International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Unlocking the Potential of Aquasomes: A Comprehensive Review on Innovative Nanocarriers in Drug Delivery and Beyond

Krishnaveni Manubolu[1], Pavan Kumar Balagani[2], Edward Raju Gope[3], Varalakshmi J[2], Raveesha Peeriga[4], Sridevi A.R.[2]*

[1]*Narayana Pharmacy College, Chinthareddypalem, Nellore-524002, Andhra Pradesh, India.*

[2]*Gokula Krishna College of Pharmacy, Sullurupeta-524121, Andhra Pradesh, India.*

[3]*Dr Samuel George Institute of Pharmaceutical Sciences, Markapur ANUCPS, Prakasam District, Andhra Pradesh, India.*

[4]*V. V. Institute of Pharmaceutical Sciences, Seshadri Rao Knowledge Village, Gudlavalleru-521301, Krishna District, Andhra Pradesh, India.*

Abstract

Aquasomes are nanoparticles fabricated from ceramics developed to enhance proteins and peptides stability, providing an adequate residence time in circulation. It consists of ceramic core coated with poly hydroxyl oligomer, on which protein and peptide drug can be adsorbed. Aquasomes preparation, characterization, and application in protein and peptide drug delivery are discussed. Microneedles are promising transdermal approach; it involves creation of micron-sized pores in the skin for enhancing the drug delivery across the skin, as their length ranged between 150 and 1500µm. The delivery system has been successfully utilized for the delivery of insulin, hemoglobin, and various antigens. Oral delivery of enzymes like serratiopeptidase has also been achieved. This article discusses the problems faced in the delivery of clinically important peptides and presents aquasomes as a reliable approach to troubleshoot them.

Keywords: Aquasomes; Delivery; Microneedles; Peptides; Proteins

Full-length article **Corresponding Author*, e-mail: drprsha@gmail.com

1. Introduction

Aquasomes are one of the most recently developed delivery system for bioactive molecules like peptide, protein, hormones, antigens and genes to specific sites. Aquasomes are spherical in shape with 60–300 nm particles size. These are nanoparticulate carrier systems but instead of being simple nanoparticles these are three layered self assembled structures, comprised of a solid phase nanocrystalline core coated with oligomeric film to which biochemically active molecules are adsorbed with or without modification. These structures are self-assembled by noncovalent and ionic bonds. The solid core provides the structural stability, while the carbohydrate coating protects against dehydration and stabilizes the biochemically active molecules. The delivery system has been successfully utilized for the delivery of insulin, hemoglobin, and enzymes like serratiopeptidase etc. This reviews the principles of self assembly, the challenges of maintaining the conformational integrity and biochemical activity of immobilized surface pairs, the convergence of these principles into a single functional composition and its application in various fields of pharmacy.

Aquasomes are nanoparticulate carrier system but instead of being simple nanoparticle these are three layered self assembled structures, comprised of a solid phase nanocrystalline core coated with oligomeric film on which biochemically active molecules are adsorbed with or without modification. Aquasomes are like “bodies of water" and their water like properties protect and preserve fragile biological molecules, and this property of maintaining conformational integrity as well as high degree of surface exposure is exploited in targeting of bio-active molecules like peptide and protein hormones, enzymes, antigens and genes to specific sites. These three layered structures are self-assembled by non-covalent and ionic bonds. These carbohydrate stabilize nanoparticles of ceramic are known as “aquasomes”. The pharmacologically active molecule incorporated by co-polymerization, diffusion or adsorption to carbohydrate surface of pre formed nanoparticles. Aquasomes discovery comprises a principle from microbiology, food chemistry, biophysics and many discoveries including solid phase synthesis, supramolecular chemistry, molecular shape change and self assembly [1].

Self assembly implies that the constituent parts of some final product assume spontaneously prescribed structural orientations in two or three dimensional space. The self assembly of macromolecules in the aqueous environment, either for the purpose of creating smart nanostructure materials or in the course of naturally occurring biochemistry, is governed basically by three physicochemical processes: the interactions of charged groups, dehydration effects and structural stability.

2. Strategies used in chemical synthesis of nanostructure

Aquasomes are self-assembled three layered nanostructures. Therefore the strategies involved in chemical synthesis of nanostructure need elaboration. The strategies normally used in the chemical synthesis of nanostructures are discussed below.

