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Hydrogel Implants for Methotrexate Obtained by Ionizing Radiation

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ABSTRACT

The characteristics of poly(2-hydroxyethyl methacrylate) matrices for the controlled release of drugs have been investigated using methotrexate (MTX) as a model drug. Radiation-induced polymerization of hydrophilic monomers (HEMA and related compounds) at low temperature (−78°C) was performed to immobilize MTX in the polymer matrix. The effect of the drug loading and different amounts of cross-linking agent on the in vitro drug release was studied in this work. The diffusion of MTX from the hydrogel matrices was proportional to the square root of the time, according to the equation proposed by Higuchi (1).

INTRODUCTION

Hydrogels have many attractive properties as therapeutic systems in order to control the drug release and the drug delivery site. The immobilization of anticancer agents in a biocompatible system, able to release the drug in the site of the tumor and to reduce the side effects, is one of their possible applications (2–7). Among the various polymers used in order to formulate hydrogels, poly(2-hydroxyethyl methacrylate), or PHEMA, is one of the most interesting hydrophilic insoluble polymers.

The objective of this work was to study the inclusion of methotrexate (MTX) and the in vitro release rate of the drug from hydroxyethyl methacrylate hydrogel implants.

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MATERIALS AND METHODS

Materials

HEMA (2-hydroxyethyl methacrylate), purity (GC): 99.3%, was obtained from Mitsubishi Rayon Co., Ltd. EGDMA (ethylene glycol dimethacrylate), purity (GC): 96.0%, was provided by Fluka Chemie AG. MTX (methotrexate) was obtained from Lederle Corporation.

Preparation of Polymeric Hydrogels

The hydrogel matrices were obtained by radiation polymerization of 2-hydroxyethyl methacrylate (HEMA) both in the absence and in the presence of ethylene glycol dimethacrylate (EGDMA) as cross-linking agent.

The matrix was prepared by mixing the monomers and the cross-linking agent in a 0.1 N sodium hydroxyde solution of MTX and freezing, at -78°C, the suspension obtained in PE tubes with an inner diameter of 3.5 mm. The polymer matrices were obtained by irradiation of the frozen mixtures by γ rays from a ^{60}Co source for 2 hr at a dose rate of 5 $kGy \cdot h^{-1}$.

In Vitro Release

For each formulation, the in vitro release study was carried out on 1 cm of the 3.5 mm diameter matrix. The results are the mean of six determinations. In vitro release was determined with the following methods:

- The flow through cell method using 1000 ml of pH 1.3 buffer as dissolution medium and a flow rate of 30 $ml \cdot min^{-1}$.
- The paddle method with 1000 ml of pH 1.3 buffer as dissolution medium and at 60 rpm.

The assay of samples was performed with a specific and validated high-performance liquid chromatography (HPLC) method.

RESULTS AND DISCUSSION

Different polymer matrices, whose formulations are given in Table 1, were prepared both with and without cross-linking agent and with increasing concentrations of MTX.

The γ-ray irradiation allowed the polymerization of the HEMA by polyaddition and the immobilization of the MTX by physical capture in the three-dimensional network. After the polymerization, a cycle of washing with purified water and sodium chloride solution was performed. No monomers were detected in the washing water.

Table 1

Characteristics of the Hydrogels Formulated

	Volume of Solvent (ml)	Volume of Monomer (ml)		Dose ($mg \cdot cm^{-1}$)
		HEMA	EGDMA	
A	4	4	0	0.6
B	4	4	0	1.2
C	4	4	0	1.8
D	4	3.8	0.2	1.2
E	4	3.6	0.4	1.2

The lack of influence of radiation on MTX was investigated by exposure of the drug to radiation. The experiment did not show any structure modification or degradation product formation.

The inclusion of MTX was a physical capture during the polymerization process and the cross-linking of monomers.

The in vitro dissolution was performed with the flow through cell method for 24 hr on all the polymers in order to determine the influence of the MTX amount and the presence of EGDMA on the drug release. The results are presented in Table 2 and displayed in Figs. 1 and 2.

