Formulation Development and Evaluation of Aceclofenac Ultrafine Ceramic Aquasomes for Treatment of Rheumatoid Arthritis

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Abstract: Aceclofenac (NSAIDs with short half-life) is associated with gastrointestinal side effects and generally cause gastritis due to frequent administration. To overcome these side effects, the aim of this research investigation was to develop aceclofenac aquasomal drug delivery system to increase its solubility and penetration for enhanced transdermal delivery. Aquasomes were formulated by co-precipitation technique by sonication method and mechanical stirring method for prolonged as well as controlled release of aceclofenac. Various concentration of sugar was used and all nanoparticles were smooth with spherical shape. The prepared formulations were characterised for various evaluation parameters like vesicle size, entrapment efficiencies, in-vitro drug release studies by using semi-permeable membrane. The SEM visualisation demonstrated the smooth, spherical, and varying sizes of the synthesised aquasomes. Formulation F3 had the highest drug loading, while formulation F4 had the lowest loading. The in-vitro release of aceclofenac from produced aquasomal formulations was shown to significantly increase (p<0.05) with an increase in the coating's sugar concentration. Aceclofenac was discovered to be released from F1, F2, and F3 at concentrations of 80.7%, 84.23%, and 86.51%, respectively. Finally, it was concluded that formulated aquasomes possess good release rate and by altering the concentration of sugar, aceclofenac release can be controlled for prolonged period of time with no side effects.

Keywords: Aceclofenac, aquasome, particle size, drug entrapment, drug release.

1. Introduction

1.1. Gross Structure and Function of the Skin

According to Chien et al., 2009, the skin is the largest organ in the body, making up about 10% of the body mass (with a surface area of 3000 inches, or roughly 1.8 metres), and it gets one-third of the blood that is in circulation. According to Graham et al., 1987, the stratum corneum, or horny layer, which is roughly 10 µm thick and made up of compressed, overlapping keratinized cells that create a flexible, robust, and cohesive membrane, makes up the epidermis itself. Skin performs protection, homeostatic, and sensing functions as follows:

i. A chemical entity's barrier features include resistance to its entry (the stratum corneum acts as a barrier);

ii. The metabolic pathway for the percentage of the entity that avoids the stratum corneum (in viable epidermis)

iii. Recognising and paying attention to harm brought on by entrance (release of inflammatory mediators in the epidermis).

iv. The evacuation of the object from the site by dermal blood supply and distribution into the bodily organs that are in charge of excreting and metabolising the object (liver and kidney, respectively).

v. The subcutaneous fat pad is used to regulate body temperature.

vi. Blood flow management by the body to influence.

Mehta et al., 2004, discovered that a chemical undergoes transdermal penetration when it partitions into stratum corneum. A topical product's usual purpose is to treat dermal problems by delivering the medication directly to the skin. (Chien et al., 1987)

1.2. Advantages of Transdermal Drug Delivery (TDDS) (Mehta et al., 2004)

TDDS can provide a constant, controlled drug input:

- It avoids factors that may affect GIT;
- It is beneficial for low bioavailability drugs;
- It reduces frequent drug dosing, which is helpful for drugs with a short half-life;
- It avoids variations in drug plasma levels (reduce side effects; useful for drugs with a narrow therapeutic index);
- It is simple and painless (increase patient compliance);

1.3 Limitations of topical systems
Not appropriate for medications that cause skin sensitization;
limited by the delivery system's surface area;
comparatively costly in comparison to traditional dose forms.

1.4. Aquasomes

The goal of aquasomes, which are three-layered self-assembled nanoparticulate delivery devices, is to successfully transport bioactives while maintaining the integrity of their conformation. Biochemically active molecules are adsorbed onto a polyhydroxy oligomer-coated central solid nanocrystalline core of these entities. Structural stability is provided by the solid core, while the biochemically active molecules are stabilised and protected from dehydration by the carbohydrate covering. The aquasome particle size ranges from 60 to 300 nm and has a spherical shape. Active pharmaceutical compounds that are adsorbently incorporated onto surfaces. The method of prolonged release aquasomes are used to deliver a mixture of targeted and bioactive chemicals. When it comes to the controlled and sustained distribution of pharmaceuticals that are not highly soluble in water, aquasomes show great promise.

Nir Kossovsky (1995) initially discovered aquasomes, which are also referred to as "Bodies of water." Aquasomes are a revolutionary delivery technology that has increased safety and effectiveness. Simple nanoparticle three-layered nanoparticle delivery system and they have the ability to make hydrophilic drugs with low water solubility much more soluble. Pharmacologically active molecules are present in the three-layered structure, which is made up of a coated core, a ceramic core, and drug-loading aquasomes.

