RESEARCH PAPER

Development of Anakinra and Zaltoprofen Loaded Liposomes for Rheumatoid Arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder characterized by persistent synovial inflammation, joint pain, swelling, stiffness, and progressive cartilage and bone destruction. Conventional treatment primarily focuses on reducing inflammation, relieving pain, and controlling disease progression; however, limitations associated with systemic drug administration, adverse effects, and inadequate drug delivery highlight the need for improved therapeutic approaches. The present study aims to develop and characterize anakinra and zaltoprofen loaded liposomes for the effective management of rheumatoid arthritis.

Anakinra, an interleukin-1 receptor antagonist, helps control inflammatory responses by inhibiting interleukin-1-mediated signaling, whereas zaltoprofen, a non-steroidal anti-inflammatory drug (NSAID), provides analgesic and anti-inflammatory effects. Liposomes are lipid-based vesicular carriers capable of encapsulating therapeutic agents and potentially improving drug delivery, stability, and release characteristics. The formulation will involve the preparation of liposomes containing anakinra and zaltoprofen using a suitable lipid-based technique, followed by optimization of formulation variables such as lipid concentration, cholesterol content, and drug-to-lipid ratio. The prepared liposomes will be evaluated for physicochemical properties, including particle size, polydispersity index, zeta potential, entrapment efficiency, drug content, morphology, in-vitro drug release, and formulation stability. Compatibility between the drugs and excipients will also be assessed using appropriate analytical techniques.

The optimized formulation will be investigated for its potential to provide controlled drug release and complementary anti-inflammatory activity. The combined delivery of anakinra and zaltoprofen may offer a promising approach for addressing inflammatory processes and pain associated with rheumatoid arthritis. Overall, this study is intended to establish a suitable liposomal drug delivery system that may improve therapeutic performance and provide a foundation for further preclinical evaluation of combination therapy for rheumatoid arthritis.

Keywords: Rheumatoid arthritis, anakinra, zaltoprofen, liposomes, lipid-based drug delivery, anti-inflammatory activity, controlled drug release.

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Introduction

The arthritis involves complex interactions among immune cells, inflammatory mediators, synovial fibroblasts, and osteoclasts. Activated T lymphocytes, B lymphocytes, macrophages, and synovial cells contribute to the production of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6). These mediators promote synovial inflammation, increase the recruitment of inflammatory cells, and stimulate the production of enzymes responsible for cartilage degradation. Persistent inflammation can

result in pannus formation, erosion of the underlying bone, joint deformities, and progressive loss of joint function. Therefore, effective management of rheumatoid arthritis requires strategies that control inflammatory signaling, reduce pain, and prevent further structural damage.[1]

The treatment of rheumatoid arthritis aims to reduce disease activity, relieve pain and stiffness, prevent joint destruction, and maintain physical function. Conventional therapeutic approaches include disease-modifying antirheumatic drugs (DMARDs), biological agents, non-steroidal anti-inflammatory drugs (NSAIDs), and corticosteroids. Conventional synthetic DMARDs, particularly methotrexate, are frequently used as initial disease-modifying therapy, while biological agents may be prescribed when adequate disease control is not achieved. NSAIDs are commonly used to alleviate pain and inflammation, whereas corticosteroids may provide short-term control of severe inflammatory symptoms in selected patients.[2]

Despite the availability of several effective medicines, important challenges remain in the treatment of rheumatoid arthritis. NSAIDs primarily provide symptomatic relief and do not independently prevent the long-term progression of joint damage. Their prolonged use may be associated with gastrointestinal irritation, ulceration, renal complications, and cardiovascular risks, depending on the drug and the patient's clinical condition. Biological agents may improve disease control in selected patients but can be associated with injection-related reactions, increased susceptibility to infections, high treatment costs, and the need for careful monitoring. These limitations have encouraged research into novel drug delivery systems that may improve drug localization, stability, release characteristics, and therapeutic tolerability.[3]

Inflammatory cytokines play a central role in the initiation and progression of rheumatoid arthritis. Among these mediators, interleukin-1 is involved in the activation of inflammatory pathways, stimulation of synovial cells, production of matrix-degrading enzymes, and promotion of cartilage and bone damage. IL-1 may also contribute to the amplification of other inflammatory mediators within the affected joints. Consequently, inhibition of IL-1 signaling represents an important therapeutic strategy for controlling selected inflammatory mechanisms involved in rheumatoid arthritis.[4]

