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ACKNOWLEDGMENTS

Y. G. Smeyers is indebted to Dr. A. Perales for valuable discussions and Prof. E. Galvez for reading the manuscript.

In Vitro Method for Detecting Precipitation of Parenteral Formulations After Injection

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Abstract □ Many injectable formulations currently on the market, including diazepam and alprazolam, utilize one or more cosolvents to solubilize the active constituents. On injection into an aqueous medium, some of these components tend to precipitate. A simple procedure is described for measuring the degree of precipitation that occurs when a solubilized drug is injected. This *in vitro* technique was used to show that alprazolam injection shows less precipitation than diazepam injection under all tested conditions, and that the precipitation observed with diazepam can be controlled by ensuring that the formulation is injected very slowly. This simple technique also can be used during preformulation development to evaluate the relative potential for precipitation of various formulations.

Keyphrases □ Diazepam—*in vitro* detection of precipitation for injectable formulations □ Alprazolam—*in vitro* detection of precipitation for injectable formulations □ Formulations, injectable—potential precipitation in aqueous media, *in vitro* detection using diazepam and alprazolam

It is often necessary to administer a drug parenterally at a concentration which exceeds its aqueous solubility. The use of water-miscible cosolvents is by far the most versatile means of increasing the solubility of drugs. Cosolvents in concentrations up to 50% v/v can produce solubility increases of several orders of magnitude for very insoluble drugs (1, 2).

In some cases, the injection of a formulation (in which the drug is solubilized by a cosolvent) into blood or some other aqueous fluid can result in precipitation of the drug (2–7). This precipitation, in turn, can result in erratic or reduced drug bioavailability, pain on injection, and/or thrombophlebitis (3–7). The amount of precipitation, and thus the severity of the above problems, often is related to the rate at which the drug is injected (3–7).

The elimination of precipitation on dilution not only can lead to a safer and more effective formulation, it can also

Table I—Some Parenteral Products Formulated with Cosolvents

Generic Name	Cosolvent Composition
Hydralazine HCl[a]	10% propylene glycol
Lorazepam[b]	80% propylene glycol 20% polyethylene glycol
Deslanoside[c]	9.8% ethanol 15% glycerin
Phenytoin sodium[d]	40% propylene glycol 10% ethanol
Dihydroergotamine mesylate[e]	6.1% ethanol 15% glycerin
Dimenhydrinate[f]	50% propylene glycol
Digoxin[g]	40% propylene glycol 10% ethanol
Chlordiazepoxide HCl[h]	20% propylene glycol
Phenobarbital sodium[i]	67.8% propylene glycol
Multiple vitamin infusion[j]	30% propylene glycol
Pentobarbital sodium[k]	40% propylene glycol 10% ethanol
Methocarbamol[l]	50% polyethylene glycol
Reserpine[m]	10% dimethylacetamide 5% polyethylene glycol
Diazepam[n]	40% propylene glycol 10% ethanol

[a] Apresoline (Ciba). [b] Ativan (Wyeth). [c] Cedilanid (Sandoz). [d] Dilantin (Parke-Davis). [e] DHE 45 (Sandoz). [f] Dramamine (Searle). [g] Lanoxin (Burroughs Wellcome). [h] Librium (Roche). [i] Luminal (Winthrop). [j] MVI (USV). [k] Nembutal (Abbott). [l] Robaxin (Robins). [m] Serpacil (Ciba). [n] Valium (Roche).

