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# An evaluation of the cold-temperature stability of two propofol formulations

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## Abstract

The current conflict in Ukraine has illustrated the problems and complexities of large-scale modern warfare in a cold-weather environment. Military operations in environments in which external temperatures can drop below -20˚C are often plagued by difficulties in transportation and supply. These factors can result in medical materiel being exposed to external temperatures for prolonged periods of time. Here, we evaluate the chemical and physical stability of two commercially available formulations of propofol (generic propofol and Diprivan) to decide if they are suitable for use in under arctic conditions. It was hypothesised that the simple molecular structures of both generic propofol and Diprivan would make them immune to cold-mediated degradation. Single particle optical sensing, laser diffraction particle size analysis and pH determination were used to assess the stability of each formulation following a series of freeze-thaw cycles. It was found that both formulations remained physically stable throughout each freeze-thaw cycle, confirming the initial hypothesis by indicating that both formulations should remain stable and physiologically active in the ambient temperatures of the arctic and near arctic environments. Further research is needed to determine the chemical stability of these formulations and to evaluate the impact of the resulting physiological consequences.

## Introduction

Propofol (2, 6 diisopropylphenyl) is a well-known sedative-hypnotic anesthetic.[1,2] Propofol is highly effective in the induction and maintenance of general anesthesia. Consequently, it has become one of the most used anesthetics in the ambulatory setting.[3] The physical properties and environmental stability characteristics of propofol are a direct result of its chemical structure.[4] Propofol is an organic hydrocarbon comprising 12 carbon atoms, 18 hydrogen atoms, and one oxygen atom ($C_{12}H_{18}O$) arranged into an isopropyl-substituted ring structure.[5,6] Large hydrocarbon ring structures typically display a high-degree of thermal stability. Propofol is a pale yellow to yellow oily substance that is poorly miscible in water and highly soluble in organic solvents.[7] Due to its solubility profile, most commercial formulations of propofol are prepared as lipid emulsions consisting of propofol as the active ingredient and soybean oil, glycerol, and egg lecithin as the carriers.[8] These emulsions are characterised by the presence of numerous propofol-containing lipid droplets ranging in size from 0.15 to 0.3 µm in diameter that are stabilised by negative charges that are imparted upon the droplets by the free fatty acids and phosphatidic acids that are present in egg lecithin.[9] These formulations typically have a pH ranging from 7 to 8.5 and tend to be milky-white due to ambient light reflection from the lipid droplets.[8] Propofol has been found to be especially stable when it is stored under nitrogen.[10] However, exposure to oxygen can lead to oxidative degradation and loss of potency.[11] Therefore, most propofol formulations are only stable for a few hours after opening at ambient temperature. Earlier studies have shown that there is a clear correlation between storage temperature and the stability of propofol formulations. For example, it has been shown that mixtures of propofol and thiopental sodium (another general anesthetic and induction agent) are stable for approximately 312 hours when stored at 4˚C and for up to 120 hours when stored at 23˚C.[11] However, the impact of low temperature on the stability of these formulations has not been adequately explored. This is significant given that military medical personnel often operate in remote and austere environments where temperature

control may be severely limited or nonexistent.[12] In the arctic and near arctic environment the use of anesthetic agents in the absence of temperature control may result in exposing them to unintentional freeze-thaw (FT) cycles.

Propofol has several anesthetic properties that make it an attractive agent for use in austere environments: it has a rapid onset of action and can induce loss of consciousness in less than 60 seconds after administration, it has a relatively short half-life, and it has a low incidence of postoperative complications.[13] Several formulations of propofol are commercially available. They vary by adding or omitting one or more compounds with antimicrobial properties.[14] Sodium metabisulfite or a sodium benzoate/benzyl alcohol combination are the antimicrobial agents most used in generic formulations (typically referred to as generic propofol or just as propofol). In contrast, brand-name formulations such as Diprivan tend to use disodium edetate (EDTA).[15] Previous studies have indicated that EDTA is the most stable additive to propofol formulations and sodium metabisulfite is the most unstable.[16] There have been very few studies on the stability of sodium metabisulfite.[17] In addition, the impact of these antimicrobial agents on the stability of the various propofol formulations has yet to be adequately studied. For example, it has not been decided whether rapid FT cycles or the types of temperature conditions found in the arctic environment have an impact on active ingredient stability and potency or impact the stability of any commercially available formulations. This information will be essential for the development of a formulary for use in extreme environments and for determining whether on-hand propofol formulations that have been exposed to arctic temperature conditions can be used safely for patient care. In addition, this information will be necessary for providers engaged in the selection of appropriate anesthetic agents for contingency operations in cold-weather environments or during humanitarian operations. Here, we evaluate the hypothesis that neither generic propofol or Diprivan should be susceptible to the potential degradative effects of sequential repetitive FT cycles due to the relative stability of the ring structured molecule and that no significant physical changes should result from this treatment.

