Journal of Advanced Zoology

ISSN: 0253-7214
*Volume **44** Issue **S-6 Year 2023** Page **72:79***

Formulation and Evaluation of ACNE Gel using Metronidazole and Doxycycline

Anchal Dwivedi[2], Shriyanshi Singh Rana[3], Vishal Dubey[1], Megha Tiwari[1*], Mayur Porwal[1], Anita Devi[4]

[1,2,4]Naraina Vidyapeeth Group of Institutions, Faculty of Pharmacy, Panki, Kanpur
[1]Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad
[3]Advance Institute of Biotech and Paramedical Sciences, Kanpur[c]

**Corresponding author's E-mail: tiwarimegha860@gmail.com*

Article History

Received: 06 June 2023
Revised: 05 Sept 2023
Accepted: 21 Nov 2023

Abstract

This research study focuses on the formulation and evaluation of an ACNE gel using Metronidazole (1%) and Doxycycline (0.5%) as active ingredients. Three types of polymers, Hydroxypropyl Methylcellulose (HPMC), Carbopol 940, and Carbopol 934 were used for gel formation. The formulated gels were evaluated for their physical characteristics, in vitro drug release, and drug-polymer compatibility. The calibration curves for both Metronidazole and Doxycycline displayed strong linearity within the concentration range of 5 to 25 µg/mL, facilitating accurate drug quantification. The gel formulations exhibited pH values of 6.0 (HPMC), 5.8 (Carbopol 940), and 5.7 (Carbopol 934), which are within the skin's natural pH range. The mean viscosity values were recorded as 205 cP (HPMC), 220 cP (Carbopol 940), and 231 cP (Carbopol 934). Spreadability and extrudability tests also yielded favorable results, suggesting ease of application. The in vitro drug release study showed a time-dependent increase in drug release, with the highest cumulative release observed from the HPMC formulation (90% at 8 hours), followed by Carbopol 940 (80% at 8 hours), and Carbopol 934 (64% at 8 hours). Fourier Transform Infrared Spectroscopy (FTIR) analysis revealed no significant interaction between the drugs and the polymers, indicating their compatibility. This study offers a promising approach to the development of effective ACNE gel formulations with Metronidazole and Doxycycline, while emphasizing the influence of the type of polymer on the drug release profile.

Keywords: *Acne, Gel Formulation, Metronidazole, Doxycycline, HPMC, Carbopol 940, Carbopol 934, Calibration Curve, pH, Viscosity, Spreadability, Extrudability, Drug Release, FTIR Analysis.*

1. Introduction

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit. It is one of the most common dermatological conditions, predominantly affecting adolescents and young adults. It involves various pathogenic factors, including sebum production, follicular hyperkeratinization, bacterial colonization, and inflammation [1]. Clinically, acne is characterized by a variety of lesions including open and closed comedones, papules, pustules, and in severe cases, nodules and cysts. While the disease is not life-threatening, it can lead to significant psychological distress and long-term scarring [2].

The management of acne is multifaceted. Various topical and systemic therapies are available, targeting the different aspects of acne pathogenesis. Among these, topical therapy is the cornerstone of acne treatment, especially for mild to moderate forms of the disease [3]. The choice of topical agent depends on the type and severity of acne, the patient's lifestyle, and the potential side effects of the medication. Antibiotics, such as metronidazole and doxycycline, have been widely used in acne management due to their antibacterial and anti-inflammatory properties [4]

Metronidazole, a nitroimidazole antibiotic, is effective against anaerobic bacteria and certain parasites. It is often used to treat rosacea, a dermatologic condition that shares features with acne. On the other hand, doxycycline is a tetracycline antibiotic that treats infections caused by susceptible gram-negative and gram-positive organisms, in addition to treating other conditions like acne and rosacea [5].

Despite the availability of various antibiotics, the therapeutic efficacy of these agents can be hindered by poor patient compliance due to local irritation or systemic side effects. Therefore, the development of new formulations that can deliver the active agent effectively while minimizing side effects is necessary [6].

