

Original Article

Unveiling Synergistic Bonds: Exploring Spectroscopic Interactions between Tween 20 and Bromocresol Green for Enhanced Applications

Asma Naz¹, Sundus Khan², Aqsa Sajjad³, Kanwal Mehreen⁴, Muhammad Sajid², Muhammad Qaiser^{2,5}, Syed Waqas Bukhari⁵, Abdul Rehman¹, Tayyebah Noreen⁴, Khadija Karim², Hafiz Muhammad Usman Abid^{2*}

¹Institute of Chemical Sciences, Bahauddin Zakariya University Multan, Pakistan.

²Department of Pharmaceutics, Faculty of Pharmacy, Bahauddin Zakariya University Multan, Pakistan.

³Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Government College University Faisalabad, Pakistan.

⁴Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Bahauddin Zakaria University Multan, Pakistan.

⁵Drug Testing Laboratory, Multan, Pakistan.

*Corresponding Author: Hafiz Muhammad Usman Abid; Email: usmanabid394@gmail.com

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ABSTRACT

Background: Surfactants and dyes interact in ways that can significantly alter the optical properties of solutions, a phenomenon with broad implications across various scientific fields, including healthcare. Understanding these interactions, especially in aqueous environments, is crucial for developing advanced diagnostic tools and enhancing drug delivery systems.

Objective: This study aimed to investigate the interactions between the non-ionic surfactant Tween 20 and the dye Bromocresol Green (BCG) in aqueous solutions. By elucidating these interactions, the research sought to explore the potential for improving the accuracy and efficiency of spectroscopic assays used in medical diagnostics and therapeutic applications.

Methods: Aqueous solutions of BCG were prepared in varying concentrations of Tween 20 surfactant. Spectroscopic analyses were conducted at two wavelengths, 510 nm and 520 nm, to observe the effects of surfactant concentration on dye absorbance. The critical micelle concentration (CMC) was determined by analyzing changes in absorbance and reciprocal absorbance (1/A) patterns. Beer's Law was applied to calculate the molar absorption coefficients, providing a quantitative measure of the interaction strength between the dye and surfactant molecules.

Results: The study found that the absorbance of BCG increased linearly with the concentration of Tween 20 up to the CMC, after which the increase continued at a different rate. At 510 nm, absorbance values ranged from 0.277 to 1.627 au as Tween 20 concentration increased from 0.005 mM to 0.017 mM, with molar absorption coefficients (ϵ) escalating from 0.483 to 2.839 ($10^3 \text{ cm}^2 \text{ mmol}^{-1}$). Similar trends were observed at 520 nm, underscoring the impact of micelle formation on dye-surfactant interactions.

Conclusion: The interaction between Tween 20 and BCG significantly affects the absorbance properties of BCG in aqueous solutions, with marked changes observed upon reaching the CMC. These findings highlight the potential of surfactant-dye interactions to enhance the performance of spectroscopic assays in healthcare settings, offering pathways to more sensitive diagnostic techniques and efficient drug delivery systems.

Keywords: Surfactant-Dye Interaction, Bromocresol Green, Tween 20, Spectroscopic Analysis, Critical Micelle Concentration, Diagnostic Assays, Healthcare Applications.

INTRODUCTION

Surfactants, with their unique amphiphilic structure comprising a polar hydrophilic head and a non-polar hydrophobic tail, play a pivotal role in facilitating the interaction between polar and nonpolar substances, thus reducing surface tension at interfaces in aqueous solutions. These compounds, whether derived from natural sources or synthesized, form thermodynamically stable colloidal aggregates known as micelles upon reaching the critical micelle concentration (CMC), a saturation point beyond which surfactant molecules transition from existing primarily as monomers to forming micelles as the concentration increases, leading to

a decrease in surface or interfacial tension to its minimum (1, 2). This behavior is influenced by various factors including the concentration and type of additives, solvent media, temperature, and the presence of other amphiphiles or solvents, which can significantly affect the formation of micelles (3). The formation of micelles, which mimic biological pellicles, not only increases the surface area but also enhances the solubility and penetration abilities of organic compounds, making surfactants effective catalysts in altering chemical reaction pathways, rates, and equilibria (4, 5). Their wide range of applications spans industries such as textiles, pharmaceuticals, food, paints, metallurgy, pulp and paper, oil, and personal care, earning them the title of "green chemicals" due to their minimal toxicity to plants and animals (6-10).

