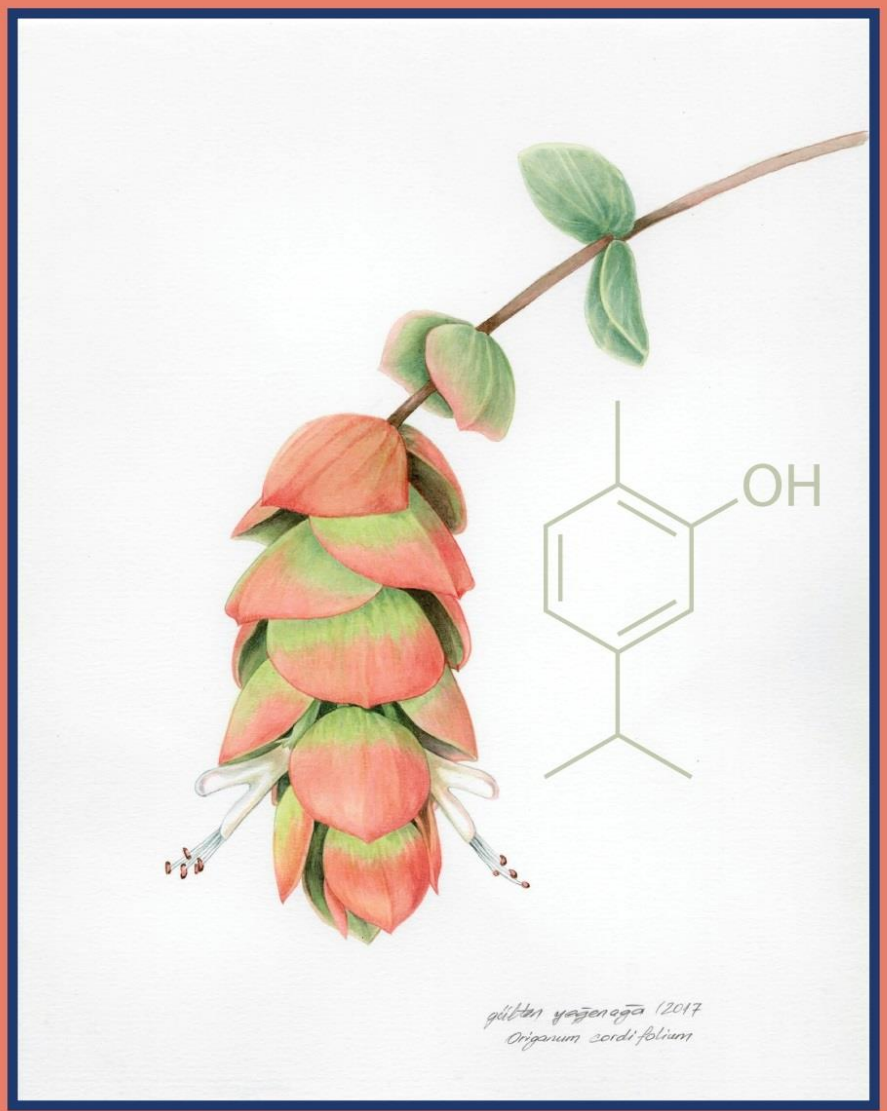
EMU
Journal of
Pharmaceutical
Sciences

ISSN: 2651-3587
Volume 6 / Issue 1

EMUJPharmSci

July 2023



Eastern Mediterranean University Press

EMU
Journal of
Pharmaceutical
Sciences

EMUJPharmSci

EDITORIAL BOARD

Origanum cordifolium* **in cover picture was illustrated by Gülten Yeğenağa

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Advisory/Scientific Board

EMU
Journal of
Pharmaceutical
Sciences
EMUJPharmSci

GUIDE FOR AUTHORS

EMU Journal of Pharmaceutical Sciences (EMUJPharmSci) covers the research on all aspects of Pharmacy presented as original articles, short reports and reviews.

EMU Journal of Pharmaceutical Sciences is published three times (March, August, December) in a year. It is an open access and peer-reviewed journal.

- Contributions to EMU Journal of Pharmaceutical Sciences must be in English.
- All manuscripts are subject to editorial review.
- The manuscripts should not be previously published or accepted for publication and should not be submitted or under simultaneous consideration for publication elsewhere.
- The manuscripts are published in the order of final acceptance after review and revision.
- If the manuscript is returned to authors for revision and the revised manuscript is not received by the editor within 2 months it will be treated as a new article.
- If the manuscript is accepted and the proof is returned to the authors, corrected proofs should be sent to the editor within 5 days.

Original articles: These are limited to 15 typewritten pages in addition to supplementary materials (schemes, tables, figures, etc.).

Short papers: Short papers are limited to 5 typewritten pages and maximum of 2 supplementary materials (schemes, tables, figures).

Reviews: They are limited to 20 pages in addition to supplementary materials (schemes, tables, figures, etc.).

- The original manuscript must be arranged as follows: Title page (including the title, authors and correspondence address), abstract, key words, introduction, materials and methods, results and discussion, acknowledgements and references.
- The reviews must be arranged as follows: Title page (including the title, authors and correspondence address), abstract, introduction, discussion, acknowledgements and references.

EMU Journal of Pharmaceutical Sciences
EMUJPharmSci

1. General Format

a) All manuscripts can only be submitted electronically via DergiPark.
b) Manuscripts should be 1,5 lines spaced and justified.
c) Use 2.5 cm margins, Times New Roman and format for A4 paper.
d) Number all pages, starting with the title page.
e) Spell out all acronyms in full at first use.
f) Make sub-headings if necessary.
g) Follow internationally accepted rules and conventions: use the international system of units (SI).

2. Before main text

A. Title page

a) The first page of the manuscript is a title page containing the following information:
b) The manuscript's full title (*Font: Times New Roman Font Size: 13*). The title must be concise and informative.
c) All authors' full names (*Font: Times New Roman Font Size: 11).*
d) The affiliation of the author(s) should be linked by superscript numbers, and listed beneath the title.
e) Corresponding author (*Font: Times New Roman Font Size: 10).* E-mail, telephone and fax number (with country and area code) of the corresponding author should be provided.
f) Ethical approval should be attached for manuscripts involving studies with human/laboratory animals participants.

B. Abstract

a) The abstract appears on its own page.
b) The abstract should be written in Times New Roman and font size 11.
c) The maximum length of the abstract is 200 words.
d) The abstract should contain the objectives, methods, results and conclusions.
e) 3- 6 key words must be provided in alphabetical order (Font: Times New Roman Font Size: 10). Separate the keywords with colon.

3. Main text

A. Introduction

(Font: Times New Roman Font Size: 12)
State the objectives of the work and provide a brief background of the literature related with the topic. The novelty and the aim of the study should be clearly stated.

B. Materials and Methods

(Font: Times New Roman Font Size: 12)

a) Give a brief and clear description of the materials and methods used. Subtitles can be given as appropriate.
b) For plant materials, herbarium name (or acronym), number, name and surname of the person who identified the plant materials should be indicated in this part of the manuscript.
c) Statistical analysis must be provided when necessary.

EMU Journal of Pharmaceutical Sciences
EMUJPharmSci

C. Results / Discussion

(Font: Times New Roman Font Size: 12)

A combined Results and Discussion section is often appropriate. Results should be concise.

Discussion should explore the significance of the results of the work.

Discussion should not repeat the results.

The main conclusions of the study should be presented.

D. Acknowledgement

(Font: Times New Roman Font Size: 10)

Supporting institutions or individuals should be briefly acknowledged just before the reference list.

E. References

i. Citation in text

(Font: Times New Roman Font Size: 12)

- Please ensure that every reference cited in the text is also present in the reference list (and vice versa).
- Unpublished results and personal communications are not recommended in the reference list.
- References in the text should be cited as: the author(s) surname and the publication date**.**

Examples:

(Sahin, 2000) – one author

(Sahin and Kosar, 2000) – Two authors

(Sahin *et al.*, 2000) – more than two authors

(Celik and Ozhatay 2000 a, b) – More than one paper in the same year by the same author (s)

(Ozhatay and Avci, 2000; Ozhatay *et al.*, 2001; Ozhatay, 2005) – listed by the earliest year first for multiple citations.

ii. Reference style

(Font: Times New Roman Font Size: 10)

- The list of references should be single-spaced.
- List the references in alphabetical order under section of "references".
- For references up to 5 authors, write the names of all authors.
- For references more than 5 authors, write the names of the first 5 and add *et. al.*
- The title of journal should be abbreviated in italics according to the style used in the National Library of Medicine's Journals in NCBI Databases.
- Volume numbers should be indicated in bold letters.

iii. Examples

Reference to a journal publication:

Ozhatay N, Kultur S, Gurdal B (2017). Check-list of additional taxa to the supplement flora of Turkey VIII. *Istanbul J Pharm* **47**(1): 31-46.

Reference to a book:

Strunk W Jr, White EB (1979). The Elements of Style. 3rd ed. New York, NY: Macmillan.

Reference to a chapter in an edited book:

Bonati A (1988). Industry and conservation of medicinal plants. In Akerele O, Heywood V, Synge H (eds). The Conservation Medicinal Plants p.141-148 Cambridge University Press UK.

Electronic resources:

World Nuclear Association (WNA) (2014). Radioisotopes in Medicine, http://www.world-nuclear.org/info/ Accessed 13.10.2014.

EMU
Journal of
Pharmaceutical
Sciences
EMUJPharmSci

4. After main text

Figures / Tables captions

- Use figures and tables when information cannot easily be stated or summarized in the manuscript itself.
- All the figures and tables must be referred to in the main body of the text.
- Tables and Figures should be numbered consequently in the order of appearance within the text, referred as “Table 1” and “Figure 1”.
- Descriptive titles should be given at the top of the tables and at the bottom of the figures.
- Figures should be prepared with the highest resolution and should be provided as a separate page following references.

Submission checklist

Check the following submission list before submit your manuscript:

- Full E-mail address, full postal address, telephone and fax number of the corresponding author.
- All necessary files have been uploaded.
- References are in the correct format for this journal.
- All references mentioned in the Reference list are cited in the text.
- All figure captions.
- All tables (including title, description, footnotes).
- For any further information please e-mail:
  emuj.pharmsci@emu.edu.tr

EMU
Journal of
Pharmaceutical
Sciences
EMUJPharmSci

CONTENTS

Research articles

Reviews

Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Evaluation of Microbiological Cleanliness of Machines/Equipment through Rinse Technique Using Statistical Process Control

Mostafa Essam Eissa[1*], Engy Refaat Rashed[2]

[1] Independent Researcher, Pharmaceutical and Healthcare Research Facility, Cairo, Egypt.

[2] National Centre for Radiation Research and Technology, Cairo, Egypt.

Abstract

To guarantee that patients receive safe therapy with predictable and acceptable medicinal properties, monitoring the quality standards in the healthcare sector and the pharmaceutical business, in particular, is essential. Medicinal products are no exception from these crucial characteristics and hence mitigation of the sources of microbial contamination is a mandatory strategy to avoid harming already-ill populations. Machines, equipment and tools that are used in the industry must be appropriately cleaned to ensure that they will not contaminate the product under processing. The study herein aimed to establish an evaluation system for cleaning efficiency through the rinse technique using Statistical Process Control (SPC) methodologies. A database was established and created for the recorded cleaning process over 20 months of the monitoring period. The control charts were constructed and evaluated from processed data using SPC software. A rare event control chart was used to track the cleaning process intervals with event probability estimated to be 0.080. Concerning the monitoring of the bioburden of rinse samples, the most appropriate fitting attribute chart is U type with Laney modification of over-dispersion to correct for tight control limits that increase alarming false points. Understanding the inspection property is inevitable for the right interpretation of trends.

Keywords

Laney correction, U chart, over-dispersion, control limits, SPC, event probability

Article History

Submitted: 31 October 2022 Accepted: 21 June 2023 Published Online: July 2023

Article Info

***Corresponding author:** Mostafa ESSAM EISSA **email:** mostafaessameissa@yahoo.com

Research Article:

Volume: 6 Issue: 1 2023 Pages: 1-12

DOI: 10.54994/emujpharmsci.1196909

INTRODUCTION

The establishment and dissemination of the quality concept are crucial for any reputable organization that strives to survive and grows in the modern competitive world (Kanji *et al.,* 1999; Zakuan *et al*., 2012). Total Quality Management (TQM) is a vision and perspective of management that focuses on long-term success through customer satisfaction (Mehra and Ranganathan, 2008). In a TQM initiative, every employee of a firm takes part in enhancing their workplace's processes, goods, and services. One of the important statistical tools is the process-behavior diagram which is used in the control and monitoring of a specific process and/or certain inspection characteristic.

One of the critical quality aspects in the healthcare industry, specifically the pharmaceutical field is the consistent safety of the delivered service or product to the final customer (Marucheck *et al*., 2011). Microbiological quality and safety are among the hot topics, especially when considering that medicinal goods are intended to be administered to populations that are sick and with health problems in most situations. This is a critical aspect to avoid any adverse events that could be stemmed from inappropriate product handling and processing.

During manufacturing and processing, medicinal products may be getting exposed to microbial contamination through the processing machines, equipment and tools themselves. Thus, it will be crucial to monitor and control their bioburden quality to ensure stability, predictability and diminishing level of contamination. The investigation herein focuses on the examination of the microbiological cleanliness of the machine/equipment that is used in the preparation of non-sterile medicinal products through rinse samples using Statistical Process Control (SPC).

MATERIALS AND METHODS

The establishment of a measurable monitoring and control system for the assessment of the microbiological cleanliness of the machines and/or equipment that are used in the medicinal sector would be crucial for microbial safety of the manufactured products to be safe for consumption by minimization of the product risk of contamination. The Total Viable Count (TVC) was used as an estimate for microbiological cleanliness which was determined following the path of previous works (Eissa, 2018; Eissa *et al*., 2022).

Study principle and goal

Evaluation of cleaning quality for specific items is usually conducted through swabbing and/or rinse methods to monitor and control the efficacy of the standard practice established in the firm chemically and microbiologically (Schmitt and Moerman, 2016). Herein, the present investigation will focus on the method used to investigate a segment of this practice which is the microbiological quality of the rinse samples that were collected from equipment/machines after cleaning according to Standard Operating Procedure (SOP). Since bioburden count is examined in this case, the monitoring was performed using an attribute control chart (Eissa *et al*., 2021). The microbial count is expressed as Colony Forming Unit (CFU) which belongs to a discrete type of dataset and quantification is usually stated as CFU/mL or CFU/100 mL and the maximum allowable limit was set at 100 CFU/100 mL (Walters, 2008; Eissa *et al*., 2022). There should be an appropriate surveillance procedure over machinery and tools to maintain control over the TVC of microbes and hence protect the processed product from exposure to high microbial populations that could be transferred to the patients or terminal users (Roesti, 2019).

Control charts selection

From this perspective, C or U charts may deem the most appropriate types for this type of analysis (Eissa, 2018). Nevertheless, previous experience in the trending and collection of this type of data rarely follows the prerequisite of Poisson distribution for proper interpretation of these types of trending charts (Laney, 2002). This challenge could lead to misleading reading and interpretation of data due to incorrect control limits with subsequent false out-of-control alarms. Thus, if the initial diagnostic probability plot test failed, a correction procedure might be necessary to adjust for data dispersion (Rinaman, 2018). With this respect, Laney's approach was sought as a reasonable means to solve this issue based on previous experience. It will be plausible to conduct a preliminary examination and visualization of the process rate by using time-interval type of chart i.e. G chart which is known as a rare event process behavior chart to determine the frequency of the cleaning operation.

Programs and software application

Bioburden data of Equipment/Machine rinse was collected and processed in the Microsoft Excel database after chronological arrangement based on dates. The study coverage period embraces 20 months which is equivalent to about 91 weeks or 635 days. The generated dataset was then analyzed using Minitab version 17.1.0 (Ryan *et al*., 2013; Rinaman, 2018). G control chart was drawn from the “Stat”

tab then going to “Control Charts” and selecting “Rare Events Charts”. The chart that illustrates the number of opportunities or days between events i.e. G chart was selected. Since the sample sizes (rinses) were variable and greater than unity, the U chart was the option of choice in this case and was selected from the same tab till reaching “Attribute Charts”. The first step here is to run a diagnostic test to determine whether to use an ordinary U or Laney trending chart. The second step is to execute the run of the most appropriate chart based on the fit to the Poisson probability plot (Ryan et al., 2013; Rinaman, 2018). The U chart monitors the average defects per unit which are herein corresponding to the TVC per 100 mL rinse or microbial count per mL may be equivalent to a Defect per Unit (DPU) using the “Assistant” tab help scheme for “Control Charts”.

RESULTS AND DISCUSSION

This study was established with the aim to disseminate the concept of the implementation of SPC techniques and methodologies to monitor, investigate, correct and improve the bioburden quality – and hence safety – in the healthcare field with a specific scope that focused on the rinse as a marker for the efficacy in the cleaning process from the microbiological point of view. The examination of microbial count data did not return any sign of out-of-specifications results with none of the rinse samples exceeding 100 CFU/mL (Steyaert, 2021). The details of the usability and the outcome of the Shewhart charts will be discussed in the following sections based on the software analysis results.

G process behavior chart output

The average interval between machine and equipment rinses is about one week. Figure 1 shows a rare event control chart that demonstrates an interval timeline of chronologically arranged machines' rinse record.

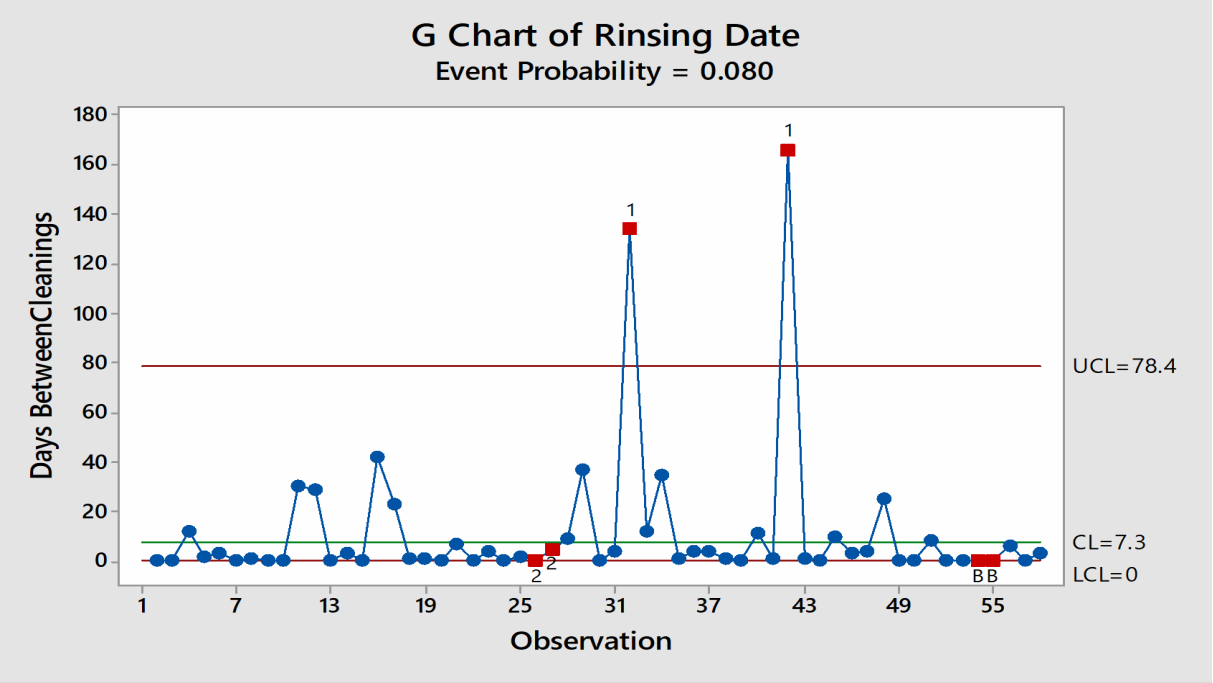


Figure 1: Rare event control chart illustrating the intervals and frequency of the machine and equipment cleaning process.

The event probability is 0.080. The event probability is the chance of an event occurring on any given day. For the machine and equipment cleaning data, the rinse process occurring on a given day is 8.0% (Park and Wang, 2022). Two points failed the Benneyan "B" test (three points in a row are equal to zero), which indicates that three or more cleaning and rinsing procedures were recorded on the same day. Alarm points "1" are showing higher than usual intervals between event dates with a "K" value greater than three standard deviations (σ) from the center line. In the same line, the red dots "2" indicate nine points on the same side of the mean line.

Checklist for reporting U trending chart

Assessment of the fulfillment of the Laney U (prime) attribute process behavior chart criteria were subjected for brief screening as the following (Minitab® Statistical Software: The Assistant, 2022):

Stability

The number of CFUs per 100 mL of rinse might not be stable. Ten (27%) subgroups are out of control (0.7% of out-of-control subgroups might be observed by chance, even when the process is stable). Out-of-control subgroups should be investigated to decide whether to omit those with special causes from the calculations.

Number of subgroups

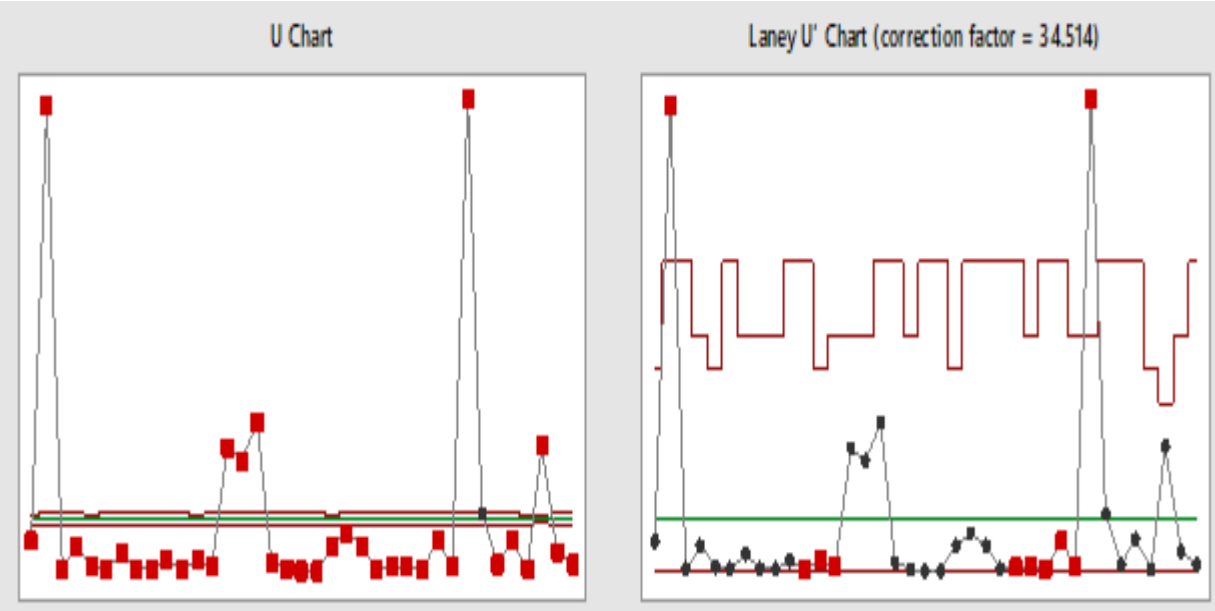
The precision of the control limits would not be of concern because more than seven subgroups are included in the calculations.