2.1. Sequential covalent synthesis

This can be used to generate arrays of co-valently linked atoms generated with well defined composition, connectivity and shape i.e. vitamin B12. It can generate the structures that are far from the thermodynamic minimum for that collection of atoms [2].

2.2. Covalent polymerization

This strategy is used for preparing molecules with high molecular weight. Here a relatively simple low weight substance is allowed to react with itself to produce molecule comprising many covalently linked monomers. For example: Formation of polyethylene from ethylene. The molecular weight of polyethylene can be high (>106 Daltons), and it is easily prepared, but the molecular structure is simple and repetitive and the process by which it is formed offers only limited opportunity for controlled variation in the structure or for control of its three dimensional shape. Polymerization indirectly provides synthetic routes to stable nanostructures e.g. phase separated polymers [3].

2.3. Self–organizing synthesis

This strategy abandons the covalent bond as required connection between atoms and relies instead on weaker and less directional bonds such as ionic, hydrogen and van der waals interactions to organize atoms, ions or molecules into structures. The different type of structures prepared by this stratergy includes molecular crystals, ligand crystals, colloids, micelles, emulsions, phase separated polymers and self assembled monolayer. Self organization is the peculiar feature of these methods. The molecules or ions adjust their own position to reach thermodynamic minimum.

By self-organization, true nanostructures can be prepared heat denaturation and stabilization is due to the effect of sugars and polyols on hydrophobic interactions [3]. The extent of stabilization by different sugars and polyols is explained by different influences on structure of water. The hydroxyl group on carbohydrate interacts with polar and charged groups of biological molecules in a manner similar to water molecules alone and preserves the aqueous structure of biological molecules like protein on dehydration. Since these disaccharides are rich in hydroxyl groups and help to replace water around the polar residues in proteins, thus maintaining their integrity in the absence of water. The free mobility associated with rich hydroxyl component creates a unique hydrogen binding subsrate that produces glassy aqueous state [4]. There are many systemic biophysical and intrinsic biophysical constraints, which tend to destabilize the drug.

3. Systemic biophysical constraints

There are physical and chemical degradative agents, which cause compositional changes and loss of spatial activity by breaking chemical bonds in the drug candidate. Such agents include UV radiation, heat, ozone, peroxide and other free radicals. Likewise mammalian body also contains certain agents viz. inflammatory, peroxides, free radicals and degradative enzymes related to serine proteases. Other than these physical and chemical degradative agents, those agents that promote dehydration also cause molecular inactivation. Since water is critical structural component of most biochemically reactive molecules, its loss leads to change in energies and results in altered molecular conformation and impaired spatial qualities. Exposure and surface immobilization often promotes dehydration. Degradative agents present in mammals can destroy rapidly complex and expensive polypeptide biopharmaceuticals, while denaturation during dehydration can impair polypeptides on long term storage [5].

4. Intrinsic biophysical constraints

The intrinsic biophysical constraint is normally posed by drug delivery system. When drug candidates are immobilized to nanoparticulate substrate, it can cause surface induced dehydration and, in turn molecular conformation. The altered molecular conformation can produce adverse or allergic reaction with suboptimal pharmacological activity. In short, biochemically active molecules lose their functional properties in either case, means in a ‘dry’ or ‘wet’ state. At the same time, a water environment is vital for molecular activity. Therefore, the challenge is to store and transport promising and useful biomolecules in the dry state without causing them to lose too much of their potential activity. In such case, aquasomes with natural stabilizers like various polyhydroxy sugars act as dehydroprotectant, maintains water like state and thereby helps to preserves the molecular conformation of bioactive molecules in dry solid state. Fungal spores producing ergot alkaloids were stabilized by sucrose rich solution. Desiccation induced molecular denaturation is reported to be prevented by certain disaccharides [6].

5. Composition of aquasomes

5.1. Core material

Ceramic and polymers are most widely used core materials. Polymers such as albumin, gelatin or acrylate are used. Ceramic such as diamond particles, brushite (calcium phosphate) and tin oxide are used.

