Role of the Device Inclination on the Synthesis of Nanocomposite Gels

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Introduction

Hydrogels are soft materials formed by the cross linking of polymer chains; their pores vary in size based upon the composition and SDS-acrylamide monomer concentration, but generally can hold a large percentage of water. These gels have a wide array of applications including cosmetics, food, biomedicine, separation media, agriculture, and tissue engineering. The cross-linking can be achieved through chemical and physical processes with synthetic and natural polymers such as polyacrylamide or agarose. Synthetic hydrogels are commonly used because of their reproducibility and greater control of the hydrogel's functions and properties in comparison to natural polymers. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a widely used method for separation of protein solutions. It is a reliable separation process because the SDS denatures but does not bind to the proteins. Yet, the acrylamide monomer is a strong neurotoxin, and research regarding lowering the concentration required to effectively separate the proteins completely has been ongoing. From previous research provided by Sarah Beth Cain, post-graduate student, further characterization of hydrogels at lower concentrations of SDS can be studied. With further characterization about the proportions, or specifically concentrations, of acrylamide and SDS in combination with physical influences on the nanocomposite polymerization by the inclination of the casting angle, better control of hydrogel properties can be attained.

Materials

Table 1: General Ingredient List for 6% Acrylamide Nanocomposite Gels

Reagent	Volume
1.5 M Tris Buffer	2 mL
Protogel (30%)	1.6 mL
Water	Varied for changes to desired nanofiller concentration
SDS (nanofiller)	Varied for changes to desired nanofiller concentration
APS (initiator)	80 µL
TEMED (accelerator)	8 µL

Methodology

Thus far, four experimental conditions identified by Cain impact the protein resolution in hydrogels. The first two conditions, the percentage acrylamide (%T) and concentration of SDS, affect the internal structure and pores of the gel composition. The other two variables, polymerization time and the casting angle of the gel, both impact the protein separation.

- The preparation of the polyacrylamide gel is accomplished first by preparing the solution with the desired percentage of acrylamide (6%) and concentration of SDS nanofillers (5-15%).
- The Handcast apparatus is assembled with clean, dry glass plates and a rubber spacer secured in the system. The gel solution is then inserted, using a pipet, between the glass plates in the gel box.
- A layer of isopropanol is added to the fill line of the glass plate to prevent dehydration. The Handcast system is aligned either to 90˚ (vertical) or 40.2˚ (inclined) while the hydrogel polymerizes. After the mixture is solidified, the isopropanol is removed, and the stacking gel layer and comb inserted.

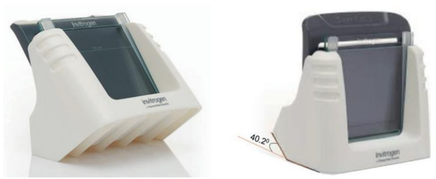

Figure 1: SureCast™ Gel Handcast System at 40.2˚ (left) and 90˚ (right)

- Following the polymerization of the stacking gel and removal of the comb, and the plates are relocated to the electrophoresis tank filled with buffer. The protein samples are then loaded into the wells with a pipet.
- The system is closed and charged with 100V and ran for 90 minutes.
- The resulting protein resolution is visible by the colored bands in the gel. Based on the relative migration of the proteins within the gel, the effects of the variables on the separation process can be measured.

Acknowledgments

We would like to gratefully acknowledge Sarah Beth Cain for her research and samples as the foundation for our project. Additionally, we would like to extend our gratitude to Anfal Haris, Dr. Pedro E. Arce, and Dr. J. Robby Sanders for their ongoing guidance, support, and contributions to this work.

Preliminary Results

Cain's thesis, "Identification of Polymerization conditions for Minimizing Requisite Concentration of Toxic Acrylamide in Nano-templated and Nanocomposite Polyacrylamide Gels Embedded with SDS Micelles", accomplished successful protein resolution of 10 proteins of varying molecular masses under 6% gel and 5% concentrated SDS. Focusing on the variations involving this nano-composition, it was discovered that the casting angle of 40.2˚ better resolved the proteins in less time in comparison to setting the gel at the typical 90˚ angle. Within 24 hours, the gel cast at 40.2˚ resolved all 10 proteins loaded, but the vertical cast only resolved 8 bands.

Table 2: Number of Resolved Proteins Based on Device Inclination and Polymerization Time

	Number of Resolved Proteins	
Polymerization Time (Hours)	40.2˚ Inclination	90˚ Inclination
2	7	7
12-18	9	7
24	10	8
48	N/A	8

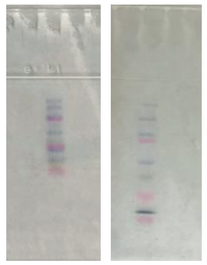

Figure 2: Protein resolution for 6% Gels with 5% SDS Polymerized 24 hours at an inclined angle of 40.2˚ (left image) and vertically at 90˚ (right image).

References

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Discussion

The methodology of this research is focused on sedimentation potential, which is a factor of electrophoresis. Sedimentation potential describes the movement of colloidal particles in a liquid, with gravity being the driving force for their motion. Without SDS denaturing the protein and creating a uniform negative charge among the proteins, the movement would be dependent on their mass to charge ratio. Based on the results depicted in Table 2, the inclination angle of 40.2˚ resolved the proteins faster than the 90˚ cast angle, but the distance between the bands of the latter was greater. This is because the force of gravity has more influence when the Handcast System is vertical, allowing even the larger proteins to get through the porous structure of the gel. This brings to question why the polymerization angle of 40.2˚ resolved all the proteins faster than at 90˚ when all other conditions are identical. The protein bands are more distinguishable from one another in the vertical gel, which could be important for analyzing specific, purified bands individually. This directs the research to future works and the possibilities of the polymerization angle.

Conclusions & Future Work

Based on the previous research and current understanding of the variables that affect nanocomposite polymerization, the concentration of SDS in hydrogels can be as low as 5% in 6% acrylamide gel. This discovery is a substantial progression towards characterizing hydrogels and making the solution less dangerous to those directly and indirectly affected by the neurotoxicity of SDS. If the protein separation can be purposely influenced by a combination of SDS nanofiller concentration, acrylamide percentage, casting angle, and polymerization time, then further research opportunities exist for each variable's individual influence on the protein migration within the gel. Our continued research with these nanocomposite gels will primarily focus on extensive analysis of how varied inclination angles of the casting device affect the internal structure of the gels and, as a result, the protein separation process. An inclination between 0-90˚ could be found to separate the proteins in less time. Additionally, as an extension of Cain's research, further attempts could be made to decrease the concentration of SDS required for complete protein resolution. Once the optimal angle of inclination for the Handcast apparatus is determined, the SDS concentration can be challenged to determine if a minimized concentration is possible under a controlled cast angle and polymerization time.