The interaction between surfactants and dyes in both aqueous and non-aqueous micellar solutions has been a subject of extensive research aimed at enhancing the dyeing process from scientific, technological, ecological, and economic perspectives. This research is crucial for understanding the thermodynamics and kinetics of the dyeing process by exploring how different dyes behave within hydrophilic surfactant systems (11). Employing various analytical techniques such as tensiometry, conductometry, surfactant-sensitive electrodes, and UV-Vis spectroscopy, researchers have delved into surfactant-dye interactions, with findings that have implications far beyond the realm of dyeing, extending to genetic engineering, paper coating, and the pharmaceutical industry, while also exploring the potential for photo galvanic effects (12, 13).

In this context, the interaction between the non-ionic surfactant Tween 20 and Bromocresol Green (BCG), a pH indicator with applications ranging from DNA agarose gel electrophoresis to various other fields, presents a significant area of study. Investigating these interactions through spectroscopy and Beer's law measurements is expected to yield insights that could lead to the development of new applications and the expansion of existing ones, thereby contributing valuable knowledge to the fields of medical and biochemical research. Understanding the physicochemical properties of BCG in the presence of surfactants like Tween 20 not only enhances our comprehension of surfactant-dye dynamics but also opens up avenues for advanced applications in areas requiring precise control over substance behavior at the molecular level (14, 15).

MATERIAL AND METHODS

In this study, the materials utilized included a non-ionic dye, Bromocresol Green (BCG), with a molar mass of 698 g/mol, and a non-ionic surfactant, Tween 20, with a molar mass of 1226 g/mol, both of which were procured from Sigma Aldrich and employed without further purification. The preparation of dye-surfactant solutions was conducted using deionized water at room temperature, specifically maintained at 25°C, to ensure consistency in the experimental conditions.

For the spectroscopic analysis, stock solutions of the non-ionic dye (BCG) and the non-ionic surfactant (Tween 20) were meticulously prepared in an aqueous medium. In creating the ternary system comprising BCG, Tween 20, and water, various concentrations of the surfactant were introduced to formulate the non-ionic – non-ionic mixed micellar solutions, aiming to investigate the interaction dynamics within this system.

The experimental methodology involved the initial preparation of aqueous dye solutions, which were then utilized for spectroscopic evaluations. The surfactant/dye matrices in water were systematically formulated by adjusting the surfactant concentration while maintaining a constant dye concentration. These solutions were allowed to reach equilibrium for a precise period before the commencement of measurement procedures. The absorption spectra of the dye, in both the presence and absence of the surfactant, were recorded using a double beam spectrophotometer (UV-1800 Shimadzu), with deionized water serving as the reference for all absorption measurements.

The principal objective of the spectroscopic study was to determine the molar absorption coefficient (α), a parameter critical for understanding the interaction dynamics within the system. To achieve this, Beer's law equation, expressed as $A = 1000 \times \epsilon \times c \times l$, where A represents the system's absorbance, ϵ denotes the molar absorption coefficient (in $\text{cm}^2 \cdot \text{mol}^{-1}$), c is the solution's concentration (in mol/L), and l is the path length of the sample (in cm), was utilized.

In addition to the meticulous preparation and experimental execution, the study adhered to stringent ethical guidelines in line with the Declaration of Helsinki, ensuring that all research practices were conducted ethically and responsibly. Data collection was rigorously carried out, followed by a comprehensive analysis using the latest version of SPSS (version 25) to ensure the reliability and validity of the findings. This analysis facilitated a deeper understanding of the interactions between the non-ionic dye and surfactant, contributing significantly to the field of medical research by providing insights into the potential applications of these interactions in medical and biochemical contexts.

