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Effect of pH on the Sorption of In-solution Diazepam into the Ethylene-Vinylacetate Copolymer Membrane

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We investigated the mechanism for diazepam sorptions into the ethylene-vinylacetate (EVA) copolymer membrane. A linear relationship was found between the amount of diazepam sorbed into the EVA membrane and the diazepam concentration when it was 5–20 μg/ml. The sorbed amount was influenced by pH. When the diazepam concentration in solution was 20 μg/ml, it was 0.17 mg/g at pH 3.2 and increased to 0.74 mg/g at pH 7.0. Moreover, the experimental data showed a similar tendency as the calculated values, suggesting that the amount of diazepam sorbed was proportional to the concentration of the diazepam molecular form.

Keywords——diazepam, sorption, ethylene-vinylacetate copolymer membrane, pH

Nitroglycerin, diazepam and other variety of pharmaceutical products have been reported to adsorb to containers made of ethylene-vinylacetate (EVA) copolymer membrane, decreasing their contents[1-2]. Additionally, the decrease of diazepam content may be influenced by pH since diazepam contains basic nitrogen atom. The pH of commercial transfusion fluid is known to be maintained at the pH range of 3.67–7.68[3], and the amount of decreased diazepam content may vary depending on the types of transfusion fluid used. Therefore, it is necessary to know relationship between the amount of decreased content and pH precisely.

This investigation was conducted in order to elucidate the mechanism of decrease of diazepam content in EVA containers. We report our results regarding the relationship between pH and the amount of decreased diazepam content.

Experiments

1. Materials

EVA containers (TERUPACK, TERUMO) containing physiological saline solution were used as transfusion containers. EVA membrane was cut appropriately just suitable for EVA containers.

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Acid Phthalate, Neutralized Phthalate, and Phosphate Buffer (all 0.2 M, USP XXII) were used as buffer. Cercine for injection (diazepam 10 mg/2 ml A, Takeda Chemical Industries, Ltd.) was used as diazepam solution for injection. Additionally, bulk diazepam substance was kindly provided by Takeda Chemical Industries, Ltd. Commercially available n-butyl benzoate (Kanto Chemical Co., Inc.) was used.

2. Quantification of diazepam

Diazepam was quantified by high performance liquid chromatography (HPLC)[4]. Column: Finepak SIL $C_{18}S$ (4.6 × 150 mm, Jasco Co.), mobile phase: acetonitrile-methanol-0.05 M ammonium acetate (5 : 5 : 4), flow rate: 1.0 ml/min., wavelength: 254 nm, and n-butyl benzoate was used internal standard substance.

3. Sorption to EVA containers

Diazepam solution (40 μg/ml) for injection was added to physiological saline solution or buffer solution (pH 7.0) in an EVA container, kept at 30°C without disturbing it, and changes of the amount of diazepam were monitored successively. Moreover, light in the laboratory was dimmed as much as possible.

4. Measurement of sorption rate to EVA membrane

EVA membrane (approximately 0.2 g) was added to 10 ml of buffer solution containing diazepam, kept at 30°C without disturbing it, and changes of the amount of diazepam were monitored successively.

The amount of diazepam sorbed to EVA membrane was calculated by the concentration of diazepam in solution. Moreover, diazepam is known to degrade in acid solution[5]. Out of pH region (pH 2.0 to pH 7.0) measured in this study, it was reported that remaining rate of diazepam dropped to 97.1% after keeping it at 30°C for 7 days when it was kept at pH 2.0[6]. Therefore, the amount of diazepam sorbed at pH 2.0 was calculated after compensating the amount of degraded diazepam.

Results and Discussions

1. Sorption to EVA containers

Table 1 showed changes of diazepam concentrations in EVA containers when the initial diazepam concentration was approximately 40 μg/ml. The amount of diazepam content decreased as a function of time in physiological saline solution and pH 7.0 buffer solution, and 24 hours later, it dropped to 74.1% & 73.9%, respectively.