Anti-diabetic polypeptides like insulin and polypeptideK have been effectively delivered by means of these ceramic nanoparticles. Promising outcomes have also been observed in the delivery of other macromolecules, including haemoglobin and other antigens. Enzymes like serratiopeptidase are being investigated for oral administration via aquasomes.

According to Anand et al., 2017, the slow antigen, which is produced in modest amounts, has been shown to improve immunity against COVID-19. Aquasomes have the ability to protect drug antigens, maintain the biological and therapeutic effects of bioactive substances, enhance the poor solubility of aqueous soluble pharmaceuticals, and maintain oxygen transport.

Patri et al., 2005, aquasomes can be used to deliver viral antigen or red blood substitutes for particular intracellular gene therapy systems. Even in situations where the rate of dissolution restricts the bioavailability, the carbohydrate stabilised in the nanoparticles due to the inclusion of additional core material in the solid ceramic
nanoparticle production process. Cherian et al., 2000, the quantity of carriers blood, proteins, polymers, or niosomes that are utilised to transport a drug to its target site. The size, content, and carrier of nanoparticles affect their stability in the stomach environment and are all important aspects of improved drug delivery absorption.

Damera et al., 2019, several research endeavours have endeavoured to enhance its solubility and augment its capacity to convey a vaccine and bioactive macromolecular substance. In the field of peptide-based vaccine administration, aquasomes are employed to shield the immune system against allergic illnesses. It is challenging to distribute proteins and peptides by mouth since coating techniques can protect against acids that aren't proteolytic enzymes. The molecule is maintained in a dry, solid state by the natural stabiliser polyhydroxy sugar act dehydroprotectant. Recent research suggests that in addition to parenteral administration, aquasomes can also be administered orally or through an alternative approach due to their particle size, which ranges from 60 to 300 nm.

When the drug loading aquasome was exposed to DSC analysis to identify proteins and carbohydrates, it revealed evenly distributed drug particles in a well-contained coating material. De La Zerda and Gambhir (2007) state that XRD was employed to determine the material's natural state whether crystalline or amorphous.

1.4.1. Advantages of Aquasomes (Goyal et al., 2009)

- The drug’s bioavailability has been enhanced
- Rules out quick degradation or insolubility.
- Both lipophilic and hydrophilic drugs can be included in aquasome formulation.
- It improves the formulation's stability.
- It decreases medication toxicity and negative effects (Gholap et al., 2011)
- The drug's extended life in systemic circulation.
- Serves as a means of administering vaccines.

1.4.2. Aquasomes Composition

1.4.2.1. Core Material

Kaur et al., 2015, core materials utilised to effectively bond coating materials such as gelatin, acrylate, and albumin. Since hydroxyapatite is easily fabricated and ceramic is biodegradable, it is also utilised in the preparation of aquasomes. A carbohydrate's high surface energy or great potential results in its structural regularity. The benefits of calcium phosphate for the body's normal occurrence. The two most popular core materials are ceramic and polymers. There is utilisation of polymers like acrylate, gelatin, and albumin. Ceramic materials are utilised, including tin oxide, brushite (calcium phosphate), and diamond particles.

1.4.2.2. Coating Material

According to Khopade et al., 2002, bioactive materials can maintain the structural integrity of proteins by creating an environment akin to water and using self-assembling calcium phosphate particles for coating. Kumar et al., 2013, chitosan, which is derived from chitin, is employed as the coating material for aquasomes and facilitates drug adsorption. Drug delivery for various routes of administration for physicochemical features in antimicrobials and biocompatibility has been available in the past few years.

Commonly utilised coating ingredients include citrate, trehalose, cellobiose, pyridoxal 5 phosphate, sucrose, and chitosan. It has been documented that carbohydrates have a significant stabilising effect and function as a natural stabiliser.

Kumar et al., 2013, disaccharide sugar, which is coupled with 1, 4- glycosidic linkage and contains glucose and galactose, is used to relieve constipation. Its mild flavour makes lactose an essential component of milk products. Lactose is utilised in the aquasome formulation's coating substance. Kutlehria et al., 2018, its naturally occurring sugar, which consists of two glucose molecules, is non-reducing and protects the medication against dehydration. Trehalose's primary purpose is to protect organisms from dying in totally dry conditions. Trehalose also protects lipid bilayer and biochemically active component in water because it interacts with water at a much higher level than bioactive substances. in order to maintain the glass transition, entrapment, and hydration process that produces flexible polar groups.
1.4.2.3. Bioactive Molecule

Kossovsky et al., 1994, protein, drug vaccines that are poorly soluble, and antigen delivery via ionic contact are all carried by the medications and therapeutic usage in the traditional aquasome manufacturing process. In order to protect the aqueous molecule from protein dehydration, the carbohydrate interacts with charged groups.

Figure 4: Drug loaded Aquasomes.