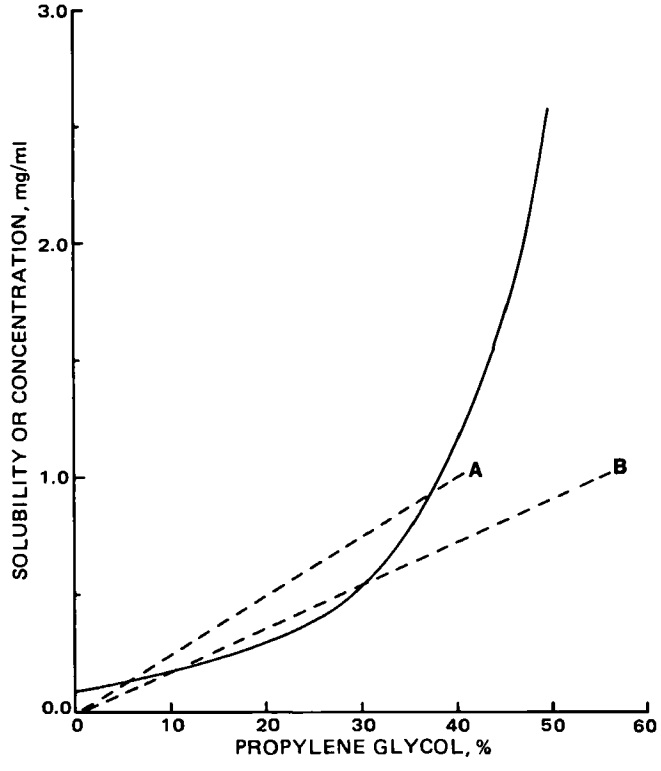

Figure 1—*Solubility (——) of alprazolam in propylene glycol–water mixtures containing 40% (A) and 55% (B) propylene glycol. The dilution lines (- - -) above the solubility curve represent conditions under which precipitation can occur.*

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Table II—Some Properties of Diazepam and Alprazolam

	Diazepam	Alprazolam
Chemical name	7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one	8-Chloro-1-methyl-6-phenyl-4*H*-*s*-triazolo-[4,3-*a*][1,4]benzodiazepine
Formula	$C_{16}H_{13}ClN_2O$	$C_7H_{13}ClN_4$
Melting point	125–126°	226–234°
pK_a	3.50[b]	2.4 @ 25°
Solubility[a]	0.0414 (20°)[c]	0.114 (25°)
Water	0.048 (25°)[b]	
Propylene glycol	15.2 (10°)[c]	42 (25°)

[a] In g/liter. [b] From N. A. Mason, S. Cline, M. L. Hyneck, R. R. Bernardi, N. F. H. Ho, and G. L. Flynn, *Am. J. Hosp. Pharm.*, **38**, 1449 (1981). [c] From M. C. Neira, F. Jiminez, and L. F. Ponce de Leon, *Rev. Colmb. Cienc. Quim.-Farm.*, **3**, 37 (1980).

ensure more meaningful evaluations of new drugs. A simple *in vitro* dynamic system for evaluating the degree of precipitation on injection of a solubilized drug is presented. The technique also can be used during preformulation development to evaluate the relative potential for precipitation of various formulations.

BACKGROUND

Many parenteral products currently on the market utilize one or more water-miscible cosolvents (Table I). The tendency for this type of formulation to precipitate when injected or diluted with an aqueous medium has been explained (2) on the basis of the following:

1. When a formulation is diluted by blood or by an intravenous drip solution, the concentrations of drug and cosolvent(s) decrease proportionally to one another.
2. The solubility of the drug in the mixed solvent decreases exponentially as the concentration of cosolvent is decreased linearly.

This frequently results in a situation in which the drug concentration exceeds the solubility, as shown in Fig. 1. The curve in Fig. 1 represents the solubilization of alprazolam in the propylene glycol–water system. The shape of this curve indicates an exponential increase in solubility with increasing cosolvent composition. This commonly encountered dependency of solubility on cosolvent composition has been explained previously (8, 9). The dashed lines show dilution curves for two 1.0-mg/ml alprazolam formulations: the upper line represents a formulation containing 40% propylene glycol (minimum needed to solubilize the drug) and the lower line represents the alprazolam formulation used in this study (55% propylene glycol). When the concentration line is above the solubility curve, the solution is supersaturated. If the formulation is diluted further, the dilution line again crosses the solubility curve, and the solution becomes unsaturated as it approaches infinite dilution. The distance between the concentration line and the solubility curve is equal to the amount of supersaturation. The length of the dilution line between the crossover points is a measure of the time that the drug will be supersaturated when it is being diluted.

It is clear from the figure that the dilution line for the formulation containing 55% propylene glycol is much closer to the solubility curve than the line for the 40% propylene glycol formulation. Consequently, there is a much lower probability of drug precipitation in the former formulation on dilution. The excess cosolvent present also helps to prevent precipitation when the formulation is cooled (as can happen during shipping in the winter season).

