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Stability of Propofol in Polystyrene-Based Tissue Culture Plates

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Abstract

Propofol has been reported to have high stability in glass and relatively high stability up to 24 hours in polyvinyl chloride-based medical plastics. Recent publications have observed the effects of propofol on cells and tissues grown in culture. Many cell culture plastics are formulated from polystyrene but we could find little information on the stability of propofol exposed to these products. We observed very little change in the concentration of propofol diluted in cell culture medium over 24 hours when exposed to glass, but substantial loss of the drug when exposed to 96-well polystyrene cell culture plates. This decrease was most rapid in the first hour but continued until 24 hours. The type of plastic used in cell and tissue culture experiments with propofol may influence the results by increasing the apparent dose required to see an effect.

Introduction

Propofol is highly lipophilic and is supplied for clinical use in a lipid emulsion to facilitate its delivery in aqueous solutions. Lipophilic drugs interact with certain plastics. Previous studies of propofol demonstrated less than 10% loss of the drug when stored in polyvinyl chloride (PVC)-based plastics for 24 hours and little or no loss when stored in polypropylene-based plastics or glass.[1–3] In the clinical setting, most storage occurs in containers manufactured from glass or polypropylene. However, many laboratory-based studies are performed using polystyrene plastics for cell and tissue culture.

There are little published data on the potential binding of propofol to polystyrene. We therefore conducted a study to assess the stability of propofol diluted in cell culture medium and exposed to 96-well polystyrene laboratory dishes, and conical tubes for up to 24 hours.

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Reprints will not be available from the authors.

DISCLOSURES:
Name: Jeffrey W. Sall, MD, PhD
Contribution: Study design, conduct of the study, data collection, data analysis, and manuscript preparation.
Attestation: Jeffrey Sall approved the final manuscript and attests to having reviewed the original study data and data analysis, the integrity of the original data and the analysis reported in this manuscript. Dr. Sall is the archival author who is responsible for maintaining the study records.
Name: Jason Leong, (No degrees held)
Contribution: Data collection
Attestation: Jason Leong approved the final manuscript and the data that was collected.
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To measure propofol concentrations we extracted medium with hexane and measured the absorbance at 273nm spectrophotometrically.

Methods

Summary

Each day that data were collected a new 10mM propofol stock solution was made by diluting pure propofol in dimethyl sulfoxide (DMSO) to facilitate further dilution into cell culture medium. Propofol was also directly diluted in pure hexane and stored in glass or PVC tubes for analysis of absorbance and preparation of a standard curve. Freshly diluted propofol was used to prepare a new standard curve each day an analysis was performed.

Materials

Pure propofol was purchased from Aldrich Chemical Company (Milwaukee, WI) and stored in a darkened glass bottle at room temperature. DMSO was purchased from the University of California, San Francisco cell culture facility. Hexanes were manufactured by Acros Organics (Distributed by VWR, New Jersey, USA). Cell culture medium (proliferation medium) has been described by Sall et al.[4] Falcon 96-well polystyrene U-bottom tissue culture plates were manufactured by Becton-Dickinson, Franklin Lakes, NJ. All other plastics (microfuge tubes and pipette tips) were polypropylene-based and procured from VWR.

UV spectrophotometry

The propofol concentration assay was performed by measuring absorbance at 273nm using a Smart Spec 3000 spectrophotometer and quartz cuvettes (BioRad). Data were collected by printing directly from the spectrophotometer or by export to a MacBook Pro computer using a keyspan serial to USB device (Keyspan USA product # USA-19QW). Preliminary experiments were performed comparing the absorbance pattern of propofol in ddH_2O, phosphate buffered saline and hexane. The absorbance pattern of DMSO in hexane was also measured. On each day that data were collected a standard curve was run by diluting propofol in hexane over a wide range of concentrations in duplicate or triplicate at each point. A linear regression was performed and unknowns were determined based on the result.

Propofol Extraction into hexane

Medium was collected and combined in a ratio of four parts medium to one part hexane before briefly vortexing. The mixture was then centrifuged at 4500 × gravity for 15 minutes to improve phase separation. The hexane phase was then collected for spectrophotometric analysis as described in section 2.2.

