileşim oranlarında Mt, HPMC ve TMZ kullanılarak altı farklı formülasyon hazırlandı. TMZ'nin salımı, farklı zaman aralıklarında simüle edilmiş mide sıvısı koşulları altında değerlendirildi. Salım testleri sonucunda, optimum formülasyonun (F-6) HPMC ile modifiye edilmiş Mt'den en iyi kontrollü TMZ salımı gösterdiği belirlendi. F-6 formülasyonu ilk 45 dakikada %50'lik hızlı bir TMZ salımı sergiledi. Devam eden periyotta, TMZ salımının 360 dakika boyunca daha yavaş gerçekleştiği ve yaklaşık %90'a kadar ulaştığı belirlendi. Korsmeyer-Peppas ve Hixson kinetiği, üretilen formülasyonların TMZ salımını açık bir şekilde açıkladı. Bununla birlikte, düzenlenmiş ilaç difüzyonunun (Fickian mekanizması) ve iyon değişim mekanizmasının varlığı, Korsmeyer-Peppas modelindeki n katsayısı değerlerinin 0,5'ten küçük olmasıyla bağlantılıdır.

Keywords: Montmorillonite; Bentonite; HPMC; Drug Release; Temozolomide

Anahtar Kelimeler: Montmorillonit; Bentonit; HPMC; İlaç Salımı; Temozolomit

1. Introduction

Montmorillonite (Mt) clay, an essential component of bentonite rock, is an exceptionally versatile and multifunctional clay mineral having a chemical formula of $(\text{Na}, \text{Ca})_{0.33}(\text{Al}, \text{Mg})_2\text{Si}_4\text{O}_{10}(\text{OH})_2 \cdot n\text{H}_2\text{O}$ (Lagaly and Dekany 2013). This mineral boasts exceptional properties that contribute to its broad applicability, such as high surface area, remarkable adsorption capacity, biocompatibility, high swelling capacity, and non-toxicity. Even in acidic medias, it demonstrates remarkable thermal and chemical stability (Çiftçi 2022, Lee et al. 2005). Mt's negatively charged surface attracts and binds positively

charged species, notably including drug molecules (Hassen et al. 2023). Its high adsorption capacity significantly enhances drug entrapment and sustained drug release in numerous formulations. Moreover, it accelerates the dissolution and enhances the bioavailability of hydrophobic drugs (Park et al. 2016). Mt acts as an efficient encapsulation agent, shielding drugs from degradation and providing control over their release rates. Given these exceptional attributes, Mt emerges as an ideal candidate for advanced drug delivery systems in the pharmaceutical industry (Park et al. 2016, Tekin et al. 2019; Çiftçi et al. 2020a). Prior studies have focused on Mt

clay as a drug carrier, highlighting its biocompatibility, biodegradability, and non-toxicity (Çiftçi et al. 2020b, Viseras et al. 2010, Yang et al. 2016, Salcedo et al. 2012, Kevadiya et al. 2012, Wang et al. 2016). However, Mt clay must be subjected to surface modification with polymers such as chitosan or hydroxypropyl methylcellulose (HPMC) to improve the drug release characteristics. HPMC, a non-toxic, biocompatible, and water-soluble polymer, is a hydrophilic cellulose derivative widely utilized in the pharmaceutical sector for the formulation of controlled-release drugs by creating a gel layer on the surface of matrix systems (Arpa et al. 2023a, Arpa et al. 2023b, Friuli et al. 2022, Novak et al. 2012). Its non-ionic nature minimizes the likelihood of drug interactions, maintaining consistent drug release profiles regardless of the pH of gastrointestinal fluids (Joshi 2021).

Temozolomide (TMZ) is an effective active ingredient in treating various cancers, especially glioblastoma. However, the efficiency of TMZ faces several limitations. The brief half-life results in swift excretion from the body, impeding its capacity to target and eradicate cancer cells (Waghule et al. 2023). Additionally, poor solubility poses challenges in dissolving TMZ in water, affecting its effectiveness. Another critical consideration is the drug's side effects, which encompass nausea, vomiting, fatigue, and reduced blood cell counts (Jatyan et al. 2023). Researchers persistently explore methods to enhance the drug's efficacy while mitigating its side effects (Rodriguez et al. 2023, Stupp et al. 2009). For this purpose, studies are being conducted on various inorganic or organic nano- or micro-sized carrier systems (Irani et al. 2017).