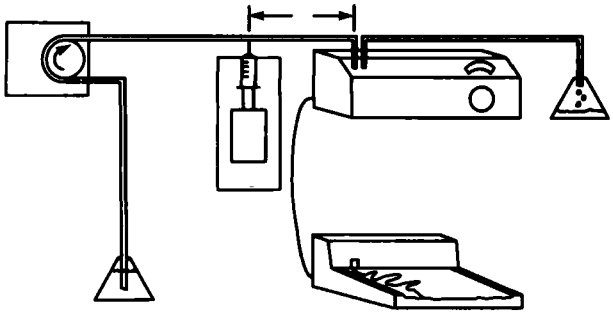

Figure 2—*Schematic of the apparatus used to detect precipitation.*

Table III—Composition of Diazepam and Alprazolam Injectable Formulations

Component	Diazepam	Alprazolam
Active ingredient	5.0 mg/ml	1.0 mg/ml
Benzyl alcohol	1.5%	—
Sodium benzoate–benzoic acid	5.0%	—
Ethanol	10%	—
Propylene glycol	40%	55%

As stated, both concentration lines fall below the solubility curve at high dilution. This indicates that any formed precipitate would redissolve on further dilution. It does not, however, give any indication as to the rapidity of the redissolution. In fact, this step is usually very slow. Therefore, it is not advisable to rely on redissolution as a means of avoiding precipitation.

Using the aforementioned technique, it is possible to estimate the precipitation potential of a drug on the basis of its solubility in water and in the cosolvents used. However, these estimates are based on equilibrium data and do not account for kinetic factors, such as crystal growth rate, fluid (or blood) flow rate, and injection rate. (The last two parameters determine the formulation dilution rate.) A more meaningful assessment of precipitation potential requires a dynamic system in which the above factors are controlled to the greatest extent possible.

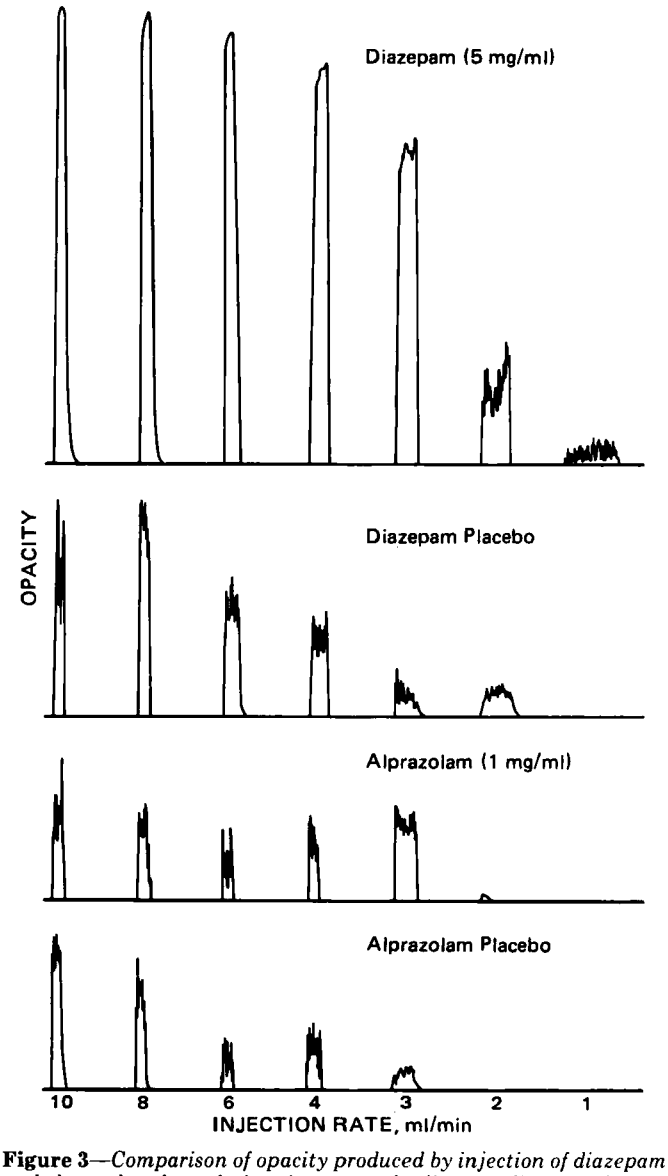

Figure 3—*Comparison of opacity produced by injection of diazepam and alprazolam formulations into normal saline at a distance of 15 cm from the flow cell.*

