LIPOSOMES FORMULATION AND ASSESSMENT

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ABSTRACT

The most sophisticated formulation for targeted and regulated medication delivery is liposomes. Liposomes work well to prolong a drug's duration of action. Liposomal formulations containing Mosapride citrate were produced and examined in this study. Usually, there are numerous ways to give these liposomes. The thin film hydration approach was used in this work to create the liposome. UV analysis and a microscope are used to assess or characterize the prepared liposome. The topic of the current study is liposomes. The primary ingredient in the liposome formulation was mosapride, a medication used to treat irritable bowel syndrome, gastritis, gastroesophageal reflux disease, and functional dyspepsia. On the gastrointestinal system, they also have an anti-inflammatory action. Dizziness, headaches, sleeplessness, nausea, diarrhea, and occasionally constipation are some of its common adverse effects. The oral mosapride citrate sustained release tablet of the current invention gradually controls the active ingredient and exhibits the same biological activity as the prior art. As a result, the mosapride liposome was effectively created.

KEYWORDS: cholesterol, cow ghee, handshaking technique, liposomes, and mosapride citrate.

INTRODUCTION

Alec Douglas Bangham, a British haematologist, discovered liposomes in 1961 at the Babraham Institute in Cambridge, England. He published his work in 1964. They were discovered while A.D. Bangham and R.W. Horne were testing a new electron microscope at the institute using a dry phospholipid and gram-negative stain.[1] They discovered the "Bag Like" arrangement, which was named "multilamellar smectic mesophase" or "Banghasomes" by A.D. Bangham. Gerald Weissman, his close colleague, recommended that these phospholipid bilayer vesicles, known as Liposomes, be more user-friendly.[2] Sustained release medication delivery makes use of physical and polymer chemistry. These polymers slowly release the drug into the biosystem and keep the drug's blood level within the therapeutic range for an extended period of time. Some of the results indicate drug permeability through the exact cellular barrier and any first-pass metabolic repercussions prior to drug entry into systemic circulation[3]. The liposome is a small bubble composed of the same substance as the cell membrane. The term liposome is derived from two Greek words: "Lipos" means fat, and "soma" means body. The membrane of phospholipids has the ability to self-assemble into small bilayer spheres. Micelles occur when a single layer sphere is produced[4]. In the pharmaceutical and cosmetic industries, liposomes carry a variety of compounds that are also utilised in the creation of cosmetics. Both hydrophilic and hydrophobic drugs are carried by the liposome.[5] Liposomes range in size from 30 nm to several micrometres and have a spherical structure. Lipids' polar heads face the internal and external aqueous phases, whereas the bilayer's non-polar tail faces each other.[6] Oral local therapy can also benefit from the liposomal preparation.[7] The most frequent gastrointestinal diagnosis made at clinic visits is gastroesophageal reflux disease (GERD).[8] The term "GERD" refers to a condition that arises when the reflux of stomach contents results in problematic symptoms and/or problems.[9] The primary issue with GERD is that the oesophageal epithelium may be exposed to stomach secretions, which could cause a histopathologic injury or trigger symptoms.[10]

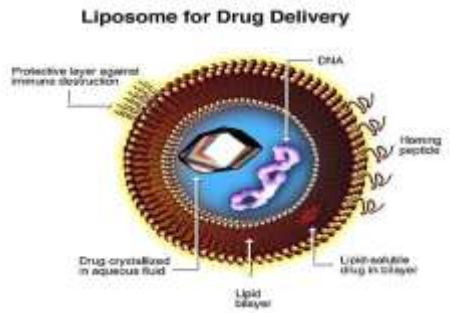


fig: internal structure of liposome[40]

