Propylene Glycol Dose-Dependent Hemolysis in Intravenous Formulations: Thresholds and Mitigation Strategies in Beagle Dogs

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Introduction

Propylene glycol (PG), a widely used solubilizing agent in preclinical intravenous (IV) formulations, exhibits dose-dependent hemolytic effects in beagle dogs. This can lead to various pathological and clinical symptoms, including high bilirubin levels, jaundice, anemia, enlarged liver and spleen, kidney damage, tachycardia, and dyspnea.[1,2] These effects compromise animal welfare and distort pharmacokinetic (PK) data. Existing common practices predominantly focus on limiting PG concentration (e.g., ≤40%) but neglect other critical factors such as injection volume and co-solvent interactions.[3] This poster investigates the hemolytic risks associated with PG dosing thresholds, co-solvent interactions, and strategies for mitigating hemolysis in beagle dogs.

Methods

Quantitative Hemolysis Assay Development

- Instrument: Thermo NanoDrop™ One (UV-Vis Spectrophotometer)
- Detection: Hemoglobin (Hb) absorbance at 540 nm

Hb is released following the rupture of red blood cells (RBCs)

Standard Curve:

- ✓ Plasma from Beagle dogs → Graded hemolysis samples
- ✓ Linear regression: Samples with different hemolysis rates have different absorbance at 540nm, the standard curve was generated by plotting the theoretical hemolysis percentage (X) against the corresponding absorbance at 540 nm (Y). Linear regression analysis yielded the calibration equation: $Y = 2.7403X + 0.1303$ ($R^2 = 0.9997$).

Study Design 1

Table 1 PG Dose-Dependent Hemolysis Threshold in Beagle Dogs

Test Article (%, w/v)	Solvent	Animal No.	Dose Volume	Dose Route	Sample Collection
25% PG	Water	3	1 mL/kg	IV bolus	30 min post-dose
30% PG	Water	3	1 mL/kg	IV bolus	30 min post-dose
35% PG	Water	3	1 mL/kg	IV bolus	30 min post-dose
40% PG	Water	3	1 mL/kg	IV bolus	30 min post-dose
Control	Saline (0.9% NaCl)	3	1 mL/kg	IV bolus	30 min post-dose

Study Design 2

Table 2 Hemolysis Risk Assessment of PG-Based Formulations with Co-Solvents

Co-Solvent (10%, w/v)	PG Conc. (%, w/v)	Solvent	Animal No.	Dose Volume	Dose Route	Sample Collection
None (Control)	30%	Water (70%)	3	1 mL/kg	IV bolus	30 min post-dose
NMP	30%	Water (60%)	3	1 mL/kg	IV bolus	30 min post-dose
EtOH	30%	Water (60%)	3	1mL/kg	IV bolus	30 min post-dose
DMAC	30%	Water (60%)	3	1 mL/kg	IV bolus	30 min post-dose
DMSO	30%	Water (60%)	3	1 mL/kg	IV bolus	30 min post-dose

Comments: NMP(N-Methyl-2-pyrrolidone); EtOH(Ethanol); DMAC (Dimethylacetamide); DMSO (Dimethyl Sulfoxide)

Study Design 3

Table 3 Hemolysis Mitigation by Isotonic Solvent

Test Article (%, w/v)	Solvent	Animal No.	Dose Volume	Dose Route	Sample Collection
40% PG	Water	3	1 mL/kg	IV bolus	30 min post-dose
40% PG	Saline (0.9% NaCl)	3	1 mL/kg	IV bolus	30 min post-dose

Results

The results (**Figure 1**) demonstrate a clear dose-dependent hemolytic effect of PG solutions. The 25% PG solution exhibited acceptable hemolysis levels (0.46%) below the critical 0.5% threshold (50 mg/dL Hb), whereas solutions containing ≥30% PG consistently exceeded this limit (0.596-0.777%). This dose-response relationship indicates that PG concentrations ≥30% can produce both visually detectable hemolysis (as defined by CAP/CLSI guidelines: Hemolysis at ≥50 mg/dL (≈0.5%) is typically visually detectable as pink/red discoloration in serum/plasma under good lighting) and quantifiable analytical interference. Consequently, formulations containing ≥30% PG require careful evaluation during bioanalytical method development to address potential matrix effects.

