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Evaluation Of Drug Release Kinetics Of Temozolomide Loaded PLGA Nanoparticles In Pluronic® F-127 Hydrogel
PLURONIC® F-127 Hidrojel İçinde Temozolomid Yüklü PLGA Nanopartiküllerinden İlaç Salım Kinetiklerinin Değerlendirilmesi

Tansel Çomoğlu. Evaluation Release Kinetics from Temozolamide Nanoparticles

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ABSTRACT

Objective: Controlled local release of temozolomide (TMZ) at the tumor site is a new strategy to get beneficial treatment of glioblastoma. Localized delivery systems, based on biodegradable polymers, are capable of slowing and controlling drug release for a certain period of time. Therefore, the main objective of the study was to investigate a new approach for encapsulating TMZ in a PLGA nanoparticle system which was then formulated in 18% Pluronic® hydrogel matrix which would provide a sustained and local delivery of TMZ.

Methods: Hydrogels are rapidly being investigated as local drug delivery methods due to their tunable characteristics and capacity to retain labile pharmaceuticals. The lack of established procedures for describing and evaluating drug release, on the other hand, offers considerable problems, impeding reliable evaluation of systems for defining drug release characteristics.

Results: In this part our study, we aimed to research drug release kinetics of TMZ nanoparticles of which have an encapsulation efficiency and particle size that range between 52-69.67% and 164.4-235.5 nm from a novel hydrogel drug delivery system.

Conclusion: The application of mathematical modeling proves to be extremely beneficial for estimating the release kinetics before the release systems are implemented. The release mechanism was found to be diffusion controlled and not accompanied by dissolution of matrix.

Key words: Hydrogel, Pluronic F-127, temozolomide, nanoparticles, drug release kinetics

ÖZ

Amaç: Tümör bölgesinde temozolomid'in (TMZ) kontrollü lokal salınımı, glioblastomanın faydalı tedavisi için yeni bir stratejidir. Biyobozunur polimerlere dayanan lokalize dağıtım sistemleri, belirli bir süre boyunca ilaç salınımını yavaşlatabilir ve kontrol edebilir. Bu nedenle, çalışmanın ana amacı, TMZ'nin sürekli ve yerel bir dağıtımını sağlayacak olan %18 Pluronic® hidrojel matrisinde formüle edilen bir PLGA nanoparçacık sisteminde TMZ'yi kapsüllemek için yeni bir yaklaşımı araştırmaktır.

Yöntemler: Hidrojeller, ayarlanabilir özellikleri ve kararsız farmasötikleri tutma kapasiteleri nedeniyle yerel ilaç dağıtım yöntemleri olarak hızla araştırılmaktadır. Öte yandan, ilaç salınımını tanımlamaya ve değerlendirmeye yönelik yerleşik prosedürlerin olmaması, ilaç salım özelliklerini tanımlamaya yönelik sistemlerin güvenilir bir şekilde değerlendirilmesini engelleyen önemli sorunlar ortaya çıkarmaktadır.

Bulgular: Yeni bir hidrojel ilaç taşıma sisteminden 164.4-235.5 nm arasında değişen partikül boyutu ve %52-69.67 kapsülleme etkinliğine sahip TMZ nanopartiküllerinin in vitro ilaç salım kinetiğini araştırmayı amaçladık.

Sonuç: Serbest bırakma sistemleri uygulanmadan önce serbest bırakma kinetiğini tahmin etmek için matematiksel modellemenin kullanılmasının çok faydalı olduğu ortaya çıktı. Salım mekanizmasının difüzyon kontrollü olduğu ve matriksin çözünmesinin eşlik etmediği bulundu.

Anahtar kelimeler: Hidrojel, Pluronic F-127, temozolomid, nanopartiküller, ilaç salım kinetiği

INTRODUCTION

Temozolomide (TMZ) is an anticancer agent with alkylating properties, included in the group of imidazotetrazine derivatives, developed in Aston University in the 1980s. TMZ was found to have antitumor activity in intracranial and extracranial tumors and has been used for glioblastoma multiforme treatment (GBM) (1). However, treatment of GBM remains a challenge, largely due to the fast degradation of TMZ, inability to deliver an effective dose of TMZ to tumors, and lack of target specificity which may cause systemic toxicity.