RESULTS

In this study, the influence of bovine serum albumin (BSA) concentration on its absorbance and the effects of Tween 20 concentration on the absorbance properties of Bromocresol Green (BCG) in aqueous solutions were meticulously analyzed through a series of

spectrophotometric measurements. The results, encapsulated in Figures 1, 2, and 3, along with Table 1, provide a comprehensive insight into the interaction dynamics between these substances.

The initial findings, as delineated in Figure 1, reveal a direct correlation between the concentration of BSA and its absorbance at two distinct wavelengths, 510 nm and 520 nm. It was observed that at a BSA concentration of 1.0 mg/mL, the absorbance was approximately 0.2 at 510 nm, increasing to about 0.3 at 520 nm. This trend continued, with the absorbance approximately doubling at a concentration of 2.0 mg/mL to about 0.4 at 510 nm and 0.6 at 520 nm, illustrating a clear proportional relationship between BSA concentration and its absorbance, thus indicating the potential for concentration-dependent changes in molecular interactions. Further exploration into the interactions between BCG and Tween 20, as detailed in Figure 2, highlighted a nuanced effect of Tween 20 concentration on the absorbance properties of BCG/Tween20/water mixtures. At both examined wavelengths, an incremental increase in Tween 20 concentration resulted in a slight decrease in the absorbance of BCG. Specifically, at 510 nm, the absorbance decreased from an initial value of 0.6 to approximately 0.45 with increasing Tween 20 concentration. This trend was mirrored in the reciprocal absorbance measurements, where a steeper positive slope was noted at 520 nm compared to 510 nm, indicating a more pronounced effect of Tween 20 at the higher wavelength.

Complementary to these observations, Figure 3 further elucidates the relationship between the reciprocal of absorbance and the concentration of Tween 20. A positive, linear relationship between the reciprocal of absorbance and the reciprocal of the sum of the concentrations of BCG and Tween 20 was observed at both 510 nm and 520 nm. This indicates that as the combined concentration of BCG and Tween 20 increases, so does the reciprocal of the absorbance, suggesting a concentration-dependent effect on the molecular environment of BCG. The relationship between the reciprocal of absorbance and the reciprocal of Tween 20 concentration alone was more complex, particularly at higher concentrations of Tween 20, indicating a nuanced interaction that affects the absorbance characteristics.

The data presented in Table 1 corroborates the observations made in the figures, revealing a strong positive correlation between the concentration of Tween 20 and the absorbance of BCG. As the concentration of Tween 20 increased from 0.005 mM to 0.017 mM, a significant increase in absorbance was noted, from 0.277 to 1.627 au at 510 nm. This was accompanied by a marked increase in the molar absorptivity of BCG, more than doubling from 0.483 to 2.839 ($10^3 \text{ cm}^2 \text{ mmol}^{-1}$), suggesting that Tween 20 significantly alters the molecular environment of the dye, thereby enhancing its ability to absorb light.

Overall, the results underscore the complex interplay between the concentration of BSA and Tween 20 and the absorbance properties of the solutions. The observed trends suggest that both the concentration of BSA and the presence of Tween 20 can significantly influence the molecular interactions within the solutions, affecting their spectroscopic properties. These findings provide valuable insights into the physicochemical dynamics of BSA and BCG in the presence of surfactants, offering potential implications for their applications in various biochemical and pharmaceutical contexts.

