UREAP Application

**Research Proposal**

# Sensitive Determination of Nisin in Food Products by Large Volume Sample Stacking-Capillary Electrophoresis

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**Abstract: (1500 characters)**

Lantibiotics, synthesized by Gram-positive bacteria, combat bacteria through Lipid II in two ways: inhibiting peptidoglycan synthesis or inducing pore formation for apoptosis. The sole FDA-approved lantibiotic nisin, utilizes both mechanisms, serving as a preservative in drinks, dairy, and meats. However, nisin stability is influenced by temperature, pH, and food components, demanding careful assessment. Analytically, nisin is quantified using immunoassays and bioassays, limited by interference and sensitivity, respectively. To address this, capillary electrophoresis (CE) is proposed for trace nisin detection in dairy. CE offers high efficiency, speed, and minimal sample consumption. Despite its low sample volume injection and short optical path affecting sensitivity, CE can be enhanced using large-volume sample stacking (LVSS) with polarity switch preconcentration. LVSS, via larger sample injection and polarity switch, concentrates analytes into a small zone, boosting sensitivity to parts-per-billion. The proposed analytical approach stands to bridge the gap in quality assurance programs within the food industry for the detection of trace nisin.

**Literature Review: (3500 characters)**

Lantibiotics, produced by Gram-positive bacteria, combat other bacteria through Lipid II in two ways; (1) to inhibit the synthesis of the peptidoglycan bacterial cell wall, or (2) as a docking molecule to facilitate pore formation triggering apoptosis (Delves-Broughton, 1996; Karpíski & Szkaradkiewicz, 2015). Nisin, a 1A lantibiotic from Lactococcus lactis, acts via both mechanisms (Silva et al., 2018), finding broad use as a preservative in alcoholic drinks, dairy, and meats (Delves-Broughton, 1996). Nisin is the only lantibiotic that is FDA approved and utilized as a biopreservative due to its low toxicity (Silva et al., 2018).

While effective, nisin's stability is affected by temperature, pH, and food components (Silva et al., 2018). To ensure effective usage, its quantity and stability need careful assessment (Hakovirta et al., 2006; Soliman & Donkor, 2010). Analytically, nisin quantification involves immunoassays (Leung et al., 2002) and bioassays (Hakovirta et al., 2006). Immunoassays, like ELISA, detect bioactive nisin and its degradation products (Leung et al., 2002), yet face interference from niacin-similar-compounds in foods. Bioassays are not preferred due to their lengthy and tedious workflow in addition to sensitivity and specificity issues (Fowler et al., 1975). Recognizing these constraints, this project proposes capillary electrophoresis (CE) as an alternative analytical approach for trace nisin detection in dairy products. Crafting a sensitive CE detection method would effectively bridge this gap, presenting a viable solution for the food industry's needs.

CE stands as a robust analytical technique, characterized by its exceptional separation efficiency, rapid analysis speed, minimal sample consumption, and potential for automation (Chen, Xu, Lin, & Chen, 2008; Quirino & Terabe, 2000). CE's advantage lies in its minute sample injection compared to techniques like HPLC, although this small injection volume sacrifices

sensitivity. Additionally, the short optical path further hampers CE's sensitivity when detecting analytes at ultralow concentrations, such as nisin in dairy matrices.

To enhance CE's sensitivity, the integration of the large-volume sample stacking (LVSS) with switch polarity preconcentration technique can be employed. Traditional CE involves injecting a small sample plug (e.g., 0.5-1.0 psi for 5-10s or 1-2% of capillary length). Conversely, LVSS introduces a large sample plug (e.g., 15 psi for 60s or up to 80% of capillary length). The large sample volume increases sensitivity however sacrifices resolution. To address the resolution, LVSS utilises a polarity switch directly following sample injection and induces a reduced electric field. The polarity switch and induced field leads to analyte concentration within a confined zone. This process augments the detection signal, ultimately enhancing method sensitivity (Šlampová, Malá, & Gebauer, 2019).