Nanoparticles can be a solution for the rapid degradation of TMZ and can specifically deliver them to GBM cells through the mediation of a tumor.

Poly Lactic-co-Glycolic Acid (PLGA) nanoparticles (NPs) have been used as efficient delivery vehicles for therapeutic agents to the brain. PLGA NPs can be produced in any desired shape and size and can trap molecules of any size (2). PLGA has been evaluated for encapsulating small anticancer agents used in nanoparticulate cancer chemotherapy (3).

Although NPs offer a number of positive properties for drug delivery, NPs have often been combined with hydrogels to further improve the therapeutic index, particularly in localized administration.

Hydrogels, like Pluronic[®], excel in controlled release applications because of their tissue compatibility and ease of dispersion in the matrix (4). Hence, we hypothesized that a hydrogel, containing nanoparticles loaded with TMZ, would provide a sustained and local delivery of the drug and destroy cancer cells that may remain in the areas that cannot be reached by tumor resection during surgical intervention in the treatment of GBM.

Pluronic F-127, is a hydrophilic polymer which is capable of holding a large amount of water and showing sol-gel transition near 37°C and the unique thermo-responsive property of the polymer is directed towards a wide area of drug delivery applications (5). In addition to Pluronic's inertness and good biocompatibility, its ability to release entrapped drug in aqueous media makes Pluronic particularly suitable as drug carriers in the controlled release of pharmaceuticals (6).

The first part of the study was conducted to formulate TMZ loaded nanoparticle formulations with PLGA by emulsion-solvent evaporation method. The PLGA nanoparticle formulations were evaluated for their particle size and size distribution, entrapment efficiency, zeta potential and in vitro drug release studies. Then, the optimum nanoparticle formulation was chosen to develop a thermo-responsive hydrogel formulation with Pluronic F-127. Also, hydrogel formulations tested for their rheological and drug release properties (7).

Many manufacturing process variables affect drug release from dosage forms. To contemplate the TMZ release mechanism from Pluronic F-127 hydrogel, and PLGA NPs, different kinetic models were considered to fit the experimental data (8).

In this part of the study, after giving a brief information about the first part of the study, it is planned to focus on the kinetics of TMZ release from nanoparticles from a Pluronic F-127 hydrogel matrix. Caccavo reviewed the trend in mathematical models used in the field of hydrogel-based drug delivery system and found that the main fit model equations used are: Zero order kinetics, first order kinetics, Korsmeyer–Peppas kinetics, Weibull and The Higuchi kinetics (9).

MATERIALS AND METHODS

Materials

Temozolomide, PLGA (acid terminated; Mw = 24.000 – 38.000; copolymer ratio 50:50), Mowiol 18-88 (Mw=130.000), and Pluronic[®] F-127 were supplied by Sigma-Aldrich. Ultrapurified water was obtained from the Milli-Q system. All other reagents and solvents used in this study were of analytical grade.

Methods

Formulation of TMZ loaded PLGA nanoparticles

The formulation method of TMZ nanoparticles was modified of Ananta from their study (7,9,10). Nanoparticles were prepared using emulsion (w/o) solvent evaporation method. TMZ and PLGA were dissolved in 1 mL dimethylformamide (DMF). The result solution was added in drops to 5 mL of PVA 5 % solution under homogenization. The organic solvent used was evaporated. NPs were collected by centrifugation and washed with deionized water to remove free TMZ. And then nanoparticles were collected from the resulting emulsion.

Particle size measurement of nanoparticles

The mean particle size of the nanoparticles was measured by Malvern Zeta Sizer (Malvern Instrument Ltd.). The dispersions were diluted with nanopure water and the sample was placed in a disposable cuvette at a count rate for 20 seconds. The particle size measurement data is reported in Table 1.

