Original Article

Drug delivery behavior of magnetic composite: In vitro study

Birnur Akkaya[1]*, Beyza Nur Yerliyurt[2]

ABSTRACT

Background

One method for delivering the medication to the intended location and achieving the desired therapeutic effect is controlled drug release. Compared to typical release systems, controlled medication release systems offer fewer potential adverse effects and can increase patient comfort by prolonging the time intervals between drug intakes. Expanded perlite modified composite were used as drug carrier materials in the current investigation because of its excellent adsorption capability. ***Aim:*** Using magnetic poly(hydroxyethylmethacrylate-expanded perlite) (m-p(hema-ep)) composite material, doxorubicin release was examined in this work.

Methodolgy

First, a magnetic p(hema-ep) composite was created for this purpose and examined using SEM, FTIR, and swelling tests. The medication doxorubicin served as a model. Tests for reusability were conducted. The synthesized magnetic p(hema-ep) composite was shown to be capable of being recycled ten times without losing effectiveness. On the C6 cell line, the material and the material that had been loaded with doxorubicin were subjected to cytotoxicity tests under magnetic field.

Results

The impacts of variables like pH, time, temperature, on drug release were examined as a consequence of the characterisation procedure. The ideal release parameters were found to be 80% release at pH 4.5, 70% release is seen at 7.4, and temperature 22 °C, under magnetic field. ***Conclusion:*** The anti-cancer medication doxorubicin (Dox) was used as a model drug because it is widely used in cancer treatment and is simple to quantify using methods like UV-visible spectroscopy. There are enhanced doxorubicin release under magnetic field.

Keywords

Composite; drug release; magnetic field; expanded perlite.

INTRODUCTION

Perlite is amorphous volcanic glass with a high water content[1,2]. When heated to a sufficient temperature (between 850 and 900 °C), it naturally forms and possesses the peculiar ability of expanding as the water held inside its structure is released, turning it into a comparatively light, white mineral. Perlite has a wide range of uses, including medicines.

Magnetic materials are significant class of nanomaterials. They are tools for diagnosis or treatment (nanotheranostics). Applications for magnetic resonance imaging can make use of magnetic nanoparticles as contrast agents. They can be further functionalized for this use by adding medicinal substances. They are employed in numerous industries, including optics, electronics, chemistry, mechanics, biotechnology, and biomedicine. Numerous potential uses of magnetic nanoparticles, including catalysis, enzyme immobilization, gene therapy, MRI, targeted drug delivery, magnetically induced hyperthermia, and cancer

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treatment, have long been researched.

The structural characteristics of both magnetic particles and polymers can be found in magnetic sensitive polymeric architectures. The polymer gains magnetic characteristics if magnetic particles are added to the structure. This characteristic allows for the quick and simple separation of magnetic polymers from mixed liquid media using an externally generated magnetic field. These polymers also have another essential function in the treatment of cancer and hyperthermia because their magnetic energies can be converted into heat energy and employed as heat generators. When compared to non-polymeric magnetic particles, polymeric magnetic particles increase the stability of the structure. Additionally, it gives the structure qualities like flexibility and swelling resistance [3]. Some magnetic fields with a good track record can be used alone or in conjunction with chemotherapy and radiation therapy to treat cancer. The goal of developing anticancer medications for cancer therapies is to limit harm to healthy cells while specifically targeting malignant cells. The creation of new anticancer medications with fewer side effects is the most difficult aspect of cancer treatment. Research has been done on magnetic fields and drug combinations to lessen cancer drug resistance [4].

Current issues and research include the creation of cancer medicines that target tumor cells an d particular cellular components (particularly cytoskeletons and organelles).

A study has synthesized material that used magnetic fields and provided anticancer technique *In vitro* and *In vivo* The research did not appear harmful to normal cells[5,6]. The slightly acidic tumor microenvironment and intracellular tumor cells have been shown to deliver anticancer medications more readily into the body when the pH is low [7–9].

Doxorubicin is an anthracycline-derived antibiotic with growth inhibition of DNA binding and nucleic acid suggestibility. [10]. The anticancer medication, doxorubicin hydrochloride, exhibits activity against a variety of cancers [11].