This study aims to develop an efficient topical acne gel formulation incorporating metronidazole and doxycycline. Various polymers, namely hydroxypropyl methylcellulose (HPMC), Carbopol 940, and Carbopol 934, have been used to evaluate the best gelling agent for the formulation. The evaluation of the formulation was carried out based on certain parameters including pH, viscosity, spread diameter, extrusion force, and cumulative drug release. Furthermore, Fourier-transform infrared spectroscopy (FTIR) analysis was performed to determine the compatibility of the active drugs with the selected polymers [6].

it is worth noting that the effectiveness of a topical gel formulation is determined not only by the properties of the active ingredients but also by the characteristics of the gel base. The gel base, composed of various polymers, plays a crucial role in determining the gel's stability, ease of application, and drug release characteristics [7].

Hydroxypropyl methylcellulose (HPMC) is a semi-synthetic, soluble cellulose ether. It is used in pharmaceutical formulations as a suspending agent, emulsifier, and thickening agent. HPMC is frequently used in gel formulations due to its ability to form a robust gel at low concentrations [8] Moreover, it has high compatibility with many active ingredients and exhibits excellent bioadhesive properties that increase the residence time of the formulation at the application site, hence improving the absorption of the active drug [9].

Carbopol, a brand name for polyacrylic acid, is another common polymer used in pharmaceutical gel formulations. It is available in several grades, including Carbopol 940 and 934, which differ in their cross-linking density and molecular weight. Carbopol polymers form clear gels, have excellent bioadhesive properties, and are capable of providing controlled release of drugs. They have been extensively used in transdermal and topical formulations due to their high viscosity and excellent stability [10].

The selection of the polymer in the formulation of a gel is a critical step. It not only affects the physical characteristics of the gel, such as viscosity and spreadability, but also influences the release of the active drug from the gel. Therefore, an effective gel formulation should possess an optimal balance of these parameters, ensuring easy application while maintaining a sustained drug release profile [11].

Furthermore, compatibility between the active drug and the selected polymer is of utmost importance. Incompatibilities may lead to changes in the physical stability of the formulation, or even worse, chemical degradation of the active ingredients [12]. Fourier-transform infrared spectroscopy (FTIR) analysis is a common technique used to study the compatibility of drug and excipients. It can provide valuable information about the possible interactions between the active ingredients and polymers by analyzing their functional groups [13].

2. Materials And Methods

The active ingredients used for the acne gel formulation were Metronidazole and Doxycycline. For the formation of the gel base, Hydroxypropyl methylcellulose (HPMC), Carbopol 940, and Carbopol 934 were used. Purified water was also used as a solvent and to adjust the final weight of the gel formulation.

Gel Formulation [15]

The gel was formulated by first dissolving the Metronidazole and Doxycycline in a minimal amount of purified water with continuous stirring until a clear solution was obtained. The respective polymer (HPMC, Carbopol 940, or Carbopol 934) was gradually added to this solution under constant stirring until a homogeneous gel base was formed. The gel base was then allowed to swell overnight. The final weight of the formulation was adjusted with purified water.

Calibration Curve [16]

The calibration curves for Metronidazole and Doxycycline were plotted to quantify these drugs in the formulation. An adequate quantity of each drug was dissolved in a suitable solvent to prepare a stock solution. Further dilutions were made from this stock solution to obtain solutions of varying concentrations ranging from 5 to 25 µg/mL. The absorbance of these solutions was measured using a UV-visible spectrophotometer at their respective maximum absorption wavelengths. The calibration curves were then plotted with absorbance on the Y-axis and concentration on the X-axis.

Evaluation of the Formulation

The prepared gel formulations were evaluated for different parameters.

1. **pH Measurement**: The pH of the gel formulations was determined using a calibrated pH meter. A sufficient quantity of each gel was dispersed in purified water to form a 1% solution. The pH of each solution was then measured [17].
2. **Viscosity Measurement**: The viscosity of the gel formulations was measured using a Brookfield viscometer. A specific quantity of the gel was taken, and the viscometer spindle was immersed into the gel. The dial reading corresponding to the 100% torque was recorded as the viscosity of the gel [18].
3. **Spread ability Test**: The spread ability of the gel formulations was evaluated by measuring the spread diameter. A fixed quantity of the gel was placed on a glass slide, another slide was placed on top of it, and a known weight was placed on the top slide. After a predetermined time, the weight was removed, and the diameter of the circular gel spread was measured [19].
4. **Extrudability Test**: The extrudability of the gel formulations was determined by measuring the force required to extrude a certain quantity of gel from a collapsible tube [20].