2. Sorption rate of diazepam and the equilibrium amount of diazepam sorbed to the EVA membrane

Fig. 1 showed the time course of diazepam sorption. Y-axis showed the amount of diazepam sorbed to EVA membrane (mg/g weight) and X-axis showed time (h). Each point was the mean value of three measurements, and coefficient of variation (C.V.) was 0.73–1.75%.

Under this experimental condition, the equilibrium was achieved approximately 96 hours later at both pH 4.0 and pH 7.0. Therefore, we determined the relationship between the amount of diazepam sorbed to the EVA membrane and the equilibrium concentration of diazepam in the buffer

Table 1. Diazepam Loss in the EVA Container at 30°C

(Residual diazepam, %)

	EVA[a)]	EVA (pH 7.0)
Initial	100.0	100.0
2 h	92.9	92.6
6 h	86.1	87.0
24 h	74.1	73.9

a) Ethylene-Vinylacetate copolymer membrane
Initial concentration: 40 μg/ml
Intravenous fluid: isotonic sodium chloride sol., USP buffer

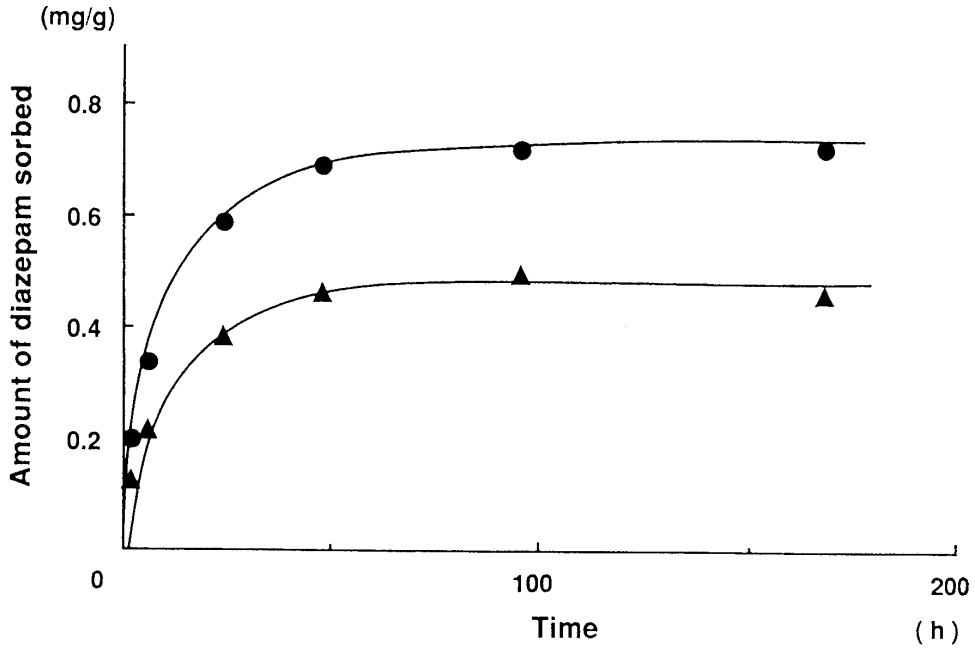

Fig. 1. The Time Course of Diazepam Sorption from the Buffer Solutions to the Ethylene-Vinylacetate Copolymer Membrane at 30°C
pH 4.0: (▲), pH 7.0: (●)

solution on the basis of the values 96 hours later, and the result was shown in Fig. 2.

As shown in this figure, the amount of diazepam sorbed to EVA membrane was higher at pH 7.0 than pH 4.0, and it demonstrated a straight dose response line when its concentration was 5–20 μg/ml.

Since it took a long time (approximately 96 hours) to reach the equilibrium and the amount of diazepam sorbed to the EVA membrane was proportional to diazepam concentration as shown in Fig. 1 or Fig. 2, the decrease of diazepam content in EVA containers was speculated to occur due to sorption of diazepam as well as adsorption to the surface of EVA membrane, that is, diazepam was sorbed to the inner portion, as was the case for isosorbide dinitrate[7] (already reported).