In a prior study, composite materials based on polyacrylamide(paa)andpolyhydroxyethylmethacrylate (p(hema)) were created and employed for removal of heavy metal ions and protein adsorption [12,13]. Expanded perlite is modified by magnetite [14]. In this study, magnetic-expanded perlite-(M-p(hema)) composite material was used for the first time in drug delivery research.

MATERIALS AND METHOD

Adrimycin 50 mg (Doxorubucin), expanded perlite, Fe_3O_4, 50 mM phosphate prepared at different pH (Na2HPO4-NaH2PO4 pH: 7.4 and pH:7.2), acetate (CH3COOH-CH3COONa pH:4,5 and pH:5.5), bicarbonate (NaHCO3-H2CO3 pH:11 and pH:9) buffers, XTT kit and various consumables required for cell culture were used.

EXPERIMENTAL STUDIES

Preparation of DOX-loaded p(hema-ep) composite

After weighing and dissolving 5 mg of DOX in 10 mL of distilled water, 1 g of p(hema-ep) was added to the mixture and swirled on a magnetic stirrer during 24 hours at room temperature. After centrifuging it for five minutes at 4000 rpm, it was allowed to air dry at ambient temperature[15].

Preparation of magnetized p(hema-ep)

2 g p(hema-ep) and 0.2 g Fe_3O_4 were mixed in 10 mL distilled water for 20 minutes at room temperature on a magnetic stirrer. Then, 100 mL of 20% $CaCl_2$ solution was added dropwise into this mixture. It was centrifuged 3 times at 4000 rpm for 5 minutes and then washed with water. It was left to dry at room temperature[16].

Uploading DOX to M-p(hema-ep)

9.105 mg of DOX and 1.821 g of M-p(hema-ep) we prepared earlier were mixed in 18.21 mL of distilled water for 24 hours at room temperature. It was centrifuged 3 times at 4000 rpm for 5 minutes and then washed with water. It was left to dry at room temperature [15].

CHARACTERIZATION OF THE MATERIAL

Fourier Transform Infrared Spectroscopy (FT-IR) (Bruker Model: Tensor II), Scanning Electron Microscope (SEM) (TESCAN MIRA3 XMU), which carried out with service procurement from Sivas Cumhuriyet University Advanced Technology Research and Application Center (CÜTAM).

DRUG RELEASE STUDIES

In order to determine the drug release profiles of DOX-EP, M-p(hema-ep) and M-p(hema-ep) +DOX particles, release studies were performed by applying time

scanning at different pH, different temperatures and presence of magnetic field. For drug release studies, an apparatus with a dialysis membrane was prepared using 10 mL injectors. The dialysis membrane was placed in the middle of the injector.

5 mL of the buffer to be used in the drug release study was added to a part of the syringe. 30 mg of the particle to be used was weighed and dissolved in the buffer solution at different pHs and added to the other part of the injector. Thanks to this mechanism, drug release studies were carried out.

TIME SCAN IN DRUG RELEASE

Drug release studies were carried out at different time intervals (5-180 minutes and then every 24 hours for 10 days) to examine the change in the amount of drug release in DOX-EP, M-p(hema-ep) and M-p(hema-ep)+DOX particles over time. 30 mg of prepared particles were weighed and put into 5 mL of 50 mM pH:7,4 Phosphate and pH:4,5 Acetate buffer solution to be used. To the other part of the prepared device, 5 mL of the buffer solution used in the drug-loaded sample was added. Samples of 3 mL were taken with the help of a different injector for certain times and measurements were made with a UV-Vis spectrophotometer (Optima SP 3000 Plus) at a wavelength of 480 nm. It was performed in duplicate in two separate buffer environments.

Determination of the Effect of pH on Drug Release Rate

To determine the effect of pH on drug release rate, DOX-EP, M-p(hema-ep) and M-p(hema-ep)+DOX particles were mixed in different buffers and pH ranges (50mM CH3COOH-CH_3COONa pH:4.5 and pH:5.5 Acetate Buffer; 50mM Na_2HPO_4-NaH_2PO_4 pH: 7.4 and 7.2 Phosphate Buffer and 50 mM $NaHCO_3$-H_2CO_3 pH: 11 and pH: 9 Bicarbonate Buffer) release studies were performed. 30 mg of drug-loaded samples were weighed and 5 mL of buffer solutions with different pH values were added. Mechanism prepared for observing the pH effect

It was kept constant at 37 °C. Measurements were taken at 5-180 minute intervals and then again, every 24 hours, 3 mL samples were taken for 15 days and measured with a UV-Vis spectrophotometer at a wavelength of 480 nm. Each procedure was performed in duplicate.