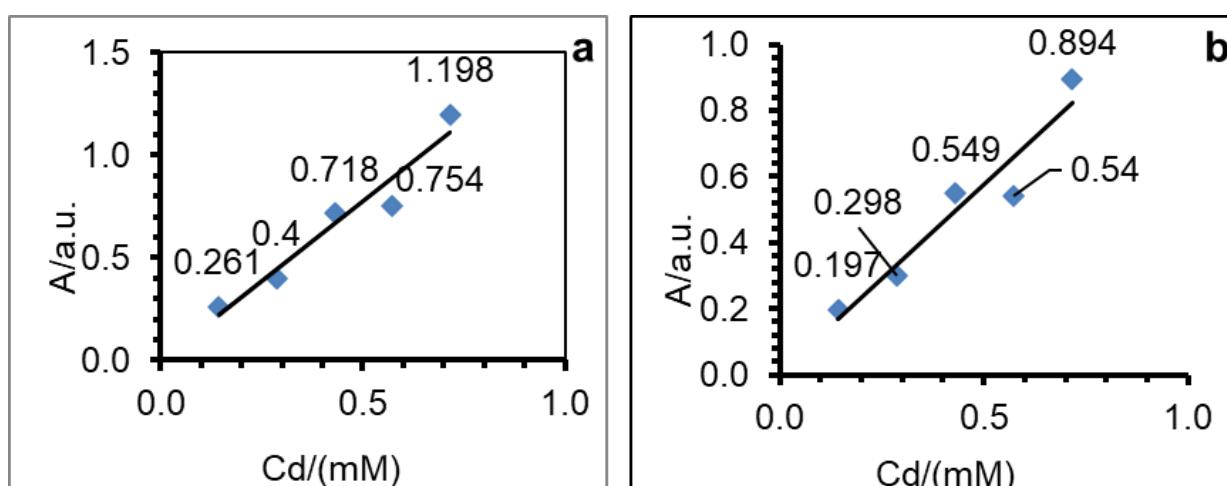


Figure 1 BCG absorbance (A) vs. concentration (Cd) at 510 nm and 520 nm (a, b).