Mosapride is a 5HT4 agonist that is selective. M1, mosapride's main active metabolite, also has 5HT3 antagonistic properties.[11] Mosapride citrate (MSP) is a selective serotonin 5-HT4 receptor agonist medication used to treat mild GERD patients for a short period of time, reducing symptoms and endoscopic recurrence.[12/13] Mosapride has been used to treat diabetic gastropathy, non-ulcer dyspepsia, chronic gastritis, and gastroesophageal reflux disease.[14] When administered orally, mosapride can be absorbed in the digestive tract at rates more than 93%. Mosapride has a quick pharmacological impact, reaching its peak concentration in the blood within 0.5 to 1.4 hours after oral administration. 6]Because Mosapride has a short half-life ranging from 1.3 to 2 hours, it swiftly fades once absorbed into the body, and so it should be delivered a few times due to the drug's short duration of action.[15] The term "functional gastrointestinal disorder"[16] (FGID) refers to gastrointestinal (GI) irregularities and symptoms that are ongoing and persistent but have no evident underlying disease. Dyspepsia.[17] indigestion is another term for stomach pain, overfullness, and bloating that occurs during and after eating. Other common symptoms include acid reflux, heartburn, and frequent burping. About 80% of dyspepsia patients are classed as having functional dyspepsia (FD), which means that their symptoms have no anatomical explanation. Functional dyspepsia is connected with a variety of hypothesised processes.[18] Liposomes are vesicles that contain nutrients and various pharmacological medications. These liposomes have various advantages, but they also have a downside, which is oxidation due to the presence of lipid as a main component.[19] When taken orally, mosapride can be absorbed in the digestive tract by more than 93%.mosapride is found in the liver, small intestine, kidney, and adrenal glands at concentrations at least ten times higher than in blood plasma, as well as in the lungs, submaxillary gland, pancreas, hypophysis, thyroid gland, spleen, and eyeballs at a concentration about half that of blood. About 80% of dyspepsia patients are classed as having functional dyspepsia (FD), which means that their symptoms have no anatomical explanation. Functional dyspepsia is connected with a variety of hypothesised processes.[20] Liposomes are used as drug delivery systems because of their excellent characteristics such as flexibility, biocompatibility, biodegradability, increasing the therapeutic index of the drug and efficacy, increasing the stability of the entrapped drug from the hospital environment, reducing side effects, providing sustained release, and acting as a reservoir of drug.[21]

MATERALS AND METHOD

Mosapride citrate (Amepurva Forum Nirant Institute of Pharmacy), cow ghee (Solapur Demart), cholesterol (Amepurva Forum Nirant Institute of Pharmacy), soya lectin, chloroform methanol, and phosphate buffer solution.

PREPRATION OF LIOPOSOMES

Handshaking method

In this way. The mosapride citrate and cow ghee were dissolved in chloroform and methanol (2:1) and dispersed across a flat bottom conical flask. The solution was then evaporated at room temperature, without disturbing it. The lipid film form was hydrated with aqueous medium phosphate buffer (pH 7.4). The flask was tilted to one side, and an aqueous medium containing the medication to be entrapped was introduced down the side of the flask. The flask was gradually returned to its upright orientation. The fluid was gently poured over the lipid layer, and the flask was let to stand at 37 degrees Celsius for 2 hours to ensure complete swelling. After swelling, vesicles are harvested by whirling the contents of the flask, yielding a milky white solution. Then, make liposomes[22].

Composition of liposomes with cow ghee.

Batches code	Cow ghee	Mosapride	Oraganic solvent(2:1)	Hydration medium(pbs)
1	100mg	10mg	10ml	10ml
2	150mg	15mg	10ml	10ml
3	200mg	20mg	10ml	10ml

Hand Shaking Method.

In this way. Mosapride citrate, cholesterol, and soyalectin were dissolved in chloroform and methanol (2:1) and dispersed over a flat-bottomed conical flask. The solution was then evaporated at room temperature, without disturbing it. The lipid film form was hydrated with aqueous medium phosphate buffer (pH 7.4). The flask was tilted to one side, and an aqueous medium containing the medication to be entrapped was introduced down the side of the flask. The flask was gradually returned to its upright orientation. The fluid was gently poured over the lipid layer, and the flask was let to stand at 370 degrees Celsius for 2 hours to ensure complete swelling. After swelling, vesicles are harvested by whirling the contents of the flask, yielding a milky white solution. Then, construct a liposome.[23]

Liposome formulation table based on cholesterol.

