MOLECULAR IMPRINTING OF PROGESTERONE

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Key Words:
Molecular Imprinting, Progesterone

ABSTRACT

Molecular imprinting involves designing synthetic polymers with customized cavities that are complementary in shape and functionality to a specific target molecule, enabling precise molecular recognition. In this study, progesterone—a naturally occurring steroid hormone essential for reproductive functions—was selected as the template to develop molecularly imprinted membranes (MIMs). Membranes were synthesized using acrylonitrile-based copolymers and functional monomers such as acrylamide, acrylic acid, and methacrylic acid via phase inversion in DMSO. Characterization was performed using FTIR, UV-Vis spectroscopy, SEM, and XRD to confirm polymer structure and membrane morphology. Binding studies revealed enhanced affinity and selectivity of the imprinted membranes toward progesterone compared to non-imprinted controls. The membranes demonstrated high reusability and selectivity even in the presence of structurally related analogues like testosterone and cholesterol. These results suggest that progesterone-imprinted membranes have strong potential for application in hormone separation and detection technologies.

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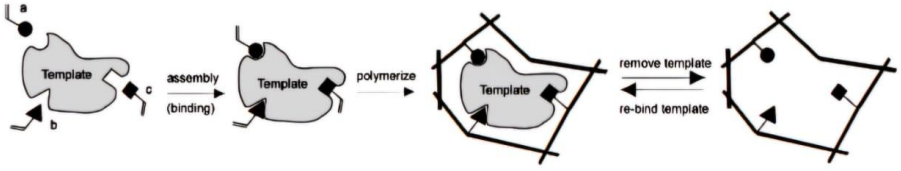
INTRODUCTION

Molecular imprinting is an adaptable approach for embedding selective recognition features within synthetic polymers, enhancing their ability to identify and bind target molecules. Over the past twenty years, molecularly imprinted polymers (MIPs) have undergone significant development and gained widespread attention due to their numerous advantages over natural receptors, enzymes, and antibodies. MIPs are known for their superior stability, cost-effectiveness, and ease of preparation. Among the most promising applications of molecularly imprinted polymers is the use of molecularly imprinted membranes (MIMs) for enantiomer discrimination in separation processes. This technique stands out because it allows for continuous separation, unlike traditional methods such as crystallization or chromatography. Additionally, molecular imprinted membranes are easily scalable and energy-efficient. Thanks to these benefits, imprinted membranes are now employed across various industries including water treatment, pharmaceutical purification, and food processing.

Polymeric network materials with the ability to recognize small molecules, particularly those exhibiting antibody-like recognition properties, are increasingly available. As a result, they are being explored as promising substitutes for biological receptors across diverse areas of analytical chemistry. An increasingly explored application is their role in the extraction and preconcentration of trace analytes from complex sample matrices. Molecularly imprinted polymers have already been applied for selective solid-phase extraction of a range of substances, including pharmaceutical drugs, drugs of abuse, and pollutants. However, before these imprinted materials can be used in any application, it is essential to remove the template molecules within the polymer matrix. The extent of template removal needed varies based on the particular

purpose for which the material is designed.

Molecularly imprinted polymers offer several distinct advantages, including low cost, durability, and long-term stability. These polymers have been applied for molecular recognition of both small molecules and macromolecules. The molecular imprinting technique is one of the simplest methods for creating synthetic macromolecules with molecular recognition capabilities. Imprinted polymers possess inherent porosity, making them ideal for a wide variety of applications. Molecular imprinting technology involves designing molecular interaction sites within materials that mimic the native binding properties of immune proteins and receptor sites. These imprinted polymers have a broad range of uses, including drug and biological derivative separation, chemical sensing, and catalysis. In contrast to living organisms, template-moulded polymers offer advantages such as lower cost, enhanced strength, stable under high thermal and pressure conditions, and superior chemical stability.



