INTERNATIONAL JOURNAL OF PHARMACEUTICAL EXCIPIENTS

Impact of polyethylene glycol polymer source variations on the performance of long-acting injectable suspensions

Nilesh Malavia[1], Saurabh Badole[1]**, Melissa Sabatella[1], Quanying Bao[1], Diane J Burgess[1]***

[1] University of Connecticut, Department of Pharmaceutical Sciences, Storrs, CT 06269 USA

Recieved: July 30 2026
Accepted: September 16 2026

Research Article

ABSTRACT

Long-acting injectable (LAI) suspensions rely on polymeric excipients such as polyethylene glycol (PEG) to stabilize suspended drug particles and modulate release behavior. While generic LAI formulations must be compositionally equivalent (Q1/Q2) to their reference listed drug, excipients sourced from different manufacturers may exhibit subtle but functionally significant differences in physicochemical properties. This study investigated the impact of PEG3350 source variability on the physicochemical properties and *in vitro* performance of medroxyprogesterone acetate (MPA) suspensions equivalent to Depo-Provera®. PEG3350 polymers obtained from four commercial sources Dow Chemicals Company (DC), Lab Alley (LA), Spectrum Chemicals (SC), and BASF Chemicals (BC) were characterized for thermal behavior, molecular weight, and molecular weight distribution using thermogravimetric analysis (TGA), gel permeation chromatography (GPC), and liquid chromatography-mass spectrometry (LC/MS). Four Q1/Q2 equivalent MPA suspensions were prepared using these polymers and evaluated for particle size, sedimentation volume, rheology, and *in vitro* drug release using a novel adapter with USP Apparatus Type IV. Physicochemical characterization revealed vendor-dependent differences in molecular weight distribution, with DC PEG3350 exhibiting the highest weight-average molecular weight and a predominance of high-MW chains, while BC PEG3350 displayed the broadest distribution and LA PEG3350 showed a bimodal profile. Despite comparable particle sizes across all formulations, these molecular differences translated into measurable differences in suspension microstructure. The DC-based formulation demonstrated the highest sedimentation volume ratio (F = 0.95) and the slowest *in vitro* drug release, consistent with stronger particle–polymer agglomerate networks formed by longer polymer chains. Conversely, the BC-based formulation produced weaker agglomerates, greater susceptibility to shear-induced particle size reduction, and faster initial drug release. These findings demonstrate that nominally equivalent PEG3350 polymers from different sources are not necessarily functionally interchangeable, and that molecular weight distribution is a critical quality attribute governing agglomerate structure, suspension stability, and drug release from LAI suspensions.

**Correspondence: d.burgess@uconn.edu
**Both authors contributed equally

KEY WORDS: Long-acting injectable suspensions; polyethylene glycol; PEG3350; excipient variability; medroxyprogesterone acetate; in vitro drug release; agglomeration; molecular weight distribution.

ABBREVIATIONS

Abbreviation	Definition
ANOVA	Analysis of variance
API	Active pharmaceutical ingredient
DSC	Differential scanning calorimetry
DTG	Derivative thermogravimetry
EO	Ethylene oxide
GPC	Gel permeation chromatography
LAI	Long-acting injectable
LC/MS	Liquid chromatography–mass spectrometry
LNP	Lipid nanoparticle
MPA	Medroxyprogesterone acetate
MW	Molecular weight
Mw	Weight-average molecular weight
NF	National Formulary
PDA	Photodiode array (detector)
PEG	Polyethylene glycol
PLGA	Poly(lactic-co-glycolic acid)
Q1/Q2	Qualitative (Q1) and quantitative (Q2) composition
q-TOF	Quadrupole time-of-flight
QSM	Quaternary solvent manager
RLD	Reference listed drug
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SEC	Size-exclusion chromatography
TFA	Trifluoroacetic acid
TGA	Thermogravimetric analysis
UPLC	Ultra-performance liquid chromatography
USP	United States Pharmacopeia

INTRODUCTION

Long-acting injectable (LAI) suspensions are sustained released parenteral drug products that have gained increased popularity in recent years due to their advantages, including prolonged drug release duration and improved patient adherence (1-3). While the number of newly developed and approved LAI suspensions has increased over the years, the development of generic equivalents remains limited. This is largely due to the complexity associated with formulation development and lack of understanding of the quality attributes affecting product performance (2).

Previously published reports have shown that particles in LAI suspensions may exist in different states such as primary particles, weak agglomerates and strong agglomerates, each of which can affect drug release differently (1). The formation of weak agglomerates is attributed to interactions between the API and polymer excipient (polyethylene glycol (PEG)). PEG is a hydrophilic polymer and as such it facilitates the dispersion of hydrophobic API into the aqueous media. However, variations in the physicochemical attributes of PEG can significantly alter its interactions with the API and, in turn, affect the overall performance of LAI suspensions. Therefore, establishing a clear relationship between the physicochemical attributes of PEG and the physicochemical properties of LAI suspensions is critical for better understanding and optimizing product performance.

To obtain approval for a generic LAI suspension, the manufacturer must demonstrate both pharmaceutical equivalence and bioequivalence to the reference listed drug (RLD) (4). Accordingly, the generic formulation must contain the same inactive ingredients (Q1) in the same quantities (Q2) as the RLD. Exceptions are permitted only for differences in preservatives, buffers, or antioxidants, provided that the applicant identifies and characterizes these differences and demonstrates that they do not impact the safety or efficacy of the proposed product (5-7). Even so, using the same ingredients sometimes can lead to major differences in product quality and final product performance. For instance, recent work by Wan et al. (8) highlighted the critical influence of polymer source variability on the performance of long- acting PLGA-based formulations. In this study, PLGA polymers obtained from different manufacturers though specified to have identical monomer ratios, inherent viscosities, and end-group chemistries exhibited notable differences in molecular weight, blockiness, and degradation behavior upon detailed characterization. Using these polymers, the authors prepared leuprolide acetate–loaded microspheres under identical formulation and processing conditions to isolate the impact of polymer source. The resulting microspheres showed significant variations in particle morphology, porosity, and *in vitro* drug release profiles, with some formulations displaying faster burst release and others showing more sustained release patterns. These findings demonstrated that nominally equivalent PLGA polymers from different sources may not be functionally interchangeable, and such differences can directly affect the Q3 attributes and release kinetics of long-acting injectable products.

Similarly, variations in PEG polymers from different manufacturers may also exist and could influence formulation performance. Although PEG is a linear polyether (9), differences in manufacturing processes, purification methods, and raw material sources can lead to variations in monomer and oligomer content, molecular weight distribution, and end-group composition. Such subtle differences may significantly affect the physicochemical behavior and performance of long-acting injectable (LAI) suspensions, particularly those relying on PEG as a stabilizing or viscosity-modifying excipient.