Sr. No	Batch Code	mosapride Drug	Cholesterol	soya lectin
1.	F1	25 mg	15 mg	100mg
2.	F2	50 mg	15 mg	100mg
3.	F3	75 mg	15 mg	100mg

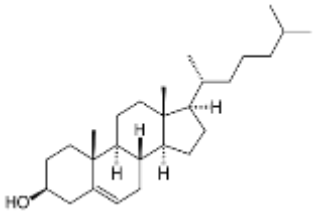
Cow ghee:

In India, cow ghee is used in traditional medicinal formulations to solubilize lipophilic medicines and increase intestine absorption. However, there have been no observations on the role of cow ghee, which is naturally high in saturated fatty acids, in carotenoid chemistry. In mice fed cow-ghee, postprandial plasma lutein levels increased significantly ($p < 0.05$) within 2 hours (Cmax-135.76 pmol/mL; AUC-592.80 pmol.h/mL) (Tmax). Cow ghee increased lutein oral bioavailability by 2.02, 1.41, and 1.66 folds as compared to the control, olive oil, and flaxseed oil. Cow ghee, which contains 69.28% saturated fatty acids, has the potential to be a lutein delivery vehicle, as indicated by increased postprandial triglyceride levels. This is the first study of its kind to reveal the effect of saturated fatty acids on the oral bioavailability of lutein in an in vivo system[24].

Cholesterol:

Cholesterol: Cholesterol is another key structural component of liposomes. It's a regularly used sterol. The addition of sterols alters the function of stability and rigidity and increases the time of circulation in the bloodstream[25.] It does not self-assemble into a bilayer structure. It is integrated into phospholipids at extremely high concentrations, up to a 1:1 or 2:1 molar ratio of cholesterol to phosphotidyl choline. The presence of cholesterol in the lipid bilayer promotes stability, forming a highly organized and rigid membrane structure[26].

Cholesterol lowers the permeability of water-soluble molecules while increasing the fluidity and stability cellular membrane. Cholesterol hindered liposome interaction and instability.[27]



Structure: cholesterol

Mosapride citrate:

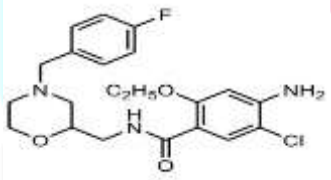
Mosapride specifically activates the 5-HT4 receptor, promoting gastrointestinal motility. The 5-HT4 agonist is a typical treatment for functional dyspepsia.[28] Mosapride is a new prokinetic drug that appears to act through a high affinity and specificity for the 5-HT4 receptor. In addition, the main metabolite has a high affinity for 5-HT3 receptors and has been shown to be a powerful 5-HT3 antagonist.[29]When administered orally, mosapride can be absorbed in the digestive tract at a rate of more than 93%. Mosapride is at least ten times more concentrated in the liver, small intestine, kidney, and adrenal glands than in blood plasma, and it is also found in high concentrations in the lungs, submaxillary gland, pancreas, hypophysis, thyroid gland, spleen, and other tissues. Mosapride has a quick pharmacological impact, reaching its peak concentration in the blood within 0.5 to 1.4 hours after oral administration.[30] Recent research has revealed the critical function of the gut microbiota in gastrointestinal disorders such as dyspepsia and infections. Dyspepsia development and persistence can be influenced by gut microbial imbalance. Furthermore, maintaining a healthy gut microbiota is critical for protecting against gastrointestinal illnesses, particularly those caused by Helicobacter pylori.[31]

Synonym:4-amino-5-chloro-2-ethoxy-N-((4-(4-fluorobenzyl)morpholin-2-yl)methyl)benzamide-2-hydroxypropane-1,2,3-tricarboxylate

Molecular formula: C21H25ClFN3O3.C6H8O7

Molecular weight: 614.02 g/mol

BCS class: II Category: gastroprokinetic agent



Structure of mosapride

Evaluation test for liposomes:

Compound microscope

UV analysis.