## Methods

Two formulations of propofol with differing preservatives were evaluated in this study. Diprivan® (NDC 63323-269-29) contains disodium edetate (EDTA) and is produced by Fresenius Kabi USA, LLC. Generic propofol (NDC 25021-608-20) contains sodium metabisulfite and is produced by Sagent Pharmaceuticals®, Schaumburg, IL, USA.

There were six groups evaluated in this study; the evaluation of each formulation included 1 control, 1 single FT cycle and 1 three repetitive FT cycle group. A total of five vials were evaluated for each formulation. One FT cycle consisted of eight hours in a -20°C freezer (Fisher Scientific Isotemp, F1821FMSA14; Asheville, NC, USA) and a 16-hour thawing period at room temperature (approximately 22°C).

### Physical appearance

The gross physical appearance of each formulation was evaluated before and after each FT cycle. The microscopic physical appearance of the emulsions were evaluated by light microscopy (Olympus CH-2 microscope). The results of gross physical evaluations were captured by photography of the sealed containers (Fuji DX7 digital camera). In these evaluations, the clarity or turbidity of the solution was determined, and the presence or absence of large particles or flocculent material was determined.

### pH

To identify pH changes that may result from the various FT cycles, a Fisher Scientific AB15 Plus pH meter was used. The meter was equipped with a combination glass/saturated calomel electrode for pH determination. This instrument was calibrated against a set of standard pH buffers (Scientific Laboratory Supplies, Nottingham) and thoroughly rinsed prior to each use.

### Zeta potential

To evaluate the zeta potential of each formulation before and after treatment, a Zetasizer Nano ZS (Malvern Panalytical) system using laser doppler velocimetry and the Henry equation was employed. In this evaluation, zeta potentials were measured on both control samples and experimental vials in a dispersant of deionized water using the electrophoretic method.

### Particle size analysis

Particle size analysis was conducted by two different mechanisms due to the size of the particles in the emulsion. Laser light diffraction was performed on a Beckman Coulter LS 13 320 LASER diffractor. This instrument calculates a volume distribution from the LASER diffraction pattern of a cloud of particles. This raw scatter data is then processed and presented based on equivalent spherical diameter. Laser light diffraction can detect particles ranging in size from 0.02µm to 3500µm. Single particle optical sensing (SPOS) was also used to quantify particles greater than 5µm in size. This was conducted on a Particle Sizing Systems AccuSizer A7000 System. Unlike laser diffraction, this method can determine both particle size and concentration of suspensions.

All samples were assessed in their original unopened containers on a single occasion after a single FT cycle and then discarded; every container was sampled once. None of the samples had evidence of free-floating oil or emulsion breakdown that would be indicative of a compromised sample prior to treatment.

## Results

To compare the thermal stability of generic propofol (which uses a sodium metabisulfite formulation) with Diprivan (which uses

an EDTA formulation) in a simulated cold-weather environment, a series of FT experiments were performed. While the gross visual examination of each formulation after 3 FT cycles (ranging from -20°C to 22°C) revealed no changes in color or viscosity, a consistent number of large particles were seen when microscopy was employed at 20X magnification (Figure 1). pH measurements showed that there was no effect of any of the FT cycles on the acidity or basicity of Diprivan but that there was a significant effect on generic propofol with pH values dropping to around pH4 at after three FT cycles in contrast to a pH of around pH6 for controls. This represents a 2-log decrease in hydrogen ion concentration indicating an increase in acidity which might result in increased pain on injection or have undetermined physiological impacts (Figure 2).

Since the two propofol formulations evaluated in this study are lipid emulsions, the zeta potential of each was determined prior to initiating the FT cycles to understand the potential for altered colloidal stability of the formulation. The zeta potential was determined to be -47.4mV for Diprivan and -49.1mV for propofol, indicating sufficient electrostatic force to support colloidal stability. The size of the particles in each formulation was estimated using laser light diffraction which showed a bimodal distribution with over 99% of the volume being less than 5µm. Significantly, this value did not change with the increasing numbers of FT cycles. Mean particle sizes did not change significantly (Table 1). SPOS revealed large globular formations in all samples regardless of the number of FT cycles ranging from 1–2% of total particles with sizes ranging from 24 to 108 µm.