1.4.3. Method Used for preparation of Aquasomes

The aquasome was made in three steps using the principle of self-assembly; the core was formed by sonication, the carbohydrate coating was applied, and the drug load was present in the core material. Lactose, sucrose, and trehalose are the coating materials utilised for delicate structures; this keeps the medicine from changing form. (Mitragotri et al., 2014)

1.4.3.1. Fabrication of Ceramic Core / Core Preparation

Rawat et al., 2008, aquasomes are made by the sonication method, which is a way of preparing ceramic cores using core material. This procedure involves precisely measuring and thoroughly mixing 50 millilitres of distilled water with calcium chloride and disodium hydrogen phosphate. Following mixing, the temperature was kept at 4°C for 22 minutes by sonication in an ultrasonic bath. After collecting the particles on the surface, the solution is filtered, and it is then dried at a temperature of 70°C in a hot air oven. According to Barroug et al., 1998, the material chosen for fabrication has a major impact on the core fabrication process. Aquasomes are mainly made from three types of core materials: brushite (calcium phosphate dihydrate), nanocrystalline carbon ceramics (diamonds), and tin oxide.

1.4.3.2. Carbohydrate Coating

Nehra et al., 2018, different processes employed in carbohydrate coating to adsorb ceramic core. Take 1 milligramme of ceramic core and mix it with reconstituted pure water. Add the lactose to the core material and fill the beaker to the full 50 ml capacity. Stir thoroughly. After sonicating the lactose and ceramic mix solution for 20 minutes, add the non-solvent (one millilitre of acetone), thoroughly agitate the mixture for another two minutes, and set the mixture aside for another 20 minutes. After that, lyophilization is applied to them in order to encourage the irreversible adsorption of carbohydrates onto the ceramic surface. Using stir cell ultra-filtration or centrifugation, the carbohydrate is eliminated.

1.4.3.3. Drug Loading

By partially adsorbing the coated particles, which provide the biochemically active molecule a solid phase, the drug is injected into coated material. (Rojas-Oviedo et al., 2007)

The medicine of choice is immobilised on the oligomer coating in the third stage. Adsorption loads the medication onto the coated particles. In order to do that, coated particles are dispersed into a solution of known drug concentration that has been produced in an appropriate pH buffer. After that, the dispersion is either lyophilized to produce the drug-loaded formulation (aquasomes) or it is stored overnight at a low temperature for drug loading. Figure
2 depicts the schematic representation of the aquasome preparation process, whereas Figure 2 provides the diagrammatic version.

1.4.3.4. Co-precipitation Method

**Patel et al., 2016**, utilising a reflux condenser and magnetic stirring, a co-precipitation process was used to manufacture the ceramic core. The temperature was kept at 75 degrees by adding 0.4 gm of diammonium hydrogen phosphate to 0.7 gm of calcium nitrate mixture while stirring continuously. After shaking or leaving the mixture for a day, precipitate forms. To keep the pH level stable, add aqueous ammonia, and then magnetically stir the liquid. The filtered precipitate is dried overnight after the solution has been thoroughly agitated, filtered, and then cleaned with distilled water. 800–900°C of heating was used to sinter the powder form in an electric furnace.

1.4.3.5. Dendrimer Method

**Patil et al., 2004**, PAMAM dissolves in a simulation of a biological fluid and is subsequently maintained at pH 7.4. After a week at 37°C to promote crystal formation, NaOH solution is added to the solution to change its pH. Eventually precipitate cores form, which are washed and filtered using distilled water. The precipitate is characterised using a range of techniques after being dried in a hot air oven and chilled for an additional night.

![Schematic representation of preparation of ceramic core.](image-url)
Figure 6: Formation of aquasomes.

1.5. Evaluation of Aquasomes

1.5.1. Size distribution

Prasanthi et al., 2010, size distribution and morphological examination of aquasomes using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). These two methods are used to analyse the coated core: electron photon spectroscopy is used to evaluate the zeta potential. The particle is determined to be negatively stained in 1% phosphotungstic acid using a TEM transmission electron microscope, and images are captured on photographic film produced by Adobe software in both clear and dark modes.

1.5.2. Structural analysis

Jain et al., 2009, FT-IR Fourier transform infrared spectroscopy can be used in the structural investigation of aquasomes to identify the sample's core material through the potassium bromide disc method. FTIR is used to analyse the identity and conformation of coating materials.

1.5.3. Crystallinity

Using X-ray diffraction (XRD) analysis, the crystalline or amorphous characteristics of the ceramic core are assessed. Based on diffractogram interpretations, this approach noticed that the calcium phosphate core is same and that the coated core is crystalline. Following coating, the polysaccharide's core contains trehalose, lactose, sucrose, and pyridoxyl-5-phosphate peak, which has decreased in intensity and become amorphous. In addition to anthrone, polysaccharide hydrolyzes to monosaccharide.

