

A 5% Glucose Infusion Fluid Provokes Significant Precipitation of Phenytoin Sodium Injection *via* Interruption of the Cosolvent Effect of Propylene Glycol.

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The precipitation of phenytoin sodium injection provoked by mixing with infusion fluids renders its use in clinical practice difficult, as rapid intravenous (i.v.) push and i.v. infusion are supposed to be avoided. As some of its aspects remain unclear, this study tried to elucidate this precipitation mechanism. In particular, this study focused on the significant precipitation induced by glucose infusion fluid. The precipitation provoked by 5% glucose infusion fluid was obviously different from the precipitation that accompanied simple pH reduction, in terms of the growth mode and morphology of crystals. In addition, the effect of glucose was partially unrelated to pH reduction. NMR measurements including a two-dimensional nuclear Overhauser effect spectroscopy (2D-NOESY) spectrum indicated the specific interaction between glucose and propylene glycol, which is incorporated into phenytoin sodium injection as a solubilizing agent. These results led to the conclusion that this interaction was crucial for the precipitation of phenytoin, as it diminished the solubilizing effect of propylene glycol, resulting in the enhancement of the crystallization of phenytoin. The determination of phenytoin solubility in aqueous solutions at different pH values revealed that phenytoin incorporated in the admixture could be dissolved completely, as long as the injection was diluted with saline or water. These findings offer a profound insight into the formulation design of phenytoin sodium injection and its use in clinical practice.

Key words phenytoin; precipitation; glucose infusion fluid; propylene glycol; 2-dimensional nuclear Overhauser effect spectroscopy

Phenytoin sodium injection is indicated for the control of status epilepticus of the grand mal type and for the prevention and treatment of seizures that occur during neurosurgery. As phenytoin is a weak-acid drug ($pK_a=8.3$)¹⁾ with low solubility at biological pH values,^{2,3)} phenytoin sodium injection is prepared using an organic cosolvent consisting of 40% (v/v) propylene glycol and 10% (v/v) ethanol, with the pH adjusted to 12.

The intravenous (i.v.) administration of phenytoin sodium has long represented a dilemma. The rate of administration is strictly limited; the delivery of the injection is recommended at a rate not exceeding 50 mg/min,⁴⁾ as a rapid i.v. push has a risk of serious adverse effects on the cardiorespiratory system.^{5–7)} In contrast, the addition of phenytoin sodium injection to an i.v. infusion is not recommended because of its low solubility and resultant precipitation; this means that continuous infusion should be avoided.

Many reports in the literature describe precipitation occurring by mixing phenytoin sodium injection with various infusion fluids.^{8–15)} The reduction in pH that accompanies the dilution with the infusion fluids is widely recognized as the principal cause of the precipitation,^{16,17)} the pH of the admixture is reduced once the phenytoin sodium injection is diluted in an excessive amount of infusion fluids. Hence, the solubility threshold of phenytoin might be exceeded, resulting in its precipitation. However, it is also well recognized that the reduction in pH is not always the sole determinant of precipitation. The influence of various factors other than the final pH of the admixture has been investigated. These factors include dilution of the phenytoin solubilizing agent by infusion fluids,^{9,10,14)} particulate matter present in infusion fluids,⁹⁾

time delay from the dilution,^{14,18)} and solvent evaporation and absorption of carbon dioxide when the solution is exposed to air.^{11,19)} Despite the best endeavors to elucidate the precipitation of phenytoin, some of its aspects remain unclear.

The purpose of this study was to elucidate the mechanism responsible for the precipitation occurring after the dilution of phenytoin sodium injection with infusion fluids. This study focused on the precipitation induced by glucose infusion fluid. The 5% glucose infusion fluid (the same as the 5% dextrose infusion fluid) is a well-known infusion fluid that induces significant precipitation of phenytoin. To the best of our knowledge, all reports in the literature describe significant precipitation after the addition of a glucose infusion fluid to phenytoin sodium injection.^{8–11,14,15)} From investigation using a glucose infusion fluid admixture,⁸⁾ Pfeifle *et al.* insisted on the existence of crucial factors that are not associated with pH reduction. Regarding other fluids, such as saline and lactated Ringer's injection, their enhancement effect on the precipitation of phenytoin is doubtful: the majority of authors rejected their ability to induce precipitation,^{8,10,14,15,18)} whereas some authors reported opposite results.^{11,12)}

In this study, first we characterized the precipitation occurring after mixing phenytoin sodium injection with glucose infusion fluids. Subsequently, we investigated the intermolecular interactions of components in the admixture using NMR. In the course of the investigation, a specific interaction between glucose and propylene glycol (PG) was identified in the admixture, which was crucial for the precipitation of the phenytoin sodium injection admixture. We also assessed the solubility of phenytoin in aqueous solutions and obtained evidence that suggests that the phenytoin sodium injection admixture is

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stable as long as it is diluted using saline or water.

Experimental

Materials Phenytoin sodium injection (Aleviatin[®]) was purchased from Dainippon Sumitomo Pharma (Osaka, Japan). 5,5-Diphenylhydantoin sodium (phenytoin sodium) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Saline (Otsuka normal saline) and 5% glucose infusion fluid (Otsuka glucose injection 5%) were purchased from Otsuka Pharmaceutical (Tokyo, Japan). Deuterium oxide (D, 99.9%), D-glucose, acetonitrile, hydrochloric acid (HCl), and sodium hydroxide (NaOH) were purchased from Wako Pure Chemical Industries (Osaka, Japan). PG was purchased from Nacal Tesque, Inc. (Kyoto, Japan). All other reagents were of chemical grade.

