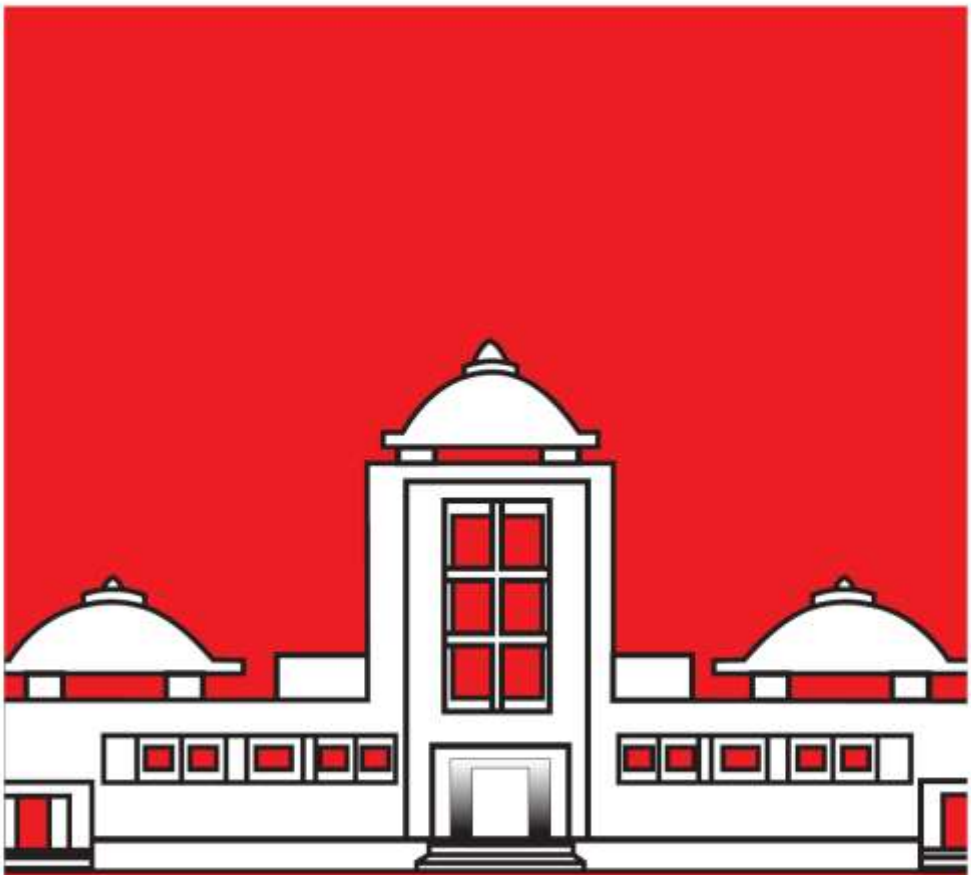
ISSN : 0973-6204

PHARMBIT

INTERNATIONAL JOURNAL

INDEXED IN "CHEMICAL ABSTRACT"

Vol 36, Jan - Dec, 2020



The Official publication of

PHARMACEUTICAL SOCIETY

DEPARTMENT OF PHARMACEUTICAL SCIENCES & TECHNOLOGY

BIRLA INSTITUTE OF TECHNOLOGY

MESRA, RANCHI, INDIA

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Contents

Remdesivir: An Anticipatory Treatment Against COVID-19

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Abstract

Remdesivir, also known as GS-5734, was discovered as an adenosine triphosphate analogue in ebola research in 2016 as a possible therapeutic agent. Its efficacy against the coronavirus family was also observed in 2017. Remdesivir is currently being researched as a possible agent for the management of SARS-CoV-2 (the virus that causes COVID-19). Remdesivir (GS-5734) is a parenteral antiviral drug having broad-spectrum pharmacological action. It was invented by a US-based biopharmaceutical company named Gilead Science with its collaboration with the Army Research Institute of Infectious Disease, USA.Remdesivir is a nucleotide analogue that acts as RNA-dependent RNA polymerase inhibitor and terminates RNA synthesis in the virus. Daily administration of the drug may accumulate in the body thus on basis of large-scale clinical trials 100 mg of dose is adjusted to avoid accumulation and ensure appropriate blood circulation in body after the first dose of 200 mg administration.

Keywords: Remdesivir, COVID-19, Nucleoside phosphate, RNA polymerase.

Introduction

Remdesivir, also known as GS-5734, was discovered as an adenosine triphosphate analogue in ebola research in 2016 as a possible therapeutic agent. Additionally, in 2017 its effectiveness toward the coronavirus family was observed. Nowadays, Remdesivir is again studied as a potential agent for SARS-CoV-2 (a virus liable for COVID-19) management. It received FDA Emergency Use Authorization on May 1, 2020. SARS-Co-2 enters in body through the S protein of ACE inhibitor available on the surface of the cell. Remdesivir is a nucleotide analogue that acts as RNA dependent RNA polymerase inhibitor and terminates RNA synthesis in the virus. Remdesivir added at the initial (I) stage is able to block further replication. It will continue to stretch three more nucleotides down to the I+III position before stopping.[1]

Its frequency of administration is once a day by intravenous route having a moderate duration of action. It may cause an increased level of transaminases and diminished its effectiveness in combination with hydroxychloroquine or chloroquine thus patient should becounseled properly for associated risk before its administration.[2]

Recommended loading dose regimen by FDA Emergency Use Authorization is 200 mg in a

day for patient ≥ 40 kg body weight or 5mg/kg in patient 3.5 to <40 kg body weight in a day followed by 100 mg for the patient with≥ 40 kg body weight or 2.5 mg/kg for patient 3.5 to <40 kg body weight in a day. Patients that do not need artificial ventilation or Extracorporeal Life Support (ECLS) should be treated for five days with the loading dose of 200 mg the first day and with the treatment being extended up to ten days if the patient does not improve.[2]

Chemical Identification

Type: Small molecule (602.6 g/mol)

Chemical formula: $C_{27}H_{35}N_6O_8P$

Chemical structure:

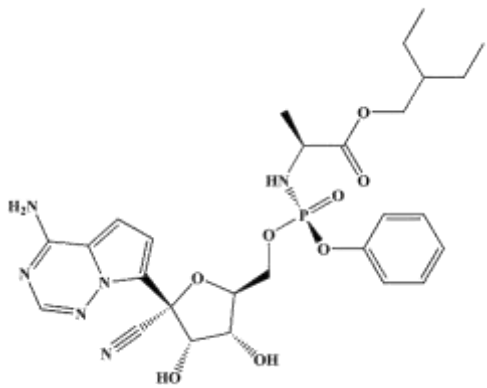

Figure 1. Chemical structure of Remdesivir

IUPACname-2-ethylbutyl(2S)-2-{[(S)-{[(2R,3S,4R,5R)-5-{4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl}-5-cyano-3,4-dihydroxyoxolanyl]methoxy}(phenoxy)phosphoryl]amino}propanoate.

Remdesivir's development

Remdesivir (GS-5734) is a broad-spectrum antiviral medication administered intravenously. Remdesivir was invented by a US-based biopharmaceutical company named Gilead Science with its collaboration with the Army Research Institute of Infectious Disease, USA. It is an RNA targeting-based therapeutic agent that was previously formulated for the treatment of hepatitis C and tested for efficacy against the Ebola and Marburg viruses but was found to be ineffective.

The beginning of the development of this molecule was based on the effectiveness of antiviral drugs targeting RNA viruses and focused on nucleoside analogue molecules. These analogues have poor cell permeability thus these nucleosides were modified to prodrugslike monophosphate, ester, and phosphoramidate which shows significant cell penetrability which further metabolized into nucleoside or phosphorylated nucleoside within the cell.[3-5] The 1′-CN modified adenosine C-nucleoside hit (GS-441524) was discovered to be highly active, as was a prodrug version of the monophosphate of GS-441524 (GS-5734, further renamed as remdesivir).[6]

Various groups evaluated antiviral activity in vitro and in vivo after establishing that GS-5734 (remdesivir) had broad activity against RNA viruses, authenticating its activity against coronaviruses.[7-9] Antiviral activity against the zoonoses, coronaviruses, SARS, and MERS, and even the emerging human coronaviruses HCoV-OC43 and HCoV-229E, which cause pneumonia, was reported.[10,11] Moreover, in a non-human primate in vivo model, de Wit et al. demonstrated that remdesivir also had therapeutic and prophylactic efficacy against MERS.[12]

Mechanism of action

Remdesivir is a nucleotide analougue and its triphosphate form produces complexes with RNA polymerase of viruses which inhibit viral RNA synthesis by chain termination mechanism of three species of coronaviruses SARS-CoV, SARS-CoV-2, and MERS-CoV (fig.2). Remedesvir triphosphate is similar to ATP which competes with nucleotide during viral RNA synthesis. Remdesivir added at the initial (I) stage can unable to block further replication. It will continue to stretch three more nucleotides down to the I+III position before stopping.[13]

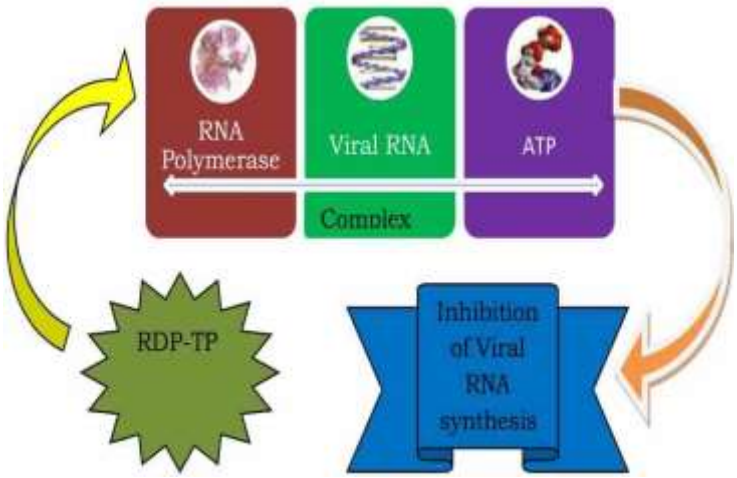

Figure 2. Mechanism of action of Remdesivir (RDP-TP: Remdesivir triphosphate)

Pharmacokinetic study

The first-pass effect of oral administration of remdesivir resulted in low bioavailability, according to pharmacokinetic studies in cynomolgus monkeys. In comparison to the control group, parenteral administration, especially intramuscular injections of 3 mg/kg body weight, had a 50% survival rate, whereas intravenously injections of 10 mg/kg body weight dose had a 100% survival rate.

In rhesus monkeys, Remdesivir rapidly decomposed into the original drug (nucleoside phosphate). Remdesivir was rapidly distributed in peripheral blood mononuclear cells (PBMCs) and easily triggered to nucleoside triphosphate, reaching a peak with a 100% survival rate.[14] As per investigation in PBMCs half-life of remdesivir is more than 35 h.[15] Daily administration of

remdesivir may accumulate in body thus on basis of large-scale clinical trials 100 mg of dose adjusted to avoid accumulation and ensure appropriate blood circulation in the body after the first dose of 200 mg administration.[16]

A single dose of 3-325 mg and 150 intravenous infusion solutions for two hours and 1 hour per day respectively reveals dose linear pharmacokinetics. Lyophilized formulation and solution of 75-150 mg over 2 hours have a similar pharmacokinetic profile. Remdesivir is generally metabolized to a triphosphate metabolite.[17]It was distributed within 4 hours to testes, epididymis, eyes, and brain on 10 mg/Kg intravenous administration in cynomolgus monkeys and 74% excreted through urine while 18% excreted through the feces.[18]

Conclusion

Seeing the covid-19 pandemic cases across the world most of the pharmaceutical companies, academics, government laboratories, and biotechnological companies have actuated their caliber to develop therapeutic agents or vaccines to overcome the above said crisis. Among the therapeutic agent against COVID-19 remdesivir have been proven for its in vivo and in vitro activity against coronavirus. Based on this fact USFDA has announced emergency use authorization of remdesivir for treatment of a hospitalized patient suffering from coronavirus. Whatever progress is made in clinical trials of remdesivir as a featured drug, it will make significant advances in the treatment of COVID-19 or will be a more promising therapy for other virus infections in the future.

Acknowledgement

All Authors are grateful to the KNIMT, FOP, Faridipur, Sultanpur for providing an excellent e-resources facility to conclude this study.

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Assessment of Prevalence of Patient Counseling Practice Among Community and Hospital Pharmacists

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Abstract

Proper dispensing of medicines encompasses the supply of correct medicine to the right patient, in the right dosage form and right quantities, in a manner to preserves the potency and quality of the medicine up to the labeled shelf life. It also includes clear and convincing counseling with appropriate follow-up action/reminders/inquiries about proper and timely administration of medicines. In achieving the objectives of rational use of medicines, patient counseling plays an important role. While counseling the patient, care must be taken to account for other prescription and/or non-prescription medicines/home remedies being consumed by the patient, as also his food and drink habits. Thus, the counseling aspect of every patient is not only different but also an individualized practice model. This study was conducted based on data collected through the circulation of questionnaires to practicing pharmacists. Analyses of the data revealed the prevalence of patient counseling practice. The results revealed that 90.58% of the pharmacists who responded are engaged in patient counseling of which 15.94 % offer partial counseling and 2.17% provide extensive counseling, while 72.46% are engaged in medication-related counseling. Results also present data analyses on various features of patient counseling, resources utilized, initiatives, reminder, and feedback steps taken by pharmacists to ensure compliance of medication regimen. Patient counseling parameters studied in this research prove to be best practice model to achieve strict adherence to the therapeutic regimen. Non-compliance with prescription instructions is a common issue across the globe. This emphasizes the role and importance of patient counseling to assure rational use of medicines.

