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ORIGINAL ARTICLE

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Formulation and Evaluation of *Tridax Procumbens* (L.) L. Herbal Soaps

M. V. Sudharani[1*], A. C. Kullayappa[1], C. Dheeraj[1], K. Bhaskar Naik[1], M. Vandana[1], P. Jamalbi[1] and V. Sravani[1]

ABSTRACT

Introduction: Traditional medicine is an important source of potential therapeutic compounds. *Tridax procumbens* has great importance in traditional medicine because of its good antibacterial and antifungal properties. The current research aims to formulate herbal soap using methanolic extract of T. procumbens leaves and evaluate its physicochemical properties.

Method: The herbal soaps are formulated using T. procumbens leaf extract. The leaves were extracted by the Soxhlet extraction method using methanol as solvent. The plant extract was evaluated for phyto constituents like saponins, phenols, alkaloids, flavonoids, tannins and steroids. Four different formulations are formulated with varying doses of plant extract and ingredients. The physical parameters like colour, odour, appearance and evaluation parameters like pH, moisture content, % alcohol insoluble matter, foam height, foam retention and % free alkali are evaluated for four formulated soaps.

Results: The plant extract consists of phyto constituents like saponins, phenols, alkaloids, flavonoids, tannins and steroids. All four formulations had good appearance, uniform colour and odour.

Conclusion: Among four soap formulations, F1 soap had the least number of impurities whereas F3 soap had stable foam. The evaluation parameters are in the limits prescribed by Bureau of Indian standards. Hence formulated soaps can further be standardised and used.

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***Corresponding author:**

Email address:
mvsudharanireddy@gmail.com

Authors' Affiliation:

[1] *Department of Pharmaceutics, Sri Krishnadevaraya University College of Pharmaceutical Sciences (SKUCOPS), Anantapur, 515003, Andhrapradesh, India.*

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Introduction

Skin is the first line of defence in the human body. The skin constitutes 15% of the total body weight, as it is the largest organ. It protects against physical, biological and chemical attacks and plays an important role in thermoregulation by preventing the loss of excess water from the body (Kolarsick et al., 2011). Crores of bacteria, fungi, and viruses reside on our skin and constitute the skin microbiota. These act as a physical barrier to prevent the invasion of pathogens. When the balance between these symbiotic bacteria and pathogens is disturbed, it may result in skin infections (Allyson et al., 2018). For many years skin problems are common ailments that are affecting humans (Akuaden et al., 2019). Common causes of skin infections are the invasion of the skin by pathogenic microorganisms. So, any substance with antimicrobial properties, which either kills the microorganism or inhibits the growth of the microorganism is essential for treating skin infections.

Nature has been a major source of herbs with immense antimicrobial potential to treat mild to severe types of skin diseases. Many ancient medical systems like Ayurveda, Siddha and Unani systems of medicine have explored the use of several herbal preparations for treating skin infections (Christudas et al., 2012). According to the World Health Organisation reports, Traditional medicine is fulfilling the primary health needs of 80% of the population. The dependency on traditional medicine is more in developing countries. The increasing desire to investigate new potential drugs in natural sources has led us to create some amazing medicines (Policepatel & Manikrao, 2013).

Herbal formulations with antifungal and antibacterial activities can be prepared from various parts of the plant like stem, leaves, roots, bark, flower, or fruit for skincare. These medicines can be applied topically or administered orally. For topical administration, these medicines are formulated in the form of cream, lotion, gel, soap, sap, or ointment (Kareru et al., 2010). Herbal soaps are one of the highly used formulations for skincare and for treating skin diseases.

Soap is a surface-active agent and it is chemically the alkali metal salt of long-chain fatty acids. When a fat or oil containing triglycerides is reacted with alkali, soap is formed by a reaction called saponification reaction (Akuaden et al., 2019). Generally, soaps are prepared by the melt and pour method, hot press method and cold press method. Oils like coconut oil, palm kernel oil, olive oil, castor oil, sunflower oil, rice bran oil and soybean oil among others are used for soap preparation. The quality of soap depends on the type of oil used, type of alkali used, its hardness, foam height, moisture content, and total fatty matter. (Sindhu et al., 2019). Herbal soaps incorporated with herbal extracts should show significant antimicrobial activity, provide conditioning to the skin, have good foam, fragrance and are gentle on the skin.

One of the potential plants with antimicrobial properties is *Tridax procumbens* (L.) L (*T. procumbens*). It is generally called coat buttons in English, jayanthi veda in Sanskrit, balapaku or gaddi chamanthi in Telugu. It belongs to the family Asteraceae. *T. procumbens* is a widely spread herb, covered with hair (Jain, 2012). It is widely spread in India up to 2400 m above sea level even though it is primarily native to tropical regions of South and North America (Vinod & Nagaraju, 2015).

T. procumbens has great importance in traditional medicine. It is used to treat colds, inflammation, anaemia and liver diseases in Central America. In Guatemala, it is used as an antibacterial, antifungal, and antiviral agent and plays an important role in the treatment of vaginitis, stomach pain, diarrhoea, mucosal inflammations, and skin infections. In India, leaf juice is used to treat wounds and bleeding. Gastrointestinal and respiratory infections, high blood pressure, and diabetes are some of the ailments for which *T. procumbens* is used significantly. The whole plant is used for the treatment of protozoal infections, like malaria, leishmaniasis and dysentery in Guatemala (Beck et al., 2018). In the West African sub-region and tropical region of the world, the leaves of the plant are used as a remedy for conjunctivitis by traditional practitioners and the native people (Pandey & Tripathi, 2014).

The methanolic extract of the leaves consists of phytoconstituents like alkaloids, flavonoids, phenols, saponins, steroids, and tannins (Kushwaha et al., 2018). The plant is rich in minerals like iron, copper, zinc, sodium and other trace minerals like phosphorus, potassium, selenium, calcium and magnesium (Vinod& Nagaraju, 2015). Phytoconstituents like luteolin, kaempferol, apigenin, catechins, myricetin, biochanin A, baicalein, quercetin, phenolic acids, vanillic acid, akuammidine, are found to be responsible for the antibacterial and antimicrobial activity of *T. procumbens* plant leaves (Ikewuchi et al., 2015).

Many studies have proved the antibacterial and antifungal potential of *T. procumben*s against human skin pathogens. According to Policegoudra et al., (2014) the methanolic extract has shown high antifungal activity against human skin pathogens like *Microsporum fulvum, M. gypseum, Trichophyton mentagrophytes, T. rubrum, Trichosporon beigelii.* Bharathi et al. (2012) reported the significant antibacterial activity of *T. procumbens* against *Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi and Escherichia coli*. According to Jain et al. (2014), methanolic extract of *T. procumbens* leaf exhibited a significant zone of inhibition against both *Fusarium oxysporum* and *Trichoderma reesei.* Kale & Dhake, (2013) investigated the antimicrobial activity of *T. procumbens* against five bacterial pathogens *S. aureus, E.*

coli, K. pneumoniae, Proteus vulgaris and *Pseudomonas aeruginosa* and reported that the methanolic extract had shown effective antimicrobial activity.

T. procumbens also exhibited anti-malarial activity when tested against chloroquine resistant *Plasmodium falciparum* using 3H – hypoxanthine assay. The results show that along with RBC protective effects the extracts of *T. procumbens* also inhibited the growth of the chloroquine resistant *P. falciparum* parasites (Appiah-Opong et al., 2011). The leaves exhibited anti-arthritic activity in complete Freud's adjuvant (CFA) induced arthritis in rats. It reduced the swelling of the rat paws and migration of leukocytes into the inflamed area (Jain et al., 2012). The aerial parts had shown hepatoprotective activity against D-galactosamine/lipopolysaccharide(D-GalN/LPS) induced hepatitis in rats. The hepatoprotective action may be mediated through the inhibition of UDP-sugar derivatives, enhancement of glycoprotein biosynthesis and stabilisation of cell membrane and inhibition of lipid accumulation by its hypolipidemic property. The hepatoprotective property of the extract may be attributed to the presence of flavonoids (Vilwanathan et al., 2005). The ethanolic extract of leaves had shown wound healing properties in streptozotocin induced diabetic and non- diabetic laboratory animals. In the excision model, animals treated with 2.5 % and 5% w/w plant extract showed significant results in wound contraction, epithelization period, and wound index (Shrivastav et al., 2020). The hydro alcoholic extract of *T. procumbens* exhibited cardioprotective activity against isoproterenol induced myocardial infarcted rats (Vadivelan et al., 2004). The acetone and ethanol extract of *T. procumbens* show anti-cancer activity on A549 (human lung cancer cell line), Hep G2 (human liver carcinoma cell line) when tested using 3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, and trypan blue dye exclusion assay method (Vishnu Priya & Srinivasa Rao, 2015). The *T. procumbens* also exhibited immunomodulatory activity (Tiwari et al., 2004), anti-hyperuricemia and antioxidant (Andriana et al., 2019). The leaves exhibited anti-inflammatory and analgesic activity when tested with formalin, acetic acid and CFA induced pain models in male C57BL6/J mice and male Sprague-Dawley rats (Prabhu et al., 2011).

Tridax procumbens leaves have good antimicrobial properties. This antimicrobial potential may help in treating the skin infections caused by certain types of microorganisms. So this study involves formulation and evaluation of herbal soap using *Tridax procumbens* leaves methanolic extract.

Methodology

Collection of Materials

The *T. procumbens* plants were collected from the Herbal Garden of Sri Krishnadevaraya University College of Pharmaceutical Sciences, Ananthapuramu, Andhra Pradesh, India. The collected plant was authenticated as *T. procumbens* (herbarium number 57417) by Botanist Prof B. Ravi Prasad Rao. The leaves were separated from the plant material. The collected leaves were washed with water and shade dried for 5 days and ground into a fine powder using a mixer grinder. The glycerin soap base is brought from Ghanshyam enterprises. Vitamin E oil is purchased from a local pharmacy. Coconut oil, Tulasi oil, and honey of different brands were purchased from the local market.

Preparation of Plant Extract

The dried leaf powder was used for the extraction of phytoconstituents. The powdered plant leaves were stored in an airtight container and the powder was extracted using methanol as solvent by the Soxhlet extraction method. *T. procumbens* dried leaf powder is weighed accurately and packed in a filter paper. The solid matrix is kept in the Soxhlet evaporator and the solvent is heated in the process of reflux. To 40 g of dried leaf powder 400 ml of methanol is used in the extraction process. Continuous extraction was done and solvent was transferred into the reservoir from the chamber. This process is continued for 8hrs and the extract is collected and concentrated using a hot water bath. Final concentrated extract is used in the formulation.

Formulation of Herbal Soap

The glassware is sterilised by dry heat sterilisation technique. 100g of glycerin soap base was weighed and melted. The glycerin soap base is prepared using coconut oil and sodium hydroxide, it is alcohol-free. In another beaker plant extract (as per formulation design), vitamin E oil and other ingredients aloe vera gel, coconut oil, honey (as per the formulation design) were mixed until all the ingredients dissolve completely. 1ml of Tulasi essential oil is added for the fragrance to the mixture. Finally, the plant extract mixture is incorporated into the melted soap base. This mixture is poured into moulds and allowed to solidify at room temperature. Four formulations were prepared. The formulation design for soaps were given in Table 1.

Phytochemical analysis of plant extract

1. Test for Flavonoids

Alkaline reagent test: Few drops of 10 % NaOH solution were added to 2 - 3 mL of extract in a test tube. Formation of intense yellow colour that becomes colourless in addition to dilute HCl indicates presence of flavonoids (Shah & Hossain, 2014).

Table 1: Formulation designs for soaps.

Ingredients	F1	F2	F3	F4
Plant extract	0.5g	1.0g	1.5g	2.0g
Soap base	100g	100g	100g	100g
Vitamin E	400mg	400mg	400mg	400mg
Tulasi oil	0.1 ml	0.1ml	0.1 ml	0.1 ml
Aloe vera gel	-	10g	-	-
Honey	5g	-	-	-
Coconut oil	-	-	-	10g
Total weight	105.9g	111.4g	101.9g	112.4g

(- means that the ingredient is not used in the formulation)

2. Test for Phenols

0.5 mL of alcoholic Ferric chloride ($FeCl_3$) solution was added to 2 mL of extract. Formation of intense bluish black colour in addition of $FeCl_3$ solution indicates presence of Phenols (Kushwaha et al., 2019).

3. Test for Tannins

Gelatin test: Gelatin solution was prepared by dissolving gelatin powder in water by heating using a water bath. To this gelatin solution 2 mL extract was added. Presence of tannins is indicated by formation of white precipitate (Kushwaha et al., 2019).

4. Test for Alkaloids

Iodine test: Few drops of dilute Iodine solution is added into 3 ml of test solution. Formation of blue colour which disappears on boiling and reappears on cooling indicates presence of alkaloids (Kushwaha et al., 2019).

5. Test for Saponins

Foam test: The extract was diluted with 20 ml of distilled water and shaken for 15 min in a graduated cylinder. Formation of foam layer indicates presence of saponins (Hossain et al., 2013).

6. Test for Steroids

2 ml acetic anhydride was added to 0.5 g of methanol extract. To this 2 ml H2SO4 was added. Colour change from violet to blue indicates presence of steroids (Kushwaha et al., 2019).

Evaluation tests

1. Examination of physical properties of formulated soap

Colour and clarity were checked by eye against white background and odour is observed. These properties were examined in all the four formulations

2. Determination of pH

5 g of soap is dissolved in 100 mL of water. The pH of the soap solution was determined using a digital pH metre (Systronics Digital pH metre MK VI). pH for four formulations was determined separately.

3. Determination of percentage free alkali

10g of sample soap was weighed using digital weighing balance (Essae Weighing balance, model- DS-852G) and taken into a beaker, 150 ml of purified water was added and boiled for 30 minutes under reflux in a water bath (SISCO water bath). The volume was made up to 250 ml in a beaker. 1ml of phenolphthalein indicator was added. It was titrated immediately with 0.1 M HCl until the solution turns colourless (Mohammed Haneefa et al., 2019).

4. Determination of foam height

0.5 g soap sample was dispersed in 25 ml purified water. It is transmitted into a 100 ml measuring cylinder and volume was made up to 50 ml with water. 25 strokes were given. It is allowed to stand till the aqueous volume is measured up to 50 ml. Foam height above the aqueous volume was measured (Ahmed et al., 2021).

5. Determination of foam retention

1% soap solution was prepared. 25 ml of 1% soap solution was taken in a 100 ml graduated measuring cylinder. The cylinder was covered and shaken 10 times. The time taken for the foam to disappear was recorded (Ahmed et al., 2021).

6. Determination of alcohol insoluble matter

Alcohol insoluble matter comprises most of the alkaline salts, such as talc, carbonates, borates, silicates and phosphates, as well as sulphates and starch, which are insoluble in alcohol under the test conditions. 5g of soap

sample was taken in a conical flask to which 50 ml of warm ethanol was added and it was shaken vigorously until the sample was dissolved completely. The solution was filtered through a tared filter paper along with 20 ml warm ethanol and dried at 1050 C for 1 hour. The weight of the dried paper was noted. (Mohammed Haneefa et al., 2019).

$$\% \textbf{ Alcohol insoluble matter} = \frac{\textbf{Weight of residue} \times \textbf{100}}{\textbf{Weight of sample}} \quad (1)$$

6. Moisture content

The moisture content is used to estimate the percentage of water present in the soap. To estimate the moisture content 5g of soap was weighed and noted as wet weight or initial weight. Using a hot air oven sample was dried at 100 to 1150C for one hour. The sample was cooled, weighed. This weight is recorded as the dry weight of the sample. Moisture content was determined using the below formula. (Mohammed Haneefa et al., 2019).

$$\% \textbf{ moisture content} = \frac{\textbf{Initial} - \textbf{Final weight}}{\textbf{Final weight} \times \textbf{100}} \quad (2)$$

Results

The formulated soaps were examined for physical parameters like colour, clarity, and odour. Evaluation parameters like pH, percentage free alkali, foam height, foam retention, and alcohol insoluble matter, Percentage of moisture content. The results for phytochemical analysis were given in Table 2, while the results of evaluation studies for the four formulations F1, F2, F3, F4 are given in Table 3.

Table 2: Phytochemical analysis of *T. procumbens*

Phytochemical analysis	Observation	Results
Test for Flavonoids (Alkaline reagent test)	Dark yellow colour was formed	+
Test for Phenol	Bluish black colour was observed	+
Test for Tannins (Gelatin Test)	White precipitate was formed	+
Test for Alkaloids (Iodine test)	Blue colour is observed and disappeared on heating	+
Test for Saponins (Foam test)	Foam was generated on shaking	+

Table 3: Evaluation parameters for formulations

Evaluation parameters	F1	F2	F3	F4
Colour	Dark green #006400	Dark green #006400	Dark green #006400	Dark green #006400
Clarity	Good	Good	Good	Good
Odour	Tulasi odour	Tulasi odour	Tulasi odour	Tulasi odour
pH	8.9	8.7	9.3	8.19
Percentage free alkali	0.68%	1.28%	1%	0.88%
Foam height	35ml	36ml	53ml	5ml
Foam retention time	13 min	100min	110 min	33 min
% alcohol insoluble matter	4%	16%	9.5%	10%
Moisture content	18%	28.4%	18.8%	23%

Discussion

The methanolic extract of *T. procumbens* consists of saponins, alkaloids, tannins, phenols, flavonoids and steroids. All the soaps have a dark green colour, good clarity, and good odour. All the soaps have pH in the range of 8-9.1. So the soaps are a little basic in nature. Percentage free alkali refers to the free or excess unreacted base (alkali) present in the formulation. These free bases cause irritation on human skin. Among four soaps F2 has a high percentage of free alkali, F1, F3 and F4 are within the range of 0.6 - 1. F1 soap has the least percentage of free alkali i.e, 0.68% when compared to other formulations. Matter insoluble in alcohol is a parameter used to determine the purity of soap (Vivian et al., 2014). According to the Bureau of Indian standards, 10% of

alcohol insoluble matter is the limit for toilet soaps of grade 2 and grade 3 (IS 2888: 2004). F1, F3, F4 have 4%, 9.5%, 10% respectively. Among all four soaps F1 has the least amount of alcohol insoluble matter i,e 4 % indicating it has the least number of impurities in it than other formulations. Moisture content indicates the presence of moisture in the sample; if it is high the formulation will get deteriorated easily. High moisture content means it releases water, which reacts with unsaponified fat in the soap and causes hydrolysis of soap releasing free fatty acids and glycerol (Vivian et al., 2014). Among the four soaps F1, F3 have low moisture content compared to F2 and F4. Foam height and foam retention time determine the foaming activity and cleaning efficacy of the soap. Among the four formulations F1, F2, F3 showed good foam heights in the range of 35 to 53 ml. All four soaps had a relatively long period of foam retention. F3 soap has foam for a longer period i.e, 110 minutes. All the four soaps showed stable foam for more than 5 minutes. All four samples had shown good foaming properties.

Conclusion

In this present study, four herbal soaps were prepared using *Tridax procumbens* leaves extract with varying quantities of ingredients and drug. All the formulated soaps had good appearance, uniform colour, good odour. F1 has the least content of free alkali (0.68%). As free bases in soap cause irritation, these should be as low as possible. When compared to other soaps F1 has least making it good when compared to other soaps. It has least amount of matter insoluble in alcohol (4%), which is very less compared to other formulations and it is also in the acceptable limit (10%) of BIS. The moisture content of the F1 soap is also less compared to other soaps, which is another parameter, which makes it better than others. F1 had shown stable foam for more than 5 min. F3 has shown stable foam than other formulations. But based on these considerations it can be concluded that F1 soap had shown better results than other soaps. Naturally *Tridax procumbens* leaves have good antimicrobial properties. The other ingredients like coconut oil, aloe vera gel, Vitamin E oil, Tulasi oil used were proven to be dermatologically safe and helps in providing additional benefits to skin like moisturising effect, and conditions skin. So, the potential use of the formulated soaps in treating skin infections can further be explored. Herbal drugs like *T. procumbens* can be formulated in the form of soaps.

But F1 had shown the best results among all for evaluation studies. It has good foaming property and the least number of impurities as per the standards of the Bureau of Indian Standards. The potential use of the formulated soaps in treating skin infections can further be explored. Herbal drugs like *T. procumbens* can be formulated in the form of toilet soaps.

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Conflict of Interest

The authors declare no conflict of interest.

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Journal of Pharmacy

ORIGINAL ARTICLE

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Spectrophotometric simultaneous analytical method validation to determine isoniazid and pyridoxine in pure and 3D printed tablet forms

Nur Suhaila Sudarman[1], Muhammad Salahuddin Haris[1,2,*]

ABSTRACT

Introduction: Isoniazid (INH) is the anti-tuberculosis drugs being used to counter tuberculosis since 1952. Patients on INH should be given daily prophylactic pyridoxine (PYR) with 10-50 mg/day to prevent the development of isoniazid-induced neuropathy. Within the framework of this research, the UV-Vis spectrophotometer is used to quantify simultaneously the drug content of INH and PYR. **Methods:** The standard curve for both INH and PYR were plotted using the concentration of 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml, and 30 µg/ml and tablets were analysed using simultaneous equation method. The proposed method was validated by analytical method validation for the linearity, specificity, accuracy, intermediate precision, limit of detection (LOD), and limit of quantification (LOQ). **Results:** A regression equation of INH standard and sample were found to be y = 0.0279x + 0.0637 and y = 0.0280x + 0.0522 obtained from the calibration curve and linear with correlation coefficient (R2) values of 0.9950 and 0.9964, respectively. A regression equation of PYR standard and sample were y= 0.0267x + 0.0723 and y = 0.0259x + 0.0806 and to be linear with R2 values of 0.9981 and 0.9962, respectively. The result of accuracy obeyed the accepted criteria of percentage recovery in between 98% to 102%. The method exhibited intermediate precision as demonstrated by relative standard deviation <2%. The LOD and LOQ of INH were 0.166 µg/ml and 0.5018 µg/ml while the LOD and LOQ of PYR were 0.122 µg/mL and 0.371 µg/mL, respectively in the pure form. In tablet dosage form, the LOD and LOQ of INH were 0.071 µg/ml and 0.215 µg/m while LOD and LOQ of PYR give the result 0.124 µg/ml and 0.375 µg/ml, respectively. **Conclusion:** This spectrophotometric simultaneous analytical method validation for INH and PYR was successfully conducted with the notion to spearhead the development of INH and PYR in a single dosage form to improve compliance among tuberculosis patients.

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***Corresponding author:**

Email address: solah@iium.edu.my

Authors' Affiliation:

[1] *Department of Pharmaceutical Technology, Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Malaysia.*

[2] *IKOP Pharma Sdn. Bhd., Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*

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Introduction

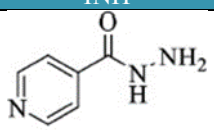
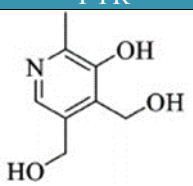
Isoniazid (INH) is a highly effective treatment for *M. tuberculosis* that the World Health Organization recommends (WHO). It serves as the main ingredient of several fixed-dose combination tablets, each of which contains two or more anti-TB drugs and has been in use since 1952 to treat tuberculosis. In addition to extending the tablet's shelf life, antioxidants and INH cocrystals may lessen oxidative stress in TB patients receiving therapy (Mashhadi et al., 2021). Pyridoxine (PYR) species are immediately inactivated by INH metabolites. In people with high-risk conditions, PYR deficiency can result in neurologic adverse effects such peripheral neuropathy. The Clinical Practice Guidelines state that daily prophylactic pyridoxine (vitamin B6) administration to INH patients with 10 to 50 mg/day is recommended to avoid the onset of isoniazid-induced neuropathy.

Pyridoxine (PYR) is a water-soluble vitamin that aids in the metabolism of carbohydrates, lipids, and amino acids. This vitamin has a significant impact on the metabolism of nitrogen-containing compounds such serotonin, dopamine, norepinephrine, gamma-aminobutyric acid (GABA), and the component of haemoglobin. In addition to encouraging the development of red blood cells, pyridoxine aids in the balance of salt and potassium. Table 1 shows the physicochemical properties of both INH and PYR, respectively (Wishart et al., 2018).

The UV-Vis spectrophotometer's fundamental principle is the absorption of light by a sample. When utilising a UV-Vis spectrophotometer, the purity of the INH and PYR samples may be measured based on how much light and its wavelength are absorbed by the samples. A sample solution is placed in a cuvette, and an Ultraviolet-visible (UV-Vis) spectrophotometer analyses the light's intensity as it passes through the solution and compares it to the light's intensity before the sample. A UV-Vis spectrophotometer's primary components are a light source, a sample holder, a dispersive device to separate the light's various wavelengths, and an appropriate detector. The visual depiction of the UV-Vis spectrum in general is the absorbance as a function of wavelength.

Within the framework of this research, the UV-Vis spectrophotometer is used to quantify simultaneously the drug content in the pure form of INH and PYR by measuring all the absorbance values for each concentration at determined wavelengths which are 263 nm of INH and 290 nm of PYR then will be calculated in the simultaneous equation method. According to Beer's Lambert law, it states that absorbance is proportional to concentration. So, this research study uses two types of modes of the UV-Vis spectrophotometer. The first one is the photometric mode. The photometric mode can help define the known wavelengths of INH and PYR by measuring the absorbance at a single wavelength or at multiple wavelengths. The second type is a spectrum mode. The spectrum mode may obtain sample spectra using wavelength scanning thus resulting in a peak wavelength of each INH and PYR as required. The UV-Vis spectrophotometer interprets the data analysis to provide the necessary information and can subsequently obtain the results.

Table 1: Physicochemical properties of INH and PYR

Properties	INH	PYR
Chemical structure		
Chemical name	Pyridine-4-carbohydrazid	4,5-bis(hydroxymethyl)-2-methylpyridin-3-ol
Molecular formula	$C_6H_7N_3O$	$C_8H_{11}NO_3$
Molecular weight	137.14 g/mol	169.18 g/mol
Melting point	171.4 °C	159 °C to 162 °C
Solubility **(at** 25 **°C)**	1.4 x 10^5 mg/L	2.2 x 10^5 mg/L
Log P	-0.70	-0.77
Half-life	0.5 to 1.6 hours for fast acetylators 2 to 5 hours for slow acetylators	15 to 20 days

Materials

INH standard (99.7% purity), PYR standard (99.9% purity), INH and PYR analytical grade powders were purchased from Sigma-Aldrich (Darmstadt, Germany). INH 300 mg 3D printed tablet and PYR 10 mg tablet were used for analysis purpose. Distilled water was used as a solvent in this experiment.

Methodology

1. Preparation of standard solution

Accurately 10 mg of INH and 10 mg of PYR standard were separately transferred into individual 100 ml volumetric flasks, then dissolved appropriately with distilled water and diluted up to the mark with distilled water to give solutions containing 100 µg/ml of INH and 100 µg/ml of PYR (Figure 1).

2. Preparation of calibration curve

The calibration curve was prepared by using the stock solution to produce six different concentrations of INH and PYR standard which are 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml, and 30 µg/ml (Figure 1). The absorbance of each concentration was acquired at the λ max using a fixed wavelength measurement mode. The calibration curve representing concentration versus absorbance was plotted.

3. Determination of Wavelength of Maximum Absorbance (λ max)

A solution containing 15 µg/ml of INH and 15 µg/ml of PYR was scanned separately using full output mode with medium scanning speed for a whole range of dual wavelengths by using a UV-Visible spectrophotometer (Shimadzu, Kyoto, Japan) ranging from 400 – 200 nm with distilled water as blank. After acquiring the spectrum, the maximum absorbance was identified.

