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EXTRACTION OF EGGSHELL MEMBRANE AND ITS APPLICATION AS A NATURAL DIALYSIS MEMBRANE FOR *EX-VIVO* DRUG PERMEATION STUDIES

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Abstract: Eggshell membrane (ESM) is a natural, protein-rich biomaterial that has gained increasing attention in pharmaceutical research due to its biocompatibility, selective permeability, and adsorption capabilities **[1–5]**. ESM serves as a versatile and cost-effective ex vivo membrane system, offering several advantages over conventional synthetic dialysis membranes **[14,15]**. Its unique fibrous architecture and biochemical composition allow realistic assessment of drug permeation, formulation–protein interactions, and controlled-release behavior. These properties make ESM an emerging platform for developing natural biomaterial-based wound dressings, topical delivery systems and therapeutic matrices.

This research paper aim on the recent advancements in ESM separation techniques and applications in the Pharmaceutical Industry for improving the separation, dissolution, and efficient development and utilization of avian eggshell membranes.

Figur: 02 Reference:15 Table:01
Keywords: Chicken eggs, eggshell membrane, protein, disulfide bond, dissolution.

1. Introduction:

Efficient separation of the eggshell membrane is crucial for its pharmaceutical application. Several techniques have been developed for isolating ESM from the mineralized shell layer, including mechanical peeling**,** acid–base decalcification**,** enzymatic digestion e.g., with papain or EDTA-assisted proteases, alkaline treatment**,** high-pressure steam processing, and physical separation via ultrasonic or microwave-assisted methods. Each method varies in cost, purity, preservation of membrane structure, and suitability for downstream applications. Mechanical separation preserves structural integrity but is labor-intensive; chemical decalcification is efficient yet may denature proteins; enzymatic methods yield high-quality membranes but are costly; and emerging technologies aim to balance efficiency with functional preservation.

The method described here treats ESM as the membrane separating donor (formulation) and receptor (receiver fluid) compartments and uses standard diffusion apparatus adapted for a flat membrane or tubular dialysis setup. Its unique combination of biocompatibility, structural integrity, and bioactive components makes it a sustainable and versatile raw material for future pharmaceutical innovations. Eggshell membranes (ESM) are increasingly recognized as a valuable biomaterial in the pharmaceutical sciences owing to their unique structural and biochemical characteristics. Composed primarily of fibrous proteins, including collagen, keratin, and glycoproteins, ESM exhibits excellent adsorption capacity**,** selective permeability, and biocompatibility**,** making it a suitable natural barrier for ex vivo studies. When utilized as an ex vivo dialysis membrane**,** ESM provides a biologically relevant platform for evaluating various formulation and drug-delivery

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parameters that are difficult to assess using synthetic membranes alone.

First, ESM allows the assessment of drug permeation and release through a protein-rich, semi-permeable barrier that closely mimics natural tissue environments. This helps in determining the diffusion behavior, release kinetics, and permeation profiles of topical, transdermal, and wound-care formulations. Second, ESM enables the investigation of interaction mechanisms between formulation components and a natural protein matrix**.** Components such as polymers, surfactants, penetration enhancers, and bioactive agents may bind, adsorb, or interact chemically with ESM proteins, providing insights into compatibility and potential biological responses.

Additionally, the use of ESM in ex vivo studies supports the development of ESM-based wound dressings and topical delivery platforms**.** Its inherent biocompatibility, moisture retention ability, and mild antimicrobial properties make it a candidate material for wound-healing applications. By evaluating a drug formulation using ESM, researchers can determine not only permeation characteristics but also the suitability of ESM as a matrix for controlled release or therapeutic agent incorporation.