In Vitro Drug Release Study [20]

The in vitro drug release study was carried out using a diffusion cell. The prepared gel was placed in a dialysis membrane, which was then placed in a beaker containing a suitable dissolution medium. The beaker was maintained at a constant temperature, and the dissolution medium was stirred at a predetermined speed. Samples were withdrawn at specified time intervals and replaced with an equal volume of fresh medium. The withdrawn samples were analyzed for drug content using a UV-visible spectrophotometer.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis [21]

The FTIR spectra of the pure drugs, individual polymers, and the drug-loaded gel formulations were recorded to investigate the possible interaction between the drugs and the polymers. The samples were finely ground with potassium bromide and compressed into discs. The spectra were then recorded using an FTIR spectrometer. The presence or absence of characteristic peaks corresponding to functional groups was observed.

The methods and protocols used in this study were designed with the aim of reducing bias and ensuring the reliability and validity of the results. The data were analyzed using appropriate statistical tools.

3. Results and Discussion

Formulation of ACNE Gel

The ACNE gel was successfully formulated with Metronidazole and Doxycycline using three different polymers: HPMC, Carbopol 940, and Carbopol 934. The gel formulations were smooth and homogenous with no visible particulates, signifying the complete dispersion of active ingredients and polymer in the gel matrix.

Calibration Curve

The calibration curves for Metronidazole and Doxycycline displayed good linearity within the concentration range of 5 to 25 µg/mL. This indicates that the absorbance is directly proportional to the concentration, which is essential for the quantification of these drugs in the gel formulations.

Evaluation of the Formulation

pH Measurement

The mean pH values of the ACNE gel with HPMC, Carbopol 940, and Carbopol 934 were found to be 6.0, 5.8, and 5.7, respectively. These values fall within the skin pH range (4.5 to 6.5), which is essential for maintaining skin integrity and minimizing skin irritation upon application.

Table-1: Mean pH Values and Standard Deviation for ACNE Gel

Polymer	Mean pH	Standard Deviation
HPMC	6.0	0.1
Carbopol 940	5.8	0.1
Carbopol 934	5.7	0.1

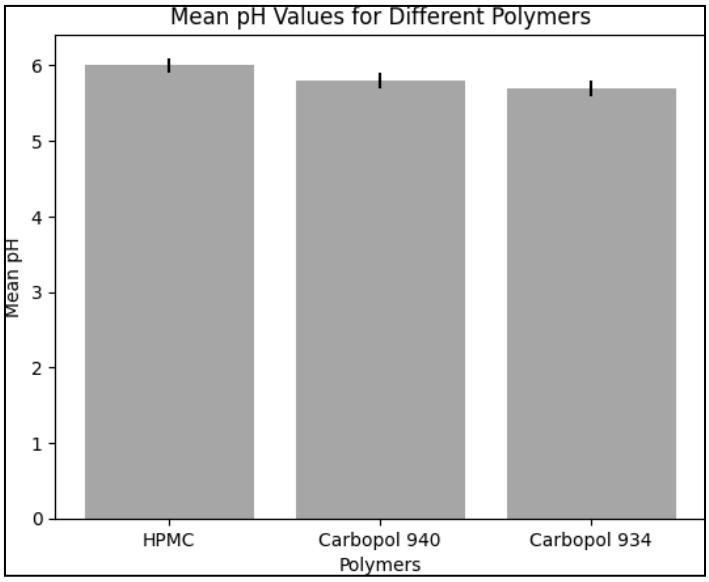

Fig 1- Mean pH Values and Standard Deviation for ACNE Gel

Viscosity Measurement

The mean viscosity values for the ACNE gel with HPMC, Carbopol 940, and Carbopol 934 were 205 cP, 220 cP, and 231 cP, respectively. These measurements reflect the consistency of the gel, which can affect the ease of application and the release of the active drug from the formulation.

