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Hydrogel formation of the pH response polymer polyvinylacetal diethylaminoacetate (AEA)

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Abstract

The effects of pH and temperature on hydrogel formation of polyvinylacetal diethylaminoacetate (AEA) and drug release were examined. When a dialysis tube containing AEA dissolved in pH 4 solution was immersed in phosphate buffer pH 7.4 at 37°C, AEA hydrogel was formed inside the tube and drug release became slow. Various porous structures in the inner region of hydrogel were observed by Scanning Electron Microscopy. The effects of temperature change on lattice distance of hydrogel were determined by dynamic light scattering. The average pore size of AEA hydrogel was also determined by the release of FITC-dextrans of various molecular weights from AEA hydrogel. AEA solution with low viscosity at pH 4 but which form hydrogel at neutral pH condition has the potential for use in controlled release of drugs applied to physiological membranes.

Keywords: pH-response polymer; Polyvinylacetal diethylaminoacetate (AEA); Hydrogel; Pore size; FITC-dextrans; SEM

1. Introduction

There has been increasing interest in evaluation as drug carriers of stimuli-sensitive polymers, which respond to environmental conditions such as temperature, solvent composition (Hrouz et al., 1981), pH, ionic strength (Rička et al., 1985) and electric field (Shiga et al., 1992). The balance of incorporation of hydrophobic and hydrophilic moieties in backbone polymers has been found to several stimuli-sensitive polymers (Serres et al., 1996). Temperature-sensitive polymers such as poly(*N*-isopropylacrylamide) and other *N*-substituted acrylamides have lower critical solution temperature (LCST) characteristics, which provide reversible high swelling at low tempera-

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ture and low swelling at high temperature (Hoffman et al., 1986; Bae et al., 1990; Dong et al., 1990). A copolymer of polyoxyethylene-polyoxypropylene, Pluronic F-127, is also a temperature-sensitive polymer, forms a hydrogel from aqueous solution above 32°C, and undergoes gel formation following rectal application (Lenaerts et al., 1987; Miyazaki et al., 1987).

On the other hand, for several pH-sensitive controlled-release systems it has also been reported that at certain pHs no drug is released, but with pH change controlled drug release can be obtained based on the swelling behavior of polymer network (Siegel et al., 1988; Brannon-Peppas et al., 1989; Kim et al., 1994). In addition, pH/temperature-sensitive hydrogels, which combine two properties by synthesis of crosslinkage in polymer, have also been studied (Dong et al., 1992). In this system, polypeptides or macromolecular drugs are loaded in hydrogel composed of *N*-isopropylacrylamide, acrylic acid and a divinyl silicone rubber at a low temperature. The hydrogel does not swell at the acidic pH of the stomach and no drug is released, but when the hydrogel reaches the intestine under the neutral pH, polymer swelling permits drug release.

Here, as a part of our development of controlled-release devices, polyvinylacetal diethylaminoacetate (AEA) was studied as a pH-response polymer. AEA is soluble in gastric juice but insoluble in water, and has been widely used for film coating and production of microcapsule membranes (Shinkuma et al., 1991; Shimano et al., 1994). In this study, we investigated the effects on hydrogel formation by AEA of changes in pH and temperature, and evaluated physicochemical properties of hydrogel and drug release behaviors.

2. Materials and methods

2.1. Materials

Polyvinylacetal diethylaminoacetate (AEA, M_w 65000) was obtained from Sankyo (Osaka, Japan). Chlorpheniramine maleate (CM) and fluorescein isothiocyanate-labeled dextrans (FDs) were purchased from Sigma (St. Louis, MO). The mean molecular weights of FDs employed were 4400, 9300, 19800, and 73100 (abbreviated: FD-4, FD-10S, FD-20S and FD-70S). All other reagents used were of analytical grade. Dialysis tubes (Dialysis Membrene, Size 36, diameter 50 mm, M_w cutoff: 10000–13000) were purchased from Wako (Tokyo, Japan), and appropriate closures were purchased from Spectrum (Los Angeles, CA).