Subgroup size

The accuracy of the investigated control limits would be acceptable because all subgroups have at least one unit.

Expected variation

The present data have more variation than expected, a condition known as over-dispersion. This causes the control limits on a U chart to be too narrow for the examined data, resulting in an increased number of false alarms (Figure 2). The Laney U′ chart corrects the control limits to account for the over-dispersion. The ratio of observed variation to the expected variation was found to be 2711.3%. 95% upper limit for ratio if the process mean is constant and its value is 140.2%. Using a U chart might result in an elevated false alarm rate. Thus, the use of the Laney U′ chart could be considered instead of the conventional one. It should be noted that the upper limit depends on the number of subgroups and the process mean.



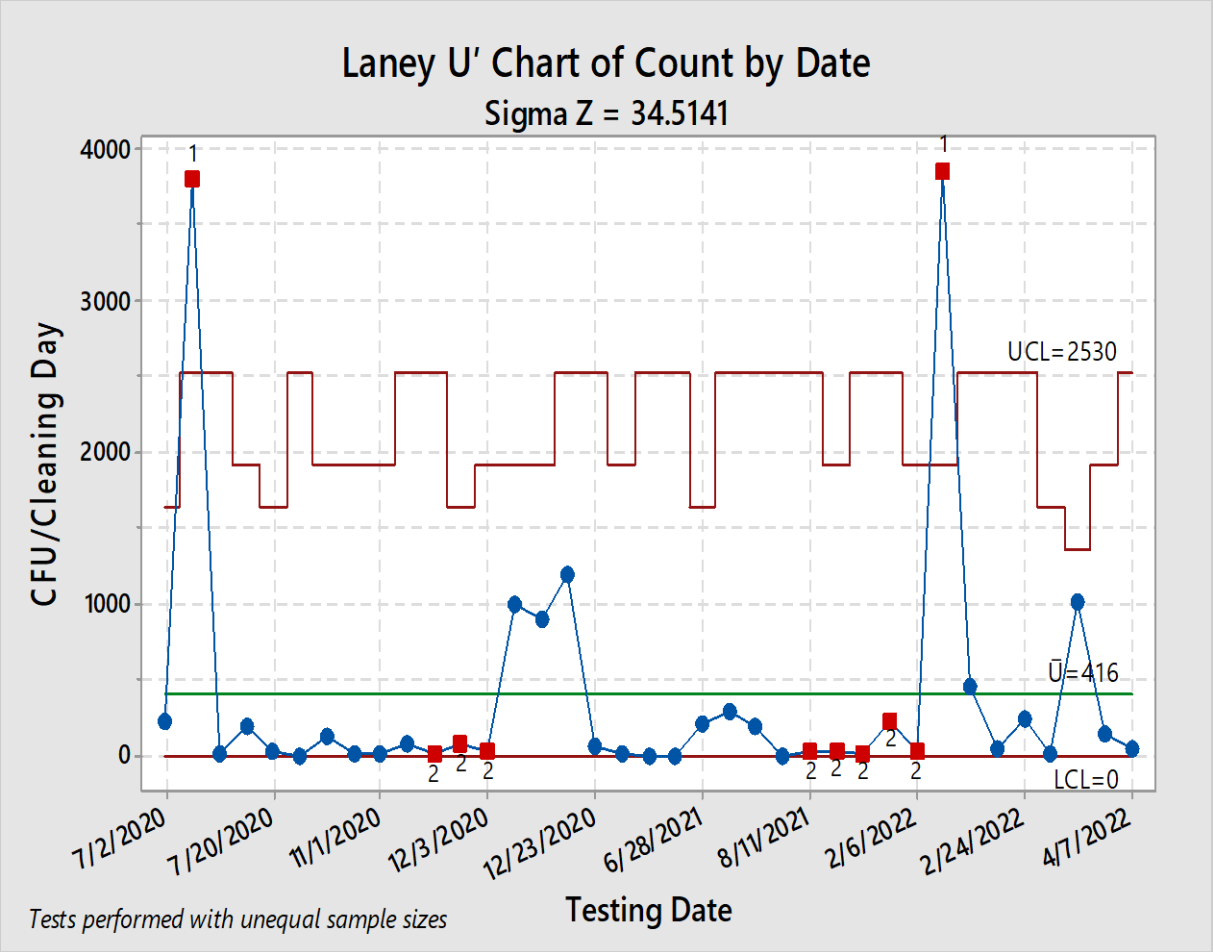


Figure 2: Analysis of the deviation of the dataset scattering from the assumed Poisson distribution and the effect on the extrapolation of the control chart.

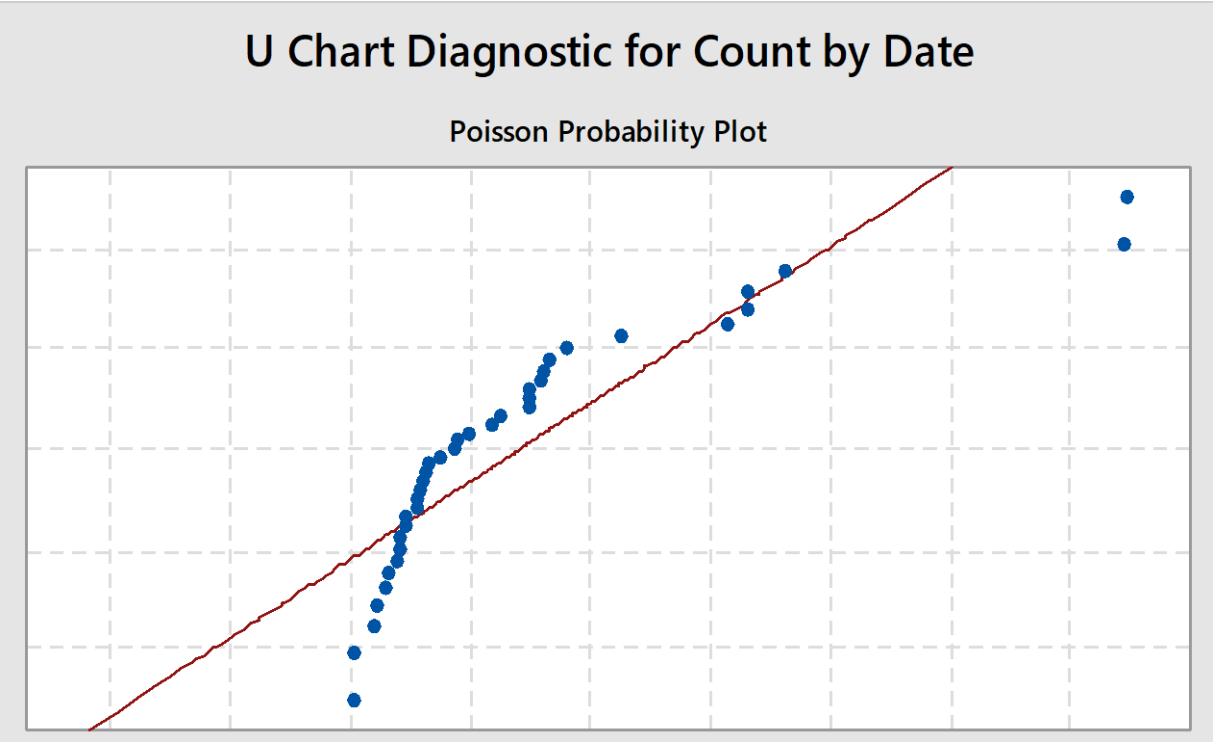
The statistical process software assesses the observed variation as a percentage of expected variation to determine which chart should be use used. The current microbiological rinse data have considerably more variation than expected (over-dispersion), which can result in an increased number of false alarms. Accordingly, the Laney U′ chart was used, which corrects this condition (Pharma Focus Asia (FPA), 2022). The use of an ordinary U chart would lead to excess variation which results in control limits that are too narrow for the existing data, which can cause an elevated false alarm rate.

Implementation of Laney U′ (prime) control chart

Laney U′ Chart imposes modification for adjustment of the excessive variations due to over-dispersion of the dataset results (correction factor (Σ Z) = 34.514). The Laney U′ chart corrects the control limits to account for the excess variation. Hence, the chart should signal appropriately (Minitab® 20 Support, 2022). Assessing the stability of the defects per unit in the investigated process and looking for patterns could help

quality officer investigators to distinguish between common and special causes. Typically, a process that exhibits only common causes has a constant defect rate. However, global trends or cyclical patterns may also be common causes. Other patterns, such as shifts and drifts, might be due to special causes. This chart has variable sample sizes which are reflected on the Upper Control Limit (UCL) as could be found in Figure 3 where it is not a fixed straight line as that for the C chart.

The stability of the microbial count in the rinse samples showed that the number of CFUs per 100 mL of the rinse may not be stable. Ten (27.0%) subgroups are out of control. Keeping in mind that 0.7% out-of-control subgroups might be found by chance, even when the process is stable. Nevertheless, the microbial count data has a single-side specification criterion towards the UCL. Hence, there is no specific lower limit as the bioburden level devolves to zero, it becomes better (Vargas *et al.*, 2022). Accordingly, the reported shift in the TVC below the average line was found to be insignificant with a minor shift - as could be found from the trending chart - and a decision was made to cancel these alarms that have been spotted by the program. Thus, the significant excursions were actually two and the rate of the out-of-control bioburden level would be 5.4%.



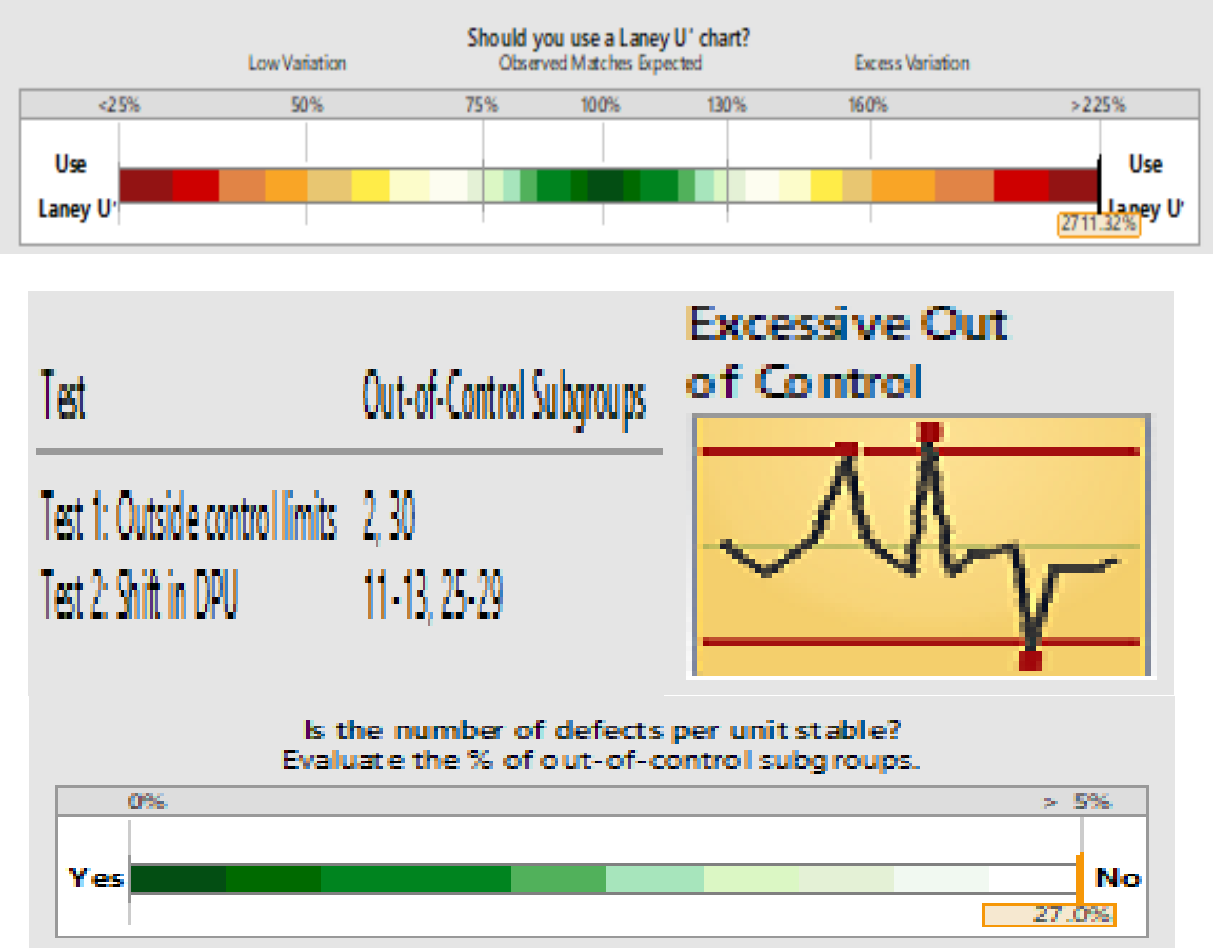


Figure 3: Laney corrected attribute chart showing the trending chart (with control limits), correction factor for over-dispersion, detected out-of-control rinse points and the defect rate.

Defects and bioburden concept in control charts

Table 1 summarizes the outcome of Figures 1 and 3 by showing the number of failed sequences from the time series order number. Benneyan alarm is unique for the G chart and showed two successive points.

Table 1: Comparison between two types of the trending charts implemented in the rinse study.

Comparison	**Process Behavior Chart**	
	G Chart	**Laney U (Prime) Chart**
Chart Type	Rare event	Attribute
Failed Test Points:		
1- One Point > 3 σ from CL: "1"	32, 42	2, 30
2- Three points in a row equal to zero: "Benneyan"	54, 55	NA
3- Nine points in a row on same side of CL: "2"	26, 27	11, 12, 13, 25, 26, 27, 28, 29

σ: Standard deviation CL: Control Limit NA: Not Applicable U (Prime) = U′

On the other hand, the type 1 and 2 warnings were found in both kinds of graphs. The first one indicates abnormal excursion while the second one is related to the drift in the monitored means of the inspection characteristics. Logically, the

attention should only be drawn to the aberrant high level of the microbial count while the very low alarming values could provide an opportunity for improvements in the present study. In the current case, two out-of-control points – (i.e. number "1" alarms) are present in each graph with an indication of unusually extended time intervals and excessive microbial count, respectively (JMP Statistical Discovery, 2022). While the frequency of cleaning might be more related to the usage of machines or equipment (and obviously can be adjusted when needed, the abnormally high microbial density could be attributed to the goodness of the cleaning process. With this respect, an investigation would be executed to determine the source of the abnormally high TVCs, even if there are no Out-Of-Specification (OOS) to avoid any adverse outcome in the future as they provide early warning signs for underlying problem(s) to be corrected and prevented through CAPA plan. Table 2 centralizes the main conceptual difference between the industrial and non-industrial inspection characteristics. By common sense, the industrial use of the U chart primarily focuses on the number of Defects per Unit (DPU) and hence the Defect per Million Opportunity (DPMO). The story is different in microbiology as there is an appreciably permissible level of the microbial count which has a specification criterion of 10000 CFU/100 mL (Moharram *et al.*, 2014). Although the aim is to maintain bioburden density as low as possible, yet the concept of industrial defect would not apply and this could be exemplified by analogous parameters Count per Milliliter (CPM) and Count per Million Opportunity (CPMO) that correspond to DPU and DPMO (PPM).

Table 2: Evaluation summary of the Laney U prime chart for the equipment and machine rinse.

Subgroup Parameters	**Record of**	**Rinse Bioburden Level**
Number of subgroups: 37	Total Rinses: 63	CFU£ per mL (CPM¥): 4
Average subgroup size: 1.703	Total CFU£: 26239	PPM§ (CPMO€): 4164921

£ Colony Forming Unit, § Part Per Million, ¥ Count Per Milliliter, € Count Per Million Opportunity

CONCLUSION

The present work showed the applicability of the trending charts in the control and monitoring of the microbiological cleanliness of the machines using the rinse technique. The pattern and excursions could be spotted and identified within the investigated time series of the process-behavior charts. While the implementation of the rare event (G) and attribute (Laney-corrected U) charts seems to be convenient for the present study, the classical understanding of the industrial defects cannot be applied herein when considering microbial bioburden data in the non-sterile

applications in the medical field. A solid comprehension is mandatory for understanding and interpretation of the trending charts by quality experts and this could be projected in the consideration of the alarming points. In the present case, Laney U′ Chart is related to the quality of cleaning microbiologically while the G chart is more likely to be linked to the operation practice in the plant which is associated with the activity and workload. The present work opens the gates for a further investigation that includes Pareto analysis to spot the major contributors in this microbial load in terms of products and machines with the possible development of a quantitative risk analysis tool that is based on the bioburden density and the frequency of the occurrence that could be extracted from the Pareto investigation.

REFERENCES

Eissa M, Rashed E, Eissa D (2022). Principal component analysis in long term assessment of total viable plate count of municipal water distribution network system in healthcare facility. *Environ Sci Technol* **5**(2): 165 - 171.

Eissa ME (2018). Microbiological quality of purified water assessment using two different trending approaches: A case study. *SJSR* **1**(3):75-9.

EİSSA ME, Rashed ER, Eissa DE (2021). Implementation of Modified Q-Control Chart in Monitoring of Inspection Characteristics with Finite Quantification Sensitivity Limits: A Case Study of Bioburden Enumeration in Capsule Shell. *El-Cezeri* **8**(3): 1093-107.

JMP Statistical Discovery (2022). Control Charts, https://www.jmp.com/support/help/zh/15.2/index.shtml#page/jmp/control-charts.shtml Accessed 19.10.2022

Kanji GK, Malek A, Tambi BA (1999). Total quality management in UK higher education institutions. *TQM* **10**(1): 129-53.

Laney DB (2002). Improved control charts for attributes. *Qual. Eng.* **14**(4):531-7.

Marucheck A, Greis N, Mena C, Cai L (2011). Product safety and security in the global supply chain: Issues, challenges and research opportunities. *JOM* **29**(7-8): 707-20.

Mehra S, Ranganathan S (2008). Implementing total quality management with a focus on enhancing customer satisfaction. *Int J Qual Reliab* **25**(9): 913-927.

Minitab® 20 Support (2022). Interpret the key results for Laney U' Chart, https://support.minitab.com/en-us/minitab/20/help-and-how-to/quality-and-process-improvement/control-charts/how-to/attributes-charts/laney-u-chart/interpret-the-results/key-results/ Accessed 17.10.2022.

MINITAB STATISTICAL SOFTWARE (2022). The Assistant, https://www.minitab.com/en-us/products/minitab/assistant/ Accessed 13.10.2022.

Moharram AM, Ismail MA, Shoreit AM, Hassan MH (2014). Biodiversity of microbiota in cephalosporin-manufacturing environments at T3A factory, Assiut, Egypt. *JBAM* **5**:1-3.

Park C, Wang M (2022). A study on the g and h control charts. *Commun Stat Theory Methods* **19**:1-6.

Pharma Focus Asia (FPA): Knowledge Bank: Pharam Articles (2022). Pharmaceutical Component Kinetics: Inventory dynamic control is crucial, https://www.pharmafocusasia.com/articles/pharmaceutical-component-kinetics-inventory-dynamic-control-is-crucial Accessed 15.10.2022

Rinaman WC (2018). Minitab. In Revival: The Handbook of Software for Engineers and Scientists p. 1028-1046. CRC Press USA.

Roesti D (2019). Calculating Alert Levels and Trending of Microbiological Data. In Roesti D and Goverde M (eds). Pharmaceutical Microbiological Quality Assurance and Control: Practical Guide for Non-Sterile Manufacturing p.329-69. John Wiley & Sons, Inc USA.

Ryan BF, Joiner BL, Cryer JD (2013). MINITAB handbook: update for release. Cengage Learning USA.

Schmitt R, Moerman F (2016). Validating cleaning systems. In Lelieveld H, Holah J, Gabrić D (eds). Handbook of Hygiene Control in the Food Industry p.587-601. Woodhead Publishing UK.

Steyaert D (2021). Cleaning validation of biologicals: Determination of a worst-case product for RTH 258 Ghent University Belgium.

Walters A (2008). A performance evaluation of the lifestraw: a personal point of use water purifier for the developing world (Doctoral dissertation, The University of North Carolina at Chapel Hill).

Vargas DA, Rodríguez KM, Betancourt-Barszcz GK, Ajcet-Reyes MI, Dogan OB, Randazzo E, Sánchez-Plata MX, Brashears MM, Miller MF (2022). Bio-Mapping of Microbial Indicators to Establish Statistical Process Control Parameters in a Commercial Beef Processing Facility. *Foods* **11**(8):1133.

Zakuan N, Muniandy S, Saman MZ, Ariff MS, Sulaiman S, Abd Jalil R (2012). Critical success factors of total quality management implementation in higher education institution: a review. *Int J Acad Res* **2**(12): 19.

Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Antimicrobial Activity Studies of 3-Substituted Indole-2-one and -thione derivatives and Molecular Docking and ADME Evaluations

Derya Doganay[1,*], Sevval Maral Ozcan Aykol[2], Ahmet Mesut Senturk[3], Sureyya Olgen[3]

[1]Health Sciences University, Hamidiye Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Istanbul, Turkey

[2]Biruni University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, İstanbul, Turkey

[3]Biruni University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, İstanbul, Turkey

Abstract

Increasing antibiotic resistance is an important problem for public health therefore new antimicrobial compounds are needed. In this study, the antimicrobial effects of 3-Substituted Indole-2-one and -thione derivatives were investigated. Antimicrobial effects of previously synthesized 18 different 3-substituted indole-2-one and 2-thione derivatives against 5 different microorganisms were investigated and the structure-activity relationships and drug-like properties of compounds were analyzed by molecular docking and in silico prediction studies. The *in vitro* antimicrobial activities of compounds were tested by microdilution method. The most active compounds were found as 2, 3, 4, 5, 6, 7, 8 at 125 µg/mL of MIC value. Compounds 2 and 3 were found to be active against *S. enterica* and compounds 4, 5, 6, 7, and 8 were found to be active against methicillin-resistant *S. aureus*. According to molecular docking studies, all compounds presented weaker binding properties than ciprofloxacin, ampicillin and gentamicin. The predicted values for molecular weight, log P, PSA, crossing the BBB, GI absorption properties and type of CYPP450 inhibition data of compounds were found promising for drug-like properties. 3-Substituted Indole-2-one and -thione derivatives can proivide an important contribution to develop alternative antimicrobial agents.

Keywords

Antimicrobial activity: Minimum inhibitory concentration: Molecular docking: Structure-activity relationship: 3-Substituted-indole-2-on and 2-thione

Article History

Submitted: 15 November 2022 Accepted: 26 May 2023 Published Online: July 2023

Article Info

***Corresponding author:** Derya Doganay **email:** ddoganay2904@gmail.com

Research Article:

Volume: 6 Issue: 1 2023 Pages: 13-26

DOI: 10.54994/emujpharmsci.1202754

INTRODUCTION

Indole is an important scaffold that shows a variety of biological activities. Among the indole derivatives, oxoindole, thioindole, isatine and spiro oxoindole frameworks containing derivatives especially cover important pharmacological activities such as antimicrobial, antifungal, anti-tumor, antiproliferative and tyrosine kinase activities useful for the treatment of cancer (Bhaskar *et al.*,2012). Isatin derivatives were found that antimicrobial activity against a variety of pathogens (Khan and Maalik, 2015). According to study of thiosemicarbazone and dispiro pyrrolidine derivatives of isatin showed that these compounds inhibit the growth of *Mycobacterium tuberculosis* (Kumar *et al.*, 2010; Banerjee *et al.*, 2011). In the another study found that isatin-3- phenylhydrazone showed antimicrobial activity compared to amoxicillin and norfloxacin against *Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Konstantinović *et al.,* 2008). Lanthanide complexes with isatin bis hydrazones derivatives were found to have antifungal properties depending on the lipophilicity of the molecules (Mohanan *et al.,* 2008). New isatin derivatives condensed with benzoyl hydrazides were found active against some bacterial strains including *M. tuberculosis* and fungi. In the another study it was found that 5,5-Disubstituted-1,2,4-Triazolidin-3-one showed good antimicrobial activity against Gram positive bacteria (Sudha *et al.*, 2015). Some mono and bis-[3,3-di(indolyl)indolinone] derivatives were assayed for in-vitro antimicrobial activity. Although they were active against *S. aureus*, they were not found active against *E. coli*, *P. aeruginosa* and *Candida albicans* (Karimi *et al.*, 2015). According to another study it is showed that Schiff bases of 1*H*-indole-2,3-dione derivatives with 4-amino-N-(5,6-dimethoxypyrimidine-4-yl) benzenesulfonamides has considerable antimicrobial activities in comparison to reference drug sulfadoxine (Singh *et al.,* 2010). Other types of Schiff bases of isatin were studied by the same research group. It was reported that these compounds have higher activity against several bacteria and fungi in comparison to norfloxacin (Pandeya et al., 2008). Sixteen substituted indole-2,3-diones hydrazones were tested for antimicrobial activity. Some compounds exhibited good inhibitory activity against *Salmonella Typhi*, *Staphylococcus haemolyticus*, *Mycobacterium paratuberculosis* 607, *Aspergillus niger*, *C. albicans*, and *Saccharomyces cerevisiae* (Piscopo *et al.*, 1986; Piscopo *et al.*, 1987). A series of spirooxoindoles obtained from isatin showed moderate to good

antimicrobial activity against several bacterial and fungal strains (Nandakumar *et al.*, 2010). 5-Fluoro and 5-iodo indole-2,3-dione derived from spiro-4-thiazolidinones were reported to be potent compounds against bacteria and fungi (Hussain *et al.*, 2016).

All the above findings of indole-2- one derivatives obtained from literature prompted us to test our previously synthesized 18 compounds against 5 different bacterial strains. The activity results were evaluated based on their structure by using computer-assisted methods. A docking study was run to explain the interaction of indole-2-one derivatives with the binding site of DNA gyrase enzymes of microorganisms. Swiss Absorption, distribution, metabolism, and excretion (ADME) (http://swissadme.ch/) prediction was also used to calculate physicochemical properties of compounds to explain drug-like properties.

MATERIALS AND METHODS

Methanol (Sigma-Aldrich) and Mueller-Hinton broth (MHB) (Difco, Detroit, USA) were used for biological assays. Autodock vina 4.2.6 was used for the calculation of receptor-ligand interactions and the 3D compound-protein docking posses were analyzed by using Pymol 4.2.6 software. The Physico-chemical data were calculated using Swiss ADME (http://swissadme.ch/) online prediction program.

In Vitro Antimicrobial Assay

Test Microorganisms

Gram-negative bacteria (*Acinetobacter baumannii* ATCC 19606, *Salmonella Typhi* clinical isolate ve *Salmonella enterica* – clinical isolate) and Gram-positive bacteria (*Staphylococcus haemolyticus* ATCC 43252, *Methicillin-resistant Staphylococcus aureus* ATCC 43300) were used to determine the antimicrobial activity of eighteen previously synthesized indole derivatives.

Determination of the minimum inhibitory concentration (MIC) by microdilution tests

MIC values of the compounds on the bacteria cultures used in the study were determined according to the broth microdilution method (Sanli-Yurudu *et al.*, 2012). 24-hour fresh cultures of all bacteria were prepared in Mueller Hinton Broth (MHB), and the bacterial concentration was adjusted as 0.5 McFarland (CLSI, 2018). The all compounds are dissolved in methanol. The dilutions of the compounds and standards in the test medium were prepared using Muller Hinton Broth at the required quantities of 1000, 500, 250, 125,

62.5, 31.25, 15.7, 7.8, 3.9, 1.95, 0.98, and 0.5 µg/mL and added onto the tested microorganisms in 96 microcell plate and incubated at 37±0.1 °C for 24 hours. In addition, a mixture containing only methanol and microorganisms was added to the microplates to examine the antimicrobial effect of methanol on microorganisms. Then 50 µL of 2,3,5-triphenyl tetrazolium chloride from a 2 mg/mL stock solution was added to each cell and MIC values of compounds were determined by colorimetric measurement. Streptomycin, gentamicin, ampicillin and ciprofloxacin (1000 µg/mL -0.5 µg/mL dilution range) were used for controls (Kang *et al.*, 2008).

Chemistry

3-Substituted benzylidene indolin-2-one and 2-thione derivatives (1-18) were previously synthesized in two steps starting from indole-2-one and indole-2-thione (Figure 1).

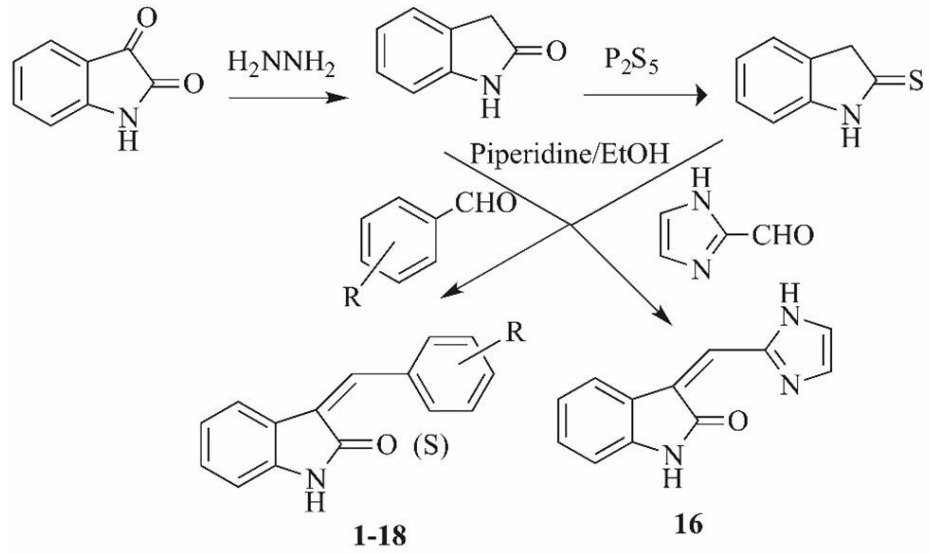


Figure 1: 3-Substituted benzylidene indolin-2-one and 2-thione derivatives tested for antimicrobial activity.

Oxoindole was synthesized by Wolff-Kishner like reduction of isatin and indole-2-thione was derived from indole-2-one by using P_2S_5. The tested compounds were synthesized by condensation of oxoindole and thioindole with several aldehydes in the presence of piperidine as a basic catalyst (Olgen *et al.*, 2005).

Molecular Docking

The crystal structures of the DNA gyrases of related microorganisms were taken from the Protein Databank (PDB, http://www.rcsb.org). The PDB ID for studied microorganisms was used as follows: *S. haemolyticus*: 2RHS, *S. thypi*: 6J90, *S. aureus*: 5CDQ, *A. baumannii*: 2XKJ, *S. enterica*: 6AEP).

After drawing the three-dimensional structure of the compounds by using ChemDraw Ultra 15.9, the energy minimizations of compounds were done by ChemBio Ultra 15.9. Then all water molecules were discarded and the polar hydrogens were added. The grid box was adjusted with a volumetric space of 30x30x30 for corresponding DNA gyrases of microorganisms. The docking study was run in AutoDock vina 4.2.6 software and the 3D compound-protein interactions were analyzed by using Pymol 4.2.6.

Calculation of molecular properties

To evaluate the structure-activity relationships of compounds, physicochemical features such as polar surface area (PSA), type of metabolizing enzymes, Blood-Brain Barrier (BBB) parameter, Lipinski's rule of five and Log P values were calculated by using Swiss ADME (http://swissadme.ch/) online software program (Swiss ADMET Prediction) (Lipinski et al., 2012).

RESULTS AND DISCUSSION

Determination of in vitro Antimicrobial Activities of Compounds

In this study, 18 different compounds were tested against 5 different microorganisms in comparison to reference drugs ampicillin, streptomycin, gentamicin and ciprofloxacin for determining antibacterial activities. The in vitro antimicrobial activities of compounds were determined by the microdilution method (CLSI, 2018) and the results were shown in Table 1. Accordingly, the activity data of the tested 18 compounds presented different profiles against the microorganisms. Compounds showed slight activity against Gram (+) bacteria of *S. haemolyticus* ATCC 43252 strain at 250-1000 µg/mL and Methicillin-resistant Staphylococcus aureus ATCC 43300 (MRSA ATCC 43300) strain at 125-500 µg/mL concentrations. They were also found active against Gram (-) bacteria of *A. baumannii* ATCC 19606 strains at 125-500 µg/mL, *S. Typhi* clinical isolate at 250-1000 µg/mL and against *S. enterica* clinical isolate at 125-250 µg/mL concentrations.

Table 1: MIC (µg/ml) values of tested compounds and standard drugs.

Results MIC (µg/ml)	*S. haemolyticus* ATCC 43252	MRSA ATCC 43300	*A. baumannii* ATCC 19606	*S. enterica* (clinical isolate)	*S. Typhi* (clinical isolate)
1	500	500	500	250	500
2	250	250	125	125	250
3	250	250	250	125	250
4	250	125	250	250	250
5	250	125	250	250	250
6	250	125	250	250	250

7	250	125	250	250	500
8	500	125	500	250	250
9	250	500	250	250	500
10	250	500	250	250	500
11	250	250	250	250	500
12	250	250	250	250	500
13	250	250	250	250	500
14	250	250	250	250	500
15	250	250	250	250	500
16	250	250	250	250	500
17	1000	500	500	250	500
18	1000	500	500	250	1000
Ampicillin	>1000	125	125	3,2	500
Gentamycin	31,25	<0,5	3,9	125	1,9
Streptomycin	500	0,9	125	62,5	31,25
Ciprofloxacin	0,48	0,97	31,25	0,48	0,48

*ND: Non Determined

As seen in Table 1 , the most active compounds showed their activities at 125 µg/mL of MIC value. Among them, compounds 2 and 3 were found to have activities against *S. enterica* and compounds 4, 5, 6, 7, and 8 were found to be active against MRSA. The reference compound ampicillin was found effective against MRSA and *A. baumannii* at 125 µg/mL and *S. enterica* at 3.2 µg/mL concentrations. Ciprofloxacin showed activity at 0.48 µg/mL of MIC value against *S. haemolyticus*, *S. enterica*, and *S. Typhi*. According to these results, it can be concluded that compounds are more active against *S. enterica* with a value of 125- 250 µg/mL than other bacteria tested in this study. In addition, the MIC values of compounds 4, 5, 6, 7 and 8 were determined in 125 µg/mL against MRSA ATCC *43300*, which represented more effective than other compounds against all tested microorganisms in the study. The MIC values of compounds 2,3,4,5,6,7,9,10,11,12,13,14,15,16 were determined 250 µg/ml against *Staphylococcus haemolyticus* ATCC 43252. Similarly, the MIC values of compounds 2,3,4,5,6,8 were determined 250 µg/ml MIC against *S. Typhi*. The MIC value of compound 2 was determined 125 µg/ml against *Acinetobacter baumannii* ATCC 19606. When the tested all compound was compared with reference drugs gentamicin and ciprofloxacin, they showed a weaker antimicrobial effect. In generally, it has seen that all compound showed a weaker antimicrobial effect compared with reference drugs gentamicin and ciprofloxacin. However, compound 2 showed similar antimicrobial effect againist *S. enterica* compared with gentamicin

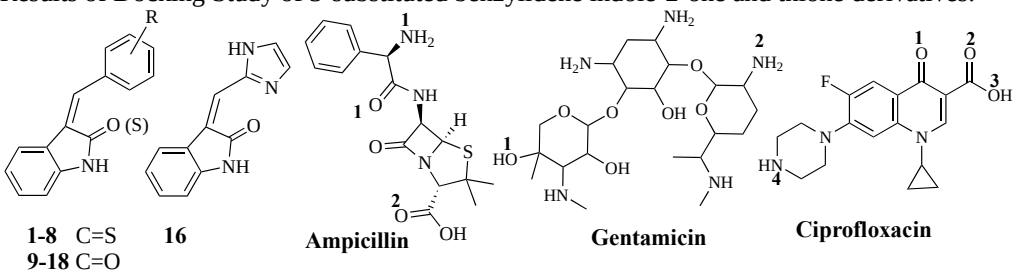
The antibacterial activity of compounds was observed better with 3-substituted benzylidene indol-2-thione derivatives than

with 3-substituted benzylidene indol-2-one derivatives.

Molecular Docking Studies

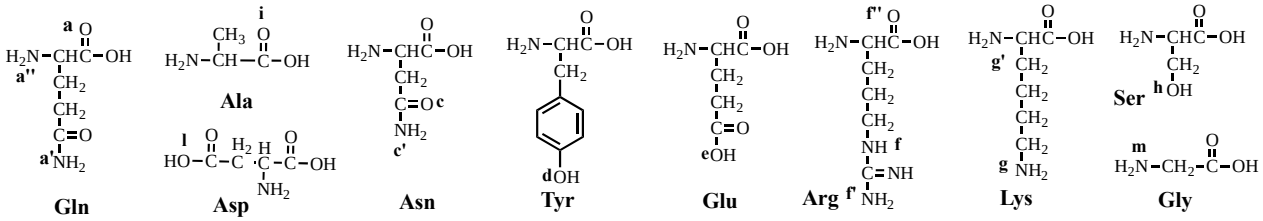
Antimicrobial efficacies of compounds were determined with molecular docking studies and the obtained best-docked poses were evaluated. To evaluate the effectiveness of compounds by molecular docking studies, the lowest binding energies, hydrogen bond capabilities, root-mean- square deviation (RMSD) value and MIC values of compounds were assessed. Since ciprofloxacin shows its effect via binding DNA gyrase, the docking studies of compounds were evaluated based on the data of biological and molecular interaction studies in comparison to ciprofloxacin**.** The best binding affinity and receptor-ligand interactions of each compound were assessed and well-established good interactions in the receptor's active pocket of the target receptor DNA gyrase of related microorganisms were shown in Table 2.

Table 2: Results of Docking Study of 3-substituted benzylidene indole-2-one and thione derivatives.



Comp No	R	Energy score	RMSD value	H-bond (distance Å)	Microorganisms
1	2-Cl	-7.00	0.11	NH of indole with e of GLU216 (2.054)	*S. heamolyticus*
2	4-COOH	-8.55	1.25	C=O with g' of LYS103 (2.107)	*S. enterica*
		-6.42	0.26	NH of indole with d of TYR1051 (1.900) C=O with m of GLY1143 (1.795)	*A. baumanii*
4	2-OH	-6.90	0.14	Ph-OH with O-l of ASP437 (2.108)	*S. aureus*
6	3-F	-7.13	0.12	-	*S. aureus*
7	4-OCH_3	-7.08	0.06	-	*S. aureus*
8	3-NO_2	-9.42	0.25	Ph-NO_2 with H-a' of GLN177 (1.842)	*S. heamolyticus*
		-9.33	0.32	Ph-NO_2 with H-c' of ASN46 (2.003)	*S. enterica*
12	3,4-DiCl	-9.02	0.22	C=O with H-h of SER174 (1.755)	*S. heamolyticus*
Amp		-12.11	0.70	O-1 with g of LYS103 (2.088) H-1 with i of ALA100 (2.095)	*S. enterica*
Gn		-10.88	1.99	H-1 with i of ALA100 (1.858) H-1 with e of GLU50 (2.143)	*S. typhi*
Cp		-7.00	0.44	O-2 with d of TYR1051 (2.155) O-2 with g' of LYS1156 (2.148)	*A. baumanii*
Cp		-10.54	0.09	O-2 with H-f' of ARG122 (1.761) O-2 with H-n of SER84 (2.176)	*S. aureus*
Cp		11.48	0.88	H-4 with O-i of ALA100 (2.151)	*S. enterica*
Cp		11.10	0.80	O-1 with H-h of SER174 (1.677)	*S. heamolyticus*

Amp.: Ampicillin; Gn: Gentamicin; Cp: Ciprofloxacin



The binding energy and RMSD value of most of the compounds were found to be very close to reference compounds ciprofloxacin. According to docking results, compounds showed better binding properties with the receptor active site of MRSA and *S. haemolyticus*. The effectiveness of compounds was evaluated based on the lowest binding energy capabilities and MIC values of compounds. The most active compounds (2, 3, 4, 5, 6, 7, and 8) were found effective at 125 µg/L concentration against *A. baumanii*, *S. enterica*, and *MRSA*. To confirm these results, docking studies were done and remarkable binding affinity values were found for compound 8 as interaction with *S. haemolyticus* at -9.42 kcal/mol and interaction with *S. enterica* at -9.33 kcal/mol.

Compound 2 showed good interaction with the receptor active site of *A. baumanii*, and *S. enterica*, with values at -6.42 kcal/mol and -8.55 kcal/mol, respectively. Good binding properties and biological activity showing compounds also presented good hydrogen-bonding ability and RMSD values and these findings were found to be mutually supportive. The reference compound ampicillin also showed good binding interaction with receptors of *S. enterica*, at -12.11 kcal/mol. This result confirms the good activity result of ampicillin with 3.2 µg/mL of MIC value against *S. enterica*. Gentamycin showed good binding property with the receptor of *S. Typhi* at -10.88 kcal/mol and also presented good activity at 1.9 µg/mL concentration. Although streptomycin did not show any interaction with the tested receptors in docking studies, it was found very active against all microorganisms in the range of 0.9-500 µg/mL concentrations. Although streptomycine is not a DNA gyrase inhibitor and does not interact with DNA gyrase active sites, it was used as a reference compound because of its strong and variety of antimicrobial spectrum. Ciprofloxacin showed good binding energies against all tested bacterial gyrase enzymes and was found as the most active compound in the range of 0.48-31.25 µg/mL. In terms of the way it binds to the receptor active site, some other compounds showed good binding properties. Figure 2 depicts the 3D structural interaction details

of the most active compounds 4, 5, 6, 7, and 8 with DNA gyrase of MRSA against *S. aureus* comparison to the reference compound ciprofloxacin.

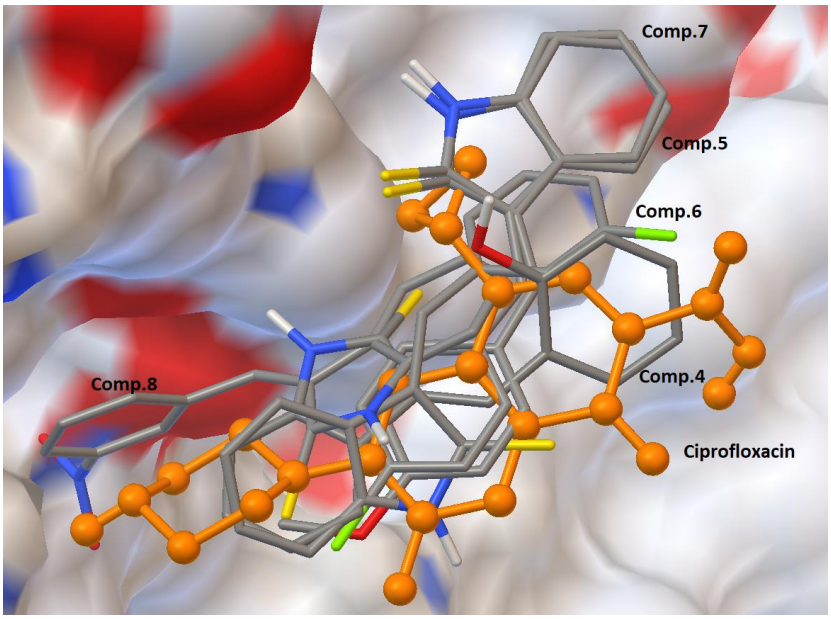


Figure 2: Three dimensional structural interaction of the most active compounds 4, 5, 6, 7, 8 and reference compound ciprofloxacin (orange) with DNA gyrase of MRSA.

