MASTERARBEIT | MASTER'S THESIS

Titel | Title

Investigation of the stability and quality of heparin 5,000 IU/mL formulations using different preservatives.

verfasst von | submitted by

Mariana Almeida Lima

angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien | Vienna, 2025

Studienkennzahl lt. Studienblatt | Degree programme code as it appears on the student record sheet:	UA 066 606
Studienrichtung lt. Studienblatt | Degree programme as it appears on the student record sheet:	Masterstudium Drug Discovery and Development
Betreut von | Supervisor:	Univ.-Prof. Dr. Lea Ann Dailey
Mitbetreut von | Co-Supervisor:	Dr. Gabriela Hädrich BSc MSc

Acknowledgments

I would like to express my sincere gratitude to Professor Dr. Lea Ann Dailey, my supervisor, for the invaluable opportunity to undertake this research project. Her guidance, support, and encouragement throughout the study process were instrumental in shaping the outcome of the project. Even the most mundane conversations with her were an opportunity to learn and grow.

The completion of this work would not have been possible without the contributions of Dr. Gabriela Hädrich, my co-supervisor. Her unwavering support and guidance served as a stabilizing force throughout the project, and her assistance was indispensable. I am grateful to her for allowing me to work under her supervision and develop my abilities with her guidance and encouragement. I would also like to thank the other master's students in the inhalation lab for their consistent support and assistance throughout the research process.

I would also like to express my gratitude to my family, and in particular my mother, for the opportunity to undertake a master's program on the other side of the world. Without her unwavering support and unconditional love, this would not have been possible. Finally, I would like to thank my partner, Victor, for all the help and support he provided throughout this journey. You are all part of this project, thank you.

Disclaimer

ChatGPT, an AI language model, was used to help organize ideas, improve writing clarity, and enhance understanding of certain concepts with the prompt “Could you explain this better [concept needing clarification]”. For example, the coloration of liquids titration method from the European pharmacopeia. All content has been reviewed and validated for accuracy and originality.

Table of Contents

1. Introduction

Heparin is a widely used anticoagulant medication for coagulation disorders. The two predominant types of heparins are unfractionated heparin (UFH) and low molecular weight heparin (LMWH), which differ in several significant aspects. The UFH possesses a higher molecular weight, needs frequent monitoring and dose adjustments, and is administered intravenously or subcutaneously. The LMWH offers more predictable pharmacokinetics, allowing for fixed-dose administration without routine monitoring. LMWH also has a longer half-life, enabling once or twice daily dosing compared to UFH's multiple daily doses. While LMWH generally poses a lower risk of complications such as heparin-induced thrombocytopenia and osteoporosis, UFH remains in use due to its rapid onset of action, shorter half-life, and easy reversibility with protamine. These properties render UFH particularly valuable in situations requiring rapid anticoagulation adjustments, such as in patients with high bleeding risk or those undergoing procedures (1,2).

The side effects of UFH are attributed to the variable biochemical composition and unpredictable pharmacokinetics of the drug (3). This phenomenon is attributed basically to the UFH being a heterogeneous mixture of polysaccharide chains with varying molecular sizes, which can contribute to inconsistencies between batches (4), and the source-dependent variation. A study conducted by Banik et al., 2021 demonstrated that pharmaceutical preparations of heparin derived from different animal sources can influence the chemical composition of the resulting drugs. For example, heparins derived from bovine and porcine sources exhibit notable differences in their 1H NMR spectra and disaccharide compositions (5). The chemical inconsistencies can lead to significant challenges in the composition of the active ingredient of unfractionated heparin between different batches. The instability of unfractionated heparin can result in inconsistencies regarding pH and the degradation of components, which causes changes in coloration and turbidity of the final product after autoclavation, and can also impact in the appearance of visible particles. Such factors significantly affect medication production and require careful attention.

Preservatives are important within the pharmaceutical industry, particularly in the context of the development and production of parenteral products. These compounds can be incorporated into pharmaceutical formulations with different objectives. One of them is to prevent microbial growth in multi-dose containers, it can inhibit the growth of microorganisms that may be introduced from repeatedly withdrawing individual doses. Another reason why preservatives may be introduced into parenteral formulations is to reduce pain on injection or discomfort for the patient and it can also contribute to the overall stability of the formulation, potentially reducing degradation of active ingredients (6). One of the principal requirements for an efficacious preservative is its capacity to act rapidly and reduce microbial populations even at low concentrations (7). Multi-dose heparin formulations and other parenteral products have been preserved with benzyl alcohol to prevent microbial growth. However, concerns have been raised about the use of this preservative, particularly in neonatal patients. A study from Hiller et al., 1986 demonstrated that benzyl

alcohol has been reported to cause neurologic deterioration and death in very low birth weight infants. Consequently, regulatory agencies have encouraged the use of alternative preservatives or the absence of benzyl alcohol to minimize potential risks associated with this substance.

Other commonly used preservatives are parabens, specifically methylparaben and propylparaben, which are typically used in combination. However, the utilization of these as preservatives has also given rise to certain concerns. Parabens have been demonstrated to induce allergic reactions, particularly when administered intravenously (9). Furthermore, there are concerns regarding the potential effects of paraben exposure in neonates, particularly in the NICU setting (10). Consequently, there is a growing reluctance to utilize parabens as preservatives, particularly in more vulnerable populations.

In the domain of parenteral formulations, pH stabilizers play a pivotal role. They are employed for multiple reasons, the foremost being the necessity to preserve an optimal pH level. This is imperative to ensure that the formulation remains within the acceptable pH ranges for the various routes of administration, thereby maintaining the pH close to physiological levels. This approach not only ensures the stability of the formulation but also contributes to the reduction of irritation at the injection site. The ability to regulate pH ensures enhanced stability, prevents the formation of undesirable precipitates, optimizes the solubility of other compounds in the formulation, and extends the shelf life of the product (11,12). The utilization of pH stabilizers must be approached with a degree of caution, as they are not without risk. It is noteworthy that certain pH stabilizers may interact with the active pharmaceutical ingredient (API) or other excipients, thereby potentially compromising the drug's stability or efficacy. It is imperative to employ these stabilizers at minimal concentrations, as elevated concentrations of stabilizers have been observed to induce local tissue irritation or discomfort upon injection, owing to the abrupt pH shift (13).

Given these complexities, this study aims to examine the impact of preservative-free formulations and the influence of pH stabilizers, such as citric acid, on the chemical and physical stability of heparin 5,000 IU/mL over 90 days. The objective is to enhance the safety, efficacy, and regulatory compliance of unfractionated heparin formulations. To this end, the study addresses the issue of batch variability and instability.