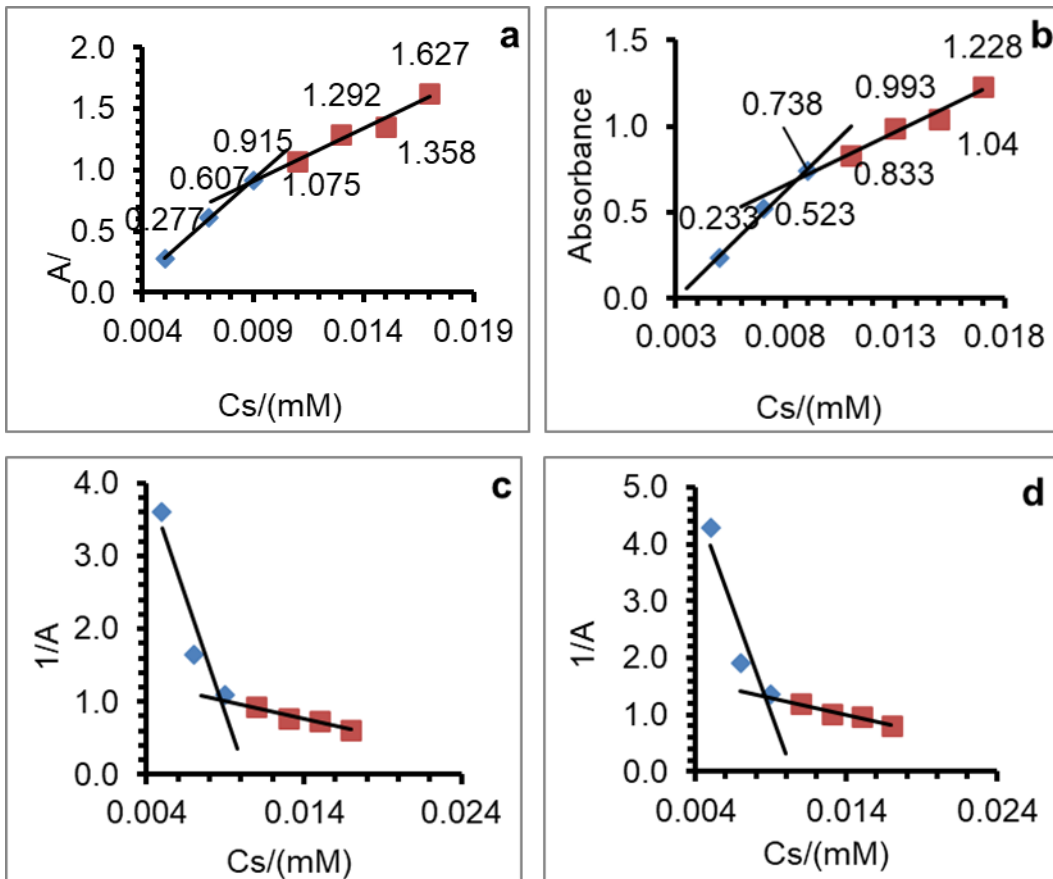


Figure 2 Tween20 concentration impacts BCG/Tween20/water absorbance (510 nm, 520 nm).

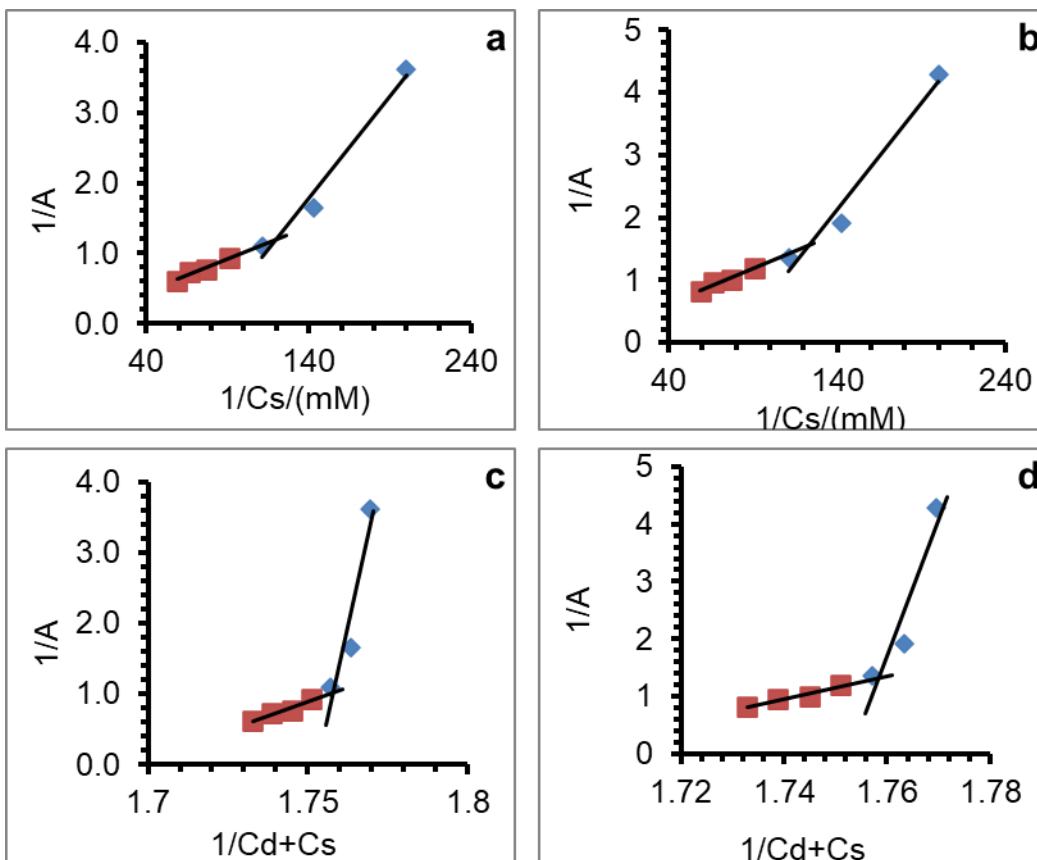


Figure 3 1/A vs. 1/(Cd+Cs) shows a linear relationship for the BCG/Tween20 system (510 nm, 520 nm).

