Optimisation and Characterisation of Cortisol-Specific Molecularly Imprinted Polymers for an Electrochemical Biosensor

Ulrich Stocker (ustocker@hm.edu), Munich University for Applied Sciences | Department of Applied Sciences and Mechatronics
Supervisors: Prof. Dr. Christian Hanshans
Annika Hangleiter, M. Sc.

Introduction

The detection of biomarkers in body fluids is crucial for disease diagnosis and monitoring. While electrochemical biosensors utilizing biological recognition elements enable decentralized point-of-care monitoring [1], high production costs and immobilization challenges often present significant drawbacks [2]. Consequently, artificial bioreceptors in the form of molecularly imprinted polymers (MIP) have emerged as promising alternatives [3]. MIPs contain specific recognition cavities and exhibit high environmental robustness, making them highly suitable for wearable sensor applications [4]. A vital target for such continuous monitoring is cortisol, a steroid hormone whose dysregulation can result in severe physiological and neurological damage [5], [6]. To realize reliable wearable MIP-biosensors for cortisol monitoring, the reproducible and efficient manufacturing of these synthetic recognition elements is essential. Therefore, this work focuses on the generation and characterization of cortisol-specific MIPs synthesized via free-radical bulk polymerization. Specifically, the present study aims to optimize an established MIP manufacturing process and to physically and chemically characterize the resulting polymer to evaluate their suitability for biosensing applications.

Materials and Methods

MIPs and NIPs were synthesized via photoinitiated free-radical bulk polymerization (Table 1). The monoliths were ground, dried, size-selected (100-200 μm), and washed to extract the cortisol template (two different washing solutions were tested). Particles were characterized via differential scanning calorimetry (DSC) and batch rebinding experiments coupled with UV-Vis spectroscopy (Figure 1).

Table 1: Chemicals used for MIP/NIP synthesis and template extraction.

Component	Chemical Used
Functional Monomer	Methacrylic acid (MAA)
Crosslinker	Ethylene glycol dimethacrylate (EGDMA)
Template	Hydrocortisone (Cortisol)
Initiator	2,2'-Azobis(2-methylpropionitrile) (AIBN)
Solvent / Porogen	Dichloromethane (DCM)
Washing Solutions	(Standard Washing) Acetic acid/Methanol (80:20) (Alternative Washing) Pure Methanol

