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CELLULOSE DERIVATIVES BASED MEMBRANES FOR BIOMEDICAL APPLICATIONS AND IMMOBILISATION OF ENZYMES

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ABSTRACT:
Polysaccharide is the correct term for cellulose. Because of the convergence of polymer science and pharmaceutical research, polymer is now often used in the creation of new kinds of drug delivery systems. Controlled or prolonged medication delivery is a primary goal of polymeric delivery systems. The planet's largest polysaccharide is cellulose. Wood and plant cells, certain bacterial and algal cells, and the only known cellulose-containing mammals, tunicates, are also potential sources. In this study, We provide three separate approaches to making membranes from cellulosic derivatives. The polymer solutions were made by dissolving cellulose acetate and nitrocellulose in DMF at a concentration of 10%. Aldrich, Dorset (UK) was sourced for the cellulose propionate and acetate-butyrate, while Sigma, Dorset (UK), was sourced for the cellulose acetate (UK). Conjugate support-enzyme activity was highest when it was applied to a cellulose acetate membrane that had been evaporated to prepare it. More crystalline and less optimal for enzyme immobilisation were the membranes produced by the immersion approach.

KEYWORDS: Cellulose, Derivative, Membrane, Immobilization, Enzymes.

INTRODUCTION

In 1838, French scientist Anselme Payen was examining various kinds of wood when he came upon a material that he knew couldn't be starch (a kind of glucose or sugar that can be stored), but which could still be broken down into its fundamental units of glucose. Since he isolated it from plant cells, he called the novel molecule cellulose, after the plant cells from which it originated. Payen extracted it from plants and figured out its molecular

structure. In 1870, the Hyatt Manufacturing Company transformed cellulose into the first economically viable thermoplastic polymer. Back in 1920, Hermann Staudinger discovered the polymer structure of cellulose. Kobayashi and Shoda first chemically synthesised the compound in 1992. Cellulose, put another way, is the natural polymer that is most pervasive worldwide. -1,4-glycosidic connections bind glucose residues together to form this structure. Both natural fibers derived from cellulosic feedstock and synthetic cellulose find widespread use in a wide range of fields, including textiles, food, and construction. Cellulose is an excellent biopolymer because it can be used in a wide variety of applications, is readily biodegradable, has a high water-retention capacity, can be renewed, and can be modified. Compared to more traditional synthetic materials, cellulosic ones are inexpensive and often regarded as environmentally preferable. Synthetic cellulose polymers for use in biomaterials may be made in two ways: chemically and biologically. Products based on cellulose may be manufactured using feedstock derived from a wide range of organisms, including plants, animals, and microorganisms. Considering the volatile fuel costs and the ongoing contribution of fossil fuel consumption to geopolitical instability and climate change, the use of cellulosic feedstocks to create biofuels has received increasing attention during the last decade. Studies of cellulose's chemical and physical characteristics have also been conducted, with an eye on creating biomaterials from the substance. Mechanical shearing or controlled acid hydrolysis of cellulose fibers results in the formation of nanoscale elongated fibrillary structures or complete rod-like crystalline particles. This is helpful because it paves the way for cellulose's macromolecular structure to be manipulated with nanoscale tunability for a broad variety of cutting-edge applications.

Cellulose is the most abundant organic molecule and polysaccharide on Earth; it is an unbranched, natural polymer made up of repeated glucose units $(C_6H_{10}O_5)_n$. It has been discovered that microfibrils of this biodegradable polymer are present in the cell membranes of the tunicate's epidermis, as well as in the cell walls of wood and plants. It can also be made by bacteria, albeit their form is a nanofiber network rather than a solid sheet. The nanoscale to macroscopic range of cellulosic materials' hierarchical structural design is exemplified by their fibril aggregates, fibrils, nanocrystallite, and nanoscale disordered domains (Fig. 1a, b). Bundles/aggregates of ultrafine fibrils form cellulose's complex multi-level structure. A number of cellulose chains compose each ultrafine fibril (Fig. 1a). Each fibril has a cross-sectional size between 2 and 20 nm, and is made up of alternating large ordered (crystalline) domains and tiny disordered (amorphous) domains (Fig. 1b). Although strong 1→4 glycosidic connections connect individual cellulose chain units, a single cellulose chain traverses several crystalline and disordered domains. Inside the fibril's crystalline region, the cellulose chains are impressively ordered. Due to its favorable physical and mechanical qualities, cellulose and its derivatives have garnered a lot of interest for use in the biomedical industry as biocompatible polymers. With its inherent hierarchical structure, cellulose creates functionality, flexibility, and high specific strength.