As seen from the figures, compounds bonded to the active site by overlapping with reference compounds. These results proved that compounds presented the parallel results due to ligand-receptor binding interactions and therapeutic potence in *in-vitro* studies.

Drug-like properties

Swiss ADME online prediction program was used to calculate physicochemical features and drug-likeness properties of tested compounds to clarify the structure-activity relationships. The predicted values such as molecular weight, log P, PSA, crossing the BBB, GI absorption properties and type of CYPP450 inhibition of compounds were shown in Table 3.

Table 3: Drug-like properties of **1-18** calculated by Swiss ADME online software program.

Comp. No	MW (g/mol)[a]	LogP [b]	TPSA [c]	BBB [d]	GI Abs.[e]	Type of CYP Inh.[f]	Rule of Five [g]
1	273.78	4.37	54.59 Å	Yes	High	CYP1A2, CYP2C19, CYP2C9, CYP3A4	Yes
2	283.34	3.34	91.89 Å²	No	High	CYP1A2, CYP2C19, CYP2C9, CYP3A	Yes
3	308.23	4.88	54.59 Å²	Yes	High	CYP1A2, CYP2C19, CYP2C9, CYP3A	Yes
4	255.33	3.41	74.82 Å	Yes	High	CYP1A2, CYP2C19, CYP2C9, CYP3A	Yes
5	273.78	4.37	54.59 Å	Yes	High	CYP1A2, CYP2C19, CYP2C9, CYP3A	Yes
6	257.33	4.15	54.59 Å	Yes	High	CYP1A2, CYP2C19, CYP2C9, CYP3A	Yes
7	269.36	3.80	63.82 Å	Yes	High	CYP1A2, CYP2C19, CYP2C9, CYP2D6	Yes
8	284.33	3.22	100.41 Å²	No	High	CYP1A2, CYP2C19, CYP2C9, CYP3A4	Yes
9	257.71	3.86	36.02 Å²	Yes	High	CYP1A2, CYP2C19, CYP2D6 ,CYP3A4	Yes
10	239.27	2.84	56.25 Å²	Yes	High	CYP1A2, CYP2D6, CYP3A4	Yes
11	302.71	3.23	81.84 Å	No	High	CYP1A2, CYP2C19, CYP2C9	Yes
12	292.16	4.34	36.02 Å	Yes	High	CYP1A2, CYP2D6, CYP3A4	Yes
13	241.26	3.60	36.02 Å²	Yes	High	CYP1A2, CYP2C19, CYP2D6 ,CYP3A4	Yes
14	268.27	2.68	81.84 Å²	No	High	CYP1A2, CYP2C19, CYP2C9, CYP2D6	Yes
15	257.71	3.82	36.02 Å	Yes	High	CYP1A2, CYP2C19, CYP2D6 ,CYP3A4	Yes
16	213.24	1.78	64.70 Å²	Yes	High	CYP1A2, CYP2D6	Yes
17	308.23	4.87	54.59 Å²	Yes	High	CYP1A2, CYP2C19, CYP2C9, CYP3A4	Yes
18	282.40	3.81	57.83 Å²	Yes	High	CYP1A2, CYP2C19, CYP2C9, CYP2D6	Yes
Ampicillin	349.40	0.88	138.03 Å²	No	Low	CYP3A4	Yes
Gentamycin	477.60	-1.6	199.73 Å²	No	Low	-	No
Streptomycin	581.57	-5.86	336.43 Å²	No	Low	-	No
Ciprofloxacin	331.34	1.10	74.57 Å²	No	High	CYP3A4	Yes

[a]Molecular weight (recommended value <500)
[b] Logarithm of the partition coefficient of the compound between n-octanol and water (recommended value <5)
[c] Polar surface area (recommended value ≤140Å²)
[d] Indicates whether the compound pass blod Brain Barrier or not
[e] Degree of Gastrointestinal Absorption
[f] Represent the ınhıbıtıon of CYP450 subtypes
[g] Indicates whether the compound obeys Lipinski's Rule of Five or not.

Except for compounds 2, 8, 11 and 14, the other compounds showed good hydrophobic properties to pass lipid barrier. Lipophilicity values of compounds were

determined lower than the recommended value of 5 which obey Lipinski's Rules (Lipinski *et al.,* 2012). Compounds also exhibited good pharmacokinetic features

such as high gastrointestinal absorption (GI), Partition coefficient (LogP), Molecular weight (MW) and PSA which all obeys Lipinski's rule of five. Similar to our study, Mendoza-Figueroa et al. investigated the antibacterial activity of fluorine-substituted indole-based imidazolines against *Escherichia coli, Staphylococcus*

aureus, Pseudomonas aeruginosa and *Listeria monocytogenes* strains by broth dilution method and they found that one substance showed the highest antibacterial effect on *S. aureus* strain with a MIC value of 80 µg/ml (Mendoza-Figueroa *et al.,* 2018). Shirinzadeh et al. investigated the antibacterial effect of new indole derivatives containing 1,2,4-Triazole, 1,3,4-Thiadiazole and carbothioamide on *S. aureus, Bacillus subtilis, E. coli* and MRSA and they reported that they had an antibacterial effect with a MIC value of 6.25 µg/ml (Shirinzadeh *et al.,* 2018). In another

study, Chodvadiya *et al.* investigated the antibacterial effect of N-methyl indole derivatives on *E. coli, S. Typhi, Bacillus megaterium, Micrococcus spp.* by agar diffusion method and they showed that 3 substances had high antibacterial effects against *S. Typhi* (Chodvadiya *et al.,* 2019). Shaker et al. investigated the antibacterial effects of 2-(4-methylsulfonyl phenyl) indole derivatives on MRSA, *E. coli, P. aeruginosa and A. baumanni* and they reported that MIC values of 3 substances were found 8, 1, 2 µg/ml against MRSA strain and MIC value of 3 substances were found 16.44 µg/ml against *A. baumannii* (Shaker et al., 2020). Doganay *et al.* investigated the antibacterial effect of 16 different indole amide derivatives on 14 different bacteria by agar diffusion and broth dilution method and found that the tested compounds were most effective (25 µg/ml) against *S. aureus* and *Aeromonas hydrophila* (Doganay *et al.,* 2022). Olgen *et al.* investigated the antibacterial effect of 3-substituted benzylidene-1,3-dihydro-indoline derivatives against *K. pneumoniae, P. aeruginosa, E. coli, B. subtilis, S. aureus* by broth dilution method. As a result of their studies, a high activity was observed (15.62 – 62.5 µg/ml) against *B. subtilis* and *S. aureus* strains (Olgen and Ozkan 2009). According to the results of the study, it was determined that the examined substances had antibacterial effects against the test bacteria, albeit in low amounts. In the

literature, it was observed that the indole derivatives tested on different bacterial strains were more effective than the indole derivatives tested in the study. In order to evaluate the structure-activity relationship of compounds, it was evaluated that the electron-withdrawing or electron-giving substituent effects might not play a role in the potency of compounds. The overall evaluation of the molecular docking and activity results indicates that the results are parallel with activity results and show that most compounds have activity against the *S. aureus*. The docking results also supported that most compounds bind the related active site with the expected properties in terms of binding energy, hydrogen bond capability, Van Der Waals interactions, and RMSD values. Since antimicrobial activity studies have not been performed on many compounds with 3-substituted benzylidene indole-2-one and thion structure in literature, it was not possible to make a comparative structure-activity interpretation of the compounds. However, the findings obtained in this study give clues about the possibility of obtaining more effective molecules when modifications with further substitutions are made on these main structures.

Several predicted descriptors such as the molecular weight, percent human oral absorption, PSA and logarithm octanol-water partition coefficient (QPlogPo/w) showed that all compounds have drug-likeness properties and good oral absorption values. As a result, it can be concluded that compounds might have good bioavailability and drug-likeness properties. All compounds tested in the study were found to exhibit less, equivalent, or greater antibacterial activities than the reference drugs used in the study.

CONCLUSION

None of indole-2-one and indole-2-thion compounds showed strong inhibitor effects against used microorganisms in this study. The presence of antimicrobial effects of structurally similar compounds in the literature and the lack of antibacterial activity in our compounds showed that small changes in the structure significantly affect the activity results. These results suggest that further studies of receptor interaction such as molecular dynamic are required to design rational novel compounds. However, it can be considered to test some of the compounds against other microorganisms in *in-vitro* studies. It is thought that the results of the study will contribute significantly to the development of antimicrobial drugs related to indole

derivatives.

REFERENCES

Banerjee D, Yogeeswari P, Bhat P, Thomas A, Srividya M, Sriram D (2011). Novel isatinyl thiosemicarbazones derivatives as potential molecule to combat HIV-TB co-infection. Eur J Med Chem **46**(1): 106-121.

Bhaskar G, Arun Y, Balachandran C, Saikumar C, Perumal PT (2012). Synthesis of novel spirooxindole derivatives by one pot multicomponent reaction and their antimicrobial activity. Eur J Med Chem **51**: 79-91.

Chodvadiya VD, Pambhar KD, Parmar ND, Dhamsaniya AP, Chhatbar PV, Ram HN, Patel PK (2019). Synthesis and characterization of N-methyl indole derivatives via desulfitative displacement by various amines and Its antimicrobial activity. World Sci. News **120**(2): 181-191.

Clinical and Laboratory Standards Institute (2018). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that GrowAerobically, Approved Standard. seventh ed. Wayne: CLSI.

Doganay D, Ozcan MS, Senturk MA, Olgen S (2022). Antimicrobial Evaluation, Molecular Docking and ADME Properties of Indole Amide Derivatives. Lett Drug Des Discov **19**(5): 387-396.

Hussain AZ, Meeran MN, Sankar A (2016). Synthesis, characterization and antimicrobialactivityof spiro-4-thiazolidione derivativesfrom 5-substituted indole-2,3-dione. Pharma Chemica **8**(2): 292-296.

Kang MS, Choi EK, Choi DH, Ryu SY, Kang IC (2008). Antibacterial activity of pyrrolidine dithiocarbamate. Fems microbiol Lett **280** (2): 250-254.

Karimi AR, Dalirnasab Z, Yousefi GH, Akbarizadeh AR (2015). Synthesis of mono and bis-[3,3-di(indolyl)indolin-2-ones] and evaluation of their antimicrobial activity. Res Chem Intermed **41**(12): 10007-10016.

Khan FA, Maalik A (2015). Advances in Pharmacology of Isatin and its Derivatives: A Review. Tropical J Pharm Res **14**(10): 1937-1942.

Konstantinović SS, Kapor A, Radovanović BC, Deak A (2008). Synthesis, X-ray and antimicrobial activity of isatin-3- phenylhydrazone. Chem Ind Chem Eng Quart **14**(1): 27-34.

Kumar RS, Rajesh SM, Perumal S, Banerjee D, Yogeeswari P, Sriram D (2010). Novel three-component domino reactions of ketones, isatin and amino acids: synthesis and discovery of antimycobacterial activity of highly functionalised novel dispiropyrrolidines. Eur J Med Chem **45**(1): 411-422.

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2012). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced drug delivery reviews, **64**, 4-17.

Mendoza-Figueroa HL, Serrano-Alva MT, Aparicio-Ozores G, Martínez-Gudiño G, Suárez-Castillo OR, Pérez-Rojas NA, Morales-Ríos MS (2018). Synthesis, antimicrobial activity, and molecular docking study of fluorine-substituted indole-based imidazolines. Med Chem Res; **27**(6): 1624-1633.

Mohanan K, Sindhu K, Rijulal G (2008). Microwave assisted synthesis, spectroscopic, thermal, and antifungal studies of some lanthanide(III) complexes with a heterocyclic bishydrazone. J Rare Earths **26**(1): 16-21.

Nandakumar A, Thirumurugan P, Perumal PT, Vembu P, Ponnuswamy MN, Ramesh P (2010). One-pot multicomponent synthesis and anti-microbial evaluation of 2'-(indol-3-yl)-2-oxospiro(indoline-3,4'-pyran) derivatives. Bioorg Med Chem Lett **20**(14): 4252- 4258.

Olgen S, Akaho E, Nebioglu D (2005). Synthesis and anti-tyrosine kinase activity of 3-(substituted-benzylidene)-1,3-dihydro-indolin derivatives: investigation of their role against Src receptor tyrosine kinase with the application of receptor docking studies. Il Farmaco; **60**(7): 497-506.

Olgen S, Ozkan S (2009). A study of 3-substituted benzylidene-1, 3-dihydro-indoline derivatives as antimicrobial and antiviral agents. Z Naturforsch C **64**(3): 155-162.

Pandeya SN, Singh BN, Shukla SK, Singh M (2008). Synthesis and antimicrobial activity of N-norfloxacin Mannich basis of isatin and its derivatives. Asian J Chem **20**(7): 5377-5382.

Piscopo E, Diurno MV, Gagliardi R, Cucciniello M, Veneruso G (1987). Studies on heterocyclic compounds: indole-2,3-dione derivatives. VII. Variously substituted hydrazones with antimicrobial activity. Boll Soc Ital Biol Sper **63**(9): 827-832.

Piscopo E, Diurno MV, Imperadrice F, Caliendo V, Nebulosi R (1986). Studies on heterocyclic compounds: indole-2,3-dione derivatives. VI. 3-Aryliminoindole-2(3H)-ones and their Mannich bases with antimicrobial activity. Boll Soc Ital Biol Sper **62**(12): 1449-1455.

Piscopo E, Diurno MV, Imperadrice F, Cucciniello M, Veneruso G (1986). Studies on heterocyclic compounds: indole-2,3-dione derivatives. V. Structure-antimicrobial activity of aromatic and heterocyclic azomethines of indole- and substituted indole-2,3-diones. . Boll Soc Ital Biol Sper **62**(12): 1441-1447.

Sanli-Yurudu NO, Kimiran-Erdem A, Arslan-Aydogdu EO., Zeybek Z, Gurun S (2012). Efficacy of colloidal silver-hydrogen peroxide and 2-Bromo-2-nitroporopane-1, 3-diol compounds against different serogroups of Legionella pneumophila Strains. Indian J Microbiol **52**(1): 54-59.

Shaker AM, Abdelall EK, Abdellatif KR, Abdel-Rahman HM (2020). Synthesis and biological evaluation of 2-(4-methylsulfonyl phenyl) indole derivatives: Multi-target compounds with dual antimicrobial and anti-inflammatory activities. BMC chemistry **14**(1): 1-15.

Shirinzadeh H, Süzen S, Altanlar N, Westwell AD (2018). Antimicrobial activities of new indole derivatives containing 1, 2, 4-triazole, 1, 3, 4-thiadiazole and carbothioamide. Turk J Pharm Sci. **15**(3): 291.

Singh UK, Pandeya SN, Jindal S, Pandey M., Srivastava BK, Singh A (2010). Synthesis and antimicrobialactivity of Schiff's and Mannich bases of 1H-indole-2,3-dione derivatives. Pharma chem **2**(2): 392-399.

Sudha RK, Kandula P, Anupama B, Chenchu L (2015). In vitro evaluation of some novel 5,5-disubstituted-1,2,4-triazolidine-3-one derivatives. International J Pharm and Life Sci **6**(4): 4417-4426.

Swiss ADMET Prediction, Swiss Institute of Bioinformatics, Switzerland, Available from: http://swissadme.ch/

Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Synthesis and cholinesterase inhibitory potential of 2-phenoxy-N-substituted-acetamide derivatives

Kiana Harati, Seyedeh Mahta Kiaei, Tina Mahdipour Amjad, Zahra Nobavar, Karar Tawfeeq Shukur, Acelya Mavideniz, Tugba Ercetin, Hayrettin Ozan Gulcan*

[1] Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, North Cyprus, Mersin 10 Turkey.

Abstract

The research studies worldwide on the identification of novel molecules having the potential to inhibit cholinesterase enzymes generated many compounds with promising results for some of them. Since there are limited number of drugs used in the treatment of Alzheimer's Disease in particular, the research studies continue. Within the scope of this study, four 2-phenoxy-N-substituted-acetamide derivatives were synthesized and their structures were identified employing spectroscopic techniques. The title molecules were further evaluated for their cholinesterase inhibitory potential in modified Ellman's method. The results displayed that the compounds have moderate activity and the simple scaffold employed might be used in future studies for more promising compounds.

Keywords

2-phenoxy-N-substituted-acetamide derivatives, Alzheimer, cholinesterase

Article History

Submitted: 29 November 2022 Accepted: 16 December 2022 Published Online: July 2023

Article Info

***Corresponding author:** Hayrettin Ozan Gulcan **email:** ozan.gulcan@emu.edu.tr

Research Article:
Volume: 6 Issue: 1 2023 Pages: 27-33
DOI: 10.54994/emujpharmsci.1211796

INTRODUCTION

Despite the fact that Alzheimer's disease (AD) was initially identified and diagnosed more than a century ago, many questions still persist, especially regarding its pathophysiological mechanisms. Dementia is a common symptom of many diseases (Gulcan and Orhan, 2021). Unfortunately, the most common type of dementia is AD-related dementia, resulting in cognitive impairment. With respect to the progressive nature of AD, dementia symptoms worsen in time as it is categorized as mild, moderate, and final stages of the disease (Pillai and Cummings, 2013).

Although the pathophysiology of the disease is too complex to fully understand, many validated and non-validated targets have been offered so far for the treatment of AD (Erdogan *et al.*, 2021). Enzymes involved in beta-amyloid cascade, kinases having function in tau-protein phosphorylation, oxidative stress mechanisms are just some of them. None of these targets studies so far ended up with promising molecules possessing disease modifying feature to stop the progression of the disease (Gulcan *et al.*, 2019). Furthermore, they have not been shown to be involved in the reconstruction of cognitive disabilities. From this perspective, cholinesterase inhibitors are still important and they are the only drugs used in the treatment of AD, beside memantine, the single representative of NMDA receptor antagonism (Pepeu, and Giovannini, 2009).

Many studies have pointed out the significance of cholinergic system in the maintenance of cognitive functions. Therefore, since 1980s, four cholinesterase inhibitors have been introduced to the clinic for the treatment of AD-related dementia. Beside the withdrawal story of tacrine, the rest three of them display diverse properties in terms of source, target, dose, pharmacokinetic and pharmacodynamic perspectives (Wilkinson *et al.*, 2004). Therefore, there has been a continuous interest on the screening of diverse structures.

In this study, the design of a previous work of our research group has been adapted on more simple molecules (Shukur *et al.*, 2021). Briefly, the 2-aryloxy-N-substituted-acetamide motif has been successfully applied in the design of multiple-target acting urolithin analogues in our previous works. This scaffold has been assessed in simple compounds, 2-phenoxy-N-substituted-acetamides. The structure of the title molecules are presented in Figure 1.

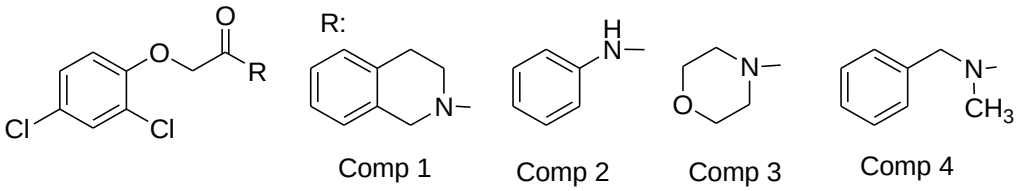


Figure 1: Title molecules.

MATERIALS AND METHODS

All reagents and organic solvents were purchased from Sigma Aldrich through the aid of local vendors. Using ethyl acetate/cyclohexane as the mobile phase (1:1, 2:1, and 1:2 ratios) thin-layer chromatography (TLC) was used to monitor the reactions on Merck aluminum-packed silica gel plates. In order to get the compounds' IR spectra, a Shimadzu FT-IR Prestige model spectrophotometer was used. 1H NMR (at 400 MHz) spectra were recorded on a Bruker-400 NMR spectrometer using tetramethylsilane as an internal standard and dimethyl sulfoxide (DMSO; d6) as a solvent; all chemical shifts were reported in parts per million (ppm, δ). The elemental analysis was performed on a Thermo Fisher Scientific Model Flash Smart CHNS elemental analyzer.

Synthesis of the ethyl 2-(2,4-dichlorophenoxy)acetate: 25mmol of 2,4-dichlorophenol was dissolved in 40mL of DMF. The solution was added 31mmol of NaH and stirred for 5 min at rt. The reaction was started through the addition of 30mmol of ethyl 2-chloroacetate into the solution prepared. At the end of 1h stirring at rt, the reaction mixture was added 50mL of distilled water. The product was extracted into the organic solution through 3 times extraction with ethyl acetate. The product was obtained via the evaporation of organic solvent at reduced pressure.

Synthesis of 2-(2,4-dichlorophenoxy)acetic acid: Ethyl 2-(2,4-dichlorophenoxy)acetate obtained in the previous step was hydrolyzed to yield out 2-(2,4-dichlorophenoxy)acetic acid. Accordingly, 20mmol of ethyl 2-(2,4-dichlorophenoxy)acetate was mixed in 5%KOH containing methanol solution. The mixture was refluxed for 3h. At the end of the reaction the organic solvent was evaporated and the residue left was added 30mL 1N HCl aqueous solution. The product was obtained through extraction with ethyl acetate (25mL x 3) and the evaporation of ethyl acetate combined fractions under reduced pressure.

Synthesis of the title molecules: In situ two steps were achieved to synthesize the final compounds. Accordingly, 20mmol of 2-(2,4-dichlorophenoxy)acetic acid was

dissolved in 25mL of dichloromethane. 3ML of thionyl chloride was added into this solution and the reaction was refluxed for 5h. At the end of the reaction time, the acyl halide (i.e., 2-(2,4-dichlorophenoxy)acetyl chloride) obtained was directly used for title molecule synthesis without further purification. Mainly, 18mmol of the appropriate amine was dissolved in 20mL of dichloromethane. Into this solution at 0ºC was added dropwise the acyl halide solution obtained in dichloromethane. The total addition was achieved in 10 min. At the end of this time, dichloromethane was evaporated. The residue was added 25mL of aqueous. The aqueous solution was further extracted with ethyl acetate (25mL) for three times. The combined organic fractions were evaporated under reduced pressure. The final compounds were obtained with column chromatography employing n-hexane/ethyl acetate (2:1) mobile phase mixture.

