

Chelating Agent Effect on the Release of Gentamicin from PEG-DA Hydrogels

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Abstract

In this study, controlled drug release devices of poly(ethylene glycol-diacrylate) (PEG-DA) hydrogels were prepared by free radical UV polymerization. PEG-DA macromere was cross-linked with different percentages of ethylene glycol dimethacrylate (EGDMA). The chelating agent, ethylene diamine tetraacetic acid (EDTA), was added at three different concentrations. Gentamicin sulphate was incorporated into the hydrogel during photopolymerization and its release kinetics was tested spectrophotometrically at 255 nm in phosphate buffer (pH 7.4). Results indicate that the presence of chelating agent leads to a prolonged drug release. Gentamicin release from hydrogel synthesized by using low chelating and cross-linking agent concentrations indicate slow release time. The effect of chelating agent concentration eliminated when the amount of cross-linking agent increased while drug release time is still higher than control group which was prepared in the absence of EDTA. It is interesting to note that the gentamicin release time prolonged when chelating agent decreased at constant cross-linking agent concentration.

Key Words: Chelating agent, PEG-DA, ethylene diamine tetraacetic acid (EDTA), hydrogel.

INTRODUCTION

Swollen networks composed of hydrophilic homo- or co-polymers, hydrogels, are suitable carriers for drug delivery [1,2]. They have a high water content and rubbery nature similar to natural tissue, which make them desirable for biomedical applications. Hydrogels have been investigated extensively for their application as carriers in diffusion-controlled release devices [3,4]. In this type of device, a drug

or protein is incorporated into the system and then released in response to a change in the environment. Poly(ethylene glycol) (PEG) is commonly used as a drug delivery matrix because it is hydrophilic, biocompatible, and relatively inert in body fluids.

UV free-radical polymerization techniques are often used to synthesize hydrogels for controlled release applications. There are several advantages to using the photo-polymerization technique, particularly in biomaterials. In general, the process is benign and the polymers can be fabricated at temperatures and pH values near physiological ranges and even in the presence of biologically active materials [3,4,5,6].

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Ethylenediamine tetraacetic acid (EDTA) is a strong chelator. Complex agents biodegradation in the environment depends on complexation. Nitrogen atom in the molecule has shown biological degradability. It has been shown that EDTA could have some influence on the properties of proteins and biological membranes [5]. Ethylenediamine tetraacetic acid (EDTA) is the most widely used complexing agent in the cosmetic and pharmaceutical industry. The molecule has six potential sites for binding a metal ion, the four carboxyl groups and the two amino groups [7]. It combines with metal ions to form stable chelates, in a 1:1 ratio regardless of the charge on the cation [7]. EDTA is a well-known chelating agent used to control the effect of metals in chemical processes for more than 50 years. Due to its excellent chelating performance it is used in many applications. It improves the efficiency of several industrial processes from pulp bleaching to the cleaning of dairies. It also improves the quality of food and many other product formulations is able to increase the quality and yield of crops [11]. Although the ferric chelate of EDTA can be oxidized to biodegradable metabolites by photodegradation [10]. There are many studies emphasizing the importance of chelation on metal bioavailability, plant uptake, toxicity, transport, adsorption, distribution and fate [9]. Many chelating agents like EDTA or phosphonates are slowly biodegradable [8].

Metal-complexing or chelating polymers refers to polymers that bind metal ions by coordinating interaction and sometimes by ionic interactions. Chelating polymers are very useful for the purpose of selective adsorption of certain metal ions from their mixtures, removal of metal ions and recovery of soluble metal ions. Adsorption of toxic metal ions using chelating polymers has the advantage of high efficiency, easy handling, availability of different adsorbents and cost effectiveness [7]. Localized delivery systems, based on biodegradable polymers

are capable of slow and controlled release of drug for a required period of time with initial burst effect to circumvent the infection. The major advantage of these systems is steady and extended release of the antibiotics directly to infected tissue without systemic toxicity [12].

Chelating agent effect on drug release was investigated in the scope of the research. Hydrogel networks containing chelating agent have been prepared by photopolymerization process. EDTA was added to the reaction medium at different concentrations.

MATERIALS and METHODS

Materials

Poly(ethylene glycol)-diacrylate (PEG-DA, Mn: 700), cross-linking agent ethylene glycol dimethacrylate (EGDMA) and 2,2-dimethoxy-2-phenyl-acetophenone (DMPA) were obtained from Aldrich Chemical Company. Gentamicin sulphate and ethylene diamine tetraacetic acid (EDTA) were purchased from Sigma Chemical Company. All other solvents and reagents were of analytical grade.

