ELSEVIER

International Journal of Pharmaceutics 137 (1996) 95–102

international journal of pharmaceutics

Controlled release of ethinylestradiol from ethylene-vinyl acetate membrane

Sang-Chul Shin*, Soo-Young Byun

College of Pharmacy, Chonnam National University, Kwangju, South Korea

Received 29 July 1995; revised 2 October 1995; accepted 19 February 1996

Abstract

The studies on the permeability of ethinylestradiol (EE) through ethylene-vinyl acetate (EVA) copolymer membrane using a two-chambered diffusion cell were carried out to develop the membrane controlled transdermal delivery system. To evaluate the effect of drug concentration in reservoir, polyethylene glycol (PEG) 400 was added to saline solution as a solubilizer and a sink condition was maintained in the receptor solution. An increased vinyl acetate comonomer content in the EVA membrane increased the drug release rate and permeability coefficient. A linear relationship existed between the permeation rate and the reciprocal of the membrane thickness. Ethinylestradiol-containing matrix was fabricated with EVA copolymer to control the release of the drug. The release of EE from EVA matrix follows a diffusion controlled model, where the quantity released per unit area is proportional to the square root of time. The release rate of drug from EVA matrix increased with PEG 400 volume fraction, temperature and loading dose.

Keywords: Ethinylestradiol; Ethylene-vinyl acetate; Transdermal delivery; PEG 400; Solubility; Permeability coefficient; Release rate

1. Introduction

In the last decades, transdermal dosage forms have been introduced as controlled delivery via skin into the systemic circulation. The usefulness of transdermal delivery systems has been demonstrated for drugs such as nitroglycerin, scopolamine and clonidine (Tymes et al., 1990).

17β-Estrogen is produced by the ovaries in premenopausal women. Postmenopausal syndrome is characterized by symptoms that are endocrinologic, somatic and psychological in nature (Laufer et al., 1983). Administration of estrogen to postmenopausal women (in dosage of 0.05–0.2 mg/day) elevates plasma estradiol concentrations into the range observed in premenopausal women at the early–mid follicular stage. So, estrogen replacement is effective for hot flushes, symptoms associated with vaginal atrophy and prevents os-

* Corresponding author.

PII S0378-5173(96)04497-3

teoporotic fractures. But oral administration of estrogen may cause side effects such as hypertension, hyperlipidemia, gallbladder disease and blood clots, etc. Therefore, the development of transdermal drug delivery of the female and male hormone without the side effects of frequent oral administration is very important.

Basic components of transdermal devices are the polymer matrix, penetration enhancers and excipients (Hadgraft, 1987). The use of a release controlling membrane is one method to regulate drug release. The use of drugs dispersed in inert polymer matrices to achieve controlled release by diffusion has had considerable attention. Among many polymers, ethylene-vinyl acetate (EVA) copolymer is a heat processable, flexible and inexpensive material (Miyazaki et al., 1982). The safety and biocompatibility of EVA copolymer are reflected in its use as a biomaterial for artificial hearts and as an antithrombogenic material.

The usefulness of EVA copolymer as a drug delivery system for pilocarpine, progesterone, hydrocortisone (Johnson, 1980), fluoride ion (Halpern et al., 1976), 5-fluorouracil (Miyazaki et al., 1984) and macromolecules such as proteins was described. However few reports have dealt with the release of contraceptive drugs from EVA copolymer matrices.

Since the first step in transdermal drug delivery systems involves controlled drug release from the dosage form, the present investigation was undertaken to determine the amounts of a potent contraceptive drug, ethinylestradiol (EE), released from EVA copolymer matrices in in vitro experiments.

2. Materials and methods

2.1. Materials

Ethinylestradiol (Hanhwa Pharm. Co., Korea) and PEG 400 were pharmaceutical grade. Ethylene-vinyl acetate copolymers of 18, 33 and 40% (w/w) VA content were purchased from Aldrich Chemical Co., Inc. (USA). Acetonitrile was HPLC grade and all chemicals were used as received.

