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ASSESSING THE STABILITY AND COMPATIBILITY OF BEVACIZUMAB IN FORMULATION WITH LAVENDER OIL

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Abstract

Background: Bevacizumab, a monoclonal antibody widely used to treat cancer and eye disorders. It inhibits tumor angiogenesis by targeting Vascular Endothelial Growth Factor (VEGF), thus inhibiting the blood supply to the tumor cell. It has issues of stability with different additives during formulation and storage. Lavender essential oil is popular due to its antioxidant and antimicrobial properties, which may provide a novel strategy for enhancing protein structure in injectable preparations.

Objective: The study aimed to investigate the fitness and stability of Bevacizumab, when combined with lavender oil. The new emulsion-based parenteral product was designed to preserve the physicochemical and biological stability of Bevacizumab even when stored over an extended period of 3 months.

Methods: Lavender oil (0.5%, 1.0%, and 2.0%) of 3 different concentrations was added to the formulation. The 3 samples were subsequently stored at refrigerated (4°C), at normal room temperature (25°C) and at accelerated temperatures of 40°C for 3 months. The stability test of all 3 samples was conducted using High-Performance Liquid Chromatography (HPLC), which determined the monomer content, the percentage of degradation, and the peak area. Various auxiliary analysis techniques, including pH determination, macroscopic analysis, particle size determination, and FTIR spectroscopy, were employed to identify any shifts in physicochemical properties.

Results: Composition containing lavender oil in low concentration was more stable at refrigeration temperatures and had low degradation rates, ascertaining its potential to become a natural preservative in antibody-derived injectable biologics. No significant alteration in pH, particle size, or FTIR spectral characteristics was observed, but showed minimal degradation under accelerated temperature conditions.

Conclusion: The physicochemical stability of bevacizumab was maintained by the incorporation of lavender oil during storage. These findings highlight that lavender oil is compatible with bevacizumab in low concentration specially at refrigeration temperature.

Keywords: Bevacizumab, Compatibility, Emulsion, FTIR, HPLC, Lavender oil, Stability

INTRODUCTION

Bevacizumab, a monoclonal antibody that inhibits tumor angiogenesis by targeting Vascular Endothelial Growth Factor (VEGF) (1). While bevacizumab is widely used to treat cancer and eye disorders such as diabetic retinopathy and age-related macular degeneration (AMD), it has issues with stability and compatibility with different additives that are utilized to maximize drug delivery (2). Bevacizumab is a clear, colorless solution administered through I.V route (3).

Bevacizumab acts by inhibiting the binding of VEGF to its cell surface receptors. This inhibition leads to a reduction in microvascular growth of tumor blood vessels and thus limits the blood supply to tumor tissues (4).



However, Lavender essential oil is derived from the flowers of the *Lavandula angustifolia* plant, is well known for its anti-inflammatory, anti-microbial, and antioxidant effects (Obanor, 2020) (5). Lavender oil is rich in bioactive molecules such as linalyl acetate and linalool (6).

The lipophilic characteristic of Lavender oil may modify the formulation aspects (pH and ionic strength), which is essential in enhancing the structural stability of monoclonal antibodies like bevacizumab. The deviations in these parameters can cause protein denaturation or even enhance aggregation (7). Since the antioxidant activity of lavender oil is associated with various antitoxic and anti-aging effects, it should be studied in detail regarding its chemical reactivity and the effect on hydrophilic regions of proteins (8).

Though bevacizumab has been a mainstay in the treatment of cancers globally, adding lavender oil both as an ancillary compound that possibly has pharmacologic effect requires extensive stability and compatibility studies. These assessments are important to ensure that active ingredients do not interact in ways that compromise the drug's efficacy or safety (9).

Bevacizumab is an anti-VEGF monoclonal antibody used extensively as a therapeutic drug against both cancers and ocular condition. The formulation of bevacizumab in a pharmaceutical product may be affected by environmental conditions, including exposure to light, temperature, or even excipients or other active compounds (10). Essential oils have become a topic of growing interest in new drug delivery systems, as they offer beneficial pharmacological and stabilizing effects (11). One of the essential oils that possess the anti-inflammatory, anti-microbial, antiseptic and antioxidant activity of lavender oil is associated with various antitoxic and anti-aging effects (12).

This study was designed to evaluate the compatibility and long-term stability of bevacizumab in formulations that contain lavender oil.

MATERIALS AND METHODS

This study was employed to evaluate the stability and compatibility of Bevacizumab combined with lavender oil under controlled conditions in Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta, Pakistan and Provincial Drug Testin Lab (DTL), Quetta, Pakistan. A total of 3 emulsion samples were analyzed focusing on parameters like appearance, pH, clarity, and protein integrity using HPLC and FTIR.

Materials used for the study was bevacizumab (Avastin®), lavender oil (pharmaceutical-grade) To enhance the clinical relevance basic tests are involve i.e. acute toxicity study, cytotoxicity assay, subchronic toxicity study and hemocompatibility assessment and I have use Lavender oil of pharmaceutical grade whose safety was already tested, Polysorbate 80, glycerol, PBS, and WFI. All materials and equipment were handled under standard laboratory protocols. Key phases include formulation preparation, pre-formulation studies, optimization, characterization, stability assessments, and physicochemical evaluations.