Comp 1 [2-(2,4-dichlorophenoxy)-1-(3,4-dihydroisoquinolin-2(1H)-yl)ethanone]: Mp: 167.3 ºC. FT-IR (major peaks) cm^{-1}: 3403, 2983, 1734, 1639. 1H-NMR (DMSO-d6) δ 7.14-6.97 (m, 6H), 6.63 (s, 1H), 4.91 (s, 2H), 4.69 (s, 2H), 3.35-2.80 (m, 4H). Anal. calc. for C17H15Cl2NO2: C, 60.73; H, 4.50; N, 4.17. Found C, 60.81; H, 4.58; N, 4.21.

Comp 2 [2-(2,4-dichlorophenoxy)-N-phenylacetamide]: Mp: 174.8 ºC. FT-IR (major peaks) cm^{-1}: 3395, 3041, 1651. 1H-NMR (DMSO-d6) δ 8.9 (bs, 1H), 7.18-6.99 (m, 7H), 6.65 (s, 1H), 4.87 (s, 2H). Anal. calc. for C14H11Cl2NO2: C, 56.78; H, 3.74; N, 4.73. Found C, 57.12; H, 3.82; N, 4.69.

Comp 3 [2-(2,4-dichlorophenoxy)-1-morpholinoethanone]: Mp: 181.0 ºC. FT-IR (major peaks) cm^{-1}: 3404, 2978, 1737, 1639. 1H-NMR (DMSO-d6) δ 7.18 (s, 1H), 7.00 (s, 1H), 6.63 (s, 1H), 4.84 (s, 2H), 3.75-3.59 (m, 4H). Anal. calc. for C12H13Cl2NO3: C, 49.68; H, 4.52; N, 4.83. Found C, 50.01; H, 4.50; N, 4.87.

Comp 4 [2-(2,4-dichlorophenoxy)-N-benzyl-N-methylacetamide]: Mp: 160.5 ºC. FT-IR (major peaks) cm^{-1}: 3412, 2928, 1737, 1637. 1H-NMR (DMSO-d6) δ 7.20-6.99 (m, 7H), 6.62 (s, 1H), 4.84 (s, 2H), 4.55 (s, 2H), 2.95 (s, 3H). Anal. calc. for C16H15Cl2NO2: C, 59.28; H, 4.66; N, 4.32. Found C, 59.65; H, 4.57; N, 4.44.

Enzyme assays

The title compounds' ability to inhibit cholinesterases was measured using a modified Ellmann's method (AChE, and BuChE) (Ellman 1958). Accordingly, each combination comprised 200 L total volume of 168 L of 50 mM Tris HCl buffer (pH 8.0), 10 L of 6.8 mM DTNB solution (0.34 mM final), 20 mM MgCl2, and 100 mM NaCl, 10 L of AChE or BuChE solution

(0.4 U/mL from Human recombinant AChE or 1.64 U/mL from Human 10 mL of either 10 mM acetylthiocholine iodide or 10 mL of 1.5 mM butyrylthiocholine iodide were added to start the reactions. Using a 96-well microplate reader, readings were taken at 412 nm following incubation for 15 minutes at 27°C (Varioskan Flash, Thermo Scientific, USA). By comparing the rates of reaction of samples relative to blank samples (DMSO and methanol) and using the formula (E S)/E100, where E is the activity of the enzyme without the test sample and S is the activity of the enzyme with the test sample, the percentage of inhibition of AChE and BuChE was calculated. Plotting the percent inhibition against the concentration of test materials allowed us to identify the concentration of test compounds and reference materials (IC50) that determined 50% inhibition of the AChE or BuChE activities. To acquire three IC50s under the experimental conditions, each concentration was evaluated in triplicate using each measurement. The mean ± standard deviation of IC50s were represented.

RESULTS AND DISCUSSION

The title compounds have been synthesized via the synthesis of 2-(2,4-dichlorophenoxy)acetyl chloride and its employment in a Schotten Bauman reaction application (Blanke, and Blanke, 1984). The general synthetic scheme is shown in Figure 2.

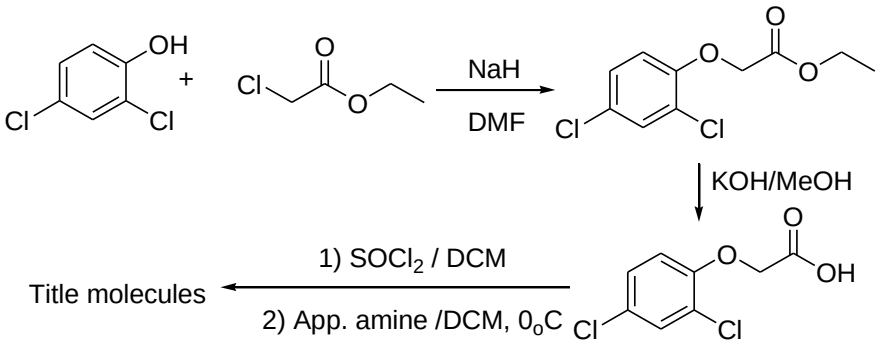


Figure 2: The synthetic scheme.

The yields of each step were in parallel with the literature findings or similar reactions. The overall (total) yields for final molecules were found higher than 40% (Gulcan *et al.*, 2003). The structures of the final molecules were identified employing IR and 1H NMR techniques. Following the synthesis work, the title molecules were assessed for their potential to inhibit cholinesterase enzymes. The results obtained are shown in Table 1.

Table 1: The potential of compounds to inhibit cholinesterase.

Compound	IC50 (AChE) (µM)	IC50 (BuChE) (µM)
Comp 1	24.5 ± 0.1	> 40
Comp 2	20.7 ± 0.8	> 40
Comp 3	22.3 ± 0.5	> 40
Comp 4	16.1 ± 0.2	29.2 ± 0.6
Donepezil	0.021 ± 0.001	8.9 ± 0.1
Rivastigmine	19.8 ± 0.3	13.4 ± 0.5

Accordingly, the title compounds were found to possess particularly acetylcholinesterase inhibitory potential. The most active compound among them was found compound 4. The N-benzyl moiety has been found an important scaffold in many cholinesterase inhibitor molecule designs. Moreover, beside compound 4, the title compounds displayed no activity against butyrylcholinesterase, implying the acetylcholinesterase selectivity of the molecules. Donepezil, one of the current cholinesterase inhibitor drugs, displayed the superior activity among the compounds tested. However, the activities of the title molecules were found comparable to the activity of rivastigmine, particularly against acetylcholinesterase enzyme.

CONCLUSION

Within this limited research work, four 2-phenoxy-N-substituted-acetamide derivatives were synthesized and their activities were assessed in cholinesterase inhibition assays. In parallel to the previous findings, the N-substituted acetamide function has been shown to be an available scaffold for the design of cholinesterase inhibitor molecules. The research warrants further work for the generation of full structure activity relationship outcomes.

DECLARATION

This manuscript has been prepared on the basis of the graduation thesis works of four EMU Faculty of pharmacy students (i.e., Kiana Harati, Seyedeh Mahta Kiaei, Tina Mahdipour Amjad, Zahra Nobavar) under the supervision of Prof. H. Ozan Gulcan. Some of the title compounds are previously synthesized for other scientific investigations and they are not original. The authors declare no conflict of interest.

REFERENCES

Blanke SR, Blanke RV (1984). The Schotten-Baumann reaction as an aid to the analysis of polar compounds: application to the determination of tris (hydroxymethyl) aminomethane (THAM). Journal of analytical toxicology, **8**(5), 231-233.

Ellman GL (1958). A colorimetric method for determining low concentrations of mercaptans. Archives of biochemistry and Biophysics, **74**(2), 443-450.

Erdogan M, Kilic B, Sagkan R I, Aksakal F, Ercetin T, *et al*. (2021). Design, synthesis and biological evaluation of new benzoxazolone/benzothiazolone derivatives as multi-target agents against Alzheimer's disease. European Journal of Medicinal Chemistry, 212, 113124.

Gulcan HO, & Orhan IE (2021). Dual Monoamine Oxidase and Cholinesterase Inhibitors with Different Heterocyclic Scaffolds. Current Topics in Medicinal Chemistry, **21**(30), 2752-2765.

Gulcan HO, Mavideniz A, Sahin MF, Orhan I E (2019). Benzimidazole-derived compounds designed for different targets of Alzheimer's disease. Current Medicinal Chemistry, **26**(18), 3260-3278.

Gulcan HO, Unlu S, Banoglu E, Sahin MF, Kupeli E, *et al*. (2003). Synthesis of new 4-(5-chloro-2-oxo-3H-benzoxazol-3-yl) butanamide derivatives and their analgesic and anti-inflammatory properties. Turkish Journal of Chemistry, **27**(4), 467-476.

Pepeu G, & Giovannini M G (2009). Cholinesterase inhibitors and beyond. Current Alzheimer Research, **6**(2), 86-96.

Pillai JA, & Cummings J L (2013). Clinical trials in predementia stages of Alzheimer disease. Medical Clinics, **97**(3), 439-457.

Shukur K, Ercetin T, Luise T, Sippl C, Sirkecioglu W, *et al*. (2021). Design, synthesis, and biological evaluation of new urolithin amides as multitarget agents against Alzheimer's disease. Archiv der Pharmazie, **354**(5), 2000467.

Wilkinson DG, Francis PT, Schwam E, Payne-Parrish J (2004). Cholinesterase inhibitors used in the treatment of Alzheimer's disease. Drugs & aging, **21**(7), 453-478.

Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Formulation development and evaluation of controlled release matrix tablets of glibenclamide

Biji Palatty Anthony[1]*, Rajendran Praveen Raj[1], Daisy Punnackal Augustine[2]

[1]Department of Pharmaceutics, St. Joseph's College of Pharmacy, Cherthala, Kerala, India.

[2]Principal, St. Joseph's College of Pharmacy, Cherthala, Kerala, India.

Abstract

The present study aimed to formulate and evaluate the controlled-release matrix tablets of Glibenclamide which is an antidiabetic drug that belongs to the second-generation oral hypoglycemics. Matrix tablets were prepared by three different polymers as sustained-release agents, using Glibenclamide as a model drug. Three polymers were selected for this study- HPMC K 15, HPMC K 100, and EC in different drug: polymer ratios. The drug was identified by FTIR spectroscopic method. The pre-compression and post-compression parameters of all formulations were found to be within acceptable limits. The release rate of Glibenclamide from matrix tablets was studied using the USP Dissolution Testing Apparatus type-I (Basket method). The formulation F6 which contained EC 50mg showed a maximum release of 99.28% in 24 hrs and revealed that EC was more effective in sustaining the drug release therefore formulation F6 was selected as the optimized formulation. The in-vitro release data of optimized formulation was fit into various kinetic models, among the different model's data of in-vitro release of best fit into Zero order kinetic model. The formulation best fit the Higuchi model and showed that drug release from the prepared matrix tablets occurs via a diffusion process.

Keywords

Glibenclamide, HPMC, EC, Controlled release, Matrix Tablet

Article History

Submitted: 6 December 2022 Accepted: 26 May 2023 Published Online: July 2023

Article Info

***Corresponding author:** Biji Palatty Anthony **email:** nisac25@gmail.com

Research Article:

Volume: 6 Issue: 1 2023 Pages: 34-55

DOI: 10.54994/emujpharmsci.1215120

INTRODUCTION

Controlled drug delivery systems have been introduced to overwhelm the drawback of fluctuating drug levels associated with conventional dosage forms. Numerous technologies have been used to control the systemic delivery of drugs. Controlled-release (CR) tablet formulations are much desirable and preferred for long-term therapy (Adepu and Ramakrishna, 2021).

The most commonly used method of modulating the drug release is to include it in a matrix system because they maintain uniform drug levels, better patient compliance, reduces the dose and side effects, increases safety margin for high-potency drugs, enhanced bioavailability, and reduce inter-patient variability. Matrix-type systems consist of drug crystals homogeneously dispersed in a matrix environment made up of cross-linked polymer (Nish *et al.,* 2012). Matrix systems can be divided into three types: Monolithic matrix, Gel forming the hydrophilic matrix, and Erodible (hydrophobic) matrix. On the basis of the retardant material used: Matrix tablets can be divided into 5 types; Hydrophobic matrices (Plastic matrices), Lipid matrices, Hydrophilic matrices, Biodegradable matrices, and Mineral matrices (Harnish *et al.,* 2011).

Glibenclamide is an anti-diabetic drug that belongs to the second-generation sulfonylurea oral hypoglycemic class. It is used to assist in the control of mild to moderately severe type 2 diabetes mellitus. Glibenclamide act by stimulating β cells of the pancreas to release insulin. Sulfonylurea increases both basal insulin secretion and meal-stimulated insulin release. Sulfonylurea also increases peripheral glucose utilization, decreases hepatic gluconeogenesis, and may increase the number and sensitivity of insulin receptors. Pharmacokinetic and Pharmacodynamic profile of Glibenclamide: duration of action; 18-24h, metabolism; hepatic, absorption (bioavailability); well absorbed, half-life; 4-6h, daily dose; 2.5 - 15mg (Brian, 2007). The main objective of the study is to formulate controlled-release matrix tablets of Glibenclamide.

The most prevalent and convenient to develop on a commercial basis are matrix-controlled release tablet formulations. As a crucial component of oral controlled-release dosage forms, matrix tablets are used. This led to the resolution of issues with traditional dose forms, such as patient non-compliance, local adverse effects, frequent administration, and variations in blood concentration levels. For

medications that are taken orally but have a short half-life and a high dose frequency, an oral controlled-release drug delivery device becomes a very viable strategy (Ajit Kulkarni *et al.,* 2017).

MATERIALS AND METHOD

Glibenclamide was obtained as gift samples from Spectrum Pharma, Hyderabad. HPMC K 15 M, HPMC K 100 M, and EC were a sample from Chemdyes Corporation, Rajkot. Magnesium stearate was obtained from Research Lab, Poona. Lactose monohydrate was obtained from Merck Limited, Mumbai. All other chemicals such as talc, starch, potassium dihydrogen Oorthophosphate, and sodium hydroxide were obtained from Laboratory equipment stores, Edappally.

Preformulation studies

A preformulation study is defined as an investigation of the physical and chemical properties of a drug substance alone and when combined with the excipients (Trevor, 2018).

Identification of drug

Drug identification was done by performing IR spectra and compared with standards.The IR spectrum of the obtained drug sample was compared with the standard functional group frequencies of glibenclamide and the drug sample was identified as glibenclamide. (Equipment used - Model: IRAFINITY-I, Manufacturer: SHIMADZU, Japan).

Pressed pellet Technique

Sample and potassium bromide in the ratio of 1:100 were placed in a clean agate mortar and triturated well and the powder mixture is compressed under 15 tonnes of pressure in a hydraulic press to form a transparent pellet. The pellet was placed in the sampling area of the FTIR spectrophotometer and scanned from 4000 to 400cm-1 and peaks obtained were identified.

Physicochemical properties of the drug

The physicochemical properties of the drug were evaluated as per the procedure of Malan *et al.,* 2002.

Physical appearance

The physical appearance of the drug was observed and compared with pharmacopoeial specifications (Indian Pharmacopoeia. 2014, Vol. 2).

Melting point

The melting point of the drug was determined using melting point apparatus.

Solubility

The solubility of the drug in water, ether, ethanol, methanol, chloroform, and alkali hydroxide solutions was determined.

Evaluation of pre-compression parameters of glibenclamide

The pre-compression parameters of glibenclamide like the angle of repose, bulk density, tapped density, Carr's Index, and Hausner'sratiowere evaluated.

Determination of drug-polymer compatibility

By IR spectroscopy: IR spectroscopy was carried out to check the compatibility between the drug and polymers. IR spectrum of drug and polymers – glibenclamide and HPMC K 15M, HPMC K 100M, EC and mixtures of drug and polymers glibenclamide- HPMC K 15M, glibenclamide- HPMC K 100M, glibenclamide- EC were recorded and compared with individual reference spectra for any spectral interferences.

Analytical methods

Determination of λmax:λmax of glibenclamide was determined in phosphate buffer pH 7.4 by scanning 10 µg/ml solution of glibenclamide in respective vehicles in the range of 200-400 nm on a UV-visible spectrophotometer. The wavelength corresponding to the peak of the spectrum was noted.

Development of standard curve of glibenclamide

Preparation of calibration curve of glibenclamide in phosphate buffer pH 7.4, 10mg of glibenclamide was accurately weighed and dissolved in a required quantity of methanol and make up to 100ml with phosphate buffer pH 7.4 (100µg/ml). Aliquots equivalent to 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, and 1.2 ml were drawn from the stock solution and made up to 10 ml using phosphate buffer pH 7.4. All these solutions were analyzed spectrophotometrically at 226 nm and absorbance was noted. A plot of absorbance Vs concentration was drawn.

Preparation of matrix tablets of glibenclamide

Glibenclamide drugs with different concentrations of hydrophilic (HPMC K 15M, HPMC K 100M) and lipophilic (EC) polymer were prepared by wet granulation technique (Shantveer *et al.*, 2010). Required quantities of all ingredients were weighed individually on an electronic balance. All ingredients were first sieved and mixed for 5 min. Then the granulating fluid was added drop by drop till a suitable mass for granulation was obtained. The wet mass granulated through sieve 16#. The granules were dried at 60°C for 1 hour in an oven. The dried granules were passed through sieve 22# and fractions of granules retained on the sieve were

discarded and then blended with talc and magnesium stearate for lubrication of granules which were then compressed on Cadmach eight station rotary tablet press using a 4 mm cone cave punches the weight of tablet adjusted to 450 mg, each tablet containing 10 mg glibenclamide and other excipients listed in table 1.

Evaluation of matrix tablets of glibenclamide

The pre-compression parameters were evaluated as per the procedure dictated by Tanbir *et al.,* 2011, Rajeshwar *et al.,* 2013 and Shantveer *et al.,* 2010. Evaluation of precompression parameters of tablet blends of controlled release matrix tablets of glibenclamide.

Angle of repose

The angle of repose was determined by the funnel method. The powders were allowed to flow through the funnel fixed to a stand at a definite height (h). The angle of repose (θ) was then calculated by measuring the height (h) and radius (r) of the heap of granules formed.

$\tan\theta = h/r$ or $\theta = \tan^{-1}(h/r)$ – Equation 1

Bulk density

A quantity of 10 g of granules from each formula, previously light Shaken for the break of any agglomerates formed, was introduced into the 100ml of measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall its weight from the hard surface from a height of 2.5cm at 2-sec intervals. The tapping was continued until no further change in the volume was noted.

Poured density

Apparent bulk density was determined by pouring a weighed quantity of powder into a graduated cylinder and measuring the volume of packing.

Poured (fluff) density = Weight of the powder / Volume of the packing

Tapped density

Tapped density was determined by the tapping method. Weighed quantity of powder was placed in a graduated cylinder and tapped until no further change in the volume of powder was noted and the volume of tapped packing was noted.

Tapped density = weight of the powder/volume of the tapped packing

Compressibility index

The compressibility of the powder was calculated by determining Carr's index and Hausner's ratio.

Evaluation of post-compression parameters of the prepared tablets.

The post-compression parameters of the prepared tablets were evaluated as per the guidelines of Sajid *et al.,* 2013, Hindustan *et al.*, 2011 and Sarika *et al.*, 2013.

Thickness and diameter

Physico-chemical properties of matrix tablets such as thickness and diameter (using a vernier caliper) were determined.

Hardness

The hardness of the tablets was tested using "Monsanto" hardness tester. In all the cases, means of six replicate determinations were taken.

Friability

Previously weighed 10 tablets were taken in Roche friabilator and the friability was checked at 25 rpm for 100 rotations. Then the tablets were dusted and reweighed and the percentage of powder eroded during 100 rotations was recorded. The resulting tablets were weighed and the percentage loss was calculated using the formula.

Initial weight – Final weight / Initial weight X 100

Weight variation

To study the weight variation, 10 tablets of each formulation were selected at random and determine their average weight. Not more than 2 of the individual weights may deviate from the average weight by more than the % deviation and none should deviate by more than twice the percentage.

Drug content

Five tablets were powdered in a mortar. From this, powder equivalent to 50 mg of the drug was taken in a 100 ml round bottom flask. It is extracted with 20 ml of phosphate buffer (pH 7.4) for ½ hour, filtered in a volumetric flask and the filtrate was made up to the mark with phosphate buffer. Further appropriate dilutions were made and the absorbance was measured at 226 nm against blank.

***In-vitro* dissolution study**

The release rate of glibenclamide from matrix tablets was studied using USP Dissolution Testing Apparatus type-I (Basket method), (Ashok Kumar Narayana *et al.,* 2001). The dissolution test was performed using 900 ml of pH 7.4 phosphate buffer, at 37 ± 0.5°C and 50 rpm. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus at different time intervals and the samples were replaced with a fresh dissolution medium. The samples were filtered and diluted to a suitable concentration with the respective medium. The absorbance of these solutions was measured at 226 nm using a UV-Visible spectrophotometer.

Kinetics of *in-vitro* drug release

To study the release kinetics of in-vitro drug release (Bhavani *et al.,* 2012), data obtained from in-vitro release study were plotted in various kinetic models: Zero order as % drug released Vs time, First order as log % drug retained Vs time, Higuchi as % drug released Vs √time, Korsmeyer- Peppas as log % drug released Vs log time and Hixson-Crowell as (% drug retained) 1/3 Vs time.

Zero-order

$Q = K_0t$ - Equation 2

Where, Q is the amount of drug released at the time, t in hrs

K_0 is the zero-order release rate constant expressed in units of concentration/time

When the data were plotted as cumulative % drug release versus time, if the plot is linear then data obeys zero order kinetics with a slope equal to Ko. This model represents an ideal release profile to achieve prolonged pharmacological action.

First order

$\text{Log } Q = K_1 t$ - Equation 3

Where Q is the percent of drug released at a time, t

K_1 is the release rate constant.

When data were plotted as log cumulative % drug remaining versus time yielded a straight line indicating that the release follows first-order kinetics. The constant K can be obtained by multiplying slope values.

Higuchi

Drug release from the matrix device by diffusion has been described by Higuchi's Diffusion equation,

$Q = K_2 t^{1/2}$ - Equation 4

Where Q is the percentage of drug release at time t

K_2 is the diffusion rate constant.

When data were plotted according to this equation, i.e., the cumulative drug released versus square root of time, yields a straight line, indicating that the drug was released by diffusion mechanism.

Korsmeyer Peppas

$Q = Kt^n$ - Equation 5

Where, Q is the percent of drug released at a time, t

K is the diffusion rate constant and n is a diffusional exponent.

