RESEARCH PAPER

Formulation of Carrageenan -Based Drug Delivery System For Sustained Release Of Letrozole In Breast Cancer Therapy

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ABSTRACT

The present study focuses on the development of a carrageenan-based drug delivery system for the sustained release of letrozole, a drug commonly used in the treatment of breast cancer. Conventional drug delivery methods often result in rapid drug release and reduced therapeutic efficiency. Hence, this work aims to design a formulation that can provide controlled and prolonged drug release. In this study, carrageenan was selected as the polymer due to its biocompatibility and good swelling properties. The formulation was prepared using a solvent casting method, where carrageenan was dissolved in an acetic acid solution and letrozole was incorporated into it. The prepared solution was cast into Petri plates, dried, and converted into powder form for further analysis. The formulation was evaluated using various characterization techniques. SEM analysis showed a porous surface structure, while TEM confirmed uniform distribution of drug particles within the polymer matrix. FTIR analysis indicated the presence of characteristic functional groups without any significant interaction between the drug and polymer. XRD results revealed the amorphous nature of the formulation, confirming reduced crystallinity of the drug. UV–Visible spectroscopy was used for drug quantification and showed a linear relationship between concentration and absorbance. The in-vitro drug release study demonstrated a gradual and sustained release of letrozole over 24 hours without any burst release. Swelling studies indicated good water absorption capacity of the formulation. The antimicrobial assay showed effective inhibition of microbial growth. Overall, the results of this study suggest that carrageenan-based drug delivery systems can serve as a promising platform for the sustained release of letrozole. The formulation developed in this work demonstrates good physicochemical properties, controlled drug release behaviour, and potential biological activity. This approach may help in improving the therapeutic efficiency of anticancer drugs while minimizing side effects.[6]

Keywords: Carrageenan, Letrozole, Drug Delivery System, Sustained Release, Breast Cancer.

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INTRODUCTION

1.1 An Overview of Drug Delivery Systems

Drug delivery systems serve a vital function in contemporary pharmaceutical research by facilitating efficient and regulated administration of medicinal substances into the body. Conventionally, drugs were administered using simple means such as orally or via injections. Despite their ease of application, such administration procedures led to variations in the concentration of the drug in the body, resulting in

inefficiencies and higher side effects. The inadequacy associated with traditional delivery systems has made way for advanced drug delivery systems. Such techniques offer higher bioavailability of drugs and enable patients to take lower doses of the substance. This ensures maintenance of a constant level of the concentration of drugs within the optimal range. There is increasing interest in developing novel drug delivery systems that use polymer materials. Natural polymers appear promising because of their superior control over drug release and enhanced biocompatibility.[1]

1.2 The Need for Controlled/Sustained Drug Delivery Systems

A common drawback with normal drug delivery techniques is the absence of a steady supply of drugs in the body. As such, the drug concentration levels tend to peak very quickly before dropping below the therapeutic levels, thereby necessitating frequent drug administration. Controlled/sustained drug delivery systems help deliver the drugs steadily at certain predetermined rates over an extended period of time. In addition, they also help minimize degradation and increase stability and efficacy. Furthermore, they enable sustained drug delivery without compromising on therapeutic benefits and minimize toxicity risks. They are therefore very useful when treating diseases that need continued medication, such as cancer.[6]

1.3 Natural Polymers in Drug Delivery

Due to their nontoxicity, biodegradability, and biocompatibility, natural polymers are being highly regarded for use in drug delivery. Natural polymers, in contrast to synthetic polymers which are chemically produced through artificial means, come from biological sources, meaning they are safe for use in pharmacy. Natural polymers, including but not limited to chitosan, alginate, and carrageenan, can all be exploited for use in drug delivery systems. These polymers can form gels, films, and matrices to encapsulate drugs and control drug release. Because of their hydrophilic nature, they can swell when exposed to water and release the drug over time. Further, natural polymers have many possibilities to be modified either chemically or physically, thus lending themselves to a plethora of drug delivery applications.[3]

1.4. Carrageenan as a Drug Carrier

Carrageenan is a substance that comes from red algae. It is very useful in the biomedical industries. The good things about carrageenan as a way to deliver drugs are that it can form gels it is safe for the body. It is not toxic. Carrageenan can form kinds of gels that hold drugs and release them slowly. The fact that carrageenan can absorb a lot of water is important for getting the drugs out of the gel. When carrageenan absorbs water, it releases the drug molecules from the gel. Carrageenan also has some good properties. It has sulphate groups that help keep the drug carrier system stable and make the drugs work better with the carrageenan. Carrageenan is a way to deliver drugs because it can release them slowly over time. This makes it ideal for treating people over a period. The way that carrageenan forms a structure makes it perfect for creating drugs that release slowly. Carrageenan is very useful, for making these kinds of drugs.[10]