Determination of encapsulation efficiency (%)

Entrapped TMZ amount in the nanoparticles was determined with our validated HPLC assay and calculated as equation below (7).

$$\text{Encapsulation efficiency \%} = \frac{\text{TMZ amount in formulation}}{\text{Added TMZ amount}} \times 100$$

Formulation design of nanoparticle loaded Pluronic F127 thermoreversible gel

The "cold method" was used in the preparation of hydrogels. Pluronic® F-127 was weighed, added to 50 mL of ultrapure water, and mixed on magnetic stirrer at 900 rpm in an ice bath until Pluronic® F-127 was homogeneously dissolved (7). Gel formulations containing 18-20 and 25 % (w / v) Pluronic® F-127 were prepared.

Physicochemical evaluation of nanoparticle loaded Pluronic® F 127 hydrogels

pH levels of produced hydrogels

The pH levels of the hydrogel formulations were measured with Eutech Instruments pH 700 (n = 3). (9).

Sol-gel transition temperature

The gelation temperatures of developed formulations were determined by a modified version of the reverse-tube method. (n = 3) (9).

Viscosity

Sol and gel viscosities of the formulations produced were measured by running the viscometer at speeds of 5-100-5 rpm. Viscosities in gel form were measured with T96 spindle in Brookfield DVII device. Measurements were repeated three times and graphs were plotted as viscosity (cP) vs. shear rate (rpm) (9).

Mechanical properties

Texture profile analysis (TPA) was performed in the TPA mode of the TA-XT2 Texture Analyzer (Surrey, UK). The formulations were transferred in gel form into 25 mL beakers. Measurements were recorded by puncturing the samples (11).

In vitro TMZ release studies

In vitro drug release experiments for all formulations were performed in a shaker bath at a temperature of 37° C ± 1° C and pH 5 buffer containing 0.1 % ascorbic acid. The amount calculated according to the data obtained from the loading capacity and which will provide the target dose is weighed and suspended in buffer with 0.1 % ascorbic acid, and for hydrogel formulations, they are suspended in Pluronic® F-127 hydrogel and attached with two-end weighted clips in the dialysis membrane tube placed in a beaker. 20 mL of buffer solution was placed in each beaker. Samples were taken at various time intervals, complete with medium, and the release medium was replaced with new medium to prevent degradation of the active substance after 24 hours.

Kinetic evaluation of in vitro drug release studies

Dissolution rate profiles were obtained by determining the amount of active substance released by conducting an in vitro release study from the formulations. In order to determine which mathematical model the active substance release profiles fit into which mathematical model, the equations of the zero-order, first-order, Higuchi, Weibull and Korsmeyer-Peppas kinetic models were applied in Excel®, statistical evaluations were made using GraphPad Prism 6® and the findings obtained were evaluated (12).

Results and Discussion

Nanoparticles were prepared by emulsion solvent diffusion method. All formulations were produced with two different TMZ amount respectively 5 and 10 mg so as to observe the effect of TMZ amount on the size and encapsulation efficiency of nanoparticles. Nanoparticles were discrete, nearly spherical with a size range of 164.4-235.5nm. Nanoparticles of 10-200 nm size are suitable for a nano-carrier systems designed for local application that will cross the blood-brain barrier in the brain and reach the tumor site. Because nanoparticles smaller than 10 nm are excreted renally and nanoparticles larger than 300 nm are removed from the body by RES (13,14). A2-10 formulation with least particle size had the maximum entrapment efficiency (Table 1). It has been determined that increasing the amount of TMZ correlates to a higher encapsulation efficiency in all the formulations (15). This effect is explained by the fact that an excess of drug leads in a more viscous dispersed phase, making mutual dispersion of the phases problematic and resulting in the formation of large particles (16). Based on these findings A2-10 formulation was selected in the preparation of hydrogel formulations. The Zeta potential indicates that it can generate enough repulsion to overcome the gravitational attraction between nanoparticles and suspension dispersion has better stability (17). If formulations were observed to evaluate the effect of the amount of TMZ on the particle properties in Table 1, it is seen that particle size, pDI and zeta potential increases where the active substance is adsorbed to the terminal carboxyl groups of PLGA nanoparticles. This is because terminal carboxyl groups are located on the surface of nanoparticles (14). In addition, this phenomenon can be explained by the production of larger particles by causing more active substances to a more viscous oil phase and making it difficult for phases to disperse into each other (16).