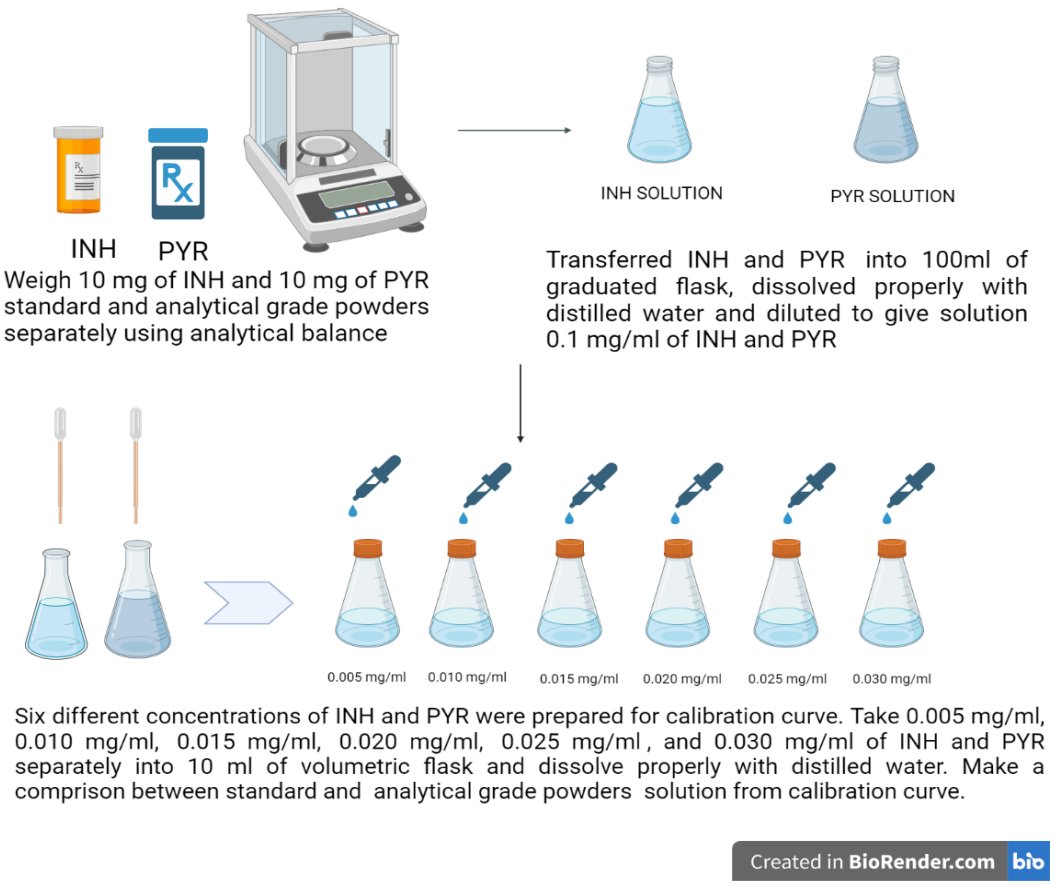


Figure 1. Preparation of stock and calibration curve solutions.

4. Simultaneous equation method

This method of analysis is based on the absorption of INH and PYR at the wavelength maximum of each other (Tilinca et al., 2017). Two wavelengths selected for the development of simultaneous equations were 263 nm and 290 nm which were lambda maximum of INH and PYR respectively. The absorbances of INH and PYR measured at selected wavelengths (Tilinca et al., 2017). Absorptivity values were calculated. The concentrations of both the drugs in mixture can be calculated by using following equations 1 and 2:

$$Cx = \frac{A_2 ay_{1-} A_1 ay_2}{ax_2 ay_{1-} ax_1 ay_2} \quad \text{Eq. 1}$$

$$Cy = \frac{A_1 ax_{2-} A_2 ax_1}{ax_2 ay_{1-} ax_1 ay_2} \quad \text{Eq. 2}$$

Where, A_1 and A_2 are absorbances of mixture at 263 nm and 290 nm, respectively.

ax_1 and ax_2 are the absorptivity of INH at 263 nm and 290 nm, respectively.

ay_1 and ay_2 are the absorptivity of PYR at 263 nm and 290 nm, respectively.

Cx and Cy are concentrations of INH and PYR, respectively.

5. Application of the proposed method for the determination of INH and PYR in tablets

The 3D-printed tablet containing 300 mg INH and 10 mg PYR was analysed by this method. An amount equivalent to 10 mg INH and 10 mg PYR of the selected tablet was weighed and dissolved in 100 ml distilled water to obtain a stock solution containing 100 µg/ml standard solution. The solution was then filtered through Whatman filter paper. INH and PYR were diluted appropriately. The absorbance of the resulting solutions was measured at 263 nm and 290 nm. The concentration of INH and PYR in the sample solution was calculated using the equation constructed from the calibration curve of each drug.Values were substituted in the respective formula to obtain concentrations.

6. Analytical method validation (AMV)

The main objective of performing analytical method validation is to demonstrate that the analytical method which is a UV-Vis spectrophotometer is suitable and adequate for its intended purpose (Patil, Patil, Chalikwar, Surana, & Firke, 2019). The validation of the developed method was carried out in terms of specificity, linearity, accuracy, precision, intermediate precision, the limit of detection (LOD), and limit of quantification (LOQ). It was validated according to the International Conference on Harmonization guidelines.

6.1 Specificity

Specificity is its ability to detect and differentiate the analyte of interest in the presence of other substances, including its related substances to guarantee character of an analyte (Patil et al., 2019). The specificity of the direct spectrophotometric method was assessed by comparing the spectrum obtained from the solvent system alone (placebo), which is distilled water, and of standard INH, PYR solution in the diluent.

6.2 Linearity and Standard Curve

In order to find the line that best fits a provided set of data, the linearity was established. This allowed for a visual representation of the relationship between the data points (Patil et al., 2019). The linearity of this method was established using six different calibration standards. Standard INH and PYR were tested at six known concentrations using a pre-determined wavelength. Every concentration's absorbance was recorded. The linearity was determined by plotting six concentrations (x-axis) of INH and PYR standard and sample against absorbance (y-axis). The equation of $Y = mX + C$ and the R^2 was developed.

6.3 Accuracy

The degree to which test results agree with the genuine value, or how closely the outcomes of the method correspond with the true value, is known as accuracy. In order to minimise potential operating errors, it is often established on samples of the material to be analysed that have been produced with quantitative accuracy. (Patil et al., 2019). Accuracy should be established across the specified range of the analytical procedure. To ascertain the accuracy of the proposed methods, recovery studies were carried at three different levels which are 80%, 100% and 120% were subjected to the determined wavelength (nm) in which 263 nm of INH and 290 nm of PYR. The percentage recovery should be in between 98% and 102% to meet the acceptance criteria.

6.4 Intermediate precision

Precision is how close individual measurements are to each other. Intermediate precision is a part of precision in which the method is tested on multiple days, instruments, and analysts to measure of the ruggedness of the method's reliability when performed in different environments (Patil et al., 2019). The intraday precision of INH and PYR was checked by assay the sample solution on same day at an interval of one hour for three hours and interday precision was carried out by estimating the correspondence responses on three different days with different preparations. According to this study, the solutions may be

analysed within 48–72 hours without negatively affecting the drug's chemical stability when urea is present. The wavelength was applied to each concentration in triplicates, and the mean and standard deviation were then calculated. To achieve the acceptance standards, the accuracy percentage of the relative standard deviation (RSD) value must be less than 2.0%.

6.5 Limit of detection (LOD) and limit of quantification (LOQ)

The lowest concentration of an analyte in a sample that can be identified and measured with suitable precision and accuracy under the specified test conditions was used for the evaluation of LOD and LOQ. The following equations 3 and 4 describe the precise calculations to estimate LOD and LOQ, respectively:

$$LOD = (3.3 \times SD)/m \qquad \text{Eq. 3}$$
$$LOQ = (10.0 \times SD)/m \qquad \text{Eq. 4}$$

SD or standard deviation in the formula was referring to the standard deviation of the absorbance values of the blank and m is the slope of the standard curve constructed previously (Ismail et al., 2016). All readings for LOD and LOQ were conducted in triplicates.

Results

1. Specificity

The identification of wavelength with maximum absorbance is needed for quantitative UV analysis. The specificity should not be tested without any blank or matrix spectrum because it does not give any reading or specified wavelength of drug content. The standard solution of INH and PYR with concentration of 15 µg/mL-1 was separately scanned in the range of 200-400 nm. The result showed that the λ_{max} was determined for each drug. The λ_{max} INH and PYR were found to be 263 nm with the absorbance is 0.519 and 290 nm with the absorbance is 0.422, respectively as shown in Figure 2a and Figure 2b. After scanned both drugs separately, then overlap the spectras and obtained the isosbestic wavelength at 280 nm as λ_{max} of common absorbance as shown in Figure 2c. Isosbestic wavelength is used when two substances of equimolar concentration show the same absorbance at particular wavelength and by using isosbestic wavelength it may record the absorbance of formulation or multi wavelength photometric mode of UV-Vis spectrophotometer.

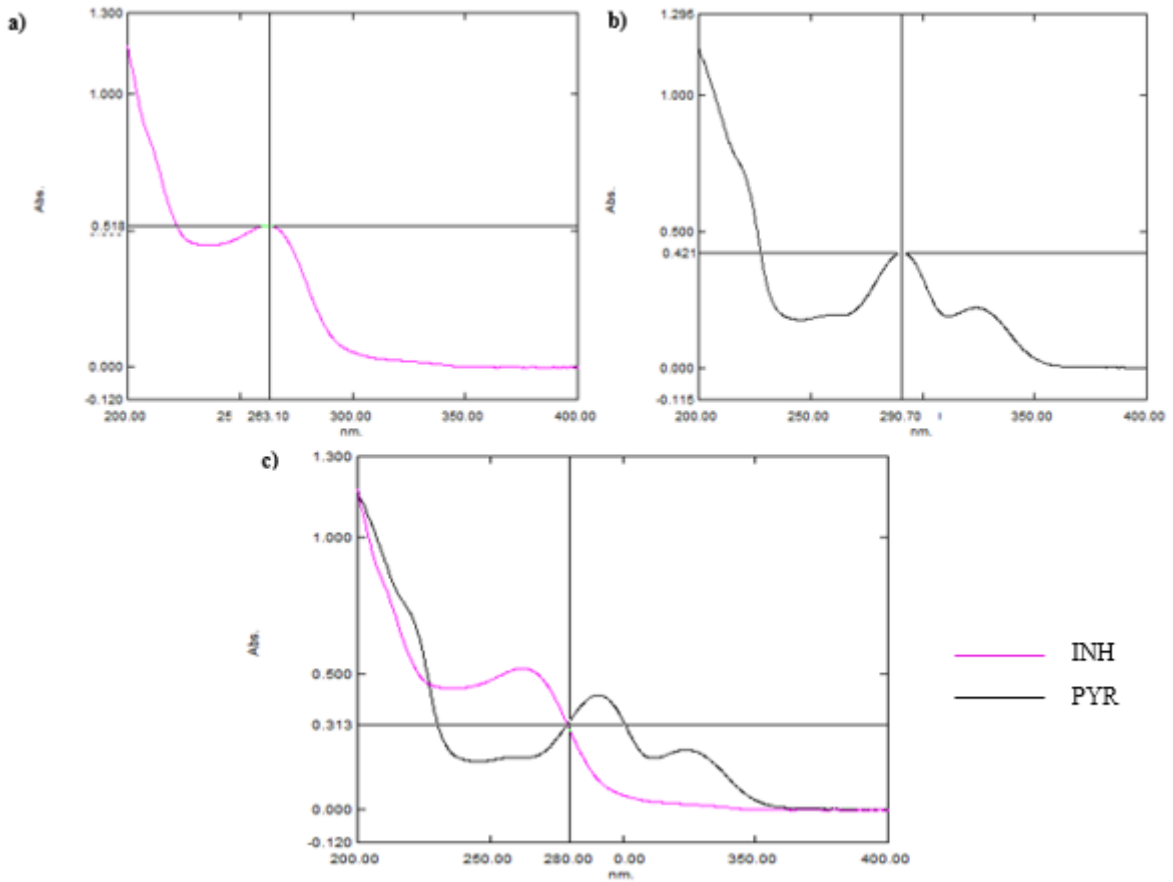


Figure 2. a) UV–Vis spectra of standard INH (λ:263 nm). and PYR (λ:290 nm). c) UV-Vis overlaid spectra of both standards (Isosbestic λ:280 nm).

2. Linearity and calibration curve

The linearity was confirmed using the absorbance values at a constant set wavelength, which is 263 nm, and the direct percentage relationship between the concentration of standard INH and sample INH. In the meantime, the linearity was established using the absorbance values at a set established wavelength, which is 290 nm, and the direct percentage relation between the concentration of standard PYR and sample PYR. Six known concentrations of both standard and sample of INH and PYR were prepared namely are 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml, and 30 µg/ml were subjected to 263 nm and 290 nm for INH and PYR to get the absorbance values for each sample. The calibration of the developed UV-Vis spectrophotometer method was in linear form with the equation and the R2 of standard INH is y = 0.0279x + 0.0637 and 0.9950 respectively while the equation and the R2 of standard PYR is y = 0.0267x + 0.0723 and 0.9981 respectively. In addition, the calibration of the developed UV-Vis spectrophotometer method was in linear form with the equation and the R2 of sample INH is y = 0.0280x + 0.0522 and 0.9964 respectively while the equation and the R2 of sample PYR is y = 0.0259x + 0.0806 and 0.9962, respectively.

3. Accuracy

In pure form, to ascertain the accuracy of the proposed methods, recovery studies were carried at three different levels which are 80%, 100% and 120% were subjected to the determined wavelength (nm) in which 263 nm of INH and 290 nm of PYR. Three samples were prepared for each level, and their absorbance was quantified using a UV-Vis spectrophotometer. The concentrations of INH and PYR in the sample solution were determined using an equation derived from the calibration curves of the respective drugs. To acquire concentrations, absorbance values were substituted in the applicable formula. Following that, the concentration was subtracted with 6 ppm, which is stated in the linearity of standard curve for the six concentrations created, and the result was represented as the obtained value. The actual value was determined using the cross-multiplication approach based on the different levels (80%, 100%, 120%), and the actual value was the fixed value for both INH and PYR. Then, the percentage recovery calculation was followed by using Eq. 5.

$$\% \text{ Recovery} = \frac{\textbf{Obtain value}}{\textbf{Actual value}} \times \mathbf{100} \qquad \text{Eq. 5}$$

Futhermore, in 3D printed tablet dosage form, the accuracy was developed by prepared six samples for each different levels which are 80%, 100%, and 120% and the absorbance was recorded to obtained the concentration of the sample. The simultaneous equation method was utilised by applying the absorbance and absorptivity values in the calculation in order to get the true concentration and then dividing it with the initial concentration. Next, it was multiplied by 100 and the result represents the percentage recovery. Overall, the percentage recovery should be in between 98% and 102% to meet the acceptance criteria, and all these accuracy results of INH and PYR for both pure form and 3D printed tablet dosage form were tabulated in Table 2 and Table 3, respectively.

Table 2: Results of accuracy for the simultaneous determination of INH and PYR in pure form

Drug	Level of accuracy	Recovery (%)	RSD (%)
INH	80	98.13	0.17
	100	98.17	0.16
	120	99.17	0.36
	80	98.13	0.17
	100	98.17	0.16
	120	99.17	0.36
	80	98.13	0.17
	100	98.17	0.16
	120	99.17	0.36
PYR	80	99.74	0.56
	100	100.33	0.15
	120	102.36	0.23
	80	99.74	0.56
	100	100.33	0.15
	120	102.36	0.23
	80	99.74	0.56
	100	100.33	0.15
	120	102.36	0.23

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Table 3: Results of accuracy for the simultaneous determination of INH and PYR in 3D printed tablet dosage form

Drug	Label claim	Sample (mg)	Actual (mg)	Accuracy (%)	SD	RSD (%)
INH	80	8	8.16	101.96	0.0010	0.40
	100	10	9.91	99.00	0.0030	1.17
	120	12	11.86	98.83	0.0006	0.27
	80	8	8.16	101.96	0.0010	0.40
	100	10	9.91	99.00	0.0030	1.17
	120	12	11.86	98.83	0.0006	0.27
	80	8	8.16	101.96	0.0010	0.40
	100	10	9.91	99.00	0.0030	1.17
	120	12	11.86	98.83	0.0006	0.27
PYR	80	4	4.02	100.52	0.0010	0.14
	100	5	4.97	99.50	0.0017	0.23
	120	6	5.99	99.87	0.0020	0.42
	80	4	4.02	100.52	0.0010	0.14
	100	5	4.97	99.50	0.0017	0.23
	120	6	5.99	99.87	0.0020	0.42
	80	4	4.02	100.52	0.0010	0.14
	100	5	4.97	99.50	0.0017	0.23
	120	6	5.99	99.87	0.0020	0.42

4. Immediate Precision

The intraday precision study of INH and PYR in pure form was carried out by estimating the correspondence responses three times on the same day with concentrations of 5 g/ml, 10 g/ml, and 15 g/ml, and the interday precision study of INH and PYR was carried out by estimating the correspondence responses three times the next day with different preparations of 5 g/ml, 10 g/ml, and 15 g/ml had been recorded in Table 4. Meanwhile, the method's intermediate precision was assessed in 3D printed tablet dosage form by assaying the sample solution on the same day at one-hour intervals (intraday precision) for three hours and on three distinct days (interday precision), as shown in Table 5. According to this study, the solutions may be analysed within 48-72 hours without affecting the chemical stability of the drug in the presence of urea. Overall, the percentage of RSD for each concentration of simultaneous INH and PYR that was subjected to the specified wavelengths of 263 nm and 290 nm was less than 2%, which passed the acceptance requirements.

5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection represents the lowest concentration of analyte that can be reliably detected. Meanwhile, the limit of quantification represents the lowest concentration of analyte that can be analysed and quantified. The LOD and LOQ of INH were found to be 0.166 µg/ml and 0.5018 µg/ml while the LOD and LOQ of PYR were found to be 0.122 µg/mL and 0.371 µg/ml respectively in pure form as shown in Table 6. Besides that, the LOD and LOQ of INH were obtained to be 0.071 µg/ml and 0.215 µg/ml respectively but however, the LOD and LOQ of PYR were obtained to be 0.124 µg/ml and 0.375 µg/ml respectively in 3D printed tablet dosage form as presented also in Table 6.

6. Assay of 3D printed tablet dosage form

The optimized method was successfully applied for the simultaneous determination of INH and PYR in the 3D printed tablet dosage forms, containing 300 mg INH and 10 mg PYR. Six samples were tested using a UV-Vis spectrophotometer and the absorbance of each sample was measured in triplicate at specific wavelengths of 263 nm and 290 nm. The mean absorbance and absorptivity of the samples were used and the amount found of tablets was calculated using the simultaneous equation method. Satisfactory results were obtained for each compound as the found amounts were in good agreement with the amount taken as indicated in Table 7.

Table 4: Results of intraday and interday precision for the simultaneous quantification of INH and PYR in pure form

Analyte (µg/ml)	SD		RSD (%)	
Intraday (n=6)	INH	PYR	INH	PYR
5	0.0006	0.0006	0.29	0.22
10	0.0010	0.0020	0.31	0.50
15	0.0006	0.0006	0.13	0.13
Interday (n=18)	INH	PYR	INH	PYR
5	0.0030	0.0006	0.37	0.31
10	0.0030	0.0006	0.54	0.26
15	0.0006	0.0010	0.07	0.33

Table 5: Results of intraday and interday precision for the simultaneous quantification of INH and PYR in 3D printed tablet dosage form

Parameters	Drug	Label claim	Sample (mg)	Actual (mg)	SD	RSD (%)
Intraday (n=3)	INH	300	10	9.91	0.0030	1.17
	PYR	10	5	4.97	0.0017	0.23
Interday (n=3)	INH	300	10	10.11	0.003	0.56
	PYR	100	5	4.92	0.004	0.65

Table 6: LOD and LOQ data of the UV-Vis spectrophotometer method for the simultaneous determination of INH and PYR in pure and 3D printed tablet forms.

Drug	Type	LOD (µg/mL)	LOQ (µg/mL)
INH	Pure	0.1660	0.5018
	3D-printed tablet	0.0710	0.2150
PYR	Pure	0.1220	0.3710
	3D-printed tablet	0.1240	0.3750

Table 7: Assay results of INH and PYR determination in tablet dosage form

Drug	Label claim	Sample (mg)	Actual (mg)	Accuracy (%)	SD	RSD (%)
INH	300	10	9.78	98.0	0.0006	0.61
PYR	10	10	10.01	100.1	0.0010	0.23

Discussion

The proposed method that used to determine simultaneous of INH and PYR in this study was the simultaneous equation method or also known as Vierordt's method. The importance of the study of the simultaneous equation method was that it allows the analysis of multicomponent drugs using different analytical techniques such as spectrophotometer, chromatography and electrophoresis. However, this study focuses more on the use of UV-Vis spectrophotometer because it is an applicable method and most scientific work has been done using this technique.

The reason why the author chose Vierord's method is that it has many advantages. For instance, this method can save time and cost effective since the absorption measurement were obtained with ease, the process was fast, and simple. Vierordt's method also had its shortcoming such as the lambda max of two drugs should be reasonable different, there must no chemical interaction between the absorbing components, and they should obey Beers law at their wavelength maximum if used in UV-Vis spectrophotometer.

Based on the result, all the validation parameters that validate the proposed method showed it was specific, linear, accurate, precise, and sensitive. It could be consistent with the other published article, where the author (Tilinca et al., 2017) also obtained the same result in the simultaneous determination of INH and rifampicin (RIF) using the same method. The assay part indicated that found amount of INH (148.84 mg) and

RIF (297.68 mg) were in good agreement with the declared amount of INH (150 mg) and RIF (300 mg). The correlation coefficient (R2) was more than 0.99, the specificity showed that 263 nm (λ_{max} for INH) and 338 nm (λ_{max} for RIF), percentage recovery in the range of 98%-102%, the RSD of an intermediate precision also less than 2%, and LOD of INH (2.60 µg/ml) and RIF (3.50 µg/ml) were showed always lesser than lowest concentration in the standard curve but the LOQ result of INH was (8.58 µg/ml) and PYR (11.70 µg/ml).

In addition, there had one article entitle simultaneous estimation of Salbutamol sulphate (SAL) and Ambroxol HCl (AMB) from their combined dosage form by UV-Vis spectrophotometer using the simultaneous equation method also discussed the same methods (Panchale, Gulhane, Manwar, & Bakal, 2020). As a result, all the validation parameters proved that the proposed method was specific, linear, accurate, precise, and sensitive. The specificity showed had two different maximum wavelength present which are 242 nm for SAL and 272 nm for AMB. The R2 of the linearity in the calibration curve showed that more than 0.99 and the percentage recovery's result still in the range between 98% and 102%.

Moreover, the RSD of intermediate precision give less than 2% in which meet the acceptance criteria and the LOD and LOQ result were 0.95 µg/ml and 0.18375 µg/ml respectively for both SAL and AMB. Finally, the author recommends in the future, the methods can be employed for routine analysis in simultaneous determination of another combination drugs and also quality control analysis.

Conclusion

In a sample that contains of two absorbing drugs like INH and PYR in which each of them absorbs at a maximum wavelength different from the other, it may be possible to determine both drugs in the pure form and 3D printed tablet dosage form by the technique of simultaneous equation method. To conclude, the described method was validated in accordance with the International Conference on Harmonisation guidelines and give a specific, linear, accurate, precise, and sensitive results for the simultaneous determination of INH and PYR from pure form and 3D printed tablet dosage form. As mentioned in the discussion part, all the results got in this work were acceptable and corresponded with the results of other published articles. Hence, the suggested approach may be directly used to quantify INH and PYR simultaneously.

Conflict of Interest

The authors declare that there is no conflict of interest.

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In vitro antimicrobial assessment of seeds of selected medicinal plants in Sri Lanka

Gowri Rajkumar[1*], Ms. Mihiri Rangika Jayasinghe[1] and Vinotha Sanmugarajah[2]

ABSTRACT

Introduction: People are suffering from many noncommunicable diseases as a result of the COVID pandemic and the stress that has followed after it. Diabetes mellitus is a complex non-communicable disease and its incidence in Sri Lanka is almost high. While a number of antidiabetic medications are available, herbal management of diabetes is encouraged due to its low side effects and efficacy. Examining the antibacterial properties of anti-diabetic plants may be highly valued because, diabetics are chronic immunocompromised individuals who are more prone to microbial infections. To focus this aim, the present *in-vitro* antimicrobial assessment carried out for the seeds of selected four medicinal plants, such as *Syzygium cumini* (L.) Skeels, *Sinapis alba* L., *Trigonella foenum-gra*ecum L. and *Nigella sativa* L. that are commonly used for diabetes management in Sri Lanka.

Materials and methods: Crude ethanol extract from the seeds has been studied for their antibacterial potential against three bacterial strains such as *Enterococcus faecalis*, *Staphylococcus aureus* and *Escherichia coli* by using agar well diffusion method in triplicates. The statistical analysis was performed using a one-way analysis of variance.

Results: The seed extract of *S. cumini* showed the highest value of zone of inhibitions (*E. faecalis*:24.70±037, S. *aureus*:16.15±1.20 and *E. coli*:10.37±1.51 mm) and *S. alba* exhibited the lowest value of zone of inhibition (1.08±2.65, 1.08±2.65, 0 mm) for all selected pathogens respectively and which were comparable to the positive control *streptomycin* (*E. faecalis*:25.45±1.18, *S. aureus*:21.08±0.26 and *E. coli*:19.37±1.35mm).

Conclusion: The result shows that *S. cumini* seed extract poses the highest antimicrobial activity in selected bacteria. Therefore, this seed is potential to be further developed as an herbal antibiotic for the management of infection in diabetes in future.

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***Corresponding author:**

Email address: gowrir@univ.jfn.ac.lk
Phone: +94773604037

Authors' Affiliation:

[1] *Department of Botany, Faculty of Science, University of Jaffna, Jaffna, 40000, Sri Lanka*
[2] *Unit of Siddha Medicine, University of Jaffna, Jaffna, 40000, Sri Lanka.*

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Introduction

The number of people with diabetes is exponentially increasing world-wide and has become an important global public health problem (Global report on diabetes, 2016; Shehab et al., 2022). Sri Lanka, despite being a developing nation, has always maintained health indices on par with most developed countries. Currently, Sri Lanka is in the midst of its worst economic crisis in history (Pathmanathan & Abhayaratna 2022). In Sri Lanka, people are suffering from many noncommunicable diseases as a result of the COVID pandemic and the stress that has followed after it. That condition gets more critical as a result of the country's economic situation. Diabetes mellitus is one of the most common endocrine disorders in Sri Lanka. 10% of the Sri Lankan population and 18% of the urban population are affected by it (Pathmanathan & Abhayaratna 2022). Diabetes mellitus is characterized by hyperglycaemia and is especially classified into two types, Type-I (Insulin dependent diabetes) and type-II diabetes (non-insulin dependent diabetes) (IDF Diabetes Atlas, 2013; AOAC, 2016; American Diabetes Association, 2019; King, 2012). The incidence of diabetes (% of the population aged 20-79) in Sri Lanka was 11.3% in 2021 (The World Bank Group, 2022). This condition may be due to the common risk factors such as genetic, environmental, different life stylishness and physical inactivity (Wu et al., 2014; Arawwawala, 2006) and prevalence of depression (Akter & Latif 2021). There is another type is gestational diabetes which mainly arises during the pregnancy (Buchanan, Xiang & Page, 2012).

The oxidative stress is a recognized pathogenic mechanism in the development and progression of diabetes which reasons owing to augmented free radical production and weakened antioxidant defences (Unuofin & Lebelo, 2020). A study mentioned that the antiglycation properties of herbal extracts and their complexes powerfully interrelated with their antioxidant capacity with that antioxidant and anti-glycation activities are associated strongly with phenol and flavonoid contents (Babich et al., 2022).

Generally, antibacterial actions are facilitated by the immune-modulating and antioxidant capabilities of medicinal plants (Aryal etal., 2021). Antibacterial action is the most significant distinctive of medical textiles, to deliver satisfactory defence against microbes, biological fluids, and infection transmission (Alihosseini, 2016). Prevention of food spoilage and food intoxication pathogens is regularly attained by use of chemical preservers. Plant extracts have been used to control food poisoning diseases and preserve foodstuff (Mostafa et al., 2018). An extensive range of biological constituents as alkaloids, flavonoids, glycosides, terpenoids, phenols, and coumarins have been stated from different parts of the plant, which are responsible for numerous biological activities as well as antimicrobial, antioxidant, and anti-inflammatory properties (Sarkar, Salauddin & Chakraborty, 2020; Phuyal et al.,2020). While a number of antidiabetic medications are available, herbal management of diabetes is encouraged due to its low side effects and efficacy. Examining the antibacterial properties of anti-diabetic plants may be highly valued because, diabetics are chronic immunocompromised individuals who are more prone to microbial infections (Hegazy et al., 2021).

For this purpose, this *in-vitro* antimicrobial assessment was performed for the ethanol extracts of four seeds of medicinal plants namely *Syzygium cumini* (L.) Skeels, *Sinapis alba* L., *Trigonella foenum-gra*ecum L. and *Nigella sativa* L. against three bacterial strains as *E. faecalis, S. aureus* and *E. coli.*

Materials and methods

Chemicals and reagents

Ethanol, streptomycin, distilled water and nutrient agar were purchased from Sigma-Aldrich. All reagents and chemicals were of analytical grade.

Collection of medicinal plants and preparation of the seed extract

The plant materials with seeds of *S. cumini*, *S. alba*, *T. foenum - gra*ecum and *N. sativa* (Table 1) were collected and botanically authenticated by a Curator of the National Herbarium Center, Department of National Botanic Garden, Peradeniya, Sri Lanka.

The fresh seeds were washed in tap water for several times to remove the soil and dust particle. Then they were air dried in thoroughly at room temperature until dried and blended to form a fine powder and stored in airtight containers at room temperature until needed for analysis.