1.5 On letrozole

Letrozole is an aromatase inhibitor that has been widely used in the management of hormone-responsive breast cancers. Letrozole acts by preventing the formation of estrogen from androgen through the inhibition of the enzyme aromatase. Letrozole lowers the estrogen concentration in the body, thereby slowing the growth and proliferation of tumour cells. Although letrozole is a potent drug, several limitations arise with its administration using traditional dosage forms. Letrozole can be rapidly metabolized and excreted from the body, causing variability in drug concentrations. Frequent dosing is required to maintain adequate drug concentrations in the body. The development of a controlled drug delivery system will address some of the limitations of the drug[4].

1.6 Drawbacks of Traditional Methods of Drug Delivery

Traditional methods of drug delivery, despite being popular and widely practiced, have several limitations that compromise their efficacy. The first and foremost drawback associated with these methods is the inability to control the release of drugs, which leads to variations in the concentration of the drug in the bloodstream. These fluctuations in drug concentration may result in either sub-therapeutic levels or drug toxicity, depending on the prescribed dosage. Moreover, repeated dosing is often necessary to maintain a constant drug concentration in the blood. Secondly, the distribution of drugs may be non-specific, thereby causing adverse effects on healthy tissues as well. Lastly, certain drugs may fail to reach their target site due to degradation during the delivery process.[5]

1.7 Need for Current Research

The inadequacies associated with conventional drug delivery systems and the strengths of polymer delivery system necessitates the need for the development of an effective drug delivery system for letrozole. There are several advantages associated with the usage of carrageenan, which is an example of a natural polymer, for drug delivery systems. In this case, the current

research will involve the design and preparation of carrageenan letrozole formulation that will facilitate sustained release of the drug. The incorporation of the drug within a polymer matrix provides control over the release rate of the drug. In addition, the current research will focus on the physical, chemical, and biological evaluation of the formulation.[11]

1.8 Scope of the Study

This study is focused on developing a sustained drug delivery system using a carrageenan-based formulation of letrozole, with evaluation of the physical properties of the formulation (SEM, TEM, FTIR, XRD) and/or by using in vitro methods, including but not limited to; drug release; swelling behaviour; and entrapment efficiency. Biological studies will be conducted on the antimicrobial and/or MTT assay to evaluate therapeutic effectiveness of the formulation. Results from this study could provide a foundation for future in vitro/in vivo studies and clinical application of a safe and effective drug delivery system for the treatment of cancer.[21]

MATERIALS AND METHODS

2.1 Materials

The formulation's materials were carefully chosen based on their suitability for pharmaceutical use and compatibility as well as on their ability to produce the product intended to help deliver the active ingredient (i.e., the drug) in a controlled manner. The primary polymer used in the formulation of the drug is carrageenan, which is highly swellable in an aqueous environment and forms a stable matrix through the hydrophilic properties of the polymer. Additionally, the reason letrozole was chosen for this study is because it has a well-established role in treating hormone-dependent breast cancer.

Throughout the study, chemicals and reagents used were of analytical grade in order to achieve reliability and precision with respect to result outcomes. For this study, distilled water was utilized as a vehicle for the production and dilution of compounds. Attention to detail was exercised in order to avoid contamination and all materials were collected and stored appropriately in temperature-controlled environments (cool and dry) to maintain material stability throughout the duration of the experimental study.

The rationale for our selection of carrageenan as the polymer utilized in this study was primarily based on its ability to swell and form a stable matrix when in contact with water, which is critical for the controlled release of drugs. In addition to providing a suitable matrix for the controlled release of drugs, carrageenan also provides excellent biocompatibility for use in pharmaceutical applications In this study, the polymer and drug were combined in a manner which supports uniform distribution of the drug and controlled release of the drug from the polymer.[15]

2.2 Instruments and Equipment

An array of analytical methods and instrumentation was employed for the preparation, characterization, and evaluation of the compositions used in this study. Each of these methods was utilized according to its appropriate application based on the analysis desired. The surface morphology of the formulation was assessed by SEM, while the internal structure was evaluated by TEM. FTIR spectroscopy was used to identify functional groups and confirm the compatibility between the drug and the polymer used to formulate it. XRD analysis was performed to determine if the drug was crystalline, while UV–Visible spectroscopy was utilized to quantify the drug. Prior to commencing the experimental methods, all analytical instruments were properly calibrated so that accurate and reproducible results could be obtained. All of the experimental work was carried out under controlled laboratory conditions in order to reduce as much error as possible. In performing the various analyses described above, care was taken to strictly follow established protocols to ensure that results were consistent. The combination of multiple analytical techniques provided an in-depth review of the surface and internal characteristics of the formulations used in this study. This information correlates the structural characteristics of the formulations with the release behaviour of the drug in vitro [16]