Fig. liposomes

Characterization of Mosapride Citrate Liposomes:

UV spectrum analyzers are commonly used to evaluate particle size in liposomes. This instrument includes Malvern PCS software. Before establishing the outcome, the sample solution must be diluted with blank solution. After dilution, the findings were obtained. The particle size must be in the nano range, although it can occasionally exceed the micron range when multilamellar vesicles are present. Microscopy and UV spectroscopy were used to determine the average particle size of liposomes administered by this program.[32/33]

To quantify drug entrapment, the amount of drug in the clear supernatant after centrifugation (w) was quantified using a UV spectrophotometer at 254 nm. This was accomplished by creating a standardized drug calibration curve. The amount of medication in the supernatant was then subtracted from the total amount utilized in the preparation (W). Effectively, (W-w) represents the amount of medicine trapped in the liposome. % Drug Entrapment = (W-w/W) x 100.[34/35]

Loading Efficiency:
The drug content in the preparation was determined by extracting it from the liposomes with 0.1M hydrochloric acid. Liposomes (50mg) were combined with 50ml of hydrochloric acid until dissolved. It was filtered through filter paper, and the drug content was determined after proper dilution. The loading efficiency (L) of the liposome was estimated as follows:
A formula.

L (%) = (Qn/Wn) × 100 Qn is the amount of drug present in the liposome, and Wn is the liposome's weight.[36]

Future Prospects:

• Liposomal encapsulation improves mosapride's solubility and stability, resulting in higher oral or transdermal bioavailability.

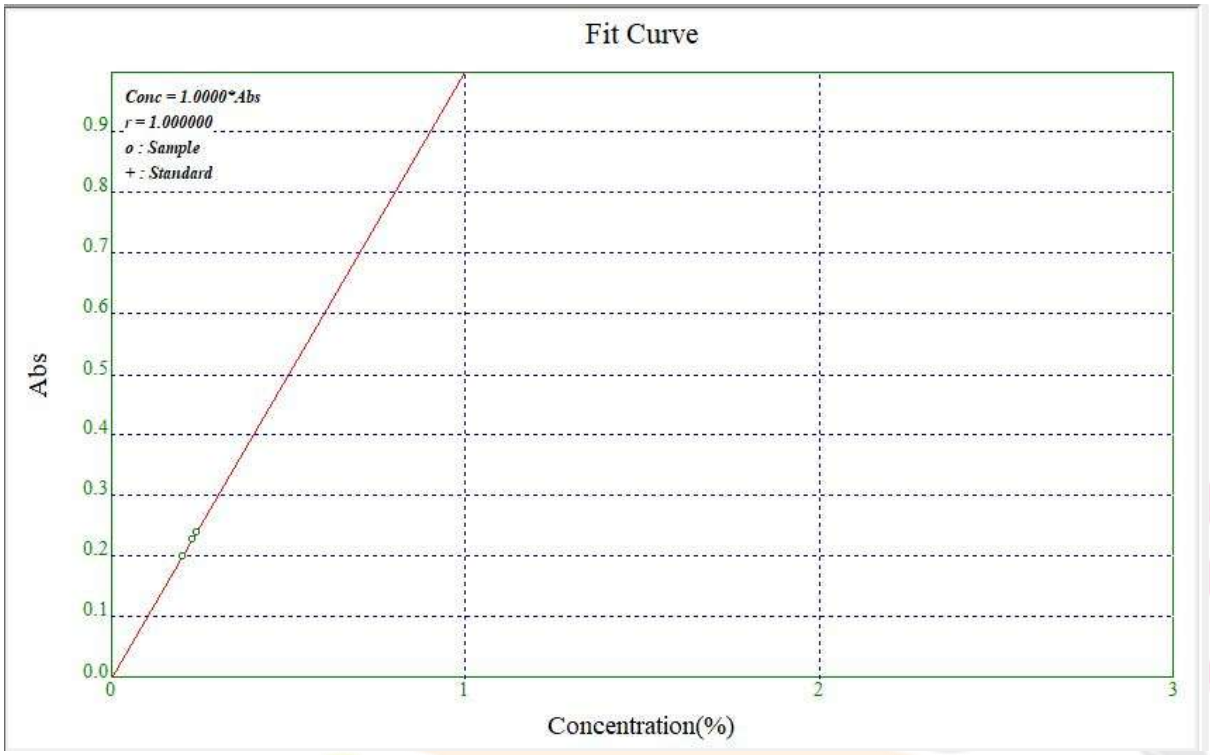
• \development of ligand-conjugated liposomes (e.g. floate pepatides) to target specific GI receptors or organs.