Table 1 Beer's law parameters calculation for Tween20/BCG system at the wavelength 510 nm

No. of Obs.	Cs (mM)	Cd (mM)	Cd/Cs	λ (nm)	A (au)	ϵ ($10^3 \text{ cm}^2 \text{ mmol}^{-1}$)
01	0.005	0.5731	114.6	510	0.277	0.483
02	0.007	0.5731	81.9	510	0.607	1.059
03	0.009	0.5731	63.7	510	0.915	1.597
04	0.011	0.5731	52.1	510	1.075	1.876
05	0.013	0.5731	44.1	510	1.292	2.254
06	0.015	0.5731	38.2	510	1.358	2.370
07	0.017	0.5731	33.7	510	1.627	2.839

Table 2 Calculation of Beer's law parameters for BCG-tween 20 system at the wavelength 520 nm.

No. of Obs.	Cs (mM)	Cd (mM)	Cd/Cs	λ (nm)	A (au)	ϵ ($10^3 \text{ cm}^2 \text{ mmol}^{-1}$)
01	0.005	0.5731	114.6	520	0.233	0.407
02	0.007	0.5731	81.9	520	0.523	0.913
03	0.009	0.5731	63.7	520	0.738	1.288
04	0.011	0.5731	52.1	520	0.833	1.453
05	0.013	0.5731	44.1	520	0.993	1.733
06	0.015	0.5731	38.2	520	1.040	1.815
07	0.017	0.5731	33.7	520	1.228	2.143

DISCUSSION

The investigation into the spectroscopic behavior of Bromocresol Green (BCG) in the presence of the non-ionic surfactant Tween 20 has elucidated significant insights into the dye-surfactant interactions within aqueous solutions. The calibration curves for BCG at 510 nm and 520 nm, as delineated in Figure 1, affirm the Beer-Lambert law's premise that absorbance is directly proportional to the concentration of the analyte in solution. This foundational principle was clearly demonstrated, with a linear increase in absorbance observed as the concentration of BCG was elevated, providing a direct verification of the law (16).

The nuanced dynamics between BCG and Tween 20 were further explored through Figure 2, which depicted a linear enhancement in the absorbance with increasing concentrations of Tween 20. This increment, particularly notable post the critical micelle concentration (CMC) threshold at 0.009 mM Tween 20, underscores the pivotal role of micellization in modulating dye-surfactant interactions. The abrupt change in absorbance at the CMC underscores the transition from monomeric surfactant molecules to micellar structures, significantly impacting the optical properties of the dye.

Moreover, the reciprocal of absorbance ($1/A$) analysis offered in Figure 2 (c and d) highlights the intricacies of dye-surfactant molecular interactions. The observed decrease in reciprocal absorbance ($1/A$) upon reaching the CMC illustrates the altered microenvironment around the BCG molecules, facilitating a more complex interaction pattern between the dye and micellized surfactant. This phenomenon suggests a nuanced balance between hydrophobic and hydrophilic interactions within the ternary system, affecting the scattering and absorption of light (17, 18).

Figure 3 further elucidates the relationship between the surfactant concentration and its effect on the absorbance characteristics of the BCG-Tween 20 system. The delineation of reciprocal absorbance ($1/A$) as a function of reciprocal surfactant concentration ($1/C_s$) not only reaffirms the CMC's pivotal role but also indicates a direct correlation between these parameters. This correlation is significant, reflecting the enhanced interaction dynamics post-CMC, which in turn influences the spectroscopic properties of the dye (19).

The empirical findings of this study resonate with previous investigations, reinforcing the notion that surfactant concentration and the formation of micelles profoundly influence the optical properties of dyes in solution. The gradual increase in the molar absorption coefficient (ϵ) with surfactant concentration signifies the enhanced capacity of the BCG molecules to interact with light, a phenomenon attributable to the more organized micellar structures trapping the dye molecules more efficiently (7, 20).