2.3 Preparation of Carrageenan-Based *letrozole* Formulation

Using a method called solvent casting, a formulation can be created for a drug delivery system based on polymers. The first step will require the use of a specified number of carrageenan (seaweed extract) in distilled water. The solution will then need to be stirred continuously for a short period to ensure that the polymer completely dissolves and a clear solution is achieved. The second step will be to weigh out an exact amount of letrozole (the active drug component) in a suitable solvent and then slowly combine the two solutions together under continuous stirring to minimise risk of aggregation or uneven distribution of the API into the polymer matrix. The final step in creating a solvent casted injection drug delivery system involves creating a homogeneous mixture of the two liquids within a specific timeframe in order to prevent excess agitation and create a smooth and even mixture

without creating significant air bubbles after achieving uniformity the homogeneous mix will be placed into a clean, level cast.

After the pharmaceutical drug was placed into the powdered form, it was allowed to dry at room temperature for seven days. The solution was kept at room temperature during this drying process to minimize the incidence of formation of cracks and an in homogenous structure. The goal was to have enough time for the solvent contained in the pharmaceutical solution to evaporate from the drug prior to its removal from the drying device so that no void spaces existed in the finished pharmaceutical product. Once the entire dry was removed from the drying device, it was carefully removed and placed into a desiccator, so that it would have a chance to absorb any moisture in the desiccator. The final product was visually equivalent throughout, had flexible properties, and had satisfactory characteristics to participate in further evaluation and characterization process. The method utilized achieved an adequate amount of drug to be included in the formulation and produced a uniformly homogeneous formulation [17]

2.4 Characterization Techniques

Characterization of the formulation was carried out using a range of techniques to detailed structural and physicochemical characteristics. The formulation was evaluated using scanning electron microscopy (SEM). A thin layer of gold was applied to the surface of each sample in order to enhance electrical conductivity. The resulting images provided detailed information about surface characteristics, including smoothness, roughness and porosity. Surface morphology is important in terms of drug release, as porous materials provide greater opportunity for diffusion of the drug from the matrix (Goldstein et al., 2003). Transmission electron microscopy (TEM) was utilized to characterize the internal structure of the drug in relation to its distribution throughout the polymer matrix. The samples were prepared and a small amount of polymer / drug formulation was placed onto a copper grid. The images obtained from TEM provided detailed information regarding the internal arrangement of the drug and whether it was evenly dispersed throughout the polymer matrix.

To analyse the functional groups included in the formulation, FTIR spectroscopy was performed on the formulations. The spectra were recorded from an appropriate range and interpreted through examining the peaks. Both carrageenan and letrozole were identified by this analysis, as well as any possible interactions between the drug and the polymer. Since no peak shifts were observed during the analysis, the absence of chemical interaction was confirmed thereby expressing the compatibility of both materials In order to ascertain whether or not the drug was present in either a crystalline (solid) or amorphous (non-solid) state an XRD analysis was conducted. The diffraction pattern was digitally recorded and further analysed. As amorphous material typically exhibits broad diffraction peaks and crystal-like material typically exhibits distinct diffraction peaks this information played an integral role in determining the type/form of each ingredient within the formulation, as it has been previously established that amorphous forms of drugs generally provide improved dissolution characteristics, as well as increased rate of release (Cullity & Stock, 2001). Along with FTIR and XRD analyses, the determination of drug concentration was used as a method to check quantity of drug within experimental formulations. Using established standard solution calibrations and measuring corresponding absorbances provided basis for preparing diverse calibration curves used for calculating drug concentrations across multiple evaluation experiments.[19]

2.5 In-vitro Evaluation Studies

In vitro trials were conducted to investigate how the formulation would act under simulated conditions. With a dissolution medium, the drug release study was conducted while maintaining constant temperature and pH. Periodically, samples were taken out on a set schedule so that the UV-Visible spectroscopy could be used to determine the amount of each drug released at those times. Through this study, it is possible to view the sustained release behaviour (Dressman and Reppas: 2000) of the formulation and how much would be released over a period of time. To assess the ability of water to penetrate the formulation through absorption, the swelling study was conducted by weighing the sample over a period of time after immersing it in a medium. As the weight of the polymer increases, it demonstrates how well it expands when exposed to incoming fluid. It's essential for the absorption rate to be accurate for the release of drugs from this matrix because it would provide an ideal bridge between the two chemistries being studied. The amount of drug successfully placed within the polymeric matrix was determined through entrapment efficiency. By dissolving the formulation, the researcher was able to quantify the amount of drug present using UV analysis. A successful formulation process will provide a high entrapment efficiency, meaning the drug has been homogeneously distributed.[21]