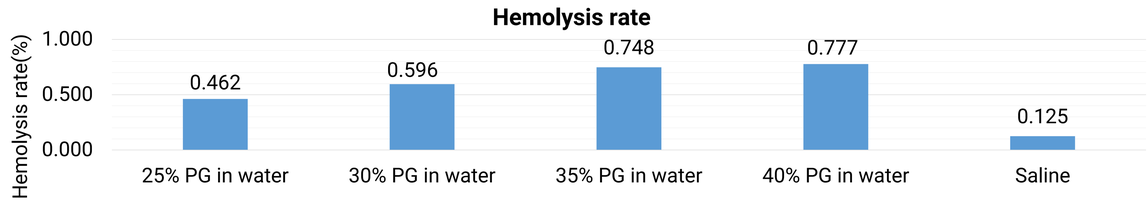

Figure 1. Hemolysis Rate of Different Concentrations of PG Aqueous Solutions

All tested 30% PG solutions containing co-solvents exceeded the 0.5% hemolysis threshold (**Figure 2**), demonstrating varying degrees of hemolytic potential and possible interference with bioanalytical assays. The co-solvents exhibited differential effects:

- NMP showed no significant hemolysis enhancement.
- EtOH, DMAC, and DMSO demonstrated progressively increased hemolysis.

Results (**Figure 3**) The addition of isotonic saline provided marked protection against PG-mediated hemolysis, achieving a statistically significant 40% reduction in hemolytic activity relative to aqueous control conditions.

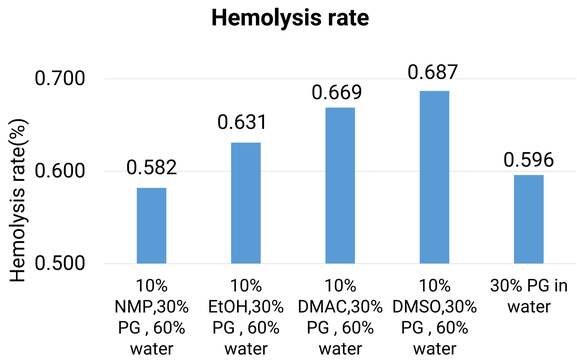

Figure 2 . Exploring Cosolvent Options

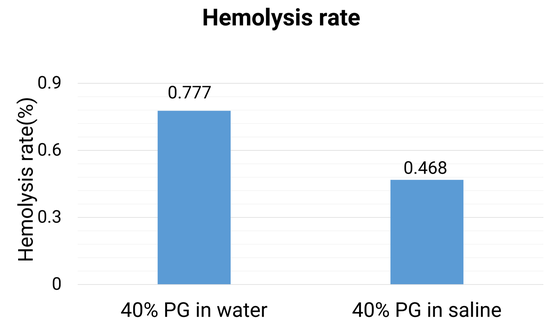

Figure 3. Mitigating PG-Induced Hemolysis with Saline

Conclusion

This study selected beagle as experimental subjects and quantitatively analyzed the release of hemoglobin (hemolysis) from red blood cell lysis under different solvent conditions using UV-visible spectrophotometry. The study established a dose threshold for PG at ≤300 mg/kg. When PG is used in combination with co-solvents at a safe ratio, interactions between solvents significantly exacerbate hemolysis, with the risk levels ranked as follows: DMSO > DMAC > EtOH > NMP, replacing DMSO/DMAC with NMP effectively reduces the degree of hemolysis. The historically used PG safety threshold (300 mg/kg) should be further lowered in formulations containing co-solvents.

Isotonic saline has a mitigating effect on hemolysis (e.g., substituting saline for pure water reduces the hemolysis rate of 40% PG from 0.78% to 0.47%), isotonic solvent adjustment is a balancing strategy to achieve solubilization while reducing hemolysis risk.

References

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[3] Shayne Cox Gad. Tolerable Levels of Nonclinical Vehicles and Formulations Used in Studies by Multiple Routes in Multiple Species With Notes onMethods to Improve Utility. International Journal of Toxicology.2016

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