5.2. Coating material

Coating materials commonly used are cellobiose, pyridoxal 5 phosphate, sucrose, trehalose, chitosan, citrate etc. Carbohydrate plays important role act as natural

stabilizer, its stabilization efficiency has been reported. Beginning with preformed carbon ceramic nanoparticle and self assembled calcium phosphate dihydrate particles (colloidal precipitation) to which glassy carbohydrate are then allowed to adsorb as a nanometer thick surface coating a molecular carrier is formed.

5.3. Bioactive

They have the property of interacting with film via non covalent and ionic interactions [7].

6. Properties of aquasomes

Aquasome possess large size and active surface hence can be efficiently loaded with substantial amounts of agents through ionic, non co-valent bonds, van der waals forces and entropic forces. As solid particles dispersed in aqueous environment, exhibit physical properties of colloids. Its mechanism of action is controlled by their surface chemistry. Aquasomes deliver contents through combination of specific targeting, molecular shielding, and slow and sustained release process. Aquasome water like properties provides a platform for preserving the conformational integrity and bio chemical stability of bio-actives.

Aquasomes due to their size and structural stability, avoid clearance by reticuloendothelial system or degradation by other environmental challenges. It is colloidal range biodegradable nanoparticles, so that they will be more concentrated in liver and muscles. Since the drug is absorbed on to the surface of the system without further surface modification as in case of insulin and antigen delivery, they may not find any difficulty in receptor recognition on the active site so that the pharmocological or biological activity can be achieved immediately. In normal system, calcium phosphate is biodegradable. Biodegradation in vivo achieved by monocytes and multicellular cells called osteoclast. Two types of phagocytosis reported, either crystals taken up alone and then dissolved in cytoplasm after disappearance of phagosome membrane or dissolution after formation of heterophagosome. Aquasomes are mainly characterized for structural analysis, particle size, and morphology. These are evaluated by X- ray powder diffractometry, transmission electron microscopy, and scanning electron microscopy [8].

7. Method of preparation of aquasomes

The method of preparation of aquasomes involves three steps. The general procedure consists of Formation of an inorganic core, followed by Coating of the core with polyhydroxy oligomer, and finally loading of the drug of choice to this assembly [9-11].

7.1. Formation of an inorganic core

It involves the fabrication of a ceramic core, and the procedure depends upon the materials selected. The two most commonly used ceramic cores are calcium phosphate and diamond.

7.2. Synthesis of nanocrystalline tin oxide core ceramic

It can be synthesized by direct current reactive magnetron sputtering. Here, a 3 inches diameter target of high purity tin is sputtered in a high pressure gas mixture of orgon and oxygen. The ultrafine particles formed in the gas phase are then collected on copper tubes cooled to 77 ^{0}K with flowing nitrogen.

7.3. Self-assembled nanocrystalline brushite (calcium phosphate dihydrate)

These can be prepared by colloidal precipitation and sonication by reacting solution of disodium hydrogen phosphate and calcium chloride.

7.4. Nanocrystalline carbon ceramic, diamond particles

These can also be used for the core synthesis after ultra cleansing and sonication. The common feature of various cores is that they are crystalline and that when they are introduced into the synthetic processes, they measures between 50-150 nm and exhibit extremely clean and therefore reactive species. Ceramic materials, being structurally highly regular, are most widely used for core fabrication. The high degree of order in crystalline ceramics ensures only a limited effect on the nature of atoms below the surface layer when any surface modification is being done, thus preserving the bulk properties of ceramics. This high degree of order also offers a high level of surface energy that favors the binding of pohyhydroxyl oligomeric surface film. The precipitated cores are centrifuged and then washed with enough distilled water to remove sodium chloride formed during the reaction. The precipitates are resuspended in distilled water and passed through a fine membrane filter to collect the particles of desired size.

7.5. Coating of the core with polyhydroxy oligomer

In the second step, ceramic cores are coated with carbohydrate (polyhydroxyl oligomer). The coating is carried out by addition of carbohydrate into an aqueous dispersion of the cores under sonication. These are then subjected to lyophilization to promote an irreversible adsorption of carbohydrate onto the ceramic surface. The unadsorbed carbohydrate is removed by centrifugation. The commonly used coating materials are cellobiose, citrate, pyridoxal-5- phosphate, trehalose and sucrose.