In the present investigation, four PEG3350 polymers were sourced from Dow Chemical Company®, Lab Alley®, Spectrum Chemicals®, and BASF Chemicals® to evaluate the potential impact of polymer source variation. Comprehensive physicochemical characterization was conducted to assess differences in polymer properties. Using these PEG3350 variants, MPA suspensions equivalent to the reference listed drug Depo-Provera® were formulated. The prepared suspensions were subsequently subjected to detailed physicochemical evaluation and *in vitro* drug release studies to understand how variations in the PEG3350 source could influence formulation characteristics and release performance.

MATERIALS AND METHODS

Materials

Medroxyprogesterone acetate (MPA, micronized, USP grade), the active pharmaceutical ingredient, was obtained from Spectrum Chemicals Manufacturing Corp., New Brunswick, NJ, USA. Preservatives methylparaben (NF grade) and propylparaben (NF grade), surfactant Tween 80 (NF grade) and sodium dodecyl sulfate (SDS), were obtained from Spectrum Chemicals Manufacturing Corp., New Brunswick, NJ, USA.

The polymer polyethylene glycol (PEG) 3350 was procured from Dow Chemical Company, Lab Alley, Spectrum Chemicals, and BASF Chemicals. All sources of PEG3350 were of USP grade except PEG3350 from Dow Chemicals.

Physicochemical characterization of PEG polymer

PEG3350 polymer from Dow Chemical Company, Lab Alley, Spectrum Chemicals, and BASF Chemicals were investigated for thermal analysis, molecular weight, polydispersity, and molecular composition.

Thermal analysis

Thermal analysis of PEG3350 obtained from Dow Chemical Company, Lab Alley, Spectrum Chemicals, and BASF Chemicals was performed using thermogravimetric analysis (TGA, TA Q500, Universal TA instruments, New Castle, DE, USA) and differential scanning calorimetry (DSC, TA Q1000 calorimeter. Universal TA instruments, New Castle, DE, USA) analysis.

For TGA analysis, the residual material from prior experiments was removed by burning the platinum pans to high temperatures. The empty platinum pans were then tared, and approximately 2.5 mg of PEG3350 from each source was loaded into the pans. Samples were allowed to equilibrate at 30º C, followed by heating to 400 °C at a rate of 10º C/min. Weight loss profiles were subsequently recorded.

Molecular weight analysis

PEG polymer standards (PSS ReadyCal-Kit GPC/SEC calibration standards, Agilent) mixed with DMF were injected into a PLgel Mixed-C column (300 mm x 7.5 mm, 5 µm particle diameter) of a gel permeation chromatography system equipped with refractive index and viscometer detectors (Agilent Technologies, 1260 Infinity II GPC/SEC System) and an isocratic pump to prepare the calibration standard for PEG showing a relationship between elution time and weighted-average molecular weight. Next, PEG3350 samples from all four sources were dissolved in DMF (dimethyl formamide) and stirred overnight for complete dissolution. These samples were eluted through the PLgel Mixed-C column (300 mm x 7.5 mm, 5 µm particle diameter) with an injection volume of 100 µl at a flow rate of 1 ml/min with DMF used as the eluent. The column and detector temperature were both set at 50º C. The data analysis was performed using Agilent software. The molecular weight (Mw) and polydispersity were calculated using the calibration curve.

Molecular fraction analysis

The separation and detection of PEG3350 from Dow Chemical Company, Lab Alley, Spectrum Chemicals, and BASF Chemicals was conducted on an Alliance Waters UPLC system (Milford, USA). The instrument configuration consisted of a quaternary solvent manager (QSM), a photodiode array detector (PDA), and a sample manager. Data acquisition and subsequent processing were carried out using Waters EMPOWER software. Chromatographic resolution was achieved with an Acquity BEH C8 column (50 mm × 2.1 mm × 1.7 µm). PEG3350 samples from four different sources were analyzed using two mobile phases: (i) water/acetonitrile, 10:90 v/v with 0.05 % v/v TFA (mobile phase A), and (ii) water/acetonitrile, 90:10 v/v with 0.05 % v/v TFA (mobile phase B). The optimized experimental conditions determined for reliable separation of PEG3350 were as follows:

Chromatographic conditions	Optimized values
Flow rate (mL/min)	0.3
Injection volume (µL)	10.0
Column oven temperature (°C)	40

A Waters Xevo G2-XS q-TOF Mass Spectrometer coupled with a UPLC system (Milford, USA) was used to identify the molecular composition of PEG3350 polymer from varying sources. The MS conditions are as follows: spray voltage 3.5 kV; cone 25 V; source gas flow 650 1/h; source temperature 350º C; and collision energy 20 V. Data analysis was performed using Mass Lynx V 4.1 (Milford, USA).

Sample preparation for molecular composition: PEG3350, sourced from Dow Chemical Company, Lab Alley, Spectrum Chemicals, and BASF Chemicals, was dissolved in LC/MS grade water containing 0.1% v/v formic acid. The dispersion was gently shaken on a shaker plate for 24 hours to ensure complete solubilization. Following this, the solution was filtered, and appropriate dilutions were prepared for analysis.

Preparation of MPA suspensions

Depo-Provera®, containing MPA as the active ingredient, was selected as the model drug product. Four suspending media were prepared by dispersing the excipients in their quantities listed in the product's package insert (10) (Table 1). The composition of all four media was identical, except for the source of PEG3350, which was varied. Using these media, four Q1/Q2 equivalent formulations of Depo-Provera® were prepared. The formulations were labelled as follows: DC (PEG3350 from Dow Chemical Company), LA (PEG3350 from Lab Alley), SC (PEG3350 from Spectrum Chemicals), and BC (PEG3350 from BASF Chemicals). The formulations were prepared by simple stirring. Briefly, 300 mg of MPA (Spectrum Chemicals) were added to a vial containing

Table 1. List of excipients along with their quantities listed in the Depo Provera® package insert

Excipient	Quantity
Methyl paraben	1.35 mg/ml
Propyl paraben	0.150 mg/ml
Polysorbate 80	2.41 mg/ml
PEG3350	28.9 mg/ml
Sodium chloride	8.68 mg/ml

2 mL of suspending medium and mixed using a magnetic stirrer at 600 rpm.

Physicochemical characterization of MPA suspensions

The MPA suspensions prepared using polymer of varying sources were characterized for particle size (with sonication, shear through pipette and syringe, and shear through shaking), rheology, sedimentation volume, and *in vitro* drug release.