Characterization of Precipitates Collected from Various Infusion Fluid Admixtures The phenytoin sodium for injection was mixed with infusion fluids and was then left at room temperature (*ca.* 21°C) for 12 h. The bottom fraction, enriched in precipitate, was withdrawn and observed using a polarizing microscope (CX41; Olympus, Tokyo, Japan). Quantification of phenytoin in the precipitate was performed using HPLC according to the protocol of Li *et al.*, with minor modifications.²⁰ The precipitates present in the admixtures were collected by centrifugation at 15400g using centrifugal filtration devices (Nanosep 3K Omega; Pall Life Sciences, Ann Arbor, MI, U.S.A.) and were then dissolved in the mobile phase. The sample solution was injected into a Shimadzu LC-20AT HPLC pump equipped with a C18 reverse-phase column (YMC-pack A-302 S-5 A, 150×4.6 mm i.d.; Yamamura Chemical Laboratories, Kyoto, Japan). A Shimadzu SPA-20A UV/VIS detector was set at 254 nm. An acetonitrile/0.1% phosphoric acid solution in water (50:50, v/v) was used as the mobile phase; the flow rate was 1.0 mL/min, and HPLC analysis was performed at room temperature. LC Solution version 1.24 SP1 (Shimadzu, Kyoto, Japan) was used as the acquisition and analysis software. The pH of the admixtures was measured using a pH meter (Horiba Navi F52; Horiba, Kyoto, Japan) at room temperature (21.6°C).

We also acquired the powder X-ray diffraction (XRD) spectra of the dried precipitates. XRD spectra were recorded on a RINT-1400 X-ray diffractometer (XRD; Rigaku Co., Ltd., Tokyo, Japan). These measurements were carried out at 40 kV, 40 mA with a CuK α source between 5° and 35° 2 θ with a scan speed of 2.0°/min in the step scan mode.

NMR Study All NMR measurements were performed using a JEOL JNM-LA500 spectrometer (¹H at 500 MHz, 11.7 T) at 30°C and ¹H chemical shifts were referenced to those of tetramethylsilane, which was used as an external standard ($\delta=0$ ppm). For the analysis, phenytoin sodium powder and precipitates collected from the 5% glucose infusion admixture were dissolved in a 1 M NaOH deuterium oxide solution. In addition, we analyzed a phenytoin sodium injection diluted with deuterium oxide or the supernatant withdrawn from a 5% glucose/deuterium oxide admixture.

Determination of the Solubility of Phenytoin as a Function of pH To prepare saturated aqueous solutions of phenytoin at different pH values, an excess amount of phenytoin sodium was suspended in purified water, and the pH was then adjusted by addition of HCl or NaOH aqueous solution. After measurement of the pH value, the undissolved phenytoin sodium was immediately removed using a centrifugal filtration device. The saturated solution was mixed with an appropriate amount of mobile phase, and the phenytoin was then quantified using HPLC. The analytical conditions used to perform HPLC were the same as those mentioned above. The solubility of phenytoin in a 10% (v/v) PG aqueous solution was also determined. The saturated aqueous solutions of phenytoin at different pH values were prepared in a similar fashion. Subsequently, 5.4 mL of each supernatant was mixed with 0.6 mL of PG. After further addition of phenytoin sodium to the solutions, the final pH and the solubility of phenytoin were measured.

Results

Characterization of the Precipitation Modes Induced by Various Infusion Fluids First, we observed the features of the phenytoin sodium injection admixtures. For sample prepa-

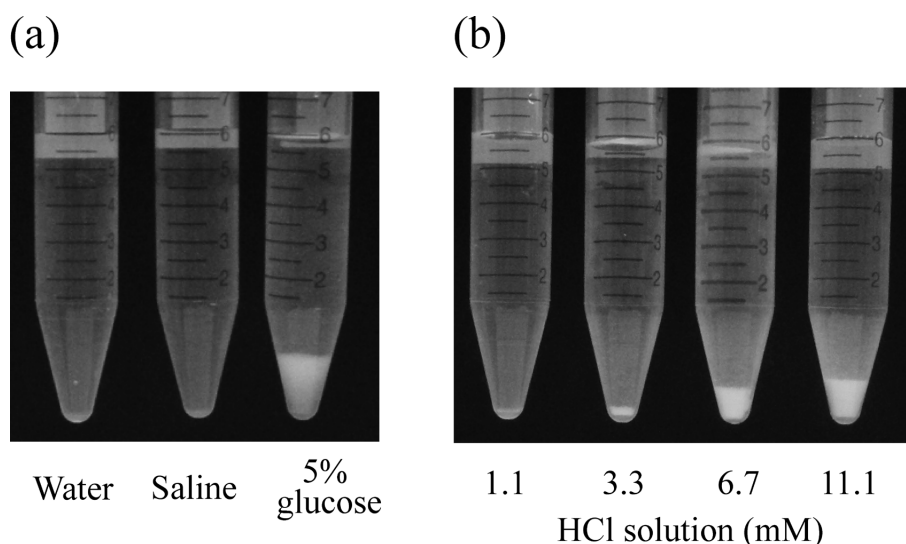


Fig. 1. Visual Aspects of Phenytoin Sodium Injection Admixtures

(a) Purified water, saline, and 5% glucose infusion fluid; (b) HCl aqueous solutions with different concentrations (1.1–11.1 mM). The dilution ratio of admixtures was 4× (1:3 ratio of injectable phenytoin to dilution fluids).

ration, phenytoin sodium injection was diluted with infusion fluids (fourfold dilution) and left at room temperature for one night. A significant amount of precipitate was observed in the 5% glucose infusion fluid admixture, whereas no precipitation was observed in the saline and water admixtures (Fig. 1a). The pH of the 5% glucose infusion fluid admixture (10.68) was much lower than that of the water and saline admixtures (11.2, 11.2, respectively; Table 1).