PROCEDURE

Events of drug-drug interactions were also performed between Bevacizumab and lavender oil before formulation procedures were carried out. These tests were conducted on the key parameters of solubility, pH stability, and partition coefficient to ensure that there are no structural changes to the drug and that it remains efficacious in an oil-in-water emulsion system.

The preparation of Bevacizumab lavender oil emulsion was carried out sequentially, and the aqueous foundation was formed by mixing glycerol (0.5 mL) and Polysorbate 80 (0.02 mL) into the phosphate-buffered saline (PBS) with pH ~7.0. Lavender oil was added at varying concentrations (0.5%, 1.0%, and 2.0%) under vigorous stirring to form a stable emulsion. Water for Injection (WFI) was then added to bring the volume close to 9.6 mL. Bevacizumab (0.4 mL of 25 mg/mL stock) was aseptically incorporated to achieve a final concentration of 1 mg/mL. The total volume was adjusted to 10 mL.

The Bevacizumab-lavender oil emulsion was developed as a sterile, injectable oil-in-water system, optimized for drug: oil: surfactant ratios to ensure physicochemical stability and protein integrity. Lavender oil was added to bevacizumab (final concentration of 1 mg/mL) at volumes of 0.5%, 1.0%, and 2.0% (w/v) to formulations with a constant concentration of 0.2% Polysorbate 80 and 5.0% glycerol, to stabilize the

emulsification and tonicity. As the aqueous phase, phosphate-buffered saline (PBS) was used, and Water for Injection (WFI) was used to complement it. Formulations were prepared aseptically in a laminar airflow hood under sterile conditions, with temperatures maintained at 2 °C to 8 °C. The resulting emulsions were transferred into sterile amber vials and stored under stability conditions to assess their stability.

PHYSICAL APPEARANCE AND COLOR EVALUATION

Physical integrity of formulation was visually inspected under ambient light and under UV light.

TURBIDITY AND CLARITY ASSESSMENT

The quantitative measurement of turbidity of a formulation was done using a nephelometric turbidity unit (NTU) device that measures the light-scattering behavior of a sample.

PHASE SEPARATION AND MONITORING

Formulations were subjected to centrifugation at 3000 rpm for 15 minutes to accelerate potential phase separation. Samples were then visually inspected for the appearance of distinct oil or aqueous layers, creaming, or sedimentation.

VISCOSITY TESTING USING CAPILLARY VISCOMETER

The test was conducted at 25°C using distilled water as the reference fluid. After calibrating the viscometer, flow times were recorded for each formulation containing 0.5%, 1%, and 2% lavender oil. The relative viscosity was calculated as the ratio of sample flow time (t_1) to reference time ($t_0 = 52.07$ sec).

PARTICLE SIZE ESTIMATION VIA OPTICAL MICROSCOPY OR IMAGE ANALYSIS

The homogenization of formulations was done first and diluted (1:10) using PBS to increase visibility. Where there is phase separation centrifugation at 3000-5000 rpm 5 minutes was used to separate the aqueous phase. Each diluted sample was added to a clean microscope slide in a small aliquot (10–20 μ L) and covered with a slip and viewed under 400x-1000x magnification. Various areas (5-10 per slide) were scanned, after particle size determination the samples of lavender oil were compared with experimental control formulation.

pH MEASUREMENT

pH measurement was conducted using pH paper strip and pH meter. The resulting value was matched with a standard chart.

FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

The biphasic system was homogenized by vortex and centrifuged, 10000 rpm with a 10-minute duration to determine the aqueous phase. The analysis was carried on three sets and these were pure Bevacizumab, lavender oil and the mixture of the formulations. The spectroscopic details recorded at attenuated total reflectance (ATR) mode of FTIR in the wavelength range of 4000–400 cm^{-1} was with a scan resolution of 4 cm^{-1} and an up to 32 scans per sample was done to give an accurate and reproducible result.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSIS

High-Performance Liquid Chromatography (HPLC) was utilized to evaluate the chemical stability of Bevacizumab, in formulations containing varying concentrations of lavender oil (0.5%, 1.0%, 2.0%). The method enabled detection of potential degradation and assessment of monomer integrity, following ICH Guidelines Q1A(R2) and Q1B.

The stability was tested by keeping track of retention time (~10.1 min) as well as the area under the curve (AUC) of the primary Bevacizumab monomer peak.

RESULTS



To evaluate the compatibility, solubility, and physicochemical behavior of Bevacizumab in oil-in-water emulsions with different levels of lavender oil (0.5%, 1%, and 2% w/v), different tests were carried out.