1.5.4. Glass Transition Temperature

According to Rakesh and Anoop (2012), the carbohydrate measure the glass-to-rubber transition in DSC.
1.5.5. Mean Particle Size and Zeta Potential

Senapati et al., 2018, zeta potential, a particle size analyzer, determines the drug-loaded aquasomes or their particle size (Zetasizer). For the purpose of measuring zeta potential. The formulation is deeply ingrained in the zeta dip cell. The study shows that the lactose process that contains carbohydrates saturates the zeta potential, lowering its value.

1.5.6. Drug Loading Efficiency

An aquasome formulation with a known drug concentration in the solution is used to load drugs. After precisely weighing the drug and allowing it to sit at 4°C for a full day, spin the liquid supernatant at a low temperature for half an hour, and then refrigerate the solution.

1.5.7. In-vitro Drug Release Study

Shahabade et al., 2009, drug-loaded aquasome formulation used in the in-vitro release investigation uses a pH 6.5 phosphate buffer and 900 cc of dissolving fluid in a USP type 1 dissolution test device.

1.6. Applications

1.6.1. Delivery of Vaccine

The disaccharide cellobiose coating on the ceramic core of aquasomes, which have a size range of 5 to 600 nm, protects the high degree of antigen distribution in drug delivery vehicles. The antigen for hepatitis B that the hydroxyapatite core contains helps the immune system fight T helper cells. According to Shirsand et al., 2012, the aquasome is used to make certain antibodies that target molecules that are either viral or immune deficiency virus antigens in the case of the Epstein-Barr virus.

1.6.2. Enzyme Delivery

Shukla et al., 2016, medication is delivered by aquasomes to a particular region where the enzyme acts as an anti-inflammatory when taken orally to prevent stomach acid or enzyme breakdown. The enzyme's biological activity on immobilised DNAse in the calcium phosphate aquasome core was observed.

1.6.3. Oxygen Delivery

Sutariya and Patel 2012, prepared formulation's oxygen-binding capabilities are examined, as is the drug's efficacy or the size of the particles in the aquasome preparation. Due to the stability of the drug-loaded aquasomes, there is a great possibility for oxygen binding or maintaining the characteristics for a month, which would sustain the toxicity at 80% of haemoglobin content.

1.6.4. Aquasomes for Insulin Delivery

Tiwari et al., 2012, large amount of insulin is available for oral administration when insulin delivery is formulated in a ceramic core dispersed in alginate polymer. Insulin has minimal toxicity and a high drug loading capacity, while the nanoparticle's high bioavailability helps it to pass through stomach-lining obstacles in the gastrointestinal tract. A novel approach to synthesis will open up possibilities for the manufacturing of insulin.
1.6.5. Gene Delivery

Wilczewska et al., 2012, studies on aquasomes shield the integral gene in a segment, preserving the membrane protein in the ceramic or film's core. The focus of research on the novel drug delivery system is on aquasomes as gene carrier systems connected to the administration of pharmacological drugs.

1.7. Aim and Objectives

Aceclofenac is delivered by aquosomal gel formulation and administered directly to the diseased region. Aceclofenac's lipophilic nature and aquasome nanosizing allow the medication to readily enter the skin and act at the desired location. Because aquasomes increase the solubility of the medicine, it is released from the target location over a longer period of time. In light of aceclofenac's low water solubility, an attempt has been made to create and develop an aquosomal drug delivery system of aceclofenac in the current study investigation with following objectives:

(i) Formation development of Aquasomes of aceclofenac;
(ii) Morphological and size analysis of formulated aquasomes of aceclofenac;
(iii) Characterization of formulated aquasomes of aceclofenac using transmission electron microscopy (TEM), scanning electron microscopy (SEM), for structural analysis, particle size, shape, solubility etc.
(iv) Release Kinetic Studies
(v) Stability Studies

1.8. Experimental / Methods

1.8.1. Pre-formulation Studies

The overall objective of pre-formulation testing is to generate information useful to the formulation and development of stable dosage form with good bioavailability. Aceclofenac drug sample was gifted by Ind-Swift Laboratories Limited, Delhi and it was authenticated/identified by FTIR and UV Spectroscopy.