As mentioned in the Introduction, as a reduction in pH is recognized as a major reason for the precipitation, we also assessed the phenytoin sodium injection solution after mixing with aqueous HCl. In this study, the precipitation observed in HCl aqueous solution admixtures was regarded as being caused by simple pH reduction. Precipitation occurred above a concentration of 3.3 mM HCl (Fig. 1b), at a pH value of *ca.* 10.7 (Table 1). The amount of precipitate increased proportionately with HCl concentration. We noted that the precipitation mode in the 5% glucose infusion fluid admixture appeared quite different from that observed in the HCl aqueous solution admixtures, despite the similar pH values. For example, after mixing with the HCl aqueous solution, the phenytoin sodium injection admixture became cloudy immediately, indicating

that the precipitate formed within a short time. In contrast, the 5% glucose infusion fluid clouded the admixture gradually (at least 30 min). The texture of the precipitates was also different. Precipitates in the 5% glucose infusion fluid admixture appeared more bulky than those observed in the HCl aqueous solution admixtures (Fig. 1). Microscopic observation revealed that the crystals formed in the 5% glucose infusion fluid admixture were longer and thicker than those formed in the HCl aqueous solution admixtures: over 300 μm in the 5% glucose infusion fluid and 100–200 μm in the HCl aqueous solution (Fig. 2).

We acquired an NMR spectrum of the precipitate collected from the 5% glucose infusion fluid admixture. As shown in Fig. 3, the spectral pattern of the precipitates agreed well with that of pure phenytoin, and no peaks derived from other components (*e.g.*, glucose, PG, or ethanol) were observed; thus, we proved that the precipitate was composed mostly of phenytoin. Moreover, we compared the XRD spectrum of the precipitate collected from the glucose infusion fluid admixture with that from HCl solution admixture to confirm whether the crystal form was the same. As shown in Fig. 4, peaks in the XRD spectra agreed well with each other, indicating the same crystal form in the situations.

Changes in precipitate amounts as a function of the glucose concentration were investigated. We prepared glucose solutions with concentrations ranging from 1.0 to 10.0% and used them to dilute the phenytoin sodium injection. The amount of precipitate collected increased proportionally to the concentration of glucose (Fig. 5). The longer and thicker crystals looked similar to those shown in Fig. 2a, and they were different from those formed in the HCl aqueous solution admixtures (data not shown). It is worth noting that the pH of the admixtures was a little higher than that at which the precipitation began to occur when mixing with the HCl aqueous solution; admixtures with 1.0, 2.5, 5.0, and 10.0% glucose solutions exhibited pH values of 10.92, 11.02, 10.98, and 10.93, respectively (Table 1). This suggests that the mechanism responsible for the precipitation induced by mixing with glucose infusion fluid is partially unrelated to changes in pH.

Intermolecular Interactions between Glucose and PG in the Admixture, as Elucidated Using NMR The NMR spectra of phenytoin sodium injection admixtures are shown in Fig. 6. Peaks corresponding to PG incorporated in the

Table 1. pH Values of Phenytoin Sodium Injection with Various Fluids

Dilution fluids ^{a)}	pH
5% glucose infusion fluid (Otsuka glucose injection 5%)	10.68
Saline	11.08
Water	11.00
HCl aqueous solution	
(1.1 mM)	10.76
(3.3 mM)	10.70
(6.7 mM)	10.64
(11.1 mM)	10.61
Glucose aqueous solution ^{b)}	
(1.0%)	10.92
(2.5%)	11.02
(5.0%)	10.98
(10.0%)	10.93

a) Phenytoin sodium injection was mixed with infusion fluids at a dilution ratio of 1:3 (injectable phenytoin to glucose aqueous solutions). *b)* Glucose solutions with different concentrations were prepared by dissolving the designated amount of glucose in purified water.

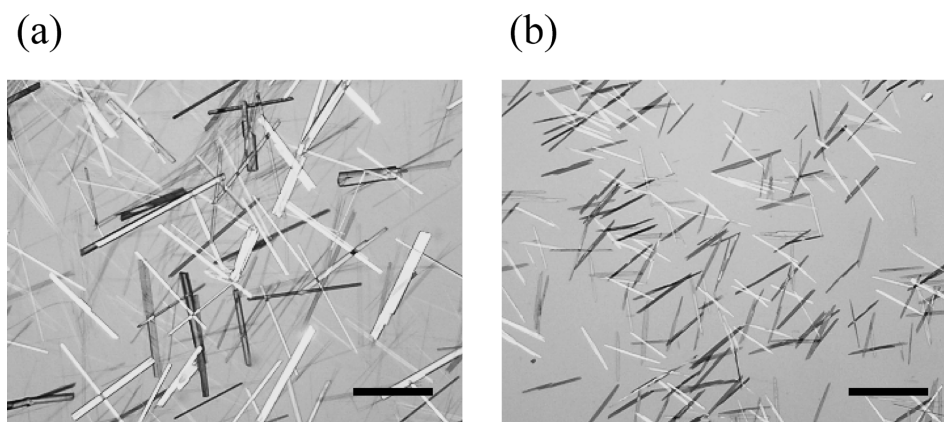


Fig. 2. Micrographs of Phenytoin Crystals from (a) a 5% Glucose Infusion Fluid Admixture and (b) an HCl Aqueous Solution Admixture. The dilution ratio of admixtures was 4 \times (1:3 ratio of injectable phenytoin to dilution fluids). Each scale bar represents 200 μm .