• Engineered sustained or delayed-release liposomal systems for reduced dosage frequency and negative effects.

• Nano-liposomes (<200nm) enhance cellular absorption and provide rapid onset effect.[39]

RESULT :

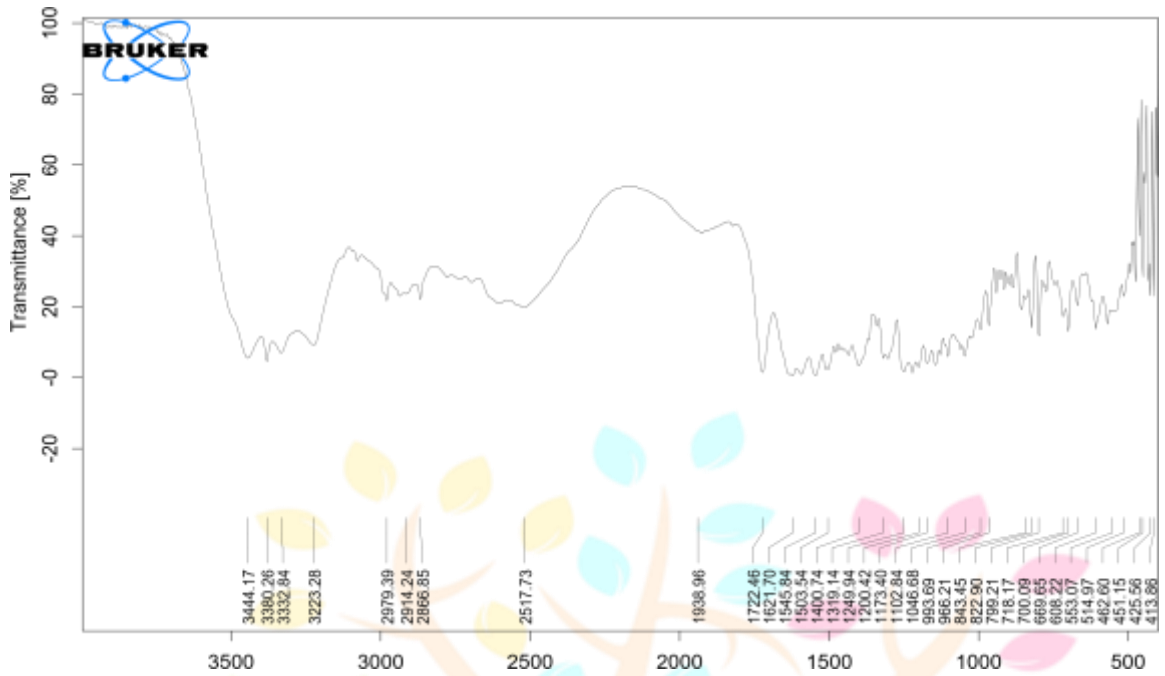
Uv spectroscopy:



- **Interpretation of mosapride loaded liposomes**

Parameter	**details**
Graph title	Fit curve
X-axis	Concentration %
Y-axis	Absorbance (Abs)
Fit line equation	Conc=1.0000 × Abs
Correlation coefficient	r=1.000000(prefect linear correlation)
Linearity	Perfect linear relationship between concentration and absorbance
Symbols in graph	O: Sample data point+: Standard data point
Working concentration range	0% to approximately 1%
Absorbance Range	0 to approximately
Interpretation	Use the equation to calcaulate unknown sample concentration by substituting absorbance values