2.2. Determination of drug solubility

An excess amount of EE was equilibrated with 5 ml of saline containing various concentrations of PEG 400 at 37°C for 24 h with constant shaking in a shaking incubator. The saturated EE solution was then filtered through a membrane filter (0.45 μm). The concentration was determined after proper dilution at 280 nm by spectrophotometer.

2.3. Preparation of EVA copolymer membranes

About 0.5–2.5 g of EVA copolymer beads were dissolved in 10–20 ml of methylene chloride or cyclohexane in a glass beaker. The vinyl acetate content of EVA copolymer varied from 18 to 40% (w/w). This polymer solution was poured onto a Teflon-coated plate and the solvent was allowed to evaporate off at room temperature overnight. The membrane was removed from the plate and dried for 2 days at room temperature in vacuo. The thickness of the membranes was measured at several points by micrometer and the mean values were obtained.

2.4. Drug permeation through EVA membranes

For the determination of steady state permeation of EE through EVA membranes, a two-chamber diffusion cell was used. Each half-cell has a volume of about 7 ml and an effective diffusional area of 0.79 cm^2. A piece of EVA membrane was clamped between the two halves of the cell and the assembled cell was placed in a shaking incubator at 37°C. A known amount of drug suspension in various concentrations of PEG 400-saline solution was poured into the donor compartment, and the same concentration of PEG 400-saline solution (without drug) was added to the receptor compartment, in order to prevent the effect of solvent permeation from the donor to the receptor side on the EE permeation through the membrane. The cell was shaken horizontally at the rate of 120 rev./min to minimize the boundary effect. The total volume of the receptor solution was removed at predetermined intervals and replaced with 7 ml of fresh solution.

The amount of drug permeated was determined by high performance liquid chromatography (HPLC).

2.5. Drug-containing matrix preparation

Devices of EVA and EE were prepared by casting process. The weighed amount of EE was dissolved in 20 ml of methylene chloride adding cyclohexane if necessary. The bead of EVA copolymer was dissolved in the drug solution. This mixture was poured onto a Teflon-coated plate and the solvent was allowed to evaporate off at room temperature overnight. The membrane was removed from the plate and dried for 2 days at room temperature in vacuo. Then a piece of matrix was cut from the membrane and weighed accurately. The drug content was calculated from the weight ratio of drug and copolymer used.

2.6. In vitro release studies

The in vitro release of EE from the EVA matrix was examined using the modified Keshary–Chien cell (Chien, 1987). A unit of EVA matrix was clamped between the cell cap and receptor cell. The diameter of the cell was 2.0 cm, providing 3.14 cm^2 effective constant area between the matrix and the bulk solution of 20 ml. PEG 400-saline solution was used as the receptor solution. The receptor was maintained at 37°C with a circulating water jacket and stirred constantly at 120 rev./min. At predetermined times, the whole solution was taken from the receptor cell and replaced with fresh solution. The cumulative amount of EE released from the matrix was determined by HPLC. The effects of volume fraction of PEG 400 in receptor solution, temperature and loading dose on drug release were also studied.

2.7. HPLC determination of EE

The concentration of EE in the samples obtained from the cells was determined by HPLC. The HPLC system consisted of a pump (Waters 501, USA), and ultraviolet (UV) detector (Waters 484, USA), a 3.9 × 300 mm stainless-steel column packed with μ-Bondapak C-18 (Waters, USA) and an integrator (D520A, Youngin Scientific Co., Ltd., Korea). The mobile phase was a combination of acetonitrile and water (50:50) and column temperature was maintained at ambient. A flow rate of 1.5 ml/min yielded an operating pressure of 1500 psi. The UV detector was operated at a wavelength of 280 nm at a sensitivity of 0.01 AUFS. Under these conditions, the EE peak appeared at the retention time of 6.5 min.