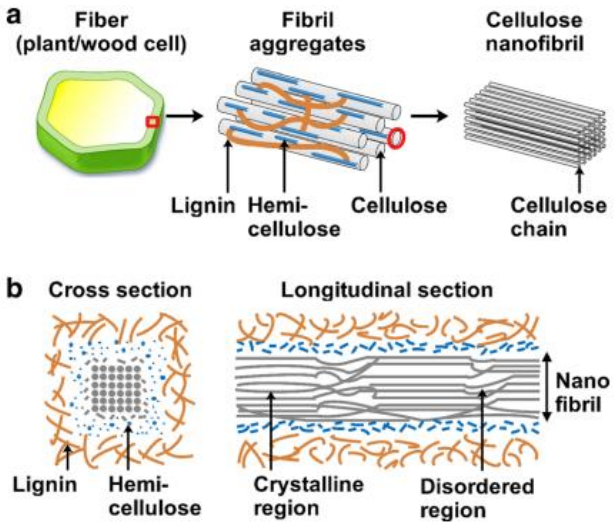

Figure 1: Microstructure of a cellulose fiber, shown schematically a. fibers, nanofibrils, and fibril aggregates found in plants and wood that are bound to lignin and hemicellulose and include cellulose chains; b. A cellulose nanofibril, embedded in a lignin and hemicellulose matrix, with its crystalline and disordered portions visible in cross section and longitudinal section

LITERATURE REVIEW

Nada, et al (2016) Several pharmaceutical and biomedical applications have made use of polymers, both natural and synthetic, for purposes such as medication targeting, imaging, drug delivery, prostheses, and tissue engineering scaffolds. Synthetic polymers have the advantage over natural polymers in a number of these uses because of their repeatability in terms of molecular weight, degradation, and mechanical properties. From a biological perspective, however, synthetic polymers typically fall short of the mark when it comes to bioactivity and biocompatibility, which may lead to unwanted side effects. In contrast, natural polymers are widely available and structurally match those found in biological extracellular matrix. Hence, the body easily accepts natural polymers, and these materials are both bioactive and biocompatible. Also, the chemical and biological characteristics of these polymers and their common derivatives are reviewed. The original form of polysaccharides may lack the necessary characteristics for a certain biological application. Consequently, This chapter also covers the polysaccharide derivatives and their mixture with other polymers for diverse biological uses.

Oglah, Mahmood & Mustafa, Yasser & Bashir, Moath & Jasim, Mahmood (2020) Curcumin, a phytochemical extracted from the rhizome of the turmeric plant, is a vivid yellow pigment. Curcumin, a compound originally extracted from this plant, has been receiving a lot of interest from medicinal experts since it was discovered. Antimicrobial, antiproliferative, antioxidant, anti-inflammatory, antidiabetic, and neuroprotective are only few of the biological properties shown by curcumin. With so many potential applications, curcumin is a viable starting ingredient for the creation of novel derivatives that might be used to treat diseases as diverse as cancer, diabetes, and Alzheimer's. This study focused on recent animal and clinical investigations that have indicated possible pharmacological activities of curcumin and its derivatives.