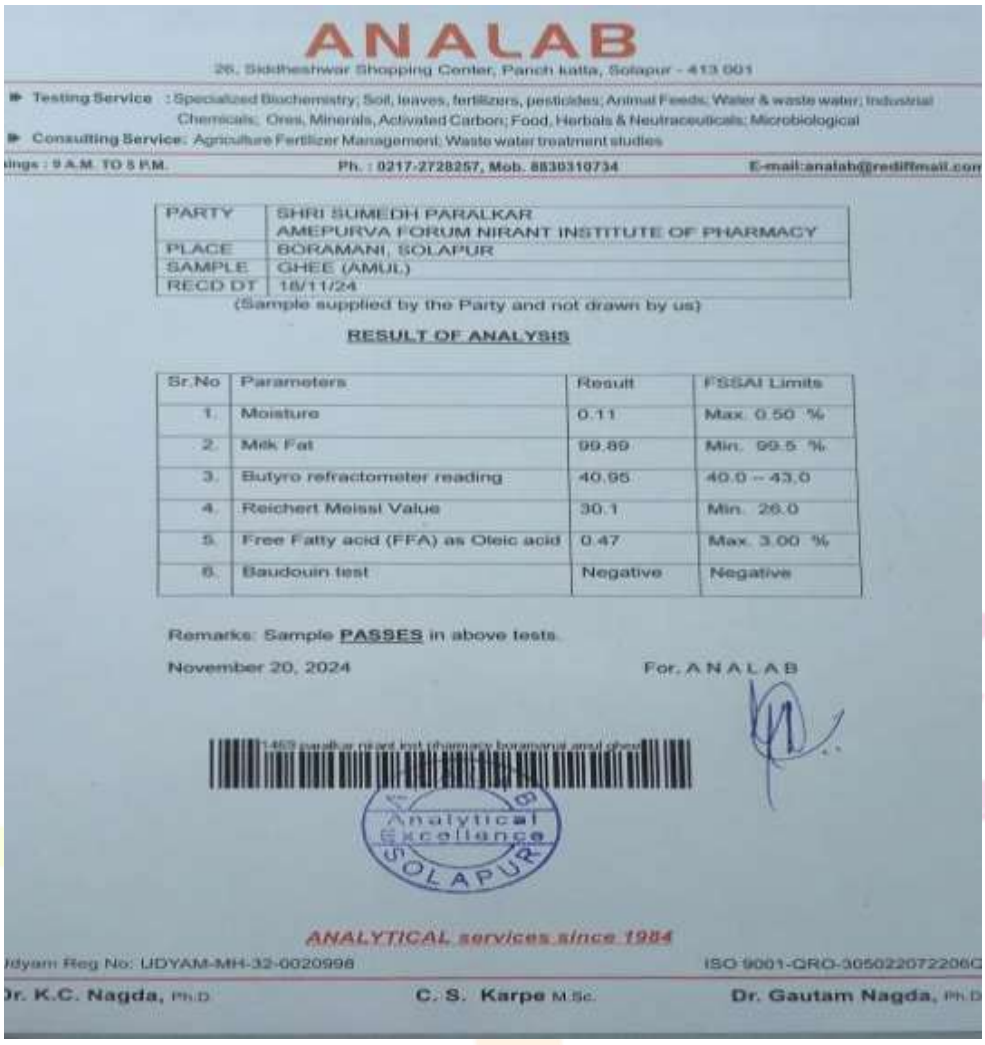
Mosapride citrate IR



Interpretation of mosapride

Wavenumber(cm^{-1})	Functional group/ Bond	Assignment
3414.17	O-H/N-H stretching	Possibly -OH or secondary amine (broad peak)
2932.26	C-H stretching	Aliphatic C-H stretch (CH_2/CH_3)
1732.30	C=O stretching	Carbonyl group (possibly ester or ketone)
1617.73	C=C or C=N stretching	Aromatic ring or imine group
1386.96	C-N stretching or CH_3 bending	Possible amine or methyl group
1200-1000	C-O stretching	Ether or ester functional groups
Around 750-600	Aromatic C-H Bending	Confirms presence of substituted aromatic rings.

Cow Ghee Test



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(Sample supplied by the Party and not drawn by us)

RESULT OF ANALYSIS

Sr.No	Parameters	Result	FSSAI Limits
1.	Moisture	0.11	Max. 0.50 %
2.	Milk Fat	99.89	Min. 99.5 %
3.	Butyro refractometer reading	40.95	40.0 – 43.0
4.	Reichert Meissl Value	30.1	Min. 26.0
5.	Free Fatty acid (FFA) as Oleic acid	0.47	Max. 3.00 %
6.	Baudouin test	Negative	Negative

Remarks: Sample PASSES in above tests.