Particle analysis

The particle size and size distribution of the Q1/Q2 equivalents of Depo-Provera® were determined using a Malvern MasterSizer 3000™ coupled with a Hydro MV dispersion unit (Malvern Analytical Instruments Ltd, UK). Prior to analysis, the dispersion unit was filled with water and sonicated for 30 seconds to remove dissolved air bubbles. Approximately 30 µL of the suspension was introduced, and the obscuration level was adjusted within the 2–20% range. The instrument recorded percentile values, including Dv10, Dv50, and Dv90. The polydispersity of the suspension was expressed as Span, calculated using the formula: (Dv90 – Dv10)/Dv50.

With sonication

The strength of interaction between the polymer and dispersed MPA particles was examined by introducing Q1/Q2 equivalent formulations (DC, LA, SC, BC) sequentially into the particle sizing well. In situ weak sonication was then applied, and particle size was recorded at 30-second intervals for a total duration of 120 seconds.

With shaking

To investigate the impact of shear on dispersed MPA particles, Q1/Q2 equivalent formulations (DC, LA, SC, BC) were filled into 3 mL plastic syringes and subjected to shear by shaking the suspensions for 60 seconds. Following this, the suspensions were introduced into the particle sizing well, and particle size was recorded.

With syringe and pipette

To investigate the impact of shear applied by syringe and pipette on dispersed MPA particles, Q1/Q2 equivalent formulations (DC, LA, SC, BC) were introduced into the particle sizing well using both pipette and syringe. For the pipette method, approximately 30 µL of suspension was pipetted and introduced into the particle sizing well and particle size was recorded. For the syringe method, suspensions were passed through syringes fitted with needles of different diameters (14G, 18G, and 22G) to simulate increasing levels of shear and introduced into the particle sizing well, and particle size was recorded.

Sedimentation volume

To determine sedimentation volume, Q1/Q2 equivalent formulations (DC, LA, SC, BC) were filled into 1 mL plastic syringes and stored in an upright position for one week. After this period, both the final sediment volume and the total volume were recorded, and the sedimentation volume was calculated.

In vitro drug release

In vitro drug release testing was carried out using a novel adapter with USP type IV apparatus (Sotax CE7 smart with piston pump CP7-35). Adapters were placed into the flow through cells and 50 µL of Q1/Q2 equivalent formulations (DC, LA, SC, BC) were introduced into the conical cavity of the novel adapter. The filter assembly was prepared by layering two glass microfilters of varying pore sizes, with the finer filter (Whatmann® 0.7 µm) placed beneath the coarser filter (Whatmann 2.5 µm). Drug release studies were conducted in 500 mL of 1% w/v sodium dodecyl sulfate solution maintained at 37 ± 0.5 °C to ensure sink conditions. As the USP type IV apparatus is a closed system, no medium loss was observed. Samples were collected at predetermined intervals and analyzed using UPLC. The method for MPA concentration analysis using UPLC was adopted from our previously published work. The F2 similarity factor was calculated using the F2 similarity factor equation.

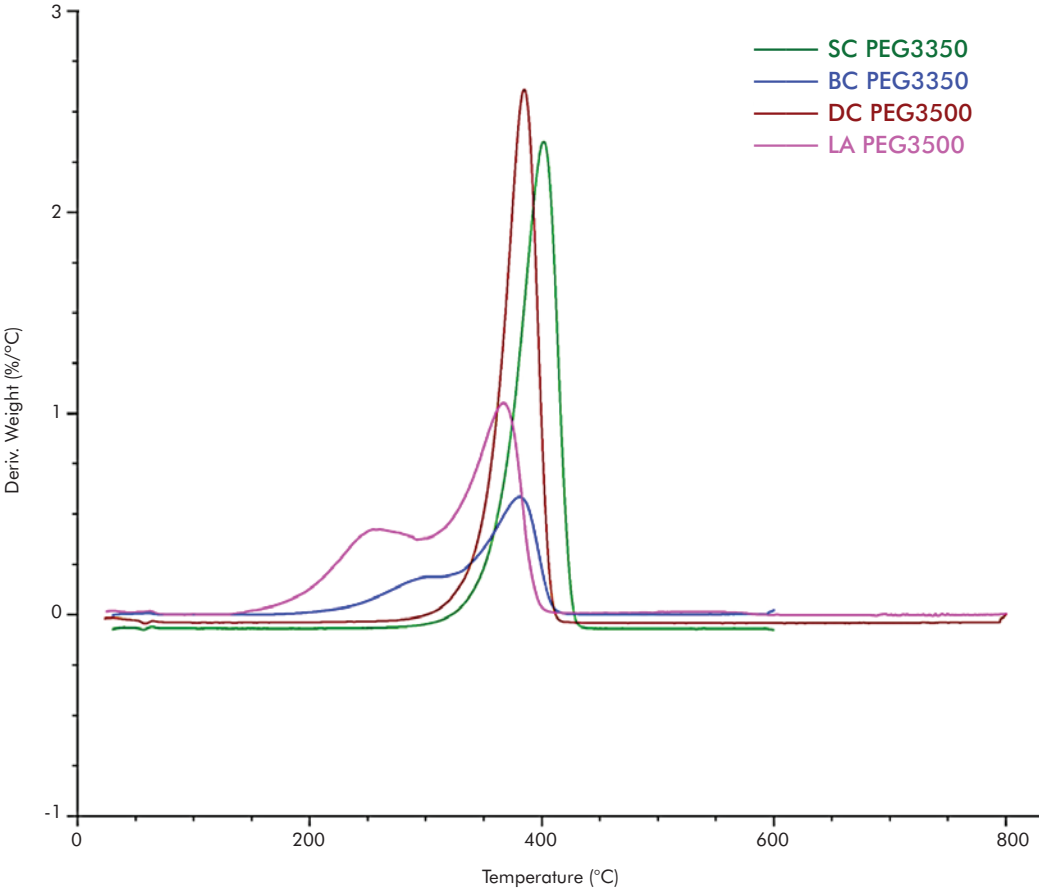
RESULTS

Thermal analysis

Thermogravimetric analysis (TGA) of PEG3350 samples obtained from different sources was performed to evaluate their thermal decomposition behavior upon controlled heating. The derivative thermogravimetric (DTG) curves revealed

that PEG3350 from both DC and SC exhibited a unimodal decomposition peak (refer fig. 1). In contrast, PEG3350 from LA displayed a bimodal DTG profile. Meanwhile, PEG3350 from BC showed a broad and shallow decomposition peak (Figure 1).

Figure 1. Graphical representation of derivative thermogravimetric analysis of PEG3350 for: (A) Spectrum Chemicals®; (B) BASF®; (C) Lab alley®; (D) Dow Chemical Company®. DTG analysis was performed using TA universal software program.