2.8. Calculations

2.8.1. Kinetics of drug permeation through membrane

The cumulative amount of drug permeating through a unit surface area (Q) can be expressed mathematically by the following relationship:

$$Q = P(C_D - C_R)t \quad (1)$$

where P is the permeability coefficient; and C_D and C_R are the drug concentrations in the donor (D) and the receptor (R) solutions, respectively.

When the drug concentration in the donor solution (C_D) is maintained at a level greater than the equilibrium solubility (i.e. $C_D > C_c$) and the drug concentration in the receptor solution (C_R) is maintained under the sink condition (i.e. $C_R \ll C_c$), Eq. (1) can be simplified to:

$$Q = P \cdot C_c \cdot t \quad (2)$$

and a constant permeation profile should result. The rate of permeation is then defined by:

$$\frac{Q}{t} = P \cdot C_c \quad (3)$$

The permeation rate was calculated from the slope of the linear region of the permeation profile. Lag time was calculated from the intercept on the time axis by extrapolation from the steady-state skin permeation profile (Morimoto et al., 1985; Morimoto et al., 1986).

2.8.2. Kinetics of drug release from matrix

A characteristic drug release profile of matrix-type drug delivery systems can be represented by the Higuchi's equation (Higuchi, 1961). The release from a planar system having dispersed drug in a homogeneous matrix should follow the relationship:

$$Q = [D(2A - C_s)C_s t]^{1/2} \quad (4)$$

where Q is the amount of drug released after time t per unit exposed area; D is the diffusivity of the drug in the matrix, A is the initial drug concentration, and C_s is the drug solubility in the matrix. The release flux was calculated from the slope of the linear region of the Q versus $t^{1/2}$ release profile. The validity of the relationships has been confirmed experimentally by a number of workers using various systems (Desai et al., 1965; Lapidus and Lordi, 1966; Farhadieh et al., 1971).

3. Results and discussion

3.1. Effect of solubilizer on the EE permeation through EVA membrane

The aqueous solubility of EE is extremely low and could be improved by addition of a water-miscible hydrophilic polymer like PEG 400 into the aqueous solution as a solubilizer for EE. PEG 400 was reported to be an excellent solubilizer for many steroids (Chien and Lambert, 1975). In the present investigation, it was observed that the aqueous solubility of EE increased exponentially with increasing volume fraction of PEG 400 in saline solution (Table 1).

When the EE concentration in the donor solution was maintained at a level greater than its equilibrium solubility, a constant permeation profile was achieved (Fig. 1). The rate of permeation (Q/t), which was measured from the slope

Table 1
Effect of PEG 400 on the permeation of EE through the EVA copolymer membranes

PEG 400 % (v/v)	Rate of permeation $\mu g/cm^2/h \times 10^{-1}$	Permeability coefficient $cm/h \times 10^{-2}$
0	7.46	4.754
10	29.83	4.329
20	74.97	3.700
30	97.79	2.035
40	338.48	1.563

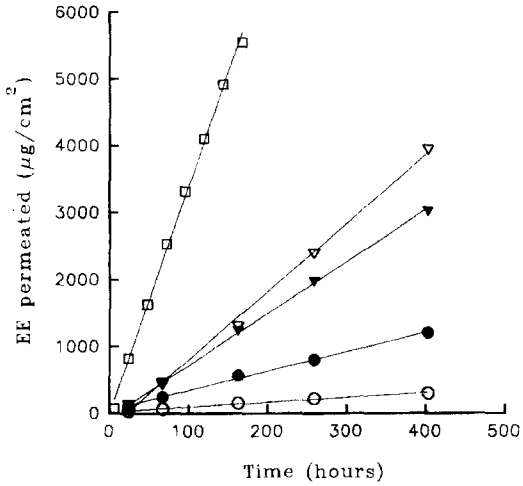

Fig. 1. Effect of PEG 400 volume fraction in saline on the permeation of EE through EVA membrane composed of 40% (w/w) VA. (○) 0%, (●) 10%, (▼) 20%, (▽) 30% and (□) 40% (v/v) PEG 400.