Ruwizhi, Ngonie & Aderibigbe, Blessing (2020) Several different diseases and conditions, including cancer, bacterial infections, diabetes, and neurological problems, have been shown to respond to cinnamic acid derivatives. The cinnamon bark is processed to extract the cinnamic acid. It is feasible to change the aforementioned functionalities with a wide range of molecules, structure-wise, it's superior to other bioactive compounds since it has a benzene ring, an alkene double bond, and an acrylic acid functional group. It has been found that the kind of substituents introduced into cinnamic acid has a significant impact on the biological efficiency of produced cinnamic acid derivatives. Some of the derivatives have shown more in vitro efficacy than the gold standard medications for treating chronic or infectious disorders, making them very interesting potential therapeutic agents. In this article, we'll go through the most up-to-date findings about cinnamic acid derivatives and their practical applications in medicine.

Trombino, S. & Cassan, R. (2015) A growing trend in the biomedical and pharmaceutical sectors in recent years has been the use of natural, biodegradable materials. The most abundant source of sustainable polymers is cellulose, is garnering a lot of attention as one of the natural polymers. It is a linear polysaccharide composed of several monosaccharide units and has many desirable features. They include hydrophilicity, biocompatibility, biodegradability, stereoregularity, and multichirality. Using its main hydroxylic groups, it may be functionalized with bioactive compounds, creating a matrix that is passive to the integrated chemicals. In addition to being cheap, safe, and biocompatible, its derivatives like esters and ethers also have a large customer base. Coatings, films, membranes, controlled-release systems, and medicines all make use of them. The most up-to-date advancements and directions in cellulose-based materials will be reviewed in this chapter. More specifically, we will examine the fabrication, characteristics, and uses of these polymers in the pharmaceutical and medical fields.

Gopi, et al (2019) Cellulose is a biocompatible, biodegradable, low-toxic organic polymer with good physical, chemical, and mechanical characteristics, making it a popular choice among researchers developing new biomedical applications. General considerations including the origins, structures, and characteristics of cellulose, nanocellulose, and other cellulose nanocomposites were the focus of this study. We surveyed the literature on the use of cellulose nanoobjects in several biological settings, including drug administration, targeted drug delivery, and wound healing. Cellulose nanoparticles used for microencapsulation and 3D printing have also been discussed. Finally, we evaluated the current status and potential uses of cellulose nanoparticles, also known as nanocellulose, in the field of biomedical engineering.

RESEARCH METHODOLOGY

The polymer solutions were obtained by dissolving cellulose acetate and nitrocellulose in DMF at a concentration of 10%. To create the membranes, a polymer solution of 5 mL is deposited onto a glass substrate and sliced to a thickness of 100 m using a knife, allowing for the deposition of films with adjustable spacing. To create a membrane, a polymer film is deposited onto a glass substrate and then immersed in a coagulation bath of deionized water, causing the polymer film to undergo phase inversion. After being precipitated, they

were given an ethanol rinse and then kept in cold deionized water to prevent any additional surface degradation or microbiological growth. The permeation studies were performed in a Sartorius apparatus using 500 mL of deionized water or ethanol at a vacuum of 10(-1) bar. To evaluate protein retention, a 500 mL volume of deionized water was used with a 10-5 M concentration of synthetic bovine serum albumin and haemoglobin, and the solution was continuously recycled for 120 minutes. The solution was analyzed using UV-Viz spectroscopy on a Camspec spectrophotometer, and samples were obtained at 10, 20, and 30 minutes. Samples were gold-coated before being scanned under an FEI microscope.

Preparation of the membranes

Glass plates were coated with tetrahydrofuran and cellulose derivative solutions using casting knives. When the solvent had evaporated at room temperature, further membranes were produced from the same solution by precipitating it in a water bath. The temperature of the water was either 5 degrees or 25 degrees Celsius.

Immobilization of Enzymes

In the dark, membrane sections were given 2 hours to react with 10 cm^3 of sodium periodate (0.5 M). After 18 hours, they were treated with 1% (w=w) hexamethylene diamine solution in a volume of 10 cm^3. The next procedure was soaking the tissue for two hours in a 5% (v/v) glutaraldehyde solution in a 0.05 M phosphate buffer at pH 7.5 in a volume of 10 cm^3. Between each phase, the membranes were given a thorough cleaning with distilled water.