Determining the Effect of Magnetic Field on Drug Release Rate

In order to examine the magnetic field effect on drug release amounts, a magnetic field (MA) generating solenoid-like device was used. 30 mg of DOX-p(hema-ep), M-p(hema-ep) and M-p(hema-ep) +DOX particles were weighed and 5 mL of buffer solution with different pH values was added to it. Drug release studies were performed at room temperature in the presence and absence of magnetic field. Solution absorbance values were measured at 480 nm wavelength with a UV-Vis spectrophotometer to determine the amount of release by taking 3 mL samples at intervals of 5-180 minutes and every 24 hours for 10 days. This process was performed in duplicate in two different buffer environments.

CYTOTOXICITY STUDIES

Culture and Passaging of C6 Glioma Cells

The C6 cell line was grown in DMEM containing 10% (v/v) FBS, 100 Units/ml penicillin and 100µg/ml streptomycin, respectively, at 37°C in a 5% CO_2 incubator until it reached 80% density. Cells confluent at the base of the flask were removed from the flasks using trypsin-EDTA solution and cell counts were performed with Thoma Slide. Experimental studies were started when the viability rate was 90% and above.

Application of XTT Analysis to Determine the Cytotoxic Effect of DOX-EP, Man-EP and Man-EP+DOX Particles in C6 Glioma Cell Line

C6 glioma cells were optimized and at the concentration determined as a result of the optimization, the cells were seeded into 3 96-well plates and incubated in an oven for 24 hours to allow the cells to adhere to the surface.

For each plate, synthesized DOX-p(hema-ep), M-p(hema-ep) and M-p(hema-ep) + DOX particles and expanded perlite and DOX as controls were determined at a concentration of 1,5 mg/mL. The C6 cell line was interacted with the samples at the determined concentration for 24, 48, 72 hours. At the end of this period, XTT solution was prepared to examine and determine the change in cell viability. 40µL of XTT solution, 80µL of clear (colorless) DMEM were added to each well and incubated at 37°C for 3.5-4 hours in a CO_2 incubator. After incubation, the wells were aspirated and measurements were made at 450 nm in a microplate reader (Thermo Multiskan FC Type 357).