The inflammatory process in rheumatoid arthritis is not controlled by a single mediator. TNF-α, IL-6, IL-1, and several chemokines contribute to a complex network of cellular signaling pathways. Blocking a specific cytokine pathway may reduce particular components of inflammation, while complementary anti-inflammatory treatments may help manage associated symptoms. This biological complexity provides a rationale for investigating combination-based therapeutic approaches involving drugs with different mechanisms of action. However, the clinical effectiveness and safety of any combination must be established through appropriate experimental and clinical evaluation.[5]

Anakinra is a recombinant human interleukin-1 receptor antagonist that competitively inhibits the biological activity of IL-1α and IL-1β by blocking their interaction with the interleukin-1 type 1 receptor. By interfering with IL-1-mediated signaling, anakinra reduces the activation of inflammatory pathways involved in the development of synovial inflammation and tissue damage. It is a biological disease-modifying agent used in selected inflammatory conditions, including rheumatoid arthritis in certain clinical settings. Its mechanism of action makes it a relevant candidate for investigating targeted modulation of cytokine-mediated inflammation.

Anakinra is a protein-based therapeutic agent with a molecular weight of approximately 17.3 kDa. Its physicochemical characteristics differ substantially from those of conventional small-molecule drugs. As a protein, it may be susceptible to degradation, aggregation, and loss of biological activity under unsuitable formulation and storage conditions. Maintaining its structural integrity during formulation and delivery is therefore an important consideration in the development of anakinra-containing pharmaceutical systems. Its relatively large molecular size also presents challenges for transport across biological barriers and may influence its distribution depending on the route of administration.[6]

Although anakinra can inhibit IL-1-mediated inflammatory signaling, its therapeutic use requires consideration of administration frequency, stability, patient adherence, and safety. Treatment may increase the risk of infections, and appropriate clinical monitoring is necessary. Incorporating anakinra into a suitable carrier system may offer opportunities to investigate its protection from environmental stress, encapsulation efficiency, and release behavior. Nevertheless, the compatibility of anakinra with lipid membranes, preparation solvents, temperature conditions, and processing steps must be carefully assessed to ensure that the protein retains its biological activity.[7]

Zaltoprofen is a non-steroidal anti-inflammatory drug that belongs to the propionic acid derivative group. It is used in certain countries for the management of pain and inflammatory conditions, including musculoskeletal disorders. Its therapeutic effects include analgesic and anti-inflammatory activity,

making it a potential candidate for addressing pain and inflammation associated with rheumatoid arthritis. Unlike anakinra, which targets IL-1 receptor signaling, zaltoprofen acts through mechanisms associated with prostaglandin-mediated inflammatory responses.[8]

Prostaglandins are lipid-derived mediators produced through enzymatic pathways involving cyclooxygenase enzymes. They contribute to inflammatory responses, pain sensitization, and local tissue reactions. By modulating prostaglandin-related pathways, zaltoprofen can help reduce inflammatory pain and associated symptoms. Its pharmacological activity may complement the cytokine-directed action of anakinra by addressing different aspects of the inflammatory process. However, the combination should be considered an investigational formulation strategy rather than an established superior treatment for rheumatoid arthritis.[9]

A combined liposomal formulation may also help investigate the release of the two drugs from a shared carrier. However, the drugs may exhibit different release rates because of their differences in molecular size, solubility, lipid affinity, and interactions with the carrier components. Consequently, simultaneous loading does not necessarily guarantee synchronized release or improved therapeutic activity. These characteristics must be established through analytical characterization, in-vitro release testing, biological activity studies, and appropriate comparative evaluation.[10]

Liposomes are spherical vesicular systems composed primarily of one or more phospholipid bilayers surrounding an aqueous compartment. Their structure resembles biological membranes and enables them to accommodate drugs with different physicochemical properties. Hydrophilic compounds may be incorporated into the aqueous compartment, whereas lipophilic compounds can associate with the lipid bilayer. This structural versatility makes liposomes useful carriers for the development of pharmaceutical formulations containing multiple therapeutic agents.[11]

Liposomes can be prepared using phospholipids, such as phosphatidylcholine, with cholesterol commonly incorporated to modify membrane packing, rigidity, permeability, and drug retention. Depending on their composition, size, lamellarity, and preparation method, liposomes may exhibit different encapsulation efficiencies, stability profiles, and drug-release characteristics. Surface modification and changes in lipid composition can further influence interactions with biological fluids and tissues. These formulation variables provide opportunities to tailor liposomal systems according to the requirements of individual therapeutic agents.[12]