Statistics and Graphs

All statistical analysis, linear regression, determination of unknowns were performed and all graphs generated using Prism 6 (GraphPad software, LaJolla, CA). Specific statistical tests are reported when they were used. All values are reported as mean ± standard deviation.

Results

Wavelength Scan and Standard Curve

Spectrum wavelength scans were performed on propofol diluted in hexane. There is a single peak in the lower wavelengths centered around 273nm that increases with increasing concentrations of propofol as reported in previous studies.[5,6] This peak was similar whether

propofol was diluted in ddH_2O, phosphate buffered saline or in hexane. Hexane alone had no absorbance at this wavelength. Propofol extracted into hexane from proliferation medium showed the expected peak at 273nm. Medium that contained DMSO but no propofol has an increased absorbance at lower wavelengths and this baseline value was subtracted from the value obtained for propofol containing medium at 273nm (Fig. 1).

A wide range standard curve was performed with propofol diluted in hexane at concentrations from zero to 2.4 mM (Fig. 2A). The change in absorbance is linear up to about 800μM. Above this value it begins to flatten. A second order polynomial curve fits these data (r^2 = 0.992). The standard curve used for all other studies presented here was generally performed from zero to 500μM and a linear regression used to calculate all unknowns. A new standard curve was performed each day of testing by diluting pure propofol in hexane at the concentrations shown (Fig. 2B). Linear regression of this example curve revealed slope = 0.00193 and r^2 = 0.991.

Extraction efficiency

Proliferation medium containing propofol was extracted with hexane in order to concentrate the propofol for measurement of absorbance. Extraction with 9 parts media to 1 part hexane yielded very high recovery rates of propofol in the hexane phase (>95%). However, the small volume of hexane meant that it frequently became contaminated with the aqueous phase when attempting to recover the hexane off the top. All subsequent extractions were performed with 4 parts medium to 1 part hexane and a recovery of 75.8±6.9% was observed.

3.3 Time-based recovery

Medium containing 50μM propofol was made fresh each day then placed in 96-well U-bottom polystyrene plates at 37°C in 5% CO_2 for the time indicated. Starting times were staggered and all plates were removed at the same time and medium extracted simultaneously for all time points. All values for a given day were made relative to the extraction of freshly made medium that was extracted immediately after preparation. Over time the amount of propofol remaining in the medium declined with a two phase exponential decay pattern (R^2 = 0.937; $Y = Span1 \cdot e^{-K_1 \cdot X} + Span2 \cdot e^{-K_2 \cdot X}$ + Plateau, where Span1= 86.04, K_1=0.02973, Span2=14.37, K_2=2.54, Plateau=~$1.371e^{-016}$; Fig. 3A). Medium extracted after one hour incubation time contained 80.3±8.6% of the original and after 24 hours 41.6±5.0% of the original medium. One-way ANOVA n=8, F = 131.2, P<0.0001, Dunnet's post test versus control for 0.5hr P<0.05, 1.0hr P<0.0001, 6.0hr P<0.0001, 24hr P<0.0001.

3.4 Recovery from glass vs polystyrene

We also compared the amount of propofol lost from the medium in polystyrene plates to the amount lost from medium stored at the same temperature in a glass vial (Fig. 3B). Medium kept in the glass vial at 37°C for 24 hours contained 93.2± 3.6% of the original amount of propofol compared to 45.3± 3.9% (student's t, n=4, P<0.0001) when kept in a polystyrene plate.

Discussion

This study demonstrates that propofol in cell culture media has poor stability over time when it is exposed to polystyrene-based plastics. Propofol had much greater stability in cell culture medium even at 37°C for 24 hours when it was kept in glass. The decrease in the concentration of propofol showed a two phase exponential decay with a rapid decrease over the first hour and slower but persistent decrease up to at least 24 hours. For longer propofol exposures one could use the area under the curve to determine the average exposure concentration.

We do not believe this is a clinical concern. No polystyrene-based syringes were identified in our hospital and a brief online review of several companies found no examples for sale for health care use. However, polystyrene plastics are commonly used in laboratory pipettes and for cell culture disposables and are likely to be used for studies that are performed on tissues such as brain slices or in cell culture models.