Fifty grams of powered materials of each seed were separately weighed and placed in 500 ml of culture bottles. As much as 150 ml of absolute ethanol was added to it and mixed well. Lid of each bottle were covered with para film. The solution was kept for 5 days with occasional shaking by using shaker at 150 rpm for 15 minutes in every morning and evening. They were filtered through Whatman filter paper No.1. The part of filtered content was concentrated by using rotatory evaporator (BUCHI, Chi Minh City, Vietnam) at 52 ^{0}C (Rajkumar, Jayasinghe & Sanmugarajah, 2021). Crude extracts were kept at 20 ^{0}C for further analysis.

Test microorganisms

Three bacterial strains were provided by the Faculty of Science, University of Jaffna were used for the antimicrobial tests, according to Table 2. All the test strains were preserved on nutrient agar slants at 4 ^{0}C and sub-cultured on to nutrient broth for 24 hours prior to testing. These bacteria served as test pathogens for this assay.

Assay of Antimicrobial activity using Agar well diffusion method

About 22.68 g of Nutrient Agar (NA) powder was dissolved in 810 ml of distilled water. Then 15 ml parts of the NA medium were poured into boiling tubes. Medium which was contained in the boiling tubes were autoclaved at 121 ^{0}C for 20-30 minutes. Then 15 mL of sterilized nutrient agar was mixed with 100 µl of bacterial suspensions inside the laminar air flow chamber. The mixture was stirred well and it was poured into sterile petri dishes separately (Dwivedi et al., 2017). After the solidification the wells were punched over the agar plates using sterile cork-borer (5mm in diameter) and 15 µL of plant extracts were added to the wells separately. The plates were incubated for 24 hours at 37 ^{0}C. Distilled water and Streptomycin (100 µg/µl) were used as the negative and the positive control respectively. After incubation the diameter of the formed inhibitory zones formed around each well were measured (mm) in four different fixed directions and recorded. Each experiment was conducted in triplicate.

Data analysis

Data were statistically analysed by one way Analysis of Variance and Tukey's multiple comparisons at probability value ($P<0.05$) using a SAS statistical package (version 9.1.3) and mean separation was performed by Least Significance Difference. Results are expressed as Mean ± SE and statistical significance was evaluated by ANOVA.

Results

This *in vitro* antimicrobial assay was done for four seeds of selected antidiabetic medicinal plants as *S. cumini*, *S. alba*, *T. foenum-gra*ecum and *N. sativa* against three selected common bacterial strains. Based on Figure 1, ethanolic seed extract of *S. cumini* exhibited the significant antimicrobial activity while the ethanolic seed extracts of *S.alba* represented minimum antibacterial activity against the all three bacteria as *E. faecalis, E. coli* and *S. auereus.*

The results showed that significant amount of inhibition zone was obtained against all the tested bacterial strains which was comparable to the positive control streptomycin.

Based on Figure 1, the ethanolic seeds extract of *S. cumini* exhibited the significant antimicrobial activity while the ethanolic seeds extract of *S. alba* showed minimum activity against all three bacterial strains at 37°C. There are also significant differences of antimicrobial activity among selected human pathogens. It showed that highest antimicrobial activity for *E. faecalis,* also it represented antimicrobial activity for both of gram negative and positive pathogens. While *S.alba* showed same antimicrobial activity against *E. faecalis* and *S. aureus*, inhibition zone is absent for *E.coli*.

Based on Table 3, moderate antibacterial activity was showed by *T. foenum-graecum* and *N. sativa* against *E. coli* & *E. faecalis and E. coli* respectively. There is no any inhibition zone against *S. aureus* by seeds extract of *T. foenum-graecum* but it showed relative values of inhibition zones against both of *E. faecalis* and *E. coli*. There is no any inhibition zone against *E. faecalis* by *N. sativa*, and inhibition zones are represented against *S. aureus* and *E. coli* human pathogens respectively.

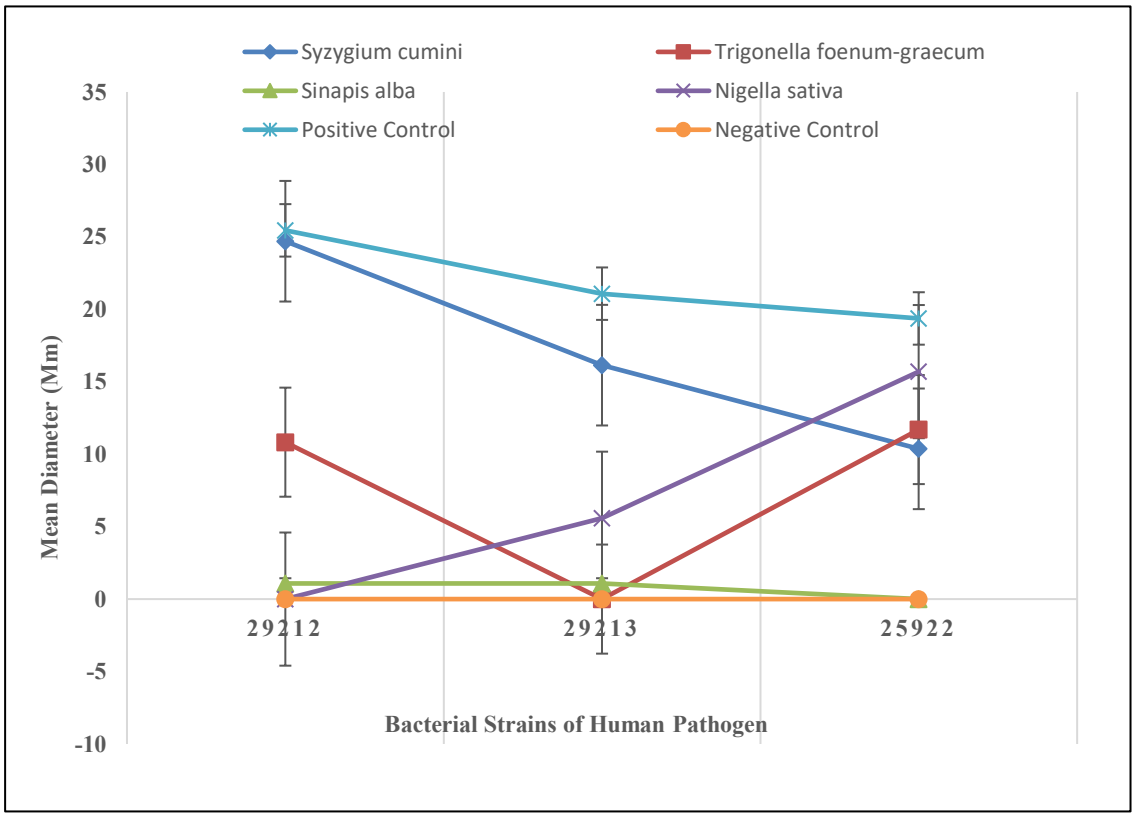
Positive and negative control are represented by *Streptomycin* and distilled water respectively. When consider about positive and negative controls, Streptomycin exhibited the highest inhibitory effect against *E. faecalis* and the lowest inhibitory effect against *E. coli*. In negative controls there were no any inhibitory zones.

Table 1: Medicinal plant seeds tested for their antibacterial activity in the study

Botanical name	Family name	Common name		
		Sinhala	**Tamil**	**English**
Syzygium cumini	Myrtaceae	*Mahadan*	*Naval*	Black Plum
Sinapis alba	Brassicaceae	*Aba*	*Kaduku*	Mustard
Trigonella foenum-graecum	Fabaceae	*Asumodhagam*	*Vendayam*	Fenugreek
Nigella sativa	Ranunculaceae	*Kaluduru*	*Karumjeerakam*	Black cumin

Table 2: Pure bacterial cultures

Name	Type	ATCC No.
Enterococcus faecalis	Gram positive	29212
Staphylococcus auereus	Gram positive	29213
Escherichia coli	Gram negative	25922



Variation of mean diameter of seeds extracts of selected medicinal plants against selected human pathogens at 37℃. Mean followed by same letters are not significantly different by LSD at 5% level, P value <0.0001

Figure 1. Antimicrobial activity of selected seeds of anti-diabetic medicinal plants

Table 3: Bacterial growth inhibition zones of selected seeds of anti-diabetic medicinal plants

Plant species	Diameter of the inhibition zones (mm)		
	29212	29213	25922
Syzygium cumini	24.7±0.37	16.15±1.20	10.37±1.51
Sinapis alba	1.08±2.65	1.08±2.65	0
Trigonella foenum-graecum	10.83±0.34	0	11.7±1.97
Nigella sativa	0	5.58±4.37	15.7±1.56
Positive control	25.45±1.18	21.08±0.26	19.37±1.35
Negative control	0	0	0

Discussion

Based on this *in vitro* study the researchers found that the ethanolic seed extract of S. *cumini* had shown highest anti-bacterial activity against selected bacterial strains. *T. foenum-graecum* seed extract also shown higher antibacterial effect against some bacterial strains. But, *S.alba* had shown least antibacterial activity. Present study results could be comparable with the previous studies which were stated that the seed phenolic extract of *S.cumini* showed antibacterial activity against tested bacterial strains (Santos et al., 2020); the methanol fraction of ethanol extract from the seeds of *S. cumini* was found to have significant antibacterial activity (Yadav et al., 2011; Patoary et al., 2014; Das, Das & Dharani, 2019). However, the present results of *S.alba* is not in line with another study which was done by the Boscaro et al., 2018. That study found that the *S.alba* seed hydroalcoholic extract was effective against *E. coli* and *S. aureus* in disc diffusion test (Boscaro et al., 2018). The hexane extract of *S. alba* seeds showed the highest anti-microbial activity (Sujatha & Mariajancyrani, 2013). Sharma et al found that the methanol extract of *T. foenum-graecum* seeds shown maximum zone of inhibition against *E. coli* and *Staphylococcus* (Sharma, Singh & Rani 2017). Another study found that the oil which was extracted from fenugreek seeds has a good antimicrobial activity against some bacteria (Sara & Abdalbasit, 2022). Further another study found that the *N. sativa* seed oil had a strong antibacterial activity significantly (P<0.01) greater inhibition zone than that of gentamicin (Forouzanfar, Bazzaz & Hosseinzadeh, 2014). Bakathir and Abbas informed that the *N. sativa* ground seeds possessed antibacterial effect against the staphylococcus (Bakathir & Abbas 2011).

Based on all of the representation of antimicrobial activity, it showed that highest antimicrobial activity is showed by *S. cumini* rather than other extracts, also when consider about phytochemical screening there is highest representation in seeds extracts of *S. cumini*. Antimicrobial and antibiotic principle are highly showed by phytochemical compounds such as alkaloids, saponins, tannins, flavonoids and steroids which are known to be biologically active (Nethathe & Ndip, 2011; Patra, 2012; Mujeeb, Bajpai & Pathak, 2014; Ali et al. 2018; Pizzi, 2021; Nek Rahimi et al., 2022). Since Gram positive bacteria's cell walls are more permeable than Gram negative bacteria, whose outer membrane has a lipopolysacharide layer that prevents some antibiotics and antibacterial compounds from penetrating, most plant extracts are thought to be more effective against Gram positive bacteria (Wintola & Afolayan, 2015). Among the all-selected medicinal plants, *S. cumini* showed in high level of antimicrobial activity. Plant produces a range of chemical constituents to protect themselves from the attack of various pathogenic micro-organisms. Substances can either prevent the growth of microorganism or kill them. It can be considered as resources for developing new drugs for various infectious diseases. Antibiotic substances are recognized to vary in concentration in different tissues of same plant, between plants of same and different species and concentration of antibiotics in plant is determined by its environment (Sushil Kumar, Bagchi & Darokar, 1997). In seeds, reserve materials are starch, fixed oils, proteins, fixed oils, fatty acids, some proteins are known to possess good antimicrobial activity and antifungal protein (Cowan, 1999).

Conclusion

This research on the antimicrobial activity of the herbs helps to develop effective herbal remedies as anti-microbial activity to reduce the infection in diabetes. The result shows that *S. cumini* seeds extract poses the highest antimicrobial activity in gram positive and negative bacteria. Therefore, this seed is potential to be further developed as an herbal antibiotic for the management of infection in diabetes in future. More detail study such as are fractionation and characterization of active phytochemicals which are responsible for the antimicrobial activity, as well as *in-vivo* activities recommended to be conducted in the future study.

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Conflict of Interest

Authors have no conflict of interest.

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ORIGINAL ARTICLE

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Pulsatile Tablet of Famotidine Using Core in Cup Method

Shreya Ajay Rajguru[1], Maimuna Fatima[1], Bandaru Hemanth Kumar[1], Shaik Farooq Ahmed[1], Veeranti Vipanchi[1], and D. Prasanthi[1*]

ABSTRACT

Introduction: The present work aims to formulate a pulsatile delivery system using a "core-in-cup" system for famotidine, a H2-Receptor antagonist prescribed for benign gastric ulcers, duodenal ulcers, gastroesophageal reflux disease and nocturnal acid breakthrough. In such a situation, pulsatile drug release is preferable, with a lag time between 3 and 4 hours.

Method: Core tablets were prepared by employing the direct compression method using HPMC K4M, sodium bicarbonate and MCC. Ethyl cellulose, HPMC K4M and Xanthan gum were used for the preparation of Core-in-cup tablets.

Results: Results: Pre-compression parameters were within the admissible limits. The *in-vitro* study indicated core tablet with 40% HPMC K4M showed 85.4± 0.15% drug release at the end of 3hrs and *in-vitro* buoyancy indicated formulation remained floating for >3hrs. Thus, 40% HPMC K4M was selected. Drug excipient compatibility studies indicated drug and excipients to be compatible. The prepared core-in-cup tablets were evaluated for hardness (6.0±0.12 to 7.0±0.12kg/cm2), thickness (3.0±0.15 to 3.5±0.13mm), weight variation (285±0.20 to 314±1.06mg), friability (0.53±0.14 to 0.65±0.12%), floating lag-time (99±0.42 to 120±0.84sec), and swelling index (120±0.56 to 030±0.60%). *In-vitro* studies indicated formulations with xanthan gum (F1 & F2) showed a lag time of 2±0.12 to 2.4±0.15hrs and percentage drug release at the 7th hour was 97±0.90% and 90±0.12% respectively. Formulations with HPMC K4M (F3 & F4) showed a lag time of 3.5±0.10 to 4.2±0.18hrs and percentage drug release at the 7th hour was 86±0.34% and 83±0.20% respectively. Model dependent kinetics depicted, F4 follows zero-order release kinetics, 'n' value of korsmeyer-peppas model indicated anomalous transport mechanism, release process being swelling controlled. Optimized formulation was found to be stable for a period of one month.

Conclusion: Pulsatile release dosage forms are more preferred than conventional dosage forms for nocturnal acid breakthrough. "Core-in-Cup" pulsatile tablets of famotidine were successfully designed to ensure drug release occurs in the morning when administered at bedtime.

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***Corresponding author:**

Email address:
prasanthidhanu@gmail.com

Authors' Affiliation:

[1]*Department of Pharmaceutics, G. Pulla Reddy College of Pharmacy (GPRCP), Mehdipatnam, Hyderabad, Telangana-500028, India*

JOP

Introduction

"Pulsatile drug delivery system (PDDS) is described as the release of a specific amount of drug molecules instantaneously in short duration immediately following a predetermined lag time i.e., no drug release period". These are time-controlled DDS developed so as to attain time-specific and site-specific delivery of drugs (Abdul & Poddar, 2004). Delivery of the drug from the body is as per the circadian rhythm of the body.

Conventional systems for the continuous release of a drug are not ideal as it results in the prompt elimination of a drug from the body, Dose administered is not maintainable within the therapeutic window whereby significant therapeutic effect cannot be achieved, usage of multiple doses may cause plasma drug level fluctuations and poor patient compliance (Adepu et al., 2021) therefore, a controlled drug delivery system is more preferable. PDDS are useful for drugs used in Chrono-pharmacological behaviour diseases (Jagdale et al., 2009; Jagdale et al., 2014). In recent times, PDDS piqued interest as it delivers drugs to suitable place, at correct time and in the appropriate amount, hence providing spatial, temporal and smart delivery, resulting in enhanced patient compliance (Arora et al., 2006; Kumar & Murthy, 2019).

Many conditions require pulsatile release such as, body functions following a circadian rhythm. (e.g.: Secretion of hormones, gastric emptying, acid secretion in stomach, etc.) (Goo et al., 1987), chronopharmacotherapy of disease exhibiting circadian rhythm in its pathophysiology (like myocardial infarction, bronchial asthma, rheumatic disease, angina pectoris, hypertension and ulcer), (Lemmer, 1999; Mali & Bathe, 2015) drugs displaying degradation in gastric fluids (e.g.: peptide drugs), drugs that cause irritation of gastric mucosa or induces nauseousness and vomitings, distal organs targeting drugs in GIT like colon (Gazzaniga et al., 1994).

Drug release from PDDS occurs either by erosion, diffusion, osmosis (Adhikari et al., 2018; Singh et al., 2012). PDDS can be broadly classified into 3 classes (Thakur et al., 2021; Jadhav et al., 2016):

1. Time controlled PDDS
2. Stimuli induced PDDS
3. Externally regulated PDDS

The class of layered tablet (Time-controlled PDDS) in which the upper part of the core is exposed instead of being completely surrounded by coating on both sides is the "inlay Tablet". Core-in-cup tablet is a type of inlay tablet developed by Danckwerts that shows zero-order drug release of aqueous-soluble and aqueous-insoluble drugs. This system comprises a matrix core which is disc-shaped subjected to compression-coated at the circumference and on one surface to create a cup on all sides of the core. Drug release occurs in sustained form from a stable surface with a constant surface area. In the case of core-in-cup tablets, the surface area of contact is less and the release of drug can be sustained completely.

Famotidine is a H2 receptor antagonist belonging to BCS class IV that binds competitively to the H2 receptors, the blocking histamine effect. The competitive inhibition marks declined basal and nocturnal gastric acid secretion and deduction in acidity, gastric volume and amount of gastric acid that is released in response to stimuli including food, caffeine, etc. (Conte et al., 1993) The gastric acid secretion normally shows circadian rhythm, but a sudden upsurge of gastric acidity is seen when the pH level is <4 for a minimum of 1 hour at midnight (2.00am to 4.00am). This physiological condition is called nocturnal acid breakthrough. In such conditions, instead of maintaining a constant plasma drug level, the release of the drug at a particular time is advantageous. (Munde et al., 2022) When the drug is administered at bedtime, it shows release after a few hours of administration (during morning hours), which is ideal in this case. (Kharwade et al., 2022; Jain et al., 2011) Earlier researchers have tried to sustain the drug release of famotidine by formulating it using various carrier systems. Mahajan and coworkers have formulated graphene oxide assisted famotidine formulations but an initial release of almost 56% in the first hour (Mahajan et al., 2019) results in immediate therapeutic effect which is not ideal in case of nocturnal acid breakthrough. Jaimini and coworkers have formulated famotidine based floating tablets which ensured site-specific release of the drug (Jaimini et al., 2007); but in case of nocturnal acid breakthrough site-specific as well as time-specific release is required which improves patient compliance and therapeutic efficacy. Based on the evaluation results of the present optimized famotidine core-in-cup tablets drug release occurs in the desired location (i.e., stomach) after predetermined lag time which is desirable in case of nocturnal acid breakthrough.

Here, in the formulation of famotidine pulsatile tablet, buoyant layer contains HPMC K4M which on contact with gastric fluid forms a gelatinous mass, cohesively binding drug release layer and effervescent component $NaHCO_3$ which upon exposure to gastric contents in the stomach liberates carbon dioxide that gets trapped in the jellified hydrocolloid resulting in the upward movement of the formulation thus imparting buoyancy. The famotidine PDDS possessing floating property is favourable as it ensures enhanced gastric residence with drug release at the expected site after the lag time which corresponds with the circadian rhythm thereby imparting enhanced patient compliance by assuring the presence of the famotidine in required quantity at the right place and the right time.

Methodology

Materials

Digital analytical balance (Contech Instruments Ltd. Mumbai, India), UV-Visible Double beam Spectrophotometer (Chemito instruments Pvt. Ltd. Mumbai, India), Dissolution test apparatus (Electrolab Pvt.Ltd. Mumbai, India), FTIR Spectrophotometer Shimadzu 8400 (Tokyo, Japan) Hot air oven Oswald world laboratory oven (India), pH meter (Elico LI 127), Tablet compression machine (Rimek Rotary Ahmedabad, India), Hardness tester (Cintex Ind. Corporation Mumbai, India).

Famotidine (Dr.Reddy's laboratories Hyderabad, India), Hydroxy Propyl Methyl Cellulose K4M (Yarrow chemical products Mumbai, India), Hydroxy Propyl Methyl Cellulose K100M (Yarrow chemical products Mumbai, India), Hydroxy Propyl Methyl Cellulose E15 (Yarrow chemical products Mumbai, India), Xanthan gum (Yarrow chemical products Mumbai, India), Ethyl cellulose (Balaji drugs, India), Sodium bicarbonate (S.D. Fine Chemicals Ltd., India), Methanol (S.D. Fine Chemicals Ltd., India), Potassium Dihydrogen Phosphate (S.D. Fine Chemicals Ltd., India), Hydrochloric acid (S.D. Fine Chemicals Ltd., India)

All the chemicals used were of analytical grade.

Drug-Excipient Compatibility Study by FTIR

Spectrum analysis of pure drug and the physical mixture was carried by FTIR using KBr pellet technique. The disc obtained was placed in an appropriate holder within an IR spectrophotometer and IR spectrum was recorded from 4000cm-1 to 500 cm-1. The spectrum obtained was observed for the presence of characteristic peaks of the respective functional group and compared for any spectral changes.

Preparation of Core Tablets

Direct compression method was employed for preparing famotidine core tablets using 6mm punch, utilizing different polymers (HPMC K4M, HPMC K100M, HPMC E15) and effervescent material (sodium bicarbonate).

Preliminary Trial formulation of core tablet:

Placebo formulations were prepared as follows, using 6mm punch powder blend is subjected to compression and checked for floating:

1. Using HPMC E15 polymer, sodium bicarbonate ($NaHCO_3$) and citric acid in different ratios as gas-generating agent.
2. Using different polymers i.e., HPMC K4M, HPMC K15M and HPMC K100M along with $NaHCO_3$ as gas-generating agent.

Table 1: Famotidine core tablet Formulation

Ingredients	C1	C2	C3	C4
Famotidine	20	20	20	20
HPMC K4M (%)	10	20	30	40
Sodium bicarbonate	5	10	15	20
Microcrystalline cellulose	82	67	52	37
Magnesium stearate	3	3	3	3

Based on the above placebo trials, the formulation containing polymer HPMC K4M and gas-generating agent sodium bicarbonate showed good floating properties. Thus, using HPMC K4M with different concentrations of sodium bicarbonate core tablets were formulated (Table 1). For formulating the core tablet, (Figure 1) the drug is thoroughly mixed with the excipients and compressed using a 6mm punch.

Evaluation of Core Tablet

A. Pre-compression evaluation

Flow properties: The prepared powder blend was evaluated for bulk density, tapped density, Hausner's ratio, compressibility index and Angle of repose (Subramanyam, 2000).

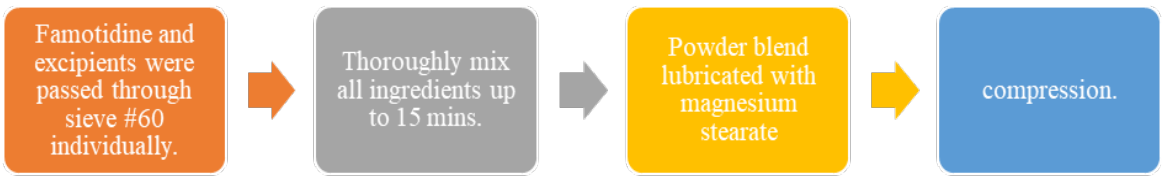

Figure 1: Preparation of core tablet.

B. Post-compression evaluation

Hardness and thickness: The hardness and thickness of core tablets were measured using Monsanto hardness tester and screw gauge respectively. (Chavda et al., 2016)

Friability: 20 tablets were correctly weighed and placed in friability testing apparatus which was operated for 100 revolutions. Then these tablets were taken out and dusted followed by reweighing tablets. The %friability calculated by

$$\% \text{ Friability} = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100$$

Weight variation: 20 tablets were weighed individually and the average weight was calculated. The individual weight was compared with the average weight.

In-vitro buoyancy studies: Tablets were taken in 200ml 0.1N HCl containing measuring cylinder and Floating lag time i.e., the time needed for a tablet to reach the surface and float and Floating time i.e., period of time during which the tablet remained floating is noted.

Determination of %drug content: Tablets were powdered in a mortar. 20mg of drug equivalent powder was weighed and dissolved in distilled water. Using a membrane filter (0.45 mm) stock solutions were filtered, diluted with 0.1N HCl and drug content analysed at 267 nm by UV spectrophotometer. (Malladi & Jukanti, 2016)

Swelling index determination: Tablets were initially weighed ($W1$) then placed in 200 ml of 0.1N HCl containing beaker and incubated at 37±1°C. For 24 h, periodically tablets were taken and carefully using paper excess surface liquid was removed. Swollen tablets were again weighed ($W2$) for calculating the swelling index (SI), (Deepika et al., 2011; Malladi & Jukanti, 2016)

$$SI = \frac{W2 - W1}{W1} \times 100$$

In-vitro dissolution studies: *In-vitro* dissolution studies were carried out using USP Type II apparatus (paddle) with 0.1N HCl as dissolution medium at 50rpm, 37±0.5°C for 3 hours. 5ml samples were taken at different time intervals and absorbance was analysed using UV visible spectrophotometer at 267nm. (Conte et al., 1993)

Preparation of famotidine core-in-cup tablets

For preparing Core-in-cup tablet ethyl cellulose was selected as an impermeable cup; (Singh et al., 2012) Xanthan gum and HPMC K4M as hydrophilic top layer. Optimized core tablets were subjected to press coating for preparing core-in-cup tablets. 11mm punch was used. (Table 2, Figure 2). Prepared Core in Cup Tablets are illustrated in Figure 3.

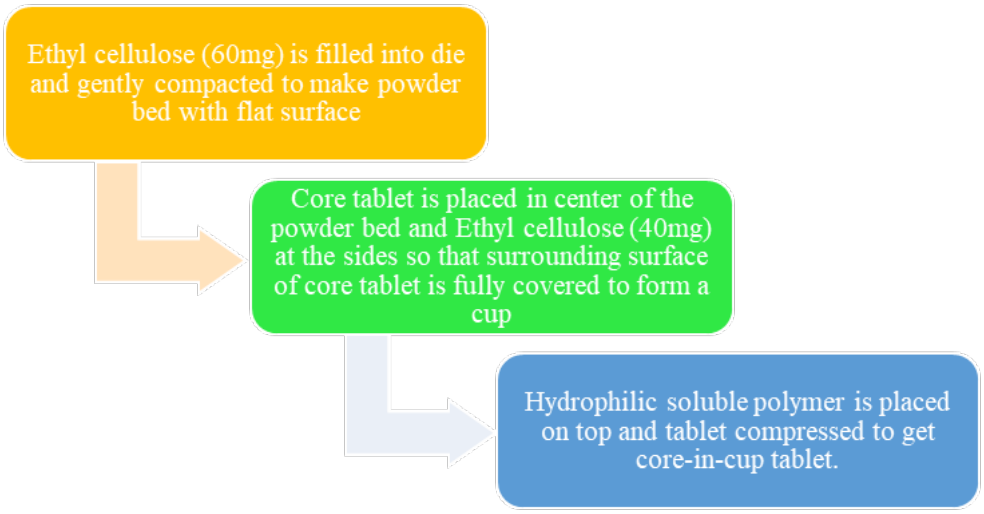

Figure 2: Famotidine Core-in-cup tablet preparation.

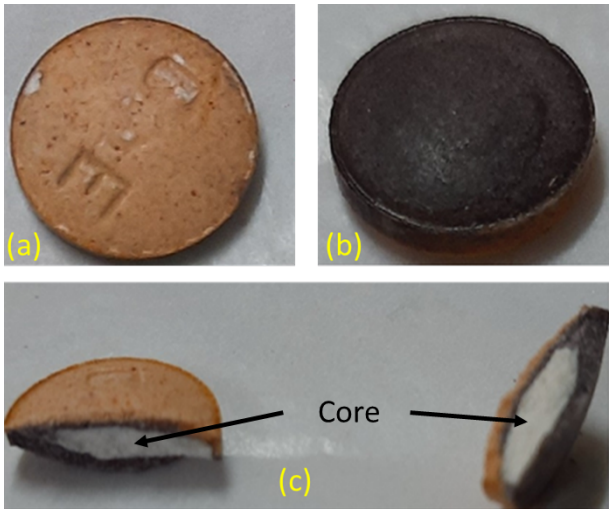

Figure 3: (a) Hydrophilic top layer (b) Cup and (c) Core part of the prepared famotidine core-in-cup tablet

Table 2: Famotidine Core-in-cup tablet Formulation

Ingredients	C1	C2	C3	C4
Core tablet	120	120	120	120
Ethyl cellulose	100	100	100	100
Sodium bicarbonate	20	20	20	20
Xanthan gum	50	75	-	-
HPMC K4M	-	-	50	75

Evaluation of Prepared Core-in-Cup Tablet

Hardness, thickness, friability, weight variation, *in-vitro* dissolution test and *in-vitro* buoyancy test was carried out as mentioned in the evaluation of core tablets.