1.8.1.1. Drug Identification

(i) FTIR
(ii) UV Spectroscopy
(iii) Melting Point (mp)
(iv) Physical Appearance
(v) Partition Coefficient

1.8.1.2. Purity Determination of Drug (Aceclofenac/ACF)

(a) Determination of Drug by Standard Curve
(b) Purity Determination of Drug by Solubility Analysis

1.8.1.3. Appearance: White crystalline powder.

1.8.1.4. Identification of Samples drug – Aceclofenac (ACF) by FTIR

Aceclofenac (ACF) (transparent disc with KBr dispersion pellets) was analysed using FTIR Spectroscopy
(Shimatzu 8400 PC-based) at 4000-400 cm intervals. KBr dispersion pellets, which are moisture-free, were used for the scanning process. In a glass mortar, 1 mg of the drug sample and the standard reference drug were separately combined with 10 mg of spectroscopic-grade KBr. The scanning was carried out in the 4000-4000 cm$^{-1}$ range. The reference medicine, conventional Aceclofenac, was compared with the drug sample, Aceclofenac (ACF). Figures 7 display the drug sample’s FTIR spectra.

![FTIR Spectra](image)

Figure 7: IR of Aceclofenac.

1.8.1.5. Identification of Aceclofenac by Ultraviolet (UV) Spectroscopy

A stock solution of aceclofenac (ACF) in ethanol and water was made, and using a Double Beam UV Spectrophotometer (Model 2202, Systronics, India), the absorbance at a wavelength between 200 and 400 nm was scanned and analysed to determine the $\lambda_{\text{max}}$ of the drug. ACF, which was precisely weighed at 100 mg, was dissolved in a small amount of ethanol (a few millilitres), and the volume was increased to 100 by adding distilled water (Stock solution A). One millilitre of this solution was put into a second, 100 millilitre volumetric flask, and the volume was increased to 100 millilitres by adding distilled water (Stock solution B). Using a Double Beam UV Spectrophotometer (Model Systronics -2202) to measure $\lambda_{\text{max}}$ (Figure 5.3-5.4). The observed $\lambda_{\text{max}}$ was 273.2 nm.

Figure 8: UV-Vis of Aceclofenac (sample).

1.8.1.6. Melting Point Analysis of Aceclofenac

Melting point (mp) is the temperature at which the pure liquid and solid exist equilibrium. MP of the aceclofenac (ACF) was determined by using theile tube method.

1.8.1.6. Solubility Determination / Analysis of Aceclofenac (ACF)

The solubility study of ACF was performed in different solvents like ethanol, distilled water, PBS, acetone etc. 10 mg of ACF was transferred in a series of different solvents (5 ml) in different conical flask. These flasks
were shaken by mechanical shaker (Hicon India) for 24 hrs with constant vibration at constant temperature (25°C).

Different solutions were then filtered and different filtrates were diluted with same / appropriate solvent and analysed by double beam UV spectrophotometer (Systronics – 2202). Quantitative estimations of ACF were carried out for each sample to calculate the solubility of ACF in different solvents. (Table 1)

Table 1: Solubility of aceclofenac (ACF) different solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>27.22 (Poorly Soluble)</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1903.28 (Freely Soluble)</td>
</tr>
<tr>
<td>PBS (pH 7.4)</td>
<td>1087.62 (Freely Soluble)</td>
</tr>
<tr>
<td>l-octanol</td>
<td>1621.06 (Freely Soluble)</td>
</tr>
<tr>
<td>0.1 N HCl</td>
<td>47.24 (Poorly Soluble)</td>
</tr>
</tbody>
</table>

1.8.1.7. Partition Coefficient Determination of Aceclofenac (ACF)

PC represents the ratio of the unionised medication dispersed at equilibrium between the organic and aqueous phases. Drug lipophilicity was measured in as octanol/water, octanol / 0.1NHCl, etc. PC characterises and determines the lipophilic/hydrophilic nature of the drug. Ascertained in the solvent system using distilled water and n-octanol. 10 milligrams of medication in 5 ml of DW and octanol. After eight hours of shaking in a vortex shaker, the two phases separated. Using a Shimadzu-1700 E UV spectrophotometer, the aqueous phase was analysed for DFS in comparison to a blank solution of reagent. The amount of drug in the octanol phase was ascertained. Value P for the partition coefficient was computed.

1.8.1.8. Compatibility Studies of Aceclofenac (ACF)

The drug ACF and polymer/surfactant compatibility was characterised using Fourier transform infrared spectroscopy (FTIR). The compatibility was verified by physically mixing ACF and Polymer in a1:1 ratio, followed by FTIR analysis of the mixture. The peaks in the mixture FTIR spectra of physical mixtures remained mostly unchanged and resembled those of pure drug FTIR (Figure 9-11).

Figure 9: Aceclofenac FTIR Spectrum.
1.8.1.9. Standard Curve Preparation of Aceclofenac

100 mg of precisely weighed aceclofenac (ACF) was dissolved in a small amount of PBS (1 mg/ml Stock solution A), and the volume was increased to 100 using PBS; After transferring 1 ml of this solution into a second 100 ml volumetric flask, the volume was increased to 100 ml using PBS (10 µg/ml Stock solution B); A variety of aliquots, or dilutions, of Stock solution B (10 µg/ml) were made in order to get concentrations of 2, 4, 6, 8, and 10 µg/ml. The absorbance was measured at 273.2 nm in comparison to a blank using a Systronics-2202 Double Beam UV Spectrophotometer; The same process was carried out again using a pH 1.2 0.1N HCl solution. and deionized water (Figure 12-14, Table 2).