2.2. Preparation of AEA solution and hydrogel

The three kinds of AEA formulations studied are listed in Table 1. AEA solution was prepared as follows: after dissolving either 0.5% CM or 0.1% FD-4 in distilled water, 7% AEA was added and the pH of the AEA solution was adjusted to 4.0 with 1 N HCl at 25°C. Each preparation was stored at 25°C prior to use in release experiments. AEA hydrogel was prepared as follows: after dissolving 0.5% CM or 0.1% of various FDs in 1/5 N phosphate buffer pH 7.4, 7% AEA was added and dissolved at 5°C. This solution formed a hydrogel on change of temperature from 5 to 25°C. CM-hydrogel preparation was stored at either 25 or 37°C overnight prior to use in release experiments. The solutions for FDs-hydrogel were poured into a 35 mm Petri dish, and hydrogel was formed change in temperature from 5 to 25°C, and stored at 25°C for 3 h prior to use in the pore size characterization experiments.

2.3. Measurement of turbidity of AEA solution

The pH response of AEA in aqueous solution was estimated by measuring turbidity at 550 nm with acid-base titration. After AEA was dissolved in distilled water at 7% and the pH of the solution was adjusted to 4.0 with 1 N HCl at 25°C, 10 ml of the solution was put in the vial with magnetic stirrer and titrated with 0.01 N-NaOH with a micropipette stirring at 25°C. The change in turbidity was monitored with a UV-Visible recording spectrometer (Shimazu, UV-265).

Table 1
AEA Formulations

Ingredient	AEA solution		AEA hydrogel				
	CM-solution	FD-solution	CM-hydrogel	FD-hydrogel			
Chlorpheniramine maleate	0.5[a]	—	0.5	—	—	—	—
FD-4	—	0.1	—	0.1	—	—	—
FD-10S	—	—	—	—	0.1	—	—
FD-20S	—	—	—	—	—	0.1	—
FD-70S	—	—	—	—	—	—	0.1
AEA	7	7	7	7	7	7	7
1 N HCl	Adjusted pH 4.0	Adjusted pH 4.0	—	—	—	—	—
Phosphate buffer solution	—	—	pH 7.4 (total 100 g)	pH 7.4 (total 100 g)	pH 7.4 (total 100 g)	pH 7.4 (total 100 g)	pH 7.4 (total 100 g)
Distilled water	Total 100 g	Total 100 g	pH 7.	—	—	—	—
Storage condition	25°C	25C	25°C, 37°C	25°C	25°C	25°C	25°C

The mean molecular weights of FDs employed were 4400, 9300, 19 800, and 73 100, abbreviated as FD-4, FD-10S, FD-20S and FD-70S, respectively.
[a] Weight percent.

2.4. Scanning electron microscopy (SEM)

A dialysis tube containing AEA in aqueous solution (pH 4.0) was immersed in phosphate buffer (pH 7.4) at 37°C. After 3 h the content of the tube was obtained and dried at 25°C for 3 h, and hydrogel formation was then observed by SEM at a magnification of 4000 or 10000.

2.5. Dynamic light scattering measurement (DLS)

The average lattice distance of AEA hydrogel was measured by dynamic light scattering (Otsuka Electronics DLS7000). Five percent AEA in pH 7.4 solution was poured into a glass tube ($\phi = 1$ mm) at 5°C, and the surrounding temperature was increased from 5 to 30°C. The intensity of dynamic light scattering was measured at various temperatures, and AEA hydrogel formation was evaluated.