PREFORMULATION STUDIES

Bevacizumab exhibited high solubility in phosphate-buffered saline (PBS, pH 7.4). Partition coefficient (Log P) studies using n-octanol/PBS and lavender oil emulsions confirmed the hydrophilic nature of Bevacizumab, with >90% retained in the aqueous phase across all conditions. Increasing lavender oil content from 0.5% to 2% did not significantly alter protein distribution, indicating no substantial protein-lipid interaction that could compromise therapeutic activity.

Visual inspection revealed no turbidity, precipitation, or phase separation after 24 hours at 25°C. Among the tested concentrations, 1% lavender oil formulations displayed the best clarity and physical uniformity, suggesting optimal oil dispersion.

Stability across a pH range of 4.0 to 7.4 was assessed using acetate, citrate, and phosphate buffers at 4°C and 25°C. Formulations below pH 5.0 showed slight turbidity and increased viscosity, while those buffered at pH 6.5–7.4 maintained transparency and emulsion integrity.

FTIR spectra showed preserved amide I and II bands (~1650 cm⁻¹ and ~1540 cm⁻¹), and HPLC analysis revealed intact monomer peaks, confirming the structural stability of Bevacizumab under near-neutral conditions.

Table I. Bevacizumab–Lavender oil emulsions

Lavender oil (%)	Temp (°C)	Appearance (Ambient/UV)	Turbidity (NTU)	Phase behavior	Overall stability
Sample A (0.5%)	4	Milky-white / No fluorescence	2.4	None	Excellent
	25	Opaque / No flocculation	2.6	None	Stable
	40	Slight haze / Minor UV spots	3.1	Minimal layering	Acceptable
Sample B (1.0%)	4	Uniform / No fluorescence	3.3	None	Excellent
	25	Uniform / No flocculation	3.5	None	Stable
	40	Mild haze	4.0	Slight surface film	Acceptable
Sample C (2.0%)	4	Translucent / No aggregates	4.2	None	Acceptable
	40	Spotty haze / Reduced clarity	7.0	Partial creaming	Mildly unstable

Table I highlights a notable drop in visual clarity observed at 2.0% oil and 40°C (score decreased from 5.0 to 3.8; $p = 0.002$), confirming that elevated oil content and temperature negatively affect the emulsion's appearance. Turbidity increased significantly with higher lavender oil concentrations, especially at 2.0% and 40°C ($p = 0.003$), indicating reduced clarity and stability of the formulation under heat stress. Creaming was observed only in the 2.0% formulation at 40°C ($p < 0.001$), with no phase separation at lower concentrations. Overall, phase separation was linked to high oil content and temperature, stressing the need for careful formulation.

VISCOSITY ANALYSIS

A clear trend of increasing viscosity with higher concentrations of lavender oil. The relative viscosities for the 0.5%, 1%, and 2% formulations were found to be 1.102, 1.195, and 1.308, respectively, compared to the reference buffer revealed in Fig. 1.

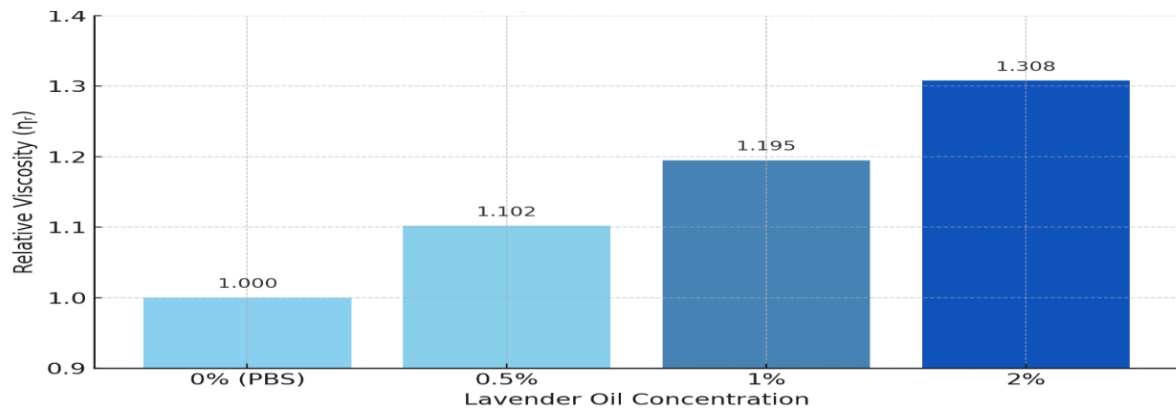


Fig. 1. Relative viscosity of Bevacizumab formulations with varying lavender oil concentrations

PARTICAL SIZE DETERMINATION

Table II breaks down microscopic analysis that show droplet size and aggregation tendencies correlated directly with the lavender oil concentration. The control sample without oil exhibited uniform spherical droplets averaging 1.25 μm in diameter. The 0.5% oil formulation showed minimal morphological change (1.40 μm), while 1% and 2% samples demonstrated moderate to significant increases in size (1.75 μm and 2.30 μm , respectively) and irregular shapes, indicative of potential coalescence and early instability. These findings suggest that the oil concentration should ideally remain at or below 1% to preserve droplet uniformity and prevent emulsion breakdown over time.