2. Materials

Heparin, Sodium chloride, Disodium Hydrogen Phosphate anhydrate, Sodium dihydrogen phosphate dihydrate, Citric acid monohydrate, Benzyl alcohol, Hydrazine sulfate, Hexamethylenetetramine, Ferric Chloride, Potassium iodide (KI), 0.1 M sodium thiosulphate (Na2S2O3), Cobalt Chloride, Copper sulfate, Filters 0.22 µm, Vials, caps and stoppers were kindly donated by Gilvasan Pharma. Methylparaben (PHR1012) and Propylparaben (PHR1010) were purchased from Sigma-Aldrich Chemie GmbH. Sterile water for injection 1000ml was purchased from B. Braun. Hydrochloric acid and sodium hydroxide were prepared using laboratory-grade materials. Buffer solutions (4.01, 7.00 and 10.01) were purchased from Xylem Analytics.

UV-vis Spectrophotometer from “Bio-Tek Instruments”, Labor-pH Meter inoLab® pH 7110 from Xylem Analytics, Autoklav HV 85L from HMC Europe, Magnetruhrwerk MR 3000 from Heidolph, Elmasonic Ultraschallreiniger P 30 H from Elma Schmidbauer GmbH, Memmert UNE 500 Trockenschrank were used during the study. Rotilabo syringe filters, PVDF, sterile, 0.22 µm, 33 mm was bought from Carl Roth GmbH. 96 Well Microplate, UV-Star, Half Area was bought from Greiner Bio-one. 96 wells-F, Surface treated, Sterile was bought from VWR.

3. Methods

3.1. Formulation preparation

Six distinct formulations were prepared. Each formulation was prepared with three distinct heparin batches called here as batches 1, 2 and 3. A total of 18 formulations were prepared, with the corresponding sample names provided in Table 1. To ease the production process, the formulations were prepared in three groups, designated as BA (comprising samples with benzyl alcohol), CA (comprising samples without preservatives, only citric acid or buffer system), and PA (comprising samples with parabens).

Table 1: Matrix of formulations. Benzyl alcohol (BA), Parabens (PA), Citric acid (CA) and Buffer system (B). Different heparin batches (1, 2 and 3).

BA		**CA**		**PA**	
BA 1	BA_CA 1	CA_B 1	B 1	PA 1	PA_CA 1
BA 2	BA_CA 2	CA_B 2	B 2	PA 2	PA_CA 2
BA 3	BA_CA 3	CA_B 3	B 3	PA 3	PA_CA 3

The formulations BA and CA were produced as follows: all containers were sterilized before use and all subsequent steps were performed under a laminar airflow (LAF) cabinet. The solid materials were weighed following Table 2, and dissolved in WFI (water for injection) and the heparin was added under stirring. The pH was adjusted to 7, and the final volume was completed with WFI. After production, the formulations were filtered using a 0.22 µm membrane filter under LAF. The non-autoclaved (NAC) formulations were stored at 4°C. The other vials were subjected to autoclave for a period of 12 minutes at a temperature of 121°C.

The process applied to Group PA was different in some aspects. The addition of methylparaben, propylparaben and heparin was done under stirring conditions. The formulation was sonicated for five minutes at 40°C, after which the pH was adjusted to 7. Following this adjustment, the formulation was sonicated for a further ten minutes at 40°C, and only after this the formulation was filtrated and the vials filled.

After autoclavation, the vials from all the groups were submitted to a series of tests according to the European Pharmacopeia. These included the coloration of liquids, the presence of visible particles, pH measurements, and the assessment of color and appearance (turbidity).

Table 2: Composition of each formulation [mg] for 1 mL.

	BA	**BA_CA**	**CA_B**	**B**	**PA**	**PA_CA**
Heparin-sodium [I.U.] 5000 I.U/mL + 3% excess	5000	5000	5000	5000	5000	5000
Sodium chloride [mg]	9	7.6	6.7	6.7	9	7.6
Disodium hydrogen phosphate anhydrate [mg]	---	2.15	1.53	1.53	---	2.15
Sodium dihydrogen phosphate dihydrate [mg]	---	---	0.65	0.65	---	---
Citric acid monohydrate [mg]	---	0.34	0.4	---	---	0.34
Benzyl alcohol [mg]	10	10	---	---	---	---
Methylparaben [mg]	---	---	---	---	1.50	1.27
Propylparaben [mg]	---	---	---	---	0.15	0.13
HCl/NaOH	Ad pH 7,0	Ad pH 7,0	Ad pH 7,0	Ad pH 7,0	Ad pH 7,0	Ad pH 7,0
WFI	Ad 1 ml	Ad 1 ml	Ad 1 ml	Ad 1 ml	Ad 1 ml	Ad 1 ml

3.2. Coloration of liquids

The test was conducted following the standards outlined in the European Pharmacopoeia 6.0 (test 2.2.2). Three primary solutions were prepared in advance: a yellow solution, a red solution, and a blue solution. The solutions were then diluted to create three standard solutions (brown, brownish-yellow, and yellow). The standard solutions were employed in the preparation of the reference solutions according to Figure 1. After 1, 30, and 90 days of the production, the vials were then compared with the reference solutions in front of a white background.

A)

Reference solution	Volumes in millilitres	
	Standard solution B	Hydrochloric acid (10 g/l HCl)
B_1	75.0	25.0
B_2	50.0	50.0
B_3	37.5	62.5
B_4	25.0	75.0
B_5	12.5	87.5
B_6	5.0	95.0
B_7	2.5	97.5
B_8	1.5	98.5
B_9	1.0	99.0

B)

Reference solution	Volumes in millilitres	
	Standard solution BY	Hydrochloric acid (10 g/l HCl)
BY_1	100.0	0.0
BY_2	75.0	25.0
BY_3	50.0	50.0
BY_4	25.0	75.0
BY_5	12.5	87.5
BY_6	5.0	95.0
BY_7	2.5	97.5

C)

Reference solution	Volumes in millilitres	
	Standard solution Y	Hydrochloric acid (10 g/l HCl)
Y_1	100.0	0.0
Y_2	75.0	25.0
Y_3	50.0	50.0
Y_4	25.0	75.0
Y_5	12.5	87.5
Y_6	5.0	95.0
Y_7	2.5	97.5

Figure 1: Reference solution preparation. A) Brown solution, B) Brownish-yellow solution, C) Yellow solution. Figures from European Pharmacopeia 6.0.

3.3. Visible particles

The test was conducted following the standards outlined in the European Pharmacopoeia 6.0 (test 2.9.20). Briefly, all the vials were observed in front of a white and a black panel for the presence of any visible particles 1, 30 and 90 days after the production.