This study's strengths lie in its systematic approach to delineating the complex interplay between BCG and Tween 20, offering a comprehensive understanding of the surfactant's impact on the dye's absorbance characteristics. However, the research is not without limitations. The reliance on specific concentrations of BCG and Tween 20 may not encapsulate the full spectrum of potential interactions in varying environmental conditions. Moreover, the study's scope was confined to aqueous solutions, potentially limiting its applicability to other solvent systems or conditions where different interaction dynamics may prevail.

In light of these findings and their limitations, future research should consider exploring a broader range of concentrations and extending the analysis to other surfactant-dye systems. Additionally, investigating the impact of varying solvent media on the surfactant-dye interactions could provide deeper insights into the molecular mechanisms governing these phenomena. Such endeavors could significantly enhance our understanding of dye-surfactant interactions, with implications for their application in diverse fields ranging from pharmaceuticals to environmental science (19-21).

CONCLUSION

The investigation into the interactions between Bromocresol Green (BCG) and Tween 20 surfactant has revealed significant insights into the modulatory effects of surfactants on dye absorbance in aqueous solutions, demonstrating the critical role of micelle formation beyond the critical micelle concentration (CMC). These findings underscore the potential of manipulating surfactant concentrations to enhance the sensitivity and specificity of spectroscopic assays, offering promising avenues for the development of improved diagnostic tools and therapeutic delivery systems in healthcare. The ability to fine-tune the optical properties of dyes through surfactant interactions opens up new possibilities for advancing medical research, particularly in the areas of drug delivery mechanisms and the design of more effective diagnostic assays, thereby contributing to the enhancement of patient care and treatment outcomes.

REFERENCES

1. Ghosh S, Ray A, Pramanik N. Self-assembly of surfactants: An overview on general aspects of amphiphiles. *Biophysical Chemistry*. 2020;265:106429.
2. Pandey K, Hanif M, Ameer N, Mahmood K, Abid U, Rana HL, et al. Comparison of Hydrophilic and Lipophilic Surfactants for the Improvement of Solubility of BCS Class II Drug by First Generation Solid Dispersion Method. *Latin American Journal of Pharmacy*. 2022;41:3.
3. Gurung J, Anjudikkal J, Pulikkal AK. Amphiphilic drug-additive systems in aqueous and organic solvent-water mixed media: A comprehensive account on physicochemical properties. *Journal of Molecular Liquids*. 2020;318:114221.
4. Kitanosono T, Kobayashi S. Synthetic organic "aquachemistry" that relies on neither cosolvents nor surfactants. *ACS Central Science*. 2021;7(5):739-47.
5. Abid U, Pervaiz F, Shoukat H, Rehman S, Abid S. Fabrication and characterization of novel semi-IPN hydrogels based on xanthan gum and polyvinyl pyrrolidone-co-poly (2-acrylamido-2-methyl propane sulfonic acid) for the controlled delivery of venlafaxine. *Polymer-Plastics Technology and Materials*. 2022;61(6):577-92.
6. Abid HMU, Hanif M, Mahmood K, Aziz M, Abbas G, Latif H. Wound-Healing and Antibacterial Activity of the Quercetin-4-Formyl Phenyl Boronic Acid Complex against Bacterial Pathogens of Diabetic Foot Ulcer. *ACS Omega*. 2022;7(28):24415-22.
7. Aleem A, Khan M, Abid U, Ishfaq MF, Rouf A, Jamshaid T. Microbial analysis of selected brands of whitening creams. *Saudi J Med Pharm Sci*. 2020;6:178-82.
8. Rizwan M, Abid HMU, Akhlaq M, Manzoor A, Ansari M, Rajpoot SR, et al. COMPARATIVE EFFICACY OF ALOE VERA (ALOBARBADENSIS) AND TURMERIC (CURCUMA LONGA) ON BURN WOUND HEALING IN RABBIT MODEL. *Journal of Population Therapeutics and Clinical Pharmacology*. 2024;31(2):526-34.
9. Abid HMU, Azeem M, Qaiser M, Faqeer MT, Abid S, Shakil M, et al. NAVIGATING THE VASCULAR FRONTIER: EXPLORING QUERCETIN-BASED DRUG DELIVERY STRATEGIES IN CARDIOVASCULAR THERAPY. *Journal of Population Therapeutics and Clinical Pharmacology*. 2024;31(1):1702-19.
10. Abid S, Abid HMU, Saleem M, Khurshid U, Afzal F, Usman M, et al. " Revolutionizing Sericulture: Unleashing the Potential of Ascorbic Acid for Optimal Silkworm Well-being and Maximized Silk Production.
11. Bhadani A, Kafle A, Ogura T, Akamatsu M, Sakai K, Sakai H, et al. Current perspective of sustainable surfactants based on renewable building blocks. *Current Opinion in Colloid & Interface Science*. 2020;45:124-35.
12. Azeem M, Hanif M, Mahmood K, Ameer N, Ramzan N, Abid U, et al. Enhanced antibacterial and antioxidant properties of chitosan-quercetin complex containing polycaprolactone microspheres for the treatment of gastroenteritis: An in-vitro and in-vivo analysis. *Materials Today Communications*. 2022;31:103780.
13. Usman Abid HM, Hanif M, Mahmood K, Abid S, Khan M, Khurshid U, et al. Exploring the Potent Combination of Quercetin-Boronic Acid, Epalrestat, and Urea Containing Nanoethosomal Keratolytic Gel for the Treatment of Diabetic Neuropathic Pain: In Vitro and In Vivo Studies. *Molecular Pharmaceutics*. 2023.
14. Ahmad M, Khan M, Asif R, Sial N, Abid U, Shamim T, et al. Expression Characteristics and Significant Diagnostic and Prognostic Values of ANLN in Human Cancers. *International Journal of General Medicine*. 2022:1957-72.