Table 2

In Vitro Release ($mg \cdot cm^{-1}$) of MTX by the Flow Through Cell Method

	Dose ($mg \cdot cm^{-1}$)	Amount Released ($mg \cdot cm^{-1}$)	
		At 8 hr	At 24 hr
A	0.6	0.21	0.27
B	1.2	0.35	0.51
C	1.8	0.46	0.71
D	1.2	0.42	0.53
E	1.2	0.41	0.54

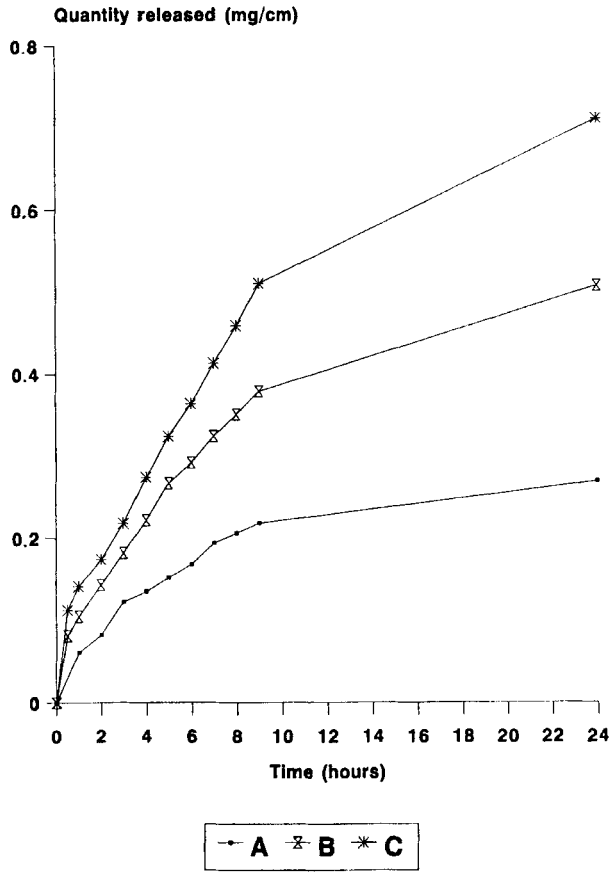


Figure 1. Dissolution kinetics of hydrogel matrices with different doses of MTX (n = 6).

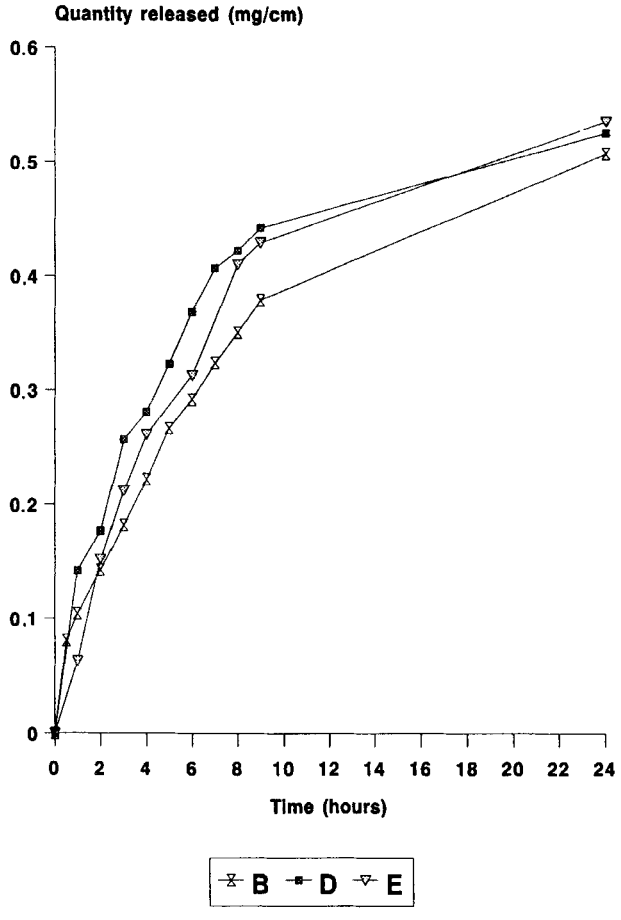


Figure 2. Influence of different amounts of EGDMA in the radiation polymerization on the dissolution kinetics of MTX from hydrogel matrices (n = 6).