Several advantages have made liposomes important in pharmaceutical research. They can improve the apparent solubilization of selected drugs, protect susceptible compounds from some environmental influences, and modify drug-release behavior. Liposomal encapsulation may also alter the distribution and clearance of a drug, depending on the formulation and route of administration. Nevertheless, liposomes may undergo aggregation, leakage, lipid oxidation, or hydrolysis during storage. Their performance depends on careful selection of excipients, manufacturing conditions, purification procedures, and storage parameters.[13]

Liposomes have been investigated as delivery systems for anti-inflammatory agents, immunomodulators, and other therapeutic compounds intended to manage inflammatory disorders. Their lipid-based structure provides a platform for modifying drug retention and release and for studying the delivery of agents with different solubility characteristics. In rheumatoid arthritis research, liposomal formulations may be evaluated for their ability to influence drug exposure, cellular interactions, and distribution to inflamed tissues. The potential outcome depends on the liposome composition, particle characteristics, administration route, and biological environment.[14]

Inflamed joints may exhibit changes in vascular permeability, immune-cell activity, and tissue architecture. These changes have motivated research into carrier systems that could alter drug delivery to inflamed tissues. However, liposomal accumulation at diseased joints is not guaranteed and varies with particle size, surface properties, circulation time, and disease severity. Therefore, claims of joint-specific targeting require direct experimental evidence rather than relying solely on the use of liposomes.[15]

For anakinra and zaltoprofen, liposomal encapsulation offers a formulation platform for studying the stability of the protein drug, incorporation of the small-molecule NSAID, and release of both agents from the same carrier. The formulation can be assessed for particle size, polydispersity, zeta potential, drug content, entrapment efficiency, morphology, in-vitro release, and storage stability. Additional biological studies are necessary to determine whether the formulation preserves anakinra activity and maintains the expected pharmacological properties of zaltoprofen.[16]

Important formulation variables include phospholipid concentration, cholesterol content, drug-to-lipid ratio, hydration medium, pH, mixing conditions, and processing time. These variables can affect vesicle formation, particle size, drug loading, membrane stability, and release behavior. The preparation method should also be evaluated for residual

solvents, unencapsulated drug removal, reproducibility, and suitability for scale-up. Where necessary, gentle processing conditions and compatible buffers should be selected to minimize the risk of protein degradation.

Optimization can be performed by preparing batches with different formulation compositions and comparing their critical quality attributes. Particle size and polydispersity index provide information about the dimensions and distribution of the vesicles, while zeta potential offers information about their surface charge under the measurement conditions. Entrapment efficiency and drug content help establish the amount of each drug associated with the formulation. Morphological assessment can provide evidence of vesicle structure, whereas in-vitro release studies help characterize the rate and extent of drug release. Statistical experimental designs may be used to identify the influence of formulation variables and determine an optimized composition.[17]

Methodology

Formulation of anakinra and zaltoprofen loaded liposomes (AZ-LMs)

Anakinra and Zaltoprofen loaded liposomes were prepared by Ethanol injection method. Phospholipid 90G and sodium cholate were dissolved in ethanol. The lipophilic drug zaltoprofen was dissolved in ethanolic phase and hydrophilic anakinra was solubilized in aqueous phase which is continuously stirred at a constant temperature of 60° C. Ethanolic solution of phospholipid 90G, Sodium cholate and zaltoprofen was injected to aqueous solution of the drug dropwise.

As the ethanolic solution comes in contact with aqueous solution, the lipid molecules tend to precipitate and forms a bilayer structure. The coarse dispersion prepared was then sonicated to obtained desired vesicle size. The final formulation was passed through a 0.45 µm membrane filter to ensure sterility and clarity, and subsequently stored under refrigerated conditions .[18]

Optimization of liposomes using central composite design

Based on the outcomes of the preliminary screening study, response surface methodology (RSM) was employed to optimize . AZ-LMs formulation, aiming to minimize vesicle size and polydispersity index (PDI), while maximizing entrapment efficiency. A three-factor, three-level Central Composite Design (CCD) was utilized for the optimization process. The independent variables selected were the amount of phospholipid (X_1), surfactant (X_2), and sonication time (X_3). The dependent variables (responses) included vesicle size (Y_1), polydispersity index (Y_2), and entrapment efficiency (Y_3). [19]