2.6. Release experiments

In order to determine the effects on hydrogel formation of AEA of changes in surrounding pH, the release kinetics of drug from AEA preparations were determined by the dialysis tube method as described by Aoyagi et al. (1988). A dialysis tube (5×3 cm) containing 3 g of AEA solution (pH 4.0) or CM-hydrogel preparation (pH 7.4) was immersed in 500 ml of phosphate buffer of various pHs. A paddle was rotated at 100 rpm at 5 cm above the bottom of the vessel. The temperature of the receivers was maintained at 25 or 37 ± 0.5°C during experiments. An aliquot of 1 ml was withdrawn at appropriate times and CM concentrations of the receivers were determined by HPLC with a reverse-phase column (NUCLEOSIL 5SA; Chemco) at 210 nm. Elution was performed with acetonitrile and phosphate buffer pH 4.0 (1:1 v/v). The retention time of CM under these conditions was 10 min.

To estimate the average pore size of AEA hydrogel, the release of FDs of various molecular weights from 7% AEA hydrogels on a Petri dish was determined. The FD concentrations of the receivers were determined by fluorescence spectrophotometry using a Diol-300 column (YMC-Pack) with a fluorescence spectrophotometer (Hitachi Model F-1050, Ex. 495 nm, Em. 515 nm). Elution was performed with 0.1 M KH_2PO_4 and 1.5 M KOH buffer pH 7.0 with 0.3 M NaCl. The retention times of FD-4, FD-10S, FD-20S, and FD-70S were 19, 18, 17, and 13 min, respectively.

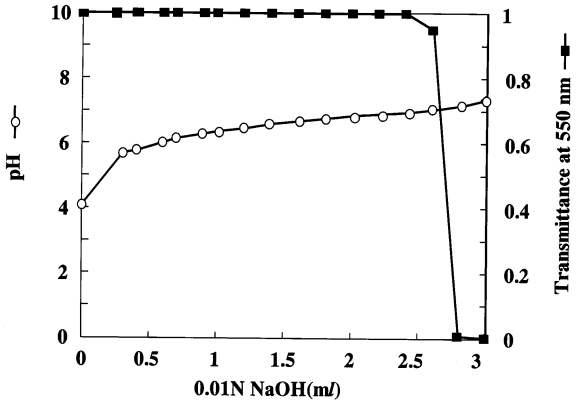

Fig. 1. Acid–base titration and change in turbidity of 7% AEA solution at 25°C.

3. Results and discussion

3.1. Effects of pH change on turbidity of AEA solution

The effects of pH on the physicochemical properties of AEA in solution were examined by acid–base titration. As shown in Fig. 1, the turbidity of 7% AEA solution, which was transparent at pH 4, changed abruptly at a pH near 7. This change appeared to be due to precipitation or hydrogel formation of AEA by pH change. These findings suggested that since the turbidity change was occurred at a pH near 7 at 25°C, the rate of titration and condition of stirring solution did not affect the hydrogel formation of AEA. Moreover, the hydrogel formation was not dependent on temperature in this titration method, when the measurement of turbidity of AEA solution was performed at 37°C, the same result was observed (data not shown).

3.2. Morphological characterization

When a dialysis tube containing 7% AEA solution (pH 4.0) was immersed in phosphate buffer (pH 7.4) at 37°C, white precipitates were observed on the inner surface of the dialysis tube immediately after initiation, and the amount of precipitate increased gradually with increase in time of dialysis. SEM photographs of the precipitate following dialysis are shown in Fig. 2. The photographs are a cross-sectional image ($\times$ 4000; left), the inner surface ($\times$ 10000; middle) and the outer surface ($\times$ 10000; right) of the precipitate. In the cross-sectional image at low magnification, the precipitate appears to be hydrogel with homogeneous polymer structure. At higher magnification