Schematic representation of imprinting process

The imprinting technique is straightforward. During polymerization, a molecular template is captured within the polymer scaffold, leaving behind molecular imprints within the cross-linked structure (Scheme I). The shape and complementary chemical features of the template molecule are preserved as cavities within the polymer. These cavities serve as binding sites for the identical template species or analogous molecules,

allowing them to adhere to the polymer matrix with precise binding affinity.

The development of membranes using polymeric imprints has introduced a novel strategy in separation techniques. Although membrane technology has seen broad application across various industries, the ability to incorporate specific recognition sites into synthetic membranes significantly enhances the transport of particular substances. Molecularly imprinted membranes are typically created through photo-co-polymerization and phase inversion techniques. Phase inversion can occur via two methods: wet phase inversion and dry phase inversion. These imprinted membranes exhibit high permeability, making them suitable for affinity filtration applications. The preparation of such membranes has been explored through immersion precipitation phase inversion, though this often results in relatively low membrane permeability. In contrast, heterogeneous membrane functionalization—achieved by grafting from the polymer membrane surface in the presence of a template—has proven to be a promising novel method. Controlling the parameters that influence the degree of functionalization is crucial for optimizing the performance of imprinted polymer composite membrane.

Progesterone is a key hormone in the human body and is classified as an endogenous steroid. This sex hormone plays a vital role in several essential processes, including the menstrual cycle, pregnancy, and embryogenesis. Additionally, it serves as a critical precursor to other endocrine steroids like sex hormones and stress-regulating corticosteroids. Beyond its reproductive role, progesterone acts as a neurosteroid influencing central nervous system function.

MATERIALS AND METHODS

1. General

1.1. Source of chemicals

High-purity reagents including acrylonitrile (AN), acrylic acid (AA), and dimethylsulphoxide (DMSO) were procured from Merck (Germany). ANand AA were purified by distillation under reduced pressure, while DMSO was distilled at 80 °C under reduced pressure (18 mm Hg). Methacrylic acid (MAA), obtained from Aldrich, was vacuum distilled to remove stabilizers. Acrylamide (AM) was purchased from SRL (India) and used without further purification. The radical initiator 2,2'-azobis(isobutyronitrile) (AIBN, Fluka, purity > 98%) was stored at low temperature until use. Progesterone (PGN), testosterone (TSN), and cholesterol were sourced from Sigma-Aldrich (Germany) and used as received.

1.2. Analysis and characterisation

a) Fourier-transform infrared (FT-IR) spectra were obtained using a Perkin-Elmer Spectrum 8400 FTIRspectrophotometer.

b) Ultraviolet-visible (UV-Vis) absorption spectra were recorded on a Shimadzu UV-Vis 2450 spectrophotometer.

c) Membrane morphology was examined via scanning electron microscopy (SEM) using a JEOL JSM-6390Amicroscope.

d) X-ray diffraction (XRD) patterns were recorded using a PANalytical X'Pert PRO diffractometer.

2. Synthesis of progesterone imprinted and non-imprinted membranes using DMSO as the solvent

For the preparation of progesterone imprinted membranes, with two

different ratios of acrylonitrile and the functional monomers acrylamide, acrylic acid and methacrylic acid were taken and dissolved in DMSO. AIBN was added as the initiator and maintained in a thermostatic bath at 50°C. Progesterone was then incorporated into the coagulated solution. Polymerization was carried out under a nitrogen atmosphere for 4 hours. Glass plates (70 × 100 × 4 mm) were used to cast membranes of uniform thickness of 250 μm. After casting, the membrane-forming solutions were immersed in a 2 L coagulation bath containing distilled water for 30 minutes at room temperature. Subsequently, the membranes were conditioned in fresh water. In the case of molecularly imprinted membranes, progesterone was isolated by methanol–acetic acid solution, and complete removal was verified spectrophotometrically at a maximum absorption wavelength (λ_max) of 242 nm. All membranes were kept in fresh water until use. Non-imprinted membranes were prepared using an identical procedure, omitting the addition of progesterone.