Ethical clearence: Not applicable

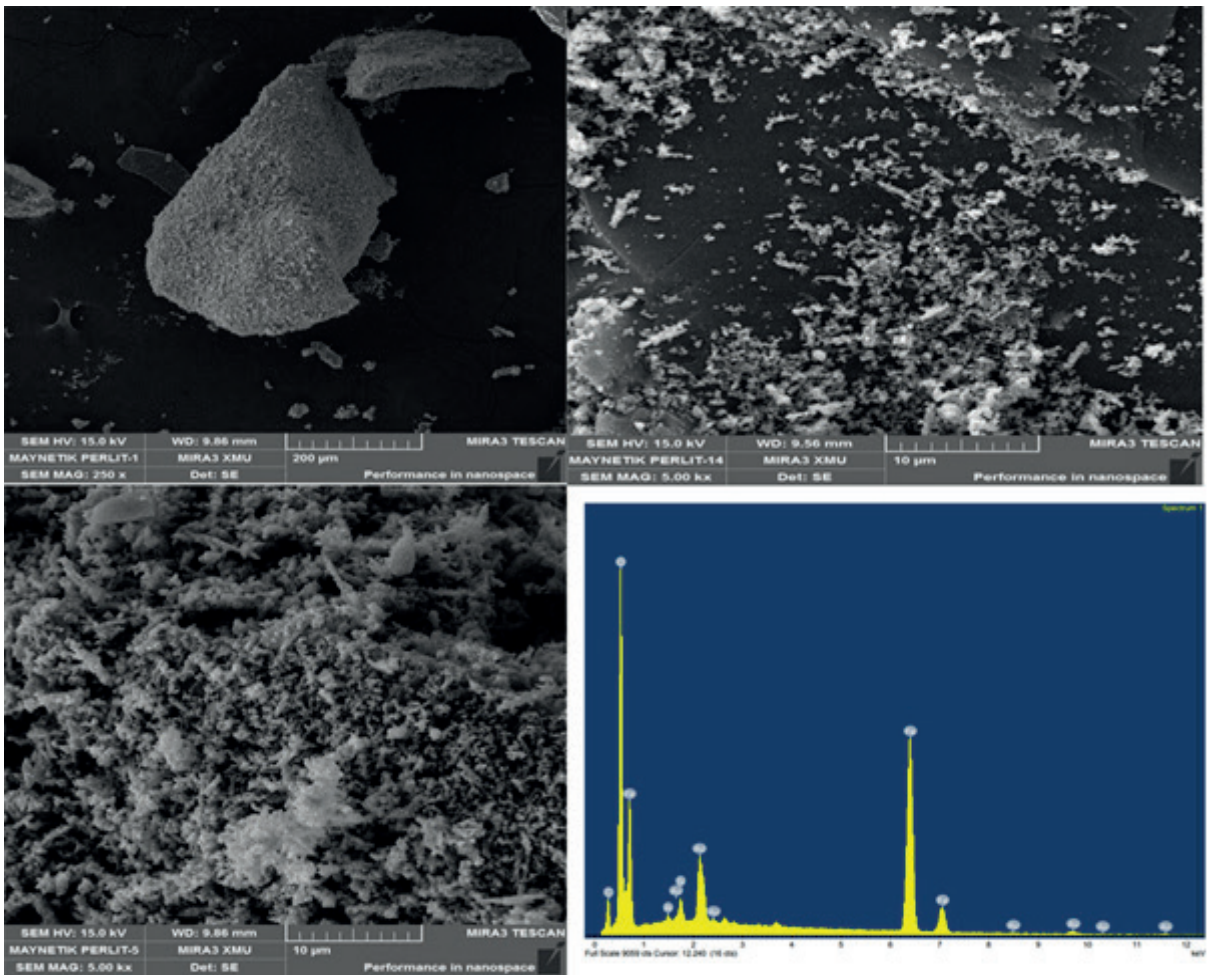

Figure 1. SEM-EDX analysis

RESULT AND DISCUSSION

SEM

SEM pictures of M-p(hema-ep) are displayed in Figure 1. It can be observed that the EP particles have a surface covered with round pores and a porous structure. To create the Man-EP compound, Fe_3O_4 was grafted onto the p(hema-ep) surface. It is significant to note that although the pulverization process disrupts the porosity structure of finely crushed expanded perlite, the laminar structure is retained [14].

It has been found that expanded perlite has an isoelectric point of 6.5, which means that it can be positively or negatively charged depending on the pH of the environment. Alkan *et al.* [17] and Ghassabzadeh *et al.* [18] found the isoelectric points 6.5 and 6.6. Alkan *et al.* [19] used the rationale that SiOH and AlOH groups can replace H+ ions to bolster their claim that silanol groups are present in EP.

FTIR-ATR

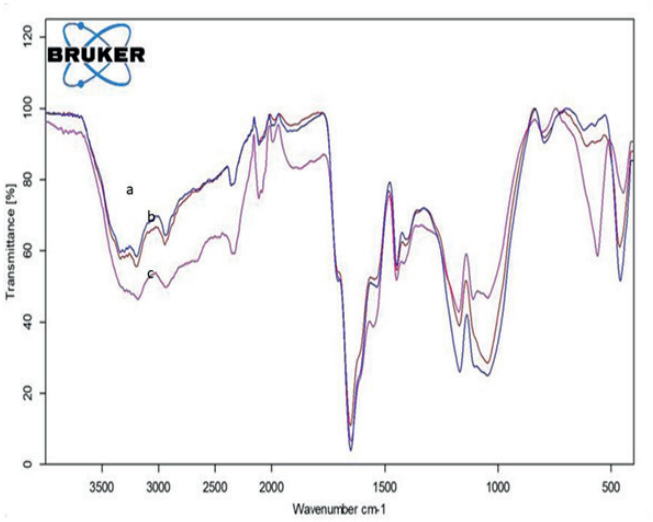

Figure 2. FTIR-ATR, a) Dox- p(ep-hema) b) p(ep-hema) c) M- p(ep-hema)