Table II. Microscopic particle size and morphology

Lavender oil concentration	Average droplet size (μm)	Morphology description
0% (Control)	1.25	Uniform spherical droplets
0.5%	1.40	Slightly enlarged, uniform
1%	1.75	Moderate coalescence
2%	2.30	Irregular, aggregated

pH MEASUREMENT

The measured pH values: Control (6.5), 0.5% (6.5), 1% (6.4), and 2% (6.3). Although there was a slight decrease in pH with increasing lavender oil concentration, the difference was not statistically significant between the control and the 0.5% or 1% groups ($p > 0.05$). A marginal significance was observed between the control and 2% group ($p \approx 0.049$), as given in Fig. 2.

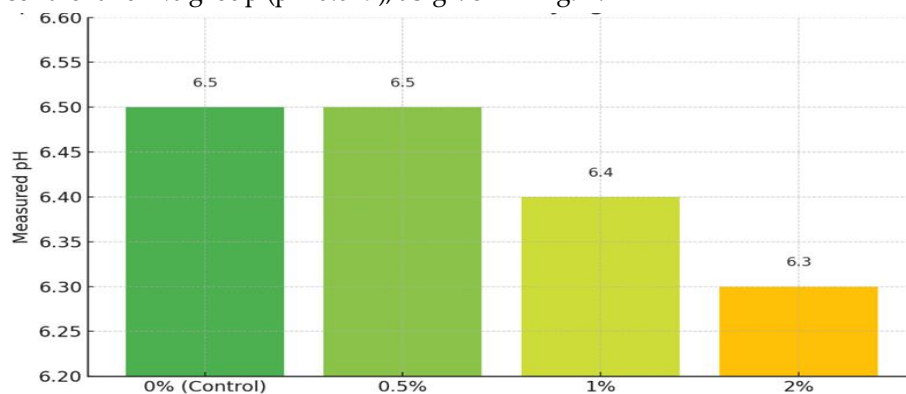


Fig. 2. pH of Bevacizumab formulation with varying lavender oil concentrations

FTIR ANALYSIS

FTIR spectra of Bevacizumab-only control (Control A) showed massive peak due to protein with broad NH/oxygen AS stretch ($\sim 3300 \text{ cm}^{-1}$), broad Amide I ($\sim 1650 \text{ cm}^{-1}$), broad Amide II ($\sim 1540 \text{ cm}^{-1}$) and broad Amide III ($\sim 1235\text{--}1300 \text{ cm}^{-1}$). These indicators confirmed that in absence of lavender oil Bevacizumab retained its native secondary structure and overall molecular integrity shown in Fig. 3.

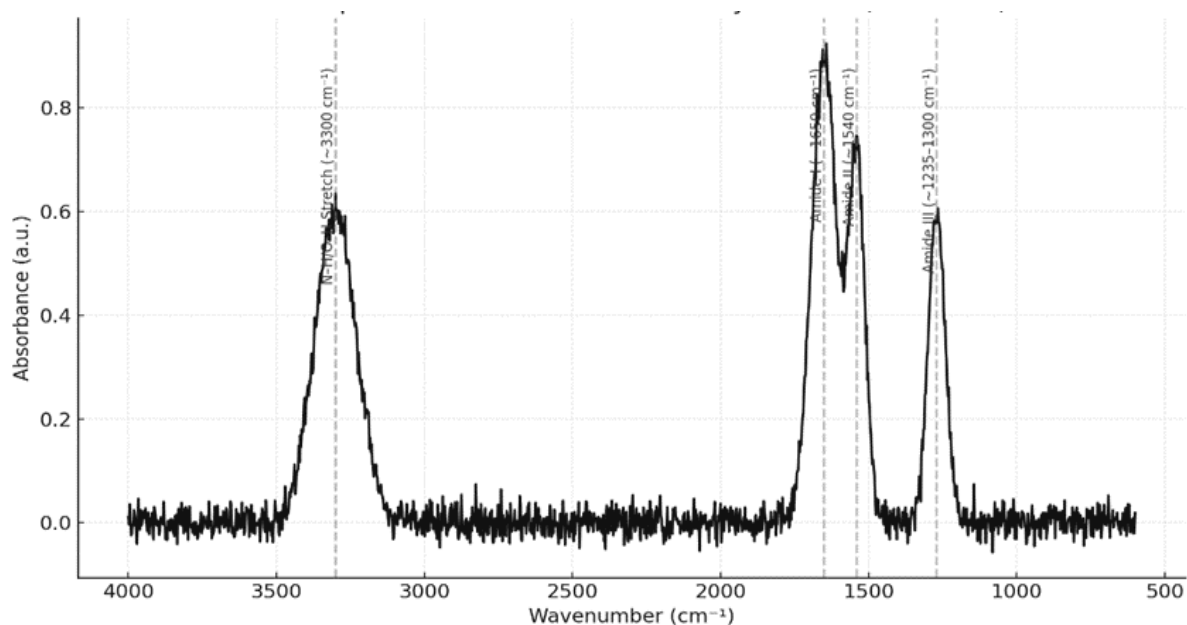


Fig. 3. FTIR spectrum of Bevacizumab only (Control A)