Water uptake and erosion studies for core-in-cup tablets: Different time points were marked on beakers used for dissolution, i.e., 0.5, 1, up to 6.5 hrs. In each beaker, 0.1N HCl was taken and maintained at 37±0.5°C. To the beaker, one tablet was added and subjected to stirring at 50 rpm. These tablets were collected after completion of the respective time and excess water from the surface was removed using filter paper. Tablets were reweighed and a gain in weight indicates water uptake. It is estimated by equation:

$$Q = 100\frac{Wf - Wi}{Wf}$$

Where Q is %liquid uptake; Wf and Wi are the weight of the hydrated sample and initial dry weight respectively. (Borgaonkar et al., 2012)

Lag time: The duration before the bursting of tablets and release of the core tablet out of press coating is lag time. This is regarded as a pre-determined off-release period.

Calculation of model-dependent kinetics: Drug release kinetics can be explained by testing various models. The mechanism of drug release rate kinetics of dosage form was analyzed by fitting the acquired release data into different release models.

Stability Studies: F4 was tested for its stability according to the International Conference of Harmonization (ICH) guidelines. At designated time intervals, tablets were assessed for their floating property, lag time, drug content and *in vitro* drug release. (Sokar et al., 2013)

Results

Drug Excipient Compatibility Studies by FTIR

FTIR spectra of both pure drug and various excipients individually and in combination are illustrated in Figure 4. The FTIR studies of the physical mixture of drug with various polymers revealed no sign of interaction. Thus, it is regarded that a combination of famotidine and polymer is appropriate for formulating a famotidine cup-in-core pulsatile delivery system. Other excipients used in the formulation are common excipients which do not offer any compatibility issues hence were not analysed by FTIR.

Preparation of Core Tablets

Trial placebos were tested for floating properties and it was observed that placebos containing HPMC K4M polymer and $NaHCO_3$ as gas-generating agent showed good floating property. Based on this, further famotidine core tablets were formulated using $NaHCO_3$ as a gas-generating agent and HPMC K4M in different percentages.

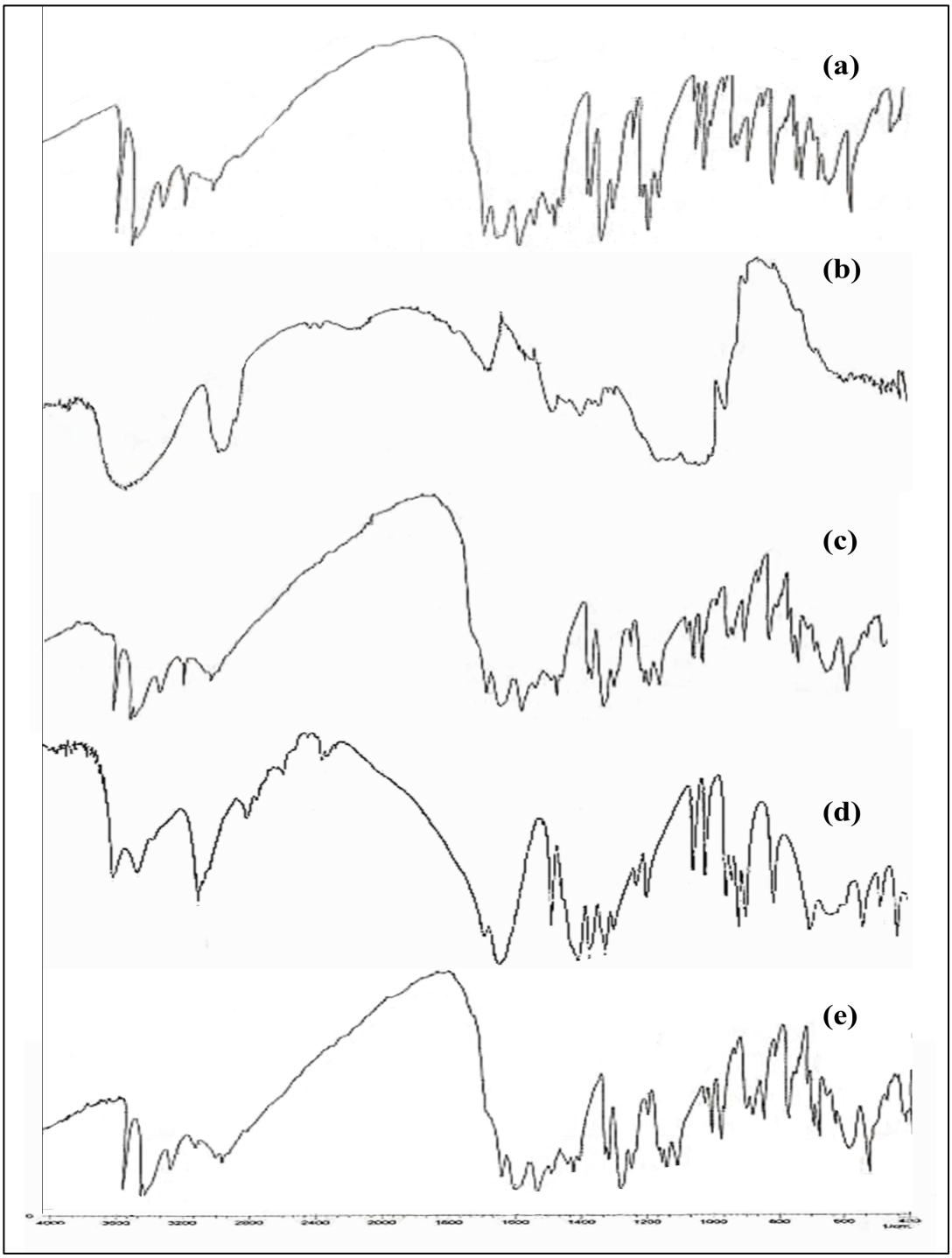

Figure 4: FTIR drug-excipient interaction studies of famotidine (a) Famotidine (b) HPMC K4M (c) Famotidine + HPMC K4M (d) Ethyl cellulose (e) Famotidine + ethyl cellulose.

Evaluation of Core Tablets

Pre-compression evaluation of the powder blend and post-compression evaluation of famotidine core tablets was carried out; the results of which are displayed in Table 3, Figure 5.

From *In-vitro* buoyancy studies, it was found that only formulation with 40% HPMC K4M remained floating for more than 3 hours. Therefore, 40% HPMC K4M was chosen for further preparation of Core-in-cup tablets.

Table 3: Evaluation of Core tablets.

Formulation	C1	C2	C3	C4
Pre-Compression				
b	0.464 ± 0.12	0.360 ± 0.14	0.482 ± 0.11	0.565 ± 0.15
Tapped density (g/ml)	0.564 ± 0.16	0.409 ± 0.11	0.554 ± 0.12	0.612 ± 0.13
Carr's Index (%)	15.0 ± 0.20	13.1 ± 0.14	12.9 ± 0.18	14.4 ± 0.16
Hausner's ratio	1.17 ± 0.13	1.13 ± 0.18	1.14 ± 0.15	1.12 ± 0.22
Angle of repose (º)	25.2 ± 0.25	21.6 ± 0.16	29.5 ± 0.35	27.9 ± 0.29
Post-Compression				
Hardness ± SD (kg/cm^2)	3.5 ± 0.12	3.8 ± 0.15	4.0 ± 0.12	3.5 ± 0.16
Thickness ± SD (mm)	2.6 ± 0.13	2.5 ± 0.15	2.0 ± 0.12	1.7 ± 0.13
Friability ± SD (%)	0.55 ± 0.19	0.59 ± 0.22	0.50 ± 0.18	0.53 ± 0.14
Drug content (%)	98.51 ± 0.52	99.36 ± 0.68	99.12 ± 0.74	98.89 ± 0.57
Weight variation (mg)	116 ± 1.20	118 ± 0.18	119 ± 1.15	118 ± 1.06
Floating lag time (sec)	125 ± 0.14	100 ± 0.19	85 ± 0.12	85 ± 0.18
Total floating time (min)	30 ± 0.16	50 ± 0.17	Up to 90± 0.13	>180±0.15

*All values represent n= 3± S.D.

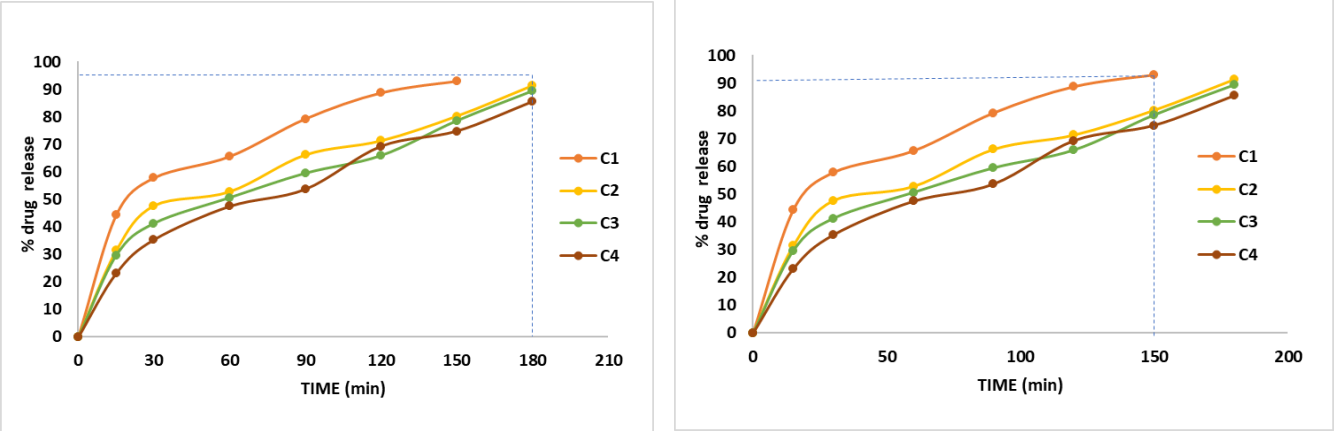

Figure 5: *In- vitro* drug dissolution of core tablets.

Evaluation of Core-in-Cup Tablets

Prepared famotidine core-in-cup tablets were assessed for various parameters Table 4, Figure 6.

With increasing concentration of xanthan gum lag time increased and it showed rapid release of the drug while with an increase in the concentration of HPMC K4M lag time extended and then followed delayed release profile.

F4 was selected as the optimized formulation from the dissolution studies as it successfully showed a lag time of about 4.2 ± 0.18 hours.

Model-dependent kinetics was performed for all four formulations to determine the release mechanism, release kinetics and transport mechanism for the drug (Table 5).

Table 4: Evaluation of Core-in-cup tablets.

Formulation	F1	F2	F3	F4
Hardness ± SD (kg/cm^2)	6.0 ± 0.12	6.5 ± 0.15	7.0 ± 0.12	6.0 ± 0.16
Thickness ± SD (mm)	3.5 ± 0.13	3.0 ± 0.15	3.2 ± 0.12	3.5 ± 0.13
Friability ± SD (%)	0.65 ± 0.12	0.59 ± 0.24	0.63 ± 0.13	0.53 ± 0.14
Drug content (%)	99.12 ± 0.31	98.71 ± 0.82	98.56 ± 1.00	99.57 ± 0.65
Weight variation (mg)	285 ± 0.20	312 ± 0.18	287 ± 1.15	314 ± 1.06
Swelling index (%)	120.8 ± 0.56	124.2 ± 0.23	130.6 ± 0.60	129.5 ± 0.37
Floating lag time (Sec.)	109 ± 0.62	120 ± 0.84	99 ± 0.42	102 ± 0.71
Lag Time (Hr.)	2.0 ± 0.12	2.4 ± 0.15	3.5 ± 0.10	4.2 ± 0.18

*All values represent n= 3± S.D.

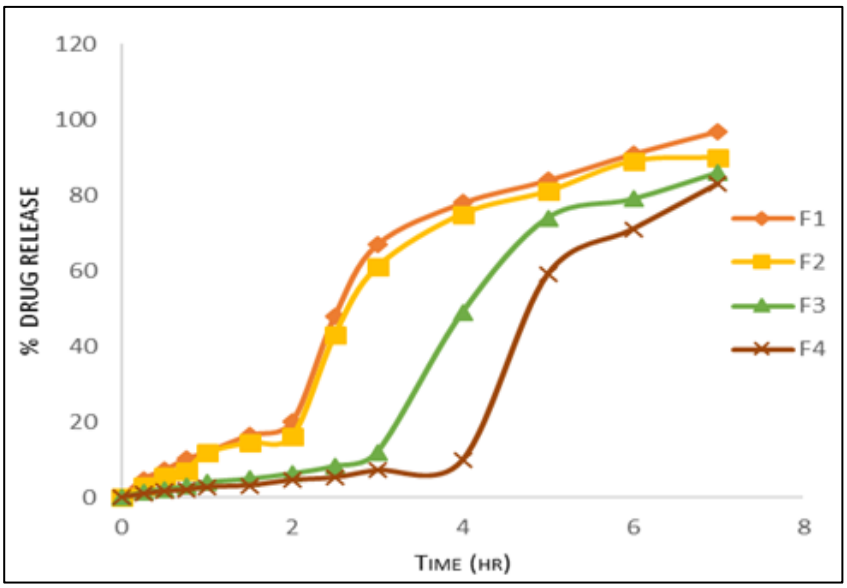

Figure 6: *In- vitro* drug dissolution of core-in-cup tablets.

Figure 5: Model-dependent kinetics.

Formulation	R^2				N	Drug transport mechanism
	Zero	First	Higuchi	Korsemeyer-peppas		
F1	0.937	0.945	0.923	0.949	1.172	Super case-II
F2	0.931	0.964	0.914	0.956	1.075	Super case-II
F3	0.915	0.909	0.778	0.900	0.661	Anomalous transport
F4	0.943	0.890	0.673	0.939	0.542	Anomalous transport

Table 6: Stability Studies for optimized tablets (F4).

Parameter	0 week	1st week	2nd week	3rd week	4th week
Floating lag Time	102 ± 0.71	100 ± 0.85	103 ± 0.52	102 ± 0.70	103 ± 0.11
Lag Time (Hr.)	4.2 ± 0.18	4.2 ± 0.28	4.2 ± 0.57	4.2 ± 0.22	4.2 ± 0.19
Drug content (%)	99.57±0.65	99.52±0.55	99.47±0.64	99.48±0.68	99.37±0.45
% Drug release	83±0.20	83±0.15	83±0.20	83±0.17	83±0.09

*All values represent n= 3± S.D.

Drug release kinetics illustrates that optimized formulation F4 follows zero-order release kinetics and the korsemeyer-peppas release mechanism. The value of release component 'n' indicates that F4 exhibit Anomalous transport and the release process is swelling controlled.

Stability Studies

According to ICH guidelines, stability study for F4 was conducted. At each sampling time (i.e., every week), F4 was assessed for its floating property, lag time, drug content and *in-vitro* drug release (Table 6).

F4 showed no substantial (P >0.05) difference in %drug release of famotidine, after 7 hrs., throughout the storage period of 1 month, when corresponded with release from the same formulation prior to storage. Additionally, no difference in drug content, floating property and lag time was observed during the span of storage.

In case of nocturnal acid breakthrough, more than maintenance of constant plasma drug level diurnal progress of disease is desired (Ravichandiran et al., 2009). Nocturnal acid breakthrough occurs in patients taking Proton Pump Inhibitors, it is basically presence of <4 intragastric pH during night time for almost 60 minutes (Tutuian et al., 2004). Nocturnal acid breakthrough follows circadian rhythm with intensity of peak more between morning hours (2:00 AM- 4:00 AM), by adding H2 receptor antagonist along with Proton Pump Inhibitors. Chrontherapeutic approach can be achieved wherein, drug release occurs after predetermined lag time creating synchrony between drug concentration in plasma and peak symptoms of Nocturnal acid breakthrough. Based on the evaluation results, the optimized famotidine core-in-cup tablets ensures drug release occurs in the desired location (i.e., stomach) after predetermined lag time which is desirable in case of nocturnal acid breakthrough.

Conclusion

Nocturnal acid breakthrough is a phenomenon that occurs at midnight. Conventional delivery systems are inconvenient for delivering famotidine, a H2 receptor antagonist due to the fact that, patients are asleep at this time, and the tablet cannot be administered when the symptoms start showing. So, oral pulsatile release dosage forms with gastric retention abilities were designed to be administered at bedtime offering drug release in the morning. Therefore, famotidine was formulated as PDDS employing a "Core-in-Cup" system.

Ethyl cellulose was selected as a cup, HPMC K4M as a hydrophilic-plug layer and sodium bicarbonate as a gas-generating agent for the preparation of core-in-cup tablets based on preliminary trials. The optimized formulation F4 was evaluated for various parameters including hardness (6.0 ± 0.16kg/cm^2), thickness (3.5 ± 0.13mm), weight

variation (314 ± 1.06mg), friability (0.53 ± 0.14%), floating lag time (102 ± 0.71sec.), swelling index (129.5 ± 0.37%), lag time (4.2±0.18hrs), *In-vitro* studies showed lower initial release after the lag time, 83±0.20% drug release was seen at the end of 7th hour. Model-dependent kinetics studies indicated F4 follows zero-order release kinetics and korsemeyer-peppas release mechanism. Based on 'n' value drug release mechanism was identified as anomalous transport. The release process is swelling-controlled. Stability studies for one month indicated no significant difference in floating property, lag time, drug content and % drug release.

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Conflict of Interest

The authors have declared that no conflict of interest exists. All products utilised for the research are normally and predominantly used products in the area of research and country. Also, the research was funded by the personal efforts of the authors and the educational institute (G. Pulla Reddy College of Pharmacy, Hyderabad, India).

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ORIGINAL ARTICLE

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Stability study of royal jelly in alginate-pectin beads

Muhammad Fitri Azhar[1], Nurul Ain Mohammad Hamdi[1], Muhammad Salahuddin Haris[1,2,*]

ABSTRACT

Introduction: The stability of royal jelly (RJ) beads is a critical aspect to ensure the product is safe, efficacious, and possesses an acceptable quality for consumers. This study aims to establish storage duration and condition to ensure the stability of RJ in alginate-pectin beads.

Methods: In this study, two types of packaging material have been chosen, namely polyethylene (PET) opaque bottles and glass containers. Samples of RJ beads were stored in four different storage conditions that include freezer, laboratory environment, real-time (30 °C, 75% RH) and accelerated (40 °C, 75% RH) stability chambers at different sampling points (0, 14 days, 1 month, 3 months). The RJ beads were characterised for physicochemical properties and 10-hydroxy-2-decenoic acid (10-HDA) content in the RJ-encapsulated beads.

Results and discussion: The colour of RJ beads in the refrigerator remained whitish grey throughout the study but colour change in room temperature (laboratory) is observable starting from 1-month time point. The particle size of RJ beads stored in accelerated stability chamber had a decreasing pattern with significance ($p < 0.05$) for both different types of storage container. No significant difference ($p > 0.05$) between sphericity coefficient values of RJ beads stored in glass and PET container in refrigerator, room temperature and real-time stability chamber at 0 month and 14-day time point. Constant peaks of 10-HDA appeared for RJ samples stored in all storage conditions at 14-day time point. Nonetheless, at 1-month and 3-months, peak area starts to show decreasing trend for beads stored in room temperature, real time and accelerated stability chambers.

Conclusion: The study showed that the RJ beads exhibited convincing stability for 3 months.

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***Corresponding author:**

Email address: solah@iium.edu.my

Authors' Affiliation:

[1] *Department of Pharmaceutical Technology, Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Malaysia.*

[2] *IKOP Pharma Sdn. Bhd., Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*

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Introduction

Stability can be defined as the ability of a pharmaceutical or nutraceutical product to withstand physical, chemical, or microbiological changes or decomposition when exposed to various environmental conditions (Association of Southeast Asian Nations, 2013). In general, the purpose of stability testing is to provide the evidence on the effect of time on the product under the influence of a variety of environmental factors, such as temperature, humidity, and light to establish a shelf life or expiry date for the pharmaceutical product hence recommending appropriate storage conditions (Association of Southeast Asian Nations, 2005).

The stability of a pharmaceutical product is complex, often being dependent on multiple physical, chemical, and microbiological factors that may or may not interact with each other (Aulton, 2018). In this stability study, two types of packaging material have been chosen, namely polyethylene (PET) opaque bottles and glass containers. Plastic and glass are the most used primary packaging materials. Glass is widely used for packaging pharmaceuticals because of its excellent barrier properties, relative inertness, and compatibility with pharmaceuticals (Polshettiwar, 2021). On the other hand, the growing use of plastics as a pharmaceutical packaging material is because of the significant advantages and consumer preference. Plastics are lightweight and shatterproof. Plastics are also easily shaped and sealed, which gives great versatility in the design of the pack (Andjelković et al., 2021).

Stability study was executed to investigate the characteristics of royal jelly (RJ) encapsulated in alginate-pectin beads in different storage conditions with varying temperatures and relative humidity (RH). In general, a drug product should be evaluated under distinctive storage conditions that test its thermal stability and, if applicable, its sensitivity to moisture or potential for solvent loss (Association of South East Asian Nations, 2005).

Alginate-pectin beads filled in PET opaque plastic bottles and glass containers were randomly sampled and later were kept in refrigerator (2-8 °C), room temperature (25 °C), a real time (30 °C and 75% RH) and an accelerated (40 °C and 75% RH) stability chambers. At different stability time points (0-day, 14-days, 1-month and 3-months), samples were taken out from each different storage condition for later characterisation. In this stability study, all the sample were characterised for physical appearance, particle size, sphericity coefficient, microscopic morphology, 10-HDA content and compression testing.

A suitable storage condition is crucial to ensure the quality of RJ as its bioactivities are primarily influenced by its storage condition. The physical and chemical constituent of RJ can change when it is stored improperly resulting in the loss of its functional properties. Therefore, it is important to evaluate the effect of storage conditions on the quality of RJ.

Materials

The RJ beads filled in PET opaque bottle and clear glass bottle were randomly sampled and stored (n=30) into four different storage conditions: in refrigerator, laboratory, accelerated and real time stability chambers. The KBF 240 accelerated and Max 1400 real time stability chambers were provided from Capromax (Selangor, Malaysia).

Methodology

The stability study of RJ beads was executed based on the pharmaceutical product guideline written in the ASEAN Guideline on Stability Study of Drug Product (2013). As mentioned earlier, RJ bead samples were stored in refrigerator (2-8 °C), laboratory (25 °C), real time (30 °C, 75% RH) and accelerated (40 °C, 75% RH) stability chambers. At predetermined stability time point: 0-day, 14-days, 1-month and 3-months, samples were taken out (n=30) from each different storage condition. The stability samples were observed and characterised for physical appearances, particle size, sphericity coefficient, microscopic morphology, 10-HDA content and compression testing. The physical appearance of RJ beads, namely under its organoleptic properties at different time points was compared to the appearance of the beads at 0-month stability time point.

1. Determination of the diameter and sphericity coefficient of RJ beads

To achieve statistical result, thirty RJ beads were randomly chosen and rinsed with distilled water after thirty minutes of gelation time. Image analysis software (Image J, National Institute of Health, USA) was used to measure the diameter of each of the RJ beads taken. Statistical data such as mean, median, and mode were generated automatically.

The sphericity coefficient (SC) of RJ beads was calculated using the following equation by Houghton & Amidon (1992) (Equation 1):

$$SC=d_{min}/d_{max} \quad \text{(Eq. 1)}$$

where, dmin and dmax are minimum and maximum Ferret's diameters of the RJ beads, respectively (Shaiqah et al., 2020). Beads with a SC value approaching 1 are considered ideal and spherical (Azhar et al., 2021).

2. 10-HDA analysis

RJ beads were immersed in the phosphate-buffer solution (PBS) with concentration 0.1 M and pH 6.8. The samples were subjected under vigorous stirring for 30 minutes until the alginate-pectin coating disintegrated. Then, 25 mL of water and methanol (1:1, v/v) were added and the suspension formed was centrifuged at 4000 rpm for 10 minutes using Rotofix 32 from Andreas Hettich GmbH & Co. (Tuttlingen, Baden-Württemberg). The 10-HDA content in the RJ beads was obtained by analysing the supernatant solution spectrophotometrically at 215 nm using HPLC (waters e2695, Waters Corporation, Milford, USA).

3. Surface morphology

Prior to SEM imaging, 10 to 15 RJ beads were rinsed with an increasing gradient of ethanol concentration of 10%, 50%, 70%, 90%, and 100% and left air-dried for 30 minutes at room temperature (25-30 °C) for proper sample dehydration. The surface morphology of the beads was evaluated by using a scanning electron microscope at 100 and 500 times of magnification (SEM, Fei, Quanta 450, ThermoFisher Scientific, Oregon, USA).

4. Compression testing

Brookfield CT3 Texture Analyser (Middleboro, USA) was used for uniaxial compression of a single RJ bead. For statistically significant results, 30 RJ beads were randomly selected from the samples that had been dipped previously in simulated gastric fluid or simulated intestinal fluid for 30 minutes. Cylindrical aluminium probe with 6 mm diameter was attached to compress the bead at 1.0 mm/s. The trigger load of 0.05 N and peak deformation of up to 50% of the initial bead diameter were set. Equation 2 was utilised to calculate Young's modulus, E (Pa).

$$\boldsymbol{E = \frac{3 \times (1 - v^2) \times F}{\sqrt{d \times H^3}}} \qquad \text{(Eq. 2)}$$

where,

d: diameter of the bead (m)
F: trigger load (N)
H: deformation of the bead (m)
v: Poisson's ratio

Results and Discussion

1. Physical appearance

RJ is whitish grey colour. It is a complex compound that consist of water (60%-70%), proteins (27%-41%), carbohydrates (30%), lipids (8%-19%), free amino acids, trace mineral, and water-soluble vitamin (Maghsoudlou, Sadeghi Mahoonak, Mohebodini, & Toldra, 2019). The colour of RJ is a critical parameter as it acts as indicator of its freshness and suitability (Zheng, Wei, Wu, Hu, & Dietemann, 2012). Figures 1, 2, 3 and 4 display the appearance of RJ beads at 0-day, 14-days, 1-month and 3-months stored in refrigerator, room temperature, real time, and accelerated stability chambers. The colour of RJ beads in both type of packaging stored in the refrigerator remained whitish grey throughout this study (Figure 1). In comparison, at 1-month and 3-months stability time points, the colour of the beads stored at room temperature turned purplish grey (Figure 2). Changing of RJ beads colour also occurred in both accelerated and real time stability chambers at 1-month and 3-months stability time point for both packaging, where the beads changed from whitish grey to dark brownish yellow (Figure 3 and Figure 4). This result is in agreement with previous studies that reported browning reaction of RJ during storage at room temperature as early 1-month storage (Chen & Chen, 1995; Qiao, Wang, Liu, & Zhang, 2018).

This effect is attributed to the Millard reaction or also known as non-enzymatic reaction where a chemical reaction occurs between amino acids present in RJ and reducing sugar that produce brown colour. Chen & Chen, (1995) proposed that browning reaction was stimulated by the higher temperature in which this reaction was sensitive to the ambient temperature and higher temperature stimulate the reaction rates. During Maillard reaction, a wide range of reaction products is formed, leading to significant alteration that affect nutritional value of nutraceuticals (Starowicz & Zieliński, 2019). In contrast, the colour change of RJ beads at room temperature (laboratory) is observable starting at 3-months. At room temperature, PET bottle is believed to reduce and delay the change of colour of the RJ beads. The opaqueness of the bottle aids in protecting the beads from direct sunlight and its subsequent heat changes. Hence, at room temperature, PET bottle can serve as the best potential candidate for the storage of RJ beads and can be considered for future recommendation in the market.