The variables and their corresponding levels used in the design. A total of 20 experimental runs were generated, incorporating factorial points, axial points to estimate curvature, and center points for replication,. Data analysis was performed using Design Expert software (version 11.0, Stat-Ease, Minneapolis, USA). A second-order polynomial equation was used to model the relationship between the independent variables and the responses:

$Y = \alpha0 + \alpha1X1 + \alpha2X2 + \alpha3X3 + \alpha12\ X1X2 + \alpha13\ X1X3 + \alpha23\ X2X3 + \alpha\ X\ 2 + \alpha\ X\ 2 + \alpha\ X\ 3$

Where, Y represents the predicted response, α_0 is the intercept (arithmetic mean of the responses of all 20 formulations), α_1, α_2, and α_3 are the linear coefficients for the independent variables X_1, X_2, and X_3, respectively, α_{12}, α_{13}, and α_{23} represent the interaction terms between pairs of variables, α_{11}, α_{22}, and α_{33} are the quadratic coefficients reflecting curvature effects. A positive coefficient denotes a synergistic effect on the response, whereas a negative coefficient indicates an antagonistic effect. Statistical significance for model terms was considered at a confidence level of $P < 0.05$.

Table 1:Variables and Levels of the CCD applied for the optimization of liposomes

Independent Variables	**Levels (Actual, coded)**				
	-1	0	1	-alpha	+alpha
X1 Phospholipid (mg)	30	40	50	23.1821	56.8179
X2 Surfactant (mg)	4	6	8	2.63641	9.36359
X3 Sonication Time (Min)	2	4	6	0.636414	7.36359
Responses	Goals				
Y1 Particle Size (nm)	Minimize				
Y2 Polydispersity Index (PDI)	Minimize				
Y3 Entrapment efficiency	Maximize				

Table 2: Experimental run with Observed response and corresponding predicted response

Run	Independent variable		
	X1	X2	X3
1	40	6	4
2	40	9	4
3	50	4	6
4	40	6	4
5	30	4	2
6	40	2	4
7	30	8	6
8	40	6	7
9	40	6	4
10	30	4	6
11	23	6	4
12	40	6	4
13	56	6	4
14	40	6	4
15	50	8	2
16	30	8	2
17	50	8	6
18	40	6	4
19	40	6	0.6

Evaluation and characterization of optimized liposomes

Vesicle size, poly dispersity index (PDI) and zeta potential

AZ-LMs were measured for the average hydrodynamic diameter, PDI and zeta potential. They were analysed using Malvern Zetasizer (Zetasizer Ver. 7.12, Serial Number: MAL1171112, Malvern Instruments ltd.). Prior to the analysis the samples were diluted up to 100 times using Milli-Q water while the system temperature being maintained at 25°C and scattering angle of 90°.[20]

Determination of entrapment efficiency

The entrapment efficiency (%EE) of AZ-LMs was evaluated using an indirect centrifugation method. The liposomal formulation was centrifuged at 18,000 rpm for 1 hour at 4 °C using a high-speed centrifuge. This procedure enabled the distinction between the unencapsulated drug and the drug entrapped within the vesicular system. The supernatant containing the unentrapped drug was carefully collected and filtered through a 0.1 μm membrane filter (EMD Millipore). Appropriate dilutions of the filtered supernatant were prepared and analyzed using the validated UV spectrophotometric method to simultaneously quantify anakinra and zaltoprofen. The entrapment efficiency (%) of the liposomal vesicles was determined.[21]

In vitro drug release

In vitro drug release study was performed and the release pattern of AZ-LMs was determined using Franz diffusion cell with an activated dialysis membrane of molecular weight 12-14kD. The activated membrane placed between the donor and the receiver compartment. The donor compartments were comprised of AZ-LMs and anakinra and zaltoprofen suspension, while the receiver compartment was saturate with phosphate buffer saline (PBS) with pH balanced to 7.4, maintained at a temperature of 37 ± 0.5 ◦C and at 100 rpm for 24 h. The sample of 2 ml was retracted from the receiver chamber at a specific interval of time, at 1, 2, 4, 6 and 24 h. Two ml of fresh PBS was added to the receiver chamber to maintain the sink conditions. The samples collected were analysed by UV spectroscopy for anakinra and zaltoprofen . The percentage cumulative drug release (%CDR) was calculated .[22]