The FTIR profile of Lavender Oil-only control (Control B) revealed clear peaks, indicating the presence of essential oil components. The presence of alkanes was characterized by strong C-H stretching vibrations at ~ 2950 and ~ 2870 cm^{-1} . C=O stretching of ester groups was another characteristic of lavender oil observed through a sharp peak at ~ 1740 cm^{-1} . Additionally, bands at 1450 – 1370 cm^{-1} were assigned to carbon monoxide bonds of methyl and methylene groups, validating the factors that make up the chemical profile of lavender oil components revealed in Fig. 4.

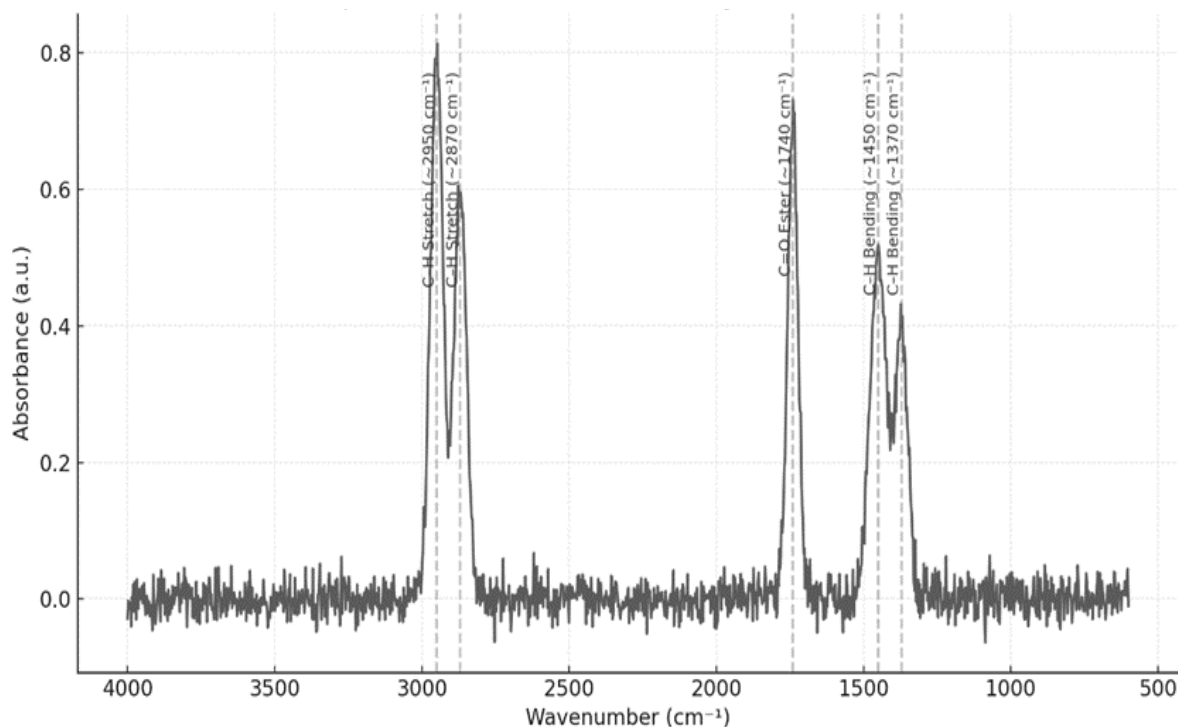


Fig. 4. FTIR spectrum of lavender oil-only (Control B)

The FTIR spectrum of Bevacizumab and Lavender Oil formulation validated the coexistence of the two components in the resultant formulation, indicating no chemical interaction. The Amide I (~ 1650 cm^{-1}) and Amide II (~ 1540 cm^{-1}) key peaks did not alter in position, as they maintained the same value, indicating that the secondary structure of Bevacizumab was not affected. Nature's peaks of lavender oil, such as the C-H stretch (~ 2950 cm^{-1}) and C=O stretch (~ 1740 cm^{-1}), were also observed, indicating its successful integration. Notably, there were no new peaks or significant spectral changes as given in Fig. 5.