The pDI value of polymeric nanoparticles is required to be less than 0.3. According to the pDI data obtained in our study (Table 1), it is seen that the pDI value of all formulations is within the acceptable range.

Smart hydrogels are attractive because of their unique sol-gel phase transitions at body temperature, biocompatibility, safety, and injectability as a solution in the body before transforming into gel matrices. In formulations containing 18%, 19% and 20% Pluronic® F-127, which showed sol-gel transition, it has been observed that the pH level increased with increasing concentration (18). According to statistical calculations, the difference between pH levels was found to be significant ($P < 0.0001$), but since the pH level of the tumor microenvironment is close to the pH level the obtained pH level is suitable for local drug administration to the tumor site (19). In our study, increased concentration resulted with a lower transition temperature (Table 2). Sol-gel phase transition temperatures of 18%, 19%, 20% and 25% Pluronic F127 containing solutions were 32°C, 30°C, 26°C and 20°C, respectively. Elasticity and cohesiveness of gels were close for all formulations.

The importance of the sol-gel transition temperature is that the formulation remains in fluid sol state at room temperature, leaving the package or injector and transforming into an in situ gel and maintaining its shape. It was observed that the gelation temperature decreased with the increase of Pluronic® F-127 concentration in the formulation, and this was confirmed by the literature (18).

It was observed that the formulations are at the bottom of the tubes in the left state at room temperature (25°C). Concentrations of 18% and above appear to be a non-flowing gel when the gels are turned upside and incubated (37°C). It was observed that the formulation containing 17% Pluronic® F-127 returned to its sol form, and formulations containing 15 to 16% Pluronic® F-127 remained in its left form. According to the results which was shown in Table 2, H18 was chosen as a proper injectable system with a gelation temperature of 32°C which leads to be a solution in the room and transits into a gel in the body (5). pH values of gels ranges between 6.80 – 6.94 which is suitable for brain application. Also, Persi revealed that acidic pH disables hypoxia adaptations of cancer cells and compromises tumor cell growth which indicates that pH values of formulations between 6.80 – 6.94 are suitable for brain cancer treatment with less damage to brain tissue (20).

The A2, A2-5, and A2-10 formulations showed a triphasic profile. In all formulations, a burst effect was observed in the first 4 hours and a plateau was detected in the following 72 hours (Figure 4). The reason for the difference in the percentage of active substance released from the formulations is thought to be proportional to the decrease in the percentage of active substance released per unit time as the encapsulation rate of the active substance increases (21). In Figure 2, the drug release of nanoparticles from the hydrogel formulations reached approximately 25% of the total TMZ in 60 days. The in vitro release profile of the active substance in the hydrogel from the nanoparticle system showed an average of 10% of immediate release in the first 12 hours, then reaching a plateau in the next 60 days. The in vitro release profile of TMZ in hydrogel showed an immediate release of 46 % in the first 6 hours and reached a plateau in the next 18 hours. The release profile of the active substance from the nanoparticle system in the hydrogel allowed the release of TMZ at lower doses over a long period of time.

Dissolution rate profiles were obtained by determining the amount of active substance released by conducting an in vitro release study from the formulations. In order to determine the active substance release profiles fit into which mathematical model, the equations of the zero-order, first-order, Higuchi, Weibull and Korsmeyer-Peppas kinetic models were applied in Microsoft Office Excel and the findings obtained were evaluated (22). The applied mathematical equations used to describe release characteristics of TMZ nanoparticles from Pluronic F127 gels were given in Table 3.

The mathematical models include: (a) zero-order model, $Q = Q_0 + k_0 t$, where Q is the cumulative release percentage, k_0 zero release rate constant, and t time; (b) first-order model, $\text{Log } C_t = \text{Log } C_0 - k_1 t / 2.303$, where $\text{Log } C$ is the cumulative release percentage, k_1 first release rate constant, t time (c) Higuchi model, $F_t = k_H t^{1/2}$, where F_t is the cumulative release percentage, k_H Higuchi release rate constant, and t time (11). (d) The formula for Weibull function is $F = 1 - \exp(-at^b)$. where F is the drug fraction released at time t , and a and b are constants. b , as a shape parameter (18). (e) Korsmeyer – Peppas model = $M_t / M_\infty = k_p t^n$ where M_t / M_∞ is the proportion of drug released at time t , k is the rate constant (Table 3) (11). As can be seen in Table 4, in vitro release kinetics of the active substance show compatibility with the Weibull kinetic model in A2, A2-5, and A2-10 formulations. The fact that all of the calculated β values are less than 1 indicates a kinetic profile where the active substance release rate occurs faster at first, then resembles the 1st degree kinetic profile and reaches a plateau (23).