The diffusion of MTX from the hydrogel was sustained and regular according to the equation proposed by Higuchi during the period of time studied (Figs. 3 and 4). Increasing the amount of MTX led to an increase in drug release. The amounts released from the matrices were not proportional to the dose—for the same contact area—according to the amount of drug included.

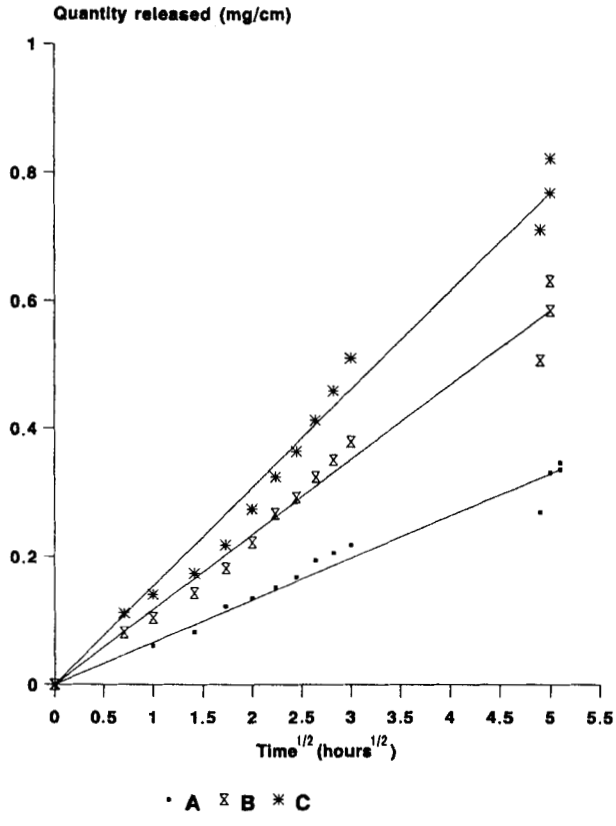
The addition of a cross-linking agent in the formulation increased the drug release. The amount of cross-linking agent was not significant in the kinetics of release.

From the results obtained, the selection of the C and E hydrogels was performed and a long-term in vitro study was realized with the rotating paddle method. The results are displayed in Fig. 5.

Whatever the hydrogel studied, the release was very incomplete and never reached 100% of the initial dose. The rate of release was quite constant for 48 hr and then became very slow. This sustained release did not seem to be due to a chemical binding of MTX with the polymer but to the fact that a large amount of drug was entrapped in the matrix.