3.4. pH measurements

The pH measurements were conducted in accordance with the standards outlined in the European Pharmacopoeia 6.0 using a pHmeter. Prior to each measurement, the pH meter was calibrated with buffer solutions with a known pH of 4,01; 7,00 and 10,01. All vials and buffer solutions were incubated for 30 minutes at a temperature of 25°C. The vials were opened, and three pH measurements were taken from n=3 vials from the same batch.

3.5. Clarity and turbidity measurements

The test was conducted following the standards outlined in the European Pharmacopoeia 6.0 (test 2.2.1). The samples for turbidity measurements were taken from the same vial as pH measurements immediately after pH determination. The Standard of opalescence (SO) was prepared on the day of the measurement. Four dilutions from the SO were prepared according to table 3.

The plate reader mode of the UV-Vis spectrophotometer was used, 250 µL of each sample was pipetted into a 96-well plate. The optical density of samples was measured at a wavelength of 510 nm. The obtained optical density (OD) values were converted to nephelometric turbidity units (NTU). A standard curve was generated by plotting OD against NTU values obtained from the known dilutions of the SO (fig. 2). The relationship between OD and NTU was determined using a linear regression equation: OD = 0.0005 × NTU + 0.0006. This equation was applied to convert the OD values of the samples into NTU.

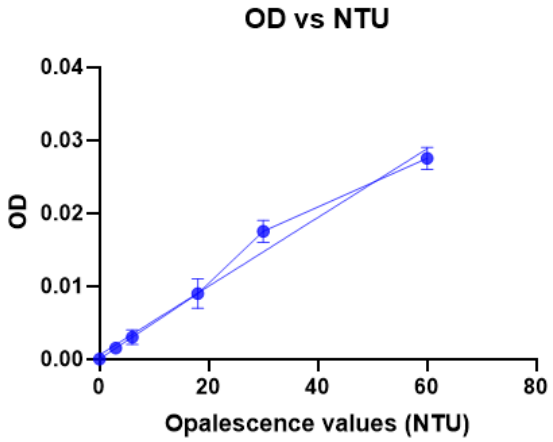


Figure 2: Graph of Opalescence values (NTU) vs OD values measured at 510 nm.

Table 3: Dilutions from standard of opalescence.

	I	II	III	IV
Standard of opalescence (SO)	5.0 mL	10.0 mL	30.0 mL	50.0 mL
Water	95.0 mL	90.0 mL	70.0 mL	50.0 mL

3.6. UV-VIS Spectroscopy

The test was performed to determine batch variability and impurities in the three different heparin batches. 5 mg of each batch of heparin was weighed and dissolved in 10 ml of distilled water with stirring. Each solution was filtered through a 0.22 µm membrane filter to remove particles. The UV-VIS spectrophotometer was calibrated using distilled water as the solvent. Each batch was read in the wavelength range 200-400 nm at a medium scan speed. All formulations before and after autoclaving were tested in the same procedure.

4. Results and Discussion

4.1. Coloration of Liquids

The NAC vials consistently exhibited a coloration indistinguishable from that of water, while the AC vials maintained a brownish-yellow coloration after autoclavation in all groups. According to the certificate of analysis provided for the heparin batches used, solutions should not exceed the intensity of reference solution 5. For BA formulations, throughout the study period, the BA 1 and BA_CA 1 were consistently identified as intensity 4, while the other formulations remained comparable in coloration (fig. 3A). For the CA, formulations CA_B 1 and B 1 were also classified as intensity 4, while the other formulations remained within intensity 5 (fig. 3B). And for the PA, formulations PA 1, PA 2, and PA 3 consistently demonstrated a coloration corresponding to Reference 6, while formulations PA_CA 1, PA_CA 2, and PA_CA 3 showed a slightly higher intensity, analogous to Reference 5 (fig. 3C). No significant changes in coloration were observed in any of the groups over time for either NAC or AC formulations.

All formulations exhibited alterations in coloration after autoclaving, with the CA demonstrating the most significant change, followed by BA and PA. This phenomenon can be attributed to the absence of a preservative in the CA formulations, which renders the solutions less stable, this way contributing to the appearance of stronger coloration.

The coloration exhibited by all AC formulations could be attributed to Maillard reactions occurring during the production of heparin, particularly during protease digestion stages lasting more than 10 hours (14). The presence of amino acids in the formulation (from heparin or other sources) provides the necessary components for the Maillard reaction to occur. (15). The presence of coloration could be indicative of impurities in the raw material. The UV spectra of pure heparin from the reference study (16) exhibit a smooth decrease in absorbance from approximately 200 to 230 nm, with no pronounced peaks. In contrast, the non-pure heparin spectra display sharp peaks around 260 nm, suggesting the presence of impurities or other UV-absorbing substances. The UV spectra for heparin dissolved in water, no peak was found in the range compatible with impurities (fig. 4). Minor variations in the UV spectra of Hep1, Hep2, and Hep3 may be attributable to batch variability inherent in UFH heparin. However, the possibility of impurities in the other components of the formulation, despite being from reliable sources, cannot be discounted.

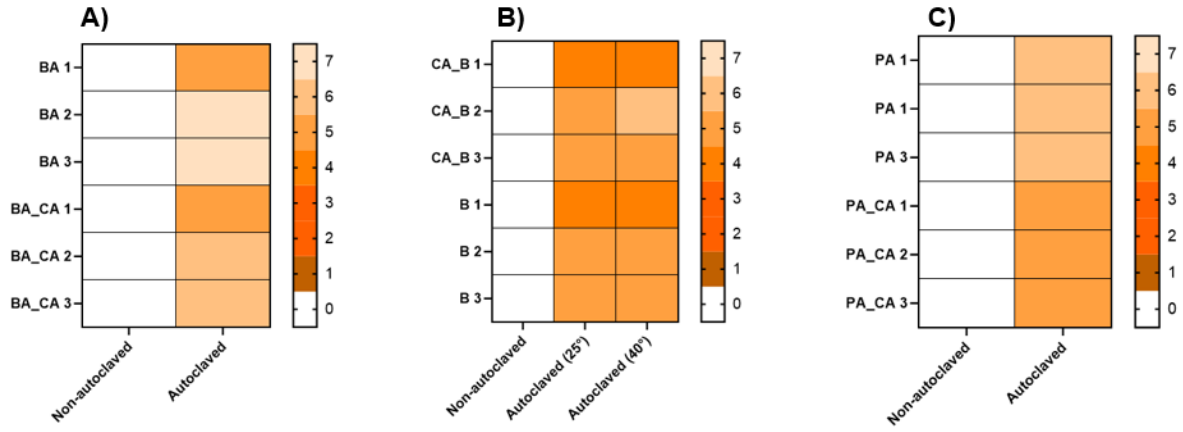


Figure 3: Heat map of coloration of liquids x Reference standards of turbidity. A) Group BA, B) Group CA and C) Group PA.