Keywords: Patient counseling, Patient compliance, Pharmacotherapy, Adverse drug reactions.

Introduction

One of the universal duties of a pharmacist is dispensing medicines on the prescription of a medical practitioner. This function is the foundation of the edifice of the practice of pharmacy. In India, Pharmacy Act, 1948 regulates the profession and practice of pharmacy[1] as enshrined in the preamble of this Act. With astounding progress and advances in pharmaceutical sciences, the application of enriched technology in the design and development of dosage forms and formulations is the order of the day. Dispensing of the pre-packed, ready-to-use medicines has

become more complex a job than ever before. Prevalence of polypharmacy practice and fixed-dose combination products poses more chances of drug interactions and adverse effects. This necessitates compulsory patient counseling for rational use of medicines and compliance with the prescription for achieving the therapeutic goal. Patient counseling enhances medication safety, efficacy, and economy by ensuring improved patient outcomes.[2] Patient compliance is directly related to therapeutic outcomes and the success of the prescription. Strictly adhering to instructions given by way of appropriate and adequate counseling, benefits every patient.

The actual design of counseling content has to be determined after examining medication history, laboratory reports, and other inputs from the patient in addition to the prescription presented for dispensing. It must be appreciated that the clinical needs of each patient are different because of underlying diseases, food habits, drinking habits, chewing or smoking, and concomitant medications of the same as other systems of medicines. Lack of patient compliance is one of the reasons for adverse drug reactions and therapeutic failures in quite a sizable number of patients. Medication counseling by pharmacists significantly improves patient's awareness about their medicines and inclination for compliance. Thus achieving the goal of therapy becomes easy.

"Drugs don't work in patients who don't take them" was once emphatically stated by C. Everett Koop, a former Surgeon General of the USA.[3] World Health Organisation (WHO) in their official document [4] emphasizes on implementation of the concept of pharmaceutical care and underscores the importance of patient counseling as depicted below –

"With the development of specific and potent synthetic drugs, the emphasis of the pharmacist's responsibility has moved substantially towards the utilization of scientific knowledge in the proper use of modern medicines and the protection of the public against dangers that are inherent in their use."

In the same breath WHO also advocates:- *"In addition to ensuring an accurate supply of appropriate products, their professional activities also cover counseling of patients at the time of dispensing of prescription and non-prescription drugs, drug information to the health professional, patients, and the general public, and participation in health promotion programs."*

The above details make patient counseling an integral part of dispensing prescriptions and the duties & responsibilities of pharmacists. The science and technology involved in patient counselling are well developed, continuously updated/enriched, and profusely utilized across the globe. Authentic modules are developed for the general guidance of practicing pharmacists. Patient counseling as a subject is very vast and advancing very fast, as can be confirmed by the volume of information pouring on. The importance of behavioral strategy and communication skills of pharmacists in providing quality counseling and inducing a sense of compliance has been underscored.[5]

Patient counseling can be classified as – drug-related counseling, dosage form-related counseling, route of administration related counseling, frequency of administration related

counseling, side effects related counseling, potential toxicity related counseling, adverse drug reaction related counseling, drug-drug interactions related counseling, drug-food interaction related counseling, drug-drink interaction related counseling, storage of medicine-related counseling, duration of therapy-related counseling, and missed dose-related counseling as few to mention.

This exemplifies the growth of patient counseling as an important discipline. As a part of practice bases research pharmacists also carry-on assessment of the effectiveness of patient counseling.[6]Each component has its importance and impact on the overall success of therapy as well as safety aspects. As a part of practice-based research, a pharmacist has to develop his own updated document for guidance in consultation and collaboration with fellow practicing pharmacists and publish that for benefit of other practitioners.

It has been reported that poor patient compliance leads to failure in meeting treatment goals and affects the quality of life of patients. Lack of adequate counseling and failure to deliver specific information on appropriate utilization[7] of the dispensed medicines deserves the attention of health care policymakers.

The importance of patient counseling is further propelled by research findings reported in the WHO Policy perspectives on Medicines[8] which the author prefers to quote:

"Worldwide more than 50% of all medicines are prescribed, dispensed, or sold inappropriately, while 50% of patients fail to take them correctly."

The Science and technology of patient counseling is very vast and a well-researched area across many countries.[9-11] In practice, most of the patients are not informed of anything beyond the dose and frequency of administration and that too orally. This proves ineffective especially in the case of illiterate patients, who ultimately forget oral instruction by the time they reach home. Common drug-drug interaction, major side effects & storage conditions intricacies are seldom passed on to the patients, or their attendant. Guidance about the missed dose is another area that is invariably missed. Encouraging feedback and assessing the effectiveness of counselling are usually absent.

In light of the above background, this research throws light on the intricacies of patient counselling intending to enlighten and encourage fellow pharmacists to appreciate the importance of the subject and involve in literature building, research, and publication of findings related to it, that will serve as a milestone in establishing professional indispensability of pharmacists. Service only speaks and quality of service advertises importance without any effort.

Methodology

Module developed

A counseling module for tablet dosage form is the most complex because of the diversity of the types of a tablet as such and the functional purposes of such tablets. In this study, the tablet was taken as a prototype dosage form because almost approximately 90% of all the clinically

used drugs are available in tablet form. Tablet favors accuracy of dose, convenient handling, useful unit dosage form, and ease of administration.

More often than not a tablet is perceived to be swallowed with water and that's all. However, for a pharmacist dispensing a tablet is a complicated job. The counseling he has to offer depends upon the formulation type and functional type of the tablet. Tablets vary in size, shape, weight, hardness, thickness, disintegration and dissolution characteristics, and other aspects, depending on their intended use and method of manufacture. According to the intended use, the functional and non-functional excipients vary. Thus, a functional change in the properties of the tablet appears by varying the excipients and manufacturing operations. Therefore, the counseling aspect for appropriate administration/consumption by the patient inevitably differs. Assorted counseling relating to the administration of few types of tablets was devised as summarized in Table 1.

Table 1. Types of tablets and counseling intricacies

Sl. No.	Type of Tablet	Counseling tips	Example
1	Compressed/Uncoated tablet	The uncoated compressed tablets are meant for disintegration and dissolution in the gastrointestinal tract for rapid absorption. It must be instructed to take it intact i.e., without breaking, crushing, or chewing; with a glass of water (200 ml).	Bactrim d.s., BRUPAL Kid, CYCLOPAM, GLYCIPHASE G2Forte, Pyrigesic 650, Vasograin
2	Sugar-coated tablet	The label of the tablet indicates the nature of the coating. The coating is done to increase aesthetic appeal, mask taste/odor, improve stability, etc. It must be taken intact i.e., without breaking, crushing, or chewing; with a glass of water (200 ml).	Fersolate, UNIENZYME.
3	Film-coated tablet	Similar to sugar-coated tablets.	Atorva 5, CEFI XL 200, Erythromycin, Amitriptyline, Diclofenac potassium, Levosiz, Montina – L, O2 (Ofloxacin & Ornidazole tablets), Valsartan tablet

Sl. No.	Type of Tablet	Counseling tips	Example
4	Enteric-coated tablet	These tablets are coated with acid-resistant polymer to protect acid-labile drugs. These tablets pass the stomach intact and after reaching the small intestine they disintegrate, dissolve, and get absorbed. This tablet must be taken intact i.e., without breaking, crushing, or chewing. Special precaution is that no alkaline drink or baking soda etc. should be taken up to 2 hours to allow the tablet to transit intact from the stomach to intestine.	Aciloc RD, Dulcolax, Encorate 500, PAN 40, Sompraz 40
5	Effervescent tablet	The effervescent tablet should be put in a glass of water and allowed to dissolve or diffuse completely and then drank.	Mucinac 600
6	Chewable tablet	These tablets are to be chewed/crushed in the mouth before swallowing with water.	Cidro, CZ Daily, Gelusil MPS, Limcee, Suvicee, Syncee plus, Vitamin A Chewable Tablet
7	Buccal tablet	These tablets are not to be swallowed with water. To be put in the buccal pouch to slowly dissolve and get absorbed through the oral mucosa.	Hydrocortisone 2.5 mg Muco-Adhesive Buccal tablet
8	Sublingual tablet	These tablets are not to be swallowed with water. To be put under the tongue to slowly dissolve and get absorbed through the sublingual mucosa.	NUROKIND – OD, Sorbitrate 5.
9	Orally Disintegrating Tablet (ODT) Orodispersible tablet Mouth Dissolving Tablet (MDT)	These tablets should be placed on the tongue with a dry hand, allowed to dissolve in saliva, and swallowed. Swallowing should be repeated several times.	ONDEM-MD4

Sl. No.	Type of Tablet	Counseling tips	Example
		No need to swallow with water or chew. Especially useful in children and geriatrics having swallowing problems and in mentally sick.	
10	Controlled-release/ Sustained-release	These tablets release the medication in a predetermined manner for a prolonged time. The tablet must be swallowed as a whole; without breaking, crushing, or chewing; with a glass of water.	Avol-300 CR, Corval CR 500, EVIC SR 400, GTN-Sorbitrate CR, Mazetol SR 200, Nitrozil 6.4, Nitogon 2.6, Nitrocontin, Nitrolong 2.6, ZEP CR 200
11	Vaginal tablet	To be inserted in the vagina with the help of a plastic inserter device, supplied with the product. Proper explanation and demonstration with the help of a sketch incorporated in the leaflet is a must. If possible, a demonstration model should be used.	Candid – V6, Canesten V6, Clotrin-V, Fungistat-V, GynosanVag, Imidil Vaginal, Surfaz Vaginal, Talsutin Vaginal, Vagid Vaginal

Strict patient compliance leads to achieving the therapeutic goal. Ultimately the prescription succeeds; therapy becomes economic and improves the patient's quality of life. This is summarized and depicted in Figure 1.

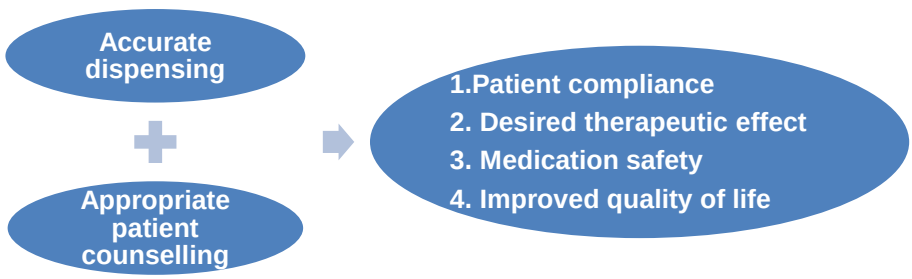


Figure 1. Depiction of Outcomes of Patient counseling

Methods

Based on the measurable parameters of patient counseling, a questionnaire was designed by the authors. The questionnaire is presented in Table 2. It was circulated among 250 practicing pharmacists across West Bengal, India. There were 138 (55.20%) responses received from hospital and community pharmacists. Data received for each of the 23 questions were properly compiled, analyzed, and interpreted.

Table 2. Questionnaire for Assessment of Patient Counseling Practice

Sl. No.	Description	Pharmacists reply
1	Patient Counseling provided	YES No Partial Extensive
2	Drug-related counseling provided	YES No Partial Extensive
3	Dosage form related counseling provided	YES No Partial Extensive
4	Route of administration related counseling provided	YES No Partial Extensive
5	Frequency of administration related	YES No Partial Extensive
6	Side effects related counseling provided	YES No Partial Extensive
7	Potential toxicity related counseling provided	YES No Partial Extensive
8	Adverse drug reaction related counseling provided	YES No Partial Extensive
9	Drug-drug interactions related counseling provided	YES No Partial Extensive
10	Drug-food interaction related counseling provided	YES No Partial Extensive
11	Drug-drink interaction related counseling provided	YES No Partial Extensive
12	Storage of medicine-related counseling provided	YES No Partial Extensive
13	Duration of therapy-related counseling provided	YES No Partial Extensive
14	Missed dose-related counseling provided	YES No Partial Extensive

Sl. No.	Description	Pharmacists reply
15	Any special aspect related to counseling	
16	Barriers to patient counseling	
17	Do patients approach/insist oncounseling?	YES No Occasionally
18	Description of Resource materials referred	
19	Description of Online Resources utilized	
20	Counseling content devised and updated.	
21	Special techniques used	
22	Feedback obtained on compliance	YES No Occasionally
23	Reminding phone call made as follow-up for compliance	YES No Occasionally

Results

Data analyses of the study revealed that although there was a response only from 138 (55.20%) of the practicing pharmacists to whom the questionnaire was sent, 125(90.57%) provided counseling and only 13(9.42%) do not provide any patient counseling while dispensing the prescription. The study further revealed that 22(15.94%) provided only partial counseling; 100(72.46%) provided compact patient counseling while only 3(2.17%) provided extensive patient counseling.