Figure 3. EP-HEMA, Dox loaded EP-HEMA and Man-EP-HEMA

Figure 2b displays the primary chemical groups found in the expanded perlite adsorbent. The material's distinctive bands were visible in the sample. Stretching vibrations of the -OH groups on the surface of the -Si-OH were suspected to be the cause of the broad band (3626 cm^{-1}) between 3450 and 3600 cm^{-1}. The bending mode of O-H from water molecules was assumed to be the cause of the band at 1641 cm^{-1}. Si-O-Si flexural vibrations have been linked to the dense band at 1 061 cm^{-1}. The bands at 783 and 454 cm^{-1} were attributed to the stretching vibrations of Si-O and Al-O, respectively. [20].

Figure 2b displays the p(ep-hema) FTIR spectra. Peaks at 3,190 cm-1 are identified as --Si-OH stretching vibrations in the p(ep-hema) composite's unique FTIR spectra structure. The tensile vibrations of Al–OH and Al–2(OH) are 1,170 cm^{-1} and 1,650 cm^{-1}, respectively, owing to the presence of silanol and hydroxyl groups that predominate. Surface silicon atoms make an effort to keep oxygen in their tetrahedral coordination. Positively charged amino acids have been shown to interact with perlite via cationic exchange interactions and hydrogen bonding via both the inner and outer layers [13]. A peak forms at 560 cm^{-1} when the FTIR-ATR spectra are compared, indicating the presence of the magnetic property of the magnetically enhanced material (Figure 2c). These findings suggest that magnetic material has been added to the p(ep-hema) composite[21].

DRUG LOADING AND RELEASE STUDIES

As pH levels rise, metal oxides on the adsorbent surface become less protonated and have a higher negative charge density. Because of the electrostatic repulsion between the positively charged doxorubicin surface and the adsorbent surface, the medicine is released at low pH levels.

Drug loading efficiency in order to find the amount of doxorubicin loaded with p(ep-hema) composite, the concentration of doxorubicin solution before adsorption, after adsorption and washing solutions were measured at 480 nm by UV spectrophotometer during the preparation of drug loaded structures. The drug loading efficiency was calculated by determining the amount of doxorubicin added at the beginning and the amount of drug loaded structures in the solution formed after preparation. The drug loading efficiency was calculated as 95%. Calibration chart was used in the calculations (Figure 3). The Dox-loaded composite acquired a red color (Figure 3). The high drug loading is due to the electrostatic attraction between the negative charge of the composite and the positive charge of Dox. Meanwhile, the swelling property of the composite facilitated drug loading. Pictures of the obtained doxorubicin loaded p(ep-hema) composite and M-p(ep-hema) composite composite are presented in Figure 3.

Investigation of Drug Release Behavior for p(ep-hema) composite and M- p(ep-hema) composite