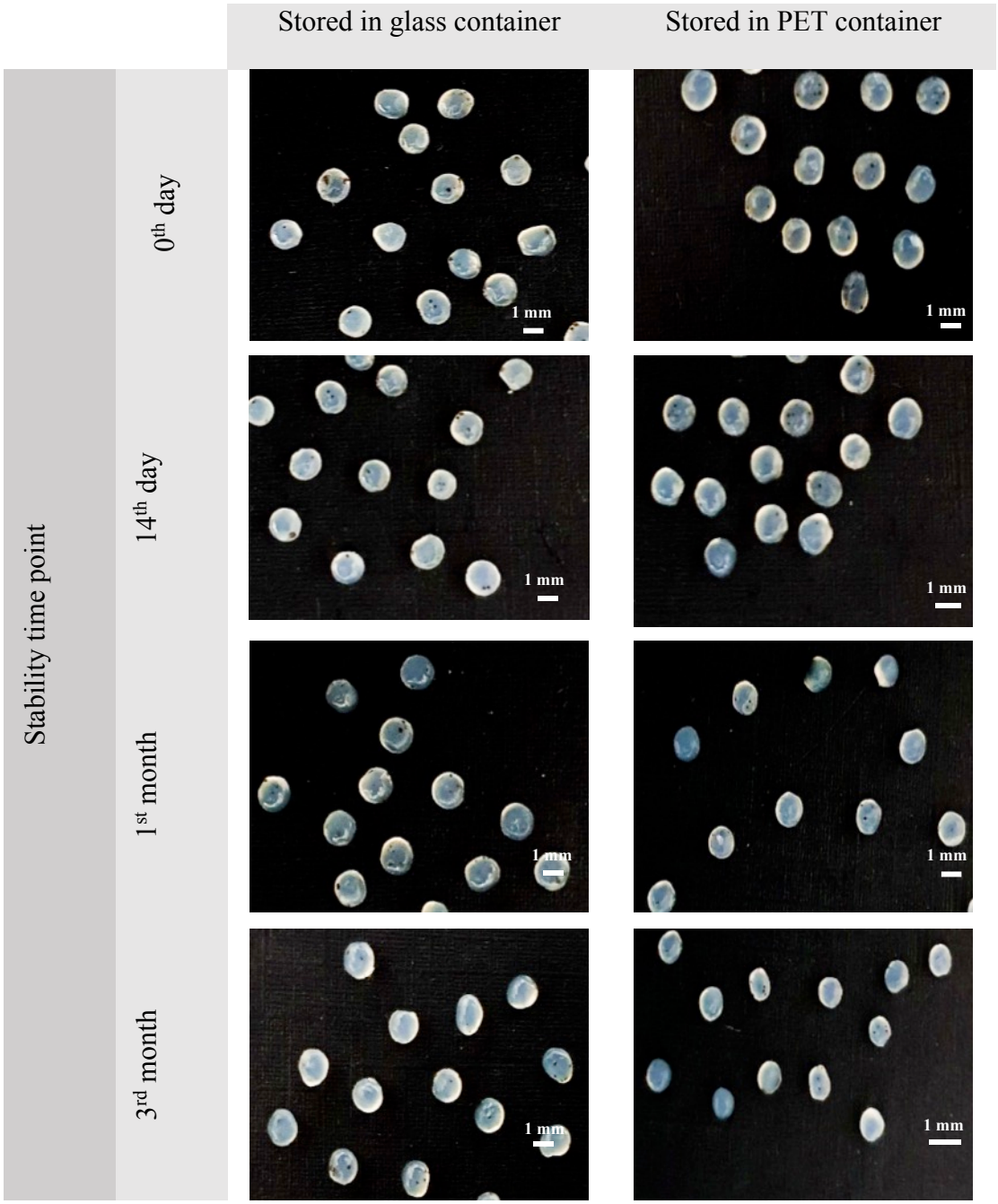


Figure 1. The physical appearance of RJ beads stored in refrigerator at four stability time points (0-day, 14-day, 1-month and 3-month)

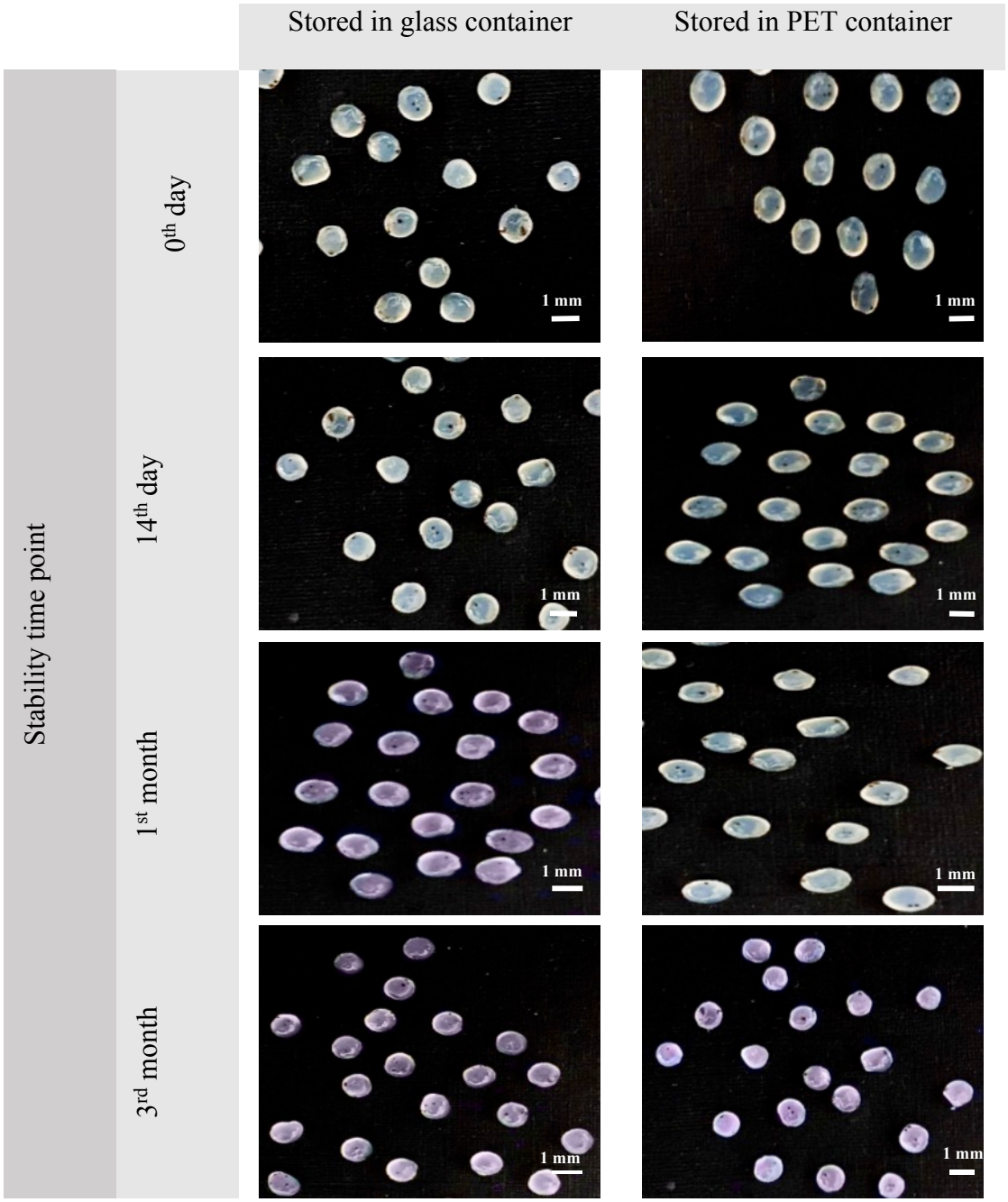


Figure 2. The physical appearance of RJ beads stored in room temperature at four stability time points (0-day, 14-day, 1-month and 3-month)

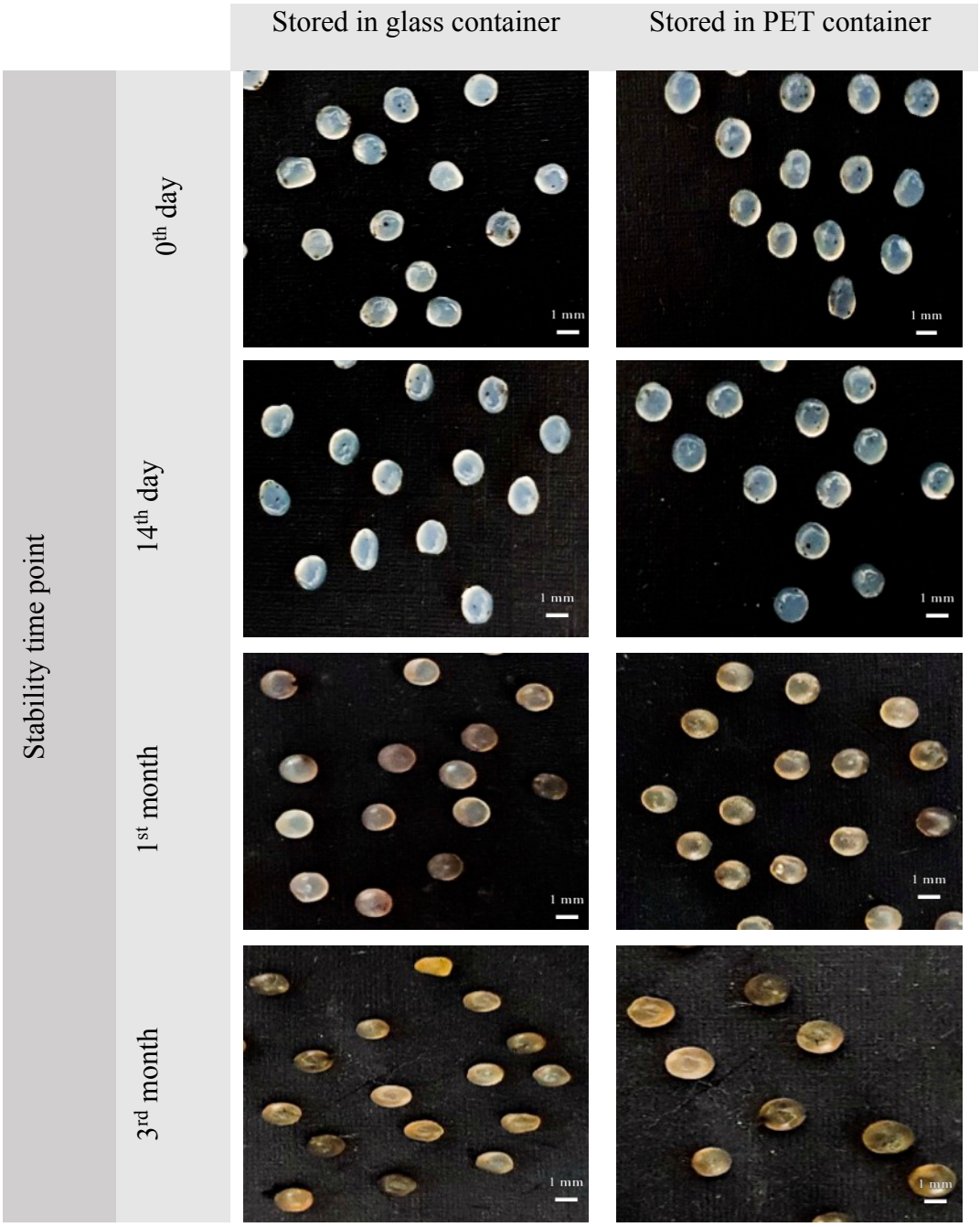


Figure 3. The physical appearance of RJ beads stored in real-time stability chamber at four stability time points (0-day, 14-day, 1-month and 3-month)

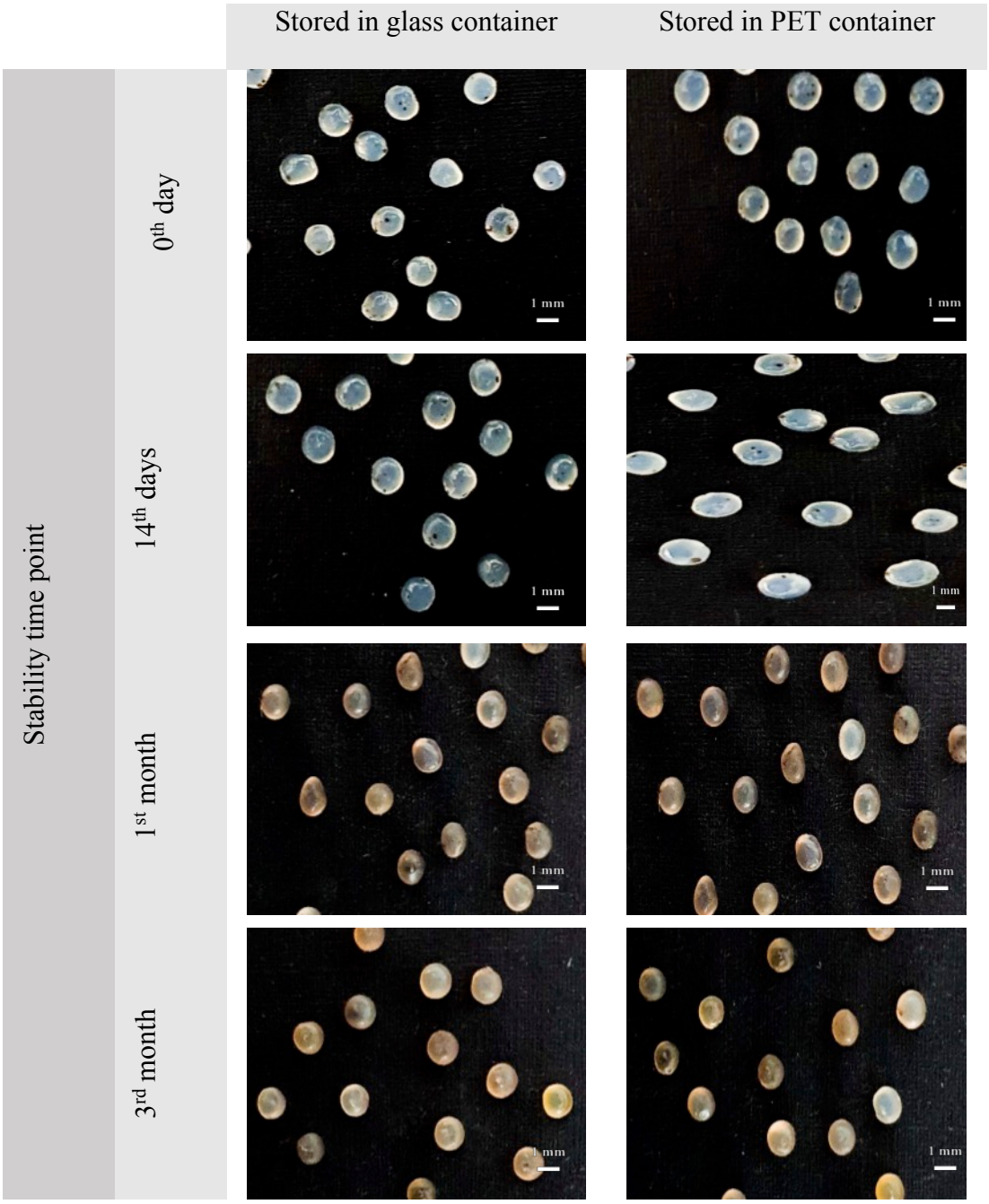
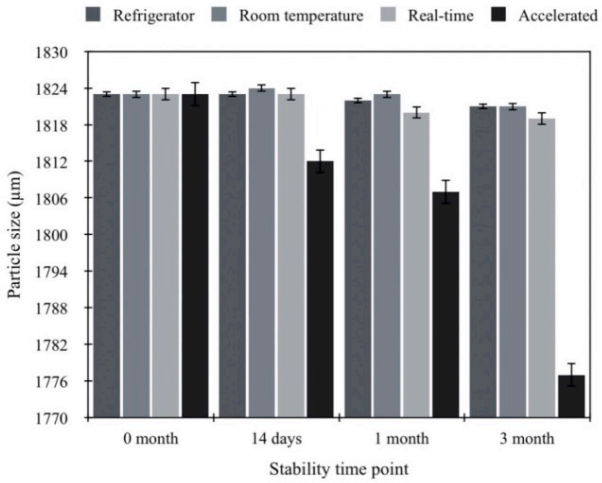


Figure 4. The physical appearance of RJ beads stored in accelerated stability chamber at four stability time points (0-day, 14-day, 1-month and 3-month)

2. Particle size

The particle size of the RJ beads was measured and analysed in four different storage conditions that include refrigerator, laboratory, real time, and accelerated stability chambers. For better overview, Figure 5 descriptively illustrates particle size of the randomly selected RJ beads (n=30) at different stability study points. The mean bead diameter ranges between 1777 ± 121 µm to 1823 ± 199 µm. Reduction of particle size was observed in all storage conditions for both types of beads container. However, only the particle size of RJ beads stored in accelerated stability chamber had a decreasing pattern with significance ($p<0.05$) for both types of storage container. The reduction in particle size of RJ beads stored in accelerated stability chambers across all stability time points might be an evident sign that 10-HDA undergoes chemical and physical degradation when exposed to higher temperature (40 °C). On top of that, occurrence of total water loss in the beads via evaporation and disruption of polymeric cross-linking of the biopolymer by high temperature explains the further reduction of the bead diameter over time (Bannikova, Rasumova, Evteev, Evdokimov, & Kasapis, 2017; Vargas, Pereira, Guimarães, Waldman, & Pereira, 2018). Nevertheless, RJ beads stored in refrigerator, room temperature and real-time stability chamber manifests insignificant size reduction ($p>0.1$) from 0-month till 3-months for both types of storage container. The statistical analysis using two-factorial ANOVA is also insignificant between all groups.

(A)



(B)

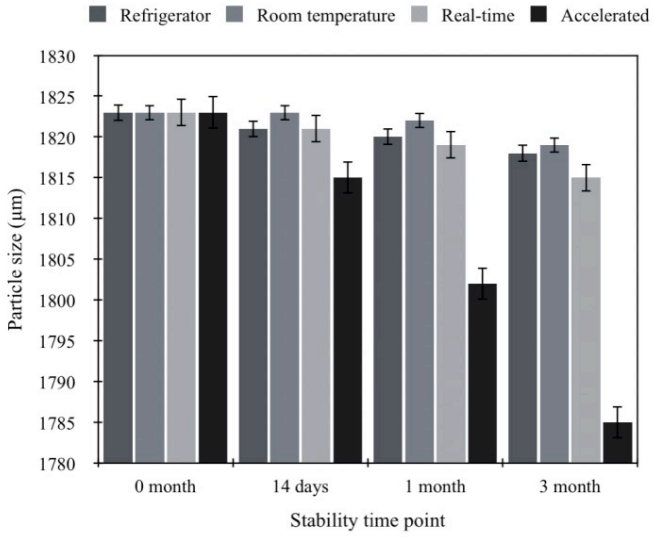


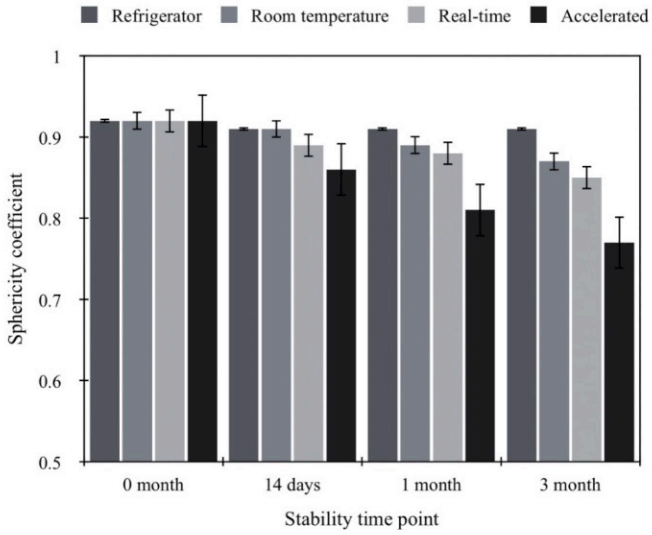
Figure 5: (A) Particle size (µm) of the RJ beads at different stability time point stored in PET container (n=30) (B) Particle size (µm) of the RJ beads at different stability time point stored in glass container (n=30)

3. Sphericity coefficient

The sphericity coefficient of RJ beads should be near to 1.0 as indication of perfect circular shape is attained during electrospraying process (Azhar et al., 2021). Figure 6 illustrates sphericity coefficient values of the randomly selected RJ beads (n = 30) in four storage conditions. All the sphericity coefficient values did not reach below 0.75. It shows that the spherical shape of the beads is maintained regardless of the storage conditions and time points. On top of that, there was no significant difference ($p > 0.05$) between sphericity coefficient values of RJ beads stored in glass and PET bottle in refrigerator, room temperature and real-time stability chamber at 0 month and 14-day time point.

However, the sphericity coefficient values of RJ beads decreased in significant manner ($p<0.05$) for both containers inside accelerated stability chambers. It ranged from 0.81 at 1 month to 0.77 at 3 months in glass container and from 0.81 at 1 month to 0.76 at 3 months in PET container. These results are in agreement with the findings that increasing storage temperature of calcium-alginate beads yields more irregular internal structure, hence decreasing the rupture strength of the beads (Jeong, Kim, Lee, Cho, & Kim, 2020). Nevertheless, the sphericity coefficient of RJ beads in both type of storage container stored in refrigerator manifests insignificant changes for 3-months stability time point indicating good physical stability of RJ beads.

(A)



(B)

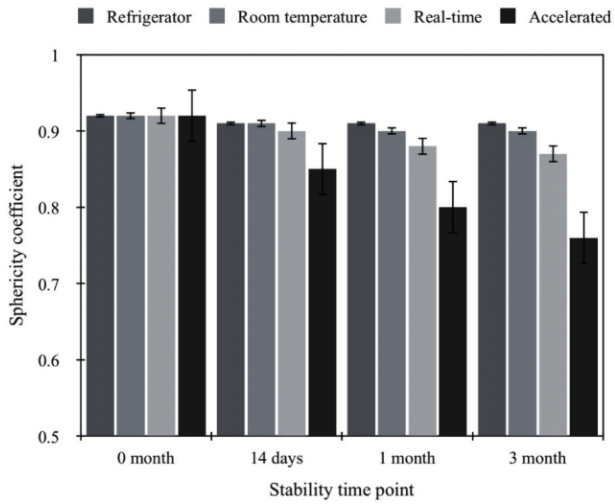


Figure 6: (A) Sphericity coefficient of the RJ beads at different stability time point stored in glass container (n=30) (B) Sphericity coefficient of the RJ beads at different stability time point stored in PET container (n=30)

4. Microscopic morphology

Figures 7 and 8 manifest the microscopic morphology of random RJ beads sample under different magnification of SEM at 0 and 3-month storage in refrigerator, room temperature, real time, and accelerated stability chambers. It is observed that microscopic morphology of the beads remained relatively indifferent after 3 months of storing in both glass and PET container.

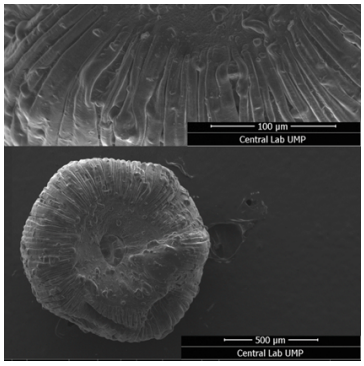


Figure 7: SEM photographs of RJ beads of selected samples at 0-month time-point (A: Surface at magnification 100 µm; B: Surface at magnification 500 µm)

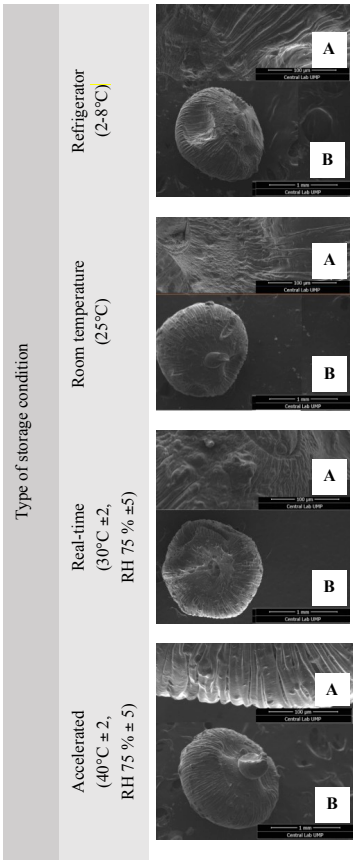


Figure 8: SEM photographs of RJ beads of selected samples at 3-month time point (A: Surface at magnification 100 µm; B: Surface at magnification 1 mm)

5. 10-HDA assay content

10-HDA is one of the main bioactive compounds of RJ that is only found in RJ in nature (Khazaei, Ansarian, & Ghanbari, 2018). Thus, the present of 10-HDA can be used as a marker to validate the freshness and quality of RJ (Antinelli et al., 2003; kim & Lee, 2010). Table 1 summarises the variation of 10-HDA content in four different conditions.

Analysis using one-way ANOVA inferred that the difference in 10-HDA content (%, w/v) in RJ beads stored in accelerated stability chamber was the only one that is statistically significant ($p<0.05$) for 1-month and 3-months compared to 0-month time point (Table 1). Both types of storage container, PET and glass type manifest similar findings with no significance ($p> 0.05$). Nonetheless, the 10-HDA content of RJ in alginate-pectin beads store in refrigerator remain the same throughout the study for both type of storage container.

This result is in accordance with the colour changes reported in this study. Changing of RJ beads colour is correlated with degradation of 10-HDA content. Maintaining required amount of 10-HDA is encouraged as a low 10-HDA content implies a low RJ activity (Muñoz, Decap, Ruiz, Arbildua, & Monasterio, 2011). The variation of 10-HDA content (mg/mL) between stability time points and type of container can be optimised by proper standardisation of the procedure in preparing the samples. In addition to the above-mentioned measure, the declining pattern of 10-HDA content occurred in the beads stored in room temperature and both real-time and accelerated stability chambers signals RJ deterioration.

Nutraceutical products, which include the formulation of RJ beads contain numerous phytoconstituents of different chemical classes (Maghsoudlou et al., 2019). These constituents may undergo various inter- as well as intra-molecular reactions under the influence of varied environmental conditions, such as heat, humidity, air and/or light during processing, formulation and storage of the material (Maghsoudlou et al., 2019; Ramadan & Al-Ghamdi, 2012).

10-HDA is a major fatty acid in RJ can degrade under the influence of heat and light which may, in turn, alter the actual content, shorten shelf-life and reduce therapeutic efficacy of the final products. Recent studies propose that 10-HDA is the leading indicator in the determination of the freshness, however, inconsistent stability of this fatty acid will cause as long-term challenge for the standardisation of RJ formulation (Shen et al., 2015). In this study, 10-HDA represents an adulteration indicator and should be

above 1.4% for fresh RJ (Abdulqader Yaslam Bazeyad, Ahmad Abdullah Al-Ghamdi, & Yehya Zaki Alattal, 2022). The values for the analysed RJ samples were within the limits proposed by the ISO RJ International Standard (International Organization for Standardization, 2016), which sets the minimum concentration of 10-HDA is 1.4% for pure RJ.

In brief, the best storage conditions to preserve critical quality attributes (CQA) of RJ beads especially the 10-HDA content itself is the refrigerator (2-8 °C) with the most practical and versatile packaging of PET. The low temperature of storage condition reduces the phenomenon of oxidation and hydrolysis reaction subsequently minimising physical and chemical degradation of RJ beads and later extending its shelf-life (Muresan et al., 2016).

6. Compression testing

Determination of degree of deformation in compression testing is crucial to assess the physical stability of the RJ beads during storage and transportation as well as its bioavailability when exposed to human gastric and intestinal environment (Rayment et al., 2009). The higher the Young's modulus being expressed in the compression testing, the higher the force needed for the beads to resist deformation phenomenon (Lee, Zhang, & Ryu, 2018).

Figures 9, 10 and 11 summarise all the Young's modulus values of RJ beads being stored in two types of containers (glass and PET) with four different storage conditions; refrigerator, room temperature, real-time and accelerated stability chambers. All the Young's modulus values were above 7 x 1000 Pa at all stability time points when the beads were tested air-dried as well as when being dipped into simulated gastric solution for 2 hours regardless the type of container used (Figure 9 and Figure 10). This proves that the encapsulation of RJ beads using alginate and pectin confers marked improvement of mechanical protection to the formulation in these both conditions. However, when the beads were subjected under simulated intestinal fluid for 2 hours, the values of Young's modulus exhibited substantial decreasing trends from 7.7 x 1000 Pa at 0 month to 2.32 x 1000 Pa at 3 month with statistical significance ($p<0.05$) (Figure 11). It was inferred that the decreasing pattern of Young's modulus is due to the reduction

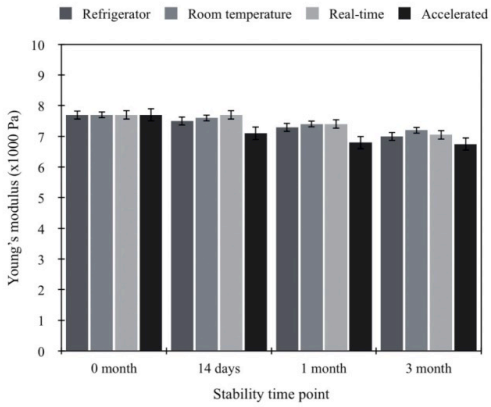
RJ beads rigidity in alkaline environment as compared to in acidic condition (Abu, Rasel, & Hasan, 2012). More open and porous polymeric network formed during rigorous swelling in alkaline environment. By increasing the dipping time of the beads in the simulated intestinal fluid, the activity contributes to the gradual reduction of the deformation and its corresponding Young's modulus values. The reason for this is that mannuronic and guluronic acids residue of alginate have pka values of 3.38 and 3.65 respectively.