Preparation of Hydrogels

PEG diacrylate precursor solutions were prepared by mixing PEG-DA (50 %) and photoinitiator (DMPA, 0.5 wt %) based on macromere weight. Gentamicin containing EDTA solutions with three different concentrations (10^{-1} , 10^{-3} , 10^{-5} M) and cross-linking agent (EGDMA, 1, 3, 5 wt %) were added to reach a final volume of 400 μ l. Photo-polymerization was initiated by a UV lamp under a light intensity of 10 mW/cm² at 365 nm wavelength. Solutions were purged with nitrogen and curing process was completed within 3-5 minutes exposure time.

Drug Release Experiments

Drug release studies were conducted with hydrogels using buffer solutions of specific pH (7.4). Each hydrogel disc which have a thickness of 1.0-1.3 mm were immersed to a glass vessel containing 5 ml of buffer solution in a shaker bath at 37°C. The stirring rate was maintained at 100 rpm. The released gentamicin sulphate amount was measured by an UV-Visible spectrophotometer at 255 nm. The concentration of the drug in the release medium at any selected time was calculated from the corresponding calibration curve, the absorption against drug concentration. The ionic strength of all buffers was adjusted to 0.13 M by the addition of KCl. The release dynamics of gentamicin sulphate were calculated as cumulative fractional release at each time.

RESULTS AND DISCUSSIONS

The focus of this work is to investigate the use of chelating agent of EDTA containing photopolymerized PEG-DA hydrogels to facilitate drug delivery.

According to the literature in this study, working percentage of DMPA was selected as 0.5% w/w and working percentage of PEG-DA was chosen as 50% w/w [13].

UV light curing was used to prepare hydrogel networks under mild polymerization conditions. Gentamicin sulphate content in each hydrogel was kept constant as 8.064 mg/disc. The concentration of chelating agent was selected as 10^{-1} M, 10^{-3} M and 10^{-5} M and the effects of these three EDTA concentrations on drug release rate were tested by changing cross-linking agent concentrations.

The cumulative release of gentamicin from hydrogels with time was as shown in Figure 1,

Figure 2 and Figure 3. The cumulative gentamicin release increased with increasing EDTA concentration when cross-linking amount was maintained to be 1% based on macromere weight. The observed cumulative release values were calculated as 37 %, 50 %, and 54 % at 10^{-5} M, 10^{-3} M, 10^{-1} M EDTA concentrations, respectively after 300 min incubation time.

Increasing cross-linking agent concentration to 3 wt% EGDMA resulted with 38 %, 38 % and 48 % drug release which correspond to increased EDTA concentrations.

High EGDMA concentration (5 wt%) gave cumulative gentamicin release between 40-47% after 300 min release in phosphate buffer of pH 7.4. Gentamicin release from PEG-DA hydrogels synthesized without EDTA was completed within 300 minutes at each cross-linking agent concentration. Results clearly indicate that addition of EDTA to hydrogel network prolonged drug release time. On the other hand, drug release up to 30 min decreased as EGDMA concentration decreases and the effect of chelating agent is evident at the lowest cross-linking agent concentration.

Table 1. Cumulative gentamicin drug release percentages for different hydrogels within 6040 min. (4 days).

Hydrogel No	Cross-Linker (%)	Chelating Agent (M)	Cumulative Release (%)
1	1	10^{-1}	100
2	1	10^{-3}	80
3	1	10^{-5}	43
4	3	10^{-1}	56
5	3	10^{-3}	43
6	3	10^{-5}	40
7	5	10^{-1}	48
8	5	10^{-3}	54
9	5	10^{-5}	44

As the ratio of the cross-linker (EGDMA) is increased, the composed hydrogels get mechanically stronger and more resistant. It was observed that the ratio of the cross-linker on the mechanic resistance of hydrogel gets more effective while the weight ratio of macromere increases. As seen in the Table 1, cross-linker percentage affected the drug release. At the same percentage of EGDMA, especially the most release was observed in the 0.1 M of EDTA concentration. The Chelating agent affected the structure of the hydrogel at most when it was in more concentrated solution. Due to the fact that the structure of hydrogel gets stronger in the hydrogel, the release of drug is hardened. Thus as the ratio of the cross-linker was increased, the gentamicin release was decreased.