Table 2: Calibration Curve of aceclofenac (ACF).
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration ACF (μg/ml)</th>
<th>Absorbance (nm)</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>D.W.</td>
</tr>
<tr>
<td>1.</td>
<td>2</td>
<td>0.009</td>
</tr>
<tr>
<td>2.</td>
<td>4</td>
<td>0.018</td>
</tr>
<tr>
<td>3.</td>
<td>6</td>
<td>0.027</td>
</tr>
<tr>
<td>4.</td>
<td>8</td>
<td>0.036</td>
</tr>
<tr>
<td>5.</td>
<td>10</td>
<td>0.44</td>
</tr>
</tbody>
</table>

**Figure 12**: Calibration curve of ACF in DW.

**Figure 13**: Calibration curve of ACF in 0.1N HCl (pH 1.2).

**Figure 14**: Calibration curve of Aceclofenac in PBS (pH 7.4).

1.9. Preparation of the Formulation of Aceclofenac Ceramic Aquasom
1.9.1. Method of Preparation of Inorganic Cores

Co-precipitation technique by sonication of 0.5 M monobasic sodium phosphate buffer solution and 0.5 M calcium chloride solution stirred by magnetic stirrer and sonication at 4°C for 2 hrs. The precipitate was separated by centrifugation at 5000 rpm for 30 min and then washed five times with distilled water to remove NaCl formed during the reaction. The precipitate was resuspended in the distilled water and passed through a 0.22 μm Millipore filter and finally dried at 60-70 °C. (Jain et al., 2000)

1.9.2. Sugar Coating

Sample of inorganic core was resuspended into distilled water and added 100 ml solution of sugar.

1.9.3. Drug Loading

A solution of 0.06 M of aceclofenac was prepared in ethanol, polyhydroxylated cores dispersed in the aceclofenac solution and finally applied mechanical agitation which was maintained for 90 min. The dispersion was filtered and dried at room temperature.

1.10. Characterisation of Formulation

1.10.1. Scanning Electron Microscope Analysis (SEM Analysis)

i. SEM (Evo-40, Zeiss, Germany) at AIRF, JNU (Delhi).

ii. SOPs were used.

iii. Stubs were then placed into fine coat ion sputter for gold coating and then samples were scanned.

1.10.2. Infrared Spectrum for Formulation Compatibility

FTIR spectroscopy was used to characterise the formulation compatibility. When the formulations were analysed using FTIR to see if the medicine and polymer were compatible, it was found that the peaks in the formulations remained the same (identical peaks seen).

1.10.3. Particle Size Analysis

In order to analyse particle size, drug loaded lyophilized nanoparticles were dispersed in de-ionised water, vortexed for 10 min and sonicated for 5 min before sampling. Particle size was determined by laser scanning light using Malvern Laser Analyser Instrument at NIPER, Mohali (Figure 15)

Figure 15: Particle Size Analysis of Different Formulations.
1.10.4. Percentage Drug Loading

To measure the amount of aceclofenac (ACF) drug loading in aquasomes, 10.0 mg of each nanoparticle formulation was suspended in 10.0 ml PBS (pH 7.4). The aquasomes were centrifuged at -40°C and 14,000 rpm for 15 minutes using a cooling centrifuge (Remi Instruments, Mumbai). The supernatant was examined for drug content using a UV spectrophotometer (Systronics 2202) after a suitable dilution with phosphate buffer solution (Figure 16).

![Graph showing drug loading percentage for different formulations](image)

Figure 16: Loading efficiency of different formulations.

1.10.5. In-vitro Drug Release Studies

Using a locally built diffusion cell, the in-vitro release of ACF from aquasomal formulation was investigated; diffusion cell's permeability area measured 1 cm², while the receptor cell's volume measured 40 ml. temperature was kept at 37±2°C; receptor compartment was continuously agitated at 100 rpm and held 40 millilitres of phosphate buffer (pH 7.4); synthetic semi-permeable membrane was placed between the donor and receptor compartments; membrane was treated with the formulation; samples taken out of the diffusion cell's sample port on a regular basis and subjected to UV analysis; The sink state was maintained and the receptor phase was promptly refilled with an equivalent volume of pH 7.4 phosphate buffer solution; experiments were carried out in triplicate.