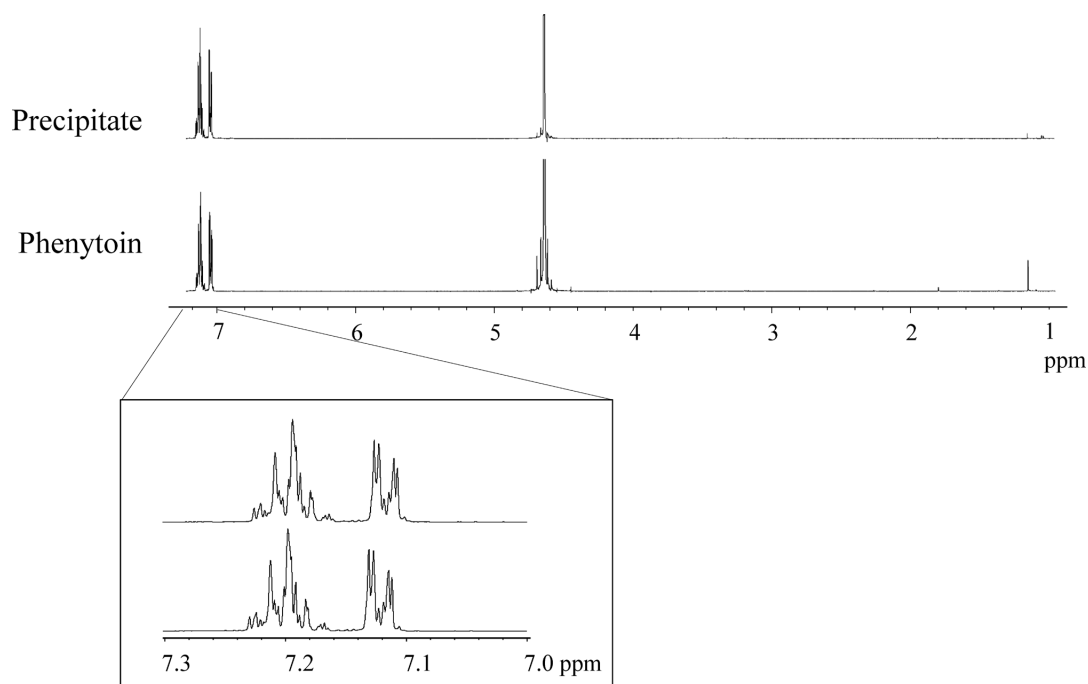


Fig. 3. $^1\text{H-NMR}$ Spectra of the Precipitate from a 5% Glucose Infusion Fluid Admixture

The precipitate that was separated was dissolved in 1 M NaOH deuterium oxide solution. Phenytoin sodium powder was also dissolved in the solution.

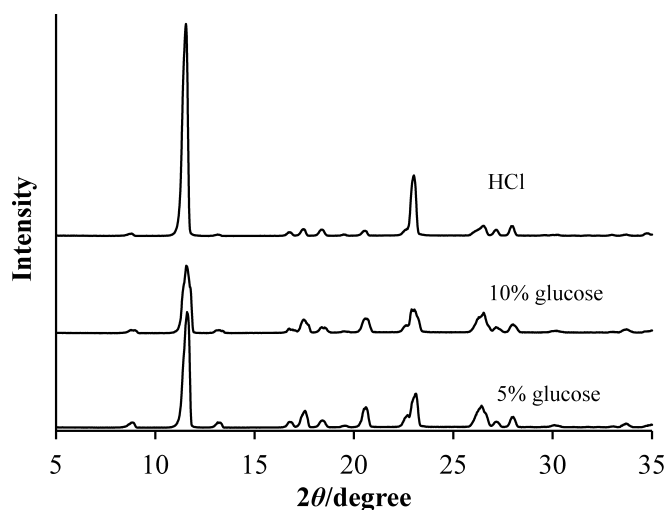


Fig. 4. XRD Spectra of Phenytoin Crystals Collected from Glucose Infusion Fluid and HCl Aqueous Solution Admixtures

The phenytoin sodium injection was mixed with 5% and 10% aqueous glucose solutions and HCl aqueous solution (11.1 mM) at a dilution ratio of 1:3 (injectable phenytoin to aqueous glucose solutions). Five percent aqueous glucose solution is the commercial product (Otsuka glucose injection 5%), and 10% was prepared by us. The XRD spectra were acquired after drying the collected crystals.

phenytoin sodium injection (3.2–3.8, 1 ppm) were shifted to a higher magnetic field by changing the dilution fluid from deuterium oxide to 5% glucose/deuterium oxide (Fig. 6). Similarly, peaks of glucose (around 5.1, 4.5 ppm) were shifted to a higher magnetic field by mixing with phenytoin sodium injection (data not shown), suggesting that glucose affected PG in the admixture.