This is a simple, semi-empiric model (Lisik and Musiał, 2019) when diffusion is the main drug release mechanism, relating exponentially the drug release to the elapsed time (t). This is used when the release mechanism is not well known or when more than one type of release phenomenon could be involved.

Hixson-Crowell

Drug release from the matrix device by diffusion has been described by the Hixon-Crowell diffusion equation;

$W_0^{1/3} - W_t^{1/3} = kt$ - Equation 6

where W_0 is the initial amount of drug in the pharmaceutical dosage form, Wt is the remaining amount of drug in the pharmaceutical dosage form at a time, and t and κ is a constant incorporating the surface-volume relation.

This expression applies to pharmaceutical dosage forms such as tablets where the dissolution occurs in planes that are parallel to the drug surface if tablet dimensions diminish proportionally in

such a manner that the initial geometrical form keeps constant all the time.

Stability study protocol

Batch selection and batch size: Stability studies were conducted for optimized formulation with a batch size of 50 tablets.

Containers and closure: The tablets were packed well in Aluminum foil and placed on an HDPE bottle.

Sampling test time point and storage conditions: The sampling plan and storage condition for the stability study were described in the following:

Storage conditions: 40° ± 2°C / 75 ± 5 % RH

Sampling point: 15, 30 days

Test parameters: The stability batch was subjected to evaluation studies like thickness, diameter, hardness, weight variation, friability, drug content, and in–vitro dissolution study.

RESULTS AND DISCUSSION

Preformulation study

Identification of drug

Drug identification was done by performing IR spectra and compared with standards.

Infra-red spectrum

The IR spectrum is a powerful analytical tool for the identification and investigation of the drug in formulation (Ouhaddouch *et al.,* 2019) and was compared with the standard functional group frequencies of glibenclamide and the drug sample was identified as glibenclamide. The IR spectrum of glibenclamide is shown in Figure 2.

Physicochemical properties of the drug

Physical appearance

Glibenclamide was found to be a white, crystalline powder. The physical appearance of the drug complied fully with pharmacopoeial specifications.

Melting point

The melting point of the drug was found to be in the range of 169-175°C, which conforms with the reported value. It indicates the purity of the drug sample, (The International Pharmacopoeia - Sixth Edition, 2016).

Solubility

The solubility of the drug in water, ether, ethanol, and dilute solutions of alkali hydroxides was examined and found to conform with pharmacopoeial specifications. The solubility of glibenclamide in various solvents is shown in Table 2.

Evaluation of precompression parameters of glibenclamide

The angle of repose for the pure drug was very less and hence the poor flow of the pure drug was exhibited. The Hausner ratio and compressibility index of the pure drug was found to be high, confirming that the drug has poor flow properties and compressibility; hence blends should be done before compression. The physical characteristics of Glibenclamide are shown in Table 3.

A good flow of powders/ granules is essential in tableting because the compressibility and flow properties of the drugs are likely to influence the compression process in the preparation of sustained-release tablets (Morin and Briens, 2013). Hence to improve the flow property the formulations were prepared by wet granulation technique to improve the flow as well as compressibility.

Drug polymer compatibility studies

IR spectroscopy was carried out to check the compatibility between drug and polymers (Adriana 2019). IR spectrum of glibenclamide, HPMC K 15M, HPMC K 100M, EC and mixtures of drug and polymers glibenclamide- HPMC K 15M, glibenclamide- HPMC K 100M, glibenclamide- EC were taken and it was found that there were no signicant change in the major functional group frequencies of glibenclamide in these combinations and values were found to be within the range. The study confirmed the compatibility of the drug with polymers. The spectra were shown in Figure 2 - 8.

Analytical methods

The analytical method development recommends the quality, purity, and specificity of the drug during the manufacturing process and hence the standard of the drug may not vary, which produces the desired therapeutic effect (Grish KT *et al.,* 2013). Hence, the λmax of glibenclamide was evaluated in the present study.

Determination of λmax of glibenclamide

The spectrum of 10µg/mL solution of glibenclamide in phosphate buffer pH 7.4 showed the peak as given in Table 4. The results showed that glibenclamide shows maximum absorbance at 226 nm therefore 226 nm was taken as λmax.

Preparation of calibration curve of glibenclamide in phosphate buffer pH 7.4.

Table 5 shows the absorbances of glibenclamide standard solutions (2-12 µg/ml) and Figure 1 shows the calibration curve at 226 nm in phosphate buffer pH 7.4. The curve was found to be linear in the concentration range of 2-12 µg/ml at 226 nm.

Evaluation of matrix tablets of glibenclamide

Evaluation of pre-compression parameters of tablet blends of

controlled release matrix tablets of glibenclamide.

The drug was blended along with other excipients and evaluated for the pre-compression characteristics such as Angle of repose, Bulk density, Tapped density, Carr's Index, Hausner's ratio. The results are shown in Table 6 and 7. The results showed that the powder blends have required flow properties for compression into tablets.

Evaluation of post-compression parameters of the prepared tablets

Post compression parameters of matrix tablets such as thickness and diameter, hardness, friability, weight variation, and drug content were determined (Sirisolla J and Ramanamurthy KV; 2015), and the results tabulated are shown in Table 8.

Physico-chemical parameters of all matrix tablet formulations were found to be within acceptable limits. The tablets were uniform in size and shape, friable, and with acceptable hardness. In determinations of tablet weights, all formulations weights were found to be within pharmacopoeia limits (Indian Pharmacopoeia. 2014, Vol. 2). Friability of the tablet was well within the acceptable range of 1% and indicates that tablet surfaces are strong enough to withstand mechanical shock or attrition during storage and transportation and until they are consumed. The average percentage deviation of all tablet formulations was found to be within the limits, and hence all formulations passed the uniformity of weight as per official Pharmacopeia. The manufactured tablets showed low weight variations and a high degree of drug content uniformity among different batches of the tablets. The drug content of all batches was found to be within 90-110%.

***In-vitro* drug release study**

Drug release is dependent on polymer properties, thus the application of these properties can produce well-characterized and reproducible dosage forms (Nokhodchi A, 2012). The *in vitro* drug release study of all formulations of matrix tablets was carried out. The results of 6 formulations were shown in Table 9 and a comparison of the *In-vitro* dissolution graph of formulations F1-F6 is shown in Figure 9. An *In-vitro* drug release study indicated that EC was more effective in sustaining the drug release, followed by HPMC K 100 and HPMC K 15, release rate is decreased with increasing concentration of polymer. The formulation F6 which contained EC in 50mg and F5 with EC in 30mg sustained the drug release for 24 hours and 19 hours respectively. The formulation F4 and F3 which contained HPMC K 100 120mg and 60mg sustained the drug release for 12 hours. The formulation F2 which

contained HPMC K 15 in 120mg and F1 with HPMC K 15 in 60mg sustained the drug release for 8 hours and 6 hours respectively.

Kinetics of *in-vitro* drug release

The formulation F6 was selected as the best formulation based on the dissolution study. The in-vitro release data was fit into various kinetic models like Zero order, First order, Higuchi plot, Peppas model, and Hixson-Crowell model. The R^2 values obtained in various kinetic models are given in Table 10. The drug release kinetics and mechanism of drug release were studied for the optimized formulation, among the different models data of *in-vitro* release of best fit into Zero order kinetic model because R^2 values in this model were more close to unity. The release patterns of glibenclamide from controlled release matrix tablets in the Zero order kinetic model are shown in Figure 10. Among the different model's data of *in-vitro* release formulation best fit into the Higuchi kinetic model, because R^2 values in this model were closer to unity. It indicated that drug release from the prepared matrix tablets occurs via a diffusion process. To explore more about the kinetic behavior, in vitro release results were further fitted into the Peppasequation and the result indicates that the drug release is controlled by more than one process. The release patterns of glibenclamide from controlled release matrix tablets in the Higuchi model are shown in Figure 11 and Korsmeyer-Peppas in Figure 12.

Stability study

Stability studies of a pharmaceutical formulation were done to determine whether environmental factors such as temperature, and humidity light affect the physiochemical and therapeutic properties of the formulation. The stability study confirms that the formulation meets its specification during the shelf life. Test parameters for optimized formulation F6 and stability study data results are given in Table 11- 13 and Figure 13. As per the in – vitro dissolution study the optimized formulation F6 was found to be more desirable than other formulations and chosen for the stability study. The formulation F6 was subjected to accelerated stability conditions at 400 ± 20° C / 75 ± 5 % RH for 30 days in a humidity cabinet (environmental test chamber – Rotek). At the time intervals of 15 and 30 days tablets were withdrawn, and evaluated for various test parameters like thickness, diameter, hardness, weight variation, friability, drug content and in – vitro dissolution study. The tablets did not show any variation in the tested parameters and the results were within the limits but showed slight variation in the dissolution profile.

Table 1. Composition of different batches of matrix tablets.

Sl.No	Ingredients (mg)	Formulation Code					
		F1	F2	F3	F4	F5	F6
1	Glibenclamide	10	10	10	10	10	10
2	HPMC K 15 M	60	120				
3	HPMC K 100 M			60	120		
4	EC					30	50
5	Magnesiumstearate	1.5	1.5	1.5	1.5	1.5	1.5
6	Talc	1.5	1.5	1.5	1.5	1.5	1.5
7	Lactose	377	317	377	317	407	387

Table 2: Solubility of glibenclamide in various solvents.

Solvent	Solubility
Water	Practically insoluble
Ether	Practically insoluble
Ethanol	Slightly soluble
Methanol	Slightly soluble
Chloroform	Slightly soluble
Dilute solutions of alkali hydroxides	Soluble

Table 3: Physical characteristics of Glibenclamide.

Sl.No	TESTS*	RESULTS
1.	Bulk Density	0.4312g/cm^3
2.	Tapped Density	0.5434 g/ cm^3
3.	Compressibility Index	20.64%
4.	Hausner Ratio	1.26
5.	Angle of Repose	30.24

*Average of three determinations ± Standard deviation

Table 4: Absorption maxima of glibenclamide.

nm	Absorption maxima (pH 7.4 buffer)
226	0.845

Table 5: Standard calibration table for glibenclamide in pH 7.4 phosphate buffer

Concentration (µg/ml)	Absorbance (226 nm)
0	0
2	0.142
4	0.286
6	0.423
8	0.569
10	0.705
12	0.845

Table 6: Bulk density and Tapped density.

Sl No:	Formulations	Bulk density* (g/ cc)	Tapped density* (g/ cc)
1	F1	0.4166±0.20	0.4587±0.17
2	F2	0.4000±0.10	0.4424±0.17
3	F3	0.3802±0.01	0.4310±0.10
4	F4	0.3602±0.45	0.4000±0.19
5	F5	0.370±0.62	0.4201±0.18
6	F6	0.390±0.48	0.4401±0.16

*Average of three determinations ± Standard deviation

Table 7: Carr's Index, Hausner's ratio and Angle of repose.

Sl. No	Formulations	Carr's Index (%)*	Hausner's ratio*	Angle of repose(^{0})*
1	F1	09.17±0.72	1.10±0.01	23.12±0.48
2	F2	09.09±0.90	1.10±0.01	23.26±0.42
3	F3	11.62±0.42	1.13±0.06	22.29±0.19
4	F4	10.00±0.53	1.11±0.05	24.22±0.24
5	F5	11.90±0.64	1.13±0.04	24.70±0.43
6	F6	11.36±0.53	1.12±0.06	24.89±0.18

*Average of three determinations ± Standard deviation

Table 8: Physico-chemical properties of matrix tablets.

Formulation	Thickness* (mm)	Diameter* (mm)	Hardness* (kg/cm^2)	Friability* (%)	Drug Content*(%)	Weight variation
F1	4.1±0.15	11.5±0.05	8.2±0.24	0.20±0.02	99.98±0.05	pass
F2	4.0±0.18	11.6±0.08	7.4±0.34	0.38±0.08	98.62±0.06	pass
F3	4.2±0.19	11.3±0.02	7.9±0.35	0.28±0.03	100.08±0.08	pass
F4	3.9±0.17	11.4±0.06	8.2±0.48	0.42±0.05	100.02±0.07	pass
F5	4.2±0.15	11.5±0.08	7.8±0.48	0.36±0.06	98.71±0.08	pass
F6	4.1±0.17	11.2±0.04	8.1±0.24	0.41±0.02	99.16±0.04	pass

*Average of three determinations ± Standard deviation

Table 9: *In-vitro* dissolution studies of formulation F1- F6.

Time (hr)	% CDR*-F1	% CDR-F2	% CDR-F3	% CDR-F4	% CDR-F5	% CDR-F6
0	0	0	0	0	0	0
1	18.45	15.32	09.90	07.65	05.85	03.15
2	40.97	30.52	20.26	16.20	10.80	06.75
3	58.54	55.54	31.75	26.11	16.66	10.35
4	77.46	71.57	41.43	33.32	22.60	14.41
5	90.08	77.48	52.47	42.78	28.37	18.46
6	99.10	86.98	64.40	49.09	33.78	23.42
7		90.99	72.16	59.00	39.98	28.37
8		97.30	81.99	66.21	44.59	31.76
9			91.07	75.67	48.64	36.48
10			94.69	84.68	54.95	40.99
11			97.33	93.24	61.71	44.59
12			98.24	98.65	66.66	48.64
13					72.97	52.48
14					78.83	55.40
15					84.23	59.46
16					91.44	64.41
17					94.15	69.37
18					96.85	75.67
19					98.65	79.28
20						84.23
21						89.64
22						93.24
23						96.85
24						99.28

CDR*- Controlled Drug Release

Table 10: Release kinetics of Formulation 6.

Formulation	Zero Order	First order	Higuchi	Korsmeyer-Peppas	Hixson-Crowell
	R^2	R^2	R^2	R^2	R^2
F6	0.997	0.739	0.925	0.805	0.899

Table 11: Test parameters for optimized formulation F6 and stability study data.

Sl. No	Test Parameters	At the end of 15 days 40° ± 2° C / 75 ± 5 % RH	At the end of 30 days 40° ± 2°C / 75 ± 5 % RH
1	Thickness	04.1±0.17	04.1±0.17
2	Diameter	11.2±0.04	11.2±0.04
3	Hardness	06.1±0.24	06.1±0.24
4	Weight variation	pass	pass
5	Friability	00.41±0.02	00.41±0.02
6	Drug content	99.16±0.04	99.16±0.04

Table 12: *In-vitro* dissolution studies of formulation F6 (After 15 days in 40° ± 2°C / 75 ± 5 % RH).

Time (hrs)	Absorbance	Amount of drug release (mg)	% drug release	Cumulative % drug release
0	0	0	0	0
4	0.12	1.53	15.32	15.32
8	0.26	3.33	33.34	33.35
12	0.40	5.04	50.42	50.45
16	0.52	6.66	66.67	66.72
20	0.68	8.69	86.91	86.98
24	0.76	9.85	98.55	98.64

Table 13: *In- vitro* dissolution studies of formulation F6 (After 30 days in 40° ± 2° C / 75 ± 5 % RH).

Time (hrs)	Absorbance	Amount of drug release (mg)	% drug release	Cumulative % drug release
0	0	0	0	0
4	0.13	1.66	16.65	16.65
8	0.26	3.37	33.75	33.76
12	0.38	4.90	49.05	49.08
16	0.53	6.61	66.15	66.20
20	0.69	8.82	88.25	88.32
24	0.76	9.85	98.55	98.64

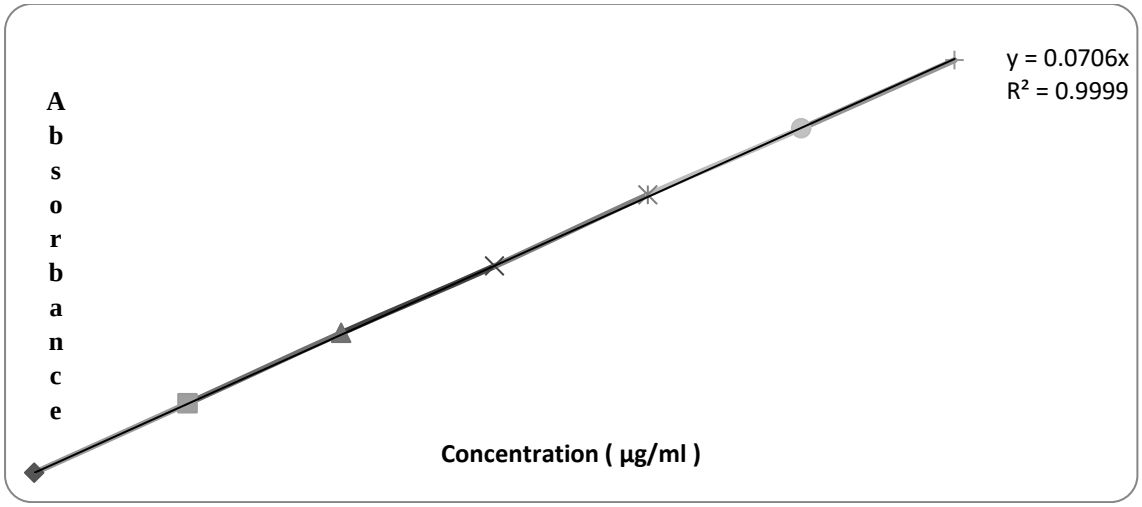


Figure 1: Calibration curve of glibenclamide in pH 7.4 phosphate buffer.

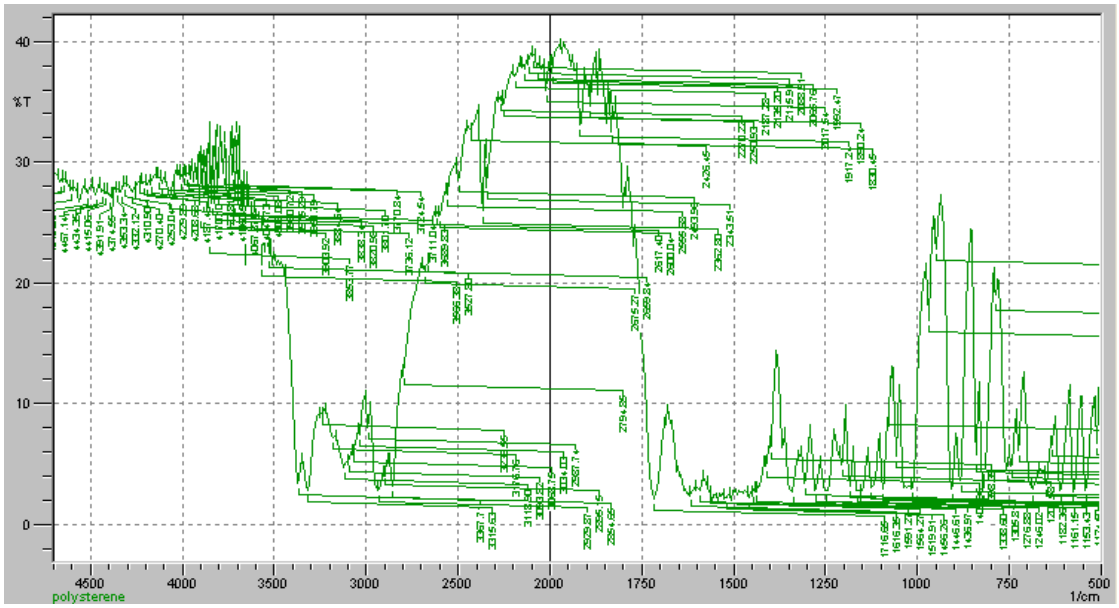


Figure 2: IR spectrum of Glibenclamide.

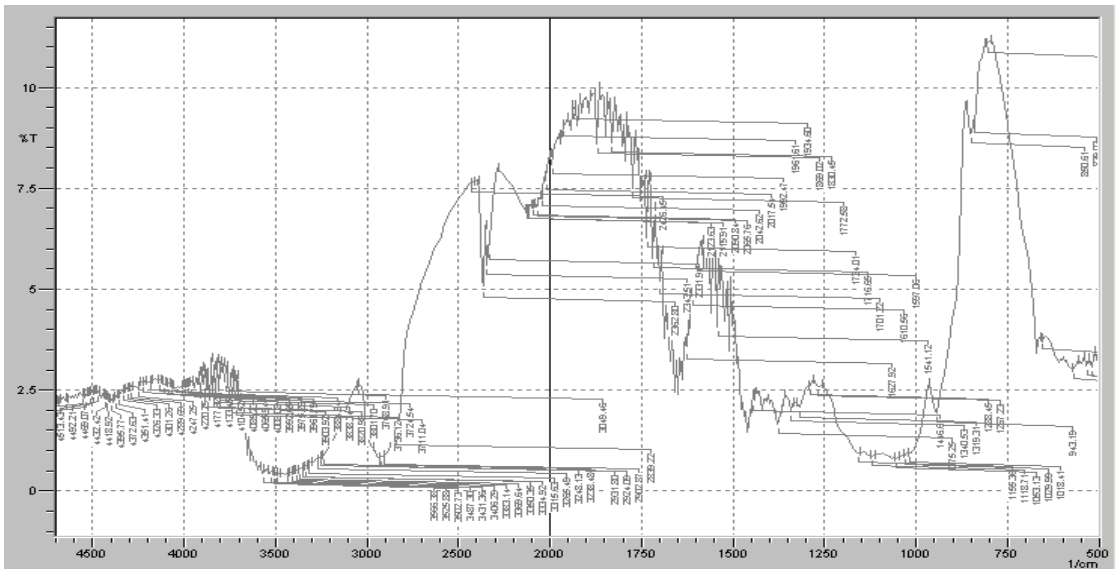


Figure 3: IR spectrum of HPMC K 15M.