7.6. Loading of the drug of choice to this assembly

The final stage involves the loading of drug to the coated particles by adsorption. For that, a solution of known concentration of drug is prepared in suitable pH buffer, and coated particles are dispersed into it. The dispersion is then either kept overnight at low temperature for drug loading or lyophilized after some time so as to obtain the drug-loaded formulation (i.e., aquasomes). The preparation thus obtained is then characterized using various techniques.

8. Fate of aquasomes

Since aquasomes are biodegradable nanoparticles, so that they will be more concentrated in liver and muscles. Since the drug is adsorbed on to the surface of the system without further surface modification as in case of insulin and antigen delivery, they may not find any difficulty in receptor recognition on the active site so that the pharmacological or biological activity can be achieved

immediately, in normal system, the calcium phosphate is a biodegradable ceramic.

Biodegradation of ceramic in vivo is achieved essentially by monocytes and multicellular cells called osteoclasts because they intervene first at the biomaterial implantation site during inflammatory reaction. Two types of phagocytosis were reported when cells come in contact with biomaterial; either calcium phosphate crystals were taken up alone and then dissolved in cytoplasm after disappearance of the phagosome membrane or dissolution after formation of heterophagosomes. Phagocytosis of calcium phosphate coincided with autophagy and the accumulation of residual bodies in the cell [12]. Monocytic activities can be modulated by many soluble factors and are increased by IFN-g (interferon gamma) or 1, 25 dihydroxy cholecalciferol. Other cytokines can also contribute to inflammatory mechanism and may be involved in the biodegradation process [13].

8.1. Characterization of aquasomes

Aquasomes are characterized chiefly for their structural and morphological properties, particle size distribution, and drug- loading capacity.

9. Characterization of ceramic core

9.1. Size distribution

For morphological characterization and size distribution analysis, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are generally used. Core, coated core, as well as drug-loaded aquasomes are analyzed by these techniques. Mean particle size and zeta potential of the particles can also be determined by using photon correlation spectroscopy [14].

9.2. Structural analysis

FT-IR spectroscopy can be used for structural analysis. Using the potassium bromide sample disk method, the core as well as the coated core can be analyzed by recording their IR spectra in the wave number range 4000–400 cm^{-1}; the characteristic peaks observed are then matched with reference peaks. Identification of sugar and drug loaded over the ceramic core can also be confirmed by FT-IR analysis of the sample [15].

9.3. Crystallinity

The prepared ceramic core can be analyzed for its crystalline or amorphous behavior using X-ray diffraction. In this technique, the X-ray diffraction pattern of the sample is compared with the standard diffractogram, based on which the interpretations are made.

10. Characterization of coated core

10.1. Carbohydrate coating

Coating of sugar over the ceramic core can be confirmed by concanavalin A–induced aggregation method (determines the amount of sugar coated over core) or by anthrone method (determines the residual sugar unbound or residual sugar remaining after coating). Furthermore, the adsorption of sugar over the core can also be confirmed by measurement of zeta potential [16].

10.2. Glass transition temperature

DSC can be used to analyze the effect of carbohydrate on the drug loaded to aquasomes. DSC studies have been extensively used to study glass transition temperature of carbohydrates and proteins. The transition from glass to rubber state can be measured using a DSC analyzer as a change in temperature upon melting of glass [17].

11. Characterization of drug-loaded aquasomes

11.1. Drug payload

The drug loading can be determined by incubating the basic aquasome formulation (i.e., without drug) in a known concentration of the drug solution for 24 hours at 4°C. The supernatant is then separated by high-speed centrifugation for 1 hour at low temperature in a refrigerated centrifuge. The drug remaining in the supernatant liquid after loading can be estimated by any suitable method of analysis [18].

11.2. In vitro drug release studies

The in vitro release kinetics of the loaded drug is determined to study the release pattern of drug from the aquasomes by incubating a known quantity of drug-loaded aquasomes in a buffer of suitable pH at 37°C with continuous stirring. Samples are withdrawn periodically and centrifuged at high speed for certain lengths of time. Equal volumes of medium must be replaced after each withdrawal. The supernatants are then analyzed for the amount of drug released by any suitable method [19].

11.3. In-process stability studies

SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) can be performed to determine the stability and integrity of protein during the formulation of the aquasomes [20].

12. Applications of aquasomes

12.1. Insulin delivery

The studies had performed on in vivo performance of various aquasome formulations of insulin was evaluated using albino rats. Prolonged reduction of blood glucose was observed with all formulations except cellobiose-coated particles. The utility of nanocarriers for effective delivery of and The optimum controlled release of insulin was also achieved. [21].