1.10.6. Stability Studies

For four weeks, the drug content of the aquasomes loaded with aceclofenac was tested for physical stability at various storage temperatures. Each sample was taken out of its various storage temperatures on the same day, and 1.0 millilitre was measured at 273.2 nm using spectrophotometry. All samples received the same amount of aliquot (0.5 ml of fresh pH 7.4), and the same investigation was conducted twice over a period of four weeks.
In present research investigation, an attempt was made to use Aquasomes drug delivery system to design an optimum aquasomal formulation to enhance the permeation of transdermal formulation aceclofenac (ACF; poorly water-soluble drug) by aquasomal formulation (characterized by safety and high therapeutic efficacy) which increase bioavailability (to reduce the daily dosing schedule of aceclofenac with subsequent improvement in patient compliance and drug safety).

FTIR analysis was performed for confirmation of their identity and purity. Aceclofenac (ACF) sample drug showed absorption peaks which were similar to reference standard drug. Absorption peaks (cm⁻¹) at 3315.07 (N-H stretching), 1718.61 (C=O stretching), 1581.81 (N-H bending), 1509.03 (C=O Aromatic stretching), 1464.35 (C=C Aromatic bending), 1311.28 (C-N), 1255.23 (C-O), and 748.51 (C-Cl) were found and both sample drug and reference drug were pure Aceclofenac (ACF).

The UV absorption maximum (λmax) for aceclofenac (ACF) was found to be 273.2 nm. (Figure 5.3-5.4). Melting point of aceclofenac (ACF) was found to be 149-151°C. (Table 5.4) ACF was found freely soluble in ethanol (1903.28 μg/ml), PBS (pH 7.4; 1087.62) and 1-octanol (1621.06 μg/ml); poorly soluble in distilled water (27.22 μg/ml) and 0.1 N HCl (47.24 μg/ml). The partition coefficient of aceclofenac (ACF) was analysed and was found to be 1.89 (reported 1.86 in n-octanol / water) and 1.44 (reported 1.56 in n-octanol / PBS (pH 7.4)). In FTIR spectrum of lactose, absorption peaks were found at 3512.29 (OH), 2946.51 (C-H stretching), 1458.85 (C-H bending), 1231.53 (C-O), 751.75 (C-H bending). In FTIR spectrum of sucrose, absorption peaks were found at 3547.57 (OH), 2932.11 (C-H stretching), 1444.42 (C-H bending), 1241.79 (C-O), 852.54 (C-H bending). FTIR spectrum of aceclofenac (ACF) with lactose showed absorption peaks at 3365.68 (OH), 3315.05 (N-H stretching), 2948.54 (C-H stretching), 1715.27 (C=O), 1505.81 (C=C stretching aromatic), 1343.41 (C-N), 1247.81 (C-O), 751.75 (C-Cl). FTIR spectrum of aceclofenac (ACF) with sucrose showed absorption peaks at 3372.78 (OH), 3307.84 (N-H stretching), 2953.29 (C-H stretching), 1711.18 (C=O), 1505.75 (C=C stretching aromatic), 1343.46 (C-N), 1251.68 (C-O), 748.51 (C-Cl). In FTIR spectral analysis, it was found that there was no change in absorption maxima of functional groups in pure aceclofenac (ACF) drug as well as drug polymer mixture hence aceclofenac (ACF) drug and polymer were compatible to each other.

The spectrophotometric analytical method was chosen to determine whether or not the medication aceclofenac (ACF) complies with Beer’s Law. The medication was produced in dilutions ranging from 1 μg/ml to 10 μg/ml, and the absorbance (λmax 273.2 nm) was measured. A standard plot of concentration vs absorbance was created, and between 1 μg/ml and 10 μg/ml of ACF, it produced a straight line that defies Beer’s Law. The drug’s purity was estimated using a standard curve plotted in distilled water, phosphate buffer (PBS; pH 7.4), and 0.1 N HCl (pH 1.2). The results are shown in Table 2, Figure 12 (DW), Figure 13 (0.1 N HCl pH 1.2), and Figure 14 (PBS; pH 7.4).
Aceclofenac ceramic aquasomes consists of an inorganic core formation which is coated with lactose forming the polyhydroxylate core that finally loaded with the drug. Subsequently, cores were coated by different concentrations of sugar such as 0.03M, 0.06M and 0.09M of lactose coating and same as variation of sucrose coating. After completion of coating, coated core were loaded with drug to prepare various formulations. Different aquasomal formulations were prepared by varying concentration of lactose and sucrose. All formulations were evaluated for various parameters like particle size, SEM and loading efficiency, in-vitro drug release analysis etc.