Molecular weight analysis

Gel permeation chromatography (GPC) analysis was performed to determine the weight-average molecular weight (Mw) of the polymer samples obtained from different commercial sources. The Mw values for the Spectrum, BASF, Lab Alley, and Dow Chemical Company samples were determined to be ~3329, ~3,268, ~3,417, and ~3,618 g/mol, respectively. The Dow Chemical Company sample exhibited the highest Mw at 3,618 g/mol, while the BASF sample displayed the lowest at 3,268 g/mol, representing an overall variation of approximately 11% across the four sources.

Molecular fraction analysis

Molecular fraction analysis was performed using mass spectrometry combined with UniDec deconvolution to resolve the polymer molecular weight distribution (Figure 2). For the DC PEG3350 sample, the polymer population was composed almost entirely of high molecular weight (MW) chains. In the SC sample, the majority of molecular chains were skewed toward higher MW fractions, although a small portion of intermediate chains was detected. The BC PEG3350 exhibited a distribution predominantly composed of high MW chains, but with a minor fraction of low MW species. The LA PEG3350 showed a similar predominance of high MW chains; however, it contained a moderate fraction of lower MW species (Figure 2).

Particle size analysis

The particle size of the prepared MPA suspensions was determined using a Malvern Mastersizer. MPA suspensions formulated with polymers from different sources did not exhibit significant differences in particle size. The mean particle sizes for the suspensions prepared with DC, LA, BC, and SC polymers were approximately 13.0 µm, 13.3 µm, 12.6 µm, and 12.1 µm, respectively (Figure 3A).

With sonication

Low-energy sonication was applied to the MPA suspensions. All MPA suspensions exhibited a significant reduction in mean particle size after 60 seconds of sonication. Extending the sonication duration beyond 60 seconds (up to 120 seconds) did not result in further reduction of particle size (Figure 3B).

Figure 2. Graphical representation of deconvoluted mass spectra of PEG3350 for: (A) Spectrum Chemicals; (B) BASF; (C) Lab alley; (D) Dow Chemical Company. Deconvolution was performed using UNIDEC deconvolution tool.

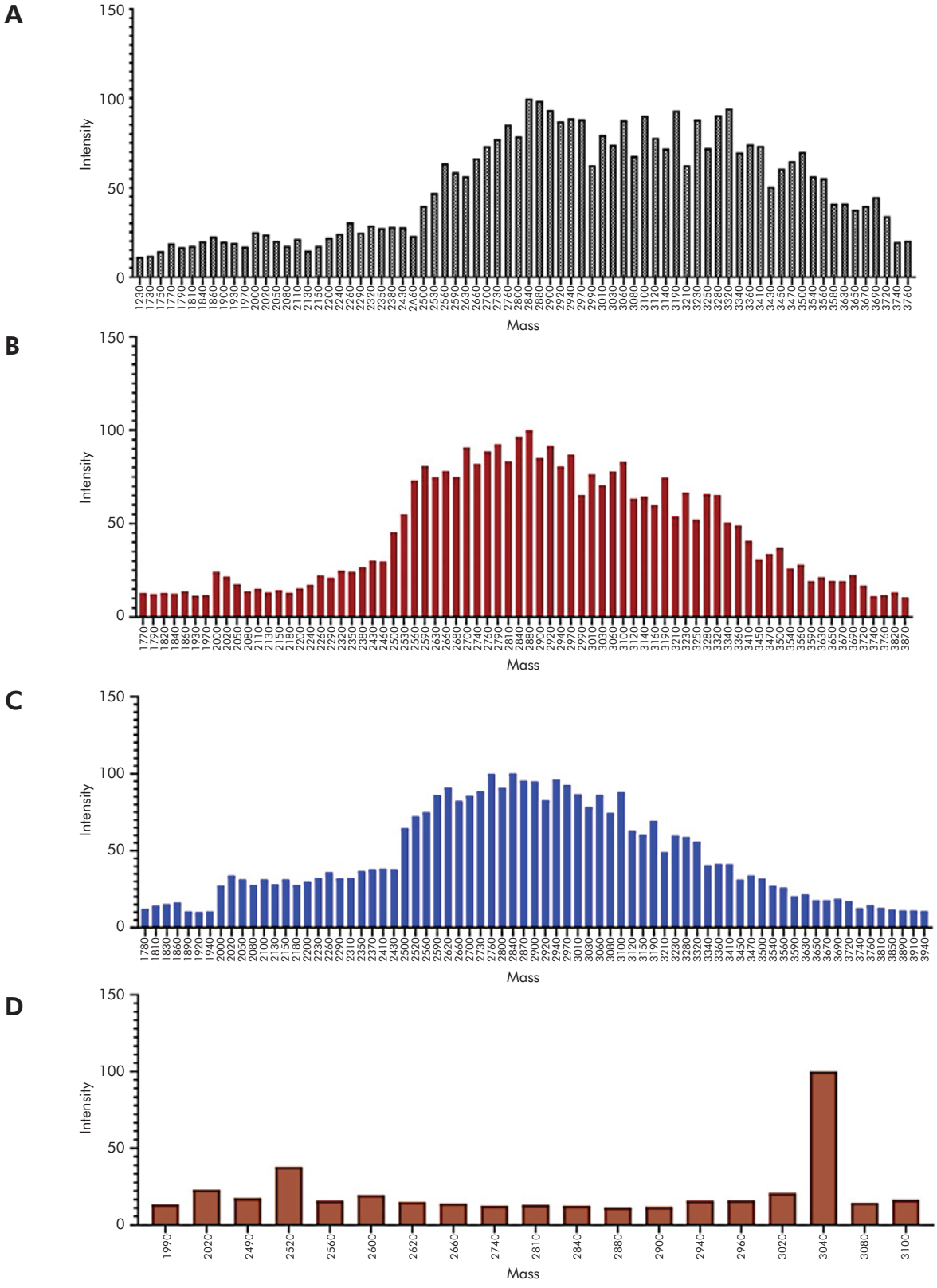
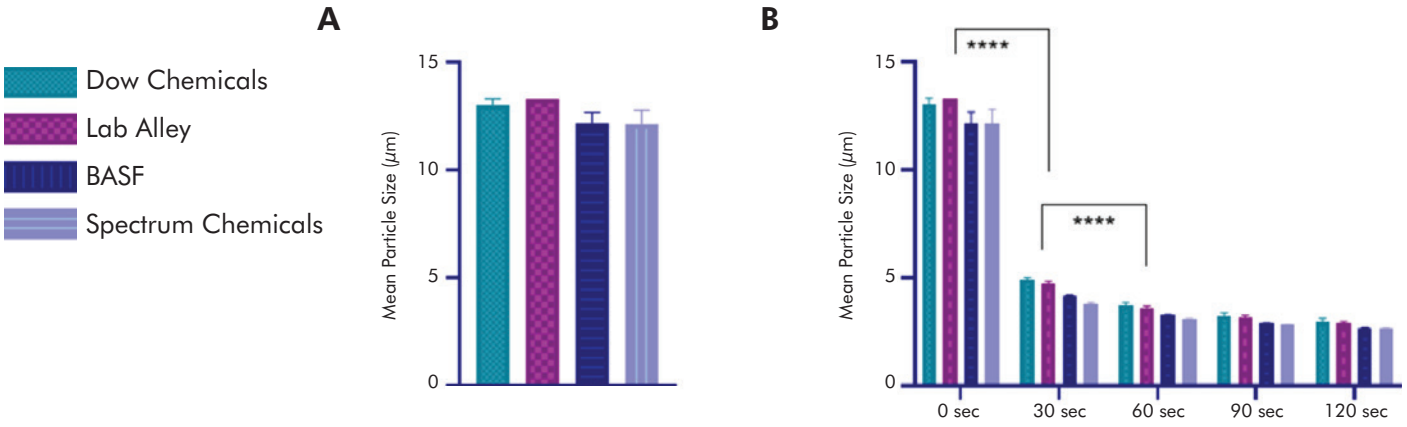