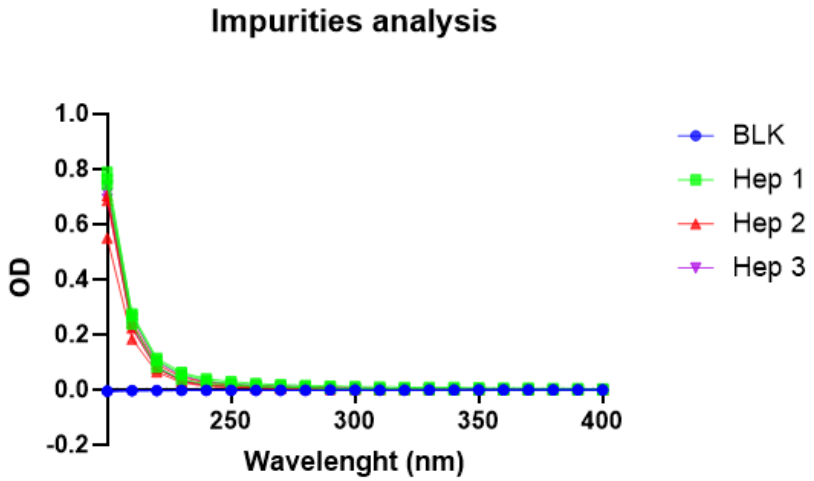


Figure 4: UV spectra of heparin batches diluted in water from 200 to 400 nm.

4.2. Visible Particles

Some particles appeared subsequent to the sterilization process, these were identified as impurities that were likely introduced during the filling process, which was conducted under LAF. It is hypothesized that the sterilization process may have dislodged or rendered visible small fibbers or lint that were present but not discernible prior to autoclaving. The presence of protein aggregation or undissolved materials is not indicated. These observations remained consistent over time. No visible particles were detected in any of the NAC vials throughout the study in any of the groups.

4.3. pH Measurements

The pH behavior varied among the BA formulations (Fig. 5). Initially, NAC formulations BA 1, 2 and 3 exhibited highly unstable pH values, making it challenging to achieve a target pH of 7. These formulations continued to display significant fluctuations, particularly after the autoclaving process. Over time, the pH levels of the NAC changed reaching a range of approximately 8 and a strong decrease to approximately 7 at 90 days. In contrast, NAC formulations BA_CA 1, 2 and

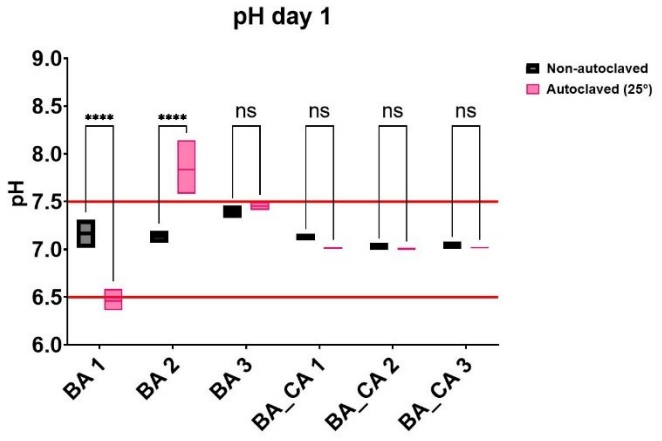
3 maintained stable pH values throughout the study. These formulations remained within the 6.5–7.5 range, showing only minimal fluctuations post-autoclaving. The AC formulations followed a similar trend, with formulation BA 1 exhibiting a slight increase in pH, while formulations BA 2 and BA 3 experienced a more substantial drop of nearly 1.0 unit at 30 days. Formulations BA_CA 1, 2 and 3 remained relatively stable, with only a slight decrease over time (Fig. 5).

The pH of all CA formulations remained within the range of 6.5–7.5 throughout the study. Initially, formulations CA_B 1 and B 1 exhibited a slight decline in pH following autoclaving but remained within the desired range. Over time, a slight elevation in pH was observed across all formulations, with NAC samples exhibiting a more pronounced increase compared to AC samples. By the final time point, all formulations exhibited a minor decrease in pH (ranging from 0.15 to 0.05), but this value falls within the range of error accepted. No substantial differences were detected between samples stored at 25°C and 40°C.

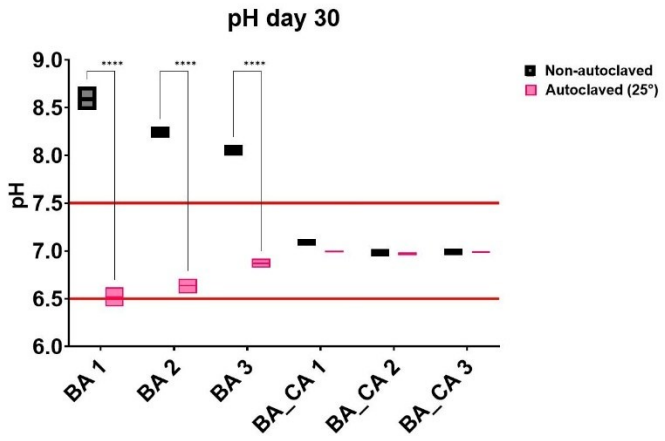
The NAC PA formulations exhibited stable pH values, remaining close to 7 throughout the study. Among the AC PA formulations, PA 1 and PA 2 exhibited greater pH instability, with a decline to approximately 6, falling outside the designated range of 6.5-7.5. Formulation PA 3 also experienced a decrease but remained within the acceptable range, although after 30 days the formulation was out of the range (Fig. 7 B). Formulations PA_CA 1, 2 and 3 showed a slight pH decline following autoclaving but consistently remained within the specified range of 6.5-7.5.

Following the process of autoclavation, some formulations from all groups exhibited a decline in pH levels. This phenomenon can be explained by the autoclaving process, which has been demonstrated to result in a modest breakdown of the heparin backbone, as evidenced by a slight reduction in molecular weight and an increase in polydispersity. This degradation process may result in the release of acidic fragments, which could contribute to a pH decrease (17). It is important to note that the same Maillard reactions that could be responsible for the observed coloration could also result in the production of acidic bioproducts (18). As expected, the function of citric acid in maintaining the stability of the formulations following autoclavation and over time has been substantiated. This assertion is evidenced by the fact that all formulations containing citric acid remained within the acceptable range of 6.5-7.5, while those not containing citric acid exhibited pH instabilities.