The nature of patient counseling provided was assessed through questions 2, 3, 4, 5, 13, and 14. The composite data of these are presented as a bar chart in Figure 2.

The extent of patient counseling related to side-effects, potential toxicities, and adverse drug reactions was studied through questions 6, 7, and 8. The composite data of these are presented as a bar chart in Figure 3.

Drug interaction-related patient counseling was studied through questions 9, 10, and 11. The composite data of these are presented as a bar chart in Figure 4.

Figure:2 - Nature of Patient counselling provided

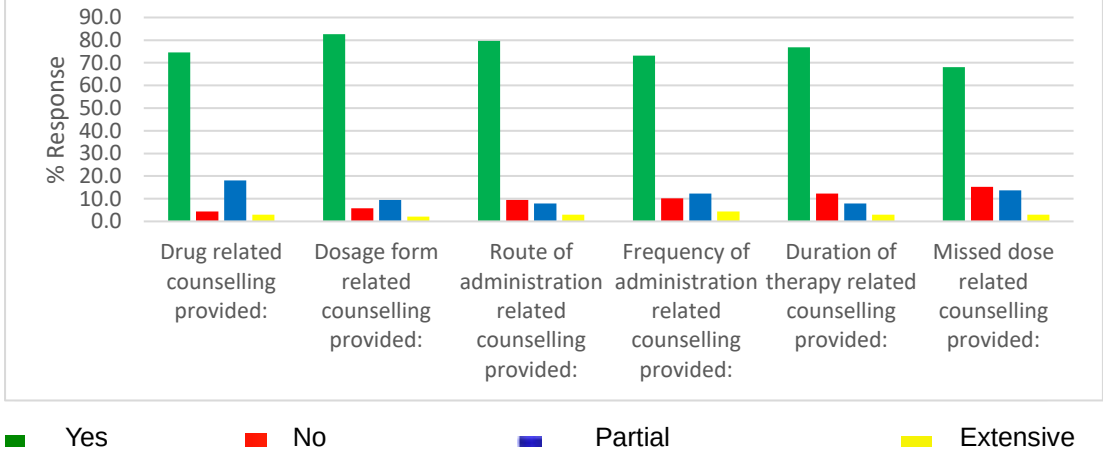


Figure:3 - Side-effects, Toxicities and ADR related counselling

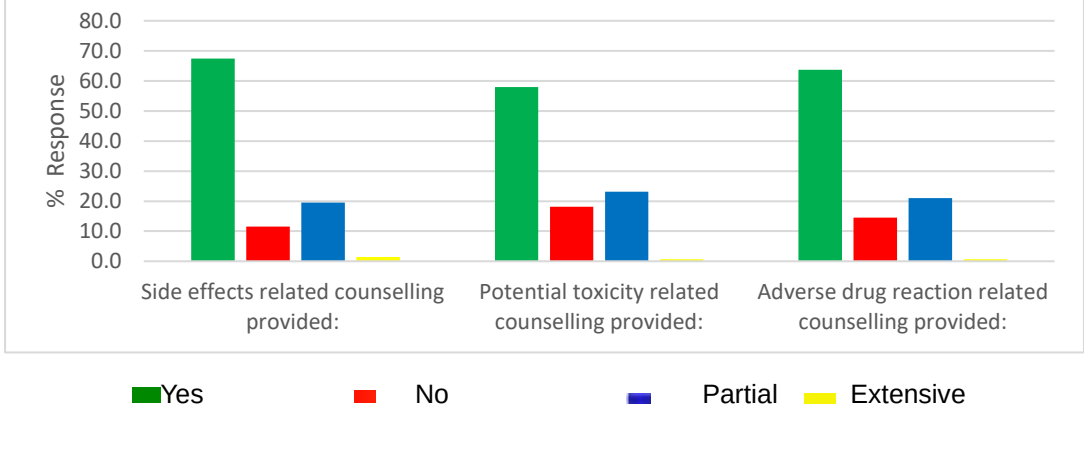
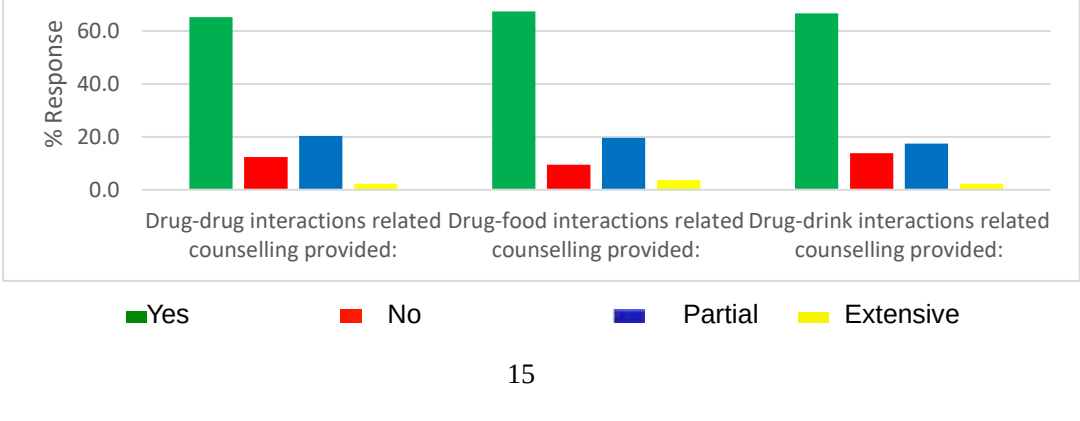
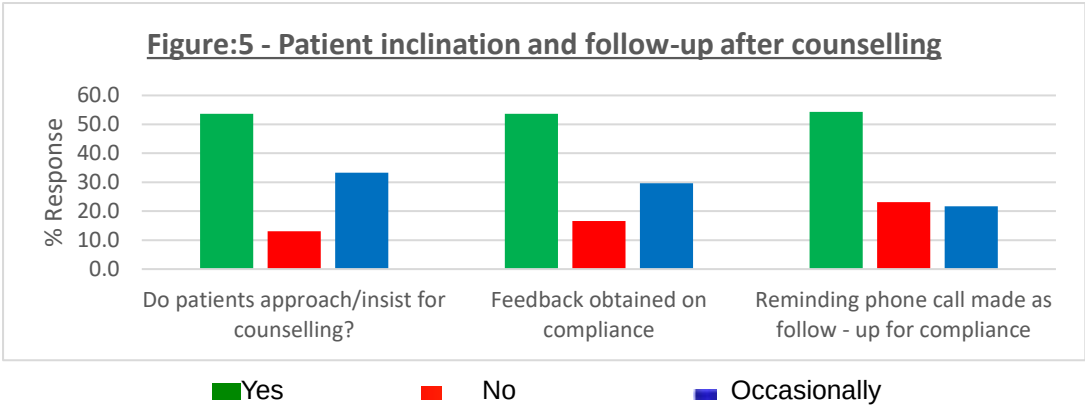


Figure:4 - Drug interaction related counselling



Patient's inclination towards counseling, feedback obtained regarding patient compliance, and reminding phone calls made as a follow-up for compliance were studied through questions 17, 22, and 23. The composite data of these are presented as a bar chart in Figure 5.



Assessment of patient counseling regarding proper storage and preservation of medicines by the patient revealed that 114(82.60%) of the pharmacists apprised the patients about proper storage of their medicines, while 10(7.20%) did not inform anything on this count. The response further revealed that 10(7.20%) gave partial information on storage while 4(2.9%) gave extensive information and encouraged the patients to meticulously follow the storage instruction to preserve the quality and potency of the medicines.

Information was also gathered regarding the resource materials referred by the pharmacists (question 18). The outcomes revealed that CIMS, research papers in the journals, Pharmacology and Pharmacotherapeutics books, and information leaflets provided by manufacturers are major resources and references utilized by the practicing pharmacists.

Online resources (question 19) are extensively utilized by Pharmacists that include google search; Drug.com; Wikipedia.com; 1mg.com; webmed.com; and journal websites.

As regards updating of counseling content (question 20) the results revealed that counseling content is periodically updated to provide the latest information to the patient.

This study revealed the absence of use of special techniques in counseling (question 21) by the participant pharmacists.

Discussion

In the present study, efforts have been made to assess the status of the practice of patient counseling in West Bengal, India. Results of the study indicate the prevalence of patient counseling among participants. Follow-up for patient compliance assessment proves to be an effective tool to encourage patient compliance. In other words, patient counseling is a means of

avoiding medication errors and improving the efficacy of prescriptions. It has been found that the participants practice patient counseling and they acquire in-depth updated knowledge of the medicines being dispensed. Wherever required or appropriate proper demonstration tools and use of cartoons to convincingly counsel the patient or his/her caregiver, need to be introduced and adopted by the participants to make it more useful, appealing, and effective than simple verbal counseling. Intermittent phone calls to remind the patient or his/her caregiver further improves outcomes and builds a strong bond between the patient and the pharmacist. This is being abundantly used in confidence-building. The findings of the present study align with the earlier report. [12]

Patient counseling delivers drug information either orally or in written form or by way of demonstration while dispensing medicines on a prescription to ensure patient compliance. Pharmacists are expected to ascertain that patients or caregivers have gained clear and complete information and they are convinced. The use of the mother tongue of the patient delivers more effective communication and that must be a priority. In the present study, all the participants provide patient counseling in the mother tongue of the patient. Patient counseling cannot be optional. It is an essential component of dispensing any prescription. Suitable steps need to be taken to encourage both patients and pharmacists about this important gateway of patient compliance and better outcomes.

Conclusion

Patient Counseling is Gateway to Patient Compliance. In the interest of safe and effective medication, and rational use of medicines, patient counseling is an inevitable and highly useful tool. Global emphasis on the rational use of medicines can be partially achieved by efficient patient counseling mechanisms. It is the most important component of the practice of pharmacy and the tower of success of pharmaceutical care. Pharmacists individually and their organizations must pay enough attention to make it a common practice in the interest of the health and happiness of patients.

Acknowledgment

The authors gratefully acknowledge the assistance rendered by Mr. Subhadeep Dey, Lecturer, Automobile Engineering Department, Coochbehar Polytechnic, Siliguri Road, Cooch Behar, West Bengal, India in the data processing.

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A Review on Gastroretentive Dosage Form for Targeted Drug Delivery

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Abstract

Oral controlled release dosage forms have wide acceptance because of their benefits, but there areissues like short gastric retention time that limit its applications. If the retention of a medicament can be increased by a certain factor, the drug solubility as well as its therapeutic efficacy increases. Gastroretentive Drug Delivery System (GRDDS) is one such system developed that helps to increase the Gastric Retention Time (GRT) by a certain factor without hampering the plasma fluctuation levels. This review aims at defining different approaches by which gastric retention can be achieved. It also lists the factors controlling the GRT and how can it be used in the future to design a novel drug delivery system.

Keywords: Gastro retentive, Gastric retention time, Controlled release dosage form, Targeted drug delivery system

Introduction

The rationale of any drug delivery system is to ensure that a required quantity of drug reaches the desired site in the body to bring about the necessary therapeutic action. To attain maximum therapeutic efficacy, delivering the drug to the target tissue in a specific amount in the desired time frame is of utmost importance. Oral ingestion is considered to be the most suited and most used route of drug administration.

Drugs having a short half-life that can be easily absorbed without any difficulty from the Gastrointestinal tract (GIT) are removed first from the blood circulation. A well-crafted controlled drug delivery system can conquer several complications associated with traditional therapy, thus enhancing the medicinal activity of a given drug by releasing the active medicament at a slower rate into the GIT and also by maintaining a persistent drug level in the serum for an extended period. In Pharmaceutical Sciences, the development of such systems for the controlled release of drugs is one of the most interesting topics and lots of research takes place in this field.

However, the bioavailability is seen to decrease due to incomplete drug release as well as shorter residence time in upper GIT, which is one of the notable sites for drug absorption. Various efforts to increase the oral bioavailability of drugs have been found to grow side by side with the pharmaceutical industry. Due to the wide variety of drugs that are increasing day by day, new techniques are thus required to develop orally active medicaments.

Anatomy and physiology of stomach

The stomach is divided into 3 segments physiologically: fundus, body, and antrum (pylorus). The fundus and body region of the proximal part serves as a storehouse for the unassimilated substances whereas, the antrum, the major site of combining, of the distal region, acts as a pump to complete the emptying of the stomach.

The fasting and the fed states produce two characteristic natures of gastrointestinal motility and secretion. As a result, depending on the stage of eating, the bioavailability of the orally administered drugs varies. It is marked by an inter-digestive series of electrical events and cycles, every 2-3hrs of the fed state, both through the stomach and small intestine. This is known as the *inter-digestive myoelectric cycle* or Migrating Motor Complex (MMC). Generally, there are 4 consecutive phases of MMC: (Figure 1)

- Phase I – basal phase, which lasts for 40-60 minutes with rare contractions.
- Phase II – pre-burst phase, which lasts for 40-60 minutes with intermittent action potential and contractions.
- Phase III – burst phase, that lasts for 4-6 minutes, including intense and regular contractions for short period. This is also known as the housekeeper wave. All the undigested materials are swept out of the stomach and small intestine because of this contraction.
- Phase IV – this occurs between the 2 consecutive cycles, phase III and phase I. It lasts for 0-5 minutes.