The release of active substance by diffusion and/or relaxation in the polymeric system is explained by the Korsmeyer-Peppas kinetics. According to the R^2 values obtained by the calculation made from the A2, A2-5 and A-10 formulations in the hydrogel, the release kinetics of the active substance are in accordance with the Korsmeyer-Peppas kinetics. This finding confirms the fact that Pluronic® F-127 hydrogel swells by absorbing water and the

active substance is released as diffuse within the hydrogel system (24). The fact that the calculated n values are less than 0.45 indicates the Fickian diffusion profile (25).

Since the formulations show compatibility with the Higuchi and 1st order kinetic model at the same time, a graph of the amount of active substance released (Q) versus the amount of active substance released (dQ/dt) in a certain time period is plotted to determine which one is compatible.

The release of water-soluble drugs from anhydrous hydrogel matrices involves simultaneous absorption of water and desorption of drug via a swelling-controlled diffusion mechanism (26). The fact that the calculated n values are less than 0.45 indicates the Fickian diffusion profile (25).

Since the formulations show compatibility with the Higuchi and First order kinetic model at the same time, a graph of the amount of active substance released (Q) versus the amount of active substance released (dQ/dt) in a certain time period is plotted to determine which one is compatible. For graphs showing biphasic characteristics, both phases and the entire profile were applied to the graph, and it was observed that the dQ / dt and Q values in all three graphs showed inverse proportion (Figure 3). This shows that the release is in accordance with the Higuchi kinetics (27).

CONCLUSION

A modified release drug delivery system of TMZ developed as a nanoparticle hydrogel served as a depot for sustained drug release and provided a rate-limiting barrier for modulation of drug release. Drug release from the hydrogel system was evaluated by means of mathematical modelling. The use of mathematical modeling turns out to be very useful for estimating the release kinetics before the release systems are implemented. The release mechanism was found to be diffusion controlled and not accompanied by dissolution of matrix. The release kinetics in H18 followed Korsmeyer–Peppas model.

UNCORRECTED PROOF

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Çizim ve Çizelgeler

Table 1. Formulation design and particle size measurement data of TMZ nanoparticles prepared by emulsion solvent diffusion method

Component	Formulation Code		
	A2	A2-5	A2-10
PLGA	2:20	5:20	10:20
DMF (ml)	1	1	1
Z-average (mV)	-13.10 ± 0.416	-13.10 ± 1.05	-4.16 ± 0.337
Size (nm)	169.30 ± 4.053	235.50 ± 24.96	164.40 ± 4.236
PDI	0.255 ± 0.014	0.362 ± 0.1	0.143 ± 0.046
Entrapment efficiency (%)	52.63	60.69	69.67

Table 2. Properties of TMZ nanoparticles loaded Pluronic® F127 gels

Hydrogel	Viscosity of hydrogel cP	Pluronic concentration %	pH	Gelation temperature °C	Elasticity	Cohesiveness
H18	22000.00 ± 1833.03	18	6.80 ± 0.01	32-33	0.9874 ± 0.0171	0.9523 ± 0.0030
H19	11600.00 ± 529.15	19	6.94 ± 0.01	28-30	0.9960 ± 0.0148	0.9176 ± 0.0000
H20	12666.67 ± 665.83	20	6.94 ± 0.01	25-26	0.9920 ± 0.0041	0.9721 ± 0.0000
H25	32966.67 ± 3194	25	6.80 ± 0.00	20-21	0.9960 ± 0.0467	0.7668 ± 0.0000

Table 3. Mathematical equations for the models used to describe release characteristics of TMZ from Pluronic® F127 gels