The release profile was plotted with time on the x-axis and cumulative drug release (% CDR) on the y-axis . The in vitro release data were analyzed using various kinetic models including zero-order, first-order, Higuchi, and Korsmeyer-Peppas models to elucidate the drug release kinetics and underlying mechanism. Linear regression analysis was performed for each model, and the coefficient of

determination (R^2) was calculated to assess the goodness of fit.[23]

Result

Optimization by Central Composite design-CCD

AZ-LMs were prepared using the ethanolic injection method. CCD was applied to optimize the formulation in terms of minimum vesicular size and PDI, and maximum entrapment efficiency. Observed and predicted vesicular size, PDI and entrapment efficiency of all the experimental trials. It was observed that vesicular size and PDI showed marked variations ranging from 197.89 ± 9nm to 476.56 ± 12nm and 0.1 ± 0.02 to 0.42 ± 0.04 respectively. Entrapment efficiency also showed variation ranging from 72.5 ± 5 % to 94 ± 6 % for Anakinra and 70.66 ± 4% to 92.8 ± 6% for Zaltoprofen. The optimal fitting model for vesicle size and entrapment efficiency data was identified as a quadratic model, while the polydispersity index (PDI) followed a linear model. The validity of these models was supported by close agreement between the observed and predicted values for all three dependent variables . Additionally, the lack-of-fit tests for each response were found to be statistically insignificant, confirming the suitability of the response surface methodology employed.

Table 3: Observed values of response in (CCD) for optimization of AZ-LMs

Run	Independent Variables			Dependent variables							
				Observed Response				Predicted Response			
	X1	X2	X3	Y1	Y2	Y3	Y4	Y1	Y2	Y3	Y4
1	40	6	4	280.8	0.28	88.5	84.24	281.65	0.24	88.42	86.20
2	40	9	4	263.4	0.28	76.8	74.1	284.88	0.35	76.79	73.86
3	50	4	6	410.56	0.14	91.66	90.32	444.14	0.33	92.85	89.16
4	40	6	4	285.9	0.26	87.01	86.23	281.65	0.24	88.42	86.20
5	30	4	2	265.06	0.34	78.12	76.2	248.58	0.08	84.73	75.69
6	40	2	4	337.36	0.21	82.65	80.45	281.65	0.24	88.42	81.45
7	30	8	6	197.89	0.16	72.5	70.87	263.77	0.27	76.44	71.18
8	40	6	7	243.8	0.1	85.54	82.21	281.65	0.24	88.42	82.24
9	40	6	4	280.8	0.24	88.5	85.34	302.99	0.17	83.24	86.20
10	30	4	6	256.41	0.13	78.01	75.25	281.65	0.24	88.42	75.65
11	23	6	4	236.14	0.22	72.89	70.66	408.48	0.25	93.36	69.91
12	40	6	4	280.8	0.24	89.51	87.52	281.65	0.13	91.02	86.20
13	56	6	4	476.56	0.25	92.34	90.5	236.54	0.24	88.42	92.01
14	40	6	4	280.8	0.24	88.6	86.34	416.22	0.24	72.02	86.20
15	50	8	2	414.58	0.36	90.85	88.32	254.66	0.36	89.83	87.39
16	30	8	2	282.92	0.36	76.5	74.22	337.16	0.13	78.68	74.63
17	50	8	6	304.71	0.17	83.7	81.22	195.30	0.22	83.50	81.19
18	40	6	4	280.8	0.24	89.2	87.68	353.90	0.16	73.31	86.20
19	40	6	3	358.52	0.42	87.9	86.76	266.67	0.41	89.20	87.49
20	50	4	2	441.67	0.33	94.5	92.8	408.48	0.32	77.41	91.95

Table 4: Outputs of regression analysis of three-level Three factor CCD of AZ-LMs

Response	Model	R2	Adjusted R2	Predicted R2	P Value
Y1 Vesicle size	Quadratic	0.9991	0.9982	0.9941	0.0945
Y2 PDI	Linear	0.9827	0.9771	0.9772	0.0967
Y3 EE%	Quadratic	0.9846	0.9707	0.9025	0.0877