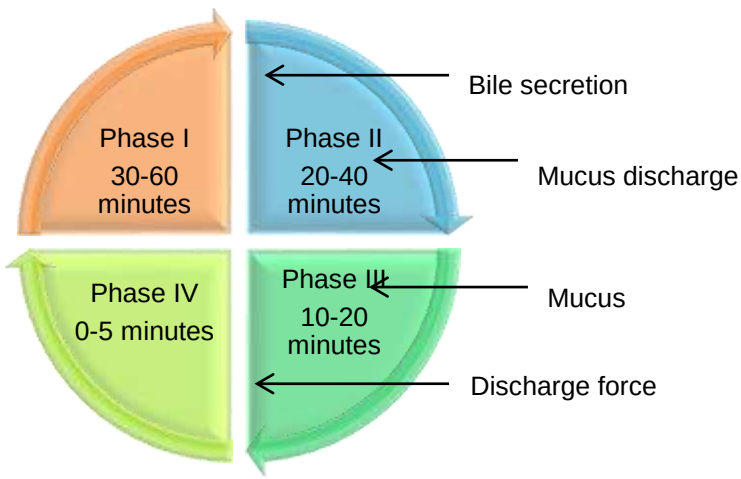


Figure 1. Gastrointestinal motility pattern

Factors affecting gastric retention

- **Impact of dosage form's shape and size** – in fed conditions, during the digestive phase, the smaller dosage form gets cleared from the stomach, and at the time of housekeeping waves, the larger units. Generally, the bigger the units, the more the gastric residence

time as the larger size prevents the dosage form to move to the intestine through the pyloric antrum.

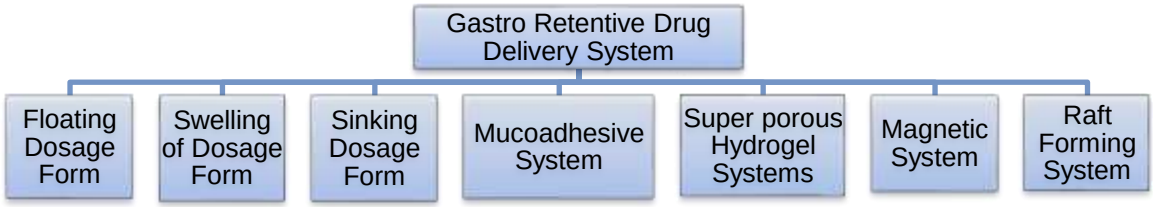
- **Dosage form's density** – for the floating behavior and also for the increased gastric residence time, the dosage form's density should be low. However, to maintain the equilibrium, with an increase in time, the floatable nature of the dosage form reduces.
- **Nature of the food and its intake pattern** – during fasting conditions, the GI motility is characterized by strong motor activity phases or by migrating myoelectric complex (MMC), every 1.5 to 2 hours. The GRT of the unit is generally very short when the administration of the formulation coincides with MMC. However, the MMC is delayed and GRT is significantly more in the fed state.
- **Effect of gender, posture, and age** – males have lower mean ambulatory GRT concerning female counterparts of the same age and race, regardless of weight, height, and body surface. The GRT of geriatric patients, especially those above 70years of age, is significantly longer. GRT can differ between the patient's supine and upright ambulatory states.
- **Type of meal** – the motility pattern of the stomach can change to a fed state due to intake of polymers that are not digestible, or due to the salts of fatty acids. This can slow down the gastric emptying rate and can also extend the release of active medicament.
- **Calorie count** – for a meal that is a good source of fats as well as proteins, increases the GRT by 4-10 hours.
- **Feedrecurrence** – if meals are given in succession, GRT increases by 400 minutes as compared to a single meal.
- **Accompanying drug administration** – floating time can be affected by several drugs like anticholinergics, opiates, and prokinetic agents.
- **Biological factors** – diseases like Diabetes, Crohn's Disease, etc. can alter the floating time.

Gastro Retentive Dosage Forms (GRDFs / Gastro Retentive Drug Delivery System (GRDDS)

This is one of the most viable proposals for attaining an extended drug release and also predictable delivery of drug in the GIT. As it can sustain within the gastric pH for longer hours, the retention time also gets controlled. Increased gastric retention thus, not only improves bioavailability but also reduces drug waste. It also improves the solvable nature of drugs that are comparatively less soluble in a high pH environment.

Approaches to Gastric Retention

Several techniques are there that are being used to enhance the gastric retention time (GRT) of a dosage form in the stomach by utilizing several ideas. This includes:



1. **Floating Dosage Form** – in this system, the dosage form is formulated in such a way that its density is lower than the gastric content which helps them to remain floatable in upper GIT for a longer time frame, keeping in mind that the gastric emptying rate must not be hampered. The drug is allowed to release slowly at a predetermined rate while the system floats on the gastric contents. The gastric retention time is also increased. They are also known as hydro-dynamically balanced systems.

   They can be classified into two systems – Effervescent System and Non-effervescentSystem.

- **Non-effervescent system –** here, the polymer either increases in size or gets attached to the mucosal layer of the GIT tract. The dosage forms like capsule/tablet, are prepared using a drug mixture along with hydrocolloids. Once they are exposed to the GI fluid, they swell up and forms a gelatinous barricade. This allows the system to remain floating in nature for a longer timeframe (Figure 2).

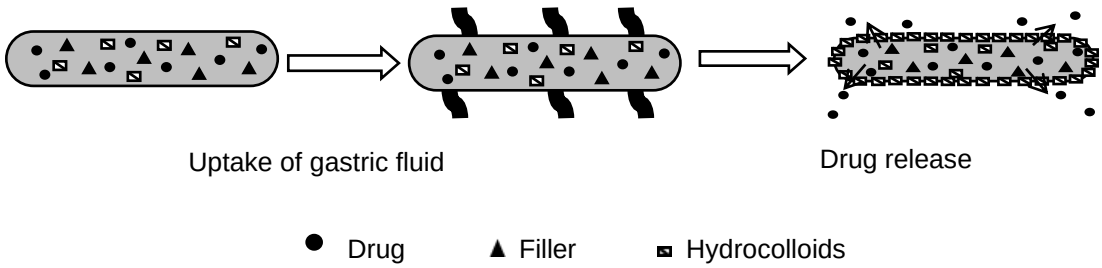


Figure 2. Working principle of the non-effervescent system

- **Effervescent system –** this system involves the use of carbonates, organic acids, and most importantly certain agents that can produce gases like carbon dioxide (CO_2). This evolved gas reduces the density of the system that helps the system to remain light. Another alternative method is there that incorporates a matrix containing a certain amount of liquid that can generate gas. This gas then vaporizes at body temperature (Figure 3).

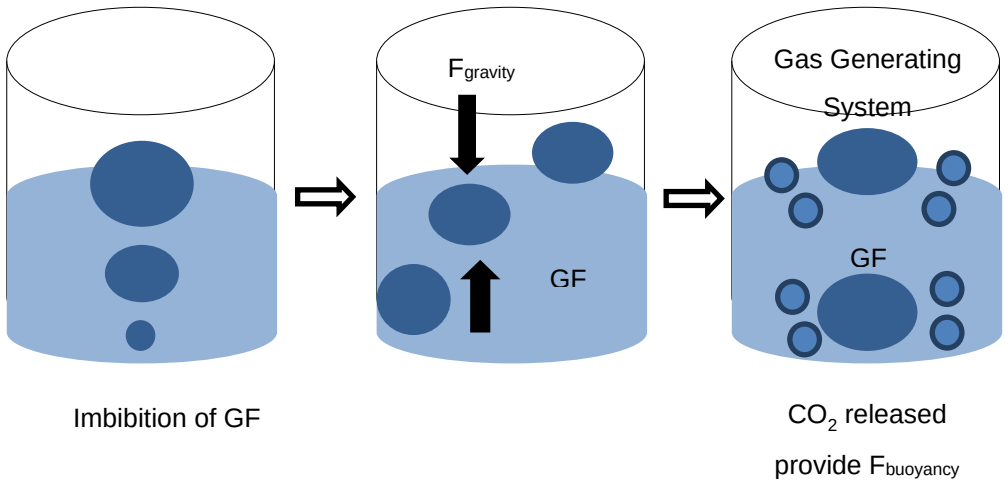


Figure 3. Working principle of Effervescent FDDS

2. **Swelling of Dosage Forms** – these are prepared with such polymers that swells up once the dosage form is swallowed. This helps in increasing the size, preventing the exit of the dosage form from the pylorus (Figure 4). Thus, the dosage form gets holds on to the stomach for an increased pattern. Such methodology is also sometimes known as a 'plug type system' as they are capable of remaining within the pyloric sphincter for a longer period.

   Certain factors are there that should be considered while designing this type of dosage form. They are:

   - A small structure so it becomes convenient to take orally.
   - An enlarged structure that can provide the necessary retention.
   - A small structure at the end, so that the removal can be easy.

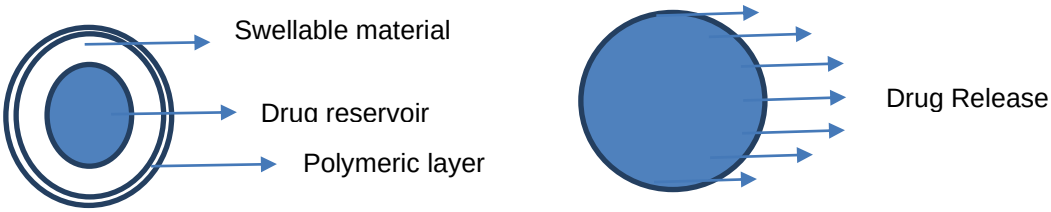


Figure 4. Swelling Dosage Form

3. **Sinking Dosage form** – unlike the previous systems, this system tends to remain in the lower part of the stomach. The principle of retention followed here is sedimentation. They are formulated as such that their density exceeds the density of the normal GI fluid, thus allowing them to hang on to the bottom of the stomach. This dosage form is found to contain a heavy core, on which the drug is coated. Certain times substances like iron powder, barium sulphate, or other appropriate substances are used that can increase the

density to a greater extent. Literature studies reveal that a density of around 2.5gm/cm^3 is beneficial for such a system. It should be formulated as such that it can withstand the peristaltic movements.

4. **Mucoadhesive System** – from the name itself, it can be understood that they tend to attach to the epithelial cell surface of the gastric layer or mucin, thus extending the gastric residence time of the system. They are generally designed for site-specific drug action, involving the use of certain polymers, like HPMC, sodium alginate, chitosan, etc. that are bio-adhesive in nature. This method is not very beneficial as it sometimes becomes problematic to target the preferred site, and the attractive force is not that strong to resist the peristaltic forces. Different principles behind the attachment of the system to the mucosal layers are:
    - Theory of wetting, which allows the polymers to extend over the mucosal layer, forming a close contact between the two.
    - The diffusion theory, which puts forward the idea of physical ensnarement of mucin strands, which are flexible in nature, or interpenetration of the mucin strands into the permeable polymer structure.
    - Another theory is there, viz. the absorption theory which proposes the Vander Waal Interaction and Hydrogen bonding between the two surfaces.
    - Electron theory, which suggests the electrostatic force of attraction among the two surfaces.
5. **Super porous Hydrogel System** – hydrogels are cross-linked polymers that have the proneness to uptake a high quantity of liquid through their open porous structure (Figure 5). They possess an average pore size of > 100µm that swells to equilibrium size once they come in contact with water. The water enters through capillary wetting. They are designed in such a manner to tend to withstand gastric contraction force.

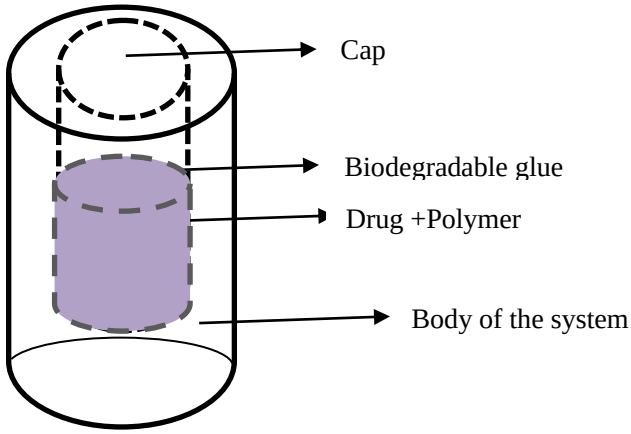


Figure 5. Super-porous hydrogel system

6. **Magnetic System** – here, a small magnet is incorporated within the dosage form. The working principle behind this system is that the dosage form contains a small internal magnet, and also a magnet is placed on the abdomen over the stomach's position. This system is though useful, but the external magnet present can compromise the patient's compliance.
7. **Raft forming System** – this is formulated by incorporating a carbonate component thattends to liberate CO_2 reacting with the gastric fluid, making them light in nature. Moreover, they have the property to form a gel that can swell and form a viscous cohesive layer. This gel is found to entrap CO_2 bubbles once they come in contact with the gastric fluid. It piles up and forms a continuous layer which is known as the raft. This raft floats above the gastric content.