For further investigation, we acquired a two-dimensional nuclear Overhauser effect spectroscopy (2D-NOESY) spectrum of the phenytoin sodium injection solution after dilution with 5% glucose/deuterium oxide. Cross peaks of 2D-NOESY represent a spatial relation among a set of hydrogen nuclei.

Key cross peaks representing the close proximity of the hydrogen of α - and β -glucose to those of PG were observed (Fig. 7). This indicates that glucose interacted with PG in the admixture.

Solubility of Phenytoin in Aqueous Solutions at a Function of pH We determined the pH–solubility curves of phenytoin in aqueous solutions. For preparing saturated solutions of phenytoin, an excess amount of phenytoin sodium powder (not phenytoin sodium injection) was suspended in water and in 10% (v/v) PG aqueous solution, and the pH values were modified by addition of appropriate volumes of HCl or NaOH aqueous solutions. The 10% (v/v)

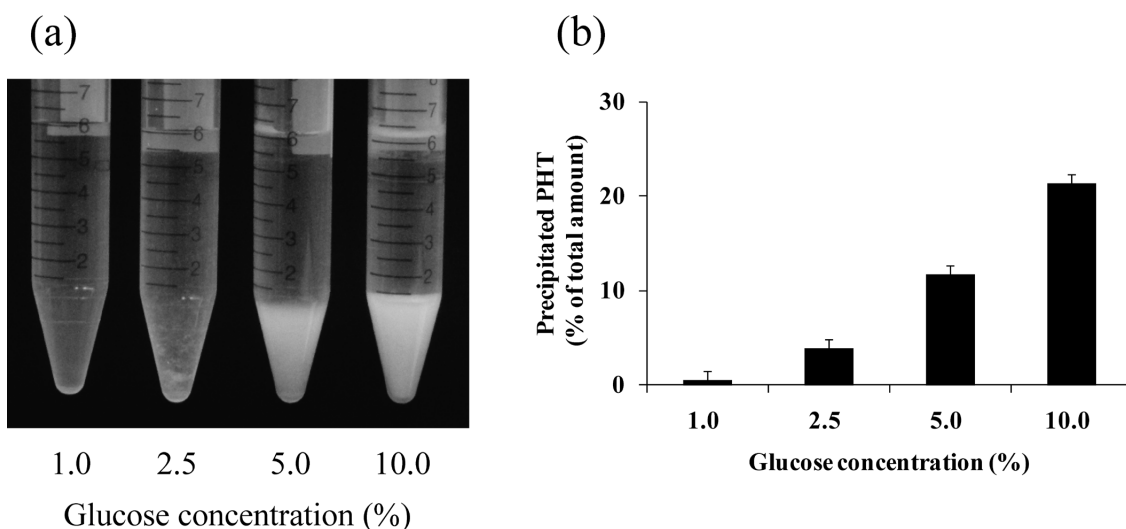


Fig. 5. Effect of Glucose Concentration in Dilution Media on the Precipitation of Phenytoin

(a) Visual aspects of glucose aqueous solution admixtures. Phenytoin sodium injection solution was mixed with glucose aqueous solutions prepared to different concentrations, ranging from 1.0 to 10.0% at a dilution ratio of 1:3 (injectable phenytoin to glucose aqueous solutions), and then the admixtures were left at room temperature for one night. (b) The amount of phenytoin precipitated in the admixtures as a function of glucose concentration. Each value represents the mean \pm S.D. ($n=3$).

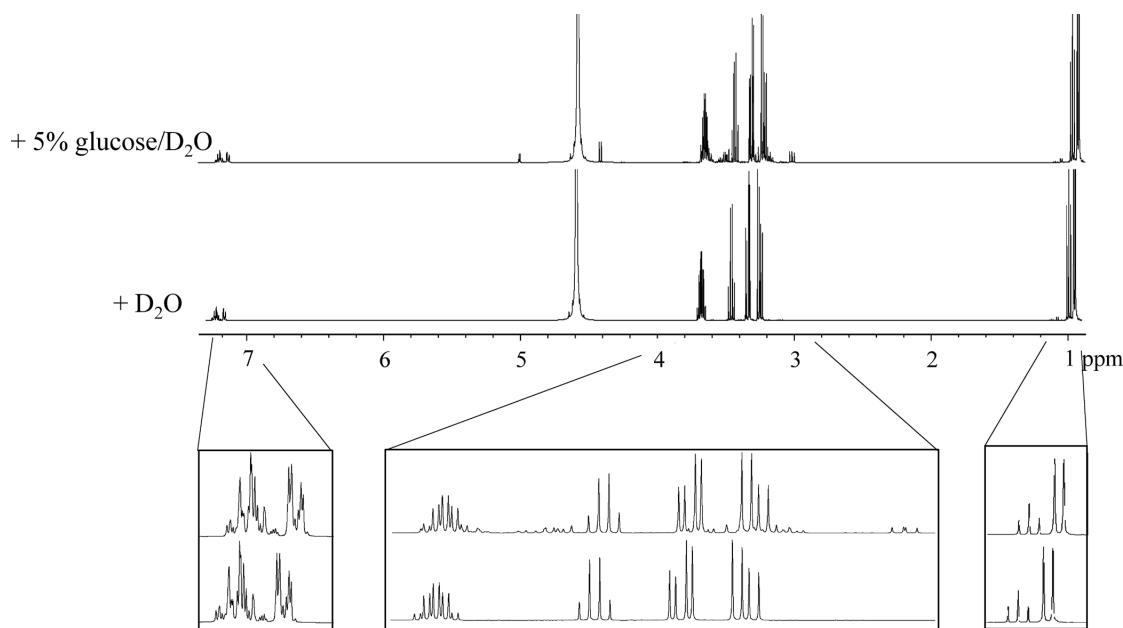


Fig. 6. ¹H-NMR Spectra of Phenytoin Sodium Injection Diluted with 5% Glucose/Deuterium Oxide or Deuterium Oxide