A)



B)



C)

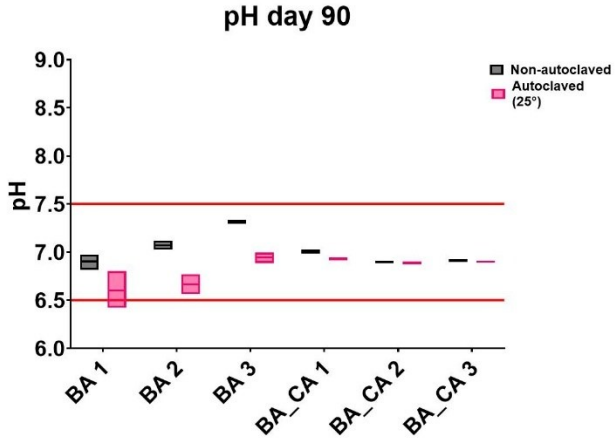
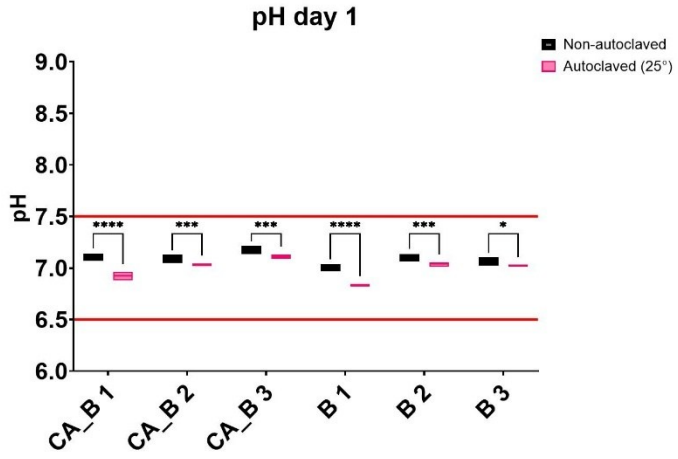
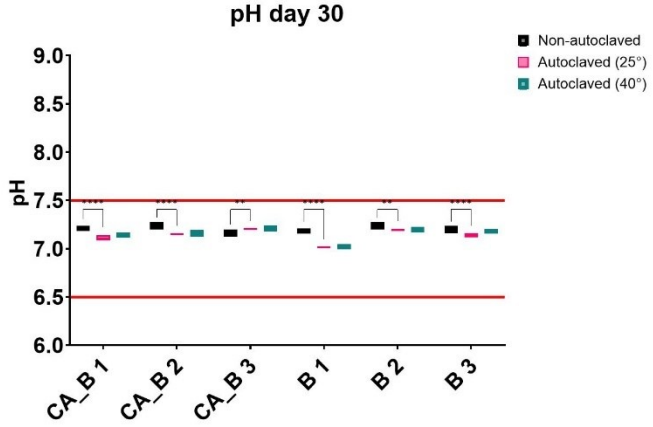


Figure 5: pH of formulations with Benzyl alcohol. A) Day 1, B) Day 30 and C) Day 90.

A)



B)



C)

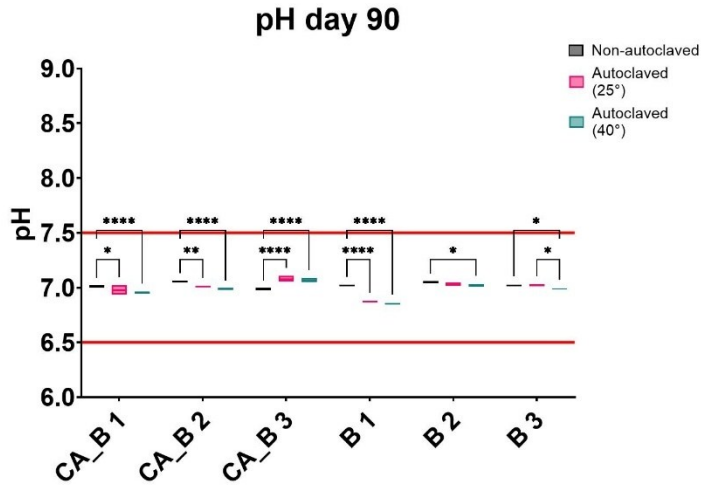


Figure 6: pH of formulations without preservative, only citric acid. A) Day 1, B) Day 30 and C) Day 90.