Advantages of GRDDS over traditional dosage forms

- ✓ **Increased Bioavailability**: for the drugs that get easily degraded within the acidic pH of the stomach, the gastroretentive drug delivery system is very helpful as it allows the drug to remain within the system for a longer period.
- ✓ **Sustained drug delivery**: generally, the drug absorption offered by the oral controlled release dosage forms is limited because of short gastric residence time. But as the GRDDS system allows the drug to sustain within the stomach for longer hours, the GRT also increases, it can thus provide sustained delivery.
- ✓ **Targeted drug delivery**: for drugs that are meant to produce a prolonged activity on the gastroduodenal wall, this system is helpful as the active medicament is released slowly into the systemic circulation, thus providing site-specific drug delivery.
- ✓ **Fluctuation of a drug in plasma concentration**: in this system, the medicament gets released slowly as compared to the traditional system where it is present at a higher concentration from the very beginning. Thus, if the drugs in the GRDDS system are given continuously to the patient, the therapeutic window remains within a narrow range as compared to that of the traditional system.
- ✓ **Pharmacokinetic benefit**: as the gastrointestinal transit time is lengthened, more medicine can be administered, resulting in enhanced relative biological availability.
- ✓ **Limited antagonistic activity at the colon**: as the drug is retained within the stomach, it reduces the chance for the drug to enter the colon. As a result, significant pharmacological actions in the colon can be avoided.
- ✓ **Patient compliance**: in such a delivery system, patient compliance is always more as the need for frequent dosing is minimized. Also, the administration is easy.

Disadvantages of GRDDS

- This is not suitable for drugs that are having issues regarding solubility as well as stability in GIT.
- For the floating dosage form, a high amount of fluid is to be ensured within the stomach for the dosage form to remain floatable.
- Drugs undergoing the first-pass metabolism are unfit candidates.
- Medicaments producing irritation in the GIT are also unfit candidates.
- This is not meant for drugs that are having stability issues in a low pH medium.

The basis for selecting suitable drug applicants for GRDDS

- Preferable half-life – an optimum half-life is desirable for this system because if the half-life is high, the release already gets controlled.
- High therapeutic index – for this formulation, drugs having a low therapeutic ratio are not preferable candidates.
- Aqueous solubility – drugs having aqueous solubility above 0.1µg/ml are considered to be good candidates for this system.
- Stability over wide pH range and GI enzymes – the active ingredient must not be affected for the pH range of 1-8 for this system. This is important for ensuring drug stability within the GI content.
- First pass clearance – for drugs undergoing extensive hepatic first-pass metabolism, the release in the desired amount is greatly hampered.
- Absorption site – generally, the suitable drug candidates are the ones that are mainly absorbed from the stomach and upper part of the GI tract.
- Drugs affecting the colon – suitable drug candidates for this system are the ones that can disturb normal bacteria growth in the colon or the ones that can degrade in the colon.

Assessment factors for GRDDS – some general parameters that are useful for evaluating the GRDDS are:

1. Normal tests like the hardness of the designed dosage form, its appearance, drug loading, uniformity of the polymer, etc. are to be checked.
2. Test like determining the floating time is also one of the parameters that are to be checked. This measures the time for which the system could remain floatable.
3. Floating lag time test that measures the minimum time that is required by the system to come to the top of the dissolution medium.
4. The swelling index is also one of the study parameters which gives the idea regarding how much the size has increased by up taking the gastric fluid content. The fluid uptake is also calculated from the difference in their weights.

Future Scopes

The development of novel technologies for drug delivery is increasing day by day and is one of the most researched topics in the present scenario. It can be easily estimated that in the coming future development of such a drug delivery system providing site-specific action is going to take place at a large number. Certain scopes where research can be carried out in the future are –

- Determining the size range of the delivery system that can provide the maximum gastric retention.
- Determining the effectiveness of the system during the fasting as well as fed stages of the stomach.
- Designing the synthesis of natural polymers that can be used for GRDDS and can provide better adhesion.
- Designing drugs to treat certain specific diseases like ulcers, cancers, etc.

Conclusion

Developing Gastroretentive dosage forms is one of the blooming topics in the pharmaceutical industry having its own benefit. Though there are certain drawbacks related to it, they are being used widely nowadays. Thus, research must go on to develop this system further overcoming all of the related difficulties.

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Identification of Hits AgainstSARS-COV-2 non-structural protein-14 (nsp-14)

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Abstract

SARS COV 2 which caused COVID 19 poses a global threat to humanity on and from the end of 2019. To date lack of effective medication creates a necessity to develop promising small molecule-based therapeutics against it. Certainly, drug repositioning reduces the drug discovery cost; nevertheless, searching of the plant kingdom may be helpful to identify effective hits. Besides SARS COV 2 spike protein, the main protease, current discovery established the potentiality of non-structural protein 14 (nsp-14) as a drug target. Total 30927 numbers of naturally occurring small molecules were subjected for molecular docking based on silico screening against SARS COV 2 nsp14. The present work reports top ten natural products exhibits promising interactions with SARS COV 2 nsp-14.

Keywords: SARS-COV 2, NSP-14, NPASS, AutoDock GPU, COVID-19

Introduction

One of the biggest risks to public health is SARS coronavirus belongs to the family of Coronaviridae and order Nidovirales[1]. For the most significant infections like SARS-CoV and MERS-CoV, this virus is responsible.[2, 3] Recently, the world has seen terrifying conditions due to the enduring outbreak of a novel SARS-associated virus SARS-CoV-2 or Coronavirus-2, also termed as COVID-19 (Coronavirus Disease-2019)[4]. The COVID-19 has been extremely affected, has spread throughout the entire globe, and has severely affected millions of people in most of the countries. As per the global statistics, 4,765,966 corona cases and 314,319 confirmed deaths have been reported for 213 countries and territories (data until 17thMay 2020)[5]. SARS-CoV-2 or COVID-19 has high infectious characteristics and is rapidly spread across the globe. It was classified as an emergency of international attention to public health by a World Health Organization (WHO) in contrast to SARS-CoV pathogenesis[6].

It is a positively strained RNA virus, containing the biggest known genomes of RNA up to 32 kb in length. The Coronaviridae subfamily, which comprises medicinal as well as veterinary viruses, may be classified into four alpha, beta, gamma, and delta coronaviruses (α-, β-, Ţ- and Ś-CoV). At least four structural canonical proteins E (envelope protein), M (membrane protein), N

(nucleocapsid protein), and S are present in the coronavirus. In addition, HE (hemagglutinin–esterase) membrane anchoring protein is expressed by viruses belonging to lineage A of beta coronaviruses. The S-Glycoprotein includes both the receptor-binding domain (RBD) and the fusion domains, making it the key protein for entering into the host cell.[7]The genome of SARS-CoV is composed of 14 ORFs. ORF1a and ORF1b include 16 nonstructural proteins (nsp), which are mostly used in replication and transcription[8]. In nsp-12, nsp12, in conjunction with nsp7, works as an RNA-dependent RNA polymerase (RdRp).[9-11] Furthermore, without affecting RNA production, the nsp7–nsp8–nsp12 complex may interact with nsp-14.[11] It is a key interaction since nsp-14 has shown itself to play an important role in lowering the occurrence of mismatched nucleotides via its exoribonuclease domain (ExoN).[12–14] Abrogation of activity nsp-14 ExoN leads to increased susceptibility to the 5-fluorouracil RNA mutagen.[15-17] The connection between nsp-14 and nsp-10 was disrupted, and replication fidelity was observed to be decreasing.As a result, the low mutational rates (10−6-10−7) of SAR SARS-CoV are related to the ExoN activity, as opposed to the overall replication fidelity of RNA viruses (10−3–10−5).

For viral replication and transcription, coronavirus (CoV) nonstructural 14 (nsp-14) protein is crucial. A significant role for preventing fatal mutagenesis is the N-terminal exoribonuclease (EexoN) domain. The C-terminal region acts as a (guanine-N7) methyl transfer (N7-MTase) for capping mRNA.[18] Both functions are yet unknown to have a molecular foundation. Here, we present crystal structures containing a complex nonstructural protein activator (nsp10) complexed with nsp14 and functional ligands information guided identification of promising binders from natural product database. Natural products are the potential source to identify lead molecules. Thus, to accelerate the drug discovery process, we significantly screened the naturally occurring small molecules' library. The present work reports top ten ligands exhibited promising binding with nsp 14 of SARS COV-2.

Materials and Method

All computational works have been conducted on a Linuxmint operating system-based computer integrated with intel i7 9700k processor and NVIDIA RTX 2070 GPU. For molecular docking GPU accelerated Autodock docking program was used.[19] The molecular dynamics was conducted by an Academic version of Desmond software.[20]Pymol 1.8 [21] and Maestro visualizer were used for analyzing and renders images. The 2D interaction diagrams were rendered by Proteins Plus (https://proteins.plus) online server based software tool.

Protein Preparation

The co crystalline coordinates of SARS-COV 2 NSP 10 complexed with NSP 14 and a ligand was retrieved from protein data bank (PDB id 6W4H). Using protein preparation wizard of maestro available with Academic Desmond, inbound water molecules, ions, and solvent molecules were stripped out. The co crystalline ligand (SAM) bound chain A which is nsp14 was extracted and

saved in PDB file format. The confined ligand binding site was considered as a chemical search space followed by populating receptor grid maps using AutoGrid4 [22] software.

Ligand preparation

The two-dimensional structures of 30927 numbers of small molecules were retrieved from natural product activity and species source database (www.bidd.group/NPASS/) in SDF file format. The openable software was used to convert all the molecules into a 3D pdbqt file format followed by energy minimization. The MMF94 forcefield was used to prepare the ligands.

Docking protocol validation and Molecular Docking

The molecular docking-based virtual screening was conducted by a GPU-accelerated AutoDock software tool. The docking protocol validation is a critical step for a precise docking-based virtual screening process. The inbound co crystalline ligand coordinates were retrieved from the Drug Bank database and prepared with Openbabel software in pdbqt format. The prepared co crystalline ligand was then docked with the SARS COV-2 nsp-14 proteins. The docked poses were then superimposed upon their native pose and RMSD was calculated. A list of all prepared 3D ligands was prepared and supplied as an input for the GPU-accelerated Autodock program. Asymptotic heuristics was set to 12000000 followed by 2500000 steps per LGA run with 42000 generations. ADADELTA was used as a local search method. For each ligand 20 round of docking simulation was conducted. From each round, the conformation was extracted and clustered. Finally based upon high negative binding energy and RMSD score the docking pose was selected. A python-based in-house script was used to perform post docking analysis and protein-ligand complex generation.

Molecular Dynamics simulation

To analyze the protein-ligand complex stability total of 50ns atomistic molecular dynamics simulation was conducted for the top-scoring ligand. The Desmond software was used for performing molecular dynamics simulations (MD). The protein-ligand system was incorporated into a 10 X 10 X 10 Å cubic box and solvated using TIP3P [23] water model. To neutralize the system 0.15M NaCl was added. The OPLS_2005 [24] force field was used to parameterize the systems. After minimizing / relaxing, the prepared system was equilibrated by the NVT-NPT ensemble equilibration method. The MD production run was conducted by recording snapshots for every 2fs. Total 5000 snapshots were recorded. The simulation interaction diagram program of the Desmond program was used to analyze the MD output.

Results

The present work reports the virtual screening of the NPASS database against the nsp-14 protein of SARS COV-2. The molecular docking method was used for the virtual screening process.[25] The docking protocol used for the virtual screening process was validated by re-docking of the co crystalline ligand. The RMSD of 1.03Å was found after superimposition (Figure 1) of docked pose upon the native pose of co crystalline ligand. The obtained RMSD value strongly infers the validity of the docking protocol.

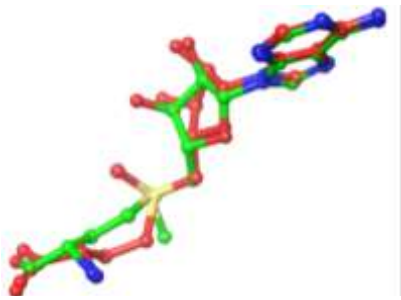

Figure 1. Red color docked pose, Green Color native co crystalline pose.

The corresponding binding energy was found to be -8.3kj/mol. Therefore, ~8.5kj/mol was set as a threshold binding energy. The in-house python script program used for post docking analysis was configured in such a way that it will short those ligands only, depicted binding energy less than 8.5kj/mol. The Structure and binding energy of the top ten ligands can be found in Figure 2 and Table 1.