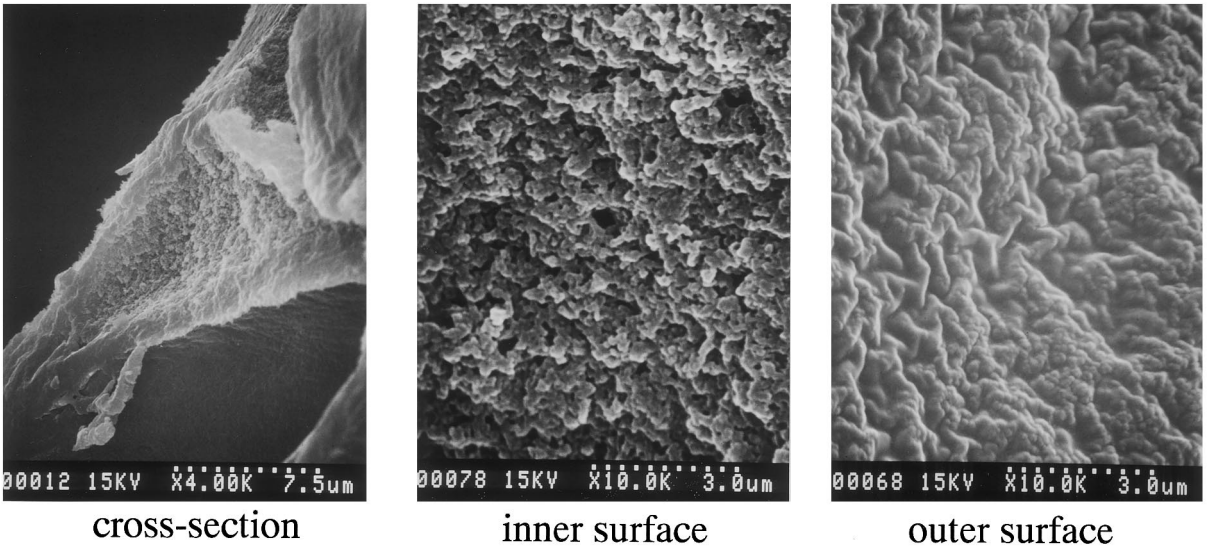

Fig. 2. SEM observation of AEA hydrogel. A dialysis tube containing 7% AEA solution (pH 4.0) was immersed in pH 7.4 phosphate buffer at 37°C. Hydrogel was formed in the dialysis tube with change in pH.

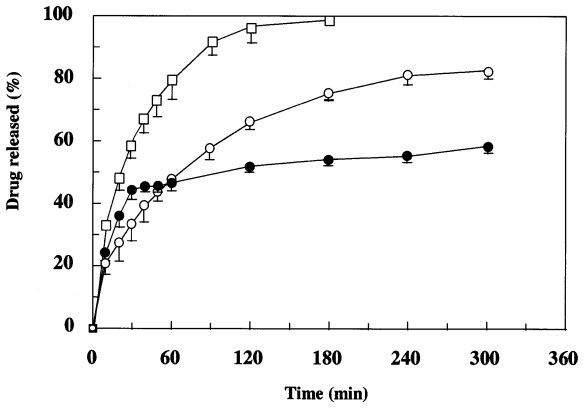

Fig. 3. Profiles of release of chlorpheniramine maleate from AEA solution. 7% AEA solution (pH 4.0) in dialysis tubes was dialyzed in pH 7.4 phosphate buffer at various temperatures. – ○ –, 25°C; – • –, 37°C; – □ –, 25°C (AEA-free solution).

(× 10000), two distinct structures are observed, a porous inner structure and a fine and flat outer surface structure. The inner surface of the hydrogel, which was in the pH 4 region, has a gel-like texture comprised of various porous structures. The texture of the outer surface of the hydrogel that faced the phosphate buffer pH 7.4 region was fine, where the hydrogel was formed along the inner surface of the dialysis tube.