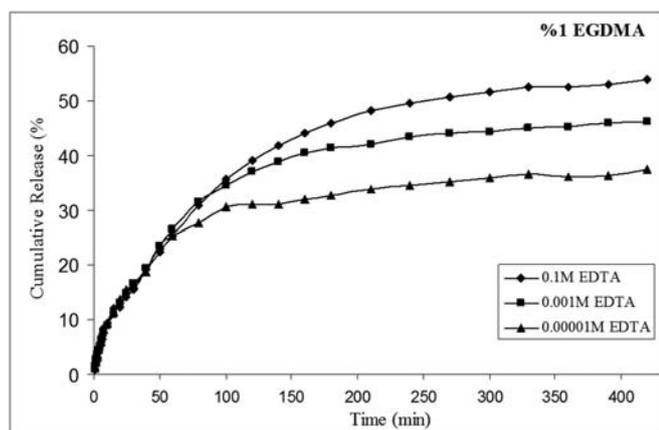


Figure 1. The effect of chelating agent on the cumulative release of gentamicin as a function of time from 50 wt % PEG-DA in the presence of 1% (wt) EGDMA.

In the experiments above, the amount of EGDMA was maintained to be %1. The graph reveals the values referring to three different EDTA molarities. The most cumulative drug release was observed at the highest EDTA concentration (0.1 M). Especially the gentamicin release was faster in the first 100 min. In different EDTA molarities, the hydrogel network was changed. The EDTA has four sites for binding. When chelating agent was added, the cumulative release was slowed. By the adding of EDTA, slow drug release was observed according to the control release. In 1% EGDMA medium, the antibiotic release were completed at 250 minutes

without EDTA (pH: 7.4, phosphate buffers). Approximately, 50% faster release was observed without addition of EDTA [14]. When 1% cross-linker was used in hydrogels with three different EDTA concentrations, as seen in this Figure 1, burst effect couldn't be observed. The gentamicin release didn't occur suddenly. In constant cross-linker percentage, drug release profiles were changed in various EDTA concentrations. The reason was that EDTA probably acts as a second cross-linker.

The drug release was faster in the first 50 minutes when EGDMA molarity was 3% with respect to the hydrogels prepared with 1% EGDMA. The drug release with hydrogels composed of 3% EGDMA was similar to %1 EGDMA. The release of gentamicin was also experimented with three different chelating agent concentrations. As it was in the previous figure, addition of EDTA led to slower drug release. The fastest release was observed when the EDTA concentration was highest. When the 3% containing EGDMA hydrogel graphic was analysed, burst effect was more evident than the 1% containing EGDMA hydrogel which can be regarded as partial burst effect. In drug delivery; early stage sudden dose wasn't desired. The highest cumulative gentamicin release was observed at 0.1M EDTA. It was thought that, EDTA behaved as a second cross-linker and because of chemical structure of EDTA why acts as an additional networking chemical.

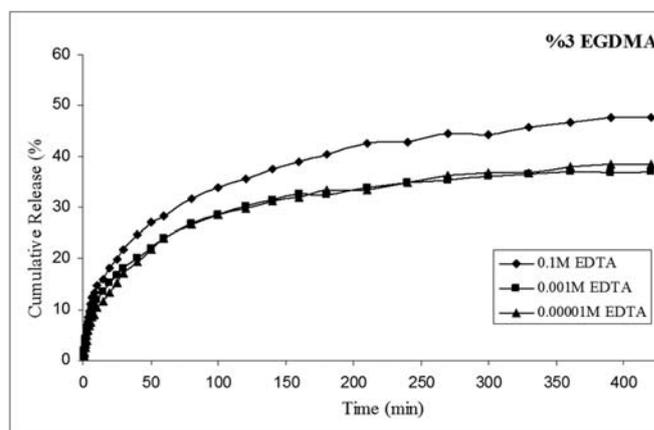


Figure 2. The effect of chelating agent on the cumulative release of gentamicin as a function of time from 50 wt % PEG-DA in the presence of 3 wt % EGDMA.

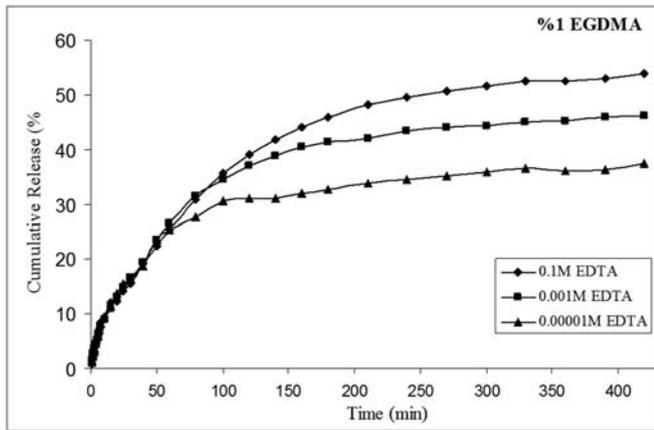


Figure 3. The effect of chelating agent on the cumulative release of gentamicin as a function of time from 50 wt % PEG-DA in the presence of 5 wt % EGDMA.