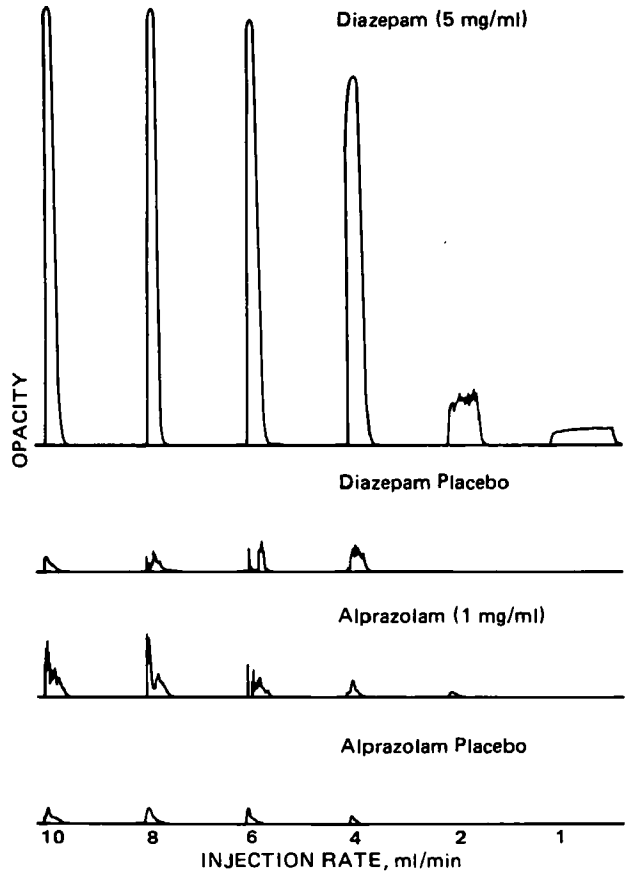

Figure 4—*Comparison of opacity produced by injection of diazepam and alprazolam formulations into normal saline at a distance of 30 cm from the flow cell.*

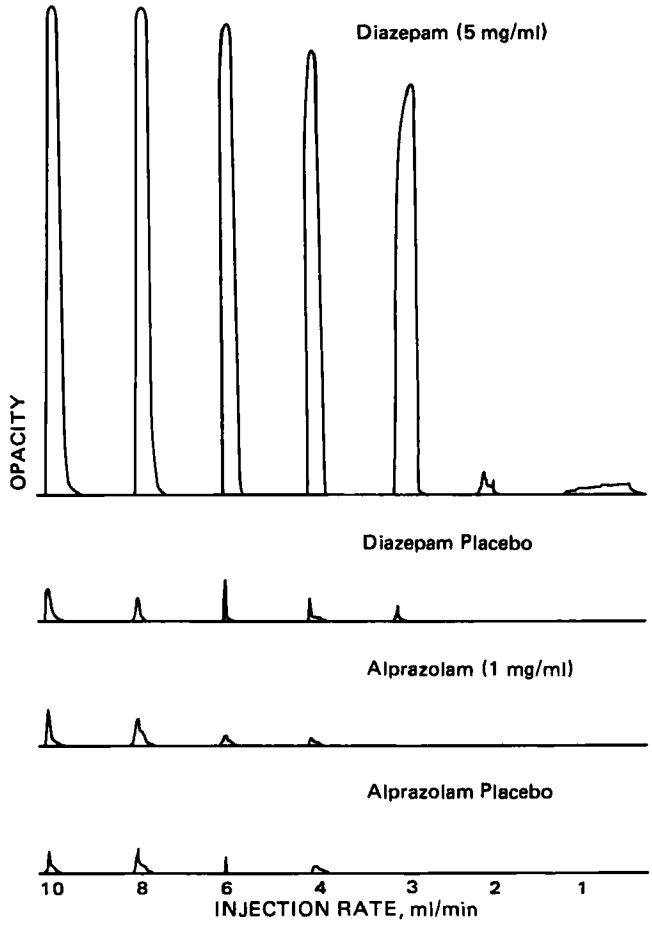

Figure 5—*Comparison of opacity produced by injection of diazepam and alprazolam formulations into normal saline at a distance of 45 cm from the flow cell.*

In this report a simple laboratory experiment is described that provides a semiquantitative evaluation of precipitation formation in a dynamic system. This is used to determine the effect of injection rate on precipitation and to compare parenteral formulations of the antianxiety drugs diazepam[1] and alprazolam[2]. Some of the important physicochemical properties of both drugs are presented in Table II.