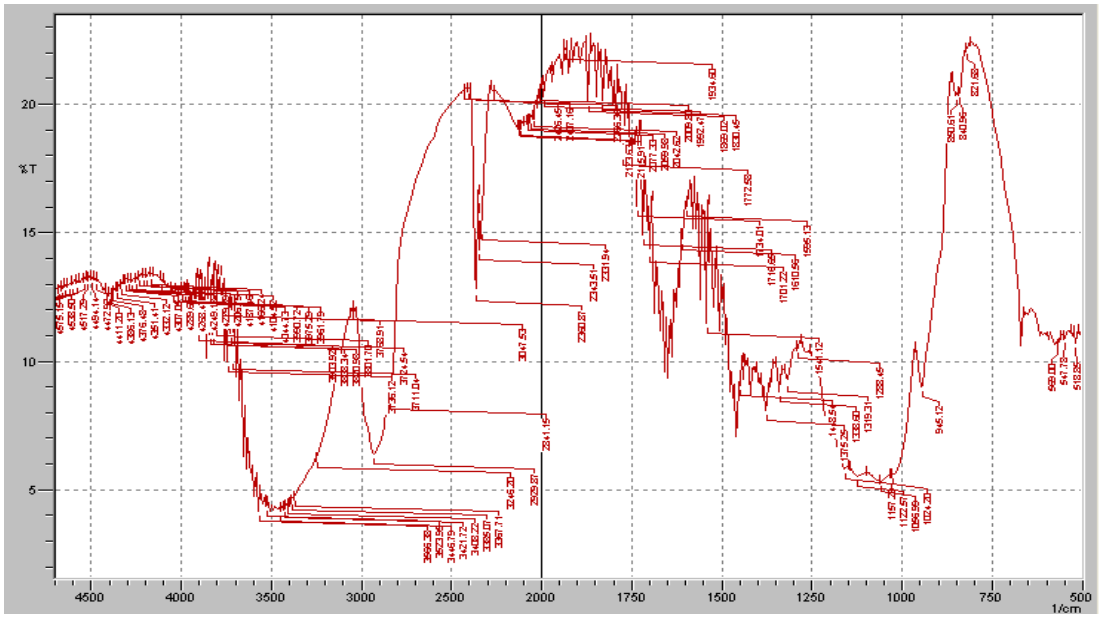


Figure 4: IR spectrum of HPMC K 100M.

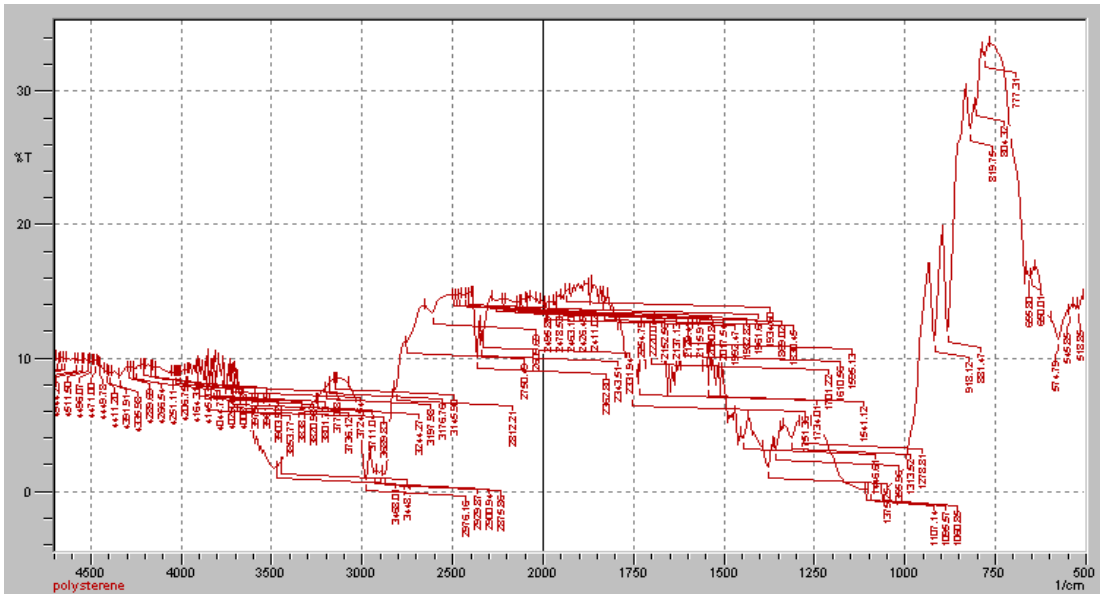


Figure 5: IR spectrum of Ethyl Cellulose.

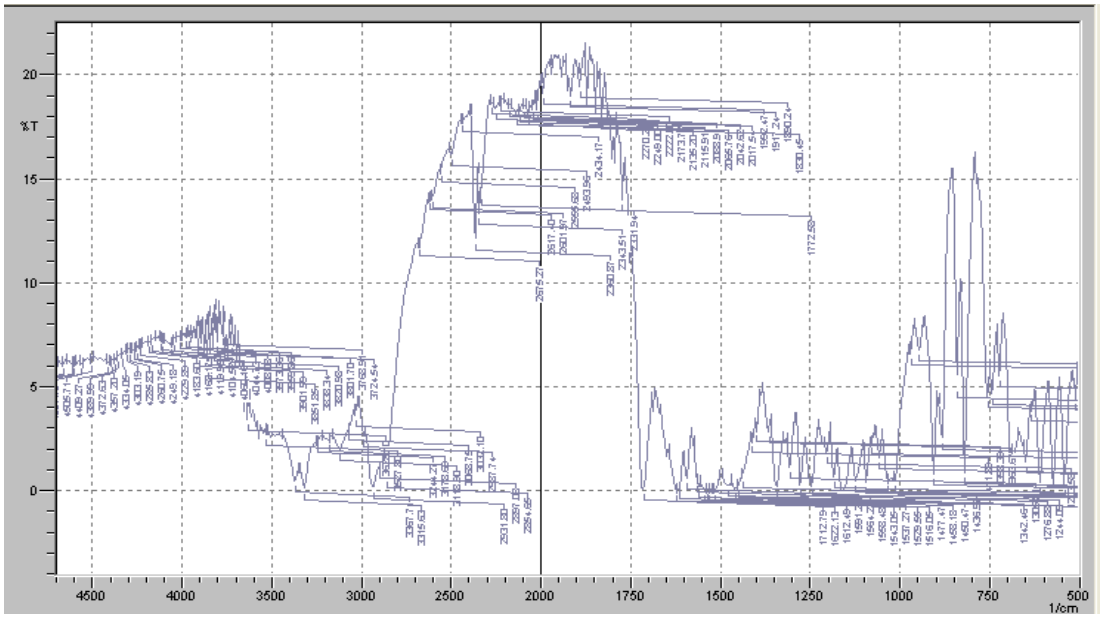


Figure6: IR spectrum of Glibenclamide - HPMC K 15M.

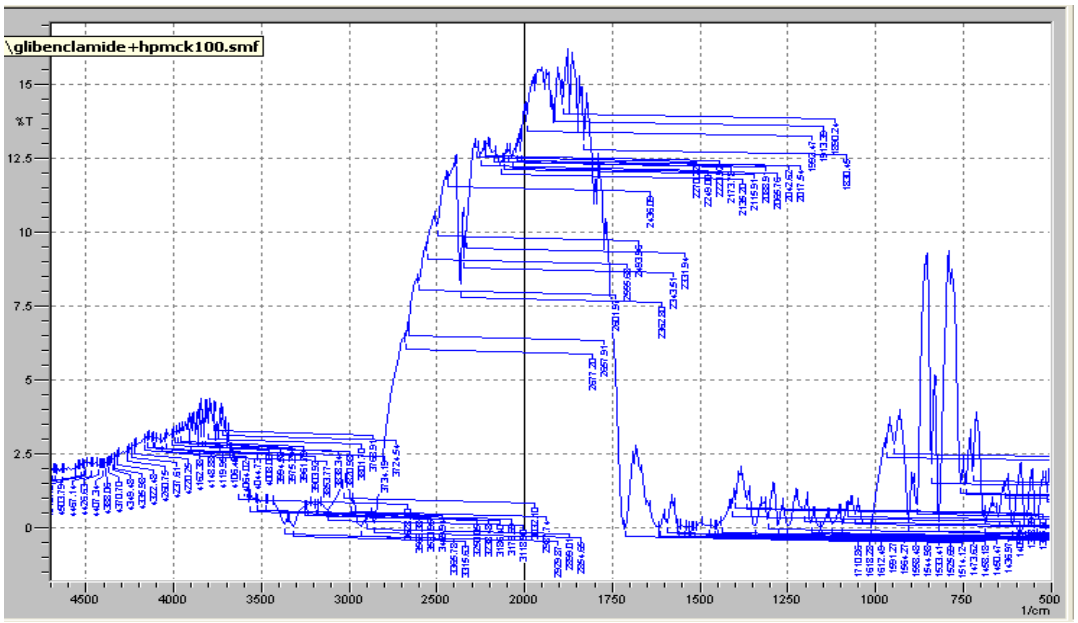


Figure 7: IR spectrum of Glibenclamide - HPMC K 100M.

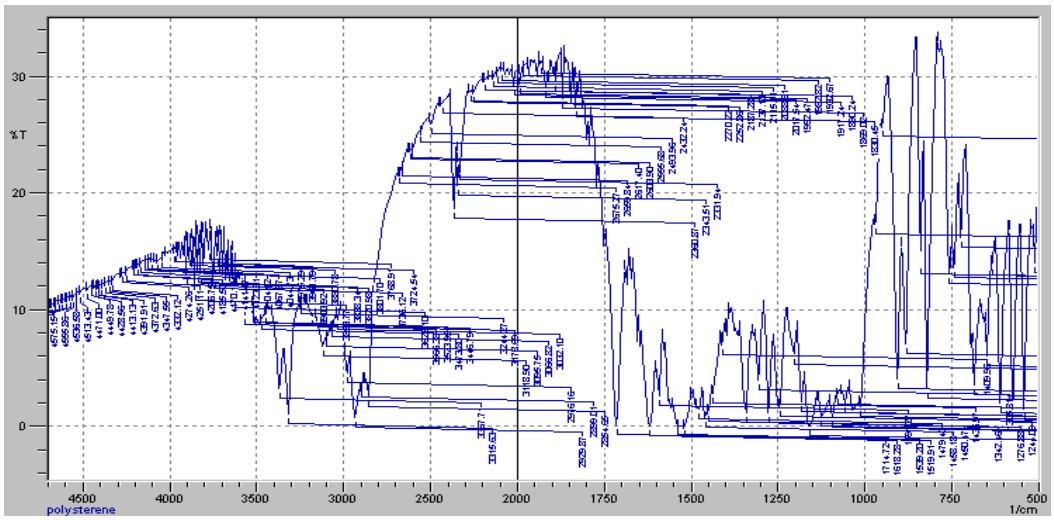


Figure 8: IR spectrum of Glibenclamide - Ethyl Cellulose.

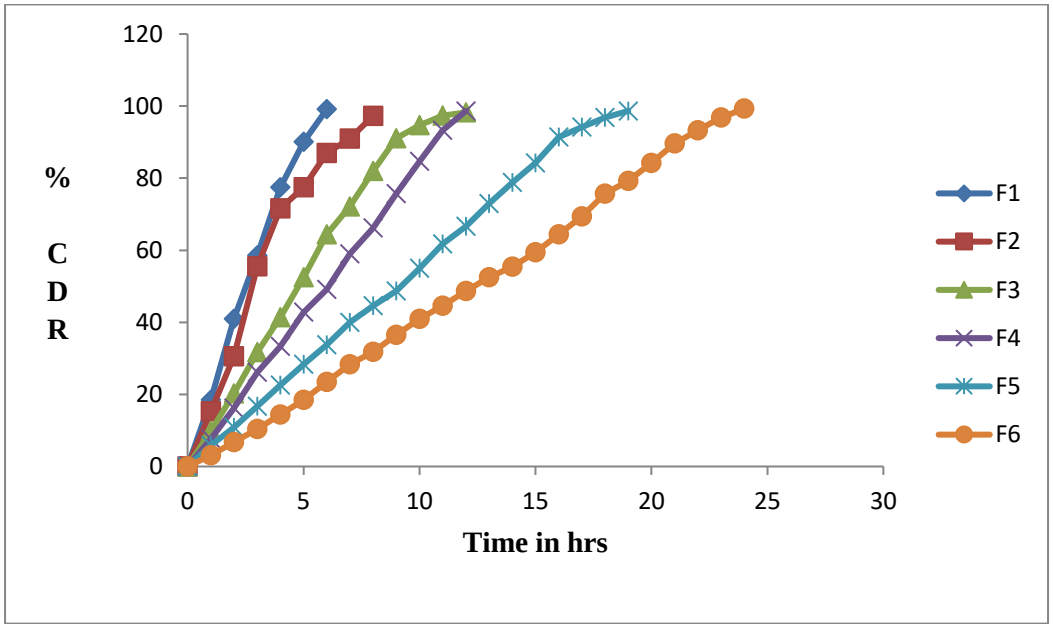


Figure 9: Comparison of In-vitro dissolution graph of formulation F1-F6.

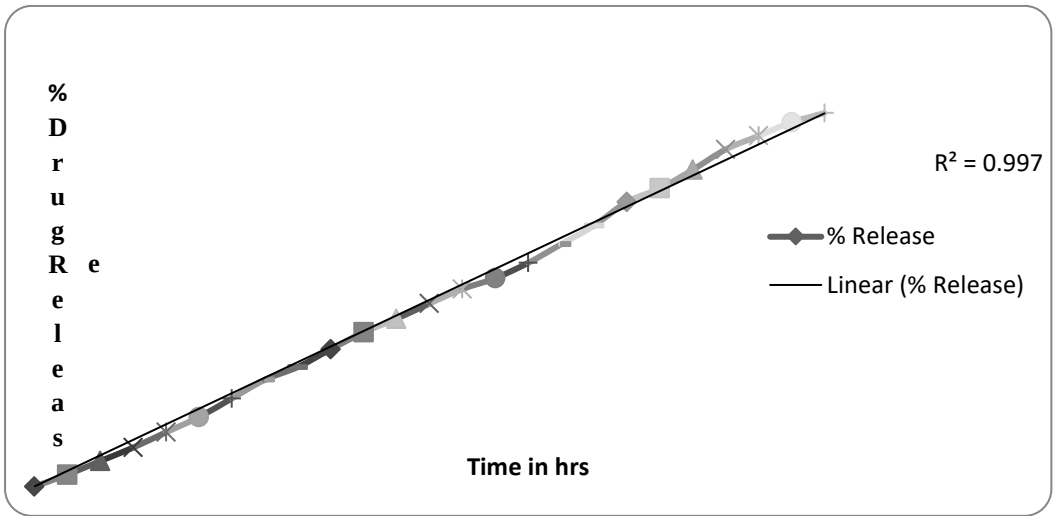


Figure 10: Zero-order plot of – Formulation 6.

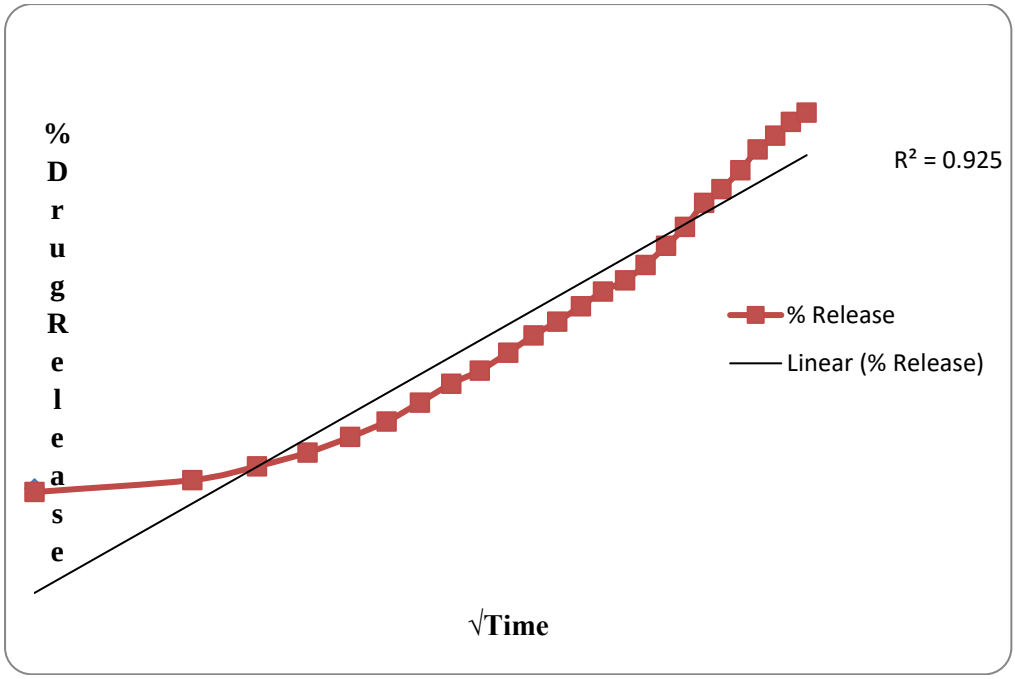


Figure 11: Higuchi plot – Formulation 6.

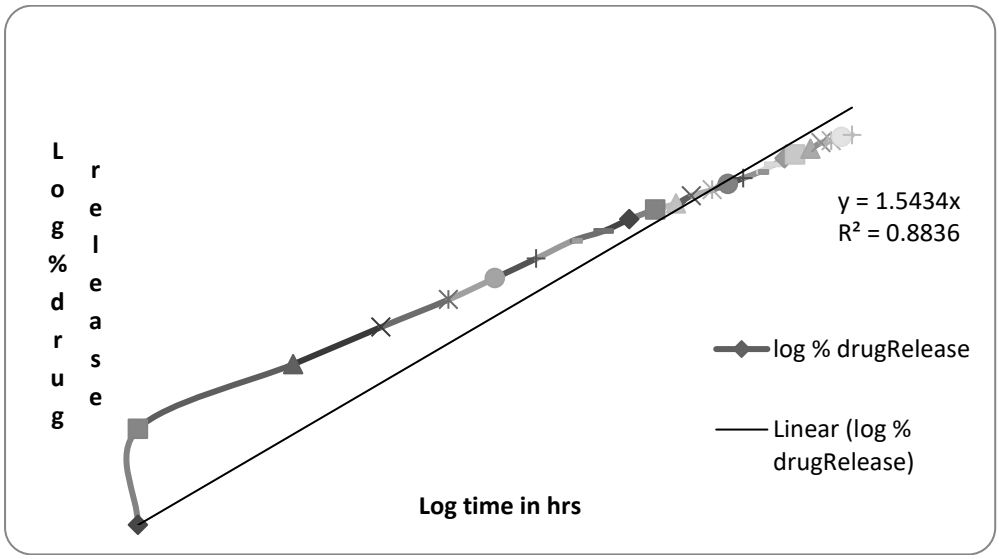


Figure 12: Korsmeyer-Peppas plot – Formulation 6.

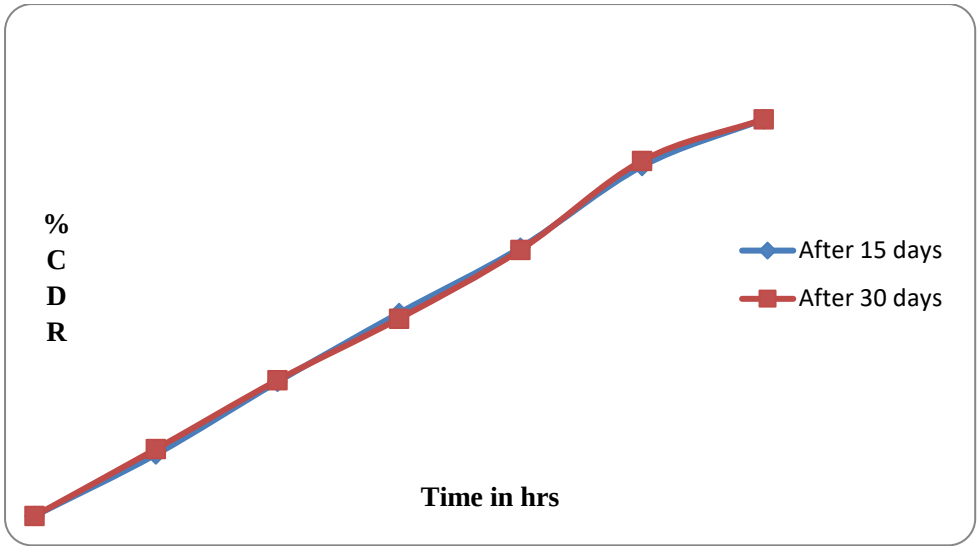


Figure 13: *In-vitro* dissolution graph of formulation F6 (After 15 & 30 days in 40° ± 2° C / 75 ± 5 % RH).

CONCLUSION

The objective of the present study was to formulate and evaluate the controlled release matrix tablets of glibenclamide. The results generated in this study lead to the following conclusions: - *In- vitro* drug release study indicated that, from the selected three polymers for this study, ie; HPMC K 15, HPMC K 100, and EC- the formulation F6 which contained EC in 50mg showed a maximum release of 99.28% in 24 hrs and revealed that EC was more effective in sustaining the drug release. The formulation F6 showed better results when compared to all other formulations and was therefore selected as the optimized formulation. The pre-compression and post-compression parameters of all formulations were found to be within acceptable limits. FTIR studies showed that there was no significant interaction between drugs and excipients. The drug release kinetics and mechanism of drug release were studied for the optimized formulation, among the different model's data of *in-vitro* release of best fit into Zero-order kinetic model because R^2 values in this model was more close to unity. The release kinetics of the formulation best fit to Higuchi model, because R^2 values in this model were more close to unity. It indicated that drug release from the prepared matrix tablets occurs via a diffusion process. To explore more about the kinetic behavior, *in-vitro* release results were further fitted into the Peppasequation and the result indicates that the drug release is controlled by more than one process. Stability studies of optimized formulation had not shown any variation in the tested parameters and the results were within the limits.

ACKNOWLEDGEMENTS

Thanks to the department of pharmaceutics, St Joseph's College of Pharmacy provided us with valuable information during the experiment. My acknowledgments to those who helped to carry out, and finish this work.

REFERENCES

Adepu S, Ramakrishna S (2021). Controlled Drug Delivery Systems: Current Status and Future Directions. *Molecules* (Basel, Switzerland) **26**(19): 5905.

Adriana IS (2019). Preformulation: The use of FTIR in compatibility studies- review. *Journal of Innovations in Applied Pharmaceutical Science* **4**(3): 01-06.

Ajit K, Trushali M, Nagesh A (2017). Application of novel natural polymer for controlling the release of fenoverine from controlled release matrix tablets. *Int J Appl Pharm* **9**(2):1-9.

Ashok KN, Hadida H, Mohamed IN, Wan AWI (2001). Development of a High-Performance Liquid Chromatographic Method for Analysis of Glibenclamide from Dissolution Studies: *Malaysian Journal of Pharmacy (MJP)*: 29-34.