3. General method for investigating template binding

After template removal, the molecularly imprinted polymer (MIP) retains specific recognition sites that enable selective rebinding of molecules with comparable structural and functional characteristics. To assess its binding performance, the MIP was incubated with a solution containing the template molecule, and the binding efficiency was quantified using UV-Vis spectroscopic analysis.

a. Binding capacity

To evaluate the selective binding capability of the imprinted polymer, its performance was compared against that of a non-imprinted counterpart. Identical amounts of each polymer were introduced into solutions containing a known concentration of the template molecule. The mixtures were sealed and agitated for 1.5 hours. UV-Vis spectroscopy was

employed to determine the template concentrations before and after incubation, with absorbance monitored at the characteristic wavelength of the template (λmax = 242 nm for PGN). The binding specificity was assessed based on the difference in template uptake between the imprinted and non-imprinted polymers.

b. **Factors affecting specific binding**

i. Concentration of template solution

The extent of specific binding exhibited by the membrane is influenced by the concentration of the target molecule in solution. To investigate the recognition capabilities of the membrane toward the template compound, batch binding experiments were conducted. Precisely weighed amounts of both the imprinted and non-imprinted polymers were incubated with template solutions of varying concentrations under agitation for 1.5 hours. Following incubation, the polymer particles were separated by centrifugation, and the residual concentration of the template in solution was measured using UV-Vis spectroscopy. The amount of bound template was determined by calculating the difference in concentration before and after polymer exposure.

ii. Time course analysis

To determine the time required to achieve binding saturation, equal masses of the imprinted and non-imprinted polymers were incubated in a solution containing a known concentration of the template molecule. The progression of template binding was monitored at defined time intervals using UV-Visible spectroscopy.

c. Selectivity study

An equal volume of a solution containing both the template and its structural analogue was added to the template-desorbed polymer, and the

difference in the extent of binding was determined spectroscopically.

d. Re-binding

To find out the extent of reusability of the imprinted polymer, the rebounded polymer was collected and washed to remove the bounded template. The complete removal of the bounded template was determined spectroscopically. It is again allowed to in contact with the template solution for fixed time. The effect of re-binding was calculated spectrophotometrically.