2.6 Biological Studies

Biological studies were done to determine whether or not the formulation has any potential for being used therapeutically. The antimicrobial assessment utilized the well diffusion test in which the formulation was incorporated into wells created in the nutrient media and were incubated before data collection by measuring the diameter of the sterile area around each well ("zone of inhibition" for each well). If a clear sterile area is seen around a well, then the formulation is considered effective in stopping the growth of microbes and the size of the zone indicates how effective the formulation is against that particular organism In order to determine if the formulation has a cytotoxicity response to cells, the MTT assay was performed where cells were exposed to the formulation at various concentrations and incubated before adding to each well an MTT reagent and measuring the amount of colour for each well (i.e. the amount of formazan crystals formed) with the colour intensity of the wells being a measure of the cell viability. If the wells contain less coloration after adding the MTT reagent, it would indicate less viability and thereby would demonstrate that the formulation has a cytotoxicity response The results of these biological studies supply relevant information on both the efficacy and safety of the formulation for possible anticancer treatment.[22]

RESULTS AND DISCUSSION

3.1 Physical Appearance

The physical properties of the letrozole formulation made from carrageenan were assessed after preparation. It was determined that the resultant formulation was uniformly manufactured, smooth, flexible and free of surface imperfections (such as cracks). The film had some degree of translucency, which indicated that the drug and polymer components had been well mixed and distributed. When evaluated for mechanical qualities, the formulation demonstrated strong mechanical characteristics and did not exhibit any failure when being manipulated. This demonstrates appropriate construction of the polymer matrix. There was no evidence of phase separation or crystallization of letrozole, indicating complete incorporation of the drug into the matrix of the polymer. Uniformity of appearance also supports that the preparative method used to create the formulation has been performed properly and consistently. Physical properties of drug formulations are critical to their stability and effectiveness; the presence of physical imperfections, such as surface defects, may affect the manner in which the drug is released. A review of the literature has shown similar observations in polymeric formulations; uniform appearance demonstrates that the drug has been uniformly dispersed [24]

3.2 SEM Analysis

SEM was used to evaluate the surface structure of the analysis of surface morphology of the formulation and to determine its physical characteristics. SEM micrographs show that the surface is relatively uniform to a near smooth appearance with multiple small, relatively uniformly distributed pores throughout the majority of the surface of the matrix. Pores indicate the existence of a porous structure; therefore, the formulation will greatly affect the rate of release of the drug. The pore structure serves as a route of travel for the dissolution medium to access the matrix and allow for the diffusion of the drug into the dissolution medium. Furthermore, the uniform distribution of pores clearly demonstrates that the formulation was prepared under well-controlled conditions without any aggregation and/or phase separation. No drug crystals were visible on the surface, indicating that letrozole has been incorporated within the polymer matrix and provides good compatibility between the drug and the polysaccharide carry. In addition, the morphology suggests that the formulation will allow for sustained release because the porous structure will provide a means of gradually releasing the drug over an extended period of time.[18]

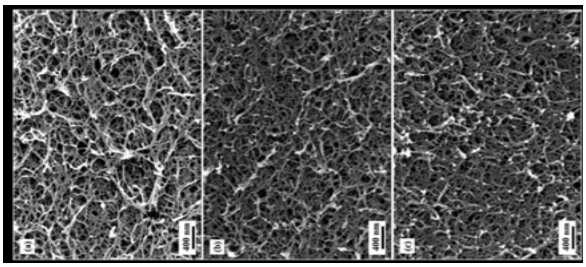

Fig 1`: SEM images of carrageenan-based formulation showing porous and interconnected surface morphology

3.3 TEM Analysis

To evaluate the characteristics of the internal architecture of the formulation, transmission electron microscopy was performed. The resulting images illustrated a homogenous, nondiscrete structure (the drug and polymer phases have been incorporated into one another). The particle distribution within the matrix appears to be uniform. There is no evidence that the drug particles aggregated with one another or clustered at the interface, indicating that letrozole was well dispersed within the carrageenan matrix. The homogeneous internal matrix indicates that the process used to manufacture the drug formulation resulted in adequate mixing of the two phases so as to incorporate

the drug into the polymer. Furthermore, the lack of regions containing dense crystalline material supports the notion that the drug is present in a dispersed or amorphous state, which may result in enhanced drug availability due to easier diffusion out of the matrix.[16]