of PG was the same as that present in the admixtures at a fourfold dilution. As a result of the experiment, pH-solubility curves fitting with good correlation coefficients were obtained at pH values ranging from 9.5 to 11.1: $\ln(y)=2.267x-22.025$ ($r^2=0.997$) for water and $\ln(y)=2.285x-21.883$ ($r^2=0.998$) for the 10% (v/v) PG aqueous solution (Fig. 8a). The area under the pH-solubility curves represents the condition in which phenytoin can be dissolved completely. The pH-solubility curve was shifted upward in parallel by the addition of PG, indicating a solubilizing effect of PG. We also assessed the solubility of phenytoin in glucose infusion fluid at different pH values. The results were concordant with the pH-solubility curve obtained in water (Fig. 8b); thus, glucose alone had no influence on the solubility of phenytoin in aqueous solutions.

We diluted the phenytoin sodium injection solution with various infusion fluids at different dilution rates, and then measured the pH of the admixtures and plotted these values over the pH-solubility curves (Fig. 9). The dilution ratio of the 5% glucose infusion fluid admixture was fixed at 4 \times , whereas those of the saline and water admixtures were changed from 4 \times to 50 \times . As the injection solution contained 46.0 mg/mL of phenytoin, the concentrations of phenytoin in these solutions became 11.5, 4.60, 2.30, and 0.92 mg/mL after 4 \times , 10 \times , 20 \times , and 50 \times dilutions, respectively. As shown in Fig. 9, the pH values of the admixtures decreased with the elevation of the ratio of dilution using saline and water. We noted that all points corresponding to the phenytoin sodium injection solution diluted with saline and water lay below the pH-solubility

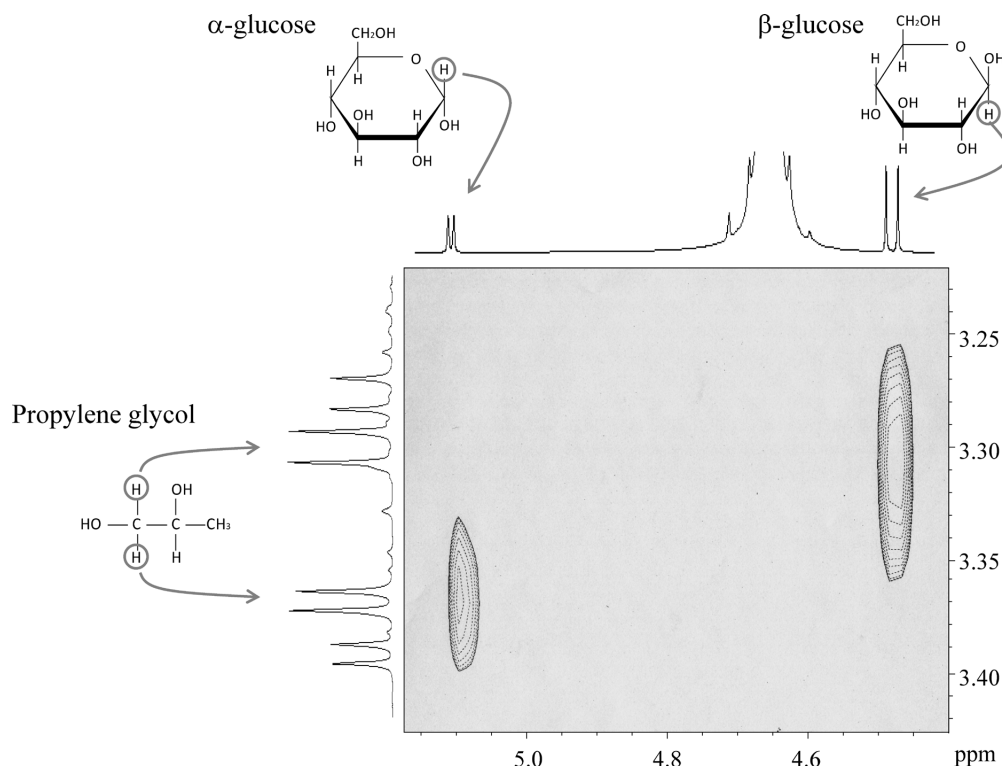


Fig. 7. 2D-NOESY Spectrum of Phenytoin Sodium Injection Diluted with 5% Glucose/Deuterium Oxide