Y4 EE%	Quadrati c	0.981 2	0.9812	0.9812	0.513 3

Effect of independent factors on vesicle size: Equation Y1 indicates the influence of phospholipid, surfactant and sonication time on vesicle size. A positive effect was applied by phospholipid on vesicle size. Increasing the concentration of phospholipid simultaneously increased the vesicle size as shown in the 3D response plot. This can be because of the fact that phospholipids cause more drug to entrap causing an increase in distance between lipid bilayers and leading to larger vesicle size . Conversely, both surfactant concentration and sonication time exhibited a negative effect on vesicle size, indicating that an increase in either parameter led to a reduction in vesicle size. Ultrasonication is a high energy process that generates cavitation bubbles leading to a decrement in vesicle size. Reduction in vesicle size can be attributed to the fact that surfactant form mixed micelles at higher surfactant levels, which are smaller in size .

Effect of independent factors on polydispersity index: Equation Y2 represents the effect of variables on PDI. PDI followed linear model exerting linear trend with sonication time with a negative effect depicting decrease in PDI with an increase in sonication time. However, phospholipid and surfactant showed the effect of low significance on PDI.

Effect of independent factors on %Entrapment efficiency: An ability of to encapsulate a significant proportion of the drug is a crucial parameter for the optimum transdermal delivery. 3D response plot shows the impact of phospholipid, surfactant and sonication time on %EE. The quadratic model was significant and best fitted for data. Y3 equation signifies the impact on %EE of Anakinra. As observed from the plot, the increase in concentration of phospholipid had a synergistic effect on %EE. This can be due to the fact that presence of more lipid provide more room for vesicle formation, ultimately increases an ambiance to accommodate high amount of drug . It was observed, low concentration of surfactant up to a level has increased % EE of the Anakinra while at higher concentrations the %EE has reduced. This is due to the solubilization and diffusion of the drug into the aqueous media . It was also reported that a higher concentration of surfactant may cause leaky points within vesicles, consequently causes %EE of the drug to decrease. Sonication time had insignificant effect on %EE as seen in the 3D response plot. Similar trend was observed in %EE of Zaltoprofen. Y4 equations signifies the effect of independent variables on %EE of Zaltoprofen. With increase in the concentration of phospholipid, the %EE has increased. Surfactant has shown increase in %EE with increase in surfactant up to a concentration after which the % EE was observed to be reduced. Sonication time does not show any significant variation on %EE of Anakinra.

Design validation

Validation of the experimental design was evaluated by the proximity of observed and predicted value which represents the observed and predicted values. It was clearly observed from the results obtained, the composition to develop an optimum formulation was established. A comparison of experimental formulation was made with predicted response generated from software. The optimized formula obtained by CCD was 40.71 mg phospholipid, 5.65 mg surfactant and 6 min sonication time. The predicted response for Vesicle size, %EE and PDI generated was 269.59 nm, 87.45% (Anakinra) and 86.203 % (Zaltoprofen) and 0.14 respectively whereas the observed responses were found to be 271.9 ± 8.45 nm, 86.12 ± 5.20% (Anakinra) and 85.65 ± 4.91% (Zaltoprofen) and 0.164 ± 0.01. From the results obtained it was observed that there is a good correlation between predicted and observed values. Therefore, AZ-LMs developed with above optimized formula were utilized in further research.

Characterization of optimized AZ-LMs

i. Vesicle size, PDI and percentage entrapment efficiency

The optimized formulation depicted an average vesicle size of 271.9 ± 8.45 nm with PDI of 0.164 ± 0.01 indicating a homogenous size distribution throughout. PDI shows how the population size is distributed within a particular sample. A PDI of 0 indicates an entirely homogeneous sample, whereas a value of 1 indicates a substantially polydisperse one. Values exceeding 0.50 indicate a broader size distribution, whereas values below 0.50 indicate a narrow distribution . The entrapment efficiency was found to be 86.12 ± 5.20% for Anakinra and 85.65 ± 4.91% for Zaltoprofen indicating good loading potential of AZ-LMs , thereby facilitating controlled and targeted drug delivery .