This study capitalizes on Mt's exceptional attributes to address the critical need for TMZ release in cancer treatments. According to the literature review, no study has been conducted to examine the release characteristics of TMZ by Mt clay. Thus, investigating an industrial raw material such as Mt, which is cheap and has good properties for drug delivery systems, as a carrier material for the drug TMZ used in cancer treatments, will contribute to the scientific literature. In pharmaceutical applications employing Mt clay minerals, the initial stage involves removing gangue minerals (quartz, calcite, opal, feldspar, etc.) from the raw clay rock using various mineral beneficiation methods, such as sedimentation, centrifugation, hydrocyclone separation, and chemical separation (Çiftçi 2021, Joshi et al. 2009, Carretero et al. 2009). The primary objective of this study was to develop formulations by integrating TMZ with pure Mt and HPMC-modified Mt. To achieve this objective, Mt clay ore (bentonite) underwent an initial purification process using sedimentation as a mineral beneficiation technique.

After the formulations, varying Mt-to-HPMC ratios were prepared, and TMZ was loaded into these formulations. The final step involved assessing the release profiles of TMZ in the simulated gastric medium.

2. Materials and Methods

2.1 Materials

This study provided commercial bentonite ore primarily comprised of Mt by Samaş Industrial Minerals Co., located in the Reşadiye-Tokat region of Türkiye. This company occupies a significant market position, and its bentonite has been thoroughly examined by numerous researchers, particularly regarding the development of clay-polymer nanocomposites (Hojiyev et al. 2017), characterization of organo-bentonites (Yeşilyurt et al. 2014, Hojiyev et al. 2016), purification of bentonites (Yeşilyurt et al. 2014, Boylu et al. 2012), and adsorption studies (Orucoglu and Hacıyakupoglu 2015). Sodium chloride (NaCl), hydrochloric acid (HCl), and hydroxypropyl methylcellulose (HPMC) were purchased from Sigma Aldrich, US. Temozolomide (TMZ), which was used as an active ingredient (drug), was purchased from TLC, Canada.

2.2 Montmorillonite Purification

The sedimentation method was employed to obtain purified Mt. This method, which is one of the mineral beneficiation techniques, is based on the separation of dispersed particles in a liquid medium according to their density and particle size. This study focused on separating clay particles below 2 µm, forming a colloidal system, from larger non-clay minerals (gangue) that settle to the bottom. Maintaining an optimal solid ratio of around 1% in the pulp is crucial to yield optimal results and minimize particle interactions. Initially, raw Mt ore was reduced in size to particles under 5 mm using a jaw crusher followed by a roller mill. Following, 10 g of the crushed sample was placed into a beaker containing 1 L of distilled water. The prepared clay water mixture was left to stand for 24 hours and then stirred using a magnetic stirrer for 60 minutes to ensure thorough dispersion. Then, the mixture was left to stand for 8 hours. Thus, larger non-clay minerals were allowed to settle to the bottom. Finally, the clay particles suspended in water above the sediment were removed by siphoning, separated from the water by centrifugation, and left to dry at 45°C for 24 hours.

2.3 Characterization Studies

The raw and purified clay samples underwent air-drying to evaluate their mineral composition. X-ray diffraction (XRD) patterns were obtained utilizing an XRD-6000 instrument (Shimadzu Co., Japon). The instrument

operated at 40 kV with Cu-K α radiation (λ : 1.54184 Å). Scans were conducted within an angular range of 2-70° with a step speed of 0.02°. To determine the elemental composition of Mt, the samples underwent a drying process at 105°C for 24 hours, followed by a 2-minute milling procedure using a ring mill. Subsequently, the

chemical composition of Mt samples was assessed using a ZSX Primus II model X-ray fluorescence (XRF) device (Rigaku Co., Japan). A Mastersizer 2000 instrument (Malvern Co., UK) was employed to determine particle size distributions using dispersions obtained before and after sedimentation experiments.

Table 1. Formulations prepared for TMZ release.