The beads are stabilised by intermolecular hydrogen bonding network in a gastric environment where the pH value (1.5 – 3.5) is lower than the pka of the uronic acid (Pawar & Edgar, 2012). Meanwhile in simulated intestinal condition as the pH rises above the pka of the polysaccharides, the beads are expected to disintegrate due to the deprotonation of the polysaccharides of alginate beads leading to electrostatic repulsion and eventually disintegration of the beads (Chuang et al., 2017; Marciani et al., 2019).

Table 4: Results of intraday and interday precision for the simultaneous quantification of INH and PYR in pure form

Storage conditions	Stability time point	0-HDA content (%, w/v) in glass container	10-HDA content (%, w/v) in PET container
Refrigerator	0 month	1.83 ± 0.1	1.83 ± 0.1
(2-8 °C)	14 days	1.83 ± 0.7	1.83 ± 0.7
	1 months	1.83 ± 0.3	1.83 ± 0.9
	3 months	1.83 ± 0.1	1.83 ± 0.2
Laboratory (25 °C)	0 month	1.83 ± 0.1	1.83 ± 0.1
	14 days	1.83 ± 0.6	1.83 ± 0.5
	1 months	1.79 ± 0.4	1.79 ± 0.7
	3 months	1.77 ± 0.6	1.77 ± 0.1
Real time	0 month	1.83 ± 0.1	1.83 ± 0.1
(30 °C ± 2, RH 75 % ± 5)	14 days	1.83 ± 0.9	1.83 ± 0.2
	1 months	1.78 ± 0.6	1.78 ± 0.3
	3 months	1.75 ± 0.2	1.75 ± 0.7
Accelerated	0 month	1.83 ± 0.1	1.83 ± 0.1
(40°C + 2, RH 75 % ± 5)	14 days	1.83 ± 0.2	1.83 ± 0.6
	1 months	1.74 ± 0.8	1.74 ± 0.1
	3 months	1.71 ± 0.1	1.71 ± 0.1

(A)



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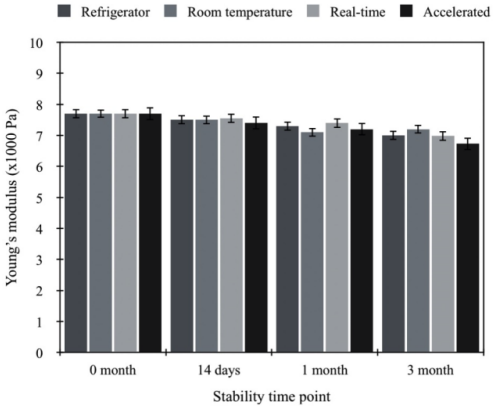
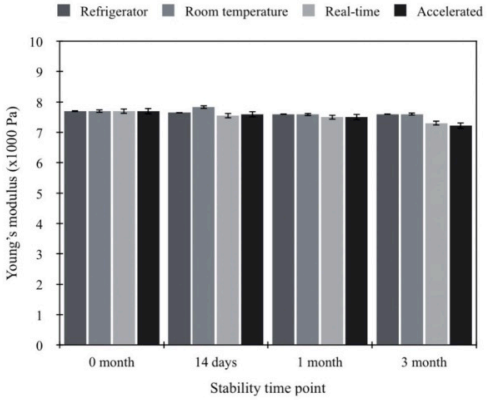


Figure 9: (A) Young's modulus of the RJ beads (air-dried) at different time point stored in glass container (B) Young's modulus of the RJ beads (air-dried) at different time point stored in PET container.

(A)



(B)

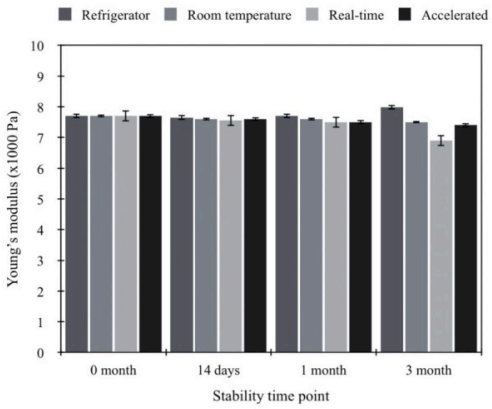
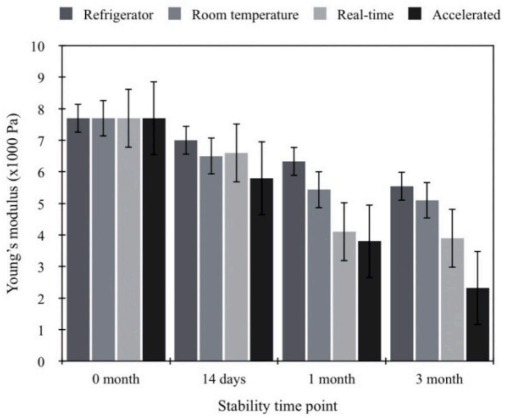


Figure 10: (A) Young's modulus of the RJ beads (dipped in simulated gastric fluid) at different time point stored in glass container (B) Young's modulus of the RJ beads (dipped in simulated gastric fluid) at different time point stored in PET container.

(A)



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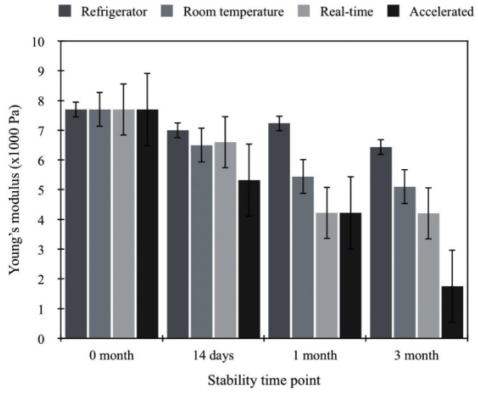

Figure 11: (A) Young's modulus of the RJ beads (dipped in simulated intestinal fluid) at different time point stored in glass container (B) Young's modulus of the RJ beads (dipped in simulated intestinal fluid) at different time point stored in PET container.

Conclusion

In conclusion, the study showed that the RJ beads exhibited convincing stability for 3 months when it is stored at low temperature. Analysis of 10-HDA content in the RJ-encapsulated beads as well as observation of its physicochemical properties that includes physical appearances, particle size, sphericity coefficient, microscopic morphology, and compression testing showed that the results are highly consistent across all stability time points. Environmental conditions especially temperature must be considered during beads preparation and storage since room temperature will accelerate physicochemical changes of RJ beads. Therefore, RJ beads must be stored at low temperature (refrigerator) to maintain the stability of formulation and provide a longer shelf life. Besides, this study also exhibits that colour changes of RJ beads is well correlated with the degradation of the 10-HDA compound. The investigation using different types of container substance proves similar results regardless of the storage conditions. In practicality, PET container offers higher superiority to the consumers compared to glass container as packaging for RJ beads due to its convenience in terms of low cost and lightweight. On top of that, opaqueness and inertness of PET container serve as additional advantages to protect the RJ beads from gradual deterioration to sunlight, heat and moisture.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Journal of Pharmacy

REVIEW ARTICLE

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An insight into the use and advantages of Carbopol in topical mucoadhesive drug delivery system: A systematic review

Nurul Ain Mohamad Hamdi[1,*], Nurul Atifah Azmi[1], Nurul Hanis Mohd Sabari[1], Ahmad Fahmi Harun[2], Muhammad Salahuddin Haris[1,3,*],

ABSTRACT

Introduction: In recent years, mucoadhesive topical application of mucous membrane has gained considerable interest among formulation researchers in advanced drug delivery systems. It has been identified as a potential route for both local and systemic drug delivery. A mucoadhesive agent is usually incorporated in the formulation to overcome the disadvantages associated with the conventional topical formulation. These disadvantages include low residence time of the medication on the site of application due to tongue movement and salivary washout in the intraoral formulation, mucociliary clearance in the intranasal application, and rapid precorneal elimination in the intraocular formulation. Carbomer or known as Carbopol is a mucoadhesive polymer that is widely studied for topical delivery of pharmaceutical agents to the mucous membrane. The use of Carbopol and its advantages in the mucoadhesive topical application has gained considerable interest with several published studies and is available in various grades. In this study, a systematic review was performed on the available literature that investigates the Carbopol application in mucoadhesive topical drug delivery. **Method:** A systematic searching strategy was performed in Scopus, ProQuest, and PubMed databases using predetermined search strings. A total of 778 articles were retrieved, however, only 25 articles met the inclusion criteria and were used for data synthesis. **Results:** The results showed that incorporation of Carbopol as mucoadhesive polymer hold multiple advantages in drug delivery namely excellent mucoadhesion effect, the prolonged residence time of the formulation, enhanced drug permeation, prolonged release of drug, pseudoplastic behaviour of the formulation, pH compatibility with all mucosal site, and biocompatible. **Conclusion:** This suggests that the incorporation of Carbopol can be an effective mucoadhesive agent for topical drug delivery systems.

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***Corresponding author:**

Email address: aenhamdi@gmail.com; solah@iium.edu.my

Authors' Affiliation:

[1] *Department of Pharmaceutical Technology, Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Malaysia.*

[2] *Department of Physical Rehabilitation Sciences, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*

[3] *IKOP Pharma Sdn. Bhd., Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*

JOP

Introduction

Mucoadhesive topical application is an interesting field among formulation researchers in advanced drug delivery systems (Duarah, Durai, & Narayanan, 2017; Kapileshwari et al., 2020; Matos et al., 2020). It is an external drug's introduction to the mucous membrane of the body part that exploits the property of bioadhesion of certain polymers (Kore, Shete, Desai, & Dnyanpeeths, 2013). Mucous membranes are found in many body parts including the eyes, respiratory tract, gastrointestinal tract, and reproductive tract (Netsomboon & Bernkop-Schnürch, 2016) that serves as a potential route for both local and systemic drug delivery (Kumar, Naik, Pradhan, Ghosh, & Rath, 2020; Srikrishna et al., 2017). Mucoadhesive drug delivery is adopted to resolve the disadvantages associated with the conventional topical formulation including low residence medication time on the site of application due to tongue movement and salivary washout in the intraoral formulation, mucociliary clearance in the intranasal application, and rapid precorneal elimination in the intraocular formulation (Netsomboon & Bernkop-Schnürch, 2016; Pagano, Giovagnoli, Perioli, Tiralti, & Ricci, 2020; Saisree et al., 2019; Sheshala, Ming, Kok, Singh, & Dua, 2019). Carbomer or is typically called Carbopol and and polyacrylic acid is a mucoadhesive polymer with the formula (CH2-CHCO2H)n that is widely incorporated for topical delivery of pharmaceutical agents to the mucous membrane (Arun Karthick, Ramya Devi, & Vedha Hari, 2018; M. N. A. Rahman, Qader, Sukmasari, Ismail, & Doolaanea, 2017; Sheshala et al., 2019; Suzilla, Izzati, Isha, Zalina, & Rajaletchumy, 2020).

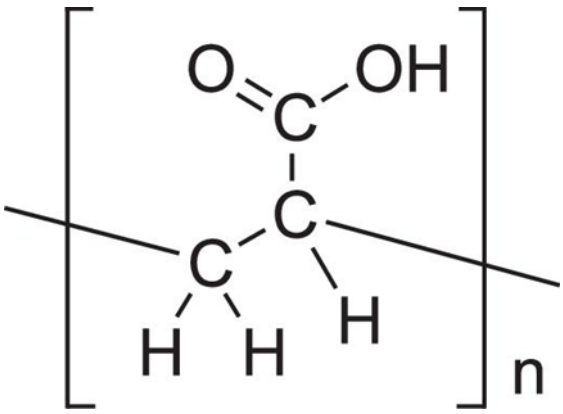

Figure 1. General structure of Carbopol polymer.

The most attractive properties of Carbopol as a mucoadhesive agent for topical application are biodegradable, bioadhesive, non-irritant, not absorbed into the body, and less expensive (Bera, Mazumder, & Khanam, 2016; Suzilla et al., 2020). Carbopol possesses several grades and is classified according to the degree of crosslinking, viscosity, and manufacturing condition, which offers flexibility in drug release profile as well as mucoadhesion (Singla, Chawla, & Singh, 2000). It is incorporated in various dosage form and various strength for topical oral, nasal, ophthalmic, and vaginal drug delivery. The main aim of this study is to systematically review the use of Carbopol according to its grade and formulation dosage form as mucoadhesive topical drug delivery particularly on the oral mucosa, nasal mucosa, ophthalmic mucosa, and vaginal mucosa based on recent studies. This review also aims to discuss the merits of Carbopol in topical mucoadhesive drug delivery.

Methodology

This section discusses the chosen method used to select articles related to the use of Carbopol in topical drug delivery. The reviewers used the PRISMA method that includes Scopus, ProQuest, and Pubmed to perform a systematic review in identifying, screening, eligibility, quality appraisal and data abstraction, and analysis.

1. The review protocol – PRISMA

This systematic review was guided by the PRISMA Statement.

2. Formulation of research question

The formulation of the research question for this review was based on PICo. It is a tool that facilitates the authors in generating relevant research question for the review. PICo is based on three key concepts namely Problem, Interest, and Context. Based on these concepts, the authors chose three primary aspects for the review namely Carbopol grade and strength based on the formulation dosage form (Problem), advantages of Carbopol in the topical application on mucous membrane such as oral, nose, eye and vagina (Interest), and drug delivery system (Context). These concepts have guided the authors to formulate the key research question namely what is the grade, strength, and advantages of Carbopol in topical mucoadhesive drug delivery system?

3. Systematic searching strategies

There are three main processes in the systematic searching strategies that include identification, screening, and eligibility.

3.1 Identification

Identification of the keywords followed by the process of searching for the synonym of the keywords was performed. The search string was developed including ((Carbopol OR caborpol OR cabormer OR cabormer OR carbapol) AND (mucosa* OR "mucosa layer" OR "mucosa membrane") AND (eye* OR ophthalmic* OR ocular* OR optic* OR oral* OR mouth* OR lingual OR periodontal OR gum* OR vagina) AND (mucoadhesive OR mucoadhesion OR bioadhesive OR bioadhesion)). The

search string was performed on three databases in which 300 articles were retrieved in Scopus, 468 articles in ProQuest, and 10 articles in PubMed.

3.2 Screening

Screening of the articles was conducted automatically based on the sorting function available in the databases according to the inclusion and exclusion criteria as shown in Table 1. A total of 615 articles was excluded after the sorting function whereas 3 duplicate articles were removed.

Table 1: Inclusion and exclusion criteria of this systematic review

Criteria	Inclusion	Exclusion
Publication timeline	2016 - 2020	2015 and before
Document type	Original research articles	Conference proceeding, chapters in book, book series, books etc
Language	English	Non-English
Nature of the study	Focus on topical drug delivery	Focus on other than topical drug delivery

3.3 Eligibility

Eligibility is the third process whereby the authors read the full text of the articles to ensure that the articles met the inclusion criteria. A total of 160 articles were assessed and 87 articles were excluded due to lack of details regarding Carbopol used on the mucous membrane in the topical application. This resulted in only 73 articles proceeded to the quality appraisal step.

4. Quality appraisal

Quality appraisal was conducted to ensure the quality content of the articles. All 73 articles were assessed individually by 3 authors and these articles were ranked to either high, medium, or low-quality article based on the predetermined criteria. The criteria were established based on the research questions. Mutual agreement between the authors was practised in this process to reduce bias. The authors concluded to only include high-quality articles hence, only 25 articles proceeded to the data abstraction and analysis step (Figure 2).

5. Data abstraction and analysis

This study used a qualitative technique to analyse the data. Data abstraction was conducted based on the research questions in which 2 of the authors categorised each article into the dosage design, the drug used, site of application, Carbopol grade and strength, and the advantages of Carbopol based on the summary of the article.

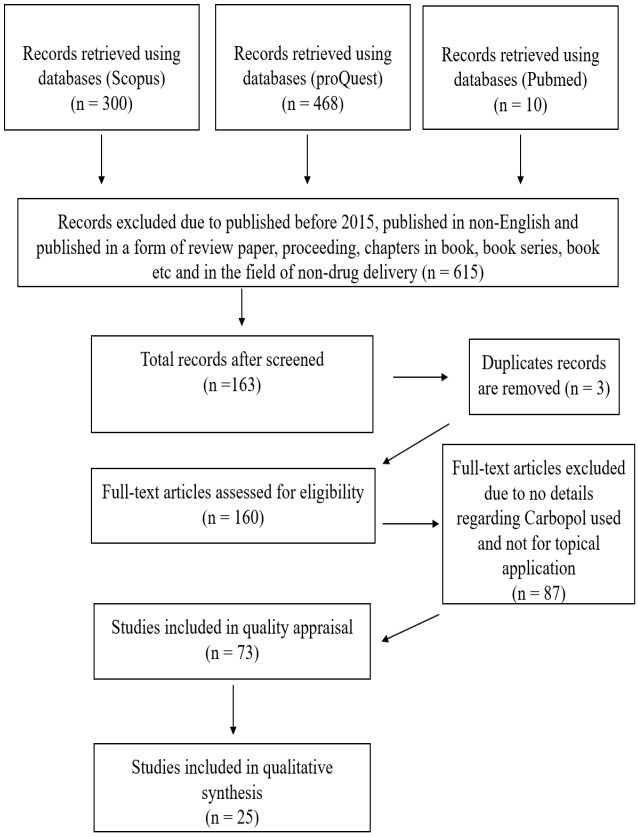

Figure 2. The PRISMA flow diagram.

Results

1. Background of the selected articles

The total selected articles were 25 of which 2 articles were published in 2020, 6 in 2019, 4 in 2018, 8 in 2017, and 5 in 2016. These articles were evaluated for Carbopol application to the oral mucosa, nasal mucosa, eye mucosa, and vaginal mucosa. The results showed that 14 articles incorporated Carbopol for oral mucosa application, 5 studies in the nasal mucosa, 5 studies in vaginal mucosa while only 1 study in eye mucosa. This review has identified 7 formulated dosages, which is in situ gel, gel, tablet, patches, film, aerosol, and wafer. There were 5 grades of Carbopol polymer were documented namely Carbopol 940, Carbopol 974P, Carbopol 934P, Carbopol 971P, and Carbopol 980. The most frequently used Carbopol grade was Carbopol 940 in which it was reported in 10 articles followed by 8 articles utilised Carbopol 934P, 6 articles used Carbopol 974P, and 1 article each used Carbopol 971P and Carbopol 980. Table 2 summarises the included studies.

Table 2: Summary of the included studies.

Year	Ref	Dosage design	Drug	Site	Carbopol type and strength	Advantages
2020	(Li, Bao, Shen, Lalla, & Burgess, 2020)	In situ gel	Bupivacaine	Oral	974P (0.08%)	Superior mucoadhesion and greater swelling
2019	(Mohamad, Abdelkader, Elrehany, & Mansour, 2019)	Tablets	Cyanocobalamin	Oral	971P (50% and 49% w/w)	Superior mucoadhesion, exhibit slow-release, good swelling rate
2016	(Patel, Prabhu, Dubey, & Kamath, 2016)	Buccal patches	Hydrochlorothiazide and atenolol	Oral	934P (100 mg – 300 mg)	Superior mucoadhesion, greater swelling, better permeation and biocompatible
2017	(Marques, Rocha, Leal, Estanqueiro, & Lobo, 2017)	Buccal gel of lipid nanoparticle	Ibuprofen	Oral	980 (1.5% w/w)	Superior mucoadhesion, higher firmness and exhibit pseudoplastic behaviour
2017	(Pham, Van Vo, Tran, Tran, & Tran, 2017)	Microemulsion-based wafer	Prednisolone	Oral	940 (1.5%)	Exhibit slow-release and superior mucoadhesion
2017	(Azeran et al., 2017)	Gel	Moxifloxacin	Oral	940 (0.3%)	Superior mucoadhesion, exhibit slow-release, and exert permeation enhancing effect
2017	(Sadeq & Rajab, 2017)	Patches	Captopril	Oral	934 (93.75mg and 18.75mg)	Superior mucoadhesion, greater swelling index, exhibit slow release and compatible
2016	(Kumria, Nair, Goomber, & Gupta, 2016)	Film	Prednisolone	Oral	940 (100mg, 75 mg and 50 mg)	Improved viscosity, superior mucoadhesion, slow and steady hydration, exhibit slow-release, and great drug permeation
2019	(Jain, Gilhotra, & Kori, 2019)	Hydrogel	L-glutamine	Oral	934P (0.25-1%)	Good consistencies and homogeneity, compatible, and exhibit slow release
2020	(T. A. Ahmed, Bawazir, Alharbi, & Safo, 2020)	Film	Simvastatin	Oral	940 (5% and 10%)	Uniform distribution, compatible (drug and excipient) superior mucoadhesion and exhibit slow release.
2017	(Ali, Sabati, & Ali, 2017)	Film	Baclofen	Oral	940 (1-5%)	Biocompatible, greater swelling, superior mucoadhesion, exhibit slow release and greater in vivo residence time
2018	(Aslani, Zolfaghari, & Fereidani, 2018)	Gel	Herbs	Oral	940 (0.5% and 1%)	Superior mucoadhesion and exhibit slow release
2017	(Calixto et al., 2017)	Liquid-crystaline system with in situ gelling	Peptide p1025	Oral	974P (2.5%)	Superior mucoadhesion and good pseudoelasticity and elasticity
2018	(Chaiprateep, Khobjai, & Noysang, 2018)	Film	Clinacanthus nutans	Oral	934P (7.5 to 24 g)	Superior mucoadhesion, increased elongation and tensile strength and greater swelling
2019	(Mahajan, Shende, Dumore, & Nasare, 2019)	In situ gel	Tapentadol	Nasal	934P 0.1% w/v	Effective gelation viscosity and gel strength, good drug release and good mucoadhesive strength

2019	(Abdelnabi, Abdallah, & Elghamry, 2019)	In situ nanovesicular gel	Buspirone	Nasal	974P (0.3% & 0.5 % w/v)	Increased muchoadhesiveness, sustained drug release, increased bioavailability and has penetration enhancing effect
2016	(Ayoub, Ibrahim, Abdallah, & Mahdy, 2016)	Microemulsion based gel (mbg)	Sulpiride	Nasal	940 (0.5% - 2%)	Uniform spreadability, increased mucoadhesive force, enhanced nasal bioavailability, has penetration enhancing effect and prolong residence time
2017	(Malekar, Gondkar, Bhairav, Paralkar, & Saudagar, 2017)	In situ nasal gel	Naratriptan	Nasal	934 0.2% w/v	Prolong residence time, better mucoadhesive property and improved bioavailability
2016	(Shelke et al., 2016)	Thermoreversible nanoethosomal gel	Eletriptan	Nasal	934 (0.4% w/v)	Increased mucoadhesive strength, prolong retention, Increased absorption and better drug permeation

2. Grade and strength of Carbopol for oral mucosa application

Oral mucosa topical application reported 6 types of dosage forms namely in situ gel, oral gel, buccal mucoadhesive tablet, patches, film, and wafer. Different concentration of Carbopol was used according to the type of formulation. Carbopol ranging from 0.25% to 1.5% was used in the oral gel formulation whereas 1.5% of Carbopol 980 was used as a single gelling polymer in nanoparticle loaded gel formulation (Marques et al., 2017). A lower concentration was reported in 1 study whereby 0.3% of Carbopol 940 was used as a single gelling agent for oral gel formulation (Azeran et al., 2017). 0.25% to 1% of Carbopol 934P was used in combination with HPMC polymer and 0.5% to 1% Carbopol 940 was used in combination with Na-CMC carbomer for intraoral gel formulation (Aslani et al., 2018; Jain et al., 2019). Carbopol 974P was used in 2 articles for in situ gel formulation with 0.08% and 2.5% in combination with pluronic polymer and as single polymer, respectively (Calixto et al., 2017; Li et al., 2020). In addition, 3 articles utilised Carbopol 940 in mucoadhesive film formulation ranging from 1% to 10% and 1 article used 7.5 g to 24 g of Carbopol 934P with the combination of various polymers such as gum acacia, sodium alginate, polymethacrylates, and HPMC (T. A. Ahmed et al., 2020; Ali et al., 2017; Chaiprateep et al., 2018; Kumria et al., 2016). 1.5% of Carbopol 940 was employed in microemulsion based mucoadhesive buccal wafer (Pham et al., 2017) and 2 articles utilised 18.75 mg to 300 mg of Carbopol 934 for buccal patches formulation (Patel et al., 2016; Sadeq & Rajab, 2017). Meanwhile, 1 study incorporated 49% and 50% of Carbopol 971P in combination with HPMC and chitosan for mucoadhesive buccal tablet formulation (Mohamad et al., 2019).

3. Grade and strength of Carbopol for nasal mucosa application

For intranasal application, 0.5% to 2% of Carbopol 940 was used as a single mucoadhesive polymer for gel formulation (Ayoub et al., 2016). Meanwhile, 3 studies used Carbopol 934P with a concentration of 0.1% to 0.4% for in situ gel formulation with a combination of other polymers such as HPMC, gellan gum, xanthan gum, and poloxamer 407 (Mahajan et al., 2019; Malekar et al., 2017; Shelke et al., 2016). Furthermore, 1 study used 0.3% and 0.5% of Carbopol 974P with a combination of HPMC for in situ intranasal formulation (Abdelnabi et al., 2019). It can be observed that the incorporation of Carbopol as the only gelling polymer requires a higher concentration of up to 2%. In contrast, a lower concentration of Carbopol is needed below 0.5% when used in combination with other polymers.

4. Grade and strength of Carbopol for vaginal mucosa application

In single gelling agent for intravaginal gel formulation, 2 articles incorporated 1% Carbopol 974P (S. S. Rahman & Ahmed, 2019; Takalkar & Desai, 2018) while another study used 0.8% Carbopol 940 (Salah, Awad, & Makhlouf, 2018). An article studied 2 grades of Carbopol namely Carbopol 934 and Carbopol 940 both ranging from 0.5% to 1% with the combination of HPMC polymer for vaginal gel formulation (Choudhury & Roy, 2016).

5. Grade and strength of Carbopol for eye mucosa application

0.1% and 0.2% Carbopol 940 was used in in situ gel ophthalmic application in combination with HPMC polymer (Kouchak, Mahmoodzadeh, & Farrahi, 2019). Based on the results, it can be concluded that HPMC was the most frequently used polymer in combination with Carbopol for topical application on the mucosal membrane. Table 3 summarises the Carbopol use according to site of application and dosage form.

Table 3: Summary of the Carbopol use according to the site of application and dosage form.

Application site	Dosage form	Carbopol type	Concentration /amount
Oral mucosa	In situ gel	974P	0.08%, 2.5%
	Gel	980	1.5%
	Gel	940	0.3% - 1%
	Gel	934P	0.25% - 1%
	Tablet	971P	49%, 50%
	Patches	934	18.75 mg - 300 mg
	Film	940	1% - 10%
	Film	934P	7.5 g - 24 g
	Wafer	940	1.5%
Nasal mucosa	Gel	940	0.5% - 2%
	Gel	934	0.1% - 0.4%
	In situ gel	974P	0.3% and 0.5%
Eye mucosa	In situ gel	940	0.1% and 0.2%
Vaginal mucosa	Gel	974P	0.8% - 1%
	Gel	934/934P	0.5% -1%
	Gel	940	0.5% - 1%

6. Advantages of Carbopol used in mucoadhesive topical drug delivery system

There were 7 advantages of Carbopol in topical drug delivery namely excellent mucoadhesion effect, the prolonged residence time of the formulation, enhanced drug permeation, prolonged release of drug, pseudoplastic behaviour of the formulation, pH compatibility with all mucosal site, and biocompatible with mucous membrane. The most reported benefit is the excellent mucoadhesion property.

Discussion

Carbopol is a mucoadhesive polymer that is extensively used in drug delivery studies. There are several types of Carbopol in which dominantly applied in a specific part of the body based on their rheological properties. The availability of the various grades of Carbopol depends on the manufacturing condition namely polymerisation and degree of cross-linking of the polymer reflected by the viscosity. Carbopol that carry the letter P after the number means that they are of high purity that makes them suitable for oral use (Mariageraldrajan, 2007; Panzade & Puranik, 2010). The summary of findings of this systematic review is summarised in Figure 3.