Table 1. Docking Score of top 10 ligands

Sl. No.	Docking Score	NPASS ID	Common Name
1	-11.38	NPC103250	*Undefined*
2	-11.18	NPC186569	Aristoloterpenate-Iv
3	-11.07	NPC183736	Capsanthin
4	-10.7	NPC102843	Fucoxanthin
5	-10.65	NPC17810	Beta Carotene
6	-10.54	NPC162440	Strychnogucine A
7	-10.32	NPC203005	O2-Natafuranamine
8	-10.23	NPC202503	Bismurrayafoline B
9	-10.16	NPC171148	Lutein
10	-10.13	NPC19631	Microcarpin

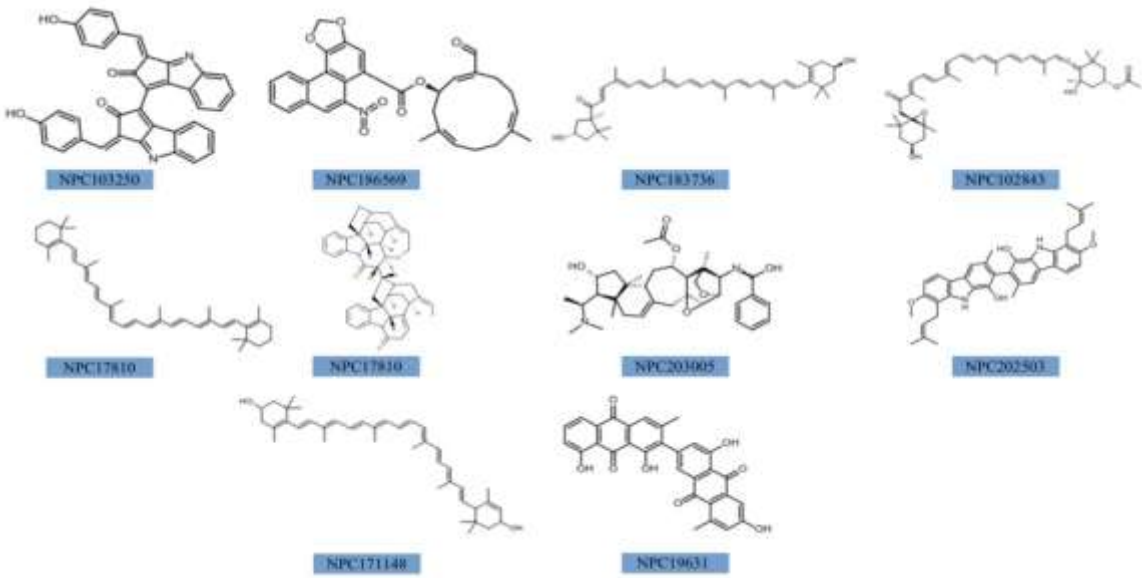

Figure 2. Structure of top 10 identified hits

Analysis of co-crystalline protein-ligand complex depicted the co-crystalline ligand SAM (S-Adenosyl Methionine, DrugBank ID: DB00118) forms hydrogen bond interaction with Gly 6879, Asp 6897, Asn 6841, Asp 6928, Gly 6869, Cys 6319 amino acid residues, and Hydrophobic interaction with Leu 6898, Met 6929. (Figure 3)

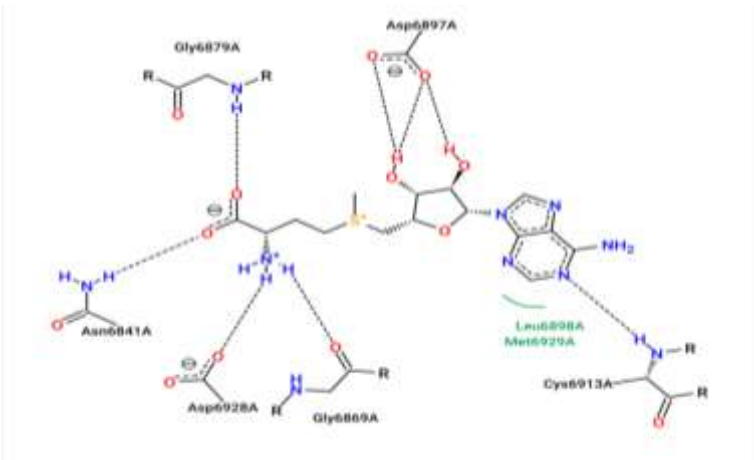

Figure 3. Interaction of SAM with nsp14

The top-ranked ligand NPC 103250 (binding energy ~ -11. 38kj/mol) shows hydrogen bond interaction with Asn 6899, Cys 6913, and hydrophobic interaction with Met 6929, Leu 6898 amino acid residues.

The second-ranked ligand NPC186569 (binding energy ~ -11, 18kj/mol), exhibited strong hydrophobic interaction with Leu 6898, hydrogen bond interaction with Asp 6897, and Asn 6899 (Figure 4). These residues are the crucial amino acid exhibited interaction with co crystalline ligand SAM. In-depth analysis of their binding orientation along with binding energy and interaction profile suggested these ligands can be considered as a potential hit against nsp-14 protein. To establish the above assumption 50ns molecular dynamics simulation was conducted using Desmond software. Various parameters like RMSD, RMSF, and interaction fraction data were calculated. The RMSD parameter calculated from molecular dynamics trajectory provides information about the geometry and stability of a macromolecule. The 50ns atomistic Md simulation of NPC103250 bound nsp14 complex to depict a stable protein backbone RMSD profile with average RMSD value ~4.0Å. The ligand RMSD value was also calculated, and the comparison plot can be found in Figure 5. The average ligand RMSD was found to be ~3.62Å. The uniform protein and ligand RMSD profile confer both of them remain in contact during 50ns simulation time.

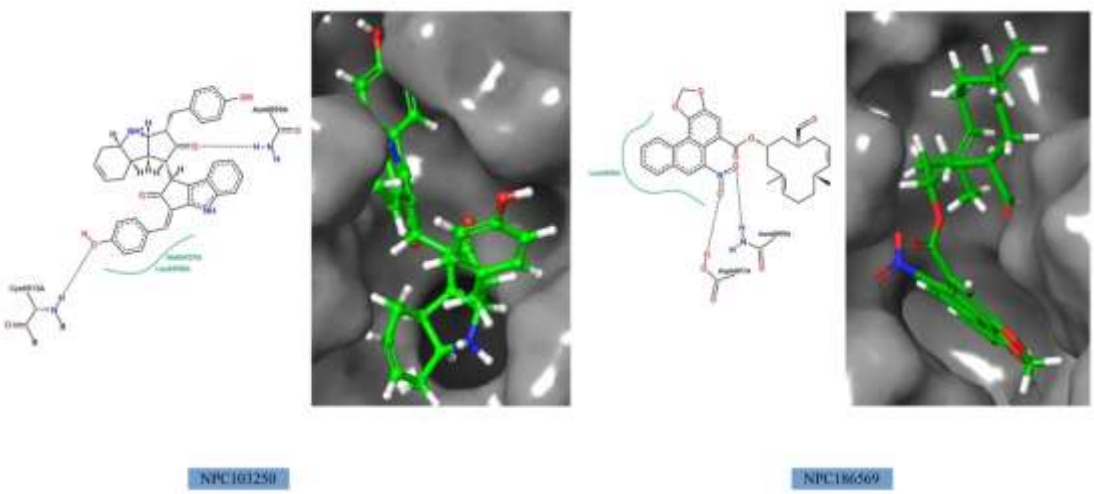

Figure 4. 2D and 3D orientation of top 2 ligands

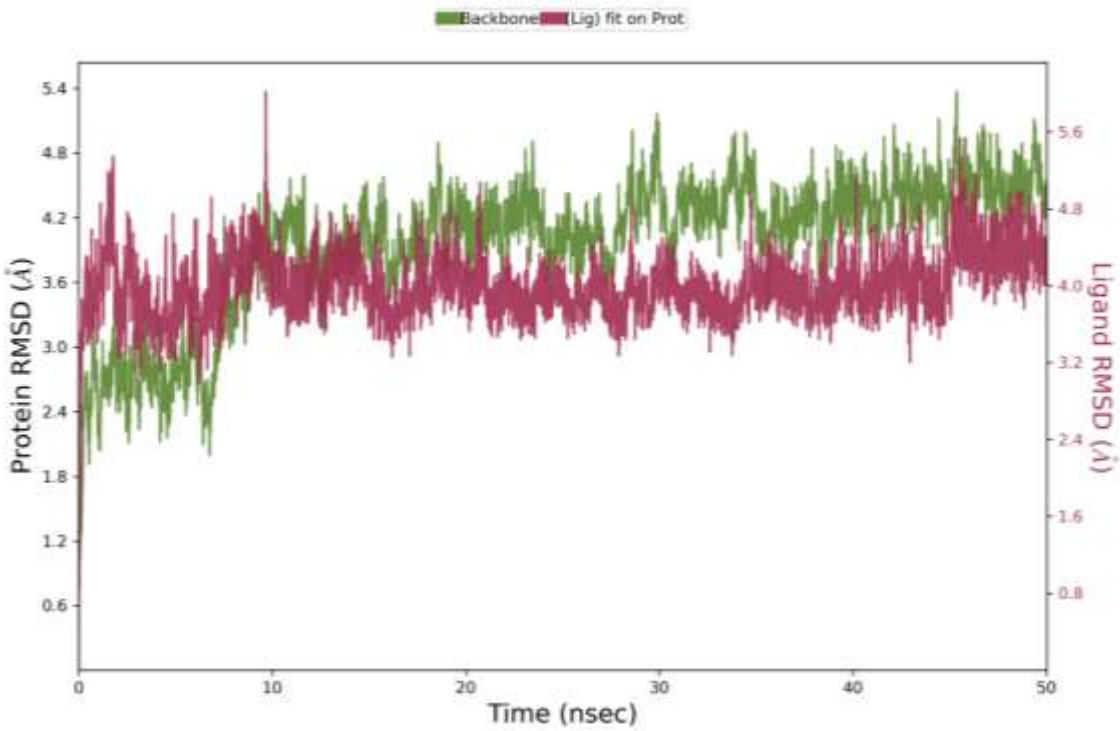

Figure 5. RMSD Profile

The RMSF and interaction fraction parameter was calculated from molecular dynamics trajectory and shown in Figure 6. From Figure 6 it can be found that the ligand NPC103250 formsa hydrogen bond with Ser 6896 during the 66%time of the simulation. Most of the time it interacts with the nsp-14 protein, through the formation of a salt bridge.

From the torsional plot of the ligand (Figure 6) it can be stated that the ligand does not undergo any significant changes during the 50ns simulation. In agreement with the above analysis, it can be stated that the ligand NPC103250 forms a stable complex with SARS COV 2 nsp-14 protein.

However, more computational analysis and biological evaluation are still required to develop new therapeutics against this virus.

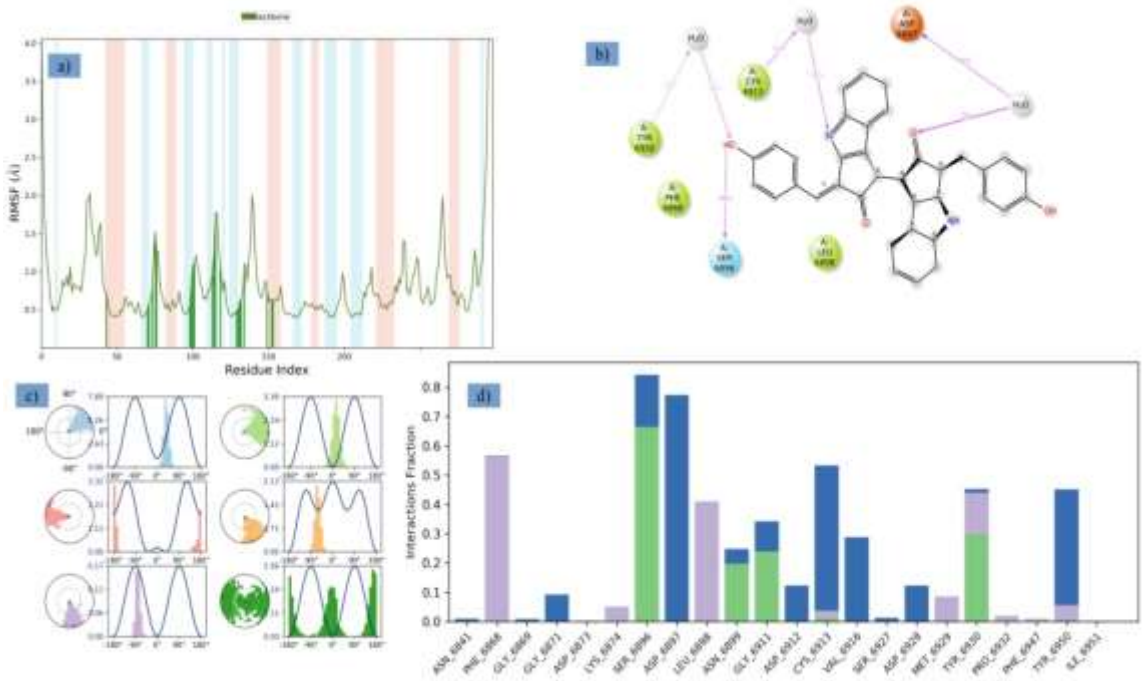

Figure 6. Protein-Ligand Interaction Profile

Conclusion

The present work reports the binding interaction of few sets of natural products identified by molecular docking-based virtual screening. The binding energy and interaction profile was used to rank the ligands. The 50ns molecular dynamics simulation of the top-ranked ligands strongly supports its characteristic as a promising hit against SARS COV 2 nsp-14. However, proper biological evaluation is required to justify its anti-SARS COV 2 property.