Formulation (Mt+HPMC+TMZ)	TMZ/Formulation	HPMC ratio (%) (based on total carrier amount)	200 mg/L TMZ solution (mL)
F-1	1/10	0	150
F-2	1/10	10	
F-3	1/10	25	
F-4	1/5	0	
F-5	1/5	10	
F-6	1/5	25	

Zeta potential (ζ) is a quantifiable measure of the electric charge on solid particles suspended in a liquid medium. It plays a crucial role in defining electrostatic repulsion forces among particles, thereby influencing the overall stability of a colloidal system (Çiftçi et al. 2020a, Soni et al. 1997). A Nano-Z model zeta-meter device (Malvern Co., UK) was employed to analyze the samples' zeta potential (ζ). For this, approximately 0.01 g of the dried and ground samples were introduced into a solution comprising 100 mL of 10-3 M NaCl. This mixture was dispersed using a magnetic stirrer for 30 minutes. After the stirring, a 5-minute settling interval allowed coarser particles to settle. A 2 mL sample was then added to the measuring cell. The ζ values were calculated automatically through the classical Smoluchowski formula, utilizing the measured electrophoretic mobility of the particles. The procedure was repeated thrice, recording outcomes with a 3-5% standard deviation. To see the ζ change depending on the medium's pH value, the prepared dispersions' pH values were adjusted using diluted HCl and NaOH solutions, and measurements were taken.

2.4 Preparation of Drug Formulations

Initially, HPMC and TMZ solutions were prepared. For this, a magnetic stirrer dissolved 200 mg of TMZ powder in 1000 mL of distilled water. This solution was then stored at 4°C. Regarding HPMC working solutions, 100 mL of distilled water was heated to 40°C on a magnetic stirrer. Then, 150 mg HPMC powder was added to the water and slowly stirred until completely dissolved. It is very important to avoid boiling the solution. The process of loading TMZ onto purified Mt followed the outlined steps in Table 1. In brief, a certain amount of purified Mt was added to 150 mL of TMZ solution. The prepared mixture was then subjected to stirring at 37°C using a magnetic stirrer. The stirring process was continued until the liquid

medium was evaporated entirely. HPMC-free formulations (F-1 and F-4) were prepared in this way. For the other formulation, a certain amount of Mt was added to 150 mL of TMZ solution, and the prepared mixture was stirred for 24 hours at 25°C using a magnetic stirrer. Then, a certain amount of the previously prepared HPMC solutions was added to the mixture, and the stirring process was continued at 37°C. At this stage, stirring was continued until the liquid medium evaporated completely. Afterward, the solid products were finely ground using an agate mortar. The resulting powders were then encapsulated into gelatin capsules.

2.5 In Vitro Release Studies of TMZ

Spectrum scans of previously prepared TMZ solutions at concentrations between 1 and 50 mg/L were performed to determine the TMZ concentration in the solutions. Scans were performed in the 200-400 nm wavelength range using a Shimadzu UV-vis Mini 1240 spectrophotometer. According to the results obtained, it was determined that the TMZ active component gave the highest absorbance at 289 nm wavelength at every concentration. Then, the calibration curve was created by reusing solutions at 1-50 mg/L concentrations and taking absorbance readings at 289 nm.

The in vitro release profiles of TMZ from the formulations were evaluated in simulated gastric fluid (comprising 80 mL 1.0 N HCl, 2.0 g NaCl, 3.2 g pepsin, and distilled water until the total volume is 1000 mL; pH: 1.2). Each capsule which contains 50 mg formulation was placed inside a dialysis membrane, containing 5 mL of simulated gastric fluid. The membranes were submerged in beakers containing 250 mL of simulated gastric fluid and maintained on a magnetic stirrer at 37°C. Then, stirring was performed at 100 rpm throughout the release period. Taking 2 mL samples from the system at predetermined

times (0.5, 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours) determined TMZ concentration using the UV-vis spectrophotometer at 289 nm wavelength. Drug release kinetics were used to evaluate the release profiles of the formulations (Çiftçi et al. 2020b, Özkan et al. 2023).

3. Results and Discussions

3.1 Characterization Studies

XRF analysis data presented in Table 2 revealed that the samples mainly comprised silicon oxide and aluminum oxide, collectively accounting for more than 81% of the composition. This composition is a strong indication of the presence of clay minerals. The $(\text{Na}_2\text{O}+\text{K}_2\text{O})/(\text{CaO}+\text{MgO})$ ratio was also determined to be 0.67. Ratios exceeding 0.33 indicate that Mt samples belong to the intermediate category, classified as sodium montmorillonite clay. The significant decrease in calcium content in the purified sample proved the effective removal of calcite minerals from raw Mt ore. The decrease in Na_2O content after beneficiation can be attributed to removing sodium-rich feldspar minerals.