3. Relationship between the amount of diazepam sorbed and pH

Relationship between the amount of diazepam sorbed and pH was shown by a solid line in Fig.

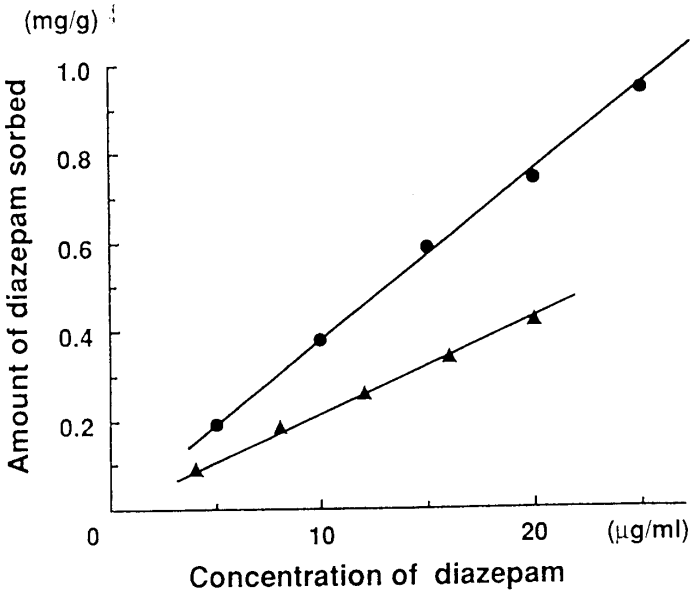

Fig. 2. The Relationship between the Amount of Diazepam Sorbed to the Ethylene-Vinylacetate Copolymer Membrane and the Equilibrium Concentrations of Diazepam in the Buffer Solutions at 30°C
pH 4.0: (▲), pH 7.0: (●)

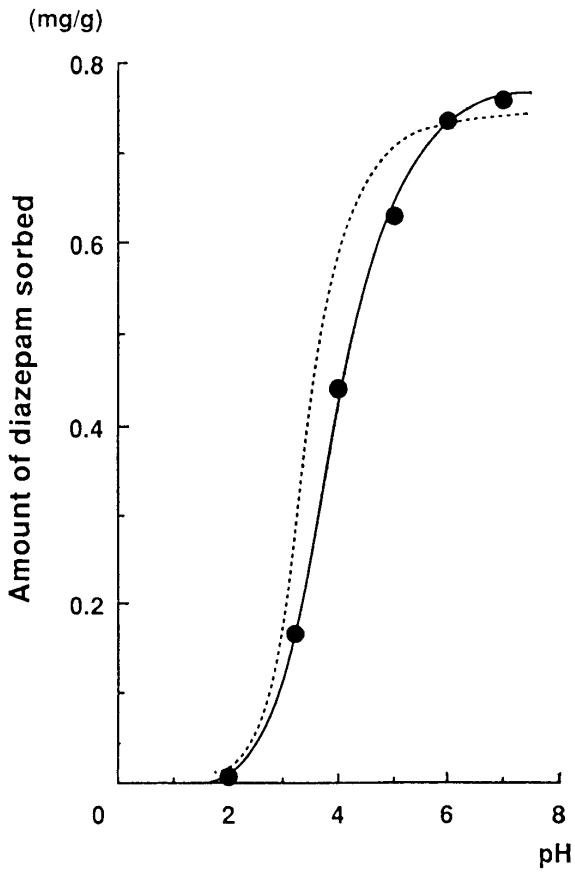

Fig. 3. The Equilibrium Amount of Diazepam Sorbed to the Ethylene-Vinylacetate Copolymer Membrane as a Function of pH at 30°C
The equilibrium concentration of diazepam in the aqueous solutions are about 20 μg/ml. The solid points represent the experimental data. The dotted line represents the calculated values, supposing that the equilibrium amount of diazepam sorbed is proportional to the concentration of diazepam molecular form.