3.3. Drug release from AEA solution with pH change

The effects of pH on release of CM from AEA solution (pH 4.0) were evaluated by the dialysis tube method. When phosphate buffer of pH 5.0 or 6.0 was used as the receiver solution, AEA solution in the dialysis tube was clear, and no hydrogel formation was observed (data not shown). As expected, CM release was fast and similar to that from AEA-free solution (Fig. 3). On the other hand, the transparent AEA solution in the dialysis tube became cloudy when phosphate buffer of pH 7.4 was used, suggesting that AEA hydrogel had formed inside the tube. This finding was consistent with those shown in Fig. 2. As hydrogel formation proceeded, CM release became slow throughout the experiment (Fig. 3). Moreover, at 37°C the initial drug release rapidly increased compared to that at 25°C, but after 30 min release became very slow. These findings suggest that the initial burst in drug release at 37°C may be related to shrinkage of the hydrogel by temperature change from 25 to 37°C. Slow release may result from acceleration of gelation of hydrogel by increase in temperature. Temperature change did not affect the rate of hydrogel formation of AEA but occur the shrinkage of hydrogel, lead to tight network formation. These findings suggest that hydrogel formation by AEA solution has pH response characteristics.

The release of FD-4 from 7% AEA solution (pH 4.0) in dialysis tube to the receiver phase of phosphate buffer (pH 7.4) at 25 or 37°C was examined. As shown in Fig. 4, the release of FD-4 from 7% AEA solution (pH 4.0) was significantly lower at both temperatures, especially at 37°C, compared to that from AEA-free solution. These findings may have been the result of the low rate of diffusion of FD-4 trapped inside the hydrogel. In addition, on SEM observation, shrinkage was especially pronounced at the outer surface of hydrogel, and the fine surface of hydrogel may prevent diffusion of FD-4 from hydrogel into the receiver phase.

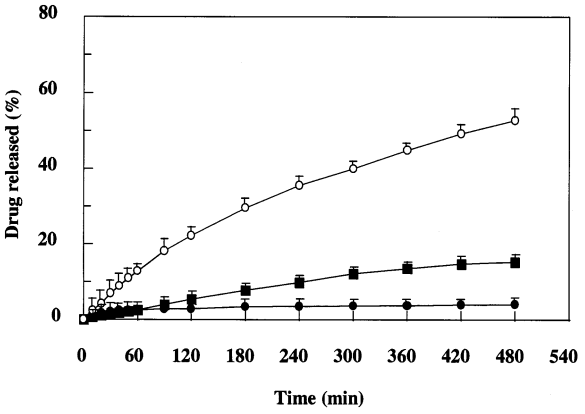

Fig. 4. Profiles of release of FITC-dextran (4400) from AEA solution. 7% AEA solution (pH 4.0) in dialysis tubes was dialyzed in pH 7.4 phosphate buffer at various temperatures. – ■ –, FD-4, 25°C; – • –, FD-4, 37°C; – ○ –, FD-4 (AEA-free solution), 25°C.

Table 2
Effects of changes in temperature on lattice distance in AEA hydrogel

Temperature (°C)	Lattice distance (nm)	Intensity (ND%)
15	246	100
20	162	3
25	108	5
30	101	25

The average lattice distance of 5% AEA hydrogel was calculated using Cumulant results of dynamic light scattering measurements with a temperature range of 15–30°C.

3.4. Effect of temperature on hydrogel formation

The thermal characteristics of AEA solution were reported by Shimano et al. (1993). However, the effect of temperature on gel structure and drug release properties of AEA hydrogel at low temperatures have not been studied in detail. We therefore examined the effect of temperature on AEA hydrogel formation by measuring lattice distances by dynamic light scattering. On increase in temperature up to 30°C, the intensity of light scattering decreased markedly between 15 and 20°C (Table 2), suggesting that the sol to gel transition temperature of 5% AEA solution is about 15–20°C. The lattice distance in the pore structure of hydrogel decreased with increase in temperature, resulting in shrinkage of the gel. Above 37°C, 5% hydrogel was so turbid and its gelling rate so rapid that DLS observation could not be performed, and therefore was not performed for the 7% AEA hydrogel preparation. The sol-gel transition temperature of 5.8% AEA solution was reported to be 11.6°C as measured by differential scanning calorimetry (Shimano et al., 1993). A similar finding for sol-gel transition temperature was obtained by DLS measurement in the present study. These findings suggest that characteristics of the hydrogel formed by pH response might be also affected by surrounding temperature.