Table 2. XRF analysis of raw and purified Mt.

Component	Content (% wt)	
	Raw Mt	Purified Mt
SiO_2	62.23	62.19
Al_2O_3	19.06	20.2
Fe_2O_3	3.63	3.99
MgO	2.7	2.93
Na_2O	2.61	2.02
CaO	2.05	0.74
K_2O	0.65	0.44
LOI	6.30	7.0

LOI: Loss on ignition

The XRD patterns, illustrated in Figure 1, revealed the composition of the initial clay sample, primarily composed of Mt minerals alongside traces of non-clay minerals like clinoptilolite (zeolite), feldspar, opal (C-T), calcite, quartz, and mica. The majority of gangue minerals in the raw Mt ore were effectively removed through the sedimentation-based beneficiation process. The Mt sample showed characteristic reflections at 2θ : 7.07° (basal space d_{001} : $12.4 \text{ \AA}$), showing the presence of Mt minerals rich in Na^+ cation (Whittig 1965). It is tough to separate non-clay minerals with particle sizes smaller than $2 \mu\text{m}$ from Mt minerals by sedimentation method. Other methods, such as chemical dissolution, must be used for this.

Particle size distribution curves (Figure 2) revealed that the raw Mt sample contained particles larger than $10 \mu\text{m}$, probably originating from gangue minerals, accounting for approximately 20–25% of the volume.

As expected, the purified Mt sample exhibited narrower size distributions ranging from 0.3 to $8 \mu\text{m}$. The results reported in the literature for purified Mt greatly agree with these results (Shah et al. 2013, Chakraborty et al. 2013). These data highlighted how the sedimentation method separates particles based on particle size differences. Nevertheless, these characteristics indicated that eliminating particles smaller than $10 \mu\text{m}$ presents considerable difficulties via sedimentation methods.

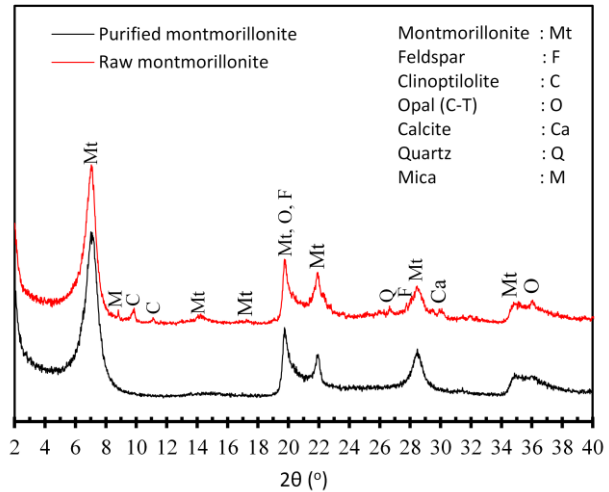


Figure 1. XRD patterns of the raw and purified montmorillonite.

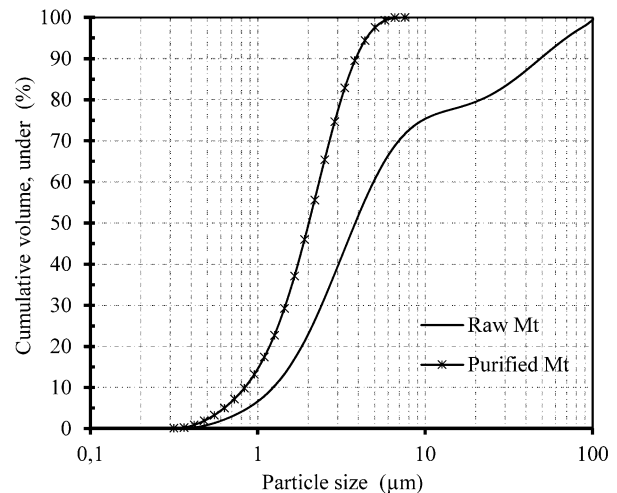


Figure 2. Particle size distributions of raw and purified Mt.