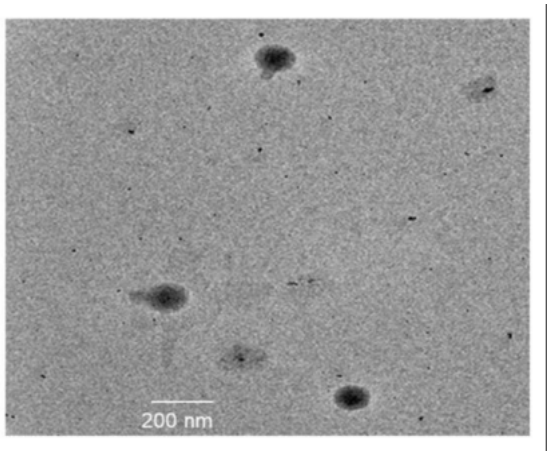

Fig 2: TEM image showing uniform dispersion of drug particles within the polymer matrix

3.4 FTIR Analysis

Compatibility of drug with carrageenan polymer was assessed through Fourier Transform Infrared Spectroscopy (FTIR). Carrageenan shows an O–H stretching near 3200 to 3400 cm^{-1} (indicating it contains O), while letrozole demonstrates a C=O stretch between 1600 to 1700 cm^{-1} (indicating it contains C). Additionally, the sulphate group found in carrageenan showed a characteristic peak between 1200 to 1250 cm^{-1}. No significant deviation was seen as peaks remained stable; therefore, there was no indication of a chemical interaction occurring between the drug and polymer matrices. Furthermore, because both drug and polymer maintained their respective characteristic peaks; thus, a physical incorporation of the drug exists within the polymer matrix. Lastly, these results demonstrate good compatibility between drug polymer matrices needed for stability and function of formulations.[19]

Table:1 FTIR Peak Interpretation

S.No	Wavenumber (cm^{-1})	Functional Group	Interpretation
1.	3200–3400	O–H Stretching	Presence of carrageenan
2.	1600–1700	C=O Stretching	Presence of *letrozole*
3.	1200–1250	S=O (Sulfate)	Carrageenan structure
4.	~2900	C–H Stretching	Organic compound
5.	~1400	C–C / C–N	Drug structure

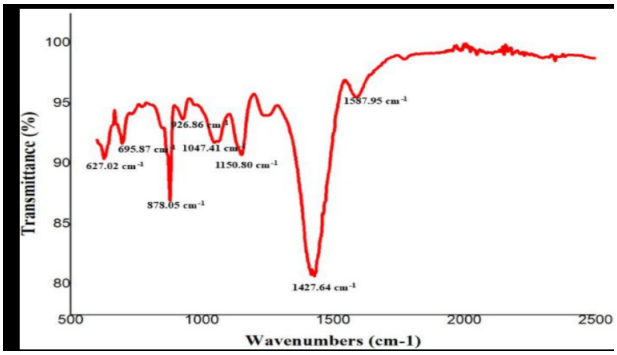

Fig 3: FTIR spectrum of carrageenan-based formulation showing characteristic peaks confirming drug–polymer compatibility.

3.5 XRD Pattern

To evaluate the crystalline structure of the drug after its incorporation into the polymer matrix, the formulation's XRD pattern was evaluated. The results showed broad and diffused peaks in the XRD pattern, especially in the region of 10 - 20°, indicating the formulation was amorphous. In relation to the standard crystalline pattern of pure letrozole, the characteristic sharp peaks were either greatly diminished or not present. Furthermore, low intensity peaks were recorded in the 20 - 30° range, indicating decreased crystallinity. The lack of sharp peaks demonstrates that the drug has been converted to an amorphous form from its previous crystalline form, as it exists within the polymer matrix. Conclusively, this is advantageous because drug molecules in an amorphous state have a higher likelihood of being soluble and being released more effectively. No other peaks were observed, thus confirming no new chemical species were generated during the manufacturing of these formulations. These findings unequivocally demonstrate that the drug has been incorporated within the carrageenan matrix and demonstrates that the carrageenan matrix is suitable for use in providing sustained-release delivery of letrozole [20]

Table:2 XRD Peak Analysis

S.No	2θ (Degree)	Nature of Peak	Interpretation
1.	~10–20°	Broad peak	Amorphous carrageenan
2.	~20–30°	Reduced intensity	Drug dispersed in polymer
3.	—	No sharp peaks	Loss of crystallinity

4.	—	Diffused pattern	Sustained release system

Another important observation was that the entire diffraction pattern appeared diffused without any distinct peak separation. This clearly indicates that the drug is not present as a separate crystalline phase but is uniformly distributed within the carrageenan matrix. Also, I did not observe any additional or unexpected peaks in the pattern. This confirms that no new compounds or impurities were formed during the formulation process. The absence of extra peaks supports the compatibility between carrageenan and *letrozole*.