Many recent studies have reported the effects of propofol on cells or tissues grown in culture.[7–12] The kind of plastic used for these experiments may affect the results by changing the concentration of propofol in the medium when exposure times are more than 30 minutes. It is possible that results from such studies may have partially overestimated the concentration of propofol required to see an effect. Our results show greater stability in glass and previous studies have shown that PVC plastics are less likely to alter propofol concentrations.[2] When plastics are required for experiments using propofol, PVC-based plastics should be considered.

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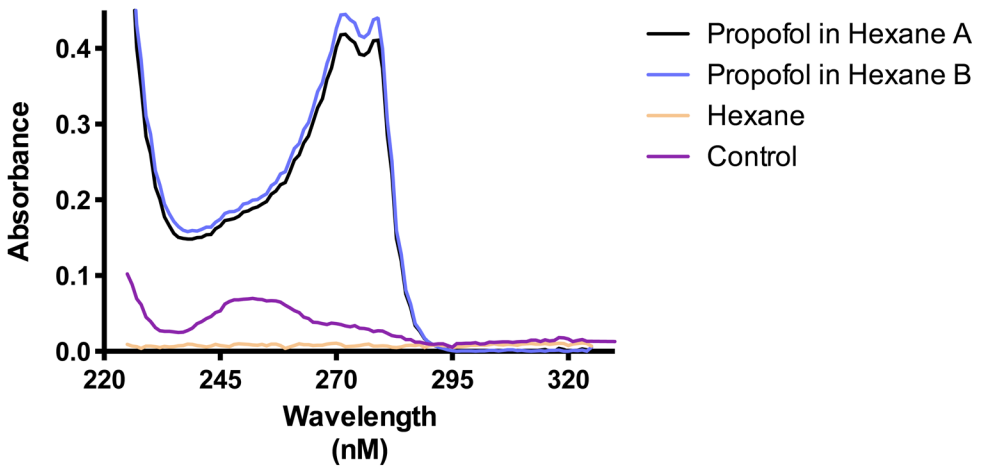

Figure 1. Wavelength Scan

A wavelength scan of propofol extracted from cell culture medium in hexane reveals a peak at 273nm. Hexane alone had no absorbance at this wavelength and medium without propofol had very low absorbance at this wavelength.