Figure 3 displays the purified Mt clay's zeta potential (ζ) profiles. These measurements reiterated the negatively charged surface of Mt clay. It is important to note that Mt clay showcased a negative surface charge even under highly acidic conditions. This occurrence can be attributed to the adsorption of hydronium ions (H_3O^+) onto the Mt.

Additionally, H_3O^+ ions were also adsorbed between the clay layers, owing to the high cation exchange capacity of Mt. While the maximum ζ value was measured around -42 mV at pH 12, the minimum value was determined to be around -29 mV at pH 2. These results showed that the adsorption capacity of Mt clay was relatively high at basic

media due to the electrostatic attraction forces against cationic species.

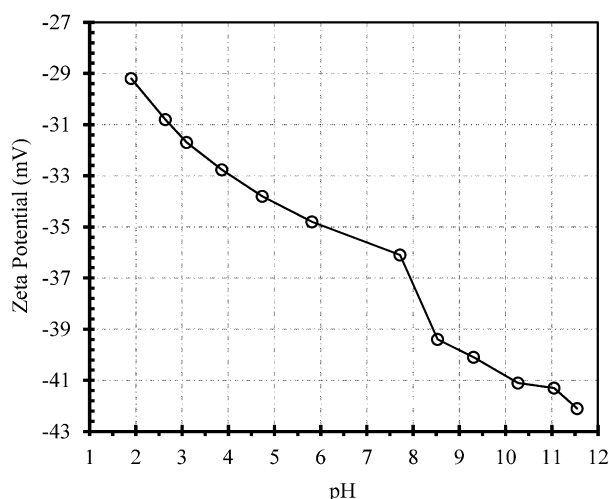


Figure 3. ζ profile of the purified Mt as a function of pH.

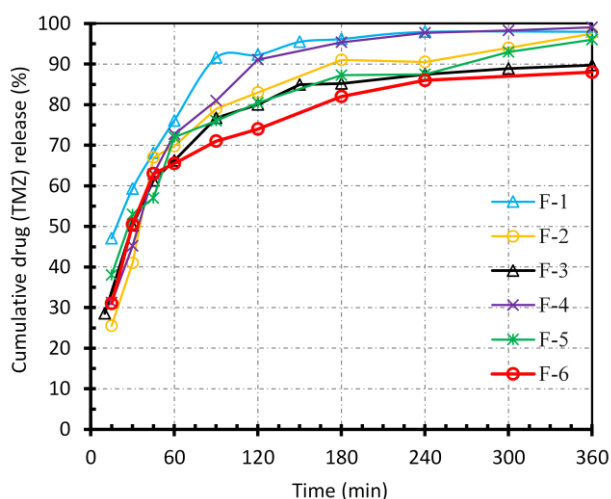


Figure 4. TMZ releases profiles of the formulations.

3.2 In Vitro Release Profiles of TMZ and Release Kinetics

The release of TMZ active ingredient from the prepared formulation is shown in Figure 4. The release profiles showed that a rapid TMZ release occurred in all formulations in the first 15 min, varying between 25% and 45%. Subsequently, rapid release continued for approximately 60 min in all formulations except F-6, and cumulative release between 65% and 75% was achieved.

The F-1 formulation's rapid release lasted 90 minutes, and cumulative TMZ release reached approximately 92%. The high rate of release in a short time in the F-1 formulation is because the carrier system was not modified with HPMC. This result proved that coating the carrier system with HPMC allows delayed release (Pani and Nath 2014, Zhou et al. 2014). Evidence of this was also seen in the F-6 formulation. Namely, it was determined that the release rate slowed down after the 45th min in the F-6 formulation

and continued for 360 min. Again, the HPMC-free F-4 formulation also exhibited a rapid TMZ release. All these data concluded that the increase in the HPMC ratio in the formulation slowed down the TMZ release.

TMZ loading ratio changed the release kinetics. A slower TMZ release was observed in formulations with high TMZ loading (1/5; TMZ/carrier system). The reason for this situation can be shown as the high driving force of the high TMZ concentration in the dispersion during the drug loading stage. Namely, as the adsorbate (TMZ) concentration increased, the mass transfer between the clay layers became more intense and vigorous. Therefore, when the TMZ concentration in the medium is much lower than the cation exchange capacity (CEC) of the clay, adsorption occurs mainly on clay surfaces. However, as the concentration increases, rapid adsorption occurs on the outer surfaces, and at the same time, slower adsorption begins to occur between the clay layers.