of Q versus t plots (Eq. (2)), was found to increase with the addition of PEG 400 up to 40% (v/v) in the saline solution. As expected from Eq. (3), the increase in the permeation rate (Q/t) was dependent upon the equilibrium solubility (C_e) of EE in the PEG 400-saline solutions. From the data on the rate of permeation and saturated concentration (equilibrium solubility) in Table 1, Fig. 2 is obtained. The low rate of permeation of EE was observed in the saline solutions containing less than 20% (v/v) PEG 400 (Fig. 2). This might be attributed to the lower equilibrium solubility of EE in receptor solutions. The effect of PEG 400 on the permeability coefficient (P) of EE across EVA membrane can be determined using Eq. (5):

$$P = \frac{Q/t}{C_e} \quad (5)$$

The results (Table 1) showed that even though the equilibrium solubility increased in higher volume fractions of PEG 400, the permeability coefficient decreased.

3.2. Effect of comonomer ratio on the drug permeation

To study the effect of comonomer ratio modifi-

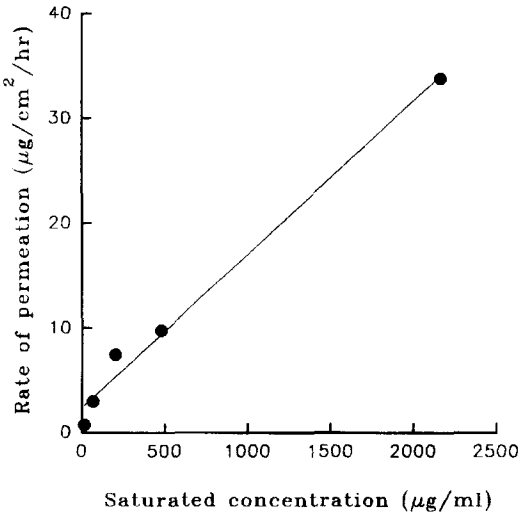

Fig. 2. Dependency of the permeation rate of EE through the EVA membrane on the saturation concentration of EE in the donor solution.

cations on drug permeation, the permeation of EE through EVA membranes composed of different VA content was investigated (Miyazaki et al., 1984).

Fig. 3 shows the representative plot of the permeation profiles of EE through the EVA copolymer membranes of various VA content at 37°C where the cumulative amount of drug permeated into the receptor solution is plotted against time. An increase in VA comonomer content increased the drug release rate and permeability coefficient.

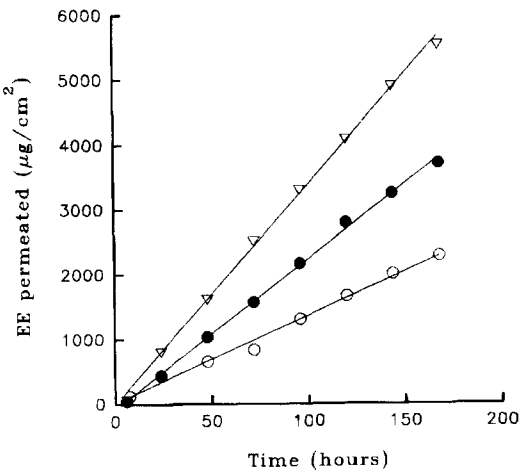

Fig. 3. Permeation profiles of EE through EVA copolymer membrane of various VA content. PEG 400 volume fraction was maintained at 40% (v/v). (○) 18%, (●) 33% and (▽) 40% (w/w) VA.