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Pharmacognostical standardization of *Murraya koenigii* leaves

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Abstract

The standardisation of medicinal plants is critical for the development of better products. It is also used to correctly identify and characterise phytoconstituents in plant drugs. However, there is still a need to emphasise the significance of crude drug standardisation. The importance of standardisation has been emphasised more than ever before as a result of changes in the current globalisation scenario. This paper aims to standardise and focus on the various uses of curry leaves (*Murrayakoenigii*). These leaves were phytochemically screened and found to contain a variety of phytoconstituents such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenol, phlobatannins, tannins, terpenoids, and quinones. Thin-layer chromatography was used to determine the presence of constituents. With the growing demand for plant-based products, it is critical to developing standardisation methods for our formulations, which have been used for centuries.

Key words: *Murraya koenigii*, Standardization, Phytochemical screening, Thin-layerchromatography.

Introduction

Plants have been an inseparable aspect of human culture since time immemorial. Plants and their parts were used as a fruit, pasture, wood, etc. or as medicinal items in different ways. A significant percentage of the population is utilizing plant-based drugs (Phytomedicines) for their primary health treatment according to the World Health Organization.[1] In a nation like India, numerous established therapeutic usage structures such as Unani, Ayurveda, Siddha, and local medical practices depend on the use of plant-based chemicals to cure many diseases to the human being as well as to the animals.[2] There are a variety of active constituents in medicinal plants having many pharmacological activities that are useful for improving safety. They not only act as an anti-infective agent but also economic and have fewer side effects.[3] Herbs' medicinal function is attributed to various stakeholders found within them. The pharmacological property of the medicinal plant is variable and differs as the active components are different. Plants are relying on many factors; among that collection of the plants is one of the sources. The plant also contains some toxic materials; therefore, evaluation of their consistency, health, and efficacy is important. Hence, proper identification and standardization of starting materials must be considered as a prerequisite for the production of the best quality of the product.[4]

The curry tree (*Murraya koenigii*) or curry leaf tree from the Rutaceae family is a tropical to the subtropical tree and is native to India. Its leaves are used in the Indian subcontinent, in several dishes. Also used in curries, the leaves are commonly called "curry leaves," but in most Indian languages they are in reality just "sweet neem leaves".[5] Leaves of *Murraya koenigii* are a common leaf spice that is used in very limited quantities for its characteristic fragrance due to the existence of volatile oil and also its ability to improve digestion. "Let food be your medicine and let medicine be your health" supporting this proverb, many scientists and medical researchers have shown tremendous interest in the field of herbal medicines as they recognise the medicinal value of certain natural products or folk medicines.[6] *Murraya koenigii* also proves to be an important Indian export product, as it produces strong foreign revenue. Various phytochemical constituents are known to be extracted from all plant parts. The plant is a rich source of alkaloids from carbazole (mahanimbine, girinimbine, koenimbine, and koenigicine). P-gurjunene, P-elemene, P-caryophyllene, and O-phellandrene are the main chemical constituents that are responsible for its strong signature fragrance.[7] The plant is especially revered for its leaves, a critical fixation for advancing edacity and processing in Indian cooking. The leaves, bark, and root of the plant serve as a tonic in stomach pain. Leaves are used indoors as part of loose intestines and are often tested for regurgitation.[8] It was documented that the carbazole alkaloids have a broader spectrum of pharmacological characteristics such as antibacterial, antioxidant, and anti-cancer.[9]

Maceration is an extractive procedure performed at room temperature. It consists of immersing a plant within an airtight jar in a liquid (water, gasoline, alcohol, etc.) for a variable duration, depending on the plant content and liquid used. Thin-layer chromatography (TLC) is a method very widely used in synthetic chemistry to distinguish molecules, assess their purity, and adopt a reaction progression. It also allows for solvent system optimization for a given separation problem. High-performance thin-layer chromatography (HPTLC) is an expeditious, reliable, sensitive, accurate, and cost-effective tool, which is commonly used to evaluate different biological compounds from medicinal plants. Thus, the HPTLC method for determining carbazole alkaloids in the leaves of *Murraya koenigii* has been established.

Materials and Method

Collection of Plant

Fresh and healthy *Murraya koenigii* (Curry leaves) were collected from the garden. The leaves were cleaned to remove unwanted materials. Leaves were then air-dried in shade; a part was then homogenized into a fine powder, and preserved for more pharmacognostic tests in air-tight jars. Half of the leaves collected were naturally dried by holding for 5 days under direct sunlight and then subjected to extraction. The microscopy study of leaves was done.

Preparation of plant extracts

Extraction of fresh and dry curry leaves was done in two different solvents viz., 100% methanol and petroleum ether each. For this, 10g fresh curry leaves were put in 250ml conical flasks and 100mL solvents viz., 100% methanol and 60-80 petroleum ether were added in respective flasks. The same procedure was repeated for dry leaves. Maceration was continued for 7 days. On the 8th day, the extract was filtered for further studies.

Physico-chemical characteristics of Murraya koenigii powder

Determination of loss on drying

The 2g of crude powder of *Murraya koenigii* leaves were taken for the determination of loss on drying. The powdered crude drugs were evaporated and dried at 105 ºC in an oven until constant weight. Amount left after drying was weighed and loss on drying was calculated. The percentage was determined based on the originally taken sample.

Determination of total ash

The 1g of *Murraya koenigii* leaves crude powder was taken for the determination of total ash value. The sample was taken in the crucible and incinerated in a muffle furnace at a temperature not exceeding 450 ºC. It was heated until free from carbon. Then the crucible was cooled and weight was measured.

Determination of Acid insoluble ash

The total ash collected was added to 25mL HCl and boiled for 5 minutes. The insoluble content was taken on a filter paper which was placed in a crucible. It was then washed with water and then heated until a constant weight was obtained. The percentage of acid-insoluble ash was calculated concerning the amount of sample taken initially.

Determination of Water-soluble ash

For the determination of water-soluble ash, the obtained ash was determined by boiled for 5 minutes with 25 mL of water. The solution was filter and insoluble matter was collected in a crucible on an ashless filter paper. It was then washed with hot water and heated for 15 minutes at a temperature not exceeding 450 ºC. The difference between the weight of the insoluble matter and the weight of ash was calculated as water-soluble ash. The percentage of water-soluble ash was calculated concerning the amount of sample taken initially.

Determination of pH

The crude powder of *Murraya koenigii* leaves was dissolved in distilled water. It was then kept in the water bath for 20 minutes. After 20 minutes, the solution was filtered and the pH of the solution was measured using a pH meter.

Characterization of Murraya koenigii Extract

Phytochemical analysis of plant extracts

The standard qualitative test procedures for the determination of the presence of various phytoconstituents was carried out by using different chemical reagents such as Wagners, Molish, Killer killani, Ferric chloride, etc.

Thin-layer chromatography study

TLC plates were prepared using glass slides and absorbent silica gel sprayed on the slides. Spotting of the sample was done 1cm above the bottom margin of the slide. After spotting, it was allowed to dry for a while and further placed in the chromatographic tank containing the solvent solution. The mobile phase benzene: methanol (9:1) accelerated the reaction and helped in eluting the sample. As the solvent reached up to the top level of the absorbent, the plate was removed and air-dried. After drying, the plates were exposed to iodine fumes which made the spots more prominent. The distance travelled by the solvent and the distances of elution of samples were recorded.

Results and Discussions

The microscopy of dried powder leaves was carried that showed the presence of microscopy of unicellular trichomes, starch, vascular bundles calcium oxalates. The extracts were collected on the 8th day of maceration to get accurate results. The extracts obtained after keeping the fresh and dry leaves of *Murraya koenigii* in different solvents comprised of characteristic colour and smell. The petroleum ether extract of both fresh as well as dry leaves of *Murraya koenigii* was yellow-green in colour and had a consistency of oily mass. It had a particular petroleum odour. The methanol extract of both fresh and dry *Murraya koenigii* leaves was dark green coloured residue.

Physio-chemical Characterization

The results of Physico-chemical characterization i.e. loss on drying, total ash value, acid insoluble ash, water-soluble ash, and pH of *Murrayakoenigii* leaves are depicted in Table 1.

Table 1: Physico-chemical characterization of *Murraya koenigii* leaves powder

S.No.	Particulars	Inference
1	Loss on drying	0.15%
2	Total ash	36.5%
3	Acid insoluble ash	1.775%
4	Water soluble ash	6.207%
5	pH	6.4

Phyto-chemical Analysis

The initial phytochemical analysis of phytoconstituents revealed the presence of alkaloids, carbohydrates, flavonoids, phenols, phlobatannins, tannins, terpenoids, and quinones. However, the absence of purple color in amino acids and protein tests concludes the absence of amino acids and proteins in *Murrayakoenigii* leaves. The results of the phytochemical analysis of *Murrayakoenigii* are shown in Table 2.

Table 2: Phytoconstituents screening of *Murrayakoenigii* leaves powder

S.No.	Phytochemical	Test	Observation	Inference
1	Alkaloids	Wagner's test	Red brown precipitate	Alkaloids present
2	Carbohydrates	Molisch's test	Violet ring	Carbohydrates present
3	Cardiac glycosides	Keller kiliani test	Brown/ violet/ green ring	Cardiac glycosides present
4	Flavonoids	Shinoda test	Red color	Flavonoids present
5	Phenols	Ferric chloride test	Deep blue color	Phenols present
6	Phlobatannins	Precipitate test	Red precipitate	Phlobatanninspresent
7	Amino acids & proteins	Ninhydrin test	Negative	Amino acids & proteins absent
8	Tannins	Ferric chloride test	Green color	Tannins present
9	Terpenoids	Salkowski test	Reddish brown precipitate	Terpenoids present
10	Quinones	Conc. HCl + extract	Yellow precipitate	Quinones present

Thin-layer Chromatography

The Thin-layer chromatography of 100% methanol extract and 60-80 petroleum ether extract of fresh as well as dry leaves of *Murrayakoenigii* witnessed 3 spots for both fresh leaves and dry

leaves methanol extract and a single spot was visible in the case of petroleum ether extract when the TLC plates were subjected to iodine fumes. The results of Thin-layer chromatography are represented in Table no. 3-5 respectively.

Table 3: Methanol extract of fresh leaves

Spot position(cm)	Distance travelled by solvent (cm)	Rf value	Day light colour of spot	Colour of spot when exposed to Iodine fumes
1.4	4.9	0.28	Yellow	Light brown
3.7	4.9	0.75	Yellow	Orange
4.4	4.9	0.89	Greenish yellow	Light brown

Table 4: Methanol extract of dry leaves

Spot position (cm)	Distance travelled by solvent (cm)	Rf value	Day light colour of spot	Colour of spot when exposed to Iodine fumes
4.8	5.0	0.96	Yellow	Yellowish orange
1.9	5.0	0.38	Faint yellow	Yellowish orange
1.5	5.0	0.31	Faint yellow	Yellowish orange

Table 5: Petroleum ether extract of fresh and dry leaves

Leaves	Distance travelled by solute (cm)	Distance travelled by solvent (cm)	Rf value	Day light colour of spot	Iodine chamber
Fresh leaves	3.9	5.1	0.76	Very faint yellow	Brown
Dry leaves	2.5	5.7	0.43	Very faint yellow	Brown

Conclusion

It can be inferred from the present sample that the coarse powder of *Murraya koenigii* leaves was dark green in color with a characteristic odour and tastelessness. The microscopy study of leaves showed the presence of unicellular trichomes, starch, vascular bundles calcium oxalates are found. However, the color of the extracts was different in different solvents. The phytochemical screening of *Murraya koenigii* leaves has shown the existence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenol, phlobatannins, tannins, terpenoids, and quinones. However, the amino acid and proteins were absent in them as evidenced in the present study.The Thin-layer chromatography results of the extracts support the presence of various phytochemical constituents in *Murraya koenigii* leaves.

Acknowledgement

The authors extend their sincere acknowledgement to the Department of Pharmaceutical Sciences and Technology, BIT Mesra for providing all necessary facilities and constant support.

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Iontophoresis: A Novel Method for Drug Permeation in Transdermal Drug Delivery

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Abstract

The limited drug molecules can be permeated to the skin due to their physicochemical limitations. Iontophoresis is a non-invasive method for physically promoting the transfer of drug molecules across the skin. This method facilitates the transfer of drugs across the skin under the impaction of electric difference in potential on topically. This technique improves the bioavailability of the drug by avoiding the first-pass effect. The current review focuses on principles, advantages, disadvantages, factors affecting iontophoresis and its uses.

Keywords: Iontophoresis, Potential, Topically, Bioavailability, First pass effect.

Introduction

The skin occupies a 1.7 m^2 body surface area. Hence it is the largest organ of the body. The skin is utilized for various administration of dosage forms on topically[1]. Drugs having short biological half-lives can be administered topical route. The transdermal drug delivery system (TDDS) offers an uninterrupted drug administration throughout a certain timeframe. Firstly drug penetrates across the stratum corneum. Then it penetrates to the layers of skin like the epidermis, dermis, and hypodermis. Topical administration of drugs is beneficial those drugs go for extensive first-pass metabolism when administered oral route. This route has more importance for potent drugs. TDDS offers less cost, convenience to patients, and a cosmetic point of view[2]. To improve drug permeability on the skin various techniques, as well as permeation enhancers, are used. Out of various methods, the iontophoresis method is the most convenient and effective one which can deliver charged molecules. This technique satisfies for improving the transdermal delivery of various drugs at lower current intensity within a short period.