Effect of pH and Magnetic Field on Drug Release Rate

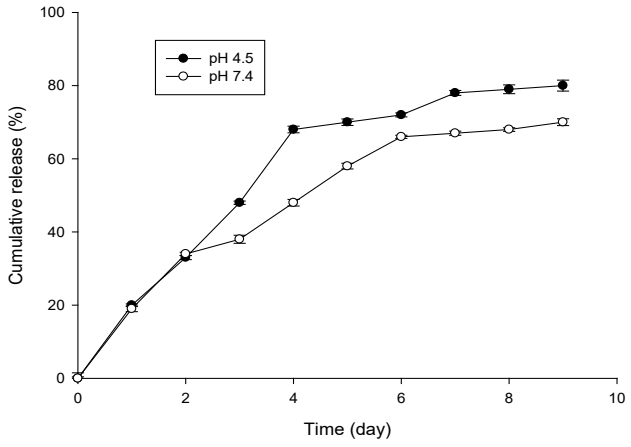

Figure 4. Cumulative release at 37 °C

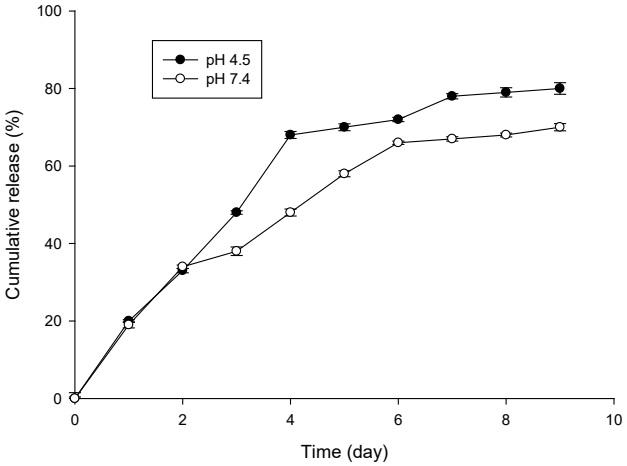

Figure 5. Cumulative release under magnetic field and at 22 °C

While there was 77% release at pH 4.5, it showed 64% release at pH 7.4 (Figure 4).

At pH 4.5, expanded perlite and M-p(ep-hema) composite are protonated (pK: 6.5). Therefore, doxorubucin, which is positively charged at this pH value, cannot interact, bind to the composite structure and is released. However, the completely negative composite at pH 7.4 interacts with the positively charged doxorubucin and does not allow the composite to be released. M-p(ep-hema) composite, on the other hand, has been shown by studies by other researchers that the positive charge of the composite structure will increase because magnetism is added to the structure, [14,21] therefore it is seen that the oscillation increases. While there is 80% release at pH 4.5, 70% release is seen at 7.4 (Figure 5).

The most sought-after characteristic for polymer systems that deliver active ingredients to the body is biological compatibility. "Biocompatibility" refers to the polymer's ability to coexist with the body without causing any harm. It is evident from Figure 6 that the novel material has a high level of biocompatibility. Following 24, 48, and 72 hours of contact, there was no discernible difference between the cells treated with composite hydrogels and the negative control groups. Additionally, over 90% of the C-6 cells remained alive, suggesting that the hydrogels were cytocompatible. EP is a non-toxic substance that has been authorized for use in biomedical applications. When combined with HEMA, it creates a non-toxic hydrogel. On top of that, it has preserved its biocompatibility feature by adding

CELL VIABILITY

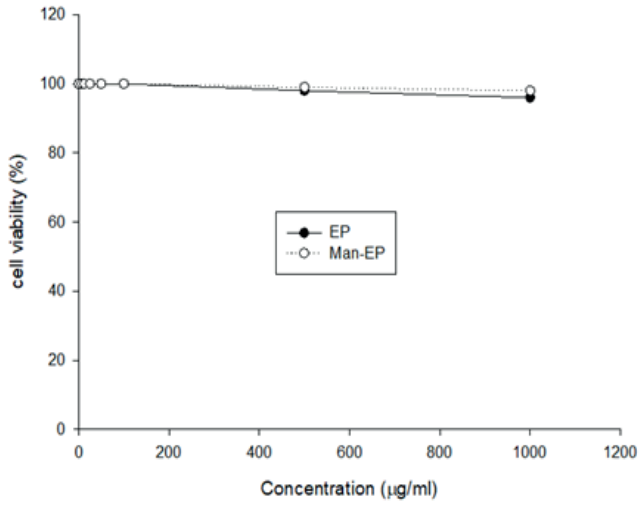

Figure 6. Biocompatibility

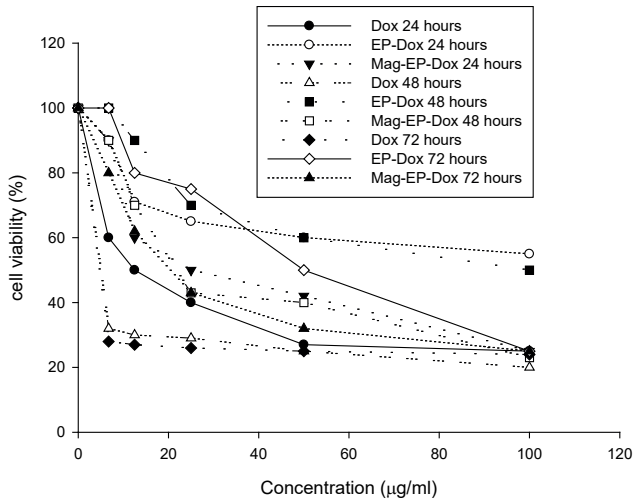

Figure 7. Cell viability

magnetite to its magnetic feature.