1. Mucoadhesive effect

Mucoadhesion is a characteristic of a dosage form that can interact with the mucous layer covering mucosal epithelial cells (Ahmed & Bhaduri, 2017). It plays an important role in the drug absorption and bioavailability (Ahmed et al., 2020). Besides this property is also important to preserve high level of drugs at the application site and to prevent expulsion of the formulation. For example, in buccal patch or buccal film, adequate mucoadhesion is prerequisite for optimal performance because low mucoadhesion would result in spitting or ingestion of the formulation (Kumria et al., 2016). Strong mucoadhesion of formulation incorporated with Carbopol were reported in various formulations types such as buccal patch, buccal film, buccal wafer, oral gel, nasal in situ gel, vaginal gel and in situ eye gel (Ahmed et al., 2020; Choudhury & Roy, 2016; Kouchak et al., 2019; Kumria et al., 2016; Mahajan et al., 2019; Malekar et al., 2017; Marques et al., 2017; Patel et al., 2016; Shelke et al., 2016). Mucoadhesive effect of Carbopol is attributed to the strong interaction that exists between carboxyl group (COOH) of Carbopol and a component of mucous membrane called mucin. Mucin are large glycoprotein expressed by epithelial membranes and are a component of the mucous secretions that covers epithelial. Mucin has a protein core with carbohydrate side chain and is the target to improve drug retention. Chemically, Carbopol polymer having abundance of carboxyl groups

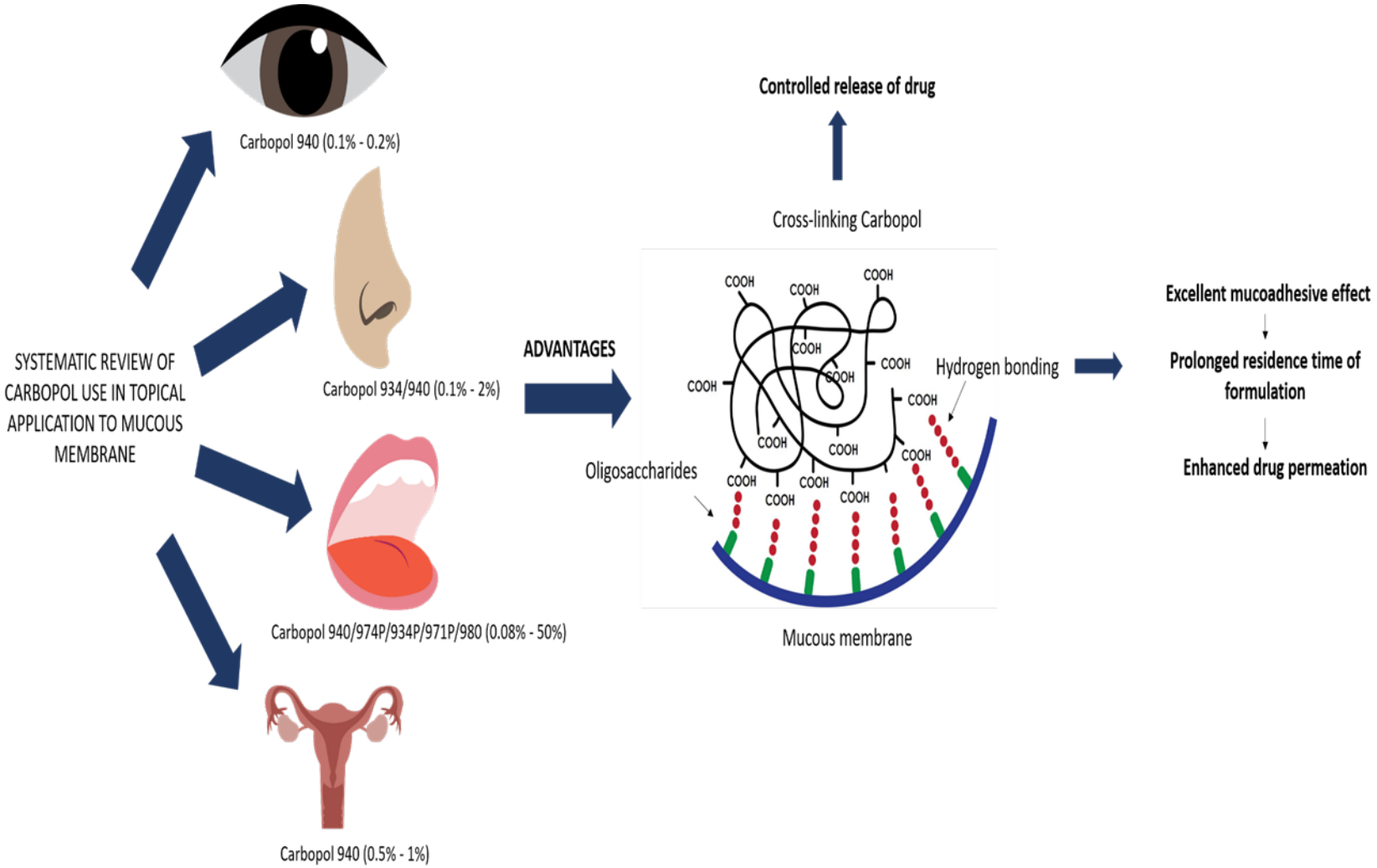

Figure 3. Summary of findings of this systematic review.

tend to form hydrogen bonding with amide group of mucin as proton accepting group (Shelke et al., 2016). Besides, studies showed that mucoadhesion of formulation were directly proportional with Carbopol concentration present in formulations (Ayoub et al., 2016). The plausible explanation is at higher Carbopol concentration, more interactions would be formed between Carbopol and mucous membrane leading to increase in mucoadhesion strength (Shelke et al., 2016). However, too strong mucoadhesive would damage the mucosal membrane (Kouchak et al., 2019).

2. Prolonged residence time of the formulation

At high Carbopol concentration, more compact lattice structure and more hydrogen bonding will be formed led to an increase in mucoadhesive strength. High mucoadhesive strength prolongs drugs retention and eventually inprove the absorption of drug across the mucosal tissue (Ayoub et al., 2016; Malekar et al., 2017).

In oral application, the retention time of prednisolone buccal wafer containing 1.5% Carbopol in ex vivo study was longer which is about 5 hours compared to formulation with lower Carbopol concentration (Pham et al., 2017). Similarly, another study found that the formulation of oral buccoadhesive films using Carbopol possesses greater mucoadhesive retention compared to without Carbopol content (Kumria et al., 2016). Dissolution of buccal film influences the retention time of the film. The high viscosity of Carbopol retard the dissolution of the film and subsequently increasing the film retention time (Kumria et al., 2016).

In nasal application, adequate mucoadhesive strength is important as this could help improving nasal drugs delivery as it prevents drainage from the nose cavity (Malekar et al., 2017). A pharmacodynamic study on the paw test was conducted to compare intranasal microemulsion based gel (MBG) over intranasal microemulsion (ME) of sulpiride. It was found that MBG has higher hind limb refraction time (HRT) values compared to ME of sulpiride. This demonstrated the role of Carbopol 940 incorporated in MBG in reducing the mucociliary clearance (MCC) and subsequently prolonging the residence time of sulpiride (Ayoub et al., 2016).

In the ophthalmic preparation, the drug's residence time plays a major concern in the formulation as it may influence the effectiveness of the drug. Prolonged residence time could result in a long duration of intraocular pressure (IOP) reduction and increase the efficiency of the in situ gel. The incorporation of Carbopol 940 (0.1 w/v) and HPMC (0.1% w/v) for in situ gel of dorzolamide HCl showed a longer and higher intra ocular pressure-lowering activity compared to dorzolamide solution and marketed drop. The prolonged residence time of the drug is attributed to its high viscosity and mucoadhesive property of the polymers. This subsequently increases the bioavailability and reducing administration frequency (Kouchak et al., 2019).

3. Drug permeation enhancing effect

Several studies have shown that the concentration of Carbopol improves the bioavailability and permeation of the drugs in certain formulations of oral and nasal application (Ayoub et al., 2016; Kumria et al., 2016; Mohamad et al., 2019). The administration of cyanocobalamine buccoadhesive tablet with 20% - 50% Carbopol 971P in combination with HPMC polymer exhibited a significant increase in the total amount of cyanocobalamin that enter systemic circulation that reflected by the estimated area under the curve (AUC) value (Mohamad et al., 2019). Similarly, enhancement in the rate and absorption of prednisolone have been reported in a study of prednisolone buccal film that use combination of HPMC and 50 mg of Carbopol 940. Higher AUC value of 2 folds was observed with buccal route compared to oral suspension formulation of prednisolone (Kumria et al., 2016). Good permeation ability of drug also reported with combination of 300 mg Carbopol 934 and HPMC in mucoadhesive buccal patch formulation of anti-hypertensive agents (Patel et al., 2016). The penetration enhancing effect are mainly due to the mucoadhesive properties as the residence time of formulation on mucosa also improved with the drug's permeation across the oral mucosa (Azeran et al., 2017).

The permeation enhancing effect of Carbopol was also reported with nasal application. Microemulsion based gel with 0.5% to 2% Carbopol 940 showed greater permeation of sulpiride compared to microemulsion after 24 hours. Carbopol exert the penetration enhancing effect by opening the tight junctions of the nasal mucosa thus promoting the transport of drugs via a paracellular pathway (Ayoub et al., 2016).

4. Prolonged release of drug

Carbopol plays important roles in sustaining the drug release for hours. Prolonged release formulation is desirable as it allows reduction of medication administration frequency and improving patient compliance (da Silva, Ferreira, Reis, Cook, & Bruschi, 2018). Various studies reported that incorporation of Carbopol polymer would retard the release of drugs from many formulations such as buccal tablet, buccal patches, buccal wafer, buccal film, in situ nasal gel and microemulsion based gel (Ali et al., 2017; Ayoub et al., 2016; Mahajan et al., 2019; Mohamad et al., 2019;

Patel et al., 2016; Pham et al., 2017). It was found that, with increase in concentration of Carbopol the release rates decrease gradually. Theoretically, drug was trapped by higher Carbopol concentration in which would exert resistance for the drug to travel through it. Additionally, drug movement area also would be limited by the density of chain structure especially at higher Carbopol concentration. Subsequently producing slower drug release rate for a longer duration (Ahmed et al., 2020; Ayoub et al., 2016; Mahajan et al., 2019; Patel et al., 2016; Pham et al., 2017).

A slow-release rate of cyanocobalamine reported from buccal tablet up to 5 hours duration (Mohamad et al., 2019). Meanwhile, longer release duration of more than 6 hours were reported with the formulations of combination antihypertensive agents (hydrochlorothiazide and atenolol) and prednisolone from buccal patches (100-300 mg Carbopol) and microemulsion based gel buccal wafer (1.5% Carbopol) respectively (Patel et al., 2016; Pham et al., 2017).

For nasal delivery, extended release of tapentadol for duration of 5 hours have been shown with in situ nasal gel formulation with 0.2% Carbopol (Mahajan et al., 2019). A longer release duration of naratriptan up to 8 hours was reported with formulation of in situ nasal gel that use combination of 0.2% Carbopol with 0.1% xanthan gum (Malekar et al., 2017).

For vaginal mucosa application, formulation of fluconazole gel with combination of HPMC and 0.5% Carbopol 940 show a constant and uniform drug release with around 80 – 85% drug release after 10th hour compared to formulation fabricated with sodium CMC and guar gum that complete the drug release within 10 hours (Choudhury & Roy, 2016). Besides, a study of nanogel of nevirapine formulated with 1% Carbopol 974P reported a zero-order kinetics suggesting the system release the drug at a constant rate for 6 hours duration (Rahman & Ahmed, 2019).

5. pH compatibility

The pH of oral cavity is maintained by the saliva with the normal pH range of 6.2 to 7.6 (Baliga, Muglikar, & Kale, 2013). It is advisable to keep the surface pH of the formulation similar to the buccal and salivary pH to minimise irritation to the oral mucosa (Kumria et al., 2016). In this review study, various forms of topical oral formulations namely buccal tablets, buccal patch, wafer, oral gel and buccal film have been successfully developed within the saliva pH value (Ali et al., 2017; Jain et al., 2019; Kumria et al., 2016; Mohamad et al., 2019; Patel et al., 2016; Pham et al., 2017; Sadeq & Rajab, 2017).

The normal nasal mucosa pH ranges between 5.5 -6.5 (England, Homer, Knight, & Ell, 1999). The pH of nasal formulation reviewed in this study were within the physiological pH of the nasal mucosa (Ayoub et al., 2016; Mahajan et al., 2019; Malekar et al., 2017; Shelke et al., 2016). The slight acidic pH is necessary for lysozyme activity (Takalkar & Desai, 2018). Lysozyme is produced in nasal secretions and is responsible for killing bacteria at an acidic pH and is ineffective under alkaline pH and could promote the nasal tissue to be vulnerable to microbial infection (Salah et al., 2018).

Similarly, the normal vaginal pH ranges between 3.8 to 5.0, which is moderately acidic. This slight acidic pH of vaginal mucosa is crucial to protect the vagina mucosa from pathogenic organism (Lin, Chen, Cheng, & Shen, 2021). Nevirapine nanoparticle loaded 1% Carbopol 974P gel and fluconazole gel (1.5% Carbopol 934) were formulated with desired physicochemical characteristics within the vaginal physiological pH (Choudhury & Roy, 2016; Rahman & Ahmed, 2019). In contrast, another study of miconazole microsponge gel was formulated with slightly higher pH which is 7 to prolong the retention of the gel at the vaginal mucosa to allow complete drug release as the maximum viscosity of Carbopol gel achieved at pH 6-7. The plausible explanation is that the neutral pH is not harmful to the vaginal mucosa as the pH of the semen ranged between 7.2-8 and also higher vaginal pH was reported in fungal infection (Salah et al., 2018).

Meanwhile, the normal pH range of tear lies between 6.5 to 7.6 (Abelson, Udell, & Weston, 1981). pH is an essential parameter of the eye's acceptance and tolerance with the formulation. With buffering capacities that tolerate the pH around 4-8, the pH of the tear is about 7.4. A pH value beyond this range will reduce the drug's bioavailability due to the stimulation of blinking and tearing (Kouchak et al., 2019). pH triggered in situ gel under was successfully formulated with combination of 0.1% carbomer and 0.1% HPMC and desired viscous gel formed under the physiological tear pH (Kouchak et al., 2019).

6. pH compatibility

Biocompatibility refers to the ability of a formulation in not causing toxicity or injury effects on living tissue (L. Guy, 1988). Carbopol is biocompatible to be administered since it does not irritate the mucous membrane. A study showed that after removal of buccal film containing (1-5% Carbopol 940), visual inspection of the mucosal tissue showed no evidence of mucosal injury to any of the polymers. No discomfort was reported by the volunteers during or after in vivo study of baclofen buccal film (Ali et al., 2017). Besides, sheep's nasal mucosa photomicrographs were observed for histopathological changes after permeation tests with in situ gel containing opioid for

nose to brain delivery. No sign of remarkable destructive effect of formulations on the treated nasal mucosa was observed (Mahajan et al., 2019). In another study, histopathological photographs were conducted where nasal mucosal membranes treated with thermoreversible gel revealed minor epithelial cell destruction, meanwhile, intact cellular integrity was seen in the untreated mucosal membrane. The physical impact caused by the application of gel and pH shock could be related to the cause of this injury. That may be attributed to the swollen aspect of Carbopol resulting in mild damage to the intact columnar cells of the epithelial cells. The absence of damage to the glands that secrete mucus, cell necrosis, and columnar cells indicated that these ethosomal thermoreversible gels were healthy for nasal mucosa and could be used to treat migraine through intranasal path (Mei et al., 2017). For the vaginal drug delivery, histopathological findings concluded an absence of vaginal mucosal irritation indicated by normal cell lining without any vaginal mucosa injury. Another study also reported no clinical symptoms of irritation involving rash, inflammation, swelling, scaling, and irregular tissue formation hence suggesting that the Carbopol-based formulation is free from any irritation (Malekar et al., 2017). Additionally, there were no signs of clinical irritation of rabbit's eye with the application of pH triggered in situ gel in the ophthalmic drug delivery (Kouchak et al., 2019).

7. Pseudoplastic behaviour

The incorporation of Carbopol in the formulations exhibit a non-Newtonian pseudoplastic behaviour with yield stress. Pseudoplastic behaviour helps to facilitate liquid flow out from its container. In the formulation of in situ gel for dorzolamide, Carbopol 940 NF and HPMC showed a lower pseudoplastic behaviour in the physiological condition compared to the non-physiological condition (Kouchak et al., 2019). Force application on the buccal mucosa causes the breakdown of the gel network structure hence making it easier to spread on the mucosa (Marques et al., 2017). In order to sustain the site-specific action for a longer period, pseudoplastic behaviour is desired for topical application (Azeran et al., 2017).

Conclusion

The result of this systematic review revealed that Carbopol 940 is the most frequent carbomer grade incorporated in mucoadhesive topical drug delivery formulation. The concentration of Carbopol polymer incorporated in a formulation varies according to the type of pharmaceutical dosage form and application site. Carbopol polymer exhibits various advantages in mucoadhesive topical drug delivery systems apart from mucoadhesive behaviour alone. Carbopol as a mucoadhesive polymer benefits drug delivery in various ways namely excellent mucoadhesion effect, the prolonged residence time of the formulation, enhanced drug permeation, prolonged release of the drug, pH compatibility with all mucosal sites, biocompatible and pseudoplastic behaviour of the formulation. Thus, it is suggested that the incorporation of Carbopol can be an effective mucoadhesive agent for topical drug delivery systems.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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REVIEW ARTICLE Open Access

Infection Control in Digital Era: Future or Futile?

Rehab Ismaeil[1], Abdul Rahman Fata Nahas[1], Mohamad Haniki Nik Mohamed[1*], Norhidayah Kamarudin[2] and Mohd Basri Mat Nor[3]

ABSTRACT

New technologies are being developed and marketed to healthcare facilities all over the world as a way to stop healthcare- associated infections. The Internet of Things and artificial intelligence have been created with a variety of capabilities to improve people's health, offer necessary services, and monitor their health The potential adoption of these technology in automated surveillance and automated hand hygiene compliance monitoring systems has a lot to offer health care systems. However, the success or failure of the use of technology will depend on the awareness of the challenge and the establishment of a strategy, goals, and processes to support technology deployment, maintenance, and training. System differences between nations and a lack of standardization in the application of digitalization in health care hinder this technology from providing the full range of potential benefits. In this review, we explore the use of technology in the areas of automated infection surveillance in healthcare-associated infection and hand hygiene compliance, with an emphasis on the difficulties in developing such technologies.

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***Corresponding author:**

Email address: haniki@iium.edu.my

Authors' Affiliation:

[1]Department of Pharmacy practice, Kulliyyah of Pharmacy, International Islamic University Malaysia (IIUM), Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.

[2] Department of Medical Microbiology, Kulliyyah of Medicine, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.

[3]Department of Intensive Care, Kulliyyah of Medicine, International Islamic University (IIUM) Jalan Sultan Ahmad Shah, 25200 Kuantan, Malaysia.

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Introduction

Digital technologies are becoming increasingly important in almost every aspect of life. A wide range of digital health technology (DHT), including Internet of things (IOT) and artificial intelligence (AI), Mobile health applications, telehealth services and big data are widely employed in health care service, and a slew of solutions have arisen to mitigate the impact of COVID-19 (Vaishya et al., 2020; Vidal et al., 2020). The COVID-19 has greatly accelerated digitalization and introduced new challenges and potential for infection prevention and control (IPC) (Javaid et al., 2020; Kalhori, et al., 2021). It has prompted healthcare systems to use new IPC technology and approaches (Vidal et al., 2020; Wang et al., 2020). which can improve the system's usability, efficacy, and level of care (Fitzpatrick et al., 2020; Torous et al., 2020).

Growing healthcare- associated infections (HAIs) reporting rates make it clearer that patient safety, healthcare quality, and preparedness for infectious disease casualties all need to be improved. As a result, surveillance measures act as the first line of defense against HAIs, highlighting the need of implementing efficient surveillance (Degeling et al., 2019; Parreco et al., 2018; Villamarín et al., 2020). The Traditional HAIs surveillance approaches are considered a sort of passive monitoring, which depends on case reporting through manual screenings however, it has shown to be time-consuming and unreliable (Du et al., 2014; Streefkerk et al., 2019). Meanwhile, with modern health care technology, monitoring can be aided by the use of the sophisticated algorithms machine learning (ML) and deep learning (DL) that built on data seek to early detection populations at-risk and keep track of an estimate of the prevalence of HAI to improve the emphasis of preventative interventions (Parreco et al., 2018; Li et al., 2019; Liao et al., 2019). By adopting automated monitoring and infectious disease detection methods, hospitals can improve the quality and safety of patient care (Streefkerk et al., 2020; Yesmin et al., 2022).

Handwashing is an easily accessible and cost-effective infection control behavior to reduce HAIs. The COVID-19 pandemic has renewed calls for increased handwashing to stop the virus's spread (Stangerup et al., 2021). Despite this the compliance is very low (Stangerup et al., 2021). Hand hygiene compliance (HHC) continues to be a global challenge, indicating that knowledge and awareness are insufficient to change behavior (Sadule-Rios& Aguilera, 2017; Clancy et al., 2021). A crucial component of multimodal techniques to enhance hand hygiene (HH) is monitoring HH. Direct observation (DO) is the gold monitoring standard for calculating HHC rates (Gould et al., 2017). However, the process is still not standardized, Hawthorne effect, which outlines how providers' behaviour changes when they realize that they are being monitored has sparked interest in new methods for checking HHC and prompted the creation of automated HH monitoring systems (AHHMSs) (Gould et al., 2017; Kelly et al., 2021; Wu et al., 2018). It can track hand hygiene compliance in "real time," avoiding the Hawthorne effect and allowing for more efficient data collecting for large groups of people (Kelly et al., 2021).

In this review, we examine the potential advantages of IPC digitalization for automated infection surveillance, predication of healthcare-associated infections and hand hygiene compliance. highlighting the challenges associated in implementing such technology.

Applications of Digital Technology in infection control

Automated Surveillance

Surveillance is crucial for infection control because it determines which prevention strategies should be given priority and enables programs to assess the success of their prevention efforts (Degeling et al., 2019; Cha & Kim, 2020). Technological developments and the gradual digitalization of health data enable more hospitals use electronic medical records (EMR) for automated HAIs surveillance. it is an innovative way to lower the infection incidence and produce novel disease control because it has been demonstrated to be more effective, reliable, lower costing and safe time in detecting infections than traditional surveillance (Streefkerk et al., 2019; Kelly et al., 2021). The efficiency of automated surveillance was revealed in many studies for instance, Real time nosocomial infection surveillance system (RT-NISS) was developed and validated in China by Du et al in 2014. The sensitivity and specificity of automatic hospital-wide HAIs surveillance system RT-NISS were 98.8% and 93.0% respectively, when compared to a manual survey of nosocomial infections (Nis) (Du et al.,2014). Study done by Blacky et al, in Vienna General Hospital showed that automated MONI-ICU (monitoring of nosocomial infections in intensive care unit (ICU) gives surveillance staff and physicians almost-real-time view of clinical markers for NIs with sensitivity, 90.3% (Blacky et al., 2011). Moreover, the InNoCBR

system, which was developed between 2010 and 2013, is an automatic HAI detection and categorization software that is commonly utilized at CHUO's Preventive Medicine (Ourense University Hospital Complex, Spain). Since its implementation at more hospitals in Galicia (Spain) in 2013, the InNoCBR system has become the standard system for HAI surveillance. InNoCBR achieves a high level of sensitivity (81.73 percent), specificity (99.47 percent), and a good positive predictive value (94.33 percent) when tested against the gold standard (Villamarín et al., 2019).

The potential impact of using AI tools in numerous aspects of healthcare is becoming more generally recognized. AI systems will be able to analyses, diagnose, and provide decision support for prevention and early intervention (Fitzpatrick et al., 2020; Li et al., 2019). ML, a subset of AI technology, can be used in clinical microbiology labs to identify and forecast diseases, enhancing patient safety. DL, a recently developed area of AI, has boosted accuracy greatly by utilizing new strategies, specialized software, and vastly larger datasets to find more complicated correlation in the data (Tobore et al., 2019). Park et al. published a study in 2021 that attempted to develop prediction models that physicians might utilize in the hospital setting to make clinical decisions based on DL and ML using laboratory data. The study found that using DL and ML might produce more accurate diagnosis findings than physicians (Park et al., 2021). AI usage for prediction or early detection of HAIs has a lot of potential in IPC (Fitzpatrick et al., 2020). For instance, the risk of nosocomial *Clostridium difficile infection* (CDI) has been predicted using ML technologies (Oh et al., 2018; Li et al., 2019). Parreco et al. investigated the effectiveness of three different ML-based models for the prediction of Central Line-Associated Blood Stream Infection (CLABSI). this study revealed that models for predicting patients with CLABSI had the highest accuracy, precision, sensitivity, and negative predictive value (Parreco et al., 2018). The quality, cost, and outcome are all impacted by the necessity of early diagnosis of these individuals (Parreco et al., 2018). The study established a non-invasive examination and inspection approach for ventilator-associated pneumonia (VAP) diagnosis using an electronic nose. The results show that an ML-based electronic nose can help patients gradually attain the idea of high-quality medical care while also improving their quality of life. 2019 (Liao et al.). The application of AI can improve patient risk assessment, provide real-time detection for more focused surveillance, and enable the development of targeted IPC interventions.

Automated hand hygiene compliance

HHC is one of the most important factors in reducing HAIs, and accurate HHC monitoring among health professionals is essential to delivering high-quality care. The gold standard method for evaluating HHC is DO and feedback, as measured by hospital auditors (Gould et al., 2017). However, the observation bias and the requirement for numerous observers over a long period of time limit its effectiveness. (Gould et al., 2017; Wu et al., 2018). With the help of automated HH-measuring technologies, IOT has a lot of potential to improve HHC. Xu and colleagues in 2021 investigate the impact of an IoT-based management system on HHC in a critical care unit. They found that although there was no decrease in NIs, the new method increased the rate of HHC among all workers (Xu et al., 2021). In another study in a hospital setting in Ontario, Canada to investigate the impact of IoT interventions on patient safety measures such as patient falls and HHC. It emphasized several key points about the use of IoT in healthcare. The HHC rates were increase in the first year followed by a reduction in the second year (Yesmin et al., 2022). Similarly, a study done by Marques et al, 2017 using IOT based automated monitoring systems in conjunction with gamification to enhance HHC among HCWs showed that there was an improving in the awareness of nurse HHC (Marques et al., 2017).

AI applications in hospitals have a significant impact on HHC. Computer vision, a type of AI, could provide a novel approach for performing more accurate and privacy-protected hand hygiene assessments. Depth pictures, which simply capture an image without allowing identification of the persons being watched or the ability to identify characteristics, are used to allay concerns about the use of video surveillance in places where

privacy is a concern (Awwad et al., 2019). In the study by Awwad et al., it was discovered that the system was more successful at identifying the supply of alcohol hand rub when it used computer vision and depth images for hand hygiene auditing. The study demonstrated that it could automate the direct observation of hand hygiene practice, which boosts clinical application and decreases privacy concerns (Awwad et al., 2019). The simplicity and accessibility of the dispenser allows for a stronger habit of hand washing, which has substantial potential benefits. Singh discovered that using a computer vision system to track use of hand sanitizer dispensers was equivalent to human observation. Given its capacity to be passive, inexpensive, privacy-safe, and sensitive, it may be helpful in attempting to eliminate an apparent recurring source of healthcare-induced harm due to its capacity to provide ongoing monitoring and feedback to clinicians (Singh & Sittig., 2020). On the other hand, hand sanitizer uses, or automatic dispenser activation counts cannot be used to assess HHC. As a result, the importance of embedding real-time feedback into AI applications to promote behaviour change has increasingly been emphasized (Lacey et al., 2020). Lacey and coworkers deployed an autonomous video auditing (AVA) system with real-time feedback at handwashing. The findings revealed that using AVA in conjunction with electronic monitoring enables for simultaneous auditing of providers' handwashing quality and quantity. But when the feedback was taken away, performance went back to normal (Lacey et al., 2020). Furthermore, according to a study done to evaluate the influence of The Sanibit electronic HH system on HHC and quality changes over time in ICU, a sensor-based platform with automated HHC and real-time feedback increased providers' HHC in an ICU (Xu et al., 2021).

Recently, the potential health benefits of wearable hand hygiene technology have received a lot of attention in the medical community. Some study has been done in hospital hand hygiene monitoring using wearable sensors (Li et al., 2019). Wearables-based systems does not require the installation of a camera, and it typically captures wrist movement data during a handwashing event using sensors, to detect handwashing steps in compliance with WHO recommendations. Wrist Wash (Li et al., 2019) is a widespread procedure involving the use of a wrist-worn platform that allows offline analysis for assessing the stages using a Hidden Markov Model-based method according to WHO criteria (Li et al., 2019). The results showed that user-dependent models had an average accuracy of 92 percent, whereas user-independent models had an average accuracy of 85 percent. However, when that assumption is relaxed, the results for relaxed performance drop considerably, from 85 percent to 69 percent (Li et al., 2019). The accuracy of a sensor wristband in monitoring adherence to WHO hand rub and handwashing guidelines was also tested by Wang et al (Wang et al., 2020). The limitations of camera-based technology were all overcome by this study. However, these are unsatisfactory in terms of gaining accuracy, reminding individuals to wash their hands, and offering feedback on the effectiveness of their handwashing (Wang et al., 2020). Over the years, a number of hand hygiene assessment systems have been developed to evaluate the quality of handwashing. The study on how a wearable device affected HHC and the quality of hand rubbing revealed that while HHC did not improve, the quality of HH activity did, with a substantial increase in both the amount of alcohol-based hand rub (ABHR) utilised and the amount of time spent rubbing hands. (Pires et al., 2021). Smartwatch-based automated systems for higher accuracy assessment handwashing quality have been developed. IWash is a smartwatch-based system for evaluating the effectiveness of handwashing that precisely identifies whether the user followed WHO guidelines or not. It uses voice to remind users to wash their hands frequently, especially as they enter the house, and to provide real-time feedback on the effectiveness of their handwashing (Samyoun et al., 2021).