Figure 3. Graphical representation of: (A) mean particle size of formulations prepared using varying sources of PEG3350; (B) mean particle size of formulations prepared using varying sources of PEG3350 equivalents with the application of in situ sonication at intervals of 30 secs for two minutes. Particle size analysis was performed using a MasterSizer™ (Malvern Instruments, UK). All data are presented as mean ± SD, n = 3. Statistical analysis was performed using a mixed-effects analysis with multiple comparisons. In (B), the reduction in particle size was significant between 0 and 30 sec and between 30 and 60 sec (****P < 0.0001).



With shaking

All MPA suspensions (DC, LA, SC, and BC) exhibited a significant reduction in mean particle size following vigorous shaking for 60 seconds. Among the four formulations, the suspension prepared with BC PEG3350 demonstrated the greatest reduction in particle size, decreasing from an initial mean particle size of approximately 12 µm to 6.5 µm after shaking (Figure 4A).

With pipette and syringe

When the suspensions were introduced using 14G and 18G needles, the measured particle sizes were comparable to those

Figure 4. Graphical representation of mean particle size: (A) initial and after shaking the suspensions for one minute, the suspensions were introduced into the particle sizing well using a pipette; and (B) after introducing the suspensions into the particle sizing well using either a pipette or syringe. Particle size analysis was performed using a MasterSizer™ (Malvern Instruments, UK). All data are presented as mean ± SD, n =3. Statistical analysis was performed using a two-way ANOVA with multiple comparisons. In (A), particle size decreased significantly after 60 sec of shaking (****P < 0.0001). In (B), differences between pipette, 14-G, and 18-G needle introduction were non-significant (ns), whereas passage through the 22-G needle produced a significant reduction in particle size (****P < 0.0001).

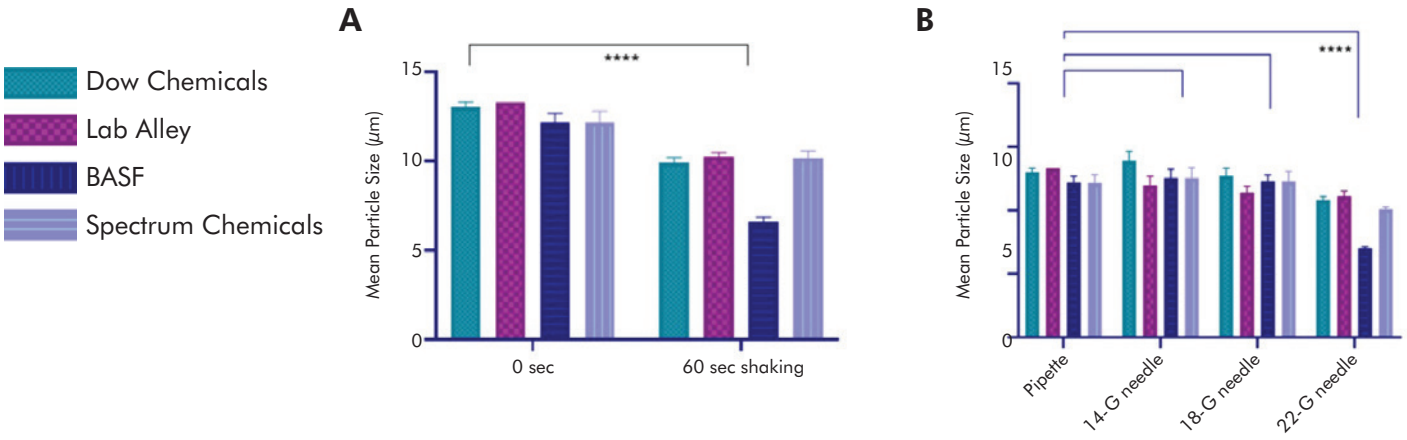


Table 2. Sedimentation behavior of Depo-Provera® Q1/Q2-equivalent LAI suspensions prepared with PEG3350 from four vendors. Sedimentation value is the ratio of final sediment volume (after 7 days) to initial suspension volume.

Polymer Source	Initial Volume	Final Volume (7 days)	Sedimentation Value
Dow Chemicals	0.96 mL	0.91 mL	0.95
Lab Alley	0.96 mL	0.81 mL	0.84
BASF	1 mL	0.81 mL	0.81
Spectrum Chemicals	1 mL	0.85 mL	0.85

obtained via pipette loading. However, when the suspensions were passed through a 22G needle, a significant reduction in mean particle size was observed. Among all samples, the MPA suspension prepared with BC PEG3350 exhibited the most pronounced decrease in particle size from approximately 12 μm to 7 μm following passage through the 22G needle (Figure 4B).

Sedimentation Volume

The sedimentation behavior of MPA suspensions prepared using PEG3350 polymers from different sources was evaluated over a 7-day period (Table 2). All formulations exhibited measurable sedimentation, with the extent varying depending on the polymer source (Figure 5). The suspension prepared with Dow Chemical Company PEG3350 retained a sedimentation volume ratio (F = 0.95) after 7 days. Suspensions prepared with Lab Alley and BASF PEG3350 demonstrated F values of 0.84 and 0.81, respectively. The Spectrum Chemicals PEG3350 suspension showed an F value of 0.85.

Rheology

All four MPA suspension formulations exhibited pseudoplastic (shear-thinning) flow behavior across the shear rate range investigated (Figure 6). At low shear rates, all formulations displayed elevated viscosity, which decreased progressively with increasing shear rate, indicating a shear-induced disruption of the weakly bound drug particle-polymer networks. Although the overall viscosity profiles were qualitatively similar

Figure 5. Visual comparison of the sedimentation study at Day 0 (left) and Day 7 (right) for the four different formulations labeled DC, LA, BASF, and SPEC.