The EVA copolymer membrane is classified as an ester-type partition membrane. The permeation through the partition membrane is governed primarily by partition of the drugs into the membrane material. Since the permeability coefficient is proportional to the product of the partition coefficient and diffusion coefficient, it may be correlated with the partition coefficient if the diffusion coefficient is considered to be of the same order of magnitude among the EVA copolymer membranes used.

On the other hand, it is known that an increase in crystallinity reduces the diffusivity of polymer (Michaels and Bixler, 1961; Donbrow and Friedman, 1975), and that the introduction of VA comonomers to a highly crystalline polyethylene decreases the crystallinity of the system (Kamath and Wakefield, 1965; Barlow and Campbell, 1967). Kamath and Wakefield (1965) predicted that EVA copolymer becomes totally amorphous if VA concentration is 43% (w/w) or greater. Therefore, the result suggested that the diffusivity of the drug within the membrane might be one of the possible factors in controlling the permeation rate through EVA membranes.

3.3. Effect of membrane thickness on the permeation rate of EE

The permeation rate (dQ/dt) is proportional to the reciprocal of the membrane thickness (h). In order to determine this permeation rate versus membrane thickness relationship, the effect of variation in membrane thickness on the EE permeation rate was studied. The result (Fig. 4) demonstrates that a linear relationship exists between the permeation rate and the reciprocal of the membrane thickness. This suggests that the permeation of EE is highly controlled by the membrane thickness.

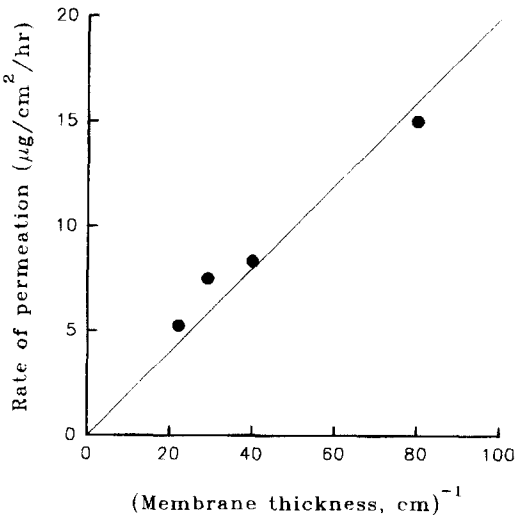

Fig. 4. Plot of steady-state flux (Q) against the reciprocal of membrane thickness ($1/h$), for the saturated solutions in 20% (v/v) PEG 400-saline. EVA composed of 40% (w/w) VA was used.

3.4. Effect of PEG 400 volume fraction on drug release

Factors determining the rate of drug release are particularly important in the design and formulation of a controlled release preparation. Thus, it is necessary to determine what factors control the release kinetics. The effect of PEG 400 content in the receiver on the release was examined as a basic step to determine an adequate PEG 400 volume fraction.

The release profiles of EE from the EVA matrix are shown in Fig. 5. The cumulative amount of EE released (Q) versus the square root of time ($t^{1/2}$) plot shows a good linearity as expected from Eq. (4). An increase in PEG 400 content increased the drug release rate from the EVA matrix.

3.5. Effect of temperature on drug release

The dependency of the drug release profile on temperature is illustrated in Fig. 6. The cumulative amount of drug released (Q) is plotted versus the square root of time. After an initial period of drug release, the release was approximately linear

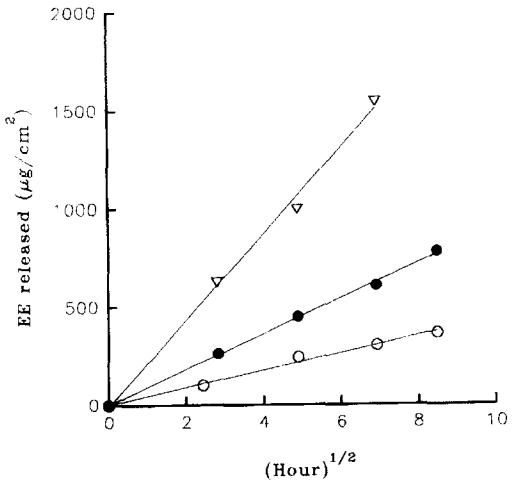

Fig. 5. Effect of PEG 400 volume fraction in receiver on the EE release from 10% (w/w) EE containing EVA copolymer matrix. (○) 0%, (●) 10% and (▽) 20% (v/v) PEG 400.