Model	Equation
Zero-order	$Q = Q_0 + k_0 t$
First order	$\text{Log } C_t = \text{Log } C_0 - k_1 t / 2.303$
Higuchi	$F_t = k_H t^{1/2}$
Weibull	$F = 1 - \exp(-atb)$
Korsmeyer-Peppas	$M_t / M_\infty = k_p t^n$

Table 4. Results of mathematical model fitting of TMZ release of nanoparticle loaded hydrogel

		A2-2	A2-5	A2-10	A2-2 in Hydrogel	A2-5 in Hydrogel	A2-10 Hydrogel
zero order	k₀	- 10.43 3	- 3.004	- 0.412	-0.032	-0.030	-0.025
	r²	0.804	0.580	0.723	0.794	0.816	0.794
	RM S	30.92 4	20.28 3	67.40 0	50.383	39.287	32.877
first order	k₁	- 0.292	- 0.061	- 0.013	-0.001	0	0
	r²	0.844	0.601	0.876	0.853	0.866	0.841
	RM S	0.018	0.008	0.026	0.007	0.005	0.005
Q→t^{1/2}	k	23.34 4	8.637	4.122	1.326	1.243	1.042
	r²	0.871	0.736	0.813	0.960	0.969	0.960
	RM S	20.33 4	12.71 4	45.47 6	9.605	6.666	6.424
Peppas	n	0.197	0.105	0.113	0.332	0.341	0.226
	r²	0.915	0.872	0.904	0.961	0.967	0.959
	RM S	0.001	0.001	0.001	0.004	0.004	0.002
Weibull	β	0.339	0.151	0.195	0.375	0.379	0.260
	r²	0.931	0.881	0.952	0.955	0.961	0.949
	RM S	0.002	0.001	0.002	0.006	0.005	0.004