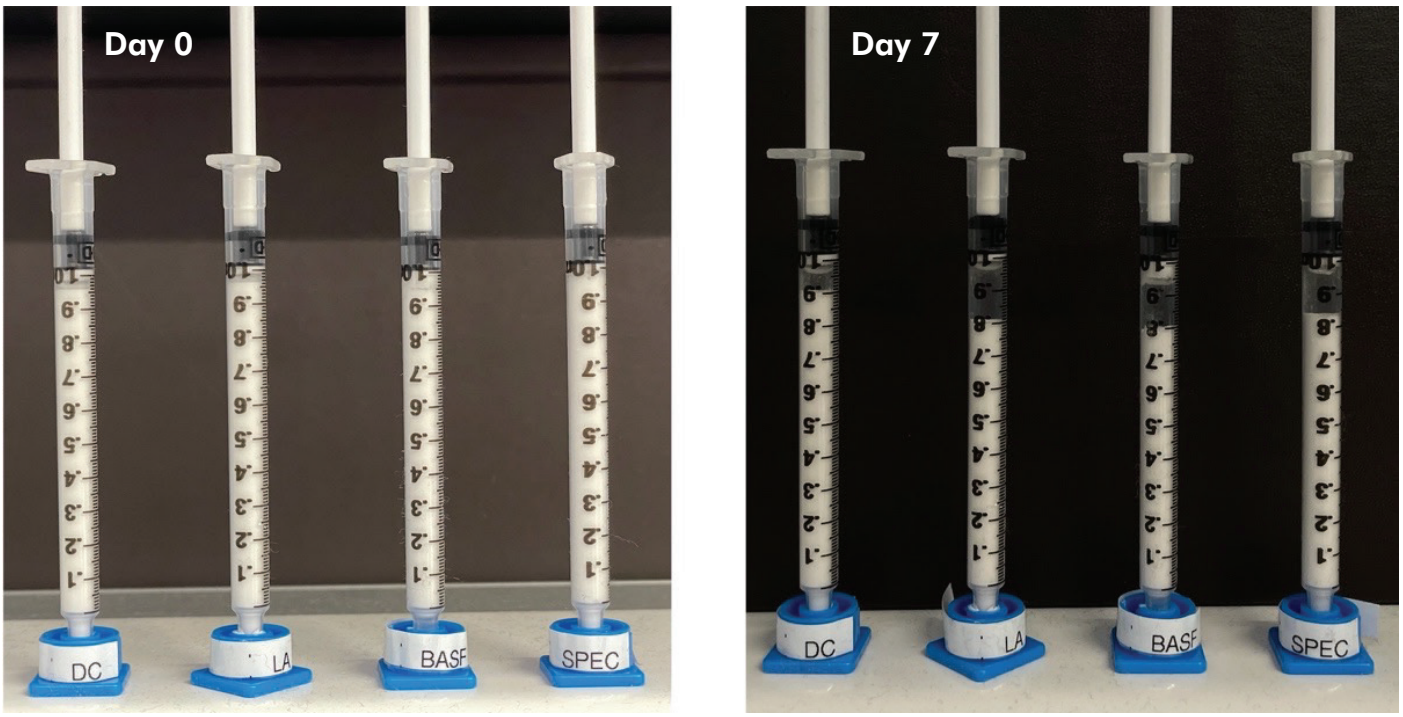


Figure 6. Viscosity profiles of MPA suspensions prepared using PEG3350 from varying vendor sources (Spectrum, BASF, Lab Alley, and Dow Chemical Company®) as a function of shear rate. Data are presented as mean ± SD (n= 3).

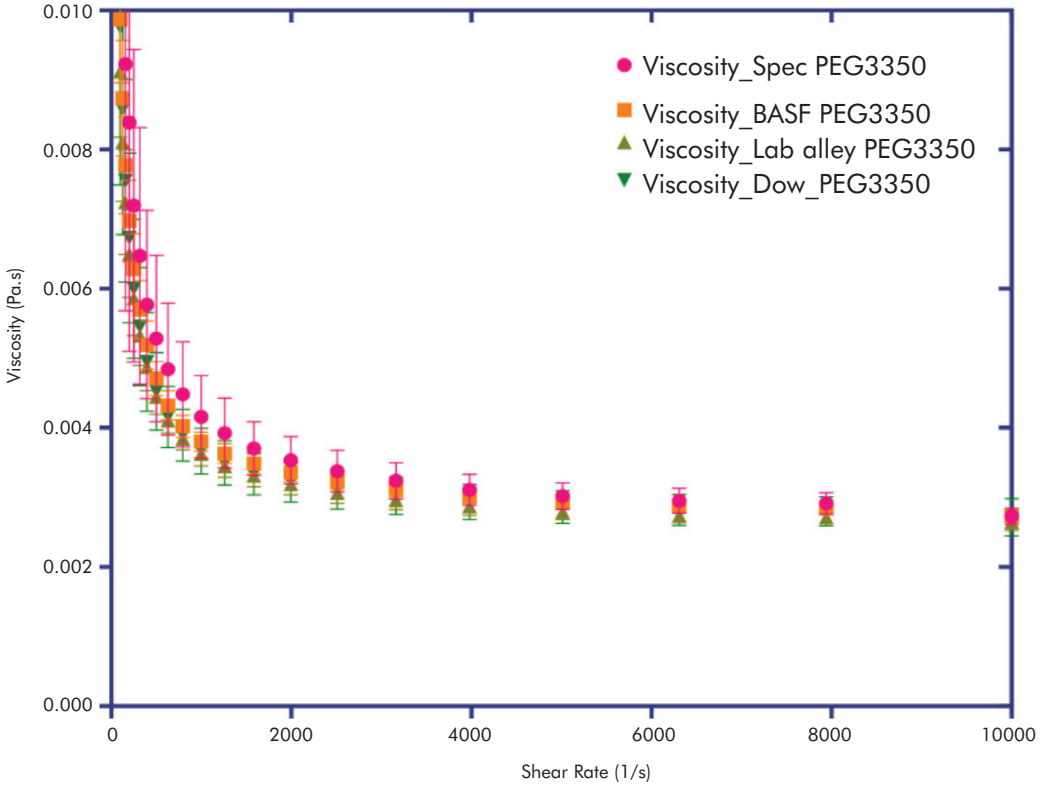
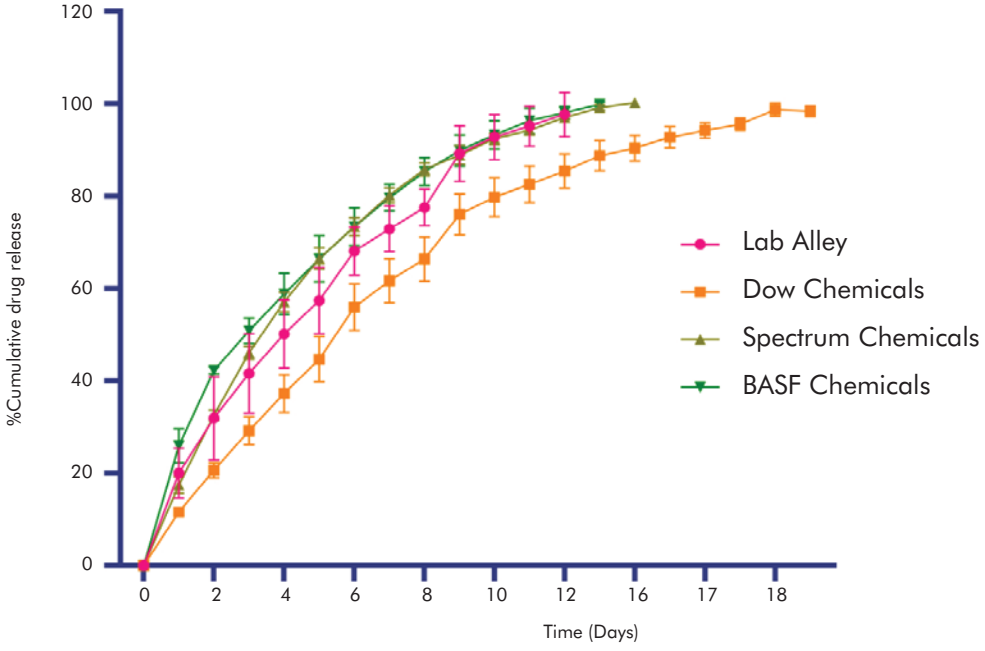