RESULTS AND DISCUSSIONS

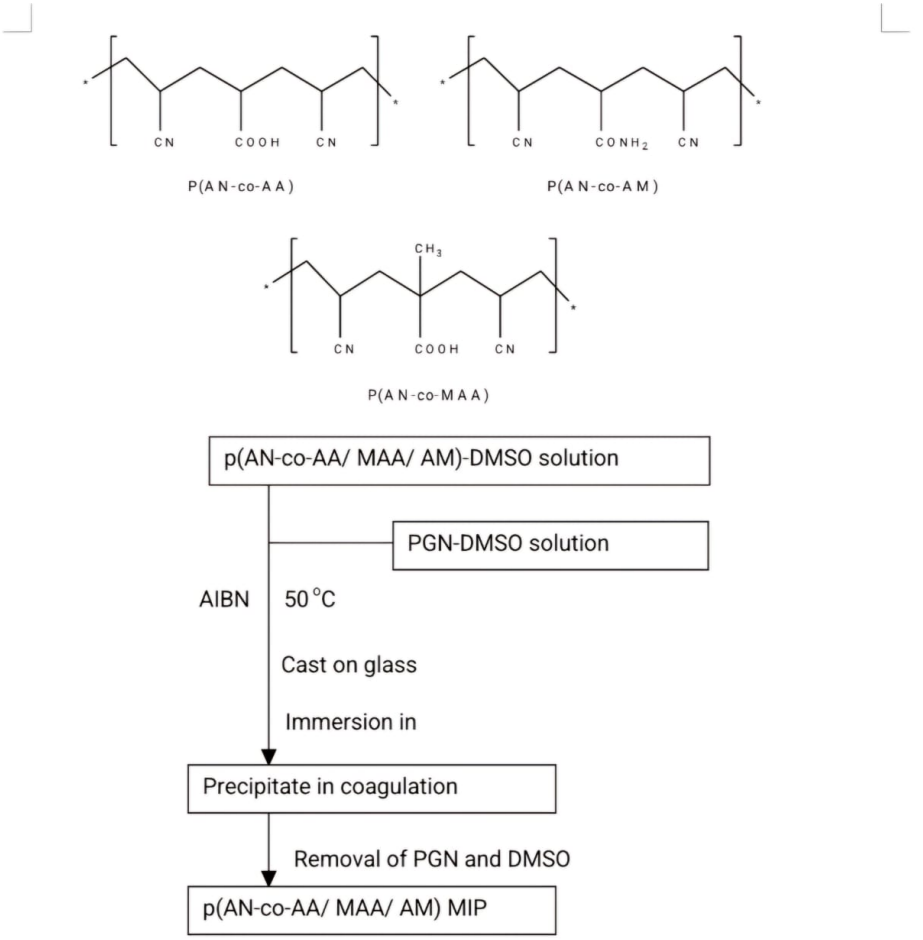
1. Molecular imprinting of progesterone

1.1. Synthesis of progesterone imprinted and non-imprinted membranes using DMSO as solvents.

Progesterone is a naturally occurring steroid hormone, biosynthesized from cholesterol through a series of enzymatic steps. While its primary site of production is the ovaries, it is also generated in the adrenal cortex, brain, testes, and placenta throughout gestation. In humans, both males and females produce progesterone, though significantly higher levels are observed in females. The hormone plays a vital role in physiological regulation and systemic circulation; however, elevated concentrations may be associated with carcinogenic potential. Due to the presence of functional groups capable of forming hydrogen bonds, progesterone can interact with polymers through amide and carboxyl moieties. The number and affinity of binding sites within imprinted membranes are influenced by the molar ratio of template to functional monomer. In this study, molecularly imprinted membranes were fabricated using a wet phase inversion method, employing acrylonitrile-based copolymers. Functional monomers such as acrylamide, methacrylic acid, and acrylic acid were

incorporated to establish selective recognition sites within the polymer matrix.

Molecularly imprinted membranes were prepared using a phase inversion approach. Due to the good solubility of progesterone (PGN) in dimethyl sulfoxide (DMSO), a polymer solution was formulated by dissolving acrylonitrile and selected co-monomers in DMSO at two different monomer ratios (95:5 and 97.5:2.5, respectively). The radical initiator AIBN was added to initiate polymerization. Progesterone was incorporated into the casting solution prior to membrane formation. The mixture was then cast onto a clean glass surface, forming a uniform film with a thickness of 250 μm. After casting, the film was immersed in a 2-liter water bath at room temperature for 30 minutes to induce phase separation. The resulting membranes were rinsed in fresh water to complete the coagulation process. To remove the template molecule, the membranes were thoroughly washed with a methanol–acetic acid solution. Finally, the membranes were housed in fresh water until further use. Control (non-imprinted) membranes were fabricated in parallel, following the same procedure but without the addition of progesterone. The synthesis and processing steps involved in the molecular imprinting technology is given in Scheme below.



Structures of co-monomers and general scheme of the synthesis of progesterone imprinted membranes

1.2. Characterisation of progesterone imprinted and non-imprinted membranes

Chemical characterisation of the PGN imprinted and non-imprinted membranes gives a clear picture about the interactions involved in the membranes. Fourier Transform Infrared (FT-IR) and UV-Vis

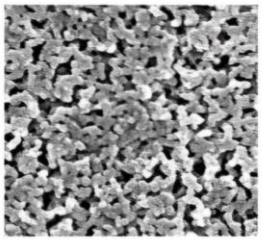
spectroscopy were employed to investigate the molecular interactions and recognition mechanism involved in the imprinting process. Additionally, SEM and XRD were used to examine the surface morphology and crystalline structure of the membrane matrix.