15. Hanif M, Ameer N, Mahmood MK, Shehzad A, Azeem M, Rana HL, et al. Improved anti-inflammatory effect of curcumin by designing self-emulsifying drug delivery system. *Drug Development and Industrial Pharmacy*. 2021;47(9):1432-8.
16. Mayerhöfer TG, Pahlow S, Popp J. The Bouguer-Beer-Lambert law: Shining light on the obscure. *ChemPhysChem*. 2020;21(18):2029-46.
17. Yang Y-Y, Niu C-G, Huang D-W, Guo H, Feng H-P, Li L, et al. Appropriate oxygen vacancies and Mo-N bond synergistically modulate charge transfer dynamics of MoO₃-x/S-CN for superior photocatalytic disinfection: Unveiling synergistic effects and disinfection mechanism. *Journal of Hazardous Materials*. 2023;445:130481.
18. Xiong S, Zeng H, Deng Y, Tang R, Wang J, Li L, et al. Unveiling the synergistic effect of internal Fe single atoms and introduced Fe₃C in Enteromorpha derived biochar with enhanced peroxy monosulfate activation property towards nitenpyram removal. *Biochar*. 2023;5(1):19.
19. Ngo QP, Nguyen TT, Le QTT, Lee JH, Kim NH. Unveiling the Synergistic Effect of Atomic Iridium Modulated Zirconium-Doped Pure Phase Cobalt Phosphide for Robust Anion-Exchange Membrane Water Electrolyzer. *Advanced Energy Materials*. 2023;13(44):2301841.
20. Sun T, Chen J, Lao X, Zhang X, Fu A, Wang W, et al. Unveiling the Synergistic Effects of Monodisperse Sea Urchin-like PdPb Alloy Nanodendrites as Stable Electrocatalysts for Ethylene Glycol and Glycerol Oxidation Reactions. *Inorganic Chemistry*. 2022;61(26):10220-7.
21. Lv X, Jiang M, Chen J, Yan D, Jia H. Unveiling the lead resistance mechanism and interface regulation strategy of Ru-based catalyst during chlorinated VOCs oxidation. *Applied Catalysis B: Environmental*. 2022;315:121592.