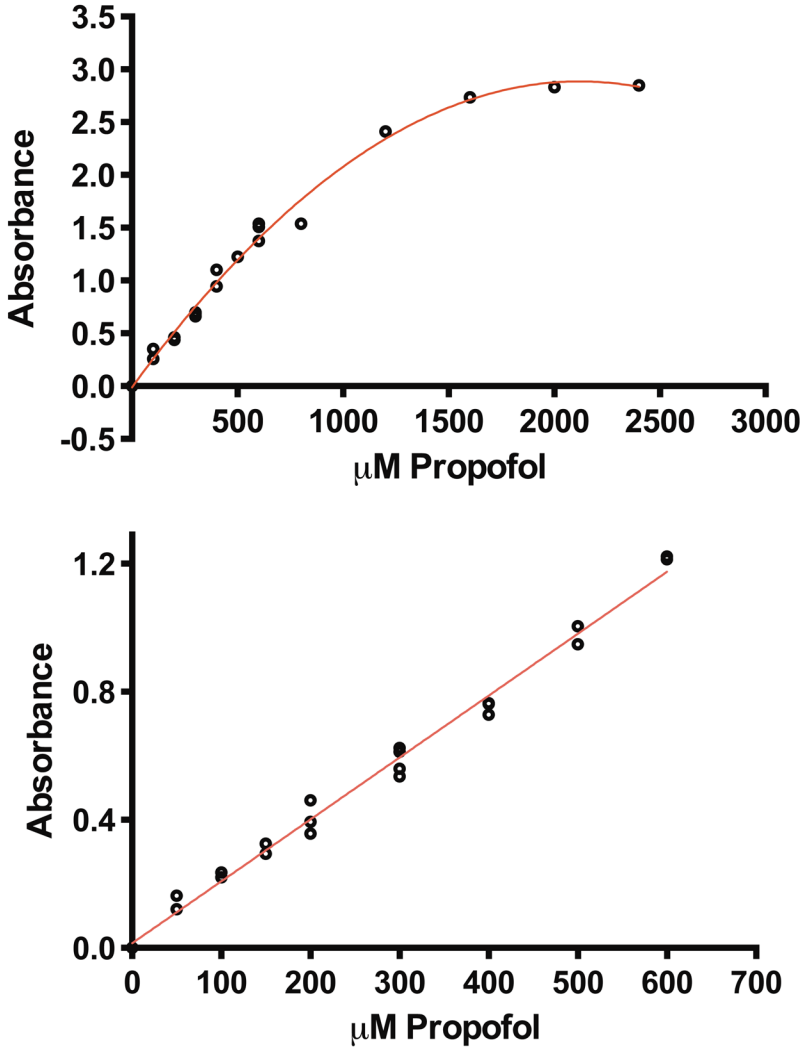

Figure 2. Concentration Standard Curve

Absorbance at 273nm was measured across a wide range of concentrations of propofol diluted in hexane. A) At very high concentrations the absorbance is no longer linear. B) Standard curves for daily experiments were typically performed from zero to 500μM. In this range the absorbance changes linearly with the propofol concentration.

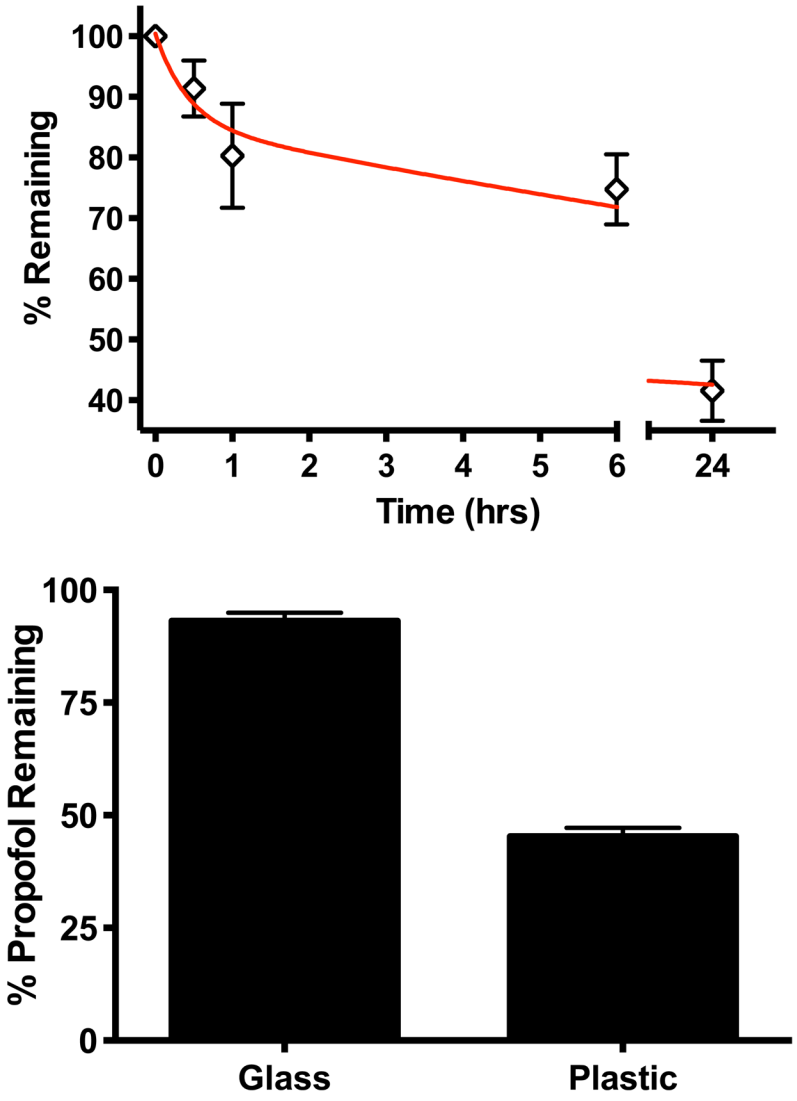

Figure 3. Propofol Concentration Decreases Over Time

A) Medium exposed to 96-well polystyrene plates for times indicated was extracted into hexane and the amount of propofol remaining relative to time zero is reported. A two-phase exponential decay curve was fit to the data. B) Medium containing propofol was kept in a glass vial or polystyrene tubes for 24 hours at 37°C in 5% CO_2. The medium was then extracted and the concentration of propofol remaining relative to that in fresh medium was determined.