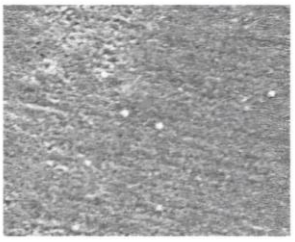
i. Fourier transform infrared spectroscopy

To confirm the incorporation of the co-monomer into the synthesized polymers, FT-IR spectroscopy was performed. A peak at 1736 cm^{-1} was detected, corresponding to the C=O stretching vibration of the carboxyl groups in acrylic and methacrylic monomers. The acrylonitrile-acrylamide polymer displayed a comparable carbonyl stretching band at 1681 cm^{-1}, characteristic of amide functionalities. Additionally, the acrylamide-based polymer exhibited distinct NH stretching bands at 3368 and 3469 cm^{-1}.

ii. Scanning electron microscope

Figure 1 illustrates scanning electron micrographs of both progesterone-template and control poly(acrylonitrile-co-acrylamide) membranes. The template-recognizing membrane exhibits elongated porous channels and a porous, foam-like upper layer. In contrast, the control membrane has a smooth, flat surface without observable cavities.





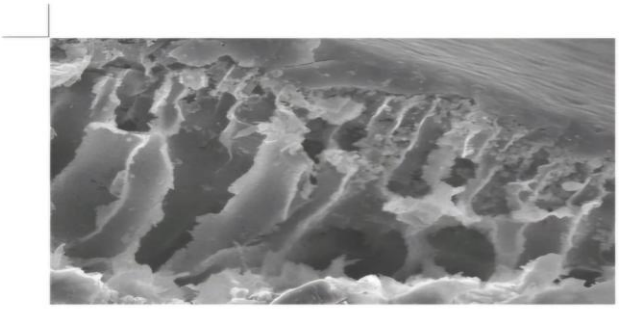


Figure 1. Scanning electron micrographs of p(AN-co-AM) membranes: (a) surface morphology of PGN-imprinted membrane, (b) surface morphology of non-imprinted membrane, and (c) cross-sectional view of the imprinted p(AN-co-AM) membrane

iii. X-ray Diffraction (XRD) Profile

The X-ray diffraction (XRD) analysis employs X-rays—electromagnetic waves with extremely short wavelengths—enables the investigation of a polymer's internal structure. By applying Bragg's equation, XRD analysis provides valuable insights into the crystallinity, chemical composition, and pattern interpretation of polymers. To assess the crystallinity of the membrane, an X-ray diffractogram was recorded. As shown in Fig. 2, the presence of distinct peaks indicates the crystalline nature of the membrane, with a prominent peak corresponding to the (210) plane observed at an angle of 72.5.

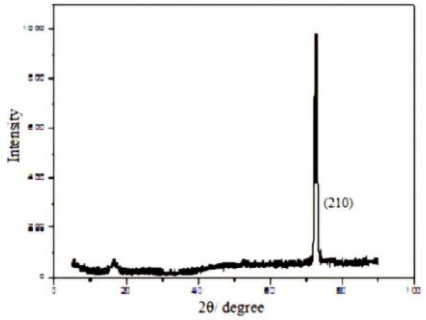


Figure 2. X-ray diffraction pattern of p(AN-co-AM)

2. Progesterone binding studies

2.1 Examination of the Selectivity of PGN-Patterned Membranes

The tailored polymer membranes demonstrate a significantly greater affinity for PGN in comparison to their non-templated counterparts, as illustrated in Fig. 3. This increased interaction is due to the presence of recognition cavities formed during the templating stage, which closely match the dimensions and structure of the PGN target. During polymerization, the functional monomer and PGN undergo conformational adjustments to achieve an optimal binding configuration, which becomes fixed within the polymer matrix. As a result, these imprinted sites retain a 'molecular memory' of PGN's shape and geometry, leading to selective and preferential recognition.