with respect to $t^{1/2}$. The steady-state rate of drug release was estimated from the slope of the linear Q–$t^{1/2}$ profile from 8 to 168 h. It should be noted that the rate of drug release increased about 2.1-fold when the temperature of the drug release

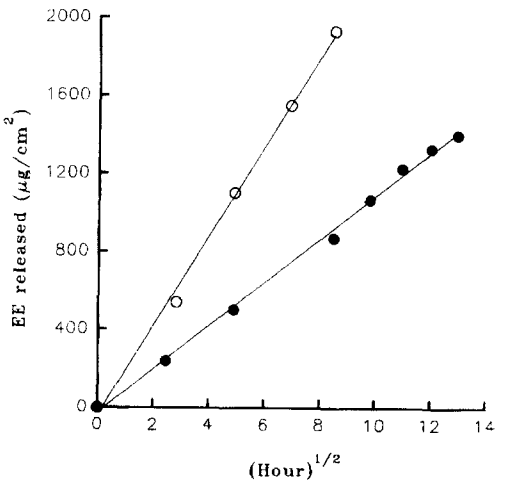

Fig. 6. Effect of temperature on the EE release from the 10% (w/w) EE containing EVA copolymer matrix composed of 40% (w/w) VA. PEG 400 volume fraction was maintained at 20% (v/v). (○) 37°C and (●) 32°C.

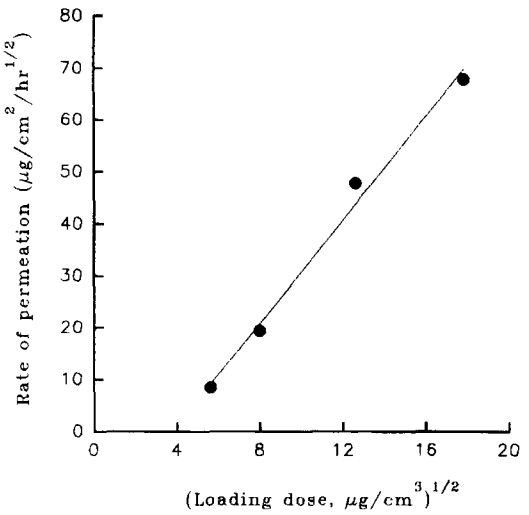

Fig. 7. Relationship of the release rate to the EE loading dose in EVA copolymer matrix composed of 40% (w/w) VA. PEG 400 volume fraction was maintained at 20% (v/v).

system was raised from 32 to 37°C. But, for practical use, a temperature of 32°C was chosen to reflect the temperature of the stratum corneum (Chien and Lau, 1976). This finding indicates that special precautions should be taken with regard to monitoring body temperature in practical applications.

3.6. Effect of loading dose on drug release

The release of EE from the EVA matrices of different drug loading over a 168-h period were studied. A plot of $Q/t^{1/2}$ versus the square root of loading dose yields a straight line (Fig. 7).

Acknowledgements

This work was supported by a grant from New Drug Development Programs of Korea.

References

Barlow, A. and Campbell, R.C., HNO_3 digestion and crystallinity of C_2H_4-vinyl acetate copolymers. *J. Appl. Polym. Sci.*, 11 (1967) 2001–2006.

Chien, Y.W., *Transdermal Controlled Systemic Medications*, Marcel Dekker, New York, 1987, pp. 132–137.

Chien, Y.W. and Lambert, H.J., Solubilization of steroids by multiple co-solvent systems. *Chem. Pharm. Bull.*, 23 (1975) 1085–1090.