Determination of water vapour sorption

When consistent weight was reached, a saturated solution of copper sulphate (98% R.H.) was used to cure the manufactured membranes at 25 degrees Celsius. The polymer was then weighed repeatedly over a period of ten minutes, one each minute. By extrapolating to zero time, a graphical representation of the original sorption capacity might be generated. Each sample was dried at 100 degrees Celsius under vacuum until its mass remained consistent. Calculating water use as a percentage is as simple as,

$\%\ \text{Sorption} = (M_i - M_f)/Mf \times 100$

Where M_i is the initial mass at zero time and M_f is the final dry mass.

DATA ANALYSIS

Differences in the membranes generated from the two polymers are readily apparent under scanning electron microscopy (Fig. 2). As compared to cellulose acetate membranes, which have a mean pore width of about 3–5 μm in their porous areas, nitrocellulose membranes are more compact. Polymer molecules interacting with non-solvent (water) explains this phenomenon; cellulose acetate makes it easier for non-solvent molecules to move around, leading to increased pore sizes.

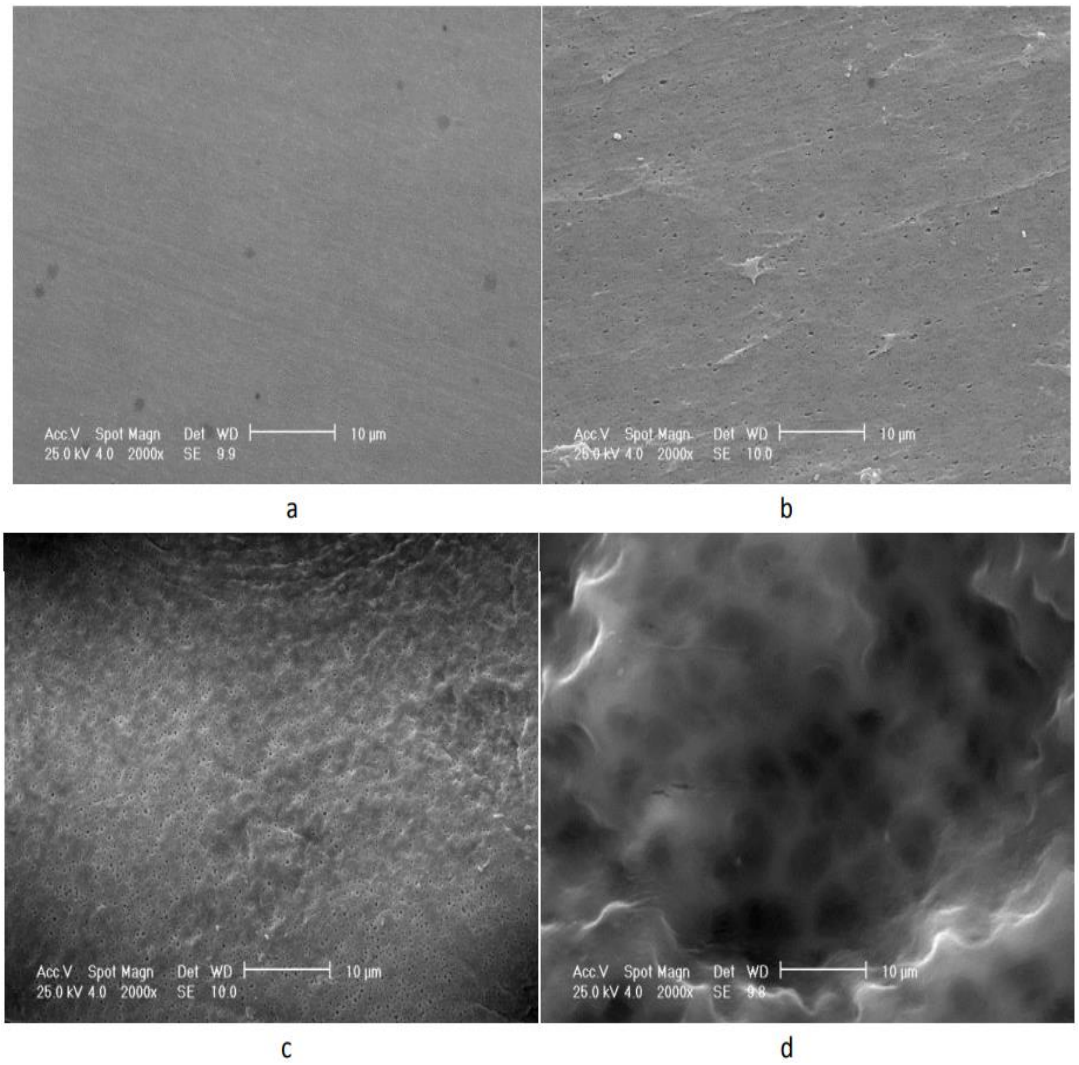

Figure 2: Scanning electron microscopy of the nitrocellulose membranes