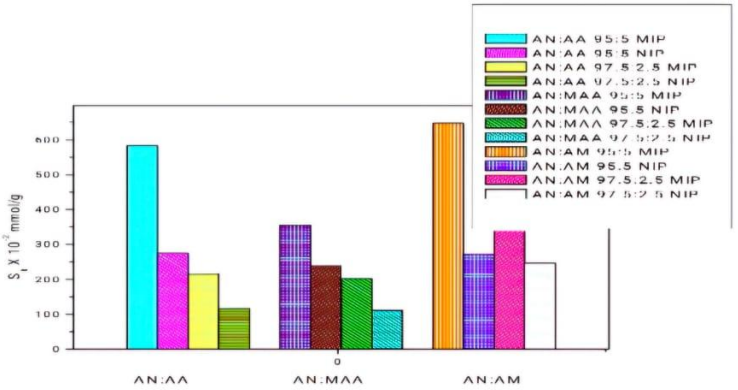


Figure 3. Specific PGN rebinding by imprinted v/s non-imprinted membranes

Among the trio of formulations containing different reactive monomer blends, the 95:5 composition demonstrates superior results. This enhanced binding is likely due to the increased concentration of functional

monomer, which facilitates stronger interactions between PGN and the polymer matrix. Comparing the co-monomers—acrylic acid, methacrylic acid, and acrylamide—the acrylamide-based system shows the greatest binding capacity. This enhanced performance is likely due to acrylamide's strong ability to form hydrogen bonds with PGN.

2.2. Optimisation of the conditions of PGN binding in DMSO

Optimizing the conditions is crucial to maximize target molecule re-binding efficiency by the functionalized polymer. For this, the polymer with the ratio of functional monomer to membrane forming segment, acrylonitrile, in maximum was used. Here we found that the membrane with 1:15 T/FM ratio has maximum binding capacity. So, the remaining studies were carried out using this polymer. The overall rebinding observed in an imprinted polymer includes both specific interactions at the recognition sites and general binding to the polymer matrix. General binding is assessed by measuring the interaction using a control polymer under identical experimental conditions. When non-target interactions prevail, they can obscure the contribution of specific binding. Therefore, optimizing the ratio of specific to non-specific binding is crucial for accurately assessing the performance of the imprinted sites.

i) Concentration Dependence

To examine the role of concentration in binding activity, progesterone solutions with concentrations ranging from 1 to 6×10^{-3} M were incubated with both imprinted and control membranes. The PGN concentration was monitored spectrophotometrically at a wavelength of 242 nm (λmax). Of the three co-monomers tested, acrylamide showed the highest binding affinity. As illustrated in Fig. 4, the highest PGN binding occurred at a concentration of 6×10^{-3} M. Notably, the control membranes also exhibited some retention of PGN, likely due to non-specific interactions

between progesterone and the nitrile groups in the polymer backbone, as well as the functional groups of the co-monomer. However, the imprinted membranes exhibited significantly greater retention. The variation in uptake between each imprinted membrane and its corresponding control reflects the selective binding capacity of the imprinted recognition sites.

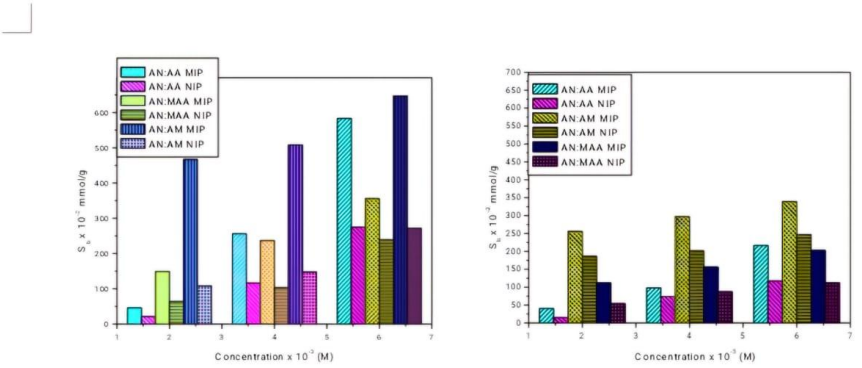


Figure 4. Effect of concentration of PGN on its rebinding by polymers with T/FM ratios (a) 95:5 and (b) 97.5:2.5