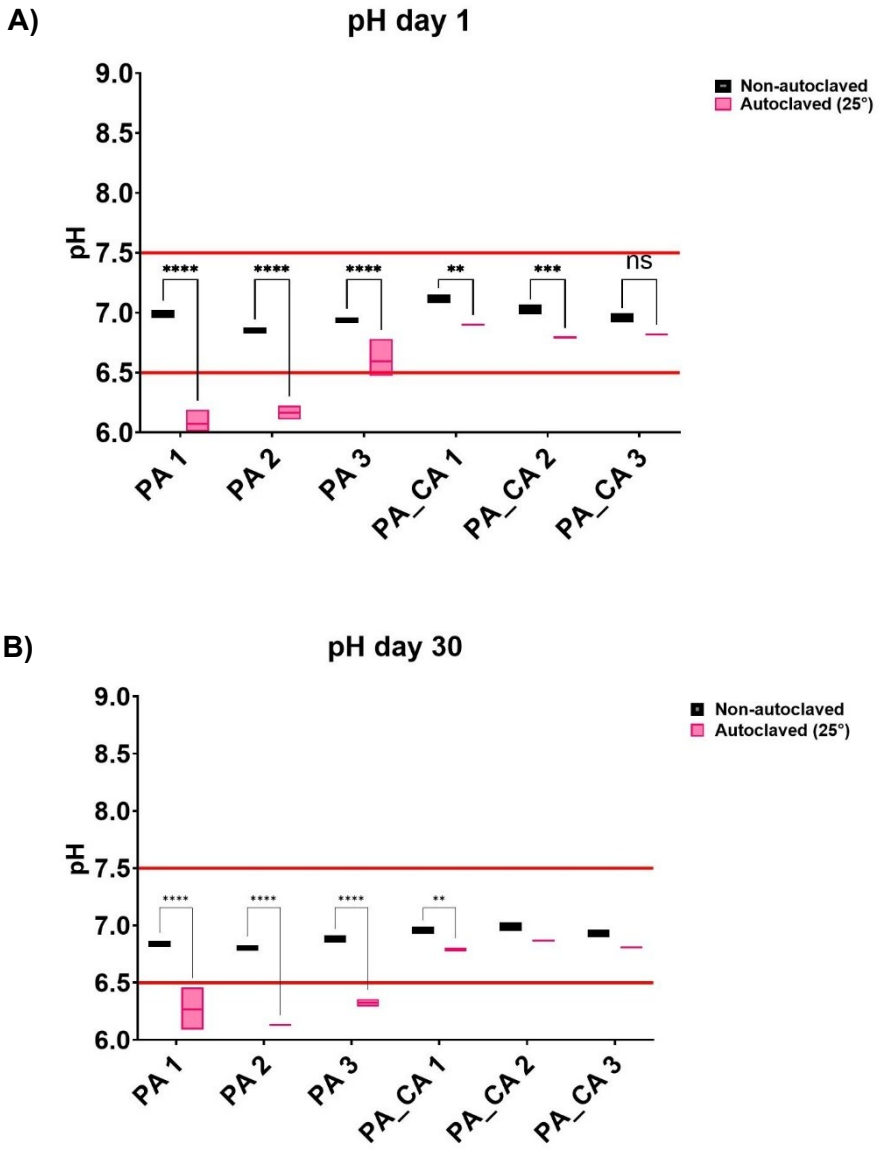


Figure 7: pH of formulations with Parabens. A) Day 1 and B) Day 30.

4.4. Clarity and turbidity measurements

The NAC formulations exhibited initial turbidity values close to zero, showing values comparable to water (fig. 7 A, B and C). The BA AC formulations exhibited greater initial variation in turbidity values. Formulations BA 1 was classified as Reference III and BA_CA 1 as Reference IV, which is the highest turbidity reference before the standard of opalescence (fig. 8A). Formulations BA 2, BA 3, BA_CA 2, and BA_CA 3 were situated within Reference II. Over time, only formulation BA_CA 1, shifted to reference III while the others remained stable. The CA AC formulations, however, showed variations in turbidity. Initially, formulations CA_B 1 and B 1 exhibited the highest turbidity, falling within Reference III. The other AC formulations were categorized within Reference II (fig. 8B). Over time, turbidity values changed depending on storage conditions.

At 25°C, all CA AC formulations remained within the same reference range. At 40°C, differences in turbidity were observed in the formulations containing citric acid. Formulation CA_B 1 shifted from Reference III to II, formulations CA_B 2 and CA_B 3 moved from Reference II to I. Formulation B 1 changed from reference III to II at day 90, while B 2 and 3 remained unchanged. The combination of citric acid and heparin may exhibit better thermal stability at 40°C, maintaining a clearer solution compared to lower temperatures where some components might be less soluble or more prone to aggregation (19). Among the PA AC formulations, PA 1 showed values similar to water at day 1 and shifted to Reference II on day 30. PA 2, PA 3, PA_CA 2 and PA_CA 3 maintained turbidity levels similar to Reference II, indicating clarity. Formulation PA_CA 1 exhibited slightly higher turbidity on day 30, falling within Reference III (fig. 8C).

Heparin has been shown to lose N-sulfo groups under thermal stress, higher temperatures and prolonged exposure can lead to increased N-sulfo group loss and decreased antithrombin-binding affinity. This degradation could manifest as turbidity changes, indicating compromised heparin quality (20). The PA formulations exhibited lower values of turbidity in comparison to Groups BA and CA, which demonstrated analogous outcomes. This phenomenon can be attributed to the enhanced compatibility of parabens with heparin relative to benzyl alcohol. The utilization of parabens has been demonstrated to enhance the overall stability of heparin formulations, thereby mitigating the potential for the formation of degradation products that could lead to turbidity (21).

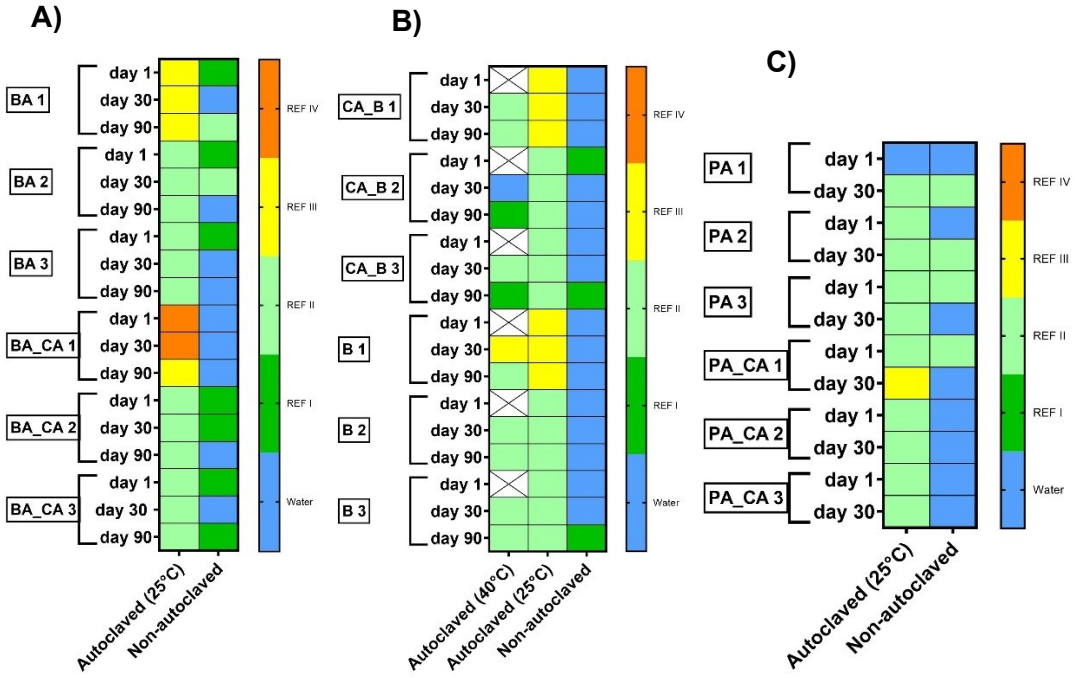


Figure 7: Heatmap of turbidity and reference standards of turbidity (NTU). A) Formulations with benzyl alcohol, B) Formulations without preservatives, and C) Formulations with parabens.

5. Conclusions

The findings of this study highlight the necessity of incorporating a pH stabilizer within the formulation to ensure stability during the processes of autoclaving and extended storage. In the absence of a pH stabilizer and a buffer system, even with meticulous aseptic preparation techniques and terminal sterilization, the formulation remains unstable across all parameters. The utilization of a preservative is thus essential to ensure stability. The batch variability of UFH was confirmed, where between three different batches, one exhibited significantly lower stability compared to the other two batches, even in the presence of benzyl alcohol. However, in the presence of parabens, the formulations demonstrated greater similarity in terms of stability after autoclaving and over time. It is not possible to rule out the possibility of the presence of impurities in the raw material, even though they were provided from trustable sources this needs to be considered.