Bhavani B, Kamala PV, Chowdary KPR (2012). Formulation and evaluation of glipizide sustained release tablets. *International Journal of Pharmaceutical and biomedical Research* **3**(1): 44-48.

Brian LF (2007). Glibenclamide. *xPharm: The Comprehensive Pharmacology Reference*, 1-5.

Girish KT, Satyawan S, Baljee VP (2013). U.V. spectroscopy technique for analysis to clarithromycin for gastric pH sensitive formulation. *Journal of Chemical and Pharmaceutical Research* **5**(12):1258-1262.

Harnish P, Dhrupesh R, Panchal UP, Tushar B, Mayur S (2011). Matrix Type Drug Delivery System: A Review. *Journal of Pharmaceutical Science and Bioscientific Research* **1**(3): 143-151.

Hindustan A, Harinath R, Sreenivasulu R, Kishore KRB, Chandrasekhar A, Krishna MCH, Sushma K, Sairam T (2016). Formulation and evaluation of glipizide prosophiscumanensis fruit mucilage and povidone sustained release matrix tablets. *International Journal of Pharmaceutical Research and Innovation* **2**: 17-21.

Indian Pharmacopoeia. 2014, Volume 2. The Indian Pharmacopoeia Commission, Ghaziabad. 1860- 1863.

Lisik A, Musiał W (2019). Conductomeric Evaluation of the release kinetics of active substances from pharmaceutical preparations containing iron ions. *Materials* (Basel) 12(5):730.

Mahajan PSC, Mahajan,.Mishra DK (2011). Valsartan release from sustained release matrix tablet and effect of cellulose derivatives. *Int J of Pharm & Life Sci* **2**(1): 521-530.

Malan SF, Chetty DJ, Plessis J (2002). Physicochemical properties of drugs and membrane permeability. *South African Journal of Science* 98: 385- 391.

Morin G, Briens L (2013). The effect of lubricants on powder flowability for pharmaceutical application. *AAPS PharmSciTech*. **14**(3):1158-1168.

Nish S, Mathew G, Lincy J (2012). Matrix Tablets: An Effective Way for Oral Controlled Release Drug Delivery. *Iranian Journal of Pharmaceutical Sciences* **8**(3): 165-170.

Nokhodchi A, Raja S, Patel P, Asare-Addo K (2012). The role of oral controlled release matrix tablets in drug delivery systems. *Bioimpacts*. 2012; **2**(4):175-87.

Ouhaddouch HA, Cheikh MOB, Idrissi M, Draoui M (2019). "FT-IR Spectroscopy Applied for Identification of a Mineral Drug Substance in Drug Products: Application to Bentonite", *Journal of Spectroscopy*.

Rajeshwar V, Sarada PS, Purnachander K, Ram-Naresh CH, Venkateswara CH (2013). Formulation and in vitro evaluation of sustained release Tablets of carvedilol. *International Journal of Research in Pharmaceutical and Nano Sciences* **2**(2): 221 - 233.

Sajid KS, Kamran AC, Rooh U, Zahid H, Majid KS, Shafiq R, Farhad U (2013). Formulation development and evaluation of domperidone sustained release matrix tablets by two different methods using guar gum as a sustaining agent. *World Appl Sci Journal* **25**(12): 1704-1712.

Sarika P, Ashutosh B, Deepak S (2013). Sustained release matrix technology and recent advance in matrix drug delivery system: a review. *International Journal of Drug Research and Technology* **3**(1):12-20.

Shantveer V, Salger L, Shivanandhiremath A (2010). Preparation and evaluation of sustained release matrix tablets of propranolol hydrochloride. *International Journal of Pharma and Bio Sciences* **1**(4): 227- 241.

Sirisolla J, Ramanamurthy KV (2015). Formulation and Evaluation of Cefixime Trihydrate Matrix Tablets Using HPMC, Sodium CMC, Ethyl Cellulose. *Indian J Pharm Sci* **77**(3):321-7.

Tanbir A, Moynul H, Ishtiaq A, & Muhammad RS (2011). Effect of granulation technique and drug-polymer ratio on release kinetics of gliclazide from methocel k15M CR matrix tablet. *International Journal of Pharmaceutical Sciences and Research* **2**(4): 1063-1068.

The International Pharmacopoeia - Sixth Edition, 2016.

Trevor MJ (2018). Preformulation Studies (Chapter 1) in Pharmaceutical Formulation. *The Science and Technology of Dosage Forms*, 1-41.

Review EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Common Treatment Formulation for Non-Scaring (Androgenetic) Alopecia: Mini Review

Jannat WH Al-Jubouri[1], Leyla B Pozharani[1], M Çelik[2,3,*]

[1]Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, TRNC

[2]Pharmaceutical Technologies International, Inc., Skillman, New Jersey, USA

[3]University of New Mexico, College of Pharmacy, Albuquerque, New Mexico, USA

Abstract

In spite of being a non-life-threatening condition, hair loss (alopecia) severely impacts the quality of life of individuals who experience it. Recent studies indicate that the number of patients suffering from alopecia globally is on the rise. Androgenic alopecia (AGA) affects both genders at all ages. Genetic factors and family history are found to greatly impact the likelihood of experiencing hair loss. Statistics reveal that during the course of their lives, 80% of men experience alopecia, while 40 to 50% of women are likely to face some form of hair shedding. AGA is characterized by frontal-temporal hair shedding in men and hair thinning of the midline part of the scalp for women. A variety of herbal formulations are available on the market to combat AGA, while only two FDA-approved medications exist at the moment: oral finasteride and topical minoxidil. Topical formulations of finasteride are still under clinical trials. Minoxidil and finasteride formulations provide effective AGA treatment for both genders. Recent concerns regarding potential side effects of these two medications have drawn interest in providing new innovative alternative formulations (nutrients, minerals and vitamins) to provide a safer treatment against AGA. This article provides a brief overview of the current and alternative AGA formulations.

Keywords

Androgenetic alopecia: Finasteride: Hair loss: Minoxidil

Article History

Submitted: 29 November 2022 Accepted: 24 January 2023 Published Online: July 2023

Article Info

***Corresponding author:** Jannat W. H, Al-Jubouri **email:** jannat.aljubouri@emu.edu.tr

Research Article:

Volume: 6 Issue: 1 2023 Pages: 56-63

DOI: 10.54994/emujpharmsci.1211611

INTRODUCTION

Hair is the mirror of human health. It is an integrated system with unique physical and chemical characteristics that reflects human well-being (Araújo *et al.,* 2011). The number of patients suffering from hair loss (alopecia) and hair thinning has recently increased globally. Alopecia is a wide spread issue that affects all genders from infancy to old age. The most prevalent type of non-scaring alopecia is androgenetic alopecia (AGA) (DeVillez, 1994). The difference between male AGA (referred to as male pattern hair loss (MPHL)) and female AGA (referred to as female pattern hair loss (FPHL) is the location of hair shedding on the scalp (Al Aboud *et al.,* 2020). Figures 1 and 2 present the difference between MPHL and FPHL. Scientific data show that 80% of men get noticeable hair loss (AGA) by the age of 80, and nearly 40-50% of women suffer from alopecia during their life course. These numbers may increase by aging (Guo *et al.,* 1998; Sadick, 2018; York *et al.,* 2020).

Figure 1. Androgenetic alopecia location in women (AGA).

Figure 2. Androgenetic alopecia (AGA) location in men.

Although AGA is an age-race-dependent genetic condition (Kelly *et al.,* 2016; Völker *et al.,* 2020), scientists have developed several approaches to address these issues, such as pharmacotherapy, surgery, and cosmetics. However, only two medications (oral finasteride and topical minoxidil) are approved by the Food and Drug Administration (FDA) for the treatment of reversible androgenetic alopecia (AGA). Currently two drugs are available in the market under various names

and formulations (Lee *et al.,* 2018). The main goal of AGA treatment is to arrest hair shedding progress, preventing any future miniaturization, and reversing the process if possible. Topical minoxidil is favored instead of oral finasteride due to its noticeable outcome and more minor side effects. Topically administered minoxidil is currently available as an OTC drug in solution form at a concentration of 2% or 5% and, foam and shampoo forms at 5% concentration. Finasteride is available in 1 mg oral dosage form, and clinical trials have been performed for 0.25% and 0.5% of the topical solution as a promising topical form (Ashique *et al.,* 2020; Kelly *et al.,* 2016; Lee *et al.,* 2018). The effectiveness of the FDA-approved drugs is, however, shown to be suboptimal, giving rise to greater interest for complementary therapies with reduced side effects, such as natural medications with potential medicinal constituents like vitamins, minerals, and oils (Kelly *et al.,* 2016; York *et al.,* 2020).

Common Causes of AGA

The main reason causing androgenetic alopecia (AGA) is heredity. However, improper lifestyle habits, hair styling abuse, oily scalp, the side effect of some medication, vitamin deficiency, hormonal changes (female pattern alopecia), chemotherapy, and pregnancy may cause alopecia (Nabahin *et al.,* 2017).

Treatment Strategies

Significant indicators for the etiology of various hair loss patterns and hair loss forms are presented in Table 1. The most common type of alopecia is androgenetic alopecia (AGA) which is a cumulative patterned hair loss attributed primarily to heredity and\or hormonal (androgen, estrogen, testosterone) disorders affecting almost 50% of the population in different severity levels (DeVillez, 1994; Kelly *et al.,* 2016; Yip *et al.,* 2011).

Table 1. Significant features and common types of non-scaring alopecia

Type	Considerable feature	Comments	Source
Androgenetic alopecia	Heredity (family history) and hormonal disorders are related factors leading to complete baldness starting from the frontal area of the scalp in both genders. It causes reduction of the hair thickness and length plus loss of hair pigmentation.	The most common type of hair loss, affecting 50% of the population.	(Kelly et al., 2016)
Alopecia areata	Hair loss in small patches on the scalp may prevent future hair regrowth in the affected areas. Alopecia areata happens due to immune system reaction against hair follicles.	Study shows 1.7% of the American population have the risk by the age of 50.	(Phillips et al., 2017)
Iron deficiency alopecia	Scalp hair shedding starts to be noticeable when the ferritin levels go under 40 mg/mL.	This case is a frequently seen especially in women during their life course.	(Almohanna et al., 2019)
Telogen effluvium	Stress and psychological\emotional state causes a significant amount of hair to fall while brushing the hair or in the shower.	A pervasive case that may affect each one of us can be resolved in 2-6 months to a normal state.	(Whiting, 1999)
Anagen effluvium	65% of the hair starts to fall after few days of chemotherapy exposure.	It is a common side effect of chemotherapy treatment.	(Phillips et al., 2017)

Pharmacological Treatment

Only two FDA-approved therapeutic agents demonstrated a significant effect in the treatment of AGA. The first drug launched on the market for the treatment of AGA was minoxidil**.** This drug was initially used as a hypertension medication for its potent vasodilator effect. The fact that balding patients experienced hair regrowth prompted the idea of a topical minoxidil formulation for treating androgenetic alopecia (AGA) in both male and female patients. Minoxidil is a prodrug that the hair follicle root converts to minoxidil sulfate; thus, the scientists proposed using this conversion topically to achieve more direct benefit while minimizing the drug's systemic potential side effects. The mechanism of action of minoxidil is not fully understood, but the fundamental mechanism on hair growth is due to its vasodilating and K+ channel opening properties, which provide more oxygen and higher blood flow to the region. The FDA has approved the 2% and 5% solution and 5% foam for long-term use, daily applied on a dry scalp once or twice a day, having visible results after 4-8 months. The procedure causes a substantial rise in existing hair diameter as well as an increase in hair weight. The drug's main side effects are demonstrated as scalp dryness, itching, and a burning sensation. Over 56 products have been introduced to the market under different brand names and application forms such as spray, liquid solutions, shampoos, and foam (Ashique *et al.*, 2020; Levy and Emer, 2013; Lolli *et al.*, 2017; Messenger and Rundegren, 2004; Varothai

and Bergfeld, 2014; York *et al.*, 2020; Zins, 1988).

The second FDA-approved drug is oral finasteride which is a selective type II 5-alpha reductase inhibitor with limited use to its systemic side effect, 5 mg dose is prescribed for prostate enlargement, and 1 mg is (oral dose) suggested to treat male pattern hair loss (MPHL). It is a life-long use medication, showing several side effects such as male feminization, especially in pregnant women with male gender fetuses, reduced libido, and erectile dysfunction. These side effects may be normalized when discontinued. Only the 1 mg dose oral Finasteride is FDA approved for AGA treatment; finasteride topical form is a potential future medication currently being tested in clinical trials (Kelly *et al.*, 2016; Varothai and Bergfeld, 2014; York *et al.*, 2020).

Other Common Pharmacological Alternatives for AGA Treatment

Dutasteride: A second-generation 5-alpha reductase inhibitor and is more potent than finasteride due to its dual- effect. It inhibits both type 1 and 2 of the 5-alpha reductase enzymes. It is a selective inhibitor which is considered an alternative for AGA treatment for the patients who did not show any response using finasteride for minimum of 8 months. Although dutasteride showed a significant increase in hair growth compared to finasteride, it is not an FDA approved drug (Gubelin Harcha *et al.*, 2014; York *et al.*, 2020).

Moreover, the main molecule causing androgenic alopecia is dihydrotestosterone (Olszewska *et al.*, 2005). Dutasteride is the only dual selective type I & II 5-alpha reductase enzyme (which converts testosterone to 5-alpha dihydrotestosterone) available in the market as an OTC drug (Nickel *et al.*, 2004).

Prostaglandins: The key function of prostaglandins is regulating the hair follicle cycle by normalizing PGD2 levels. There is an increase in the PGD2 levels in AGA, which has a considerable impact on hair loss. A previously made study showed that 0.03% bimatoprost (prostaglandin analog) lotion applied continuously for 12-16 weeks gives a tremendous increase in diameter & number of the hair. There is significant number of clinical trials to confirm this activity (Kelly *et al.*, 2016; Levy and Emer, 2013).

Physical Treatment Method

New therapies emerged with a positive result in the last decades to treat hair loss and AGA, having a vast population due to its direct effect on the desired area and less time consuming. Table 2 presents the available physical treatment options.

Table 2. Physical treatment options (Sadick, 2018).

Treatment Type	Description
Platelet-rich-plasma (PRP)	Taken from the patient's blood then go through a centrifuge machine; this concentrated number of platelets are injected intradermally to the affected.
Micro-Needling	Tiny little needles injected directly to the stratum corneum consist of a combination of drugs like minoxidil and some vitamins and growth factors.
Cytokines	Introduced to the AGA treatment list is very similar to the PRP in terms of the low number of side effects; it focuses on injecting growth factors like KGF into the skin.
Low-level-laser therapy (LLLT)	The near-infrared laser works on enhancing and regenerating the tissues; LLLT was proved in 2011 as a safe method for treating different types of alopecia.

Other Alternatives for the Treatment of AGA

Herbs, probiotics, minerals, and enzymes are all examples of natural ingredients sold as nutritional supplements which do not require FDA approval (Hosking *et al.*, 2019). Table 3 summarizes the natural source alternatives.

Table 3. Summary of natural source alternatives for AGA treatment.

Natural alternative	Comments	Reference
Vitamin A	Vitamin A is essential for healthy hair.	(Ashique *et al.*, 2020)
Vitamin D	Women with FPHL showed lower levels of vitamin D than usual.	(Almohanna *et al.*, 2019)
Vitamin C	Vitamin C deficiency is commonly associated with body hair abnormality.	(Almohanna *et al.*, 2019)
Vitamin E	Vitamin E has an antioxidant effect that can minimize oxidative stress throughout the scalp.	(Ashique *et al.*, 2020)
Iron	Iron deficiency is the most widespread nutritional deficiency in the world strongly associated with hair loss, especially in MPHL patients.	(Almohanna *et al.*, 2019)
Zinc	Alopecia is an eminent indication of a zinc deficiency significant regrowth occurring with zinc supplementation therapy.	(Ashique *et al.*, 2020)
Selenium	Selenium supplements improved dispersed hair growth.	(Bates *et al.*, 2000)
Onion juice	The entity of sulfur and phenolic compounds are responsible for hair regrowth.	(Sharquie and Al-Obaidi, 2002)
Rosemary oil	The procedure is quite similar to minoxidil in increasing blood circulation and the delivery of oxygen to the area.	(Ezekwe, 2020)
Green tea	Work as 5-alpha reductase.	(Ashique *et al.*, 2020)
Saw Palmetto	It is a non-selective 5-alpha reductase.	(Ashique *et al.*, 2020)

CONCLUSION

AGA is a psycho-social condition, which has a massive impact on psychological health and social acceptance. The therapy for AGA is determined by a variety of factors, including effectiveness, concerns, and costs. The goal is to limit and, if possible, reverse the alopecia process. Currently, only two synthetic drugs have been approved by the FDA for androgenetic alopecia treatment (oral finasteride, topical

minoxidil). Some side effects have been reported during the use of oral finasteride, particularly sexual dysfunction; this may cause significant worry and have a negative influence on patients' quality of life. Topical finasteride has been suggested of being a possible alternate option for minimizing systemic side effects. Although dutasteride is the only dual-effect treatment showing a significant increase in hair count and hair quality compared to finasteride, the lack of clinical trials on dutasteride efficacy is the main reason for being not FDA-approved yet. Besides hair transplantation, several new physical treatment approaches were found to be effective for the treatment of AGA. Moreover, various natural supplements that might also assist in preventing hair loss. Treatment of AGA is essential, relying on the fact that the number of patients increases by almost 5% every year. This mini-review summarizes the most widespread causes of alopecia and their chemical/physical treatment solutions and the natural source alternatives for AGA.

REFERENCES

Nabahin A, Eloun AA, Naser SSA (2017). Expert System for Hair Loss Diagnosis and Treatment, *Int. J. Eng. Inf. Syst.* **1** :160–169.

Messenger AG, Rundegren J (2004). Minoxidil: Mechanisms of action on hair growth, *Br. J. Dermatol.* **150**: 186–194

Al Aboud AM, Zito PM (2019). Alopecia, Conn's Handb. *Model. Hum. Aging*, 751–762.

Hosking AM, Juhasz M, Atanaskova MN (2019). Complementary and Alternative Treatments for Alopecia: A Comprehensive Review, *Ski. Appendage Disord*. **5**: 72–89.

Whiting DA (1999). Dermatopathology of common hair problems, *J. Cutan. Med. Surg*. **3**: 2–13.

Lolli F, Pallotti F, Rossi A, Fortuna MC, Caro G, Lenzi A, *et al.* (2017). Androgenetic alopecia: a review, Endocrine **57**: 9–17.

Zins GR (1988). The history of the development of minoxidil, *Clin. Dermatol.* **6**: 132–147.

Almohanna HM, Ahmed AA, Tsatalis JP, Tosti A (2019). The Role of Vitamins and Minerals in Hair Loss: A Review, *Dermatol. Ther. (Heidelb).* **9**: 51–70.

Bates JM, Spate VL, Steven MJ, Germain DL, Galton VA (2000). Effects of selenium deficiency on tissue selenium content, deiodinase activity, and thyroid hormone economy in the rat during development, *Endocrinology* **141**: 2490–2500.

Völker JM, Koch N, Becker M, Klen A (2020). Caffeine and Its Pharmacological Benefits in the Management of Androgenetic Alopecia: A Review, *Skin Pharmacol. Physiol.* **33**: 153–169.

York K, Meah N, Bhoyrul B, Sinclair R (2020). Treatment review for male pattern hair-loss, *Expert Opin. Pharmacother*. **21**: 603–612.

Sharquie KE, Al-Obaidi HK (2002). Onion juice (*Allium cepa* L.), a new topical treatment for alopecia areata, *J. Dermatol*. **29**: 343–346.

Yip L, Rufaut N, Sinclair R (2011). Role of genetics and sex steroid hormones in male androgenetic alopecia and female pattern hair loss: An update of what we now know, *Australas. J. Dermatol.* **52**: 81–88.

Levy LL, Emer JJ (2013). Female pattern alopecia: Current perspectives, *Int. J. Womens. Health* **5**: 541–556.

Olszewska M, Rudnicka L (2005). Effective treatment of female androgenic alopecia with dutasteride, *undefined*.

Ezekwe N, King M, Hollinger JC (2020). The Use of Natural Ingredients in the Treatment of Alopecias with an Emphasis on Central Centrifugal Cicatricial Alopecia: A Systematic Review, *J. Clin. Aesthet. Dermatol*. **13**: 23.

Sadick NS (2018). New-Generation Therapies for the Treatment of Hair Loss in Men, *Dermatol. Clin.* **36**: 63–67.

Nickel JC (2004). Comparison of clinical trials with finasteride and dutasteride. *Reviews in urology*, **6**(9): 31–39.

Araújo R, Fernandes M, Cavaco-Paulo A, Gomes A (2011). Biology of human hair: Know your hair to control it, *Adv. Biochem. Eng. Biotechnol*. **125**: 121–143.

DeVillez RL (1994). Androgenetic Alopecia in the Female, *Arch. Dermatol.* **130**: 303.

Ashique S, Sandhu NK, Haque SN, Koley K (2020). A Systemic Review on Topical Marketed Formulations, Natural Products, and Oral Supplements to Prevent Androgenic Alopecia: A Review, *Nat. Products Bioprospect*. **10**: 345–365.

Varothai S, Bergfeld WF (2014). Androgenetic alopecia: An evidence-based treatment update, *Am. J. Clin. Dermatol.* **15**: 217–230.

Guo S, Lilly P, Siervogel RM, Cam P, E Lea and P. D, P r e v a l e n c e o f M a l e, 0512 (1998), pp. 1330–1332.

S.W. Lee, M. Juhasz, P. Mobasher, Ekelem C, Mesinkovska NA (2018). A Systematic Review of Topical Finasteride in the Treatment of Androgenetic Alopecia in Men and Women, *J. Drugs Dermatol*. **17**: 457.

Phillips TG, Slomiany WP, Allison R (2017). Hair Loss: Common Causes and Treatment, *Am. Fam. Physician* **96**: 371–378.

Gubelin W, Harcha J, Barboza M, Tsai TF, Katsuoka K, *et al.* (2014). A randomized, active- and placebo-controlled study of the efficacy and safety of different doses of dutasteride versus placebo and finasteride in the treatment of male subjects with androgenetic alopecia, *J. Am. Acad. Dermatol*. **70**: 489-498.e3.

Kelly Y, Blanco A, Tosti A (2016). Androgenetic Alopecia: An Update of Treatment Options, *Drugs* **76**: 1349–1364.