The imprinted membrane prepared with a 95:5 ratio of acrylonitrile to acrylamide showed the most effective overall affinity for progesterone, along with the strongest selective interaction. This improved binding performance can be attributed to interactions between the slightly acidic hydroxyl group of progesterone and the electron-donating amide groups present in acrylamide. The selective binding is a result of recognition cavities formed during imprinting, which possess both structural and chemical compatibility with the target molecule.

ii) Effect of time

The imprinted membrane reached its saturation point after 80 minutes, compared to 60 minutes for the non-imprinted (control) polymer, as

shown in Fig. 5. This difference reflects the effective and specific recognition capabilities of the imprinted membrane. Initially, the imprinted membrane bound significantly more progesterone than the control, and this difference grew until the template molecules fully occupied the complementary binding sites. The delayed saturation in the imprinted membrane is also due to the time required for PGN to access the specifically arranged recognition sites. In contrast, the control polymer lacks such structured sites, allowing PGN to interact more readily with randomly distributed binding region.

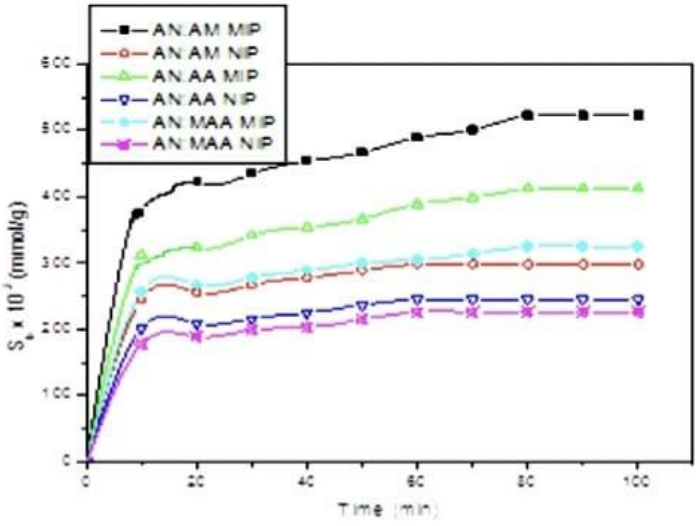


Figure 5. Temporal analysis of binding in imprinted and reference polymers

iii) Desorption and regeneration studies

To evaluate the reusability of the template-engineered membranes and facilitate effective template removal, elution and reusability studies were

performed. For molecular imprinting to be considered economically and practically viable, the ability to regenerate and reuse the membranes without significant loss of performance is essential. In this study, membrane regeneration was achieved by treating the saturated membranes with a methanol–acetic acid mixture, which efficiently restored their binding capacity. The regenerated membranes were subjected to multiple binding cycles and exhibited no substantial decline in performance. Desorption analysis indicated that 90.8% of the initially retained progesterone (95.3%) was successfully eluted using the regeneration solvent (Fig. 6). While a minor reduction in desorption efficiency was observed—from 90.8% in the first cycle to 87.3% by the third—the overall binding performance remained comparable to that of freshly prepared membranes over three successive cycles, demonstrating the robustness and reusability of the imprinted system.