Another result observed from the release profiles of the formulations was that the increase in the HPMC additive ratio decreased the cumulative TMZ release from the Mt for all formulations. This can be explained by the fact that some TMZ molecules in the depths of the clay layers do not have enough energy to overcome the HPMC cover layer. In this case, it can be said that HPMC molecules establish strong physical bonds with the surfaces between the Mt layers.

As a general result of the obtained TMZ release profiles, the F-6 formulation was selected as the most suitable formulation for treating various cancers by exhibiting the slowest release (360 min, 90% cumulative release).

Drug release kinetics are calculated to examine how quickly and by what mechanisms a drug is released from a carrier system (tablet, capsule, nanoparticle, implant, etc.). This analysis is critical in determining the therapeutic efficacy, safety, and quality of the pharmaceutical formulation. Release data were applied to different kinetic models to determine the release kinetics of TMZ-loaded formulations. The measured correlation coefficients (r^2) and "n" values of the kinetic models are given in Table 3.

As seen in Table 3, TMZ release of all formulations was explained by Korsmeyer-Peppas and Hixson kinetic models. In the Korsmeyer-Peppas (K-P) model, a "n" coefficient value ranging from 0.43 to 0.85 indicates a non-Fickian release mechanism that is time-dependent (Fickian) (Chakraborty et al. 2013, Çiftçi et al. 2020; Arpa et al. 2024).

Table 3. In vitro, drug release parameters obtained using different kinetic models.

Formulation	Zero-order (r ²)	First order (r ²)	Higuchi (r ²)	Hixson-Crowell (r ²)	Korsmeyer-Peppas n	(r ²)
F-1	0.8072	0.7534	0.9138	0.7729	0.3437	0.9992
F-2	0.7433	0.6198	0.8636	0.6616	0.7762	0.9681
F-3	0.7564	0.6264	0.8807	0.6714	0.6213	0.9642
F-4	0.8162	0.7088	0.9229	0.7470	0.6237	0.9921
F-5	0.8193	0.7372	0.9197	0.7662	0.4314	0.9642
F-6	0.7409	0.6187	0.8628	0.6506	0.4692	0.9229

4. Conclusions

The commercial bentonite ore was effectively purified through sedimentation, capitalizing on the particle size difference between montmorillonite clay (Mt) and non-clay minerals. The applied method obtained pure Mt clay with a purity level of at least 90% and a recovery rate of 67.5%.

As a result of in vitro drug release tests, it was determined that HPMC-containing Mt formulations had revealed a more delayed release of TMZ. Thus, it was decided that the F-6 product, with a 25% HPMC additive and TMZ/carrier material ratio kept constant at 1/5, was the most suitable formulation. The F-6 formulation achieved a delayed release of 90%, lasting 6 hours. The Korsmeyer-Peppas and Hixson kinetic models elucidated the release of all formulations by TMZ.

This study presented a novel approach for regulated drug release, utilizing the unique properties of Mt clay and HPMC. It presented potential solutions to the issues provided by TMZ and unveiled interesting pathways for cancer treatment. The study constitutes a preliminary step for further investigation and enhancement of treatment techniques for GBM (glioblastoma multiforme) patients.

Declaration of Ethical Standards

The authors declare that they comply with all ethical standards.

This study is derived from a master thesis (thesis number 10499249) under the supervision of Assoc. Prof. Dr. Hakan ÇİFTÇİ by Firoozeh PANAHIANLARKEY on the date of 27.02.2024, titled "Controlled temozolomide release with HPMC-modified montmorillonite".

Credit Authorship Contribution Statement

Author-1: Supervision, conceptualization, investigation, methodology, visualization, writing – original draft, review, and editing.

Author-2: Conceptualization, investigation, data curation, visualization, writing – original draft.

Declaration of competing interest

The authors reported no potential conflict of interest.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

Acknowledgment

This study was supported by Afyon Kocatepe University, Scientific Research Projects Coordination, Afyonkarahisar, Türkiye (Grant No: 23.FEN.BİL.04).

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