When the graphs of cytotoxic studies (Figure 7 and Figures 8; a,b,c) were evaluated, it was seen that Doxorubicin loaded Man-EP composite was more cytotoxic. It has been observed that the cytotoxic effect is dose and time dependent. There are many different works that have been done with EP and Man-EP so far. [20,22–25]. However, in this study, the release of doxorubicin with Man-EP was realized for the first time.

COMPARISON WITH LITERATURE

The goal of Berçem Dilan Hanolu's thesis on improved liposomal doxorubicin (DOX) release was to create more effective liposomal DOX transport and release systems. DOX loading and % release from liposomes were studied after liposomes were made using alkaline solutions comprising tris, sodium carbonate, ammonium chloride, and ammonium sulfate. 80% loading efficiency was actually achieved. At room temperature, it was discovered that the release rate was less than 13%. It was discovered that the amount of DOX released from liposomes can vary based on the amount of pH-dependent ammonia (NH_3) present in the medium. A crucial parameter was discovered to be temperature. At temperatures higher than the phase transition temperature, it has been noted that the lipid promotes the release of DOX [26].

T. Feng *et al.*; in their study they observed that the release of DOX under acidic conditions reached an optimal level, indicating the pH-responsiveness of La/Tm-MOF@d-SiO2[27].

Tekin and colleagues focused on altering perlite and sepiolite and analyzed the altered materials' surface structure. The number of surface hydroxyl groups dropped when the perlite was heated to 325 °C, but no discernible changes to the perlite's structure were seen following the H_2SO_4 solution treatment. The number of surface hydroxyl groups dropped when the perlite was heated to 325 °C, but no discernible changes to the perlite's structure were seen following the H_2SO_4 solution treatment. Compared to unmodified samples, AHM-modified perlite exhibited a greater number of negative zeta potential values. Surface charge significantly changed when DMDCS was used to modify UP, EP, and sepiolite[28].

Zincir et. al.; He made a study called Boron Removal from Waste Water with Modified Perlite. This work; It was carried out in order to find an effective and economical boron removal method. Boron removal by adsorption method using modified perlite from a synthetically prepared boron solution was investigated by intermittent work. In order to increase the adsorption capacity of perlite, a modification process was applied to increase the exchangeable ions and make the surface charge positively charged. As a result of the experiments, they observed that the boron removal from wastewater was the highest at a pH value of 7-9, at a