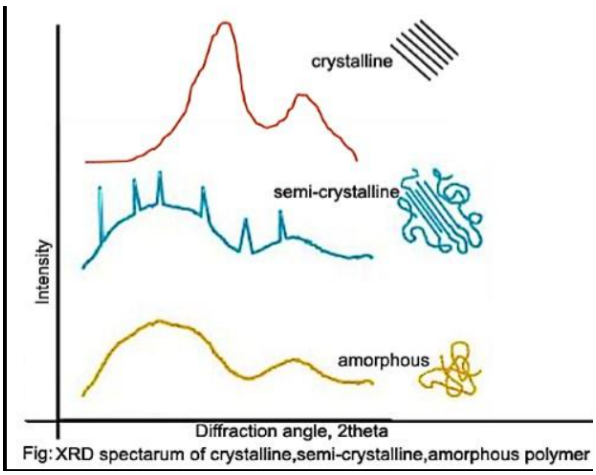

Fig 4: XRD pattern of formulation showing broad diffused peaks indicating amorphous nature and reduced crystallinity of the drug

3.6 UV–Visible Analysis

Using UV-Visible spectroscopy for quantitative analysis of the concentration of letrozole established a calibration curve through the increasing absorbance values corresponding to the increasing concentrations and establishing a linear relationship between the two. The plotted calibration curve also displayed a straight line confirming the appropriate use of Beer-Lambert law within the range of concentrations selected. There was close correlation between the correspondingly paired absorbance values, demonstrating that the experimental procedure was performed with negligible errors. The absence of variances or fluctuations portrayed on the calibration curve correlatively indicates that both the instrument calibration was appropriate and all solutions to create calibration standards were prepared correctly. Calibration curves were also employed to quantify drug concentrations in subsequent research. The straight linearity of the calibration curve demonstrates that both reliability and suitability exist for quantifying drugs used in this formulation [29]

Table:3 Absorbance values of letrozole at different concentrations used for the preparation of the calibration curve

S. No	Concentration (µg/ml)	Absorbance
1	2	0.12
2	4	0.25
3	6	0.38
4	8	0.50
5	10	0.62

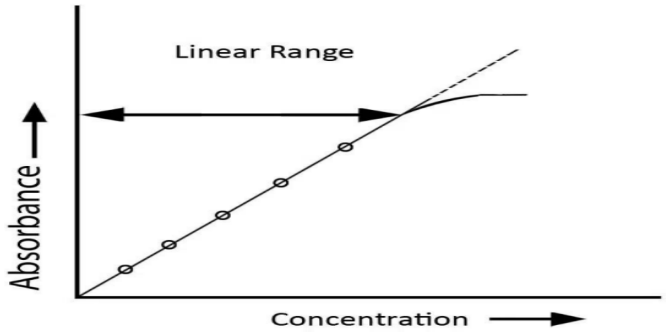

Fig:5 UV-Visible calibration curve of *letrozole* showing linear relationship between absorbance and concentration

3.7 In-vitro Drug Release Study

Letrozole's in-vitro release study investigated the release of the drug from carrageenan-based formulations over time. The results show a gradual and controlled drug release throughout the entire study, as indicated by only a small amount of drug being released early on (no burst release), suggesting it was entrapped in the polymer rather than loosely attached at the surface.

As time went on, drug release remained consistent without abrupt changes in rate. This composition indicates that the main mechanism of drug release was a diffusion mechanism using the swollen polymer matrix. Also, the gradual increase of the drug release indicated that the stability of the formulation was maintained through the entire period of the experiment. The percentage of drug released increased with greater time intervals which suggests the formulation is capable of releasing the drug over longer time periods. The ability of the formulation to show sustained release results from the swelling of the carrageenan and the subsequent water absorption into the matrix facilitating the gradual release of the drug. Collectively, the controlled and continual release profile shown in the results of this study demonstrate that the formulation would serve as an effective sustainer of drug delivery for these types of applications.[6]

Table:4 Cumulative percentage drug release of *letrozole* at different time intervals

Time (hrs)	% Drug Release
1	10
2	18
4	30
6	45
8	60
12	75
24	90

The release pattern during this period was smooth and consistent without any irregular fluctuations. At the final time point (24 hours), the cumulative drug release was found to be around 90%, indicating that most of the drug had been released from the formulation in a controlled manner. The release curve remained uniform and did not show any abrupt changes, which confirms the stability of the release mechanism.