Challenges

Data accessibility and the availability of sufficiently large data sets with high-quality and reliable data for data analytics are the initial barriers to the effective implementation of DT (Gianfrancesco et al., 2021). Healthcare professionals have spoken about difficulties with the healthcare system's data quality, including a high workload, enormous amounts of unstructured

data, a lack of diagnostic code sensitivity, and data extraction, that have an impact on the quality of the data and lead to an inaccurate assessment of the patient's current condition (Ni et al., 2019). Non-standard reporting, a lack of clarity, and a lack of validation are further implementation obstacles (Beam, Manrai, & Ghassemi, 2020). ML outcomes may become less capable of classifying or identifying comparable patterns in new data, depending on how the data was gathered and the learning algorithms were created (Conway., 2016; Park et al., 2021). Liu et al in 2019, provided a users' guide to improving research methodologies and training healthcare professionals particularly clinicians, on the fundamentals of ML, the necessity of efficient ML model validation, and effective methods for integrating ML models into clinical practice. this will make studies more credible and understandable, which will increase user confidence (Liu et al., 2019). Software developers, hospital IT employees, epidemiology experts, and IPC specialists must work closely together for the development and validation phases of data. This guarantees clinical applicability and permits the interpretation of results (Sittig et al., 2020).

The benefits of using technology must be evaluated against the serious ethical and legal concerns. Large-scale patient medical record use and sharing raises concerns about confidentiality and privacy by increasing the possibility of unlawful use, accessibility, and potential abuse of personal data (Kalkman et al., 2019; Char et al., 2020). Furthermore, even when patients have been given assurances of secrecy and privacy, there is a higher possibility that their personal information will appearing on social media when databases are breached (Gilbert et al., 2019). Digital tracking with the intention of conducting public health surveillance, have been found to be closely linked to serious privacy concerns (Zhao et al., 2021). It has been recognized as posing a significant risk to further disclosures of sensitive information. The ethical responsibility of the patient must be respected when using technology in healthcare to bolster the value of privacy (Zhao et al., 2021).

Online resources for infection prevention recommendations are abundant and easy to access. Internet connectivity issues negatively impact data availability and quality (Pollett et al., 2017). Lack of Internet connectivity, along with low Internet quality and stability, greatly hinder the use of digital technologies for infection control.

In Low- and Middle-Income countries (LMICs) with underfunded health systems and fewer providers, it might be crucial to concentrate on digitalization deployment (Jones et al., 2021). Hospitals frequently struggle with staffing and financial issues related to IPC programs. Lack of IPC training for staff members and inadequate adherence to IPC principles could exacerbate these problems (Lowe et al., 2021; Jones et al., 2022). Evaluating the potential and difficulties of implementing technology advancements is crucial to enhance assessments, development, ethics, usage, and monitoring technology techniques that might reduce the local burden in LMICs (Kruse et al., 2019; Jones et al., 2022).

Discussion

The potential use of digital technology in IPC could significantly reduce the risk of the spread of infectious diseases. However, it is limited by the awareness of the IPC challenge, objectives and processes that relate to the health system (Singh & sittig, 2015; Lowe et al., 2021). The effective use of technology to boost safety and infection control is essential for improving healthcare. Therefore, when providing care for their patients, all healthcare professionals must use technology correctly and completely. Inaccurate or inadequate data causes faulty predictions and improper outcomes. Reliability depends on the surveillance system's accessibility and connectivity to the completeness, and validity of EMR data (Streefkerk et al 2020; Gianfrancesco et al., 2021) in-addition to consideration of the ethical issues posed by applying ML in healthcare is necessary (Char et al., 2020). A systems-level approach is required for the optimum suited digital system's strategy, one that connects infection detection with case isolation, follow-up, and monitoring while also selecting the best interventions to control the infection (Beam, Manrai, & Ghassemi, 2020). Automated surveillance systems require monitoring systems to find problems and their causes, as well as ongoing maintenance and quality control, to assure improvements. Both the software and the methods need to be evaluated and perhaps updated in order to deliver safe and effective care (Siting et al., 2020).

The development of AHHMSs provided reduction in observation bias and offer the chance to continuously monitor and enhance hand hygiene procedures (Pires et

al., 2021; Lacey et al., 2020). The ability of any technology to be successfully embedded into the healthcare system is crucial. However, concerns over privacy are known to have an impact on HCWs' attitudes regarding automated monitoring (Awwad et al., 2019; Wang et al., 2020). They believe that these systems violate their right to privacy leads to refuse to change HH practices (Conway et al., 2016). The limitations of the algorithm must be considered before implementing AHHMS. The ML algorithms used to determine compliance with hand hygiene recommendations may produce inaccurate classifications that impair system accuracy (Wang et al., 2020). Moreover, the deployment of AHHMS at a healthcare facility is expensive requires infrastructure upgrades and maintenance expenses (Conway et al., 2016). The hospital system must be improved before addressing HHC to reduce HAIs. To accomplish this, future automated HH challenges should be addressed through additional research on the impact of systems on HH practices, the implementation of novel training and educational initiatives, and the determination of the best monitoring methods for improving monitoring outcomes.

Conclusion

Although the advent of digitalization in infection control holds out the prospect of real improvements in global public health, there are still many obstacles in the way, including issues with people's awareness of digital infection surveillance systems, their knowledge of HHC, and their concern for their privacy on maintaining data integrity and security. The healthcare system must weigh the benefits and drawbacks of these developing technologies to select the appropriate digital tools for their particular requirements and budgetary constraints additionally, the limitations of their infrastructure and organizational culture. Moreover, research are needed to determine whether using clinicians and healthcare experience in the context of enhancing infection control with digital technology is appropriate.

Conflict of Interest

There are no conflicts of interest.

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Journal of Pharmacy

ORIGINAL ARTICLE

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A comparative study: Impact of screen time on sleep quality among university students and school children

Nurul Qistina Mohd Kamaruzihan[1] and May Khin Soe[2,*]

ABSTRACT

Introduction: Screen time has been found to affect sleep quality negatively. Despite numerous studies proving that poor sleep quality and excessive screen time is prevalent among school children and university students, a comparative study on both age groups is yet to be explored extensively so far. This study focused more on school-aged children and university students, as they are often associated with sleep deprivation. The main objectives are to assess the association between screen time and sleep quality among schoolchildren and university students and to compare the effects of screen time on sleep quality between both age groups. **Method:** This study was conducted in Kulliyyah of Pharmacy, IIUM Kuantan, and six primary schools around Kuantan involving 100 undergraduate pharmacy students and 100 primary schoolchildren aged 10 to 12 years old. The participants were assessed using a self-administered online questionnaire consisting of demographic background, electronic device use, and Pittsburgh Sleep Quality Index (PSQI). The result was analysed using SPSS 23.0 software— descriptive analysis and Chi-Square test to determine the association between duration of screen time and sleep quality. **Results:** The mean duration of screen time among the participants is 5.5 hours (± 0.102). 56.5% participants have poor sleep quality. The PSQI score for UG students is significantly higher (mean score 6.7 ± 2.741) compared to children (mean score 5.54 ± 2.812) respectively (*p* value=0.001). The duration of screen time is weakly related to sleep quality. However, respondents with excessive screen time of more than 12 hours have a higher mean PSQI score. **Conclusion:** The findings revealed that majority respondents have poor sleep quality, independent of screen time. Further research with larger sample size is suggested for clearer comprehensive results.

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***Corresponding author:**

Email address: may_soe@iium.edu.my

Authors' Affiliation:

[1] *Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Malaysia.*

[2] *Department of Basic Medical Sciences, Kulliyyah of Pharmacy, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*

JOP

Introduction

It is a common phenomenon to see people carrying their smartphones everywhere, including going to the bed. The term "screen time" is referred to the activities done in front of a screen, such as watching television, working on a computer and laptop, or playing video games, which also includes spending time on social media (Kaneshiro Neil, 2019). Excessive screen time can be defined as the time spent in front of the screen for more than recommended. The recommended screen time is ideally 2 hours per day but it may vary according to the demographic background such as age group and occupation (LeBlanc et al., 2017).

Excessive screen time is often associated with short sleep duration and poor sleep quality. For example, university students often use laptops to do their assignments until late at night. Adolescents often use smartphones to access social media and to play games. According to a study done by Bhat et al. (2018), younger participants use electronic social media before bedtime more than older participants (Bhat et al., 2018). This finding is associated with insomnia and sleep disturbance on the particular group of population. According to Ohayon et al. (2017) on the National Sleep Foundation's sleep quality recommendation, several key indicators for good sleep quality include sleep latency of up to 15 minutes, fewer awakenings, and reduced wake after the sleep onset (Ohayon et al., 2017). The recommended sleep duration for schoolchildren aged 6-13, teenagers aged 14-17, and young adults aged 18-25 according to the National Sleep Foundation is 9-11 hours, 8-10 hours, and 7- 9 hours respectively (National Sleep Foundation Recommends New Sleep Times | Sleep Foundation, 2015). Hence, the recommended sleep duration is longer for younger ages. However, not all are aware of the importance of maintaining a good sleep quality that is essential for growing children and truly practicing it.

This study mainly focuses on school-aged children and university students that are categorised as young adults as they are often associated with sleep deprivation and poor sleep quality. A study done among college students at King Abdulaziz University on the relationship between sleep quality and the level of Internet addiction resulted in 54.4% of the participants having poor sleep quality (Abdulrahman Khayat et al., 2018). Students were well-aware of the importance of getting optimal sleep duration and quality, but very few were actually practising it or making it a priority habit (Dowdell & Clayton, 2019). This might lead to several consequences that may affect their health and academic performance. One factor that may influence sleep quality is excessive screen time, which is supported by many studies. Night-time phone usage is commonly associated with sleep quality where first-year university students reported having a very short duration of sleep compared to the recommendations (Whipps et al., 2018). The presence of smartphones nearby before sleep might disturb their sleep pattern. Moreover, a previous study found that sleep deprivation was caused by media device use, whereby 38.4% students agreed that the major cause of their sleep deprivation was internet addiction (Ranasinghe et al., 2018). As there are many studies proving that poor sleep quality and excessive screen time are prevalent among schoolchildren and university students, it is important to determine the association between them and whether excessive screen time influences their sleep quality in a negative way. This study aims to assess the association between the duration of screen time and sleep quality among schoolchildren and university students, to determine the effects of excessive screen time on sleep quality, and to compare the effects of a longer duration of screen time on sleep quality between schoolchildren and university students. The hypotheses suggested in this study is excessive screen time causes poor sleep quality in schoolchildren and university students.

Methodology

A cross-sectional study was conducted quantitatively among schoolchildren aged ten to twelve years old from primary schools in Kuantan and undergraduate pharmacy students in IIUM Kuantan Campus. The participants were assessed using a close-ended questionnaire, which consists of the demographic background of the participants, electronic device use, and the Pittsburgh Sleep Quality Index (PSQI). There are seven components in the PSQI which are subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbance, use of sleep medication, and daytime dysfunction. The questionnaire was provided in English and Malay. After receiving approval from IIUM Research Ethics Committee, this study was subsequently approved by the Ministry of Education and Pahang State Education Department. The survey was distributed by their respective teacher to the primary school students with the permission of the school headmasters. Parental consent was needed for schoolchildren to participate in this study. All participants were required to submit their consent form before answering the survey and their participation was voluntary. The result was analysed using SPSS 23.0 software— descriptive analysis and Chi-Square test to determine the association between screen times and sleep quality and to compare the result between schoolchildren and university students. The sample size was calculated with 95% confidence interval, 80% power and 0.29 odds ratio, the minimum recommended sample size is 98. Therefore, 200 (100 primary schoolchildren and 100 university students) were selected randomly as study subjects.

1. Inclusion criteria

1. Primary school children aged 10 to 12 years old, studying in a school in Kuantan, Pahang, able to write and understand English or Malay language, consented by their parents or guardian.

2. Undergraduate pharmacy students currently studying in Year 1, 2, 3, or 4 in Kulliyyah of Pharmacy, able to read and understand English.

3. Participation in both populations is voluntary.

2. Exclusion criteria

1. Children with significant physical and mental disabilities or chronic illness, age below 9 and above 12, or did not receive consent from their parents.

2. Students from other Kulliyyah in IIUM Kuantan, postgraduate students, students who cannot read nor understand English, students with chronic illness and physical or mental disabilities.

Results

1. Demographic background

Table 1 shows the descriptive statistics of the socio-demographic factors for both students and children. The mean age for the undergraduate (UG) students and children is 20.60 (±1.303) and 11.13 (±0.825) years old, respectively. 100% participants from the university are Malay while 91% from the school and minority of them are from other races.

The calculated mean BMI for both groups are 21.33 (UG students) and 19.23 (children). The basal metabolic rate (BMR) is calculated from the formula shown in Figure 1.

The mean BMR for UG students is 1411.33 (±168.07) kcal per day while for children is 1233.71 (±161.99) kcal per day. Majority of the UG students are lightly active (47%) while majority of the children are moderately active (40%). Total calorie requirement is calculated from the formula shown in Figure 2 with the calculated mean of 1859.97 (±281.08) kcal for UG students and 1738.18 (±311.03) kcal for children.

Table 1: Socio-demographic factors of participants

Socio-demographic factors		UG Students	Children
		n (%)	n (%)
Age* (years)		20.60 (±1.30)	11.13 (±0.83)
Gender	Male	26 (26.0)	46 (46.0)
	Female	74 (74.0)	54 (54.0)
Race	Malay	100 (100.0)	91 (91.0)
	Chinese	0	2 (2.0)
	India	0	6 (6.0)
	Others (Melanau)	0	1 (1.0)
BMI*		21.33 (±3.90)	19.23 (±4.68)
Height* (cm)		159.23 (±8.12)	142.44 (±13.03)
Weight* (kg)		54.10 (±3.90)	39.10 (±11.21)
BMR*		1411.33 (±168.07)	1233.71 (±161.99)
Activity factor level	Sedentary	43 (43%)	26 (26%)
	Lightly active	47 (47%)	32 (32%)
	Moderately active	10 (10%)	40 (40%)
	Very active	0	2 (2%)
	Extremely active	0	0
TCR* (kcal)		1859.97 (±281.08)	1738.18 (±311.03)

Notes: *Expressed in means ± SD
Abbreviations: BMI, body mass index; BMR, basal metabolic rate; TCR, total calorie requirement; PSQI, Pittsburgh Sleep Quality Index.

As for the activity factor level, 47% of UG students are lightly active followed by 43% sedentary and 10% moderately active. Meanwhile, 40% of schoolchildren are moderately active followed by 32% lightly active, 26% sedentary, and 2% very active.

$$BMR_{men} = 66.5 + (13.75 \times \text{weight in kg}) + (5.003 \times \text{height in cm}) - (6.775 \times \text{age in years})$$

$$BMR_{women} = 655.1 + (9.563 \times \text{weight in kg}) + (1.850 \times \text{height in cm}) - (4.676 \times \text{age in years})$$

Figure 1: Harris-Benedict equation for calculation of BMR

Total Caloric Requirements (TCR) = BMR x Activity Factor

- Little/ no exercise: BMR x 1.2
- Light exercise: BMR x 1.375
- Moderate exercise (3-5 days/week): BMR x 1.55
- Very active (6-7 days/week): BMR x 1.725
- Extra active (very active and physical job): BMR x 1.9

Figure 2: Calculation for TCR

2. Pattern of smartphone usage

The most used electronic devices among the participants are smartphones (81%), followed by computer/laptop (10%), tablet/iPad (4.5%), television (4%), and video games (0.5%) (Table 2). All UG students own a smartphone while 81% of schoolchildren own it. The mean age of first using smartphone for UG students is 13 years old, while for children is 8 years old. UG students spend average time using smartphone more than children do, which is 5-6 hours per day and 3-4 hours per day, respectively.

Most children spend more than 2 hours on electronic devices for academic purposes, but most UG students spend more than 2 hours for social media and entertainment. Other purposes of using electronic devices mentioned by the participants from the survey are online shopping, work-related purpose, reading e-books and online news, searching information, and communication. Majority of UG students (98%) and children (57%) used smartphone in the last hour before bedtime.

Most of UG students take smartphones to bed with them (88%) while majority of children (60%) are not. In addition, majority use smartphone as alarm clock, to text message and browse social media before sleep.

Table 2: Pattern of smartphone usage among participants

Questions		UG Students n (%)	Children n (%)	Total %
What type of electronic devices you use the most?	Smartphone	84 (42%)	78 (39%)	81.0
	Television	1 (0.5%)	7 (3.5%)	4.0
	Computer/ Laptop	10 (5%)	10 (5%)	10.0
	Tablet/ iPad	5 (2.5%)	4 (2%)	4.5
	Video Games	0 (0%)	1 (0.5%)	0.5
Do you own a smartphone?	Yes	100 (50%)	81 (40.5%)	90.5
	No	0 (0%)	19 (9.5%)	9.5
Age of first using a smartphone*		13.81 (±2.39)	8.8 (±2.53)	

Average time spent per day using smartphone (hours)			5.5	3.5	
Purpose of using electronic devices and daily usage	Academic	No use	0 (0%)	0 (0%)	0.0
		<30 minutes	12 (6%)	4 (2%)	8.0
		30 min-1 hours	20 (10%)	13 (6.5%)	16.5
		1-2 hours	20 (10%)	21 (10.5%)	20.5
		>2 hours	48 (24%)	62 (31%)	55.0
	Social Media	No use	0 (%)	20(10%)	10.0
		<30 minutes	1 (0.5%)	17 (8.5%)	9.0
		30 min-1 hours	13 (6.5%)	19 (9.5%)	16.0
		1-2 hours	33 (16.5%)	29 (14.5%)	31.0
		>2 hours	53 (26.5%)	15 (7.5%)	34.0
	Video Games	No use	47 (23.5%)	19 (9.5%)	33.0
		<30 minutes	21 (10.5%)	23 (11.5%)	22.0
		30 min-1 hours	11 (5.5%)	24 (12%)	17.5
		1-2 hours	13 (6.5%)	20 (10%)	16.5
		>2 hours	8 (4%)	14 (7%)	11.0
	Entertainment	No use	1 (0.5%)	17 (8.5%)	9.0
		<30 minutes	2 (1%)	22 (11%)	12.0
		30 min-1 hours	10 (5%)	27 (13.5%)	18.5
		1-2 hours	33 (16.5%)	21 (10.5%)	27.0
		>2 hours	54 (27%)	13 (6.5%)	33.5

	Others	No use	41 (20.5%)	38 (19%)	39.5
		<30 minutes	23 (11.5%)	30 (15%)	26.5
		30 min-1 hours	15 (7.5%)	19 (9.5%)	17.0
		1-2 hours	13 (6.5%)	4 (2%)	8.5
		>2 hours	8 (4%)	9 (4.5%)	8.5
Electronic devices used in the last hour before bedtime	Smartphone		98 (49%)	57 (28.5%)	77.5
	Television		1 (0.5%)	44 (22%)	22.5
	Computer/ Laptop		25 (12.5%)	2 (1%)	13.5
	Tablet/ iPad		12 (6%)	5 (2.5%)	8.5
	Video Games		3 (1.5%)	3 (1.5%)	3.0
Do you take your smartphone and/or tablet to bed with you?	Yes		88 (44%)	40 (20%)	74.0
	No		12 (6%)	60 (30%)	26.0
Do you use your smartphone and/or tablet as your alarm clock?	Yes		92 (46%)	60 (30%)	74.0
	No		8 (4%)	40 (20%)	24.0
Do you text or use a messaging app before sleep?	Yes		57 (28.5%)	79 (39.5%)	68.0
	No		43 (21.5%)	21 (10.5%)	32.0
Do you play games on your smartphone and/or tablet before sleep?	Yes		21 (10.5%)	61 (30.5%)	41.0
	No		79 (39.5%)	39 (19.5%)	59.0
Do you use social media on your smartphone before sleep?	Yes		92 (46%)	61 (30.5%)	76.5
	No		8 (4%)	39 (19.5%)	23.5

Notes: *Expressed in means ± SD

3. Sleep quality

A PSQI score of more than five indicates poor sleep quality. 56.5% participants have poor sleep quality. The mean global score for UG students is higher compared to children where their mean score is 6.73 and 5.54, respectively (Table 3). Other components that are significantly higher among UG students are Component 1 (subjective sleep quality) and Component 7 (daytime dysfunction).

The association of gender, age group (UG students and children), and screen time with sleep quality were determined using Chi-Square Independence test (Table 4). The result shows an association between age group and sleep quality, (p=0.004). Meanwhile, there is no significant association found between gender and screen time with sleep quality.

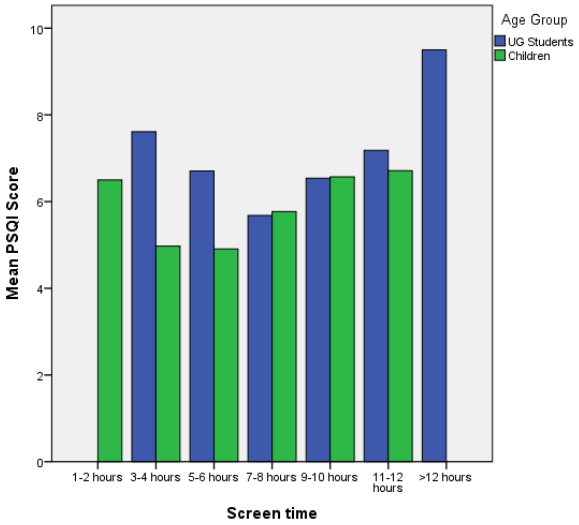

Figure 3: Graph association between screen time and sleep quality.

Table 3: Scores of each component of the Pittsburgh Sleep Quality Index (PSQI) and the Global PSQI Score

PSQI	UG Students Mean±SD	Children Mean±SD	*P*-value
Component 1: Subjective sleep quality	1.44 (0.857)	0.81 (0.775)	.000[a]
Component 2: Sleep latency	1.07 (1.008)	0.82 (0.821)	.108
Component 3: Sleep duration	1.57 (0.924)	1.56 (0.701)	.765
Component 4: Sleep efficiency	0.32 (0.634)	0.46 (0.881)	.538
Component 5: Sleep disturbance	1.05 (0.500)	1.06 (0.565)	.975
Component 6: Sleep medication	0.09 (0.473)	0.09 (0.429)	.749
Component 7: Daytime dysfunction	1.19 (0.720)	0.74 (0.705)	.000[a]
Global Score	6.73 (2.741)	5.54 (2.812)	.001[a]

[a] Significant at p<0.05

Table 4: Association of gender, age group, and screen time with PSQI sleep quality using Chi-Square test.

Variables	Pearson Chi-Square Value	df	Asymptomatic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Gender	0.634[a]	1	.426	.460	.258
Age group	8.972[a]	1	.004	.004	.002
Screen time	7.825[b]	6	.251		

a. 0 cells (0.0%) have expected count less than 5.
b. 2 cells (14.3%) have expected count less than 5. The minimum expected count is 0.87.

4. Other parameters

Of 29 respondents who are overweight, 15 of them are having poor sleep quality, while 7 out of 13 respondents who are obese have poor sleep quality. An Eta Coefficient test was used to determine whether there is an association between BMI and sleep quality. Thus, the following null hypothesis was tested:

H0 = There will be no association between BMI and respondents' sleep quality.

There was no significant association between BMI and sleep quality, $\eta = 0.011$. Thus, we cannot reject the null hypothesis and no significant association was found between BMI and respondents' sleep quality.

Discussion

1. Pattern of electronic device use

We found out that most respondents used smartphones the most, followed by computer and laptops. Previous studies showed that smartphones and laptops were the most used electronic devices among UG students and adolescents (Dowdell & Clayton, 2019; Ramesh et al., 2020). While all UG students owned a smartphone, 40.5% children in this study were using their parent's smartphone. In this study, it is observed that UG students spent more screen time compared to the children.

A previous study was conducted on the average time spent on electronic devices among schoolchildren. It was found that they spent 5 hours per day on electronic devices, indicating that children are exposed to excessive screen time (Unplagan et al., 2018). The prevalence of excessive screen time was also undeniably high among university students (Demirci et al., 2015). Most of the respondents in our study spent more than 2 hours on electronic devices for academic purposes, by which the children spent more for academic compared to UG students. This is reasonable in this era of COVID-19 pandemic where all of the teaching and learning method has been switched to online learning. Social media becomes the second lead purpose of using electronic devices which the UG students preceded the children.

Similarly, a study found that social media was used as a reference material to obtain current information in order to stay updated (Nazir et al., 2020). They tend to check their smartphone before sleep, or using it as an alarm (Dowdell & Clayton, 2019; LeBourgeois et al., 2017). Our study observed majority of them take their smartphone to bed, whether as their alarm or to check messaging app and social media before bed, or to play video games. Pharmacists could play an important role in educating the children on the effect of excessive screen time on sleep quality by campaigning at school as a community project. Educating the parents is also important to provide strict control on the children's screen time.

2. Evaluation of sleep quality

The mean PSQI global score for UG students and children suggested poor sleep quality for both groups. However, the results were just slightly over the threshold value (PSQI global score >5). This study found that majority of the respondents have poor sleep quality. This result is similar with a study by Ramesh et al. (2020) which found that 43.5% of the participants had good sleep quality while 56.43% of the participants had poor sleep quality. Our study observed a significant difference of the PSQI global score between UG students and children with a higher score among students, indicating UG students have worse sleep quality than children do.

In support with these findings, a study done by Nurismadiana & Lee (2018) on the prevalence of sleep quality among university students in a public university found that the prevalence of the students having poor sleep quality is higher among undergraduate students. Students are sleep deprived due to the tight academic schedule, academic pressure, and lack of supervision from parents (Dowdell & Clayton, 2019).

3. Sleep quality and screen time, gender, BMI

There is weak evidence of association between duration of screen time and sleep quality ($p=0.251$). In support for this finding, previous studies found no significant relation between duration of screen time before bed and sleep quality among medical students (Ramesh et al., 2020; Yeluri et al., 2020). They also found no association between genders, passive screen time, and sleep quality.

However, another study by Hrafnkelsdottir et al. (2020) found that greater screen time was associated with irregular sleep pattern in terms of bedtime, wake time, rest duration, and sleep duration among adolescents. There is no significant relation between daytime dysfunction and screen time in this study. However, respondents with excessive screen time of more than 12 hours have a higher mean PSQI score, specifically for the component of daytime dysfunction. Similar to a previous study, majority of their respondents experienced poor sleep quality and daytime lethargy because of excessive smartphone use at late-night (Syed Nasser et al., 2020).

Daytime sleepiness might affect the student's academic performance because they are unable to focus during the class. Sleep disturbance among adolescents are closely related to excessive screen time (Greever et al., 2017; Tao et al., 2017). Excessive screen time can disturb sleep quality although the exact mechanism is unknown. It is proposed that excessive screen time can disrupt circadian rhythm (Blume et al., 2019; Lely et al., 2014), delay bedtime onset (Dowdell & Clayton, 2019; Ghekiere et al., 2019), reduce sleep duration (Boonluksiri, 2018; Whipps et al., 2018), and increase daytime sleepiness

(Hershner & Chervin, 2014).

This study found no significant difference of sleep quality between genders, which is similar to previous study that found lack of consistency in gender differences concerning sleep quality (Farah et al., 2019). We found weak association in terms of BMI, activity level, and sleep quality, which is not in agreement with previous studies where they found association between sleep quality and BMI specifically in overweight and obese respondents (Krističević et al., 2018; J. Wang et al., 2019). Sleep quality might affect body weight because sleep deprivation leads to hormonal imbalance, which promotes weight gain through the production of leptin and ghrelin, hormones that control appetite (Beccuti & Pannain, 2011).

This study used a self-administered questionnaire to assess the screen time and sleep quality, thus recall bias could not be avoided. In addition, it limits the in-depth interpretation of the data, as the study method is a quantitative study. Further study in assessing qualitative aspects should be conducted to study more on the impacts of excessive screen time on sleep quality and other health-related parameters. Next, since the children need to use recall techniques to answer the questionnaire, bias could not be excluded. COVID-19 pandemic has also put a strain on this research. Data collection is limited to online survey for the participants in Kulliyyah of Pharmacy and limited contact with schoolchildren with strict standard of procedure to prevent the virus transmission. Further similar research with larger sample size focusing on children and adolescents is suggested for clearer comprehensive results.

Conclusion

Overall, this study found a promising significant finding for the association between duration of screen time and sleep quality among UG students and children. The majority of respondents developed poor sleep quality with a higher prevalence among UG students, specifically in the aspects of subjective sleep quality and daytime dysfunction, but both components are independent of screen time. This study highlights the effects of excessive screen time on sleep quality and the importance of having good sleep quality for the children and university students.

Conflict of Interest

The authors declare no conflict of interest in the journey of publishing this research article.

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