Figure 7. Graphical representation of in vitro drug release profiles of MPA suspensions prepared using PEG3350 from varying vendor sources. Drug release studies were performed in 1% w/v SDS dissolution medium at 37 ± 0.5°C and a flow rate of 8 mL/min. Data are presented as mean ± SD (n = 3).



across formulations, subtle differences in low-shear viscosity were observed. Among the evaluated formulations, the suspension prepared with PEG3350 from Lab Alley (LA) exhibited the highest viscosity at low shear rates, whereas those formulated with PEG3350 from Dow Chemical Company (DC) and BASF (BC) displayed comparatively lower values. The suspension containing PEG3350 from Spectrum Chemicals (SC) showed an intermediate viscosity profile (Figure 6). These rheological differences were most pronounced under low-shear conditions and progressively diminished at higher shear rates, where the viscosity profiles of all four formulations converged.

In vitro drug release

In vitro drug release studies of the MPA suspensions were conducted using a novel adapter setup with a USP Apparatus Type IV (flow-through cell system). Despite all four formulations exhibiting comparable particle size distributions and being Q1/Q2 equivalent to the reference listed drug, Depo-Provera®, distinct differences in the release profiles were observed depending on the polymer source. The MPA suspension prepared with PEG3350 from BASF (BC) demonstrated a slightly faster initial drug release over the first five days compared to suspensions formulated with PEG3350 from

Spectrum Chemicals (SC) and Lab Alley (LA) (Figure 7). In contrast, the formulation containing PEG3350 from Dow Chemical Company (DC) exhibited the slowest release rate among all samples (Figure 7). The F2-value for the pairwise comparison of formulations is given below. (Table 3)

Table 3. F2-value for the pairwise comparison of formulations

Pairwise comparison	F2 similarity factor	Profile
DC vs SC	38.2	Dissimilar
DC vs LA	46.3	Dissimilar
DC vs BC	35.4	Dissimilar

DISCUSSION

LAI suspensions are dispersed systems stabilized with surfactant and polymeric stabilizers. Hydrophilic polymers such as PEGs are commonly used in the formulation development of these drug products to confer dispersibility of the hydrophobic API in the aqueous medium (11) (12) (4). PEG is synthesized through ring-opening polymerization of ethylene oxide (EO) via a process called ethoxylation (13); nearly all commercially available PEG3350 is produced by the anionic route with an alkaline catalyst. Although the fundamental synthesis method is similar across manufacturers, differences in process control, catalyst handling, purification steps, and raw-material quality can introduce variability. Such variability may affect the relative proportions of monomeric, oligomeric, and higher-molecular-weight polymeric species present in the final PEG product.

In the current study, PEG3350 samples obtained from different vendors were evaluated using physicochemical characterization techniques to understand their compositional and structural differences. Thermal analysis revealed notable differences in molecular weight–dependent degradation behavior among the PEG3350 samples from different vendors. PEG3350 from Spectrum and Dow Chemical Company exhibited unimodal DTG profiles, indicative of relatively narrow molecular weight distributions and uniform thermal degradation behavior. In contrast, the PEG3350 sample from LA displayed a distinctly bimodal DTG pattern, suggesting the presence of two major polymer fractions lower-molecular-weight oligomers that degrade at lower temperatures and higher-molecular-weight chains that decompose at higher temperatures. Whereas PEG3350 sourced from BASF showed a broad, shallow decomposition peak, reflecting a much wider molecular weight distribution containing low-, medium-, and high-molecular-weight species. Furthermore, the molecular weight distributions of all samples fell within a relatively narrow range, indicating comparable overall chain lengths across suppliers, though the Dow sample exhibited the highest Mw and the BASF sample the lowest, representing an approximately 11% variation.

To further understand the differences in molecular chain populations among PEG3350 samples from different vendors, LC/MS-based molecular fraction analysis was performed. The PEG3350 from Spectrum displayed a positively skewed distribution, indicating that most molecular species were concentrated at the higher molecular-weight end of the spectrum, consistent with its narrow, unimodal thermal degradation profile. In contrast, samples from BASF and LA showed distributions centered predominantly in the medium molecular weight range, suggesting a more balanced population of intermediate-length polymer chains, in line with the broad and bimodal thermal profiles observed for these samples, respectively. The DC sample contained a substantial proportion of higher molecular-weight fragments, consistent with a molecular weight distribution extending toward longer polymer chains. Collectively, these LC/MS findings align with the thermal degradation profiles, reinforcing that vendor-to-vendor variability arises from differences in polymerization processes, which ultimately influence the molecular architecture of PEG3350.