Table-2: Mean Viscosity Values and Standard Deviation for ACNE Gel

Sample	Mean Viscosity (cP)	Standard Deviation (cP)
HPMC	205	4.1
Carbopol 940	220	4.1
Carbopol 934	231	3.1

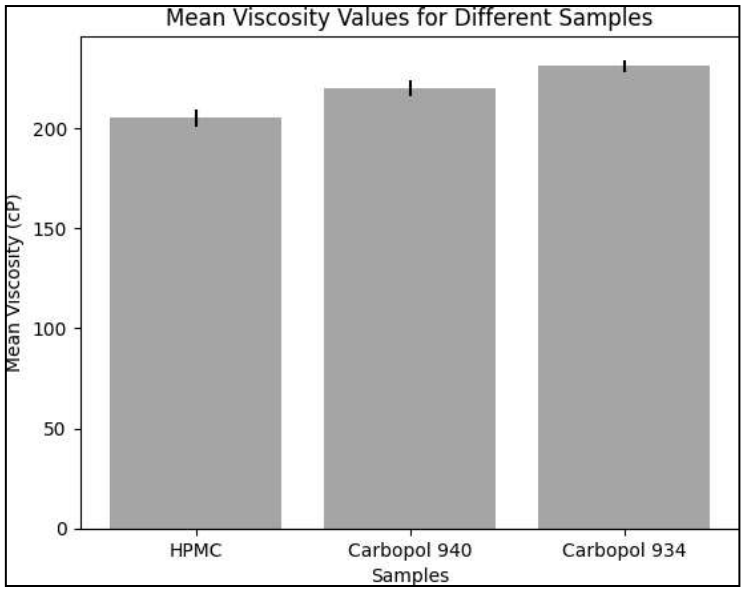

Fig. 2- Viscosity of the Formulations

Spread ability Test

The mean spread diameters of the ACNE gel with HPMC, Carbopol 940, and Carbopol 934 were 31.0 mm, 29.0 mm, and 27.3 mm, respectively. A higher spread diameter signifies better spreadability, which can influence the patient's compliance to the treatment.

Table-3: Mean Spread Diameter and Standard Deviation for ACNE Gel

Sample	Mean Spread Diameter (mm)	Standard Deviation (mm)
HPMC	31.0	1.0
Carbopol 940	29.0	1.0

Sample	Mean Spread Diameter (mm)	Standard Deviation (mm)
Carbopol 934	27.3	0.5

Extrudability Test

The mean extrusion forces for the ACNE gel with HPMC, Carbopol 940, and Carbopol 934 were 10.3 N, 11.7 N, and 9.3 N, respectively. These values indicate the force required to extrude the gel from the container, which can affect the ease of application.

Table-3: Mean Extrusion Force and Standard Deviation for ACNE Gel

Sample	Mean Extrusion Force (N)	Standard Deviation (N)
HPMC	10.3	0.6
Carbopol 940	11.7	0.5
Carbopol 934	9.3	0.5

In Vitro Drug Release Study

The cumulative drug release from the ACNE gel with different polymers showed a time-dependent increase. After 8 hours, the gel with HPMC exhibited the highest drug release (90.0%), followed by Carbopol 940 (80.0%) and Carbopol 934 (64.0%). This suggests that the type of polymer can significantly influence the drug release profile from the gel.

Table-4: In Vitro Drug Release Results for ACNE Gel with Different Polymers

Polymer	Time (hours)	Cumulative Drug Release (%)
HPMC	1	12.5
	2	25.0
	4	50.0
	6	75.0
	8	90.0
Carbopol 940	1	10.0
	2	20.0
	4	40.0
	6	60.0
	8	80.0
Carbopol 934	1	8.0
	2	16.0
	4	32.0
	6	48.0
	8	64.0