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CONCLUSIONS:

Liposomal compositions with various lipid cholesterol and cow ghee molar ratios were produced and tested. The evaluation experiments found that increased cholesterol concentrations (soyalectin stiffness of the bilayer) resulted in more regulated release of Mosapride citrate. As a result, the combination employed in F1 is the best for achieving more sustained release, and liposomal formulation of mosapride citrate would be a good approach to provide a more uniform release profile. Mosapride citrate has various side effects. Mosapride citrate's side effect is reduced as a result of its liposomal composition. Mosapride's bioavailability increases as a result of this innovative formulation.

REFERENCES.

1.Bangham AD, Horne RW. Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope. J Mol Biol. 1964;8:660–710.

2. Weissmann G, Sessa G, Standish M, Bangham AD. Abstracts. J Clin Invest. 1965;44:1109–16.

3. Chien YW, Lin S. Drug delivery: controlled release. In: Swarbrick J, Boylan JC, editors. Encyclopedia of Pharmaceutical Technology. 3rd ed. Vol. 2. New York: Marcel Dekker Inc.; 2007. p. 1082–3.

4. Dua JS, Rana AC, Bhandari AK. Liposome: method of preparation and application. Int J Pharm Stud Res. 2012;3(2):14.

5. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, et al. Liposome: classification, preparation, and applications. Nanoscale Res Lett. 2013;8:2.

6. Umbarkar M, Thakare S, Surushe T, Giri A, Chopade V. Formulation and evaluation of liposome by thin film hydration method. J Drug Deliv Ther. 2011–2021; Open Access to Pharmaceutical and Medical Research.

7. Pistone S. Formulation and evaluation of polysaccharide and liposome-based nano systems for improved targeting to the oral cavity [dissertation]. Oslo (Norway): University of Oslo, Department of Pharmaceutics, School of Pharmacy; 2016. p. 11.

8. Peery AF, Dellon ES, Lund J, Crockett SD, McGowan CE, Bulsiewicz WJ, et al. Burden of gastrointestinal disease in the United States: 2012 update. Gastroenterology. 2012;143:1179–87.

9. Vakil N, van Zanten SV, Kahrilas P, Dent J, Jones R. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. Am J Gastroenterol. 2006;101:1900–20.

10. Kahrilas PJ, Lee TJ. Pathophysiology of gastroesophageal reflux disease. Thorac Surg Clin. 2005;15:323–33.

11. Tack J, Camilleri M, Chang L, Chey WD, Galligan JJ, Lacy BE, et al. Systematic review: cardiovascular safety profile of 5-HT(4) agonists developed for gastrointestinal disorders. Aliment Pharmacol Ther. 2012;35(7):745–67. doi:10.1111/j.1365-2036.2012.05011.x. PMID: 22356640; PMCID: PMC3491670.

12. Ruth M, Finizia C, Cange L, Lundell L. The effect of mosapride on oesophageal motor function and acid reflux in patients with gastro-oesophageal reflux disease. Eur J Gastroenterol Hepatol. 2003;15:1115–21.

13. Ruth M, Hamelin B, Rohss K, Lundell L. The effect of mosapride, a novel prokinetic, on acid reflux variables in patients with gastro-oesophageal reflux disease. Aliment Pharmacol Ther. 1998;12:35–40.

14. Micromedex [Internet]. Available from: http://www.micromedex.com.

15. Mosapride sustained-release formulation that provides pharmacological clinical effects once a day. Worldwide patent applications. JP2015562936A.

16. Hyams J, Hyams JS, Saps M, et al. Childhood functional gastrointestinal disorders: child/adolescent. Gastroenterology. 2016;150(6):1456–68.

17. Ishizuka H. Carlyle's nervous dyspepsia: nervousness, indigestion and the experience of modernity in nineteenth-century Britain. In: Neurology and Modernity: A Cultural History of Nervous Systems, 1800–1950. London: Palgrave Macmillan; 2010. p. 81–95.

18. Olendzki BC, Silverstein TD, Persuitte GM, et al. An anti-inflammatory diet as treatment for inflammatory bowel disease: a case series report. Nutr J. 2014;13(1):1–7.