12.2. Oral delivery of acid labile enzyme

The studies were made on the use of a nanosized ceramic core– based system for oral administration of the acid-labile enzyme serratiopeptidase. The enzyme was protected by further encapsulating the enzyme-loaded core into alginate gel. These aquasomes were found to be protecting the structural integrity of enzymes so as to obtain a better therapeutic effect [22].

12.3. As oxygen carrier

The oxygen-binding properties of the aquasomes were studied and compared to those of fresh blood and hemoglobin solution. The aquasome formulations neither induced hemolysis of the red blood cells nor altered the blood coagulation time [23].

12.4. Antigen delivery

The adjuvants generally used to enhance the immunity to antigens have a tendency either to alter the conformation of the antigen through surface adsorption or to shield the functional groups. In some studies the aquasomes were proposed to have superior surface immutability, in that they protect the conformation of protein structure and present it in such a way to immune cells that it triggers a better immunological response [24-26].

Reference

[1] A.R. Yadav, S.K. Mohite. (2020). Aquasomes as a self-assembling nanobiopharmaceutical carrier system for bio-active molecules. Research Journal of Topical and Cosmetic Sciences. 11(2): 89-94.

[2] J.S. Chaudhary. (2018). Aquasomes; A new approach for delivering therapeutics: an overview. Asian Journal of Pharmaceutics (AJP). 12(02).

[3] A.R. Yadav, S.K. Mohite. (2020). Aquasomes as a self assembling nanobiopharmaceutical carrier system for bio-active molecules. Research Journal of Topical and Cosmetic Sciences. 11(2): 89-94.

[4] S. Dhar, N. Kolishetti, S.J. Lippard, C. O. Farekhzad. (2011). Targeted delivery of a cisplatin prodrug for safer and more effective prostate cancer therapy in vivo. Proceedings of the National Academy of Sciences. 108: 1850–1855.

[5] B. Irving. (2007). Nanoparticle drug delivery systems. Pharmaceutical Biotechnology. 24: 58–62.

[6] L.H. Reddy, R.S.R. Murthy. (2004). Pharmacokinetics and biodistribution studies of doxorubicin loaded poly (butyl cyanoacrylate) nanoparticles synthesized by two different techniques. Biomedical Papers of Medical Faculty of University Palacky, Olomouc, Czech Republic. 161–166.

[7] B. Devarakonda, R.A. Hill, W. Liebenberg, et al. (2005). Comparison of the aqueous solubilization of practically insoluble niclosamide by polyamidoamine (PAMAM) dendrimers and cyclodextrins. International Journal of Pharmacy. 304: 193–209.

[8] E. Merisko-Liversidge, G.G. Liversidge, E.R. Cooper. (2003). Nanosizing: a formulation approach for poorly-water-soluble compounds. European Journal of Pharmaceutical Sciences. 18: 113–120.

[9] Z. Luo, J. Jiang. (2012). Ph-sensitive drug loading/releasing in amphiphilic copolymer PAE–PEG: integrating molecular dynamics and dissipative particle dynamics simulations. Journal of Controlled Release. 162: 185–193.

[10] N. Kossovsky, A. Gelman, E.E. Sponsler, H.J. Hnatyszyn, S. Rajguru, M. Torres, et al., (1994). Surface-modified nanocrystalline ceramics for drug delivery applications. Biomaterials. 15: 1201–1207.

[11] R.S. Pandey, S. Sahu, M.S. Sudheesh, J. Madan, M. Kumar, V.K. Dixit. (2011). Carbohydrate modified ultrafine ceramic nanoparticles for allergen immunotherapy. International Immunopharmacology. 11: 925–931.

[12] J.R. Kanwar, G. Mahidhara, R.K. Kanwar. (2012). Novel alginate-enclosed chitosan-calciumphosphate-loaded iron-saturated bovine lactoferrin nano- carriers for oral delivery in colon cancer therapy. Nanomedicine. 7: 1521–1550.

[13] D. Singh, S. Singh, J. Sahu, S. Srivastava, M.R. Singh. (2014.) Ceramic nanoparticles: recompense, cellular uptake and toxicity concerns. Artifical Cells, Nanomedicine, and Biotechnology. 17: 1–9.