Figure 1: Vesicle Size of optimized AZ-LMs using Malvern Zeta sizer

ii. Zeta potential

Zeta potential is a salient indicator of the stability of the nanosized vesicles. Possession of higher electric charges (positive or negative) by nanoparticles prevents aggregation due to the development of strong repulsion forces among the particles. Zeta potential of our optimized AZ-LMs was found to be -28.83 . The negative charge is essential for the stability of the formulation. Because of predominating negative electrostatic charge, the particles exhibit higher affinity for the cellular membrane. Even though the cell membrane has large negatively charged domain, the uptake of these negatively charged particles is due to the occurrence of non-specific interaction process in cells

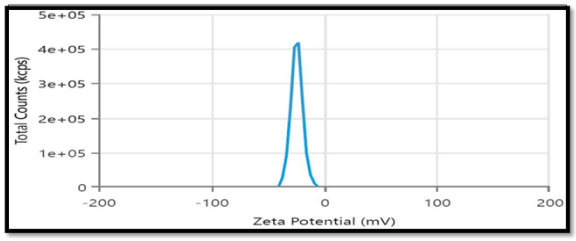


Figure 2: Zeta Potential of optimized AZ-LMs

iii. In vitro drug release

In vitro drug release of optimized AZ-LMs was contrasted with the drug suspensions. A biphasic release pattern was observed with AZ-LMs. Initially, a rapid release of both the drug; Anakinra and Zaltoprofen was observed (24.74 ± 2.02% and 28.62 ± 1.15% respectively) up to 4 hours followed by the sustained release phase for up to 24 hours (releasing 78.35 ± 1.12% and 75.72 ± 1.01% respectively). On the other hand, the suspension of Anakinra and Zaltoprofen showed 83.33 ± 1.55% and 83.25 ± 0.96% release respectively within 4 h of the study. The rapid initial release as observed in AZ-LMs can be due to the free drug lingering over the surface of the vesicles. The un-entrapped drug could pass the membrane due to release interface while entrapped drug diffuses at a slow rate.

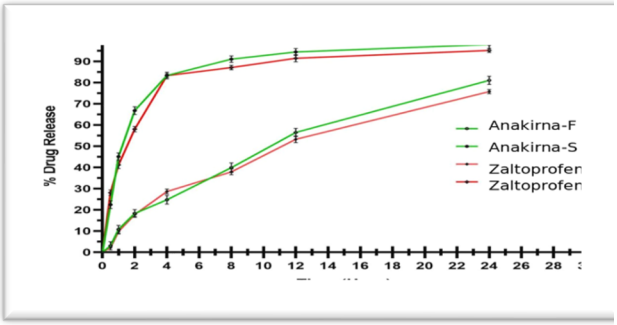


Figure 3: *In vitro* Release of Anakinra and Zaltoprofen suspension and Formulation

Mechanism of drug release of AZ-LMs was best fitted to the equations of Higuchi diffusion model which exhibits highest regression coefficient in comparison to other kinetic models . Drug release from the formulation was established due to diffusion in a slow and sustained release manner through the membrane . The findings are consistent with the release profile explanation, as the Higuchi model describes drug release from modified-release dosage forms such as transdermal systems, which aligns with the delivery approach employed in study

Conclusion

The development of anakinra- and zaltoprofen-loaded liposomes represents a promising pharmaceutical approach for the potential management of rheumatoid arthritis. Anakinra acts as an interleukin-1 receptor antagonist, helping to inhibit cytokine-mediated inflammatory responses, whereas zaltoprofen provides analgesic and anti-inflammatory effects. The combination of these drugs in a liposomal delivery system may offer an opportunity to investigate complementary therapeutic actions while modifying drug encapsulation, stability, and release characteristics. Liposomes, owing to their phospholipid bilayer structure and ability to accommodate drugs with different physicochemical properties, provide a suitable platform for exploring the combined delivery of a protein-based biological agent and a small-molecule anti-inflammatory drug.

The proposed formulation development and optimization process includes the selection of suitable lipids and excipients, preparation of liposomes, and evaluation of important parameters such as particle size, polydispersity index, zeta potential, entrapment efficiency, drug content, morphology, in-vitro drug release, and stability. Particular attention should be given to maintaining the structural integrity and biological activity of anakinra while ensuring satisfactory incorporation and release of zaltoprofen. Comprehensive characterization will help establish the pharmaceutical quality, reproducibility, and suitability of the optimized formulation.

In conclusion, anakinra and zaltoprofen loaded liposomes may provide a valuable platform for further investigation into combination drug delivery for rheumatoid arthritis. The formulation has the potential to support the development of improved drug delivery strategies; however, its therapeutic advantages cannot be established through formulation characterization alone. The research may therefore contribute to the advancement of lipid-based delivery systems for the management of rheumatoid arthritis, subject to confirmation through appropriate experimental and clinical studies.

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