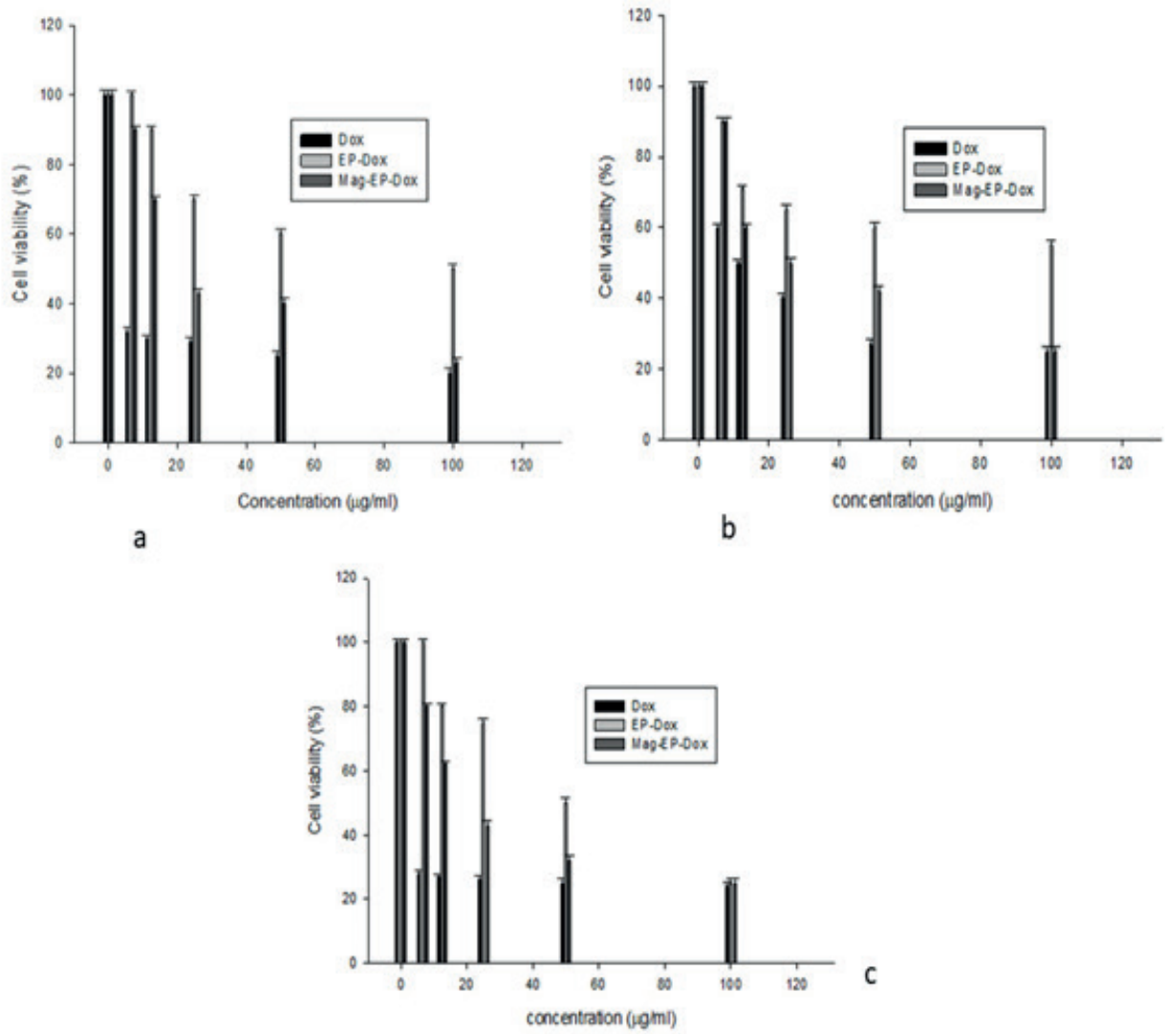

Figure 8. 24 (A), 48 (B), 72 (C) hours xtt experiments

contact time of 15 hours, at a gallic acid concentration of 0.05 mmol/L. Under these conditions, an adsorption rate of approximately 50% was achieved. As a result of the experiments and the results, it was concluded that clay minerals such as perlite can be used for removing pipes from water with various modification processes [29].

In his thesis titled Controlled Drug Release from Interpolymeric Complexes, Yıldız *et al.* employed expanded perlite particles with a high adsorption capacity as drug carrier material. The anti-cancer medication doxorubicin was chosen as the model drug. Drug-loaded expanded perlite particles were given different drug release characteristics using interpolymeric complexes. The enlarged perlite was surrounded by interpolymeric complexes made of two amino acid-based polymers (poly-L-histidine and poly-L-lysine) that had differing charges. Comparative investigations were conducted on drug release from expanded perlite samples that were loaded with drugs and those that were interpolymeric complexes. The ideal release parameters were found to be 37 °C, pH 5.5, and a time limit of up to 21 days. The study's findings indicate that the interpolymeric complex's existence influences the drug release profile in a good way and makes it appropriate for more regulated, pH-sensitive, and delayed release [15].

CONCLUSION

In addition to the drugs originated from plants, animals and minerals [30] the use of magnetic fields with anticancer drug and drug delivery systems has increased in recent years. In this study, magnetic EP-HEMA composite material was used to look at the release effect of the model drug doxorubicin. Studies conducted under magnetic field have partially responded, and it has been observed that the oscillation has increased. Composite materials used in drug release systems are expected to be environmentally sensitive. Although the perlite used in this study structurally showed drug release in the acidic region, it also showed magnetic field sensitivity with the inclusion of magnetic properties in its structure.

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Conflict of Interest: None

Authors's contribution: Data gathering and idea owner of this study: BA Study design: BA, Data gathering: BY Writing and submitting manuscript: BA Editing and approval of final draft: BA

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