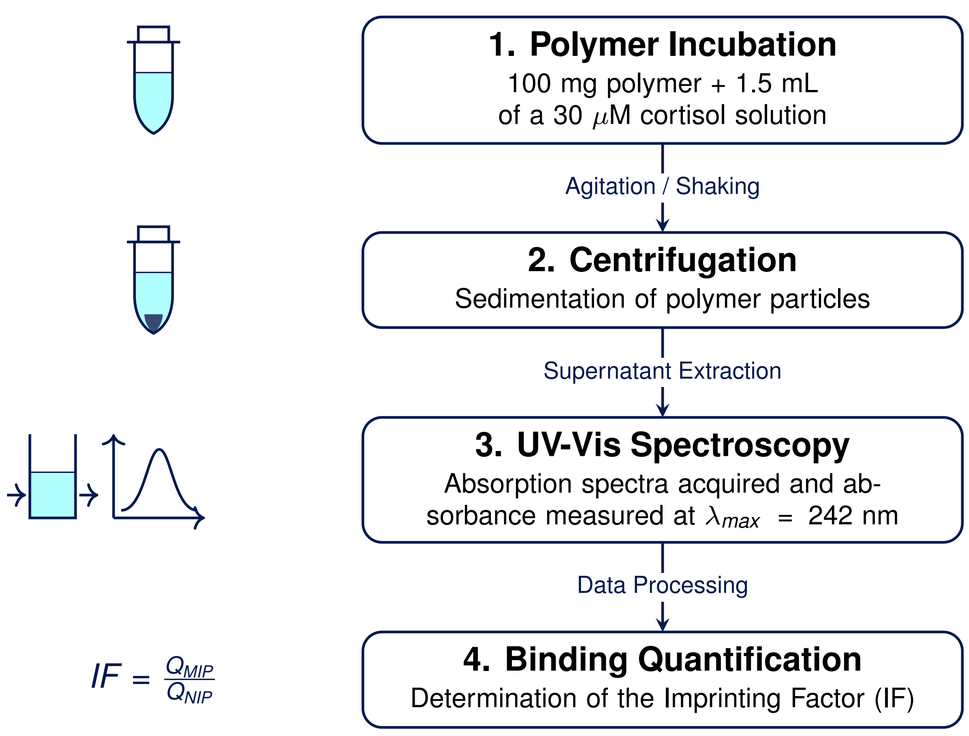

Fig. 1: Flowchart detailing the general batch rebinding experiment procedure with coupled UV-Vis quantification at 242 nm.

Process Optimization

Building upon existing methodologies at the BioMed Laboratory [7], the MIP generation process was optimized for higher throughput and better particle homogeneity.

Table 2: Comparison of standard vs. optimized MIP post-processing procedures.

Process Step	Standard Methodology	Optimized Methodology
Polymerization	No mixing or shaking.	**Orbital Shaking:** 200 rpm; significantly improved monolith homogeneity.
Grinding	Manual mortar grinding (12.5 min/sample).	**Mechanical Ball Milling:** Reduced to 6 min/sample; yielded more uniformly sized particles.
Drying	Desiccator with silica gel (24 h).	**Oven:** Dried at 70 °C (15 h); enabled efficient overnight processing.

Results

Thermal Stability

- Differential scanning calorimetry (DSC) revealed the absence of a glass transition (T_g) across all tested samples (MIPs were heated up to 500 °C and NIPs up to 350 °C) (Figure 2).
- This indicates a highly crosslinked, rigid polymer matrix capable of withstanding extreme environmental stress and high-temperature processing.

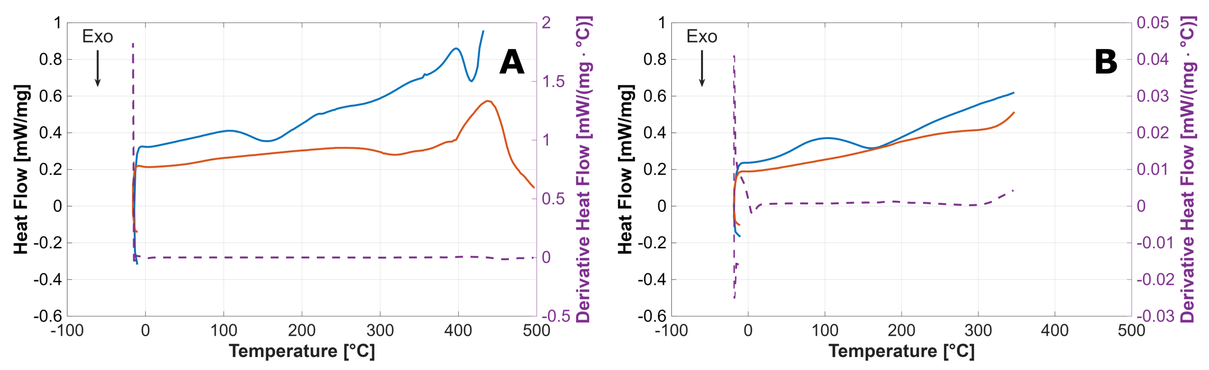

Fig. 2: DSC thermograms of a MIP (A) and NIP (B) sample. Each plot includes the first heating pass (solid blue line) and the second heating pass (solid orange line). The first derivative of the second heating pass (dashed purple line) is plotted against the secondary y-axis to facilitate the identification of thermal transitions such as the glass transition temperature (Tg).

Leaching and Potential Template Bleeding

1. Acetic Acid Leaching (Standard Washing)

- Batch rebinding experiments of samples using an 80:20 (v/v) acetic acid-methanol wash resulted in strong photometric interference. Residual acetic acid leached from the polymer, completely masking the cortisol absorbance signal at 242 nm (Figure 3A).