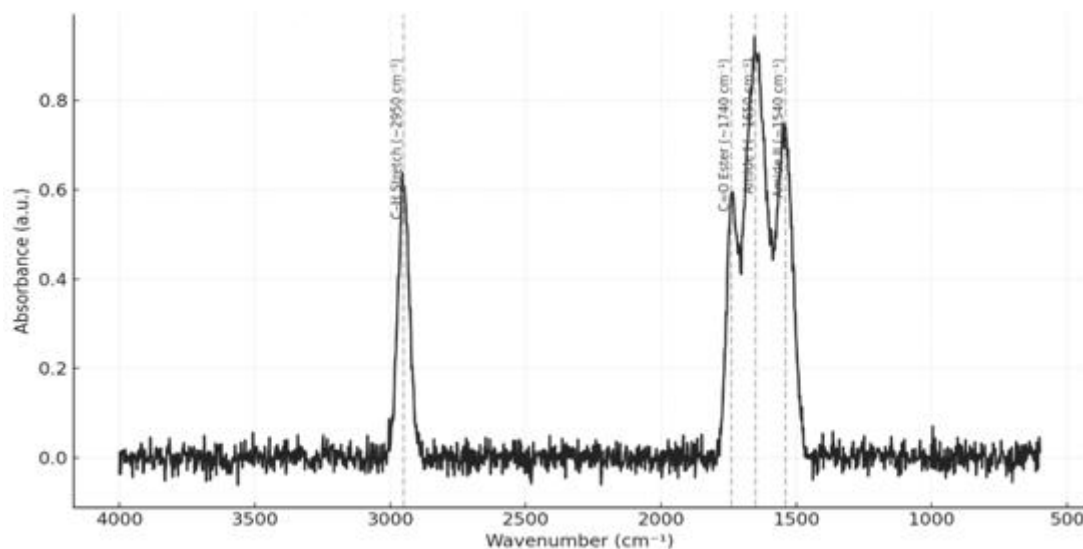


Fig. 5. FTIR spectrum of Bevacizumab-lavender oil formulation

The formulation maintained its structural integrity and did not undergo chemical disruption or interaction between the Lavender oil and Bevacizumab indicated in Table III.

Table III. FTIR Spectral analysis of Bevacizumab-lavender oil formulation

Spectral band (cm ⁻¹)	Component	Assignment	Presence in final formulation	Interpretation
~1650	Bevacizumab	Amide I (C=O stretching in peptide backbone)	Present	Indicates preserved protein secondary structure
~1540	Bevacizumab	Amide II (N-H bending + C-N stretching)	Present	Confirms no denaturation or unfolding of protein
~2950	Lavender oil	C-H stretching (alkyl chains)	Present	Confirms incorporation of lavender oil
~1740	Lavender oil	Ester carbonyl (C=O stretching)	Present	Indicates ester components from lavender oil
New/Unexpected peaks	—	—	Not detected	No chemical interaction or new compound formation observed
Shifts in amide peaks	Bevacizumab	Amide I & II	None detected	No covalent binding or structural alteration of protein

HPLC ANALYSIS

HPLC Chromatogram of Bevacizumab standard (1mg/mL), exhibits a narrow Gaussian peak at 10.1 min as given in Fig. 6. HPLC chromatogram for the Bevacizumab + 0.5% lavender oil formulation (Fresh sample), exhibiting a well-defined monomer at around 10.1 min. So this shows that Bevacizumab is formulate able, and structurally stable shown is Fig. 7. At refrigerated storage temperatures (2–8°C), the resulting formulation had the highest monomer peak area under the curve (AUC) (14,300 mAU·s) and the lowest degradation (~2.5%) as an assurance of perfect chemical stability as tabulated in Table IV.

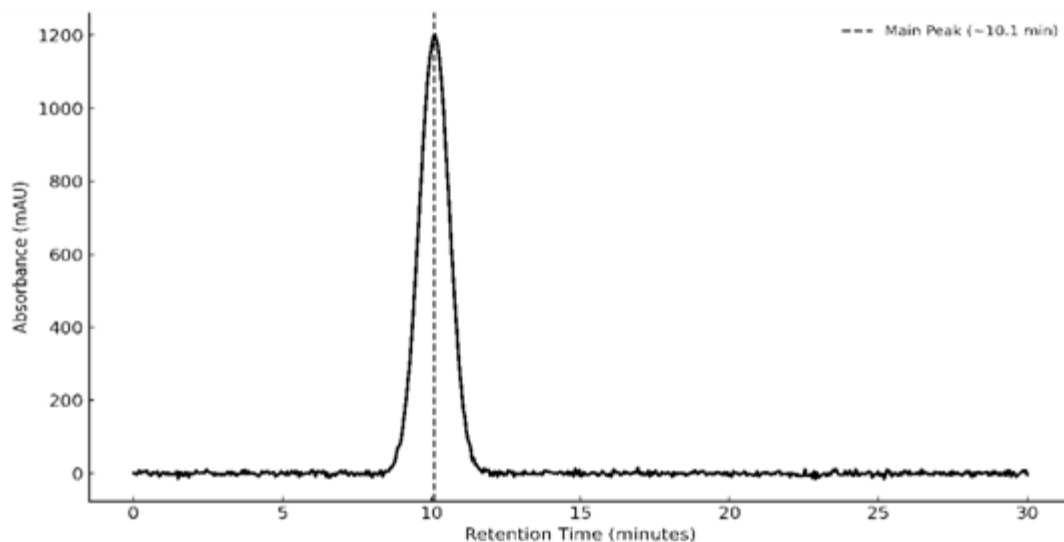


Fig. 6. HPLC Chromatogram of Bevacizumab standard (1mg/mL)