CONCLUSION

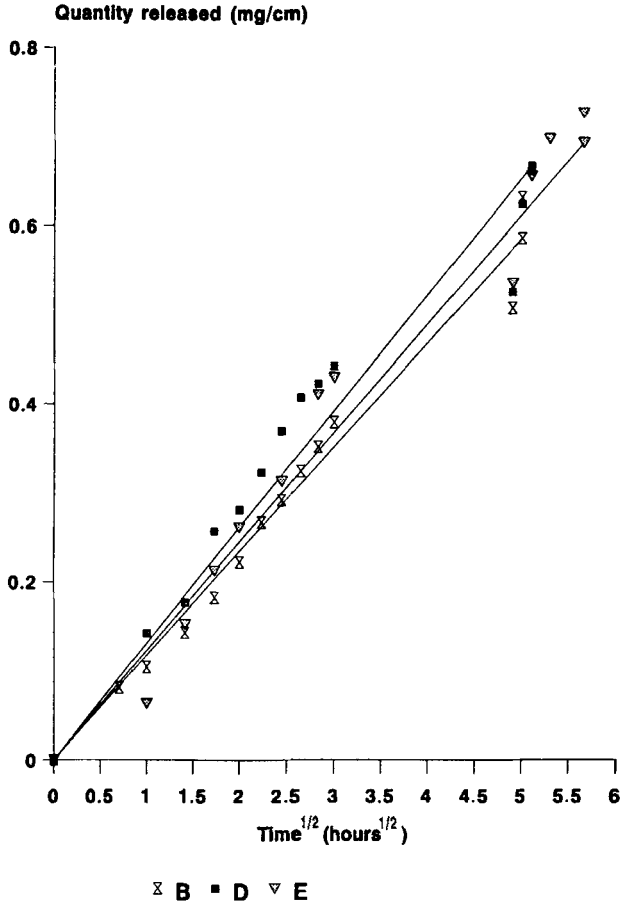
The results of this first study showed that the polymerization of HEMA by radiation in presence of MTX



Key

	A	B	C
Constant	0	0	0
Standard error *Y* estimate	0.019343	0.027684	0.033765
R squared	0.963548	0.975187	0.979652
No. of observations	13	13	13
Degrees of freedom	12	12	12
X coefficient(s)	0.065848	0.117046	0.153673
Standard error of coefficient	0.001766	0.002848	0.003473

Figure 3. Linear relationship between the quantity released from hydrogel matrices with different doses of MTX and the square root of the time.



Key

	B	D	E
Constant	0	0	0
Standard error of *Y* estimate	0.027684	0.048323	0.049393
R squared	0.975187	0.934593	0.963562
No. of observations	13	13	13
Degrees of freedom	12	12	12
X coefficient(s)	0.117046	0.130661	0.122414
Standard error of coefficient	0.002848	0.004411	0.003574

Figure 4. Linear relationship between MTX released from hydrogel matrices with varying content of EGDMA and the square root of the time.

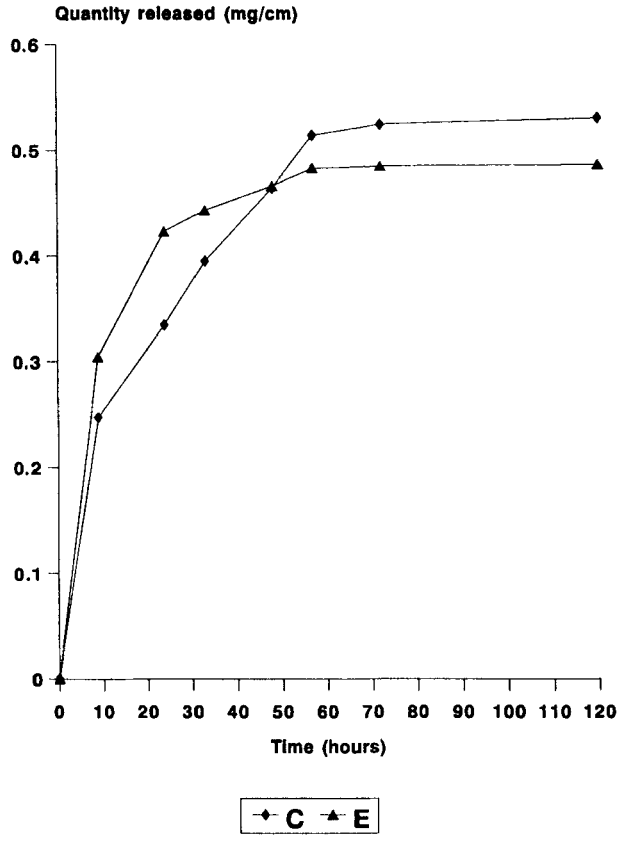


Figure 5. Dissolution kinetics of hydrogel matrices with the rotating paddle method (n = 6).

solution allowed the obtention of a hydrogel-based sustained-release dosage form. The in vitro dissolution study showed a sustained but incomplete drug release.

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