2. Potential Template Bleeding (Alternative Washing)

- Batch rebinding experiments of samples washed with pure methanol removed the acetic acid interference but revealed potential template bleeding, where residual cortisol is leaking from the MIP cavities during incubation (Figure 3B).
- This bleeding overshadowed actual rebinding events, yielding a mathematically contradictory negative imprinting factor ($IF = -1.1$).

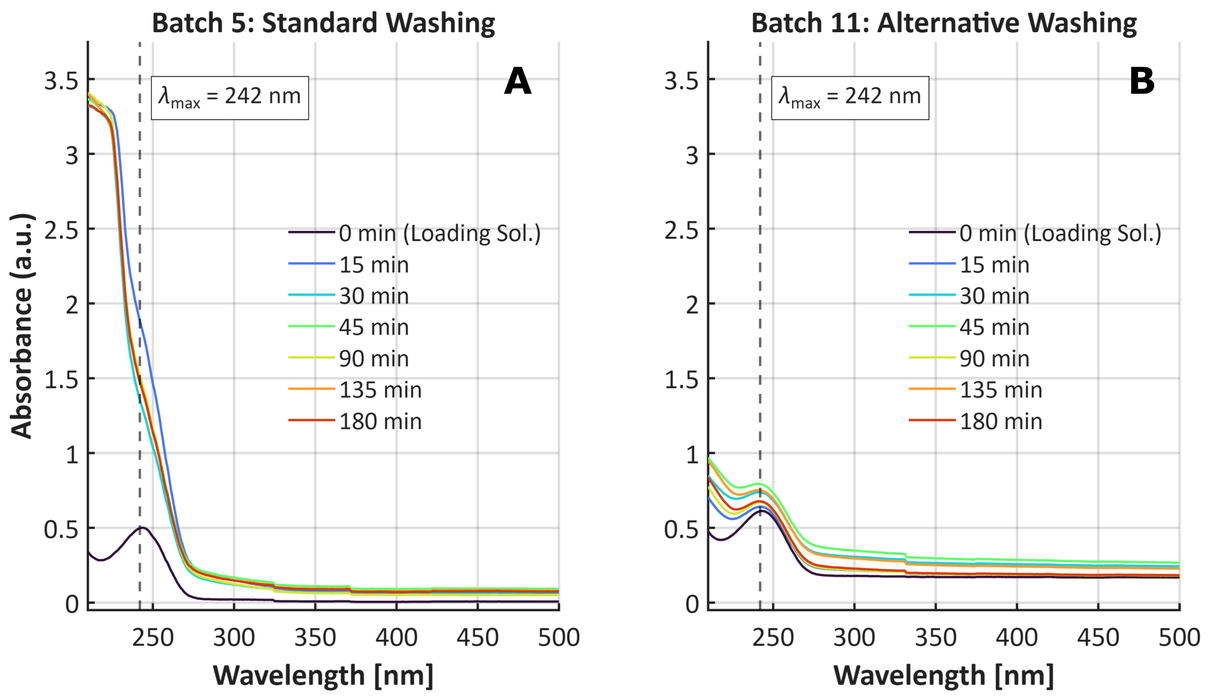

Fig. 3: UV-Vis absorption spectra (210–500 nm) of supernatants of MIP samples. (A) Standard washing shows acetic acid interference. (B) Alternative washing removes interference but reveals potential template bleeding.

Conclusion

The optimization of the MIP generation process was achieved, resulting in reduced post-processing time and increased particle homogeneity. Thermal analysis confirmed the absence of a glass transition, validating high-temperature oven drying without compromising the structural integrity of the MIPs. Batch rebinding experiments revealed that acetic acid leaching overshadowed the cortisol binding signal. Consequently, an alternative pure methanol washing procedure was identified as vastly superior, effectively mitigating chemical signal interference. Furthermore, potential template bleeding was identified, resulting in MIPs unable to specifically rebind the template. Future characterizations must therefore employ rigorous template extraction and analytical techniques, such as chromatography, to better physically separate the analyte from the MIP matrix.

References:

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