The results for water and ethanol fluxes obtained corroborate this finding; the cellulose acetate membrane recorded the greatest fluxes. Water flows more easily through the polymer than ethanol does because of the polymer's hydrophilic nature. Slowing flow rates over time may be attributed to the membrane's structural stability, which occurs when the water acts onto the polymer layers, compacting and concentrating them. After membrane stabilization, The fluxes of water (10150 L/m2 h) and ethanol (2521 L/m2 h) via cellulose acetate membranes were higher than those of water (7921 L/m2 h) and ethanol (1840 L/m2 h) through nitrocellulose membranes, respectively.

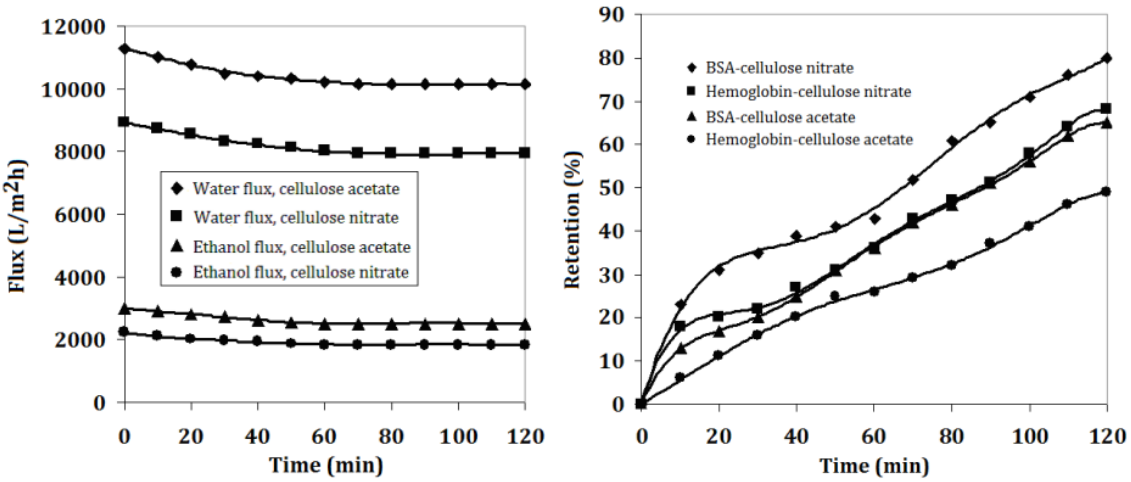

Figure 3: Water and ethanol flow rates for synthesized membranes

Nitrocellulose membranes performed better than cellulose acetate membranes when it came to retaining proteins from an aqueous solution. This occurs due to non-covalent interactions between nitro groups in the polymer and amine functional groups in the protein structure. The size of the two proteins accounts for the retention differential between BSA and hemoglobin. This research lends credence to the usage of such membranes in clinical settings, such as hemodialysis, where their inherent biocompatibility as polymers derived from cellulose eliminates the need for any further processing. Moreover, the membranes may be applied to other implantable materials for osseointegration procedures, such as those utilized in dentistry and orthopedics. The breakdown of cellulose derivatives releases glucose molecules that do not interfere with biological functions, which is a benefit.