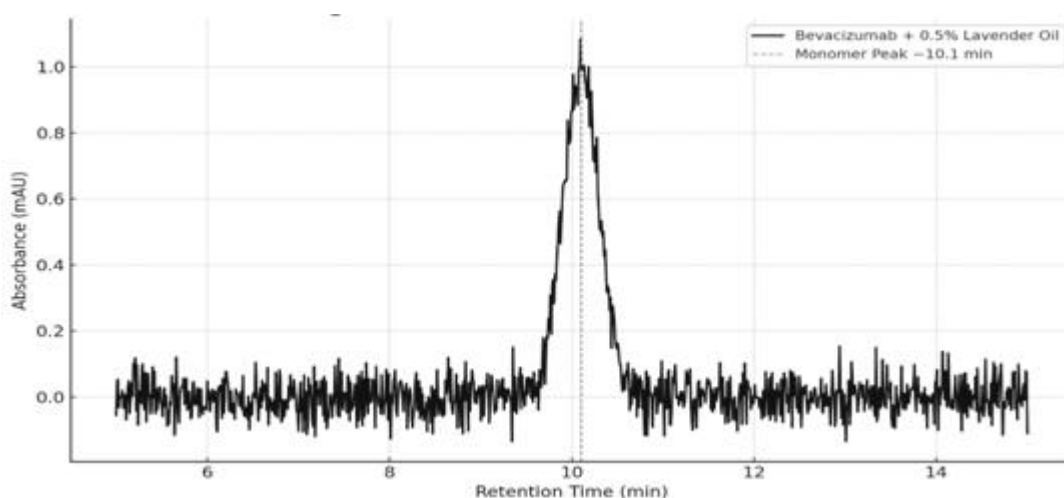


Fig. 7. HPLC chromatogram for the Bevacizumab + 0.5% lavender oil formulation

Table IV. HPLC results of Bevacizumab + lavender oil formulation under various storage conditions (30 Days)

Storage condition	Temperature	Monomer AUC (mAU.s)	Retention time (min)	% degradation	Observations
Refrigerated (Control)	4°C (2–8°C)	14,300	~10.1	~2.5%	Minor secondary peak observed; primary peak dominant; excellent stability
Room temperature	25 ± 2°C	13,600	~10.1	~4–5%	Slight reduction in monomer AUC; degradation peak present; >90% monomer retained
Accelerated	40 ± 2°C	12,800	~10.1	~8–10%	Multiple degradation peaks; broader monomer peak; evidence of aggregation

At intermediate temperature (25 ± 2°C), the minor decreases in monomer AUC (13,600 mAU.s) and increases in degradation (4-5%) were observed. Although a degradation peak appeared, the formulation still retained over 90% of the monomer content, satisfying ICH stability criteria. Thus, the formulation was considered stable under room temperature for the tested duration.

In contrast, the accelerated condition (40 ± 2°C) resulted in a noticeable decrease in monomer AUC (12,800 mAU.s) and broader peak shape, indicating partial aggregation or structural alteration. Multiple

degradation peaks were observed, and total degradation increased to ~8–10%. These findings suggest compromised stability under stress conditions, likely due to thermal sensitivity of the protein or interactions enhanced at higher temperatures.

Table V. Temperature variability assessment (3 months)

Storage condition	Lavender oil conc.	Results
Refrigerated (Control)4°C (2–8°C)	0.5% (w/v) LEO	Stable
Room temperature 25 ± 2°C	1% (w/v) LEO	Moderately stable
Accelerated 40 ± 2°C	2% (w/v) LEO	Unstable

Table VI. Summary of t-test statistical analysis for Bevacizumab–lavender oil formulation stability

Parameter	Effect of lavender oil	Key findings
Turbidity	Increased turbidity with higher oil concentration, especially at 2.0% and 40°C	Statistically significant rise at 2.0% across all temperatures ($p < 0.05$) Highly significant at 40°C: 7.0 NTU vs 4.0 ($t = 7.21$, $p = 0.003$)
Visual appearance	Reduced visual clarity and opacity at 2.0%, especially under 40°C	Visual score dropped from 5.0 to 3.8 ($t = 7.35$, $p = 0.002$) Significant decline at high oil and temperature; marginal effect overall
Phase separation	2.0% oil caused creaming at 40°C; no effect at 0.5% or 1.0%	Instability significant at 2.0% ($p < 0.001$); temp-induced changes also evident No significant overall difference among all formulations ($p = 0.087$)
pH	Slight decrease in pH with increasing oil concentration	Statistically significant ($p = 0.049$)
Viscosity	Viscosity increased with lavender oil content	Strongly significant thickening effect at 2.0% ($t = 29.83$, $p < 0.0001$)
Particle size	Increased droplet size with oil concentration; significant from 1.0% onwards	$p = 0.022$ at 1%, $p = 0.003$ at 2%; no change at 0.5% ($p > 0.05$)
HPLC stability	Oil had no major effect at low temp; degradation occurred at high temp	Monomer loss significant at 40°C ($t = 15.01$, $p = 0.0002$)
FTIR compatibility	No changes in Amide I or II bands with increasing oil	No significant structural change ($p = 0.069$)

Table V shows the summary of t-Test statistical analysis for Bevacizumab-Lavender oil formulation stability. These findings support the conclusion that no structural denaturation or significant shift in protein secondary structure occurred due to lavender oil addition.