2.Physical Structure and Chemical Composition of Eggshell Membranes:

The eggshell membrane is a fibrous layer that surrounds the egg white and extends into the papillae of the eggshell. Structurally, it is divided from the inside outward into three parts: the **limiting membrane (LM)**, the **inner eggshell membrane (ISM)**, and the **outer eggshell membrane (OSM)**. Together, these layers have an overall thickness of approximately **70 µm**. Adjacent to the outer membrane lies the **mineralized eggshell layer**, which is composed of papillae, fenestrae, and vertically oriented crystal structures. Both the inner and outer ESM are made up of layers of membrane fibers measuring about **25 µm** in length.The typical pore size of the eggshell membrane ranges from **1 to 10 µm**.Some studies report **smaller micropores (<1 µm)** located within denser fibrous regions, and **larger pores (up to 15 µm)** in loosely interwoven areas **[5,13]**.Figure 1 presents an artistic illustration of the eggshell, highlighting both the inner and outer layers of the ESM in the lower section **[1,5]**. The image clearly shows that the diameter of the membrane fibers increases progressively from the inner region toward the outer region. Eggshell membrane (ESM) is a natural biopolymer primarily composed of structural and functional proteins, accounting for nearly 90% of its composition. It contains a diverse range of over 470 protein species, including collagen, keratin, elastin, and antimicrobial glycoproteins. The membrane also comprises small amounts of lipids (2–3%), carbohydrates (1–2%), and trace minerals such as calcium, magnesium, phosphorus, and sulfur. These biochemical components contribute to ESM's unique properties, including high tensile strength, selective permeability, biodegradability, and biological activity, making it a promising material for pharmaceutical, biomedical, and environmental applications.

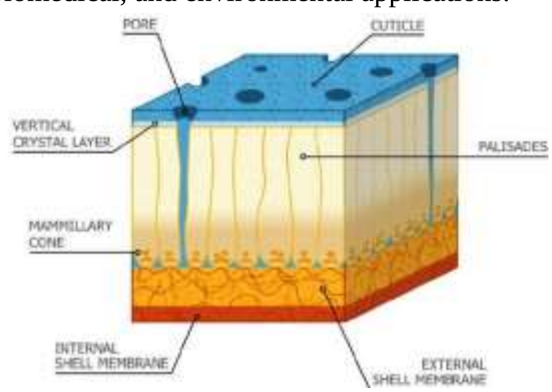

Figure:1 artistic rendition of cross section view of eggshell

3. Progress in Eggshell Membrane Separation Technology

Because the outer eggshell membrane (ESM) is firmly embedded within the papillae of the mineralized shell layer, effective separation of the membrane from the calcified matrix is a necessary step prior to any dissolution or extraction processes . Various separation methods have been developed to achieve this, and the commonly used techniques are summarized in **Table 1**. Broadly, eggshell membrane separation technologies can be classified into **physical**, **chemical**, and **enzymatic** approaches **[7–12]**.

TABLE 1 Typical shell membrane separation technology statistics.

Type	Separate characteristics	Membrane recovery rate	References
Physical method	By crushing, ultrasonic irradiation treatment and sieve separation, the difference in specific gravity between eggshell membrane and eggshell is used for recovery.	85–95%	**[7–9]**
	Using pressurisation and heating, the conditions are above 100°C and 1.2 Mpa.	High separation efficiency and high egg film purity	
	Cyclone airflow sorting devices.	94.00%	
	Method and apparatus for separating egg shell and eggshell membrane.	High separation efficiency and high egg film purity	
Chemical method	Hydrochloric acid volume 20 mL, reaction time 19 min, hydrochloric acid concentration 6.5 mol/L.	89.21%	**[10,11]**
	Separation time was 36.25 min, separation temperature of 48–96°C, hydrochloric acid concentration of 3.68 mol/L.	96.52%	
	3 times excess CH_3COOH solution.	93.00%	
Enzymatic method	Alkaline protease and Papain.	98.00%	
	50 μg/mL Proteinase K treatment for 48 h.	Unclean removal of the outer eggshell membrane	
	0.20 g/100 mL alkaline protease treatment for 2 h.	Optimizing egg film properties	

A.Physical Method

Physical separation of the eggshell membrane mainly relies on the differences in mechanical strength and specific gravity between the shell and the membrane. In this approach, the eggshells are first mechanically crushed and then mixed with water, allowing the shell fragments and membrane pieces to naturally stratify and separate. One commonly reported technique uses variations in specific gravity to isolate the membrane through a combination of crushing, ultrasonic treatment, and sieving, achieving a recovery efficiency of 85–95%. In another study, a cyclone airflow cleaning system was developed, which resulted in a membrane recovery rate of over 94% and a cleaning efficiency exceeding 96% under optimized conditions . Additionally, dissolved air flotation (DAF) technology—based on differences in water solubility at varying pressures—was applied to ESM separation. This device was capable of recovering approximately 96% of the membrane material. All these mechanical separation processes use water as the primary medium, which helps reduce operational costs, conserve resources, and minimize calcium loss from the shell. However, subsequent acid or alkaline treatments are often required to further enhance membrane purity and recovery efficienc **[7–9]**.