Membrane characterization

All of the membranes produced by evaporation were characterised using scanning electron microscopy. Cellulose acetate (CA), cellulose propionate (CP), or cellulose acetate-butyrate (CAB) may be used to create symmetric and almost impermeable membranes (CAB). Cellulose acetate membranes are made by submerging them in water, which causes the layers to separate and create a non-symmetric membrane with a thick top layer and an open structured sub-layer. If the coagulation bath temperature is increased from 5 to 25oC, the solvent will be quickly replaced by the non-solvent, resulting in a more porous surface layer.

Involvement of Water in Membrane Interaction

Table 1 displays the results of measuring the diffusion coefficient between water and cellulose acetate. This number is about three times larger than the value measured for cellulose propionate, and is on the same order of magnitude as the results reported by other authors. This result, together with the values found for the water sorption capacity and contact angle, may be explained by the increased concentration of hydrophobic alkyl groups in the cellulose propionate.

Table 1: Membrane water interaction

Membrane	Water Sorption (%)	Contact Angle (°)	Diffusion Coefficient $\times 10^{10}$ (cm^2=s)
CA	13:6 ± 0:5	73 ± 3	163:0 ±5:4
CA-5	11:8 ± 0:9	63 ± 2	282:0 ± 1:0
CA-25	12:1 ± 0:2	55 ± 1	∞
CP	7:7 ± 0:1	78 ± 3	58:2 ±2:8
CAB	4:6 ± 0:4	79 ± 1	104:0 ± 4:4

Both the increasing number of alkyl groups and the presence of two types of substituent groups must be taken into account when thinking about cellulose acetate butyrate. The diffusion coefficient and the water sorption capacity are both expected to decrease as the number of alkyl groups increases. The contact angle value should likewise go up. However, cellulose acetate butyrate has a better diffusion coefficient than cellulose propionate. This is likely due to the presence of dual substituent groups that work together to expand the membrane's void space. Diffusional pops are a useful conceptual tool when talking about the movement of sub-molecular particles. As the temperature of the polymer near the permeate compound becomes high enough, a volume big enough to enable the permeate to migrate to a new site inside the polymer briefly opens, a phenomenon known as a "diffusional pop." With all that more space, molecules of the permeate may more easily diffuse through the polymer's pores. The CA-5 and CA-25 membranes have the highest diffusion coefficients and the lowest contact angles (better wetting). This is because these membranes have a less closed structure. The water infiltrated the CA-25 membrane so quickly that the diffusion cell could not accurately detect the diffusion coefficient value. Hence, in Table 1, we see the value ∞.

CONCLUSION

We can say that in the biomedical industry, where their use has direct implications in tissue engineering, wound healing, and medication delivery, interest in bio-based materials is growing. Phase inversion of a polymer solution in DMF was used to create membranes from cellulose derivatives in this study. One of the most common organic substances found in the world, cellulose may be obtained from a wide variety of different places. As its characteristics may be altered, cellulose is a great starting point for creating novel biomaterials. The produced membranes vary in their permeability to water and ethanol and their capacity to retain proteins, as well as in their surface shape. While cellulose acetate membranes allow for more water flux, nitrocellulose membranes retained proteins well, suggesting they are a viable option for hemodialysis and other separation operations. The natural origin of the polymer and the membrane's affinity for proteins suggest that

implant coverings for osseointegration procedures are a viable prospective use. Enzymes that are immobilized in membranes maintain their activity and stability based on properties including crystallinity, hydrophilicity, and permeability. To investigate what variables affect the behavior of immobilized enzyme systems, cellulose-derivative membranes may serve as a useful model.

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