DISCUSSION

This study explored the physicochemical stability and compatibility of Bevacizumab when formulated with varying concentrations of lavender oil under diverse storage conditions. Multiple analytical techniques including HPLC, FTIR, microscopy, pH analysis, and viscosity testing were employed to determine whether lavender oil compromises, enhances, or maintains the integrity of Bevacizumab. The results demonstrate that Bevacizumab remained chemically and physically stable when formulated with lavender oil at lower concentrations ($\leq 0.5\%$ w/v), particularly under refrigerated conditions. The emulsions

retained their clarity, pH, and uniformity over the three-month study period, with minimal turbidity or phase separation. These findings align with established protein formulation studies which emphasize the protective role of cold-chain storage in maintaining monoclonal antibody (mAb) stability (13). Notably, higher lavender oil concentrations ($\geq 1\%$) introduced visible instability under accelerated stress conditions (40°C), including phase separation, turbidity, and increased particle size suggesting a concentration-dependent destabilizing influence when exposed to heat (14).

Viscosity testing revealed a moderate yet concentration-dependent increase as lavender oil levels rose. The relative viscosity remained within acceptable limits for subcutaneous administration. However, beyond 1% oil content, the injectability may become less favorable (15).

Formulations with 0.5% lavender oil maintained small, uniform droplets ($\sim 1.4 \mu\text{m}$), whereas 2% oil samples exhibited larger, irregular, and aggregated particles ($\sim 2.3 \mu\text{m}$), reflecting early signs of coalescence and instability.

The pH measurements were used to measure periodically to test both possible degradation and chemical incompatibility (16). The pH of the formulations remained within the acceptable range of 6.3 to 6.5 across all oil concentrations. Given that Bevacizumab exhibits optimal stability in a pH range of 6.0–7.0, these results confirm that lavender oil did not compromise buffering capacity (17).

Fourier transform infrared (FTIR) spectroscopy is a widely used analytical technique that assesses the chemical composition of a drug formulation, identifying potential interactions between components that may have occurred (18). From a molecular interaction standpoint, FTIR analysis revealed no significant shifts in Amide I or Amide II bands, indicating the preservation of the secondary and tertiary structures of Bevacizumab throughout the study.

The HPLC results serve as a cornerstone of the chemical stability assessment. At 4°C , Bevacizumab retained over 97.5% of its monomeric form even after 30 days, confirming excellent stability. Room temperature storage yielded a slightly reduced monomeric AUC ($\sim 95\text{--}96\%$), while accelerated conditions (40°C) resulted in a decrease to $\sim 90\text{--}92\%$ and appearance of multiple degradation peaks.

CONCLUSION

This study explored the physicochemical stability and compatibility of a Bevacizumab–lavender oil oil-in-water emulsion for parenteral administration under various storage conditions. Using analytical tools such as HPLC, FTIR, microscopy, viscosity, and pH analysis, the research demonstrated that lavender oil can be safely incorporated at concentrations up to 0.5% (w/v) without significantly compromising the stability or structural integrity of Bevacizumab. Formulations at this level maintained clarity, acceptable viscosity, stable pH (6.0–7.0), and showed minimal degradation under accelerated temperature conditions.

However, increasing the oil concentration to 1% or higher led to pronounced instability, especially at 40°C , including turbidity, phase separation, protein aggregation, and elevated degradation. These effects were particularly evident in the 2% oil group over three months of storage.

Recommendations:

As a result, it is recommended to limit lavender oil concentration to $\leq 0.5\%$ and store the formulation at $2\text{--}8^\circ\text{C}$ to ensure long-term stability and suitability for injection. While lavender oil offers potential therapeutic benefits, including anti-inflammatory or permeation-enhancing properties, further studies—such as in vivo safety, cytotoxicity, microbial testing, and ICH-compliant stability trials—are needed to fully validate this novel delivery approach.

Future Implication:

This research provides a foundation for future development of enhanced protein formulations using functional excipients like essential oil.

Limitations of the Study:



- a) Limiting the extent of any conditions tested, and so limiting the study: it could fail to cover all possible real-world situations.
- b) The test will only be performed with the concentration of lavender oil therefore; any generalization is limited to other compositions.
- c) The study duration may not be adequate to evaluate long-term stability as well as compatibility during the entire period approach.
- d) In vitro testing via chemical interaction only (no biological effect or in vivo potential).

Conflict of interest:

There is no conflict of interest in this study.

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Authors' contribution:

FM Performed the experiments and wrote the manuscript; QI Supervised and Provided technical outputs; SAS Provided relevant literature; SM Provided some agars; AK Critical analysis; AH Proof reading.

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