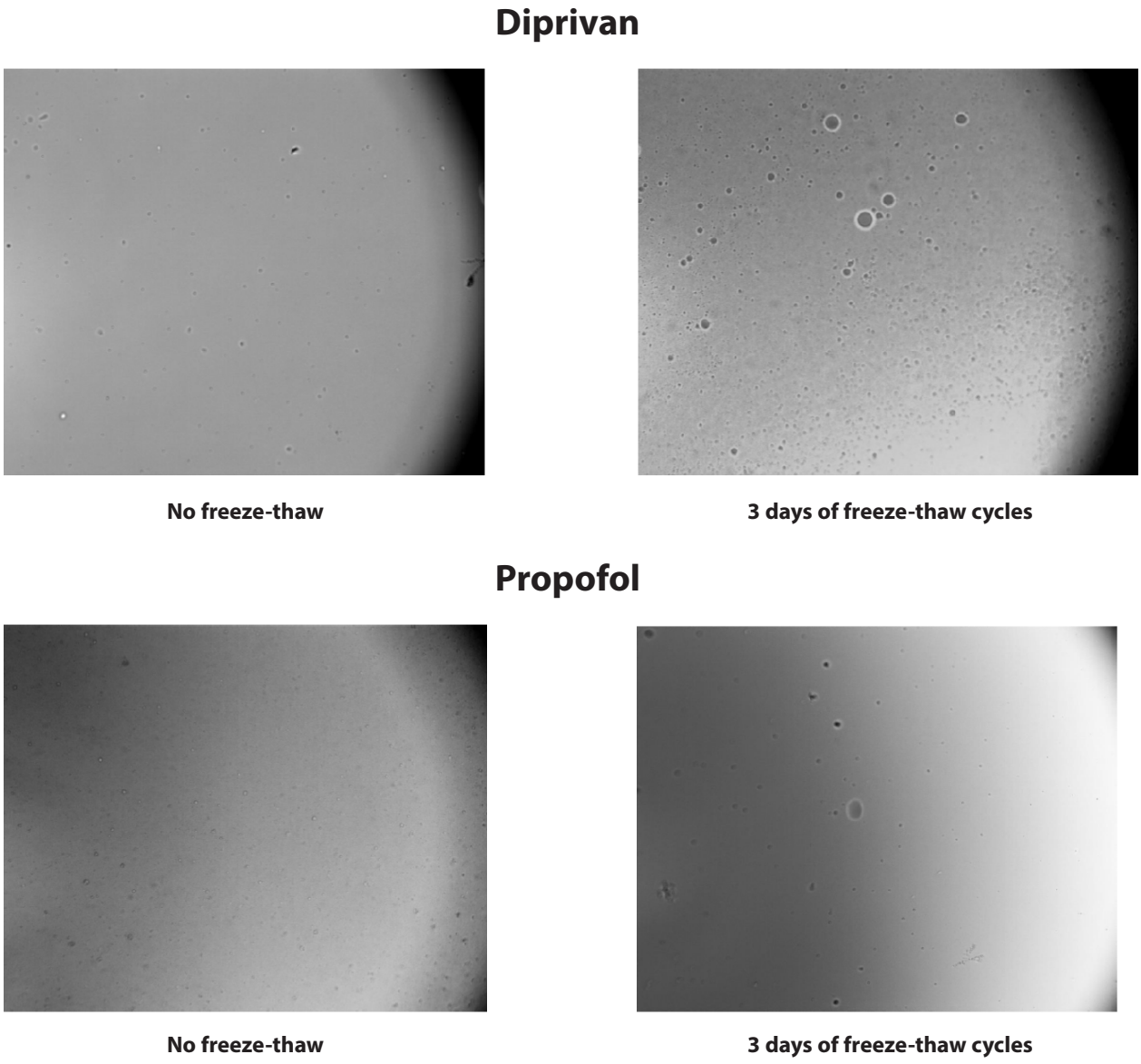


*Figure 1. 20X magnification of diprivan and generic propofol prior to the initiation of the freeze-thaw cycle and after three freeze-thaw cycles (one freeze-thaw per day).*