The visualization by SEM showed that formulated aquasomes were smooth and spherical shape in different sizes. Particle size of all formulations of aceclofenac (ACF) were found to be 257.4 μm (F1), 232.4 μm (F2), 212.6 μm (F3), 261.7 μm (F4), 224.5 μm (F5), and 210.7 μm (F6) (particle size was nanosize. The FTIR of formulations shows that there was no interaction between the drug and sugar in solid particle (formulation) and peaks were similar with the reference standard. Therewas no change in the peak behavior of functional groups of drug sample and sugars (lactose and sucrose). Hence, drugs and polymers were compatible to each other. The drug (ACF) loading efficiency of aquasomes was found in the range of 67-81 % and it was attributed to the adsorption of drug depend on the concentration of lactose or sucrose layer. Loading efficiency was found to be maximum in F3 and decreased in following order F3>F2>F1>F6>F5>F4 i.e. loading efficiency was increased as the concentration of lactose and sucrose increased (directly proportional).The maximum drug loading was found in formulation F3 (81.39) and lowest loadingin F4 (67.82). Studies on in vitro permeation consistently provide insightful data regarding the behavior of the substance in vivo.

The amount of substance that can be absorbed depends on how much of the drug has infiltrated. Semipermeable dialysis membranes were used for in-vitro drug release experiments. In various formulations, the medication released in the following order: F3>F6>F2>F1>F5>F4. The F3 formulation demonstrated the largest drug release, or 86.51%, and the F4 formulation demonstrated the minimum drug release, or 69.48%, after 30 hours, based on the drug release order of the various formulations.

Drug release was higher in F3 formulation due to the higher concentration of sugar and respectively drug loading. Increased concentration of sugar in the coating of aquasomes had produced more spherical and smooth aquasomes due to which the drug loading was also increased. With increase in concentration of sugar in coating, a significant increase (p<0.05) was found in in-vitro release of aceclofenac (ACF) from prepared aquasomal formulations. It was found that 80.7%, 84.23% and 86.51% of aceclofenac (ACF) was released from F1, F2 and F3 respectively whereas lactose was used as coating material in the concentration of 0.03M, 0.06M and 0.09M respectively. This clearly indicated that the aceclofenac (ACF) release from the prepared formulations was increased with increased concentration of lactose as a core coating material. Similar results were also obtained with sucrose as core coating material where a significant increase (p<0.05) was found in in-vitro release of aceclofenac (ACF) from F4 (0.03M), F5 (0.06M) and F6 (0.09M) where 69.48%, 72.48% and 85.58% aceclofenac (ACF) was released respectively. The enhancement in the release of aceclofenac (ACF) from nanoparticles was supported by the hydration process which occurred very rapidly due to smaller size of nanoparticles. The F3 was the best formulation among all formulated aquasomes formulations and drug permeation profiles were studied in triplicate and mean data were considered finally.

The mechanisms of aceclofenac (ACF) release from aquasomes were analysed using zero order, first order, Higuchi, and Korsmeyer-Peppas models. The release rate profiles of the intended formulations have been prepared, along with the curve fitting findings. Slopes were examined and regression coefficients for several drug release kinetics models. The models exhibiting the highest regression coefficient were deemed the best suitable for the drug release process. Formulations F1 and F6 followed the Peppas model, according to the data from the different models, with r2 values of 0.985, 0.986, and n values of 1.271 and 1.150, respectively. Because n values were greater than 0.89, the release can be explained by the super case 2 transport mechanism, which involves non-fickian transport.

First order release kinetics were followed by formulations F3, F4, and F5, with r2 values of 0.983, 0.983, and 0.978, respectively, while formulation F2 followed release kinetics (zero order) with a r2 value of 0.978. The stability studies were carried out for formulation F1 and F4 at different temperature like refrigeration temperature (2-8°C), room temperature (25±2°C) and at 45°C for 4 weeks and after evaluation of each work data, the data shows that at 2-8°C formulation F1 has 88.3% remained and for F4 formulation it was 87.8% remaining. At 25±3°C, F1 has remained 82.2% and formulation F4 was remained 81.4%. At45°C, 76.3% and 73.5% were remained for formulation F1 and F4 respectively.

So, there was nominal degradation at room temperature (stable) whereas higher drug diffusion or leakage took place at high temperature (45°C) after 4 weeks. After 4 weeks, best stability was found at refrigeration temperature
(2-8°C). So aceclofenac (ACF) aquasomal formulations should be stored at 2-8°C (refrigerator). Formulation F1 showed better storage stability than formulation F4 (either at room temperature or at refrigeration temperature).

Conclusions

Formulation F3 had the highest drug loading, while formulation F4 had the lowest loading. The in-vitro release of aceclofenac from produced aquasomal formulations was shown to significantly increase (p<0.05) with an increase in the coating's sugar concentration. Aceclofenac was discovered to be released from F1, F2, and F3 at concentrations of 80.7%, 84.23%, and 86.51%, respectively. Finally, it was concluded that formulated aquasomes possess good release rate and by altering the concentration of sugar, aceclofenac release can be controlled for prolonged period of time with no side effects.

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