The effect of temperature on the kinetics of drug release from CM-hydrogel preparation (pH 7.4) was evaluated at 25 and 37°C. The release of CM from CM-hydrogel preparation stored at 25 or 37°C is shown in Fig. 5. The release of CM from CM-hydrogel stored at 37°C was slow, and only 10% release of CM was observed at 6 h. However, the release from CM-hydrogel stored at 25°C was fast, and exhibited saturable kinetics, and 60% CM release was observed at 6 h. As expected from the results of DLS measurement, hydrogel shrinkage occurred with change in temperature from 25 to 37°C. During hydrogel shrinkage, the geometry of the micropores of hydrogel, including tortuosity and porosity, changed, resulting in decrease in pore size and slow release of CM from hydrogel preparation.

3.5. Average pore size of hydrogel

As noted above, AEA hydrogel formed in the dialysis tube method exhibits two distinct geometrical structures, a porous inner phase and a fine outer phase. A fine outer phase of hydrogel may be formed by contact with membrane of the dialysis tube. Estimation of the average pore size should require a homologous geometrical structure. AEA hydrogel was therefore prepared by the method of temperature change, which does not require a dialysis tube, and pore size was evaluated by measuring the release of FDs of various

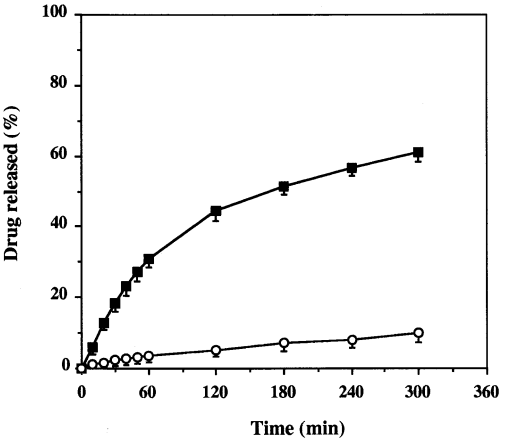

Fig. 5. Profiles of release of chlorpheniramine maleate from CM-hydrogel. Drug release was evaluated in pH 7.4 phosphate buffer at 37°C by the dialysis tube method. – ■ –, CM-hydrogel stored at 25°C; – ○ –, CM-hydrogel stored at 37°C.

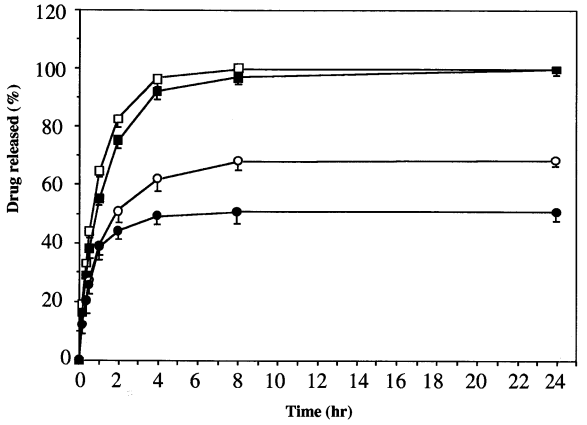

Fig. 6. Profiles of release of FITC-dextrans from FD-hydrogel. Drug release was evaluated in pH 7.4 phosphate buffer at 37°C. – □ –, FD-4; – ■ –, FD-10S; – ◦ –, FD-20S; – • –, FD-70S.