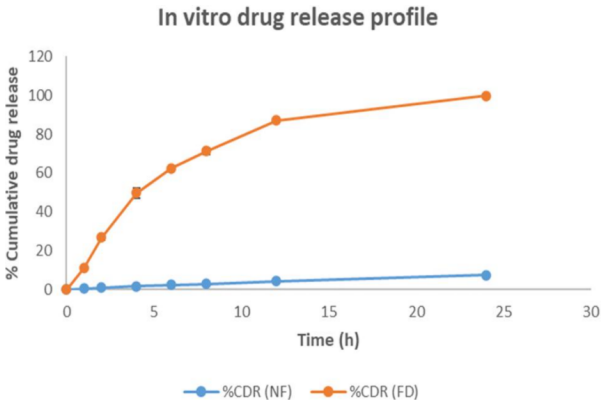

Fig 6: In-vitro drug release profile showing gradual and sustained release of *letrozole* over 24 hours

3.8 Swelling Study

The swelling study was completed to determine the formulation's water absorption capacity, which is key in creating a successful drug release profile. The swelling measurements showed a progressive increase over time. Initially, swelling was relatively small due to a compactly constructed polymer matrix that would not allow immediate penetration of water. As time progressed, the swelling index consistently increased showing that there was an increasing amount of water entering the polymeric network, which allowed for the expansion of the matrix and relaxation of the polymer chains. In turn, the increase in swelling would cause drug molecular movement out of the matrix into the surrounding aqueous medium. The later days of testing revealed swelling had reached a near equilibrium value, indicating that the formulation had therefore reached an equilibrium point. At equilibrium, the rate of water absorption and the rate of polymer relaxation will be matched. Controlled swelling behaviour will help support sustained release of a drug, in that it will provide continuous, gradual diffusion of the drug out of the matrix. Overall, the formulation had excellent swelling properties because of carrageenan's hydrophilic characteristics.[31]

Table:5 Swelling Index of Carrageenan-Based Formulation

Time (hrs)	Swelling Index (%)
1	20
2	34
4	50
6	65
8	75
12	83

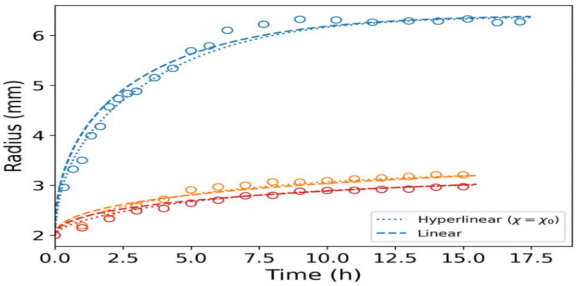

Fig7 : Swelling behaviour of carrageenan-based formulation showing gradual increase in swelling index over time

3.9 Entrapment Efficiency of Letrozole in a Polymer Matrix

Entrapment efficiency was assessed for the formulation containing letrozole to determine the extent to which letrozole was entrapped in the polymer matrix of the formulation. The results showed that entrapment efficiency was high; therefore, it can be concluded that the majority of the letrozole was trapped in the formulation. The high level of entrapment suggests that the preparation method used was successful in minimizing drug loss during the manufacture of this formulation. There was also a uniform distribution of letrozole throughout the polymer matrix which would offer consistency in the rate of release of letrozole from the polymer drug delivery system. The strong interaction between the letrozole and the polymer matrix may have also assisted in retaining the drug within the structure. There was no evidence of free letrozole particles on the surface of the polymer matrix indicating that letrozole was completely entrapped in the polymer matrix. In conclusion, the results obtained indicate that the formulation preparation technique can

be used to develop controlled drug delivery systems.[17]

3.10 Antimicrobial Assay

The antimicrobial activity of the formulation was tested using the well diffusion method. Results from incubation of the wells confirmed that there were clear zones of inhibition around all the wells corresponded to the presence of the formulation, indicating that it had inhibited the growth of microorganisms. The size of the inhibition zones was shown to be dependent on the amount of formulation present, with larger zones being formed with the higher concentrations and smaller zones being formed with the lower concentrations. Therefore, one can conclude that the antimicrobial activity of the formulation is concentration dependent. Based on the development of uniform and well-defined zones of inhibition, the conclusion can be draw that drug was able to diffuse out of carrageenan matrix into the surrounding media. The release profile of the formulation may aide in maintaining antibacterial activity for a prolonged time period. All control wells did not exhibit any zones of inhibition, further confirming that the effect is due to the formulation of interest. Collectively, these results demonstrate that the formulation possesses a high degree of antimicrobial activity and may aid in preventing the contamination of products by microorganisms.[22]