[14] K.K. Tan, G.H. Tan, B.S. Shamsul, et al., (2005). Bone graft substitute using hydroxyapatitescaffold seeded with tissue engineered autologous osteoprogenitor cells in spinal fusion: early result in a sheep model. Medicinal Journal of Malaysia. 60: 53–58.

[15] S.S. Kim, M.S. Park, O. Jeon, et al., (2006). Poly(lactide-co-glycolide)/hydroxyapatitecomposite scaffolds for bone tissue engineering. Biomaterials. 27: 1399–1409.

[16] R.K. Roeder, M.M. Sproul, C.H. Turner. (2003). Hydroxyapatite whiskers provide improved mechanical properties in reinforced polymer composites. Journal of Biomedical Materials Research. 67: 801–812.

[17] H. Zhang, B.W. Darvell. (2010). Synthesis and characterization of hydroxyapatite whiskers by hydrothermal homogeneous precipitation using acetamide. Acta Biomaterials. 63216–3222.

[18] R. Peeriga, K.P. Adarapu, A. Kurama, N. Mohammed, L.R. Atmakuri, D. Kumar. (2022). Insilico Investigation on Phytoconstituents in Pamburus missionis S. for Antioxidant Activity. Pharmacognosy Research. 14(3): 246-250.

[19] R. Peeriga, C.K. Bonnth. (2016). Pharmacognostical Investigation and Preliminary Phytochemical Screening of Leaves of Myxopyrum Smilacifolium B. Pharmacognosy Journal. 8(2): 159-164.

[20] K.B. Chandrasekhar, R. Peeriga. (2017). Evaluation of the antiarthritic activity of ethanolic leaf extract of Myxopyrum smilacifolium Blume. International Journal of Research in Medical Sciences. 3(10): 1-12.

[21] R. Peeriga, K.B. Chandrasekhar. (2017). Antiarthritic activity of leaf extracts of Pamburus missionis Swingle. International Journal of Pharmaeuatical Sciences and Research. 8(2): 1-5.

[22] P. Raveesha, B.P. Kumar, G.E. Raju, R. Narayanan, J. Mohammed, Y.L. Chakradhar. (2023). Findings of Steroids, Coumarins and Saponins in Ipomea sagittifolia (Burn. f.). Leaf Extract by HPTLC Fingerprints. International Journal of Chemical and Biochemical Sciences. 24(4): 132-140.

[23] P. Raveesha, Afsar Shaik, S. A. Mohamed Shiek Arabath, Ch. Madhavi Latha, Srinivasa M. Narayana. (2023). Assessment of Steroids in ethanolic stem extract of Morning glory by High Performance Thin Layer Chromatography.

International Journal of Chemical and Biochemical Sciences. 23(3): 361-366.

[24] . Raveesha, S. A. Mohamed Shiek Arabath, A. Narayana Rao, Y. Ankireddy, KVB. Ganesh. (2023). Exploring the Coumarins from Stem Extract of Ipomea Sagittiofolia Burm. f. by High-Performance Thin Layer Chromatography. International Journal of Chemical and Biochemical Sciences. 23(3): 268-273.

[25] R. Peeriga, A. Shaik, M. Gude, T. Sonti, M. Cheboina, B.R. Penumala, G. KVB. (2023). Analyzing Phenols and Triterpenoids in Dechaschistia Crotonifolia Leaves Using HPTLC Fingerprinting. Tuijin Jishu/Journal of Propulsion Technology. 44(3): 605-612.

[26] R. Peeriga, A. Keerthi Priyanka, K. Akhila, Md. Naseemunnisa, A. Lakshmana Rao, Deepak Kumar. (2022). Insilico Investigation on Phytoconstituents in Pamburus missionis S. for Antioxidant Activity. Pharmacognosy Research,.14(3), 246-250.

[27] R. Peeriga, A. Shaik, P. Kolli, A. Veeroji, A. Kandula, P. Varma, M. Maragani, J. Chintalapudi, S.A. Yenduri, A. Kodali, P. Gunturu. (2022). Computational Study of Phytoconstituents in Myxopyrum Smilacifolium Blume against Inflammatory Mediator TNF-α. Journal of Drug & Alcohol Research. 11 (12): 1-5.