molecular weights from the hydrogel. The release of FDs of various molecular weights from FD-hydrogel (pH 7.4) at 37°C is shown in Fig. 6. Release was complete within 8 h for FD-4 and FD-10S. On the other hand, 70 and 50% release were observed at 8 h for FD-20S and FD-70S, respectively, and these percentages remained almost the same up to 24 h. It was found that the higher the molecular weight, the slower the rate of release. Some of the FD molecules appeared to be trapped inside the hydrogel. A strong correlation between dextrans molecular radius and molecular weight was reported by Dong et al. (1994), described by the equation:

$$\log(R) = 0.46 \log(M_w) - 0.46$$

where R is the radius, and M_w the molecular weight of FDs. The relationships between drug released (%) at 24 h and logarithm of molecular weight are shown in Fig. 7. The molecular weight not permeating through the hydrogel was calculated to be 560 kDa from the X-intercept of the regression line ($r = 0.950$), and pore radius was presumed to be 15 nm. These values yield a pore size of 30 nm for 7% AEA hydrogel. The marked decrease in release for FD-4 shown in Fig. 4 appeared to be due to the characteristics of the outer surface of hydrogel formed on the inside of the dialysis tube.

The schematic illustration of drug release process in AEA system is shown in Fig. 8. AEA in acidic solution forms a hydrogel when pH is increased to around neutral in in vitro experiments. During this process, drug dissolved in water phase can be loaded into the hydrogel phase, and slow release kinetics can be obtained. The AEA system is useful not only for low molecular weight molecules but also macromolecules, such as peptides and proteins, in obtaining controlled release of drugs. Further research will focus on efficient application of this system to physiological pH conditions, such as those in the nasal mucous membranes.

4. Conclusion

SEM observations suggested that AEA in solution becomes a hydrogel on change in pH, and that temperature change also accelerates network formation in the hydrogel. The release profile of AEA solution consisted of two phases: the initial drug release was very rapid, but after hydrogel formation the release of drug loaded in hydrogel was very slow and sustained compared with that of AEA-free solution. AEA solution with low

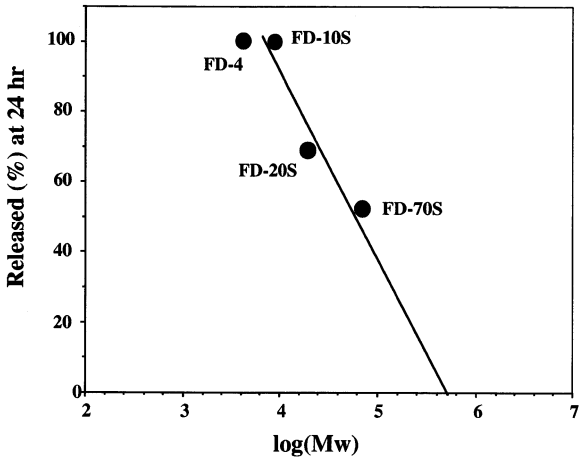

Fig. 7. Relationships between molecular weight and percent of FITC-dextrans released from FD-hydrogel at 24 h. The molecular weight not permeating through the hydrogel was calculated to be 560 kDa, from the X-intercept of the regression line ($r = 0.950$).

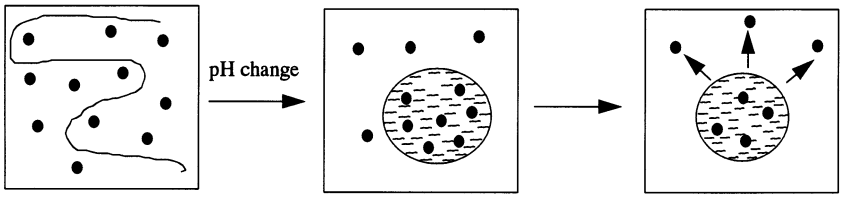

Fig. 8. Schematic illustration of drug release process in AEA system, ●. Drug molecule; wavy line: polymer in solution; lined circle, hydrogel.

viscosity at pH 4 but which forms hydrogel at pH 7.4 has the potential for use in controlled release of drugs applied to physiological membranes.

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