This type of experiment is not intended to replace *in vivo* testing. It can, however, provide an early warning as to whether a precipitation problem should be anticipated.

EXPERIMENTAL

Methods—The apparatus used in this study is shown schematically in Fig. 2. It was developed to provide a reasonable simulation of the events that occur when a drug formulation solubilized with a cosolvent is injected into the venous system or into an intravenous drip tube.

A peristaltic pump provided flow of an aqueous phase at a rate of 30 ml/min through a 2.50-mm tube and a 2.0-mm quartz flow cell. The aqueous phases used were 0.9% sodium chloride (normal saline) in distilled water and 5.0% dextrose in deionized water (D5W). Drug formulation or placebo solution was injected into the tubing through a 20-gauge needle inserted 15, 30, or 45 cm upstream of the flow cell. A syringe pump[3] was used to control the rate of drug injection. The injection rate varied from 1.0 to 10 ml/min, but the total volume injected was kept constant at 1.0 ml. Injection rates of 10, 8, 6, 4, 3, 2, and 1 ml/min were attained by injecting 1 ml of solution in 6.0, 7.5, 10, 15, 20, 30, and 60 sec, respectively. The appearance of drug precipitate was detected by measuring the optical transmittance through the flow cell in a spectrophotometer. For both diazepam and alprazolam formulations, any absorption at 400 nm was attributed to the opacity caused by the passage of precipitate through the flow cell. Changes in absorbance were monitored on a strip-chart recorder (see *Results*). This study was conducted at room temperature (20–23°).

Materials—The sodium chloride, dextrose, propylene glycol, ethanol, sodium benzoate, benzoic acid, benzyl alcohol, and distilled water were USP quality and were obtained from commercial sources. Alprazolam injectable was prepared by dissolving the drug in propylene glycol and then adding the required quantity of water. Diazepam injectable[4] was purchased. The composition of the diazepam and alprazolam injectable formulations are given in Table III.

RESULTS

The recorder tracings generated by injecting diazepam and alprazolam formulations and their respective placebos into normal saline at distances of 15, 30, and 45 cm from the flow cell are shown in Figs. 3, 4, and 5, respectively. Those obtained by injecting the same formulations into D5W at a distance of 30 cm from the flow cell are shown in Fig. 6.

Replacing the following aqueous fluid with diazepam vehicle and injecting diazepam formulation produced no opacity under any of the above conditions. Likewise, injecting alprazolam into its own vehicle under the above conditions produced no opacity.

It is clear from Figs. 4 and 6 that there is no significant difference between the results obtained in normal saline at 30 cm and the results obtained in D5W at the same distance. Changing the tubing diameter had no significant effect on the opacity observed. Changing the rate of flow of the fluid into which the formulations were injected produced an effect similar to changing the distance from the injection site to the flow cell. The following trends are apparent in each of Figs. 3–6:

1. Short distances between the injection site and the flow cell tend to produce higher opacity readings.
2. The faster the injection rate, the greater the opacity.
3. The diazepam formulations show the greatest degree of opacity under all tested conditions.

[1] Valium; Roche, P.R.
[2] Xanax; The Upjohn Co., Kalamazoo, Mich.
[3] Sage.
[4] Valium Injectable; Roche, P.R.