## Discussion

The lack of gross visual changes to either of the propofol formulations treated with up to three FT cycles provides evidence that they are both relatively stable with respect to repeated low temperature exposure. However, this result stands in contrast to the observation of large droplets in each formulation that was seen with light microscopy at 20X magnification. This result shows that potentially physiologically relevant physical changes occur in the suspension after three FT cycles. Further experimentation will be necessary to determine the nature of these changes. It is well known that aggregations of particles in liquid suspension can occur via numerous chemical and physical mechanisms with varying degrees of physiological relevance.[19] It is possible that the results of the laser diffraction particle size analysis and single-particle optical sensing indicate that gross visual observation is inadequate to evaluate the quality of a suspension and that despite the microscopic observation of large droplets in the suspension, there may be no overall change in the numbers or sizes of the particles in solution. The incongruence of these results may further be explained by the fact there are limitations to laser diffraction particle size analysis. These limitations are imposed by the assumed index of refraction of the material of interest, and the light scattering properties of the particles, and the chemical and physical properties of the solution.[19] Overall, the results suggest that both propofol formulations are thermally stable with respect to the types of rapid freezing and thawing that can be expected with use in the arctic environment under field conditions. In this regard, these results confirm the initial hypothesis that rapid FT cycles should have a negligible effect on the stability of propofol formulation. Interestingly, they contrast with previous studies showing significant coalescence of particles in the suspension after just one FT cycle of a generic propofol formulation.[8] This discrepancy might be due to the handling or storage of the propofol formulations in the previous study, as care was taken in this study to ensure proper handling and storage of each of the vials. Since the zeta potential values found in this study are greater than +/-10mV from the previously reported +/-30mV standard, they indicate well-stabilised suspensions with a low probability of aggregation.[18] This indicates that both formulations should be stable and that the lipid emulsions would not be expected to degrade during temperature shifts.[20,21] The observed reduction in the pH of generic propofol after three FT cycles requires further investigation (Figure 2). Since the experimental tests happened immediately after the termination of each FT cycle, it is unclear if this change would reduce the stability of the emulsion over time.[22] Although not previously reported, it is conceivable that prolonged reductions in the pH of the formulation could upset the electrostatic stability of the emulsion resulting in the aggregation of particles and a decrease in the potency of the anesthetic. It is also possible that a decrease in pH might result in an increase in pain upon injection.[23] It may also be possible that extreme pH shifts may result in the formation of metabolic acidosis and may require alternate dosing.[24] Further studies are necessary to determine the nature of this pH shift and to find the resulting physiological or pharmacodynamic consequences.

*Table 1. The mean particle sizes of droplets found in the lipid emulsions of Diprivan versus generic propofol before and after the initiation of several freeze-thaw cycles.*

| Treatment | Sample | Mean particle sizes |
|---|---|---|
| Control | Diprivan | 0.127 ± 0.16 |
| FT 1 Cycle | Diprivan | 0.137 ± 0.17 |
| FT 3 Cycles | Diprivan | 0.122 ± 0.16 |
| Control | Propofol | 0.14 ± 0.16 |
| FT 1 Cycle | Propofol | 0.133 ± 0.16 |
| FT 3 Cycles | Propofol | 0.136 ± 0.17 |

A common theme in extreme weather conditions is the strain it places on logistics. It is important for medical personnel to carefully consider operational requirements while planning operations in cold weather environments. This is especially true when the acquisition, transportation, and storage of material is not guaranteed as would be expected in austere or out-of-hospital settings. Temperature-sensitive medications are continually at risk of freezing in extreme cold-weather conditions. It is therefore paramount for anesthesia providers to anticipate

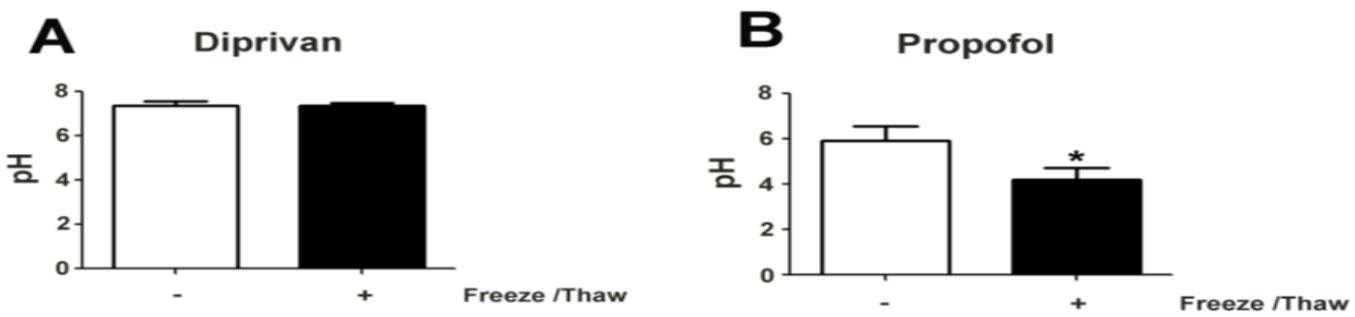


*Figure 2. The pH changes that occur after three FT cycles of Diprivan versus generic propofol. This data represents five samples of each formulation with the mean and standard deviation shown. The asterisk indicates a statistically significant pH changes as identified by the Student's T-test at the 0.01 level of significance.*

these situations and to plan accordingly. Encouragingly, the findings from this study have indicated that both Diprivan and propofol remain stable after undergoing a series of FT cycles and thus should be suitable for use in out of hospital, field operations in the arctic and near arctic environments. Future studies will involve the use of animal models to more accurately evaluate the physiological impact of repeated FT cycles and the use of mass spectrometry and other chemical methods to determine whether structural changes or degradation products can be identified and evaluated.

## Prior presentation

Poster presentation at NATO Human Factors in Cold Weather Medicine Symposium

## Conflict of interest

The authors declare no conflicts of interest.

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## Institutional review board

This study did not use human subjects or animals.

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