Future studies should individually assess all formulation components to rule out potential contaminants. Additionally, microbiological tests should be conducted to confirm sterility and evaluate whether the pH stabilizer alone can prevent microbial growth. Investigating alternative autoclaving conditions (e.g., different temperatures and durations) would provide further insights into optimizing UFH formulation stability. Further research could explore alternative salts and pH stabilizers to evaluate their interactions with heparin and their potential impact on formulation stability. Addressing these factors would improve the reliability, safety, and efficacy of UFH formulations, ultimately benefiting clinical applications.

LIST OF FIGURES

LIST OF TABLES

REFERENCES

1. Junqueira DR, Zorzela LM, Perini E. Unfractionated heparin versus low molecular weight heparins for avoiding heparin-induced thrombocytopenia in postoperative patients. Vol. 2017, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2017.
2. Saltissi D, Morgan C, Westhuyzen J, Healy H. Comparison of low-molecular-weight heparin (enoxaparin sodium) and standard unfractionated heparin for haemodialysis anticoagulation. Nephrology Dialysis Transplantation. 1999;14:2698–703.
3. Francis JL, Groce JB. Challenges in variation and responsiveness of unfractionated heparin. In: Pharmacotherapy. 2004.
4. Hirsh J, Anand SS, Halperin JL, Fuster V. Guide to Anticoagulant Therapy: Heparin A Statement for Healthcare Professionals From the American Heart Association [Internet]. 2001. Available from: http://www.circulationaha.org
5. Banik N, Yang S Bin, Kang TB, Lim JH, Park J. Heparin and its derivatives: Challenges and advances in therapeutic biomolecules. Vol. 22, International Journal of Molecular Sciences. MDPI; 2021.
6. Pereira BMP, Tagkopoulos I. Benzalkonium chlorides: Uses, regulatory status, and microbial resistance. Vol. 85, Applied and Environmental Microbiology. American Society for Microbiology; 2019.
7. Uzdrowska K, Górska-Ponikowska M. Preservatives in cosmetics technology. Vol. 12, Aesthetic Cosmetology and Medicine. INDYGO Zahir Media; 2023. p. 73–8.
8. Hiller JL, Benda GI, Rahatzad M, Allen JR, Culver DH, Carlson C V., et al. Benzyl alcohol toxicity: Impact on mortality and intraventricular hemorrhage among very low birth weight infants. Pediatrics. 1986;77:500–6.
9. Soni MG, Burdock GA, Taylor SL, Greenberg NA. Safety assessment of propyl paraben: A review of the published literature. Vol. 39, Food and Chemical Toxicology. 2001. p. 513–32.
10. Iacobelli S, Commins M, Lorrain S, Gouyon B, Ramful D, Richard M, et al. Paraben exposure through drugs in the neonatal intensive care unit: a regional cohort study. Front Pharmacol. 2023;14.
11. Nakamura K, Tanaka T, Saito K, Yokohama S, Sonobe T. Stabilization of minodronic acid in aqueous solution for parenteral formulation. Int J Pharm. 2001 Jul;222:91–9.
12. Marino D. https://drug-dev.com/formulation-forum-considerations-in-formulation-development-of-injectable-solutions/. FORMULATION FORUM - Considerations in Formulation Development of Injectable Solutions.
13. Fujita M, Ueda T, Handa T. Generation of formaldehyde by pharmaceutical excipients and its absorption by meglumine. Chem Pharm Bull (Tokyo). 2009 Oct;57:1096–9.
14. YAMAMOTO R, BELLOMO EG, KIM Y, VYAS R. Method for enhanced heparin quality. USA; WO 2016/13741 A1, 2016.
15. Lund MN, Ray CA. Control of Maillard Reactions in Foods: Strategies and Chemical Mechanisms. Vol. 65, Journal of Agricultural and Food Chemistry. American Chemical Society; 2017. p. 4537–52.
16. Lima MA, Rudd TR, de Farias EHC, Ebner LF, Gesteira TF, de Souza LM, et al. A new approach for heparin standardization: Combination of scanning UV spectroscopy, nuclear magnetic resonance and principal component analysis. PLoS One. 2011;6(1).
17. Beaudet JM, Weyers A, Solakyildirim K, Yang B, Takieddin M, Mousa S, et al. Impact of autoclave sterilization on the activity and structure of formulated heparin. J Pharm Sci. 2011;100:3396–404.
18. MENZIES AR, BENOLIEL DM, EDWARDS HE. The Effects of Autoclaving on the Physical Properties and Biological Activity of Parenteral Heparin Preparations. Journal of Pharmacy and Pharmacology. 1989;41:512–6.
19. O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Royal Society of Chemistry. 2013;416.

20. Fu L, Li L, Cai C, Li G, Zhang F, Linhardt RJ. Heparin stability by determining unsubstituted amino groups using hydrophilic interaction chromatography mass spectrometry. Anal Biochem. 2014 Sep;461:46–8.
21. Bookstaver PB, Rokas KEE, Norris LAB, Edwards JM, Sherertz RJ. Stability and compatibility of antimicrobial lock solutions. Vol. 70, American Journal of Health-System Pharmacy. American Society of Health-Systems Pharmacy; 2013. p. 2185–98.

APPENDIX

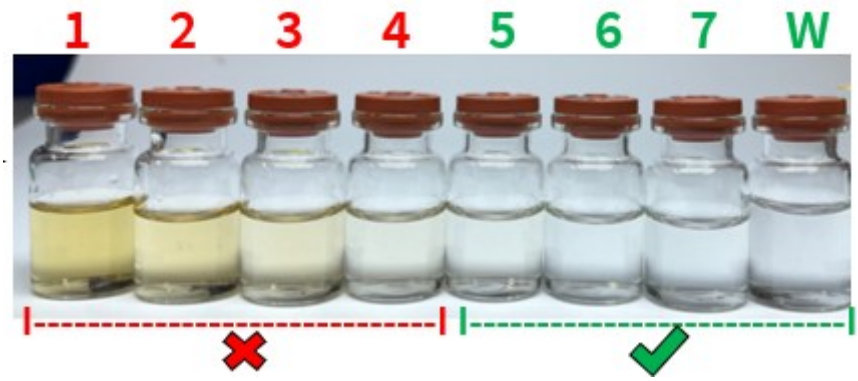


Figure 9: Range of reference solutions Brownish yellow. 1-4 are out of the expected range, 5-7 are in the expected range. W refers to water.