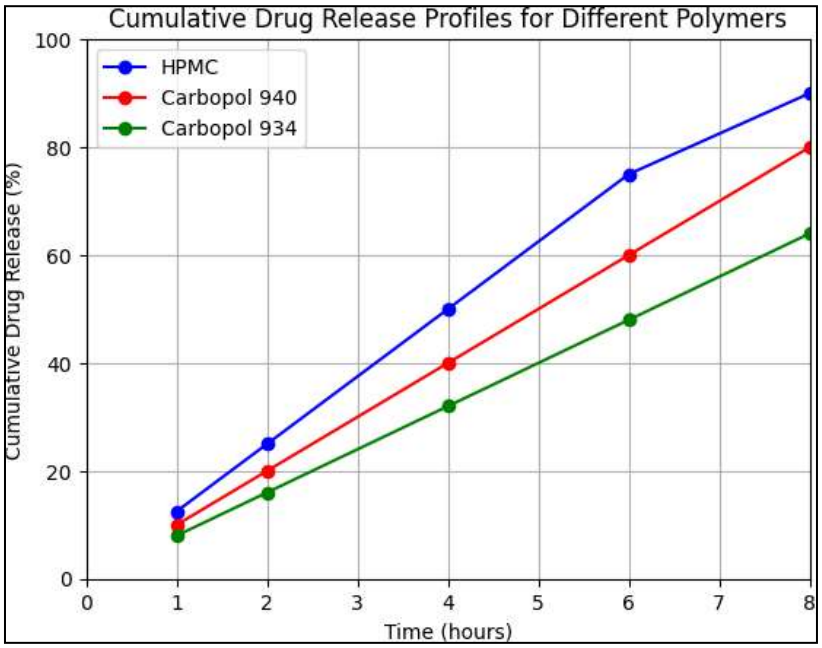

Fig. 3- In Vitro Drug Release

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR spectra of the drug-loaded gel formulations were compared with the spectra of the pure drugs and individual polymers. The characteristic peaks of Metronidazole and Doxycycline were observed in the spectra of the drug-loaded gel formulations, suggesting the presence of these drugs in the formulations. Moreover, there were no significant shifts or disappearance of the characteristic peaks, indicating the compatibility of the drugs with the selected polymers.

The aim of this study was to formulate and evaluate an ACNE gel using Metronidazole and Doxycycline as active ingredients and Hydroxypropyl methylcellulose (HPMC), Carbopol 940, and Carbopol 934 as the gelling agents. The findings suggest that the developed ACNE gel formulations exhibit promising physical and chemical properties for effective topical treatment of acne.

The gel formulations were successfully prepared with a uniform distribution of the active ingredients and polymers, resulting in smooth and homogenous gels. This is of particular importance as the homogeneity of the formulation can significantly influence the release of active ingredients, and consequently, the therapeutic effectiveness of the formulation.

The calibration curves for Metronidazole and Doxycycline were linear within the tested concentration range, facilitating the accurate quantification of these drugs in the gel formulations. The calibration curve is a vital tool in pharmaceutical analysis, serving as the reference for determining the amount of drug substance in a given sample. The strong linearity observed for both Metronidazole and Doxycycline indicates that these drugs can be accurately measured in the formulation, which is critical for quality control purposes.

The pH values of the gel formulations were found to be in accordance with the natural skin pH (between 4.5 and 6.5). Products with pH values outside this range may disrupt the skin's natural acid mantle, leading to skin irritation or dryness. Therefore, the observed pH values suggest that these formulations are likely to be well-tolerated upon topical application.

In terms of the viscosity of the gel formulations, higher viscosity generally leads to longer residence time on the skin, potentially leading to improved drug absorption. However, if a gel is too viscous, it may be difficult to apply and spread over the skin. The measured viscosities of the gel formulations in this study are expected to ensure easy application while providing a sufficient residence time on the skin for effective drug delivery.

The spreadability and extrudability of the gel formulations were also evaluated. These parameters are crucial for patient compliance as they affect the ease of application. The formulations exhibited satisfactory spreadability and extrudability, suggesting that they would be user-friendly.

The in vitro drug release profiles showed that the type of polymer significantly influences the release of the active drugs from the gel. The gel with HPMC demonstrated the highest cumulative drug release

after 8 hours. The ability to control drug release through the selection of the polymer type is a valuable aspect in topical drug delivery, where sustained release is often desired for prolonged therapeutic effect.

Lastly, the FTIR analysis revealed no significant interaction between the drugs and the polymers, suggesting that these components are compatible in the formulation. This is essential for maintaining the stability and efficacy of the gel formulation over time.

While the current study provides promising preliminary findings, further research is necessary to optimize the formulation and validate its therapeutic efficacy. This could involve exploring different concentrations of active ingredients and polymers, conducting stability studies under various conditions, and performing in vivo efficacy and safety studies. Despite these future research directions, this study serves as a critical foundation for the development of effective ACNE gel formulations with Metronidazole and Doxycycline.