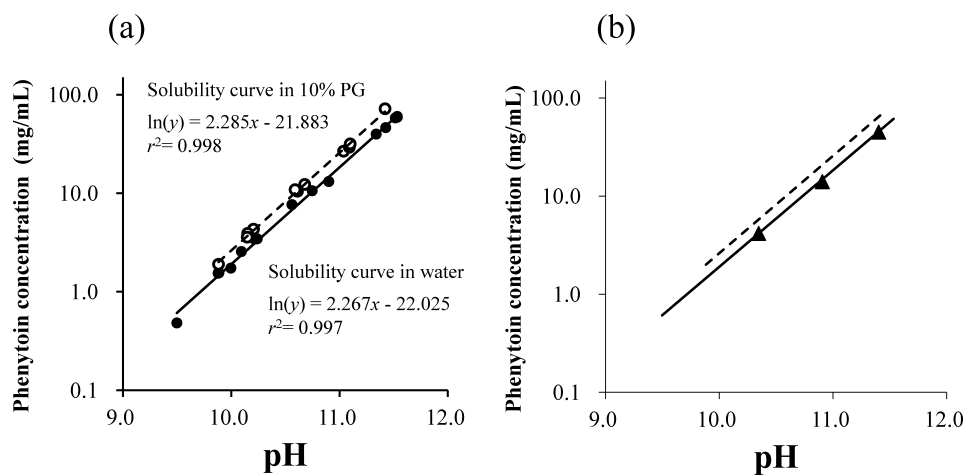


Fig. 8. (a) Solubility of Phenytoin in (●) Water and (○) 10% (v/v) PG Aqueous Solution at Different pH Values

Saturated aqueous solutions of phenytoin at different pH values were prepared by suspending an excess amount of phenytoin sodium powder (not phenytoin sodium injection solution), and their pH and the solubility of phenytoin were measured at 21.6°C. The solid and dotted lines in (a) and (b) represent the pH–solubility curves of phenytoin in water and in 10% (v/v) PG aqueous solution, respectively. (b) Solubility of phenytoin in (▲) 5% glucose infusion fluids.

curve in water. This indicated that the phenytoin incorporated was dissolved completely in the admixture, even if PG exerted no solubilizing effect. The point corresponding to the 5% glucose infusion admixture was below the pH–solubility curve in 10% (v/v) PG aqueous solution, whereas it was above the curve in water; thus, as long as the solubilizing effect of PG is working fully, the phenytoin incorporated can be dissolved in the admixture completely.

Discussion

Our findings clarified that the precipitation of phenytoin provoked by the 5% glucose infusion fluid is different from

that caused by simple pH reduction. A comparison of the HCl aqueous solution admixtures revealed that precipitation in the 5% glucose infusion fluid admixture occurred more slowly and the resultant crystals were longer and thicker (Fig. 2). The NMR analysis revealed that the precipitate was a crystal of phenytoin (Fig. 3). It is well known that the crystalline habit of phenytoin is significantly influenced by the physical conditions of formation and growth rate.²¹⁾ However, to the best of our knowledge, there is no report that phenytoin has polymorphic crystals. In accordance with this, the XRD spectral pattern of the precipitate collected from the glucose infusion fluid admixture was the same as that from the HCl solution

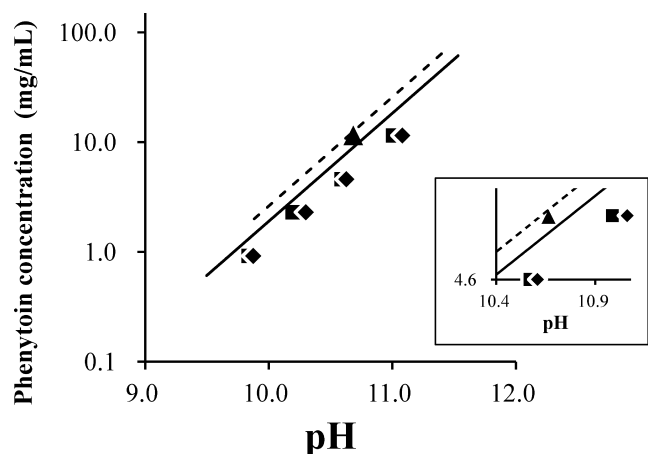


Fig. 9. Change in pH Values of the Phenytoin Sodium Injection after Dilution with (▲) 5% Glucose Infusion Fluid, (◆) Saline, and (■) Purified Water

The concentration of phenytoin in the injection after 4 \times , 10 \times , 20 \times , and 50 \times dilution was 11.5, 4.60, 2.30, and 0.92 mg/mL, respectively. The solid and the dotted lines represent the pH-solubility curves of phenytoin in water and 10% (v/v) PG aqueous solution shown in Fig. 8a.

admixture, suggesting that they only differ in terms of crystal habit (Fig. 4). The change in crystal habit was probably because of gentle crystallization caused by mixing with glucose infusion fluid. This gentle crystallization is thought to retard the termination of crystal growth,²¹⁾ resulting in the formation of longer and thicker crystals. The amount of crystals was affected substantially by glucose concentration. In addition, it appears that some part of the precipitation was not associated with pH reduction.

To investigate the intermolecular interactions between the components present in the admixture, we acquired ¹H-NMR and 2D-NOESY spectra of the phenytoin sodium injection admixtures (Figs. 6, 7). The results of the NMR study suggested that glucose interacted with PG in the admixture. As PG was incorporated into the injection as a solubilizing agent, we speculate that this interaction is critical for the significant crystallization of phenytoin provoked by the glucose infusion fluid. One mechanism that could explain this phenomenon is as follows: once phenytoin sodium injection is mixed with the glucose infusion fluid, the mobility of PG is restricted by an interaction with glucose, resulting in interruption of its solubilizing effect. Based on our findings, this interaction should develop gradually and be unrelated to changes in pH.