The drug release with 5 % EGDMA differed from the previous ones as can be seen in Figure 3. The drug release trend was different due to the increase in the EGDMA ratio which greatly resulted with a complex hydrogel network structure. The cumulative drug releases were almost the same for 0.1 and 0.001 EDTA molarities. However, the faster release trend in the first 200 minutes was common for all three concentrations. When the 5% EGDMA containing hydrogels were examined, burst effect was more distinct than other hydrogels which were containing 1 and 3% EGDMA. The reason of burst effect increase may be the concentration of cross-linker. The drug release was upsurge first 15 min.

In general, drug release increases as cross-linker and chelating agent concentrations increases. This was an unexpected result. The main reason of this situation is greatly due to cross linking between EGDMA and EDTA and low bounding between PEG acrylates leads to high mesh size formation. But it is important to not that drug release rate can be altered by the addition of chelating agent and there is an optimum chelating agent concentration value at each cross-linking agent amount.

CONCLUSION

Gentamicin loaded PEG-DA hydrogels were synthesized with photopolymerization in the presence of chelating agent EDTA. Results indicate that the presence of chelating agent leads to a sustained drug release profile. Gentamicin release from hydrogel synthesized by adding small chelating and crosslinking agent concentrations indicate slow drug release. The effect of chelating agent concentration eliminated when the amount of crosslinking agent increased while drug release time is still higher than control group which was prepared in the absence of EDTA. It is interesting to note that the gentamicin release time prolonged when chelating agent decreased at constant crosslinking agent concentration. The rate of antibiotic release in pH: 7.4 (phosphate buffer) is faster in least EGDMA concentrations. The addition of natural and synthetic chelating agents to hydrogels and their effect to drug release need to be examined and interpreted extensively.

REFERENCES

1. Peppas N.A., Ward, J.H. Preparation of controlled release systems by free – radical UV polymerizations in the presence of a drug, *Journal of Controlled Release*, 711: 183-192, 2001.
2. Kanjickal, D.G., Lopina, S.T., Modeling of drug release from polymeric delivery systems—a review, *Crit. Rev. Ther. Drug Carr. Syst.* 21: 345–386, 2004.
3. Scott, R.A., Peppas, N.A., Highly crosslinked, PEG-containing copolymers for sustained solute delivery, *Biomaterials*, 20: 1371-1380, 1999.

4. Hoffman, A.S. Hydrogels for biomedical applications. *Advanced Drug Delivery Reviews*, 43: 3-12, 2002.
5. Nguyen, K.T., West, J.L., Photopolymerizable hydrogels for tissue engineering applications, *Biomaterials*, 23: 4307–4314, 2002.
6. Kost, J., Langer, R., Responsive polymeric delivery systems, *Advanced Drug Delivery Reviews*, 46: 125–148, 2001.
7. Francis, S., Varshney, L., Studies on radiation synthesis of PVA/EDTA hydrogels, *Radiation Physics and Chemistry*, 74: 310 – 316, 2001.
8. Otto J. Grundler, Arnold T.M van der Steen, Joel Wilmot., Overview of the European Risk Assessment on EDTA, *ACS Symposium Series 910*, 336-347, 2005.
9. Bernd Nörtemann, Biodegradation of Chelating Agents: EDTA, DTPA, PDTA, NTA and EDDS, *ACS Symposium Series 910*, 150-170, 2005.
10. Brend Nowack, Jeanne M. Van Briesen, Chelating Agents in the Environment, *ACS Symposium Series 910*, 1-18, 2005.
11. Bucheli-Witschel, M., Egli, T. Environmental fate and microbial degradation of aminopolycarboxylic acids, *FEMS Microbiology Reviews*, 25: 69-106, 2001.
12. Changez M., Burugapalli K., Koul V., Choudhary V., The effect of composition of poly (acrylic acid)- gelatin hydrogel on gentamicin sulphate release: in vitro, *Biomaterials*, 24: 527-536, 2003.
13. Anseth K.S., Bryant S.J., The Effects of scaffold thickness on tissue engineered cartilage in photocrosslinked poly(ethylene oxide) hydrogels, *Biomaterials*, 22: 619-626, 2001.
14. Ayhan F., Özkan S., Gentamicin Release from Photopolymerized PEG Diacrylate and PHEMA Hydrogel Discs and Their In Vitro Antimicrobial Activities, *Drug Delivery*, 14: 433-439, 2007.