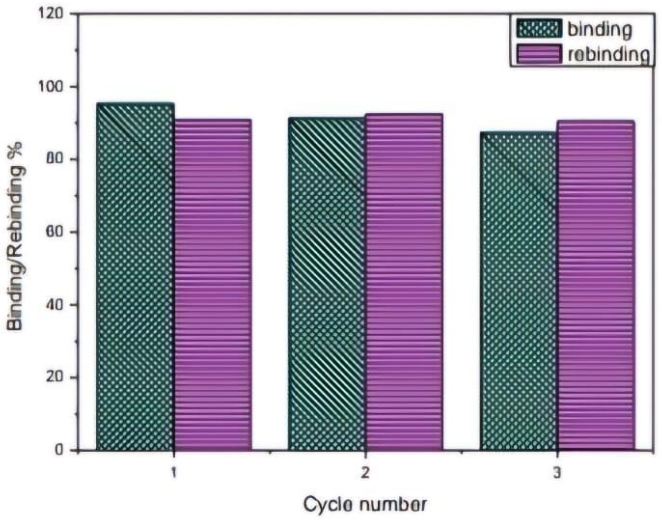


Figure 6. Recycling of PGN imprinted membrane

2.3. Selective Recognition Analysis

To evaluate the selective recognition capabilities of the PGN-imprinted membranes, binding experiments were conducted using structurally related compounds, including testosterone and cholesterol (Fig. 7). As shown in Fig. 8, the imprinted membranes exhibited significantly stronger affinity for progesterone compared to the other compounds. This enhanced selectivity is attributed to the formation of recognition motifs within the membrane that are uniquely structured in both geometry and chemical functionality to the PGN mode. These findings demonstrate that the imprinted membranes effectively and selectively adsorb progesterone. Among the tested systems, the acrylonitrile–acrylamide polymer matrix exhibited the highest selectivity for the template.

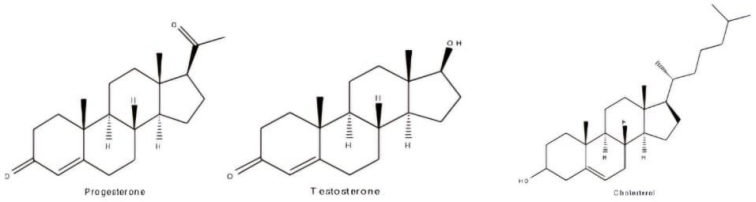


Figure 7. Structures of PGN and the competing analytes

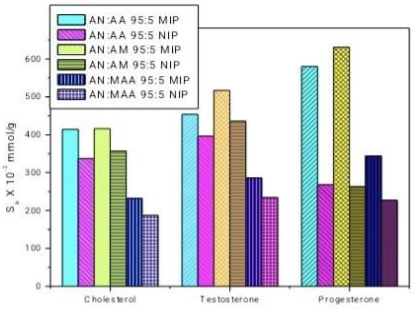


Figure 8. Competitive binding study with cholesterol and testosterone

CONCLUSION

Imprinted polymer technology provides a versatile approach for isolating and purifying specific compounds based on their structural characteristics. We can prepare molecular imprinted membranes for the purpose and it is more effective than bulk imprinted polymer. By phase inversion technique we can prepare imprinted membranes and its effective studies can be done. The template selected was progesterone which is an important hormone of human body.

In this current study molecular imprinted membranes of progesterone were prepared and its characterisation studies were done. Using FT-IR studies the effective polymerisation was confirmed. SEM and XRD analysis were done. Using the UV-Vis spectroscopy the binding studies were conducted and optimum binding conditions were found out. The selectivity study was done.

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Acknowledgment

First and foremost, I thank Almighty who guides me throughout the journey of my life and for being an unfailing support throughout my project.

I wish to express my sincere gratitude to my guide Dr. Anju Augustine, Assistant Professor, Department of Chemistry, K.E. College, Mannanam

for the whole hearted support and encouragement given throughout the course period.

I also reveal my sincere gratitude to Dr. Jollyamma Mathew, Head of Department of Chemistry, K.E. College, Mannanam who provided all the necessary support for doing the project.

I also express my sincere thanks to all other teachers in the Dept. of Chemistry for their constant inspiration for the successful completion of this work.

Once again, I wish to extend my thanks to teachers, family & friends for their love and support throughout my work..