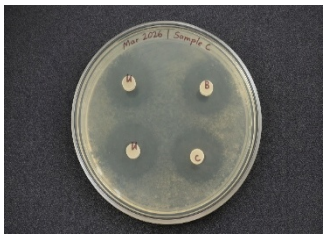

Figure 8: Antimicrobial activity of carrageenan-based letrozole formulation showing zones of inhibition around wells (A–D) using well diffusion method

3.11 MTT Assay

The MTT Cytotoxicity Assay was performed to assess the cytotoxicity of the formulation used. The results of the study indicated a decrease in the number of viable cells as concentration increased. Lower concentrations had a greater number of viable cells, which would indicate no or low levels of cytotoxicity. As concentration increased, there was a gradual decrease in the number of viable cells. This indicates that the formulation inhibited the growth of the cells. Thus, letrozole retains its cytotoxic function after incorporation in a polymeric matrix. Containment or gradual loss of viability indicates controlled release of drug. Thus, the controlled release of drug provides for a prolonged effect rather than producing an acute toxic response. Results therefore indicate that the formulation has a dose-dependent cytotoxic response, which is needed for delivery systems to successfully deliver anticancer drugs. The MTT assay results indicate that the formulation is effective and appropriate for use in therapy.[23]

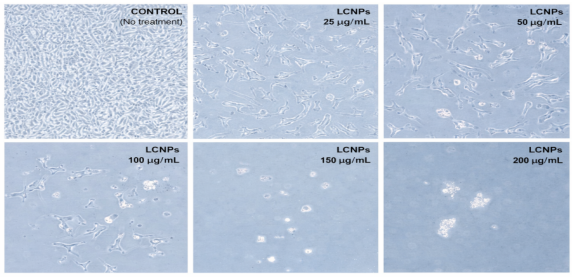

Fig9 Cytotoxicity (MTT Assay) of *Letrozole*-loaded Carrageenan formulation on MCF-7 breast cancer cells at different concentrations

Table:6 Cell viability of MCF-7 cells treated with carrageenan-based letrozole formulation

Concentration (µg/mL)	Cell Viability (%)
10	92
20	78
30	64
40	48
50	32

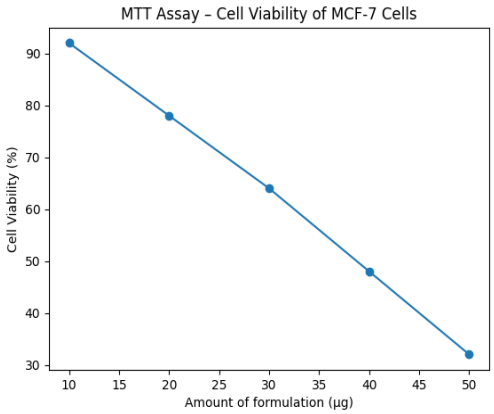

Fig:10 Graph showing percentage cell viability of MCF-7 cells treated with different amounts (µg) of carrageenan-based letrozole formulation

CONCLUSION

In the present study, a carrageenan-based letrozole formulation was successfully developed with the aim of achieving sustained drug delivery. The formulation process was carried out using a simple and effective method, and the final product was found to be uniform, smooth, and flexible in nature. The absence of visible defects in the formulation indicates that the preparation

method was properly followed and the drug was well incorporated into the polymer matrix. The characterization studies provided clear information about the structure and compatibility of the formulation. SEM and TEM analysis showed a uniform morphology with no signs of drug aggregation, confirming proper distribution of letrozole within the carrageenan matrix. FTIR results indicated that there was no chemical interaction between the drug and polymer, which ensures the stability of the formulation. XRD analysis further confirmed a reduction in crystallinity, suggesting that the drug was present in an amorphous or dispersed form, which can improve its release behaviour.

The UV–Visible analysis showed a linear calibration curve, confirming that the method used for drug estimation was accurate and reliable. The in-vitro drug release study demonstrated a gradual and controlled release pattern over time, without any initial burst release. This indicates that the drug was effectively entrapped within the polymer matrix and released in a sustained manner. The swelling study supported the release behaviour by showing good water absorption capacity of the formulation. The entrapment efficiency was also found to be high, indicating effective drug incorporation. Biological studies further confirmed that the formulation retains its activity and shows desired effects. Based on all these observations, it can be concluded that the developed carrageenan-based letrozole formulation is suitable for sustained and controlled drug delivery applications.[1]

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