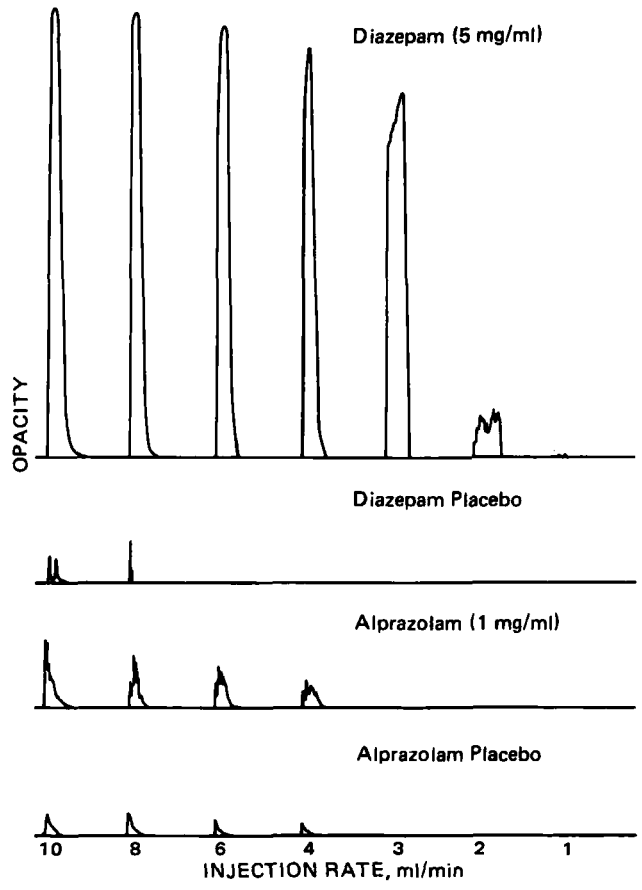

Figure 6—*Comparison of opacity produced by injection of diazepam and alprazolam formulations into 5% dextrose at a distance of 30 cm from the flow cell.*

DISCUSSION

Distance from Injection Site—In all cases, the placebo formulations produced little or no opacity when injected at distances of 30 and 45 cm from the flow cell. At 15 cm, however, the more rapid injection rates produced measurable changes in the transmission of light through the cell. Since the placebo formulations contained no material that is insoluble in the flowing aqueous fluid, the apparent opacity cannot be due to precipitation. The decrease in light transmission through the flow cell after rapid injection of placebo formulations at short distances is due to Schlieren patterns. These wavy patterns form when two liquids of different refractive indices are partially mixed; scattering light and, thus, reducing transmission. They are less prevalent at longer distances, which provide more time for the liquids to mix completely before passing through the flow cell. (This effect can be easily demonstrated by adding glycerin or propylene glycol to water. At first there are striations of Schlieren patterns in the mixture. As mixing progresses, these patterns disappear and the solution becomes clear.)

Injection Rate—Under all of the conditions considered, no opacity was produced when the formulations were injected at a rate <1.0 ml/min. At this slow rate of addition, the formulation is diluted very rapidly by the flowing aqueous phase. At more rapid injection rates, the formulation is diluted more slowly. The dilution ratio is equal to the rate of injection divided by the rate of flow of the aqueous fluid. If the formulation is injected at a rate of 1.0 ml/min and the fluid flowrate is 30 ml/min, the dilution ratio is equal to 30; if the injection rate is increased 10-fold, the dilution ratio is reduced 10-fold (to 3.0). When the dilution ratio is large and the dilution is rapid, there is little time for nucleation and crystal growth and, thus, less likelihood of precipitation. Rapid injection and the accompanying slow dilution provide more time for the formation and growth of crystal nuclei.

An additional explanation for the effect of injection rate on the amount of precipitate formation is as follows. The segment or plug of fluid containing the injected formulation tends to remain more or less intact as it flows through the tube to the flow cell: there is only poor lateral mixing in the narrow tube. The magnitude of precipitation in the plug of diluted formulation will depend on the degree of supersaturation in the plug. If the plug is highly supersaturated, the potential for precipitation is greater than if the drug concentration is only slightly above its solubility. Of course, if the concentration of drug in the plug is below the solubility limit, there is no potential for precipitation. This ideal situation is achieved when the drug is injected very slowly and the dilution ratio is very large. These results clearly show the advantage of slow intravenous administration of solubilized drugs.

Comparison of Diazepam and Alprazolam—It is obvious from Figs. 3–6 that diazepam has a high tendency to precipitate when injected, and that alprazolam shows little or no tendency to precipitate. This is not due to differences in pK_a (Table II) since both drugs are essentially completely un-ionized at physiological pH. The difference results from a combination of several factors: (*a*) the concentration of diazepam is much higher than that of alprazolam in the injection formulations, (*b*) diazepam is less soluble in water than alprazolam, and (*c*) the formulation of diazepam studied does not have excess cosolvent (as does the alprazolam formulation). This *in vitro* study indicates that formulation of a solubilized drug should be designed so that the solubility of the drug is not exceeded as it comes into contact with an aqueous environment. Based on the results of this study, excess cosolvent should be added to protect the drug against the solubility-reducing effect of the aqueous medium into which it is injected.

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