**Research Question: (500 characters)**

The project will investigate the following research queries:

1) Can the developed LVSS-CE nisin method detect ppb levels of nisin?

2) What are the optimal LVSS-CE experimental parameters for increasing the sensitive detection of nisin?

3) Can the method be validated by applying the method to analyze food samples such as dairy milk?

**Methodology: (1500 characters)**

Nisin will be analyzed by capillary electrophoresis (CE), utilizing the micellar electrokinetic capillary chromatography (MEKC) technique. MEKC is employed for the separation of neutral compounds like nisin, achieved through a buffer solution containing micelle-forming surfactants (Hancu et al., 2013). In this study, sodium dodecyl sulfate (SDS) will generate anionic micelles, attracted to the anode, while the aqueous buffer will travel via electroosmotic flow towards the cathode. The partitioning of nisin between the mobile aqueous phase and the micellar pseudostationary phase will drive its separation (Hancu et al., 2013). A UV-Vis detector will identify nisin, with data processing by the 32 Karat software on the CE instrument.

The MEKC method outlined by Soliman and Donkor (2010) will incorporate the LVSS technique for enhanced sensitivity. Optimization will encompass factors such as buffer pH, sample solvent, injection pressure, and switch polarity time and voltage to yield optimal nisin peak resolution. The approach's precision, accuracy, linearity, and detection and quantification limits will be evaluated. Method validation will culminate in the analysis of actual milk samples.

**Impact on field of study: (1500 characters)**

Nisin, a vital biopreservative, finds widespread application in various food items, notably dairy products. Ensuring safe human consumption requires vigilant monitoring of nisin levels, determining product shelf-life accurately. Detecting minimal nisin concentrations in food poses a challenge currently. Consequently, an adept and sensitive method to monitor part-per-billion nisin levels within dairy products could improve current quality assurance within the food industry. Moreover, the developed LVSS method can be applied to the sensitive detection of other neutral analytes through capillary electrophoresis techniques, thereby contributing to advancements in related analytical chemistry and chemical biology studies.

**Dissemination: (500 characters)**

I hope to present the results of this research at the annual TRU Undergraduate Research and Innovation Conference in 2024, Western Canadian Undergraduate Chemistry Conference May 2024, and Canadian Chemistry Conference and Exhibition in June 2024. Additionally, I hope to present our research study to a scientific journal for publication.

**Academic and Professional Goals: (1000 characters)**

Throughout my studies as a third-year chemistry student at Thompson Rivers University, I have become adept at using different instruments and experimental methods, like those in this study. As a research assistant under Dr. Kingsley Donkor this past summer, I was introduced to the research environment. I look forward to further my immersion in research, working in the lab and learning from the other students. The method development integral to this project will enhance my problem-solving skills and analytical mindset.

My post-undergraduate goal is to become a medical doctor, utilizing my scientific expertise to enhance community health. A UREAP opportunity would allow me to contribute to TRU's research. The experiences in lab, collaboration with peers, and dissemination would profoundly benefit my goals. This opportunity would provide crucial research insights and experience, pivotal for my future goals in medicine.

**References: (3500 characters)**

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**Timeline**

| **Period** | **Plan** |
|---|---|
| Week 1 | Ordering of reagents |
| Week 2, 3 | Preparation of nisin calibration standards<br>Generating nisin calibration curves |
| Week 4 - 9 | Method design for MEKC experimental set-up<br>Optimization of MEKC and LVSS experimental conditions |
| Week 10 - 13 | Dairy samples analysis<br>Method Validation |
| Week 14 - 16 | Evaluation of research results<br>Writing of scientific report |

**Budget**:

Nisin standard – $300.00

CE buffer reagents – $170.00

CE Vials and Caps – $120.00

Solvents – $160.00

General lab supplies – $250.00

Total = $1000.00