3. Y-axis indicated the amount of diazepam sorbed when the equilibrium amount of diazepam was 20 μg/ml, and X-axis indicated pH. The amount of diazepam sorbed increased as pH increased. The amount of diazepam sorbed at pH 3.2 was 0.17 mg/g and 0.74 mg/g at pH 7.0.

Equation (1) or equation (2) showed relationship between the diazepam concentration in solution (S) and molecular form (B) or ionized form (BH^+). Additionally, it has been reported that pKa of diazepam was 3.38[8].

$$S=[B]+[BH^+] \quad (1)$$

$$S=[B]\{1+\text{anti log}\,(pKa-pH)\} \quad (2)$$

S: Concentration of diazepam in the solution (7.02×10^{-5} M, 20 μg/ml)
B: Molecular form
BH^+: Ionized form
pKa: 3.38

The relationship between pH and the concentration of molecular form was calculated on the basis of equation (1) and (2) when diazepam concentration in solution was 20 μg/ml, and the dotted line in Fig. 3 showed the amount of diazepam sorbed (the calculated values), supposing that the amount of diazepam sorbed was proportional to the concentration of molecular form.

As shown in this figure, both the experimental data and the calculated values of the amount of diazepam sorbed showed similar tendency. The result indicated that sorption of diazepam to EVA membrane was closely related to the concentration of molecular form in solution.

Additionally, it has been known that diazepam in solution exists in pH dependent equilibrium of molecular form and ionized form with a open ring structure[5].

Thus, the relationship between sorption of diazepam to EVA membrane and pH can be illustrated in Chart 1. We speculated that molecular form of diazepam in aqueous phase and molecular form in organic phase (EVA membrane) might be in equilibrium with certain partition coefficient.

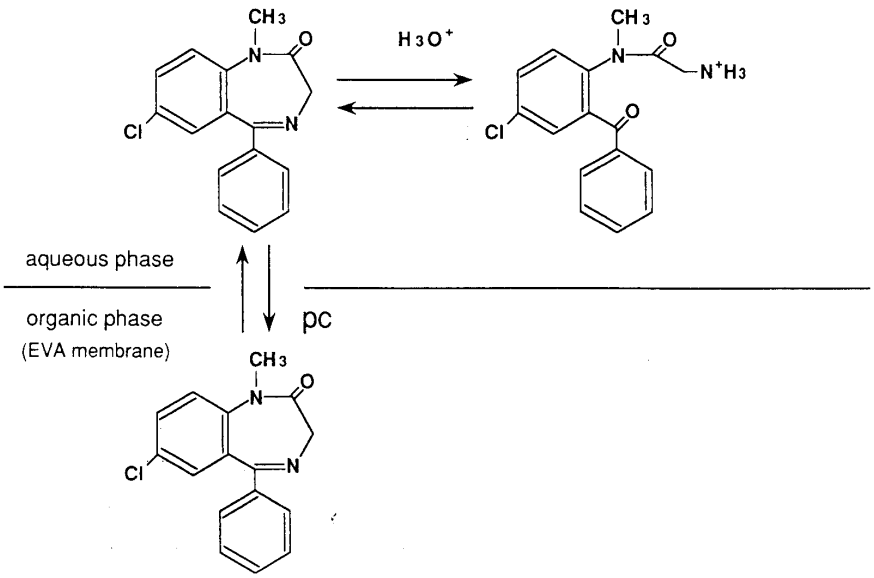

Chart 1

Conclusions

Diazepam was sorbed to EVA membrane, and the amount sorbed was proportional to its concentration. Additionally, the amount of diazepam sorbed was influenced by pH. When diazepam concentration in solution was 20 μg/ml, it was 0.17 mg/g at pH 3.2 and increased to 0.74 mg/g at pH 7.0. Moreover, the experimental data showed similar tendency as the calculated values, supposing that the amount sorbed was proportional to the concentration of molecular form.

In conclusion, our results suggested that sorption of diazepam to EVA membrane was caused by partition of molecular form of diazepam in organic phase (EVA membrane) and aqueous phase.

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