4. Conclusion

It In conclusion, this study demonstrates the successful formulation and comprehensive evaluation of an ACNE gel using Metronidazole and Doxycycline as active ingredients, and Hydroxypropyl methylcellulose (HPMC), Carbopol 940, and Carbopol 934 as the gelling agents. The well-dispersed, homogenous gel formulations showed desirable characteristics that are conducive to patient use and efficacy.

The calibration curves for Metronidazole and Doxycycline provided a linear relationship between absorbance and concentration, which is critical for accurate quantification of these drugs in the formulations. The gel formulations showed pH values compatible with skin pH, a vital aspect for maintaining skin integrity and minimizing irritation upon application. Moreover, the viscosity of the gel formulations was found to be optimal, which would ensure ease of application while maintaining the effective release of the active drugs.

The results of the spreadability and extrudability tests were in line with the desirable characteristics of a topical gel formulation, indicating that the formulations would be easy to apply and would provide an acceptable sensory feeling to the patient. Importantly, the in vitro drug release study showed a steady, time-dependent increase in drug release, demonstrating the potential of these formulations for sustained drug delivery, enhancing treatment effectiveness.

The FTIR analysis revealed compatibility between the active drugs and the selected polymers, an essential consideration to ensure the stability and efficacy of the formulation. The characteristic peaks corresponding to the functional groups of the drugs and polymers were evident in the FTIR spectra of the drug-loaded gel formulations, signifying the preservation of chemical integrity and compatibility.

Taken together, the results from this study suggest that the developed ACNE gel formulations with Metronidazole and Doxycycline, using HPMC and Carbopol polymers, exhibit promising physical and chemical characteristics for effective topical treatment of acne. However, further studies, including stability testing under different conditions and in vivo efficacy studies, would be necessary to confirm the therapeutic potential of these formulations.

This study contributes to the current understanding of ACNE gel formulation and provides a framework for the further development and optimization of such formulations. It underlines the importance of careful selection of active ingredients and polymers, and rigorous evaluation of the formulations, in the pursuit of effective topical treatments for acne.

References:

1. Daniele, S. G., Kim, S. R., Grada, A., Moore, A. Y., Suozzi, K. C., & Bunick, C. G. (2023). Truncal acne and scarring: A comprehensive review of current medical and cosmetic approaches to treatment and patient management. *American Journal of Clinical Dermatology*, *24*(2), 199-223.
2. Sadati, M. S., Yazdanpanah, N., Shahriarirad, R., Javaheri, R., & Parvizi, M. M. (2023). Efficacy of metformin vs. doxycycline in treating acne vulgaris: an assessor-blinded, add-on, randomized, controlled clinical trial. *Journal of Cosmetic Dermatology*.
3. Drake, L., Reyes-Hadsall, S., Barbieri, J. S., & Mostaghimi, A. (2022). New developments in topical acne therapy. *American Journal of Clinical Dermatology*, *23*(2), 125-136.
4. Daniele, S. G., Kim, S. R., Grada, A., Moore, A. Y., Suozzi, K. C., & Bunick, C. G. (2023). Truncal acne and scarring: A comprehensive review of current medical and cosmetic approaches to treatment and patient management. *American Journal of Clinical Dermatology*, *24*(2), 199-223.
5. Delans, K., Kelly, K., & Feldman, S. R. (2022). Treatment strategies, including antibiotics, to target the immune component of rosacea. Expert Review of Clinical Immunology, 18(12), 1239-1251.