19. Marripati S, Umasankar K, Reddy PJ. A review on liposome. Int J Res Pharm Nano Sci. 2014;3(3):159–60.

20. Kawakami K, Yoshikawa T, Moroto Y, Kanaoka E, Takahashi K, Nishihara Y, et al. Preparation of fenofibrate solid dispersion using electrospray deposition and improvement in oral absorption by instantaneous post-heating of the formulation. Int J Pharm. 2013;450(1):123–8.

21. paralkar s, Thorat Y, Tiwari B, Gadade V, Gadade S. Review On A Comprehensive Study Of Developing And Evaluating Liposomes.int j create Res Thoughts [internet].2025 mar;13(3). available form: https://www.ijcrt.org.

22. Goswami P, Changmai A, Barakoti H, Choudhury A, Dey BK. A brief review on liposomal drug delivery system. J Pharm Adv Res. 2018;1:362–8.

23. Jithan AV, Swathi M. Development of topical diclofenac sodium liposomal gel for better anti-inflammatory activity. Int J Res Pharm Nano Sci. 2010;3(2):986–7.

24. Bhat I, Madhura RJ, Badanthadka M, Mamatha BS. Cow ghee as an efficient carrier to improve oral bioavailability of lutein. [Journal name missing – please provide for complete reference.]

25. Sanarova E, Lantsova A, Oborotova N, Orlova O. Liposome drug delivery. J Pharm Sci Res. 2019;11(3):1148–55.

26. Li X, Chen D, Le C, Zhu C, Gan Y, Hovgaard L. Novel mucus-penetrating liposomes as a potential oral drug delivery system: preparation, in-vitro characterization, and enhanced cellular uptake. Int J Nanomedicine. 2011;6:3151–62.

27. Ejiogu DC. Formulation and evaluation of etodolac niosomes by modified ether injection technique. J Pharm Res. 2016;1:1–6.

28. Toru H. Meta-analysis of the effects of prokinetic agents in patients with functional dyspepsia. J Gastroenterol Hepatol. 2007;22:304–10.

29. Ruth M. The effect of mosapride, a novel prokinetic, on acid reflux variables in patients with gastro-oesophageal reflux disease. Aliment Pharmacol Ther. 1998;12:35–40.

30. Ong IJ, Loo KY, Law LNS, et al. Exploring the impact of Helicobacter pylori and potential gut microbiome modulation. Prog Microbes Mol Biol. 2023;6(1):a0000273.

31. Mutalib NSA, Wong SH, Ser HL, et al. Bioprospecting of microbes for valuable compounds to mankind. Prog Microbes Mol Biol. 2020;5(1):a0000088.

32.Lankalapalli S, Tenneti VK, Adama R. Preparation and evaluation of liposome formulations for poorly soluble drug itraconazole by complexation. Scholars Res Libr Der Pharm Lett. 2015;7(8):3.

33. Moghimipour E, Salami A, Monjezi M. Formulation and evaluation of liposomes for transdermal delivery of celecoxib. Jundishapur J Nat Pharm Prod. 2015;10(1):2–3.

34. Ghule MM, Bhoyar GS. Formulation and evaluation of modified liposome for transdermal drug. J Dev Drugs. 2018;7(1):2–3.

35. De A, Venkatesh DN. Design and evaluation of liposomal delivery system for L-Asparaginase. J Appl Pharm Sci. 2012;2(8):112–7.

36. Abd-Allah FI. Development, characterization and ex vivo evaluation of various liposome-encapsulated aceclofenac formulations. Br J Pharm Res. 2016;9(4):7.

37.Kim DY, Kim JH, Yoon YH. Pharmacokinetics and bioavailability of mosapride citrate in humans. Int J Clin Pharmacol Ther. 2001;39(5):226-30.

38. Nagarwal RC, Singh PN, Kumar R. Chitosan nanoparticles: a promising system in novel drug delivery. Chem Pharm Bull (Tokyo). 2011;59(12):1410-8.

39.Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov. 2005;4(2):145-60.

40.Lonkar VG, Shinde AD. Formulation and evaluation of liposomal drug delivery system of metformin hydrochloride. Pharmacophore [Internet]. 2016 [cited 2025 May 21];7(4):280-291. Available from: http://www.pharmacophorejournal.com/