The physicochemical characterization of the Q1/Q2 equivalent formulations to Depo-Provera® revealed notable differences in particle–polymer agglomerate behavior depending on the PEG3350 vendor source. These differences in molecular architecture were further reflected in the rheological behavior of the resulting suspensions. All four formulations exhibited pseudoplastic flow behavior, with high viscosity at low shear rates arising from the formation of a flocculated particle network stabilized by weak interparticle interactions and polymer chain entanglements. The LA formulation, prepared with PEG3350 which had a bimodal molecular weight distribution, exhibited the highest low-shear viscosity, consistent with greater specific surface area, enhanced polymer adsorption, and a more extensive interparticle network. Formulations prepared with DC and BASF PEG3350 displayed comparatively lower low-shear viscosities, in agreement with their larger particle sizes and reduced surface interactions, while the Spectrum formulation showed intermediate behavior. Importantly, these viscosity differences diminished at high shear rates, indicating that the PEG3350 source primarily

influences suspension microstructure and interparticle interactions rather than bulk flow properties under high-shear conditions.

These differences in interparticle network strength were further manifested in agglomerate behavior. Formulations prepared with PEG3350 from DC, which contained a higher proportion of long-chain molecular fragments, formed agglomerates with greater structural integrity, as reflected in improved flocculation behavior and higher sedimentation values, suggesting the formation of stronger, more cohesive particle–polymer networks. In contrast, formulations containing BASF PEG3350 produced weaker agglomerates, as evidenced by the pronounced reduction in particle size observed when the suspension was introduced through a 22G needle. The sonication studies further demonstrated that all suspensions contained loosely bound agglomerates susceptible to low-energy disruption, reaching a dispersion equilibrium after just 60 seconds, with no further breakdown beyond that point regardless of polymer source. Similarly, shaking studies confirmed that BC-based agglomerates were the most susceptible to mechanical disruption, consistent with weaker interparticle networks.

The *in vitro* release data demonstrated that these differences in particle–polymer agglomerate structure can significantly influence drug release behavior from LAI suspensions. Conventional *in vitro* release methods often fail to capture these differences since they require dispersing the suspension directly into the release medium (14) which disrupts agglomerate architecture and minimizes the functional contribution of polymeric excipients. As a result, the influence of excipient–particle interactions are effectively lost. In the current study, a previously reported (3) novel adapter design was employed, which preserved the agglomerate structure during testing and enabled a more mechanistic assessment of release behavior. Using this approach, the formulation prepared with PEG3350 from Dow Chemicals Company (DC), which exhibited stronger particle-polymer agglomerates and correspondingly higher network resistance at low shear, showed slower drug release. This behavior was attributable to enhanced polymer-particle interactions within the agglomerate matrix that slow the liberation of individual particles. Conversely, formulations prepared with BASF PEG3350, which formed weaker agglomerates and displayed lower initial viscosity, exhibited faster initial drug release due to rapid disintegration of agglomerates, leading to quicker particle liberation and accelerated early-phase release. These findings highlight that subtle variations in PEG3350 molecular weight distribution propagate through suspension microstructure as evidenced by rheological, agglomerate, and release data and ultimately alter the drug release profile of LAI suspensions.

As a widely used pharmaceutical excipient, PEG3350 is subject to a USP–NF monograph (15), which specifies an apparent weight-average molecular weight within 3015–3685 g/mol determined by GPC/SEC. Notably, the approximately 11% variation in Mw observed across vendors falls within this compendial range, yet the samples differed substantially in the modality and breadth of their molecular weight distributions. This highlights a key limitation of the current monograph: because both weight-average molecular weight and polydispersity are bulk descriptors, they define the central tendency and spread of the distribution but not its shape. Consequently, PEG3350 lots that are fully compliant with compendial specifications may still differ in the relative abundance of specific oligomeric and polymeric fractions. As shown in this study, such differences propagate into suspension microstructure and drug release. These findings suggest that distribution-level characterization beyond compendial requirements may be warranted when selecting PEG3350 for LAI suspensions and in Q1/Q2 sameness assessments.

While the present study focuses on LAI suspensions, the underlying principle that PEG functional performance can be governed by molecular weight distribution rather than nominal molecular weight alone is likely relevant to other PEG-containing drug products. This may be particularly important for PEG conjugates, which have attracted renewed interest in recent years. In PEGylated proteins, peptides, and small molecules, PEG chain length and dispersity directly influence hydrodynamic size, circulation time, and pharmacokinetics, and lot-to-lot variability in molecular weight distribution could contribute to conjugate heterogeneity and, potentially, to variability in immunogenicity, particularly in light of growing concerns regarding anti-PEG antibodies (16). Similarly, in PEG-lipid–containing nanoparticle systems such as lipid nanoparticles (LNPs), the chain length of the PEG moiety modulates particle size, colloidal stability, and PEG-shedding kinetics, thereby affecting circulation and delivery (17). In each of these applications, the vendor-dependent variability characterized here for PEG3350 could translate into differences in critical quality attributes, reinforcing the broader need for distribution- resolved PEG characterization wherever polymer architecture is mechanistically linked to product performance.

CONCLUSIONS

This study demonstrates that PEG3350 sourced from different vendors, though nominally identical and compendially compliant, is not necessarily functionally interchangeable in long-acting injectable suspensions. Comprehensive physicochemical characterization revealed meaningful vendor-to-vendor differences in molecular weight, polydispersity, and molecular fraction distribution, with weight-average molecular weights spanning an approximately 11% range and thermal degradation profiles varying from unimodal (Dow, Spectrum) to bimodal (Lab Alley) and broad (BASF). These differences reflect underlying variability in polymerization and purification processes rather than any deviation from grade specifications. Critically, these compositional differences propagated through the suspension microstructure of Q1/Q2 equivalent formulations of Depo-Provera®. PEG3350 with a higher proportion of long-chain species (Dow) produced stronger, more cohesive particle–polymer agglomerates, evidenced by higher sedimentation volume and greater shear resistance, whereas PEG3350 with a broader, lower molecular-weight-skewed distribution (BASF) formed weaker agglomerates that were readily disrupted by shear. These structural differences translated directly into distinct *in vitro* release behavior, with the Dow formulation exhibiting the slowest release and the BASF formulation the fastest early-phase release, despite comparable particle size distributions and identical qualitative and quantitative composition. Importantly, these performance differences were only resolvable using a novel USP Apparatus IV adapter that preserved agglomerate architecture during testing; conventional dispersion-based methods would have masked the functional contribution of the polymeric excipient. Taken together, these findings establish PEG3350 source as a critical material attribute for LAI suspensions and underscore that Q1/Q2 sameness alone does not guarantee equivalence in product microstructure (Q3) or release performance. This has direct implications for generic LAI development, where excipient source variability should be characterized and controlled to ensure consistent, bioequivalent product performance.

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ACKNOWLEDGEMENTS

This work was financially supported through the Pfizer Distinguished Chair of Pharmaceutical Technology, Department of Pharmaceutical Sciences, University of Connecticut.