6. Callender, V. D., Baldwin, H., Cook-Bolden, F. E., Alexis, A. F., Stein Gold, L., & Guenin, E. (2022). Effects of topical retinoids on acne and post-inflammatory hyperpigmentation in patients with skin of color: a clinical review and implications for practice. American journal of clinical dermatology, 1-13.
7. Saraiva, S. M., Crespo, A. M., Vaz, F., Filipe, M., Santos, D., Jacinto, T. A., ... & Araujo, A. R. (2023). Development and Characterization of Thermal Water Gel Comprising Helichrysum italicum Essential Oil-Loaded Chitosan Nanoparticles for Skin Care. Cosmetics, 10(1), 8.
8. Albiero, M., Fullin, A., Villano, G., Biasiolo, A., Quarta, S., Bernardotto, S., ... & Morpurgo, M. (2022). Semisolid Wet Sol–Gel Silica/Hydroxypropyl Methyl Cellulose Formulation for Slow Release of Serpin B3 Promotes Wound Healing In Vivo. Pharmaceutics, 14(9), 1944.
9. Hamdi, N. A. M., Azmi, N. A., Sabari, N. H. M., Harun, A. F., & Haris, M. S. (2023). An insight into the use and advantages of Carbopol in topical mucoadhesive drug delivery system: A systematic review. Journal of Pharmacy, 3(1), 53-65.
10. De, R., Mahata, M. K., & Kim, K. T. (2022). Structure-Based Varieties of Polymeric Nanocarriers and Influences of Their Physicochemical Properties on Drug Delivery Profiles. Advanced Science, 9(10), 2105373.
11. Iemtsev, A., Hassouna, F., Klajmon, M., Mathers, A., & Fulem, M. (2022). Compatibility of selected active pharmaceutical ingredients with poly (D, L-lactide-co-glycolide): Computational and experimental study. European Journal of Pharmaceutics and Biopharmaceutics, 179, 232-245.
12. Murillo-Fernández, M. A., Montero-Zeledón, E., Abdala-Saiz, A., Vega-Baudrit, J. R., & Araya-Sibaja, A. M. (2022). Interaction and Compatibility Studies in the Development of Olmesartan Medoxomil and Hydrochlorothiazide Formulations under a Real Manufacturing Process. Pharmaceutics, 14(2), 424.
13. Moore, L. E., Vucen, S., & Moore, A. C. (2022). Trends in drug-and vaccine-based dissolvable microneedle materials and methods of fabrication. European Journal of Pharmaceutics and Biopharmaceutics.
14. Marwah, M. K., Shokr, H., Sanchez-Aranguren, L., Badhan, R. K. S., Wang, K., & Ahmad, S. (2022). Transdermal delivery of a hydrogen sulphide donor, ADT-OH using aqueous gel formulations for the treatment of impaired vascular function: an ex vivo study. Pharmaceutical Research, 39(2), 341-352.
15. Reçber, T., Timur, S. S., Kablan, S. E., Yalçın, F., Karabulut, T. C., Gürsoy, R. N., ... & Nemutlu, E. (2022). A stability indicating RP-HPLC method for determination of the COVID-19 drug molnupiravir applied using nanoformulations in permeability studies. Journal of pharmaceutical and biomedical analysis, 214, 114693.
16. de Souza, C. C., Lisboa, T. P., de Oliveira, W. B. V., Muñoz, R. A. A., Matos, M. A. C., & Matos, R. C. (2023). Simple strategy for the detection of the amoxicillin antibiotic in different matrices using a low-cost paper electrode. Talanta, 253, 124050.
17. Gorantla, S., Puppala, E. R., Naidu, V. G. M., Saha, R. N., & Singhvi, G. (2023). Hyaluronic acid-coated proglycosomes for topical delivery of tofacitinib in rheumatoid arthritis condition: Formulation design, in vitro, ex vivo characterization, and in vivo efficacy studies. International Journal of Biological Macromolecules, 224, 207-222.
18. Rompicherla, N. C., Joshi, P., Shetty, A., Sudhakar, K., Amin, H. I. M., Mishra, Y., ... & Alhumeed, N. (2022). Design, Formulation, and Evaluation of Aloe vera Gel-Based Capsaicin Transemulgel for Osteoarthritis. Pharmaceutics, 14(9), 1812.
19. Ullah, I., Alhodaib, A., Naz, I., Ahmad, W., Ullah, H., Amin, A., & Nawaz, A. (2023). Fabrication of Novel Omeprazole-Based Chitosan Coated Nanoemulgel Formulation for Potential Anti-Microbia; In Vitro and Ex Vivo Characterizations. Polymers, 15(5), 1298.
20. Gulfam, M., Jo, S. H., Jo, S. W., Vu, T. T., Park, S. H., & Lim, K. T. (2022). Highly porous and injectable hydrogels derived from cartilage acellularized matrix exhibit reduction and NIR light dual-responsive drug release properties for application in antitumor therapy. NPG Asia Materials, 14(1), 8.
21. Gudipati, M. (2022). Formulation and evaluation of biodegradable polymer triple layer coated multiparticulate system for colon targeting. International Journal of Research in Pharmaceutical Sciences and Technology, 3(3), 48-55.