B.Chemical Method

Chemical separation of eggshell membranes works by targeting the **fibrous connections** between the outer membrane and the mineralized papillae of the shell. When chemical reagents act on these bonding fibers, the membrane detaches from the shell surface. In one study, a roller mill was used followed by sieving through a 2.5 mm steel mesh, resulting in **79.0% membrane separation** when combined with acid treatment. Another investigation employed **hydrochloric acid** for chemical detachment and used **response surface methodology** to optimize process parameters. The regression model predicted a maximum membrane recovery of **97.81%** at an HCl concentration of **3.68 mol/L**. However, the actual experimental recovery was **96.52%**, showing a relative error of **1.29%**, indicating that further refinement of the process is possible.Although acid or alkali treatments typically yield **high recovery rates**, they also lead to dissolution of the eggshell and may compromise the **biological activity** of the membrane **[10,11].**

Figure:2 separation of eggshell membranes by using hydrochloric acid

C. Enzymatic Method

Among all separation techniques, enzymatic treatment provides the highest recovery rate of eggshell membrane (ESM). This method relies on specific enzymes that hydrolyze the peptide bonds in the fibrous network linking the ESM to the outer membrane and the mineralized layer of the shell. Researchers have utilized enzymes such as alkaline protease and papain to detach and isolate the membrane, and their performance was compared with physical and chemical approaches. Findings indicated that enzymatic treatment yielded the maximum recovery of ESM—up to *98.9%*—when different enzyme formulations were applied. However, preparing the enzyme solution correctly and controlling operational conditions are crucial for achieving optimal results**[12]**.

4. Materials and Methods

In this research, fresh chicken eggshells were collected from a local hennery. Hydrochloric acid, Deionized water was used for aqueous solution preparation**[14,15]**. Prior to use, all solutions prepared were filtered through a 0.22 μm membrane. To prepare a dialysis membrane from an eggshell membrane, the eggshell is first thoroughly cleaned by removing any dirt and adhering egg white. After cleaning, the decalcification step is carried out by immersing the shells in an acid solution such as hydrochloric acid (HCl). These chemicals dissolve the calcium carbonate layer of the shell, gradually releasing the inner eggshell membrane**[3–5]**. Once the mineral shell is completely dissolved, the freed membrane is carefully separated from the remaining shell fragments using tweezers. The extracted membrane is then rinsed several times with distilled water to remove residual acid or egg components, yielding a clean and intact eggshell membrane suitable for use as a natural dialysis membrane**[10,11]**.

5. Conclusion

The eggshell membrane (ESM) represents a versatile, sustainable, and biologically relevant biomaterial with significant potential in pharmaceutical research and formulation development. Advances in ESM separation technologies—including physical, chemical, and enzymatic methods—have substantially improved the efficiency, purity, and structural preservation of the extracted membrane. Among these techniques, enzymatic treatment consistently provides the highest recovery rate and optimal membrane integrity, while physical and chemical methods offer practical alternatives depending on cost, scalability, and application requirements.

Furthermore, the application of ESM as a natural dialysis membrane in ex vivo permeation studies demonstrates its unique functional advantages over conventional synthetic membranes. Its protein-rich composition, fibrous microstructure, and semi-permeable nature allow for realistic evaluation of drug permeability, release kinetics, and formulation–protein interactions. These characteristics make ESM a promising platform for the development of novel drug-delivery systems, wound dressings, topical formulations, and controlled-release matrices.

Overall, the integration of efficient separation technologies with innovative pharmaceutical applications positions ESM as an emerging biomaterial of high scientific and industrial relevance. Continued research and optimization will further enhance its utility, supporting the development of eco-friendly, cost-effective, and biocompatible solutions in modern pharmaceutical science.

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