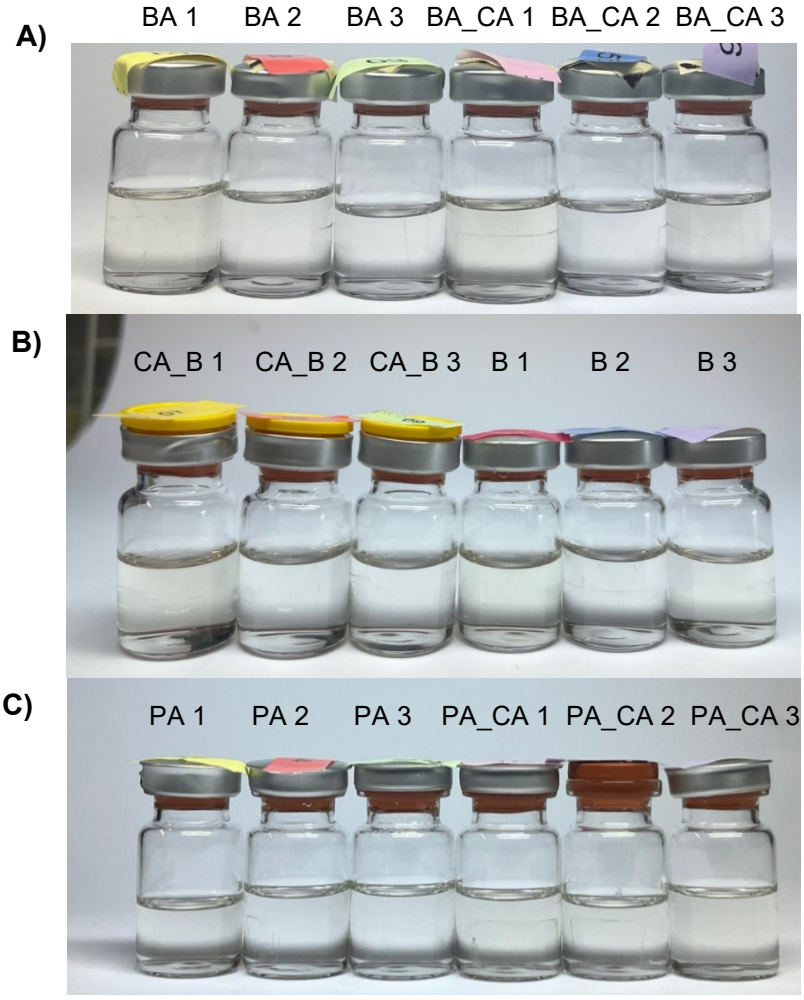


Figure 2: All formulations. A) Formulations with benzyl alcohol, B) Formulations without preservatives and only citric acid, and C) Formulations with parabens.

I. Abstract

Unfractionated heparin (UFH) is a widely utilized anticoagulant agent. However, its inconsistency between different batches poses significant challenges in pharmaceutical formulations. This study investigates the impact of preservatives and pH stabilizers on the chemical and physical stability of UFH formulations over a period of 90 days. A range of formulations, incorporating and excluding preservatives (benzyl alcohol and parabens) and pH stabilizers (citric acid), were meticulously prepared and assessed for various stability parameters. These parameters encompassed, coloration of liquids, clarity and turbidity of formulations, pH measurements, and the presence of visible particles. The results obtained demonstrate that formulations without pH stabilizers demonstrate significant instability, with pH values fluctuating beyond the acceptable range (6.5–7.5). Following autoclaving, turbidity and color changes were observed in all formulations. The samples devoid of preservatives exhibited stronger coloration. Paraben-based formulations demonstrated superior stability, maintaining consistent pH levels and reduced turbidity variations. No significant changes were observed over time. UV-VIS spectroscopy was employed to assess batch variability among the heparin batches, while simultaneously ruling out the presence of significant impurities. The findings emphasize the critical role of pH stabilizers in maintaining stability, particularly during sterilization and long-term storage. The study also highlights the importance of preservative selection, showing that citric acid alone is not sufficient to maintain stability. Furthermore, the study demonstrated that formulations containing parabens exhibited superior stability in comparison to those comprising benzyl alcohol. Future research should explore alternative stabilizers, optimize autoclaving conditions, and conduct microbiological assessments to further enhance UFH formulations for clinical use.

II. Zusammenfassung

Unfraktioniertes Heparin (UFH) ist ein weit verbreitetes Antikoagulans. Seine Inkonsistenz zwischen verschiedenen Chargen stellt jedoch eine große Herausforderung für pharmazeutische Formulierungen dar. In dieser Studie wird der Einfluss von Konservierungsmitteln und pH-Stabilisatoren auf die chemische und physikalische Stabilität von UFH-Formulierungen über einen Zeitraum von 90 Tagen untersucht. Eine Reihe von Formulierungen mit und ohne Konservierungsmittel (Benzylalkohol und Parabene) und pH-Stabilisatoren (Zitronensäure) wurden sorgfältig hergestellt und auf verschiedene Stabilitätsparameter untersucht. Diese Parameter umfassten die Färbung der Flüssigkeiten, die Klarheit und Trübung der Formulierungen, pH-Messungen und das Vorhandensein von sichtbaren Partikeln. Die Ergebnisse zeigen, dass Formulierungen ohne pH-Stabilisatoren eine erhebliche Instabilität aufweisen, wobei die pH-Werte außerhalb des akzeptablen Bereichs (6,5-7,5) schwanken. Nach dem Autoklavieren wurden bei allen Formulierungen Trübungen und Farbveränderungen beobachtet. Die Proben ohne Konservierungsmittel wiesen eine stärkere Färbung auf. Formulierungen auf Parabenbasis wiesen eine überlegene Stabilität auf, indem sie konstante pH-Werte und geringere Trübungsschwankungen beibehielten. Im Laufe der Zeit wurden keine signifikanten Veränderungen beobachtet. Die UV-VIS-Spektroskopie wurde eingesetzt, um die Chargenvariabilität zwischen den Heparinchargen zu bewerten und gleichzeitig das Vorhandensein signifikanter Verunreinigungen auszuschließen. Die Ergebnisse unterstreichen die kritische Rolle von pH-Stabilisatoren bei der Aufrechterhaltung der Stabilität, insbesondere während der Sterilisation und Langzeitlagerung. Die Studie unterstreicht auch die Bedeutung der Auswahl der Konservierungsmittel und zeigt, dass Zitronensäure allein nicht ausreicht, um die Stabilität zu erhalten. Darüber hinaus zeigte die Studie, dass Formulierungen, die Parabene enthielten, im Vergleich zu solchen mit Benzylalkohol eine höhere Stabilität aufwiesen. Zukünftige Forschungsarbeiten sollten alternative Stabilisatoren erforschen, die Autoklavierbedingungen optimieren und mikrobiologische Bewertungen durchführen, um UFH-Formulierungen für den klinischen Einsatz weiter zu verbessern.