Chien, Y.W. and Lau, E.P.K., Controlled drug release from polymeric delivery devices IV: In vitro-in vivo correlation of subcutaneous release of norgestomet from hydrophilic implants. *J. Pharm. Sci.*, 65 (1976) 488–492.

Desai, S.J., Simonelli, A.P. and Higuchi, W.I., Investigation of factors influencing release of solid drug dispersed in inert matrices. *J. Pharm. Sci.*, 54 (1965) 1459–1464.

Donbrow, M. and Friedman, M., Enhancement of permeability of ethyl cellulose films for drug penetration. *J. Pharm. Pharmacol.*, 27 (1975) 633–641.

Farhadieh, B., Boradkin, S. and Buddenhagen, J., Drug release from methyl acrylate-methyl methacrylate copolymer matrix I: Kinetics of release. *J. Pharm. Sci.*, 60 (1971) 209–211.

Hadgraft, J., *Transdermal Drug Delivery*, Marcel Dekker, New York, 1987, pp. 298–300.

Halpern, B.D., Solomon, O., Kopec, L., Korostoff, E. and Ackerman, J.L. In Paul, D.R. and Harris, F.W. (Eds.), *Controlled Release Polymeric Formulations*, American Chemical Society, Washington, DC, 1976, pp. 135–146.

Higuchi, T., Rate of release of medicaments from ointment bases containing drug in suspension. *J. Pharm. Sci.*, 50 (1961) 874–875.

Johnson, J.C. (Ed.), *Sustained Release Medications*, Noyes Data Co., Park Ridge, 1980, pp. 96–97;

Kamath, P.M. and Wakefield, R.W., Crystallinity of ethylene-vinyl acetate copolymers. *J. Appl. Polymer. Sci.*, 9 (1965) 3153–3160.

Lapidus, H. and Lordi, N.G., Some factors affecting the release of a water-soluble drug from a compressed hydrophilic procedure. *J. Pharm. Sci.*, 55 (1966) 840–843.

Laufer, L.R., Defazio, J.L., Lu, J.K.H., Meldrum, D.R., Eggena, P., Sambhi, M.P., Hershman, J.M. and Judd, H.L., Estrogen replacement therapy by transdermal estradiol administration. *Am. J. Obstet. Gynecol.*, 146 (1983) 533–540.

Michaels, A.S. and Bixler, H.J., Flow of gases through polyethylene (and rubbery polymers). *J. Polym. Sci.*, 50 (1961) 413–439.

Miyazaki, S., Ishii, K., Sugibayashi, K., Morimoto, Y. and Takada, M., Antitumor effect of ethylene-vinyl acetate copolymer matrices containing 5-fluorouracil on Ehrlich Ascites carcinoma in mice. *Chem. Pharm. Bull.*, 30 (1982) 3770–3775.

Miyazaki, S., Takeuchi, S., Takada, M. and Ishii, K., Factors affecting the release patterns of 5-fluorouracil from ethylene-vinyl acetate copolymer matrices. *Chem. Pharm. Bull.*, 32 (1984) 1633–1636.

Morimoto, Y., Sugibayashi, K., Hosoya, K.-I. and Higuchi, W.I., Effect of the absorption enhancer, Azone, on the transport of 5-fluorouracil across the hairless rat skin. *J. Pharm. Pharmacol.*, 37 (1985) 578–580.

Morimoto, Y., Sugibayashi, K., Hosoya, K.-I. and Higuchi, W.I., Penetration enhancing effect of Azone on the transport of 5-fluorouracil across the hairless rat skin. *Int. J. Pharm.*, 32 (1986) 31–38.

Tymes, N.W., Shah, V.P. and Skelly, J.P., In vitro release profile of estradiol transdermal therapeutic systems. *J. Pharm. Sci.*, 79 (1990) 601–602.