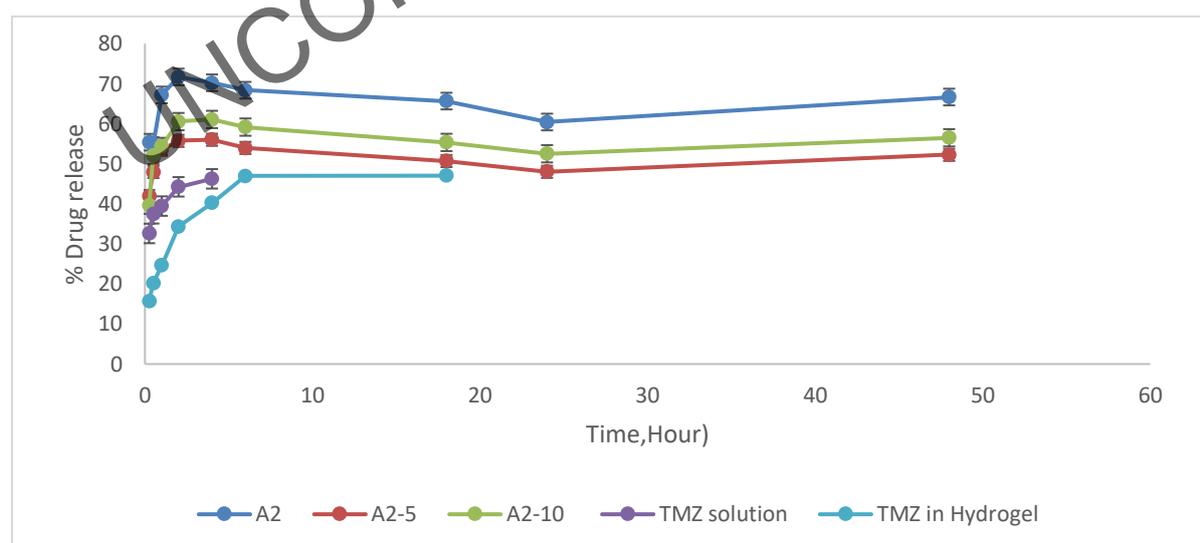


Figure 1. In vitro release of TMZ from nanoparticles

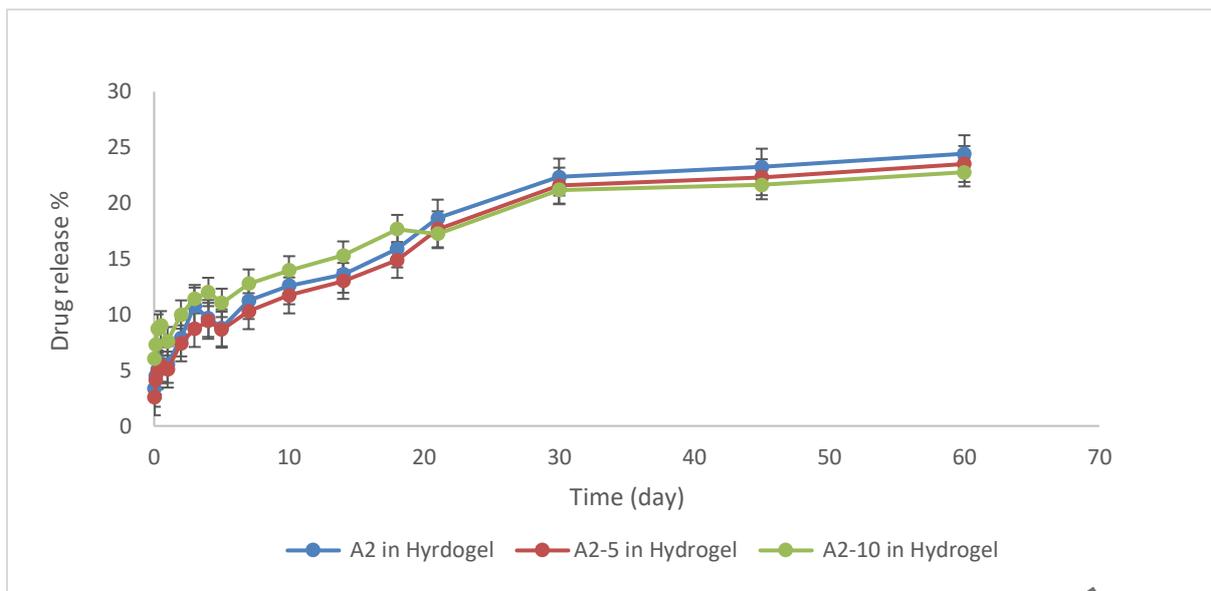


Figure 2. In vitro release of TMZ from nanoparticle loaded hydrogels

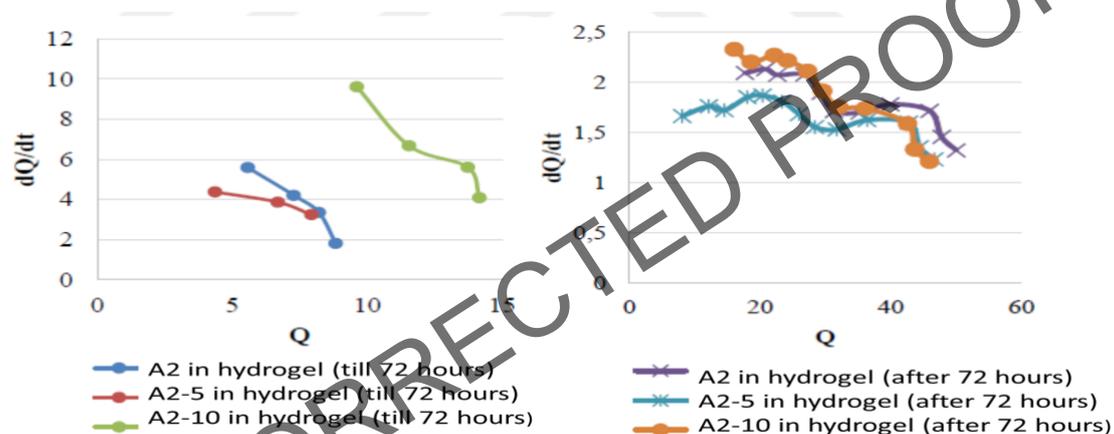


Figure 3. dQ/dt - Q plots of nanoparticle formulations in hydrogel