Structureofskin

The skin protects the body from the extraneous surroundings from foreign particles and microorganisms. The main function of the skin is protection[3,4]. It has 3 layers such as epidermis, dermis, and hypodermis. It is shown in Figure 1.

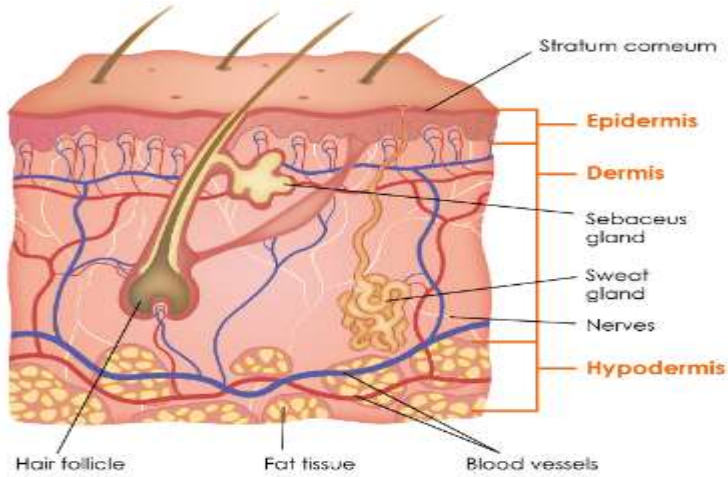

Figure 2. Anatomy of the skin

1. **Epidermis –** It is the extreme layer of the skin. The thinnest epidermal[5] layer is found on the eyelids (0.5 mm), and the thickest epidermal layer is found on the palms of the hands and soles of the feet (1.5 mm). The epidermis is multi-layered and comprises epithelial cells and the viable epidermis. The composition of epidermal tissue is of keratin protein, melanin pigment, Langerhans cells, and Merkel cells.

2. **Dermis –** The thickness of the dermis is nearly 2–3 mm thick. It constitutes collagen fibers and elastin fibers that offer toughness and elasticity to the skin. Blood vessels give nutrients to dermal tissue and epidermal tissue. The dermal layer constitutes macrophages, lymphatic vessels, and nerves.

3. **Hypodermis/Subcutaneous layer –** It is the innermost layer of the skin. It constitutes a meshwork of fatty cells which is abundant in this layer with nearly 50% body fat. It is an intermediate layer between the skin and the underlying tissues of the body. Functions[7,8] associated with this layer are protection, insulation of heat, and conduction of nerve signals.

Iontophoresis

Iontophoresis a noninvasive technique insists current range of 0.1 to 1.0 mA/cm^2 which takes charged ions across the skin by the effect of electricity and it makes easy permeation of ionic drug molecules into the skin by its potential difference. Positively charged ions are repelled by the anode chamber; similarly, negatively charged ions are repelled by the cathode chamber. Hence the iontophoretic technique[9-12] is a result of the principle of electrostatic repulsion "like charges repel and opposite charges attract each other". The drugs in the form of ions easily cross skin by the repulsive nature of similar charges. Therefore, drugs of anionic nature[13] penetrate the skin by

incorporation of the negatively charged electrode, and also drugs of cationic nature penetrate the skin by incorporation of positively charged electrode[14,15].

Advantages[16-18]

1. Injectable risk is avoided by ionophoretic topical delivery.
2. The ionized and unionized drug molecules can be delivered by iontophoresis.
3. If toxicity arises, the drug delivery can be easily terminated.
4. The amount of drug which is delivered can be controlled.
5. It avoids problems occurred in oral drug delivery like incomplete drug absorption and metabolism.
6. It decreases inter and intra subjects' variations as the drug delivery rate is more dependent on applied current.
7. Iontophoresis is a non-invasive method that is a substitute for chemical enhancers.

Disadvantages[19,20]

1. If the drug brings out irritation topically iontophoresis is not preferable.
2. The delivery rate of drug quantity is generally 5 to 10 mg/hr.
3. Pigmentation may arise by some drugs after the implementation iontophoresis method.
4. The Iontophoresis method creates differences in pH of drug molecules between 4 to 7.3.
5. Higher current strength causes pain, flaming impact, and necrosis of the skin.
6. It cannot achieve a high drug level in blood.

Principle

The permeation of charged drug molecules into surface skin is increased by the iontophoresis technique by the incorporation of an electric current. The electric field generates force externally to ions in such a way that, it can penetrate within the skin, as a result of which the drug permeability is enhanced. The movement of ions occurred across the skin by the assistance electrical energy showing the basic principle of electricity[21,22] that is like charges repel each other and opposite charges attract. The drug is kept beneath an electrode of a similar charge of the drug, and in another electrode opposite charge to the drug is kept on the surface body surface behaving as a neutral site. A current lower the level of the patient's pain threshold is applied and it confesses it to flow for an accurate length of time. There is an increase of electrical current showing penetration of the drug into the skin surface by repulsion of similar charges and attraction of opposite charges. It is shown in Figure 2.

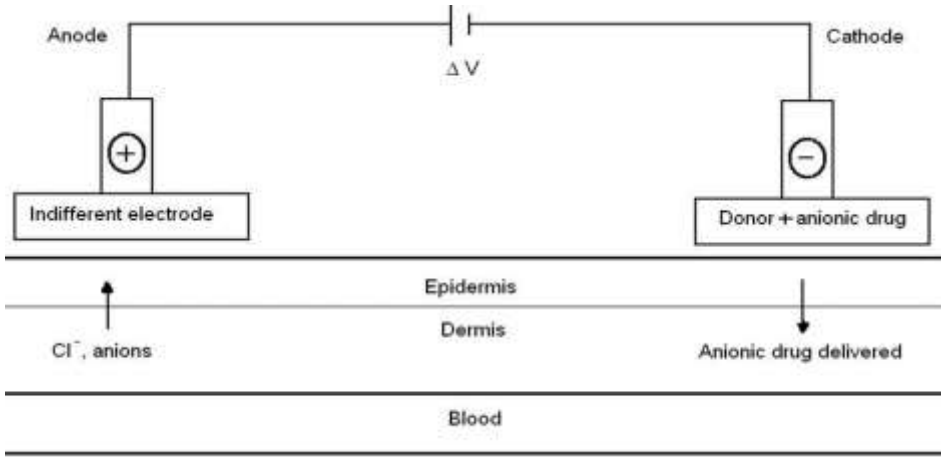

Figure 2. Principle of Iontophoresis

Factors affecting iontophoresis

Iontophoresis technique is influenced by many factors such as –

1. Physicochemical properties of the drug
2. Formulation factors
3. Experimental factors
4. Biological factors

Physicochemical properties of the drug[23-26]

- **Molecular size and molecular weight –** Molecular size depends upon permeability coefficients of drugs on the skin surface. Molecular size is inversely proportional to the permeability coefficient of drugs. Similarly, molecular weight depends on diffusion flux. By keeping all conditions constant, the transport of drugs is inversely proportional to molecular weight.
- **Charge –** Iontophoretic transport relies on electrical charge on drugs which gives information about the iontophoresis mechanism.
- **Polarity –** The polarity of drugs can influence the generation of flux. It is reported that the hydrophilic nature of drugs is suitable for optimum flux creation. Flux is inversely proportional to the lipophilicity of drugs.
- **Concentration –**The concentration of drugs in the donor chamber is directly proportional to the flux for a considerable current strength application. But at higher concentrations of drug in the donor chamber is not dependent on flux.
- **The salt form of the drug –** Drugs havingnon-identical salt forms generates divergent specific conductivities. The salt form of drugs can be selected based upon specific conductance. The estimated quantity of the drug relies on its salt form which gives information regarding the drug solution pH.

Formulation factors[27,28]

- **pH –** There is a role of donor solution on the skin pH. The drug solution promotes permeation above pH 4. As a result of which the ionization of –COOH groups occurs in the membrane promoting the transfer of cationic ions. It is observed that the ionization weakly acidic drugs occur above the pKa value of drug pH.
- **Ionic strength –** The permeability coefficient of the drug is directly proportional to its ionic strength. It is observed that peptides' ionic strength is high at low concentrations.
- **Presence of co-ions –** Co-ion is defined as an ion of a similar charge of a non-identical type. Co-ions can be generated by the incorporation of buffers in the donor compartment. The characteristics of co-ions are movable and lesser in size comparing to drug ions. The competitiveness arises among the drug and the co-ion due to the existence of co-ions which consequences a decrease of current and the flux of the drug.

Experimental factors[29,30]

- **Current strength –** Generally, the current strength of 0.5 mA/cm^2 is considered the highest iontophoretic current that can be applied to individuals. But it is shown that flux is directly proportional to current up to the limited current range for patients as well as time also up to 3 mins. Increasing the time there is a chance of skin irritancy.
- **Current density –** The amount of current transferred per unit surface area of the skin is known as current density. The current must be adequate to offer a good delivery rate of the drug in such a way that it should be devoid of deleterious reactions to the skin.
- **Pulsed current** – Direct current (DC) is directly proportional to time which is a consequence decrease in flux due to its polarization on the surface of the skin. It is controlled by the implementation of pulsed DC. Pulsed DC is can be transferred in the form DC in a manner of periodicity.
- **Duration of application –** Drug transfer relies on the duration of current applied to the skin. The permeation of the drug is directly proportional to the time applied.
- **Electrode materials –** The electrode substances utilized by iontophoresis must be non-toxic to the skin and abundantly movable while applied to the skin surface. Generally, Al, Pt, and Ag/AgCl electrodes are utilized for iontophoresis.

Biological factors[31,32]

- **Intra and inter-subject variability –** Intra and inter-subject variation for drug delivery is minimized by iontophoresis which was the major drawback in the passive absorption process. From clinical observations, reported minute variation in the flux rate among males and females.
- **Regional blood flow –** The contribution of dermal blood measures drug absorption in course of iontophoresis.

- **Condition of skin –** The skin environment influences the penetration of drug molecules during iontophoresis. *Invivo* passive diffusion of methyl salicylate shows following order: abdomen> forearm> instep> heel.

Applications of iontophoresis[33-38]

- **Topical delivery –** The rate of drug delivery on the skin can be controlled by using iontophoresis. The current strength can be altered to manage drug permeation. Hence it is a novel and effective technique to target drugs in the body locally as well as systematically.
- **Treatment of hyperhidrosis/ hyperhidrosis –** Hyperhydrosis is a state that most frequently outcomes in unrestricted sweating in certain body parts like hands and feet. Mostly well-known treatment for this situation is tap water iontophoresis. The method utilizes a little current which is penetrated via tap water for temporary cessation of sweat glands.
- **Diagnostic applications –** The Iontophoresis technique can be utilized for diagnosing various disorders. For example, the drug pilocarpine generates extraordinary production of sweat to allow an adequate quantity of sweat that can be gathered and analyzed. It is especially used for diagnosing cystic fibrosis.
- **Ophthalmology –** The Iontophoresis technique is a good method to deliver drugs as well as antibiotics into the eye. The main problem of this method is electrode touches the eye directly. Various drugs can be targeted to the eye by this method such as atropine, scopolamine, sulfadiazine, gentamycin, fluorescein, etc.
- **Non-invasive monitoring of glucose –** Noninvasive monitoring of glucose can be done by using the electro-osmosis technique a mechanism related to iontophoresis. The glucose moves in the opposite direction which is opposite to iontophoresis. Hence it is known as reverse iontophoresis. This phenomenon was utilized in Gluco Watch® Biographer (Cygnus Inc., Redwood City, CA, USA) within situ glucose sensor.
- **Peptide delivery –** Many peptides can be administered bypassing first-pass metabolism and gastrointestinal degradation by this method showing good bioavailability.

Iontophoretic devices

Iontophoresis devices release small amounts of drugs for a given time. The device operates at a steady voltage. Therefore, the current may be changed, depending upon the resistance of the skin.

The characteristics of good iontophoretic devices are safe, convenient, reliable, cost-effective, and portable[39, 40]. It may be of the disposable or reusable type. Some iontophoretic devices are shown in Table 1.

Table 1: Some Iontophoretic Devices

Iontophoretic Devices	Manufacturers	Drugs	Applications
Phoresor	Iomed Inc.	Lidocaine, Epinephrine	Dermal anesthesia
Active Tek	ActivaTek Inc.	Fentanyl HCl	Postoperative pain management
Lidosite®	Vysteris Inc.	Lidocaine	Anesthetic
E-Trans	ActivaTek Inc.	Fentanyl HCl	Postoperative pain management
IomedPhoresor® II	Iomed Inc.	Botulinum	Hyperhidrosis

Conclusions

The electrically operated permeation improvement offered by the iontophoresis technique overcomes the barrier present on the skin and showing better results for targeting various drug molecules. Iontophoresis is used for various skin disorders and other medical conditions. It is a better substitute for injectables. However, more research is required to find out the application of modern therapy.

Acknowledgments

The authors would like to acknowledge the contributions of the Pharmaceutics Department, College of Pharmaceutical Sciences, Puri, Odisha, India for providing the necessary facilities to carry out the review work.

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- Chapter in a book: Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2 ed. New York: Raven Press; 1995. p. 465-78.

ILLUSTRATIONS

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