This study determined pH-solubility curves of phenytoin. As well as water, we employed 10% (v/v) PG aqueous solution as a medium. That is because the phenytoin sodium injection solution contains a large amount of PG as a solubilizing agent; the 10% (v/v) of PG was the same concentration as that present in the admixtures at a fourfold dilution. As shown in Fig. 8, pH-solubility curves with highly significant correlation coefficients were calculated. Dill *et al.* determined that phenytoin is soluble in water at 25°C to the extent of 14 μ g/mL over a pH range of 1–7, an extent of 165 μ g/mL at pH 9.1 (in borate buffer), and an extent of 1.52 mg/mL at pH 10.0 (in sodium hydroxide).²⁾ Our pH-solubility curve analysis led to the calculation of a solubility of 1.90 mg/mL in water at pH 10.0; this similar value obtained indicates the validity of this experimental approach.

Using pH-solubility curves, we examined whether phenytoin incorporated in the injectable product (Aleviatin[®]) could be dissolved completely after dilution with infusion fluids. It is worth noting that the data point of the phenytoin sodium injection diluted fourfold with 5% glucose infusion fluid was located between the pH-solubility curves (Fig. 9). This means that in a 5% glucose infusion admixture, the solubilizing effect of PG is necessary for dissolving the whole amount of phenytoin. In reality, a significant amount of precipitate was observed in the admixture (Fig. 1), indicating that a considerable proportion of PG appears not to work as a solubilizing agent. For further information, glucose in itself did not affect the solubility of phenytoin, because the solubility of phenytoin in the 5% glucose infusion fluid agreed well with that observed in water (Fig. 8b). These results strongly support the mechanism mentioned above, in which glucose infusion fluid contributes indirectly to the precipitation of phenytoin sodium injection *via* interference with the cosolvent effect of PG.

In contrast with the 5% glucose infusion fluid admixture, all data points of the saline and water admixtures lay below the pH-solubility curve in water (Fig. 9). This means that the whole amount of phenytoin incorporated was dissolved in the test admixtures completely, even if the solubilizing effect of PG was abolished. Accordingly, no precipitation was observed for at least 24 h in any of the test admixtures stored in sealed tubes (data not shown). In a preliminary experiment, we investigated further a saline admixture at a dilution ratio of 100 \times . Although the exact pH value was not determined, because of its unstable pH, no precipitation was observed for at least 24 h in this admixture (data not shown). Many authors stated that phenytoin sodium injection in saline, but not in glucose infusion fluid, is stable for hours;^{8,10,14,15,18)} our results agree well with this contention.

These findings advocate strongly that phenytoin sodium injection diluted with saline and water is a stable solution, and is probably applicable in clinical practice settings without precipitation problems. However, before drawing this conclusion, we have to address the fact that some authors reported precipitation in saline and lactated Ringer's injection admixtures.^{11,12)} According to the literature, these experiments were conducted under open-system conditions; thus, the precipitation was thought to be caused by the evaporation of solvent or reduction of pH because of absorption of carbon dioxide from the air. In a preliminary experiment, we confirmed that a similar precipitation occurred in those conditions. Namely, we monitored a droplet of the saline admixture by placing it on a glass slide (open system) and observed significant crystallization occurring within 2 h after the mounting (data not shown). Therefore, we should keep in mind that precipitation may occur in any admixture. However, we think that the precipitation that occurred under open-system conditions is not a serious concern for clinical practice, as the preparation administered *via* i.v. infusion is hardly exposed to air; thus, the surroundings are thought to be close to those present in sealed tubes, rather than those of the open system. We applied the saline and water admixtures to the i.v. infusion device and flowed these fluids, and confirmed that no precipitation was observed in the line and infusion bag throughout the experiment (data not shown). Thus, we believe that phenytoin sodium injection can be administered together with infusion fluids *via* i.v. infusion, as long as saline or water is used for the dilution.

In addition to its small contribution as solubilizing agent, PG carries a risk of severe adverse effects on the cardiorespiratory system and potentially causes fatalities.^{7,14)} Excessive i.v. administration of PG is known to be hazardous. Louis *et al.* reported that transient hypotension, a brief period of apnea, and bradycardia were observed in epileptic model cats infused rapidly with PG at a dose of 0.5 to 1 mL/kg.⁷⁾ These authors also noted that similar cardiorespiratory events occurred after the rapid infusion of phenytoin sodium injection (10 to 25 mg/kg).⁷⁾ Because many of these events were prevented by phenytoin, they mentioned that the adverse effects of a rapid i.v. infusion of phenytoin sodium injection were mostly produced by PG. PG is also associated with a risk of inflammation of the walls of veins caused by phenytoin crystals (*i.e.*, phlebitis).²²⁾ Once phenytoin sodium injection is administered into the bloodstream, PG interacts preferentially with blood glucose rather than phenytoin, leading to the formation of phenytoin crystals. Therefore, in our opinion, the PG cosolvent system of the phenytoin sodium injection needs to be reviewed.

Conclusions

We found that glucose interacts with PG in phenytoin sodium injection admixtures. This was the main mechanism involved in the significant precipitation provoked by the glucose infusion fluid. This study is the first technical report of the indirect involvement of glucose in this type of precipitation. We also demonstrated that phenytoin sodium injection diluted with saline and water is a stable solution; thus, it can probably be applied to clinical practice without concerns regarding precipitation problems. This study provides a profound insight into the formulation design of phenytoin sodium injection and its use in clinical practice.

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