Novel and eco friendly staining method for proteins separated by polyacrylamide gel electrophoresis

Contestant: Ms. Snehal Poojary, Ms. Latika Anchan
Expert: Dr. Jayashree Pawar, Dr. Kalpita Mulye, Ms. Zahera Momin
Department of Biotechnology and Microbiology, V.P.M's B.N. Bandodkar College of Science, Thane- 400601

AIM:

To develop an environment friendly and less time consuming method for staining of proteins separated by Polyacrylamide gels.

Polyacrylamide gel electrophoresis (PAGE) is one of the commonly used techniques for the separation of proteins based on their molecular weight, charge and conformation. One of the several steps in PAGE is detection of protein following its separation by electrophoresis. The most commonly employed method for this purpose involves the use of Coomassie Brilliant Blue (CBB) dye. The present study focuses on the development of an eco friendly and less time consuming method for detection of proteins in polyacrylamide gels by using plant extract.

MATERIALS AND METHODS:

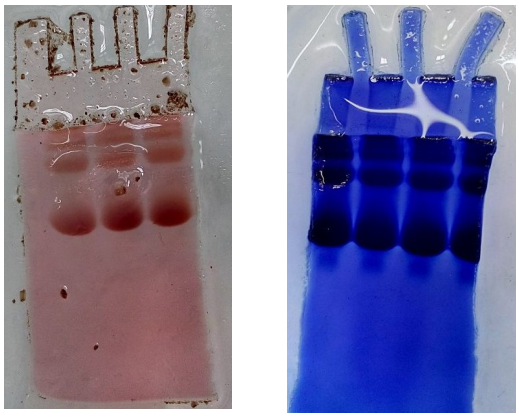
1. ***Preparation of plant extract*:**
   Aqueous extract was prepared and dried. The powdered extract was used for further study.
2. ***Preparation of protein sample, gel loading buffer, polyacrylamide gel and detection of proteins:***
   For Native PAGE 15% resolving gel, 5% stacking gel, Gel loading buffer, conventional (containing Commassie Brilliant Blue) staining and destaining solutions were prepared as per Sambrook, J., and Russell, R.W.(2001). Bovine serum albumin (BSA) (10%) was used as protein sample.
3. ***Staining of polyacrylamide gels using plant extract:***
   For preparation of staining solution, CBB was replaced with plant extract (0.025gm/ml). The proportions of solvents were kept same as the conventional staining solution i.e. methanol, distilled water and glacial acetic acid in 5:4:1 ratio. The gel was kept immersed in the staining solution overnight.
4. ***Determination of concentration of active colouring component in the plant extract:***
   The concentration of pigment as active colouring component in plant extract was determined colorimetrically and calculated using appropriate formula.
5. ***Stability of active colouring component in dried extract after 2years:***
   The stability of active colouring component in plant extract stored for 2 years was determined colorimetrically.
6. ***Formulation of staining protocol for plant extract:***
   Standardization was carried out by varying the concentration of different components of the conventional staining solution.
   6.1. Variation of glacial acetic acid concentration: 10% and 20% glacial acetic acid was solely used as staining and destaining solution.
   6.2. Variation of methanol concentration: Varied concentration of methanol (5%, 10%, 20%, 30%, 40% and 50%) was used in staining solution containing glacial acetic acid (10%) and distilled water.
   6.3. Variation in concentration of plant extract: The staining process was carried out by varying the concentration of plant extract (1X and 2X) in staining solution.
   6.4. Staining time variation: Staining was carried out using staining solution containing 0.05gm/ml of plant extract and methanol, distilled water, and glacial acetic acid in 1:8:1 ratio. Time of staining was varied (1 hour, 2 hour, 5 hours and 18 hours) followed by destaining and result was compared with conventional staining.
7. ***Formulation of destaining protocol for plant extract:***
   7.1. ***Variation in destaining solution:*** Following solutions were used to destain gel stained by plant extract:
      1) Methanol and distilled water (1:9)
      2) Glacial acetic acid and distilled water (1:9).
      3) Methanol, distilled water and glacial acetic acid (1:8:1).
      4) Distilled water.

   7.2. Destaining time variation: Stained gels immersed in destaining solution were observed after 30 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes and 180 minutes.
   The results were compared with conventional CBB staining and destaining method.
8. ***Sensitivity of the plant extract for protein staining:***
   To determine sensitivity of active staining component for protein, different concentrations (i.e. 100mg/ml, 10mg/ml, 1mg/ml, 0.1mg/ml, 0.01mg/ml) of protein were run on polyacrylamide gel and stained with plant

extract. The conventional CBB method was kept as control.

RESULTS:

1. ***Staining of polyacrylamide gels using plant extract:***
   Crude plant extract added in conventional staining solution was able to stain separated protein bands on PAGE gels. Distinct bands of stained proteins were observed with similar intensity as compared to CBB staining with lesser background staining



(a) (b)

Image 1: (a) PAGE gel stained with plant extract. (b)PAGE gel stained with CBB.

2. ***Determination of concentration of the active staining component in the plant extract:***
   The concentration active staining component in freshly prepared crude plant extract was 3.19 g/L.
3. ***Stability of dried extract after 2 years****:*
   The concentration of active staining component in 2 year old plant extract was 3.14g/L and its concentration in freshly prepared plant extract was 3.19 g/L. Hence active staining component remain stable even after 2 years when stored at 4^0 C.
4. ***Formulation of staining protocol for plant extract:***
   4.1 Variation of glacial acetic acid: Proteins separated on PAGE gels were stained with staining solution comprising of plant extract dissolved in 10% and 20% glacial acetic acid, 10% glacial acetic acid containing staining solution was found to be more efficient as compared to 20% glacial acetic acid.
   4.2 ***Variation of methanol concentration****:* Stability of plant pigment dissolved in 10% glacial acetic acid with different concentrations of methanol was determined colorimetrically. The plant extract was more stable in solution containing 10% glacial acetic acid and 10% methanol.

Concentration of methanol	**Concentration of Active staining component (g/L)**					
	Day 1	Day 2	Day 3	Day 4	Day 8	Day 11
5%	1.722	2.828	2.865	2.946	1.707	0.623
10%	3.127	3.200	2.976	2.964	2.612	1.573
20%	1.722	2.912	3.181	3.608	3.407	-
30%	1.225	3.209	2.946	3.131	3.503	1.894
40%	2.647	2.893	2.878	3.131	2.790	1.359
50%	2.819	3.172	3.050	3.102	2.813	1.433
0%	1.845	2.523	2.300	2.560	1.431	0.074

Table 1: Stability of plant extract in different methanol concentration.

4.3 Variation in concentration of plant extract**:** The gel when stained with staining solution containing 2X of plant extract showed the presence of more clear and distinct band as compared to the gel stained with the staining solution containing 1X of plant extract.

4.4 ***Variation with time:*** Gel staining under different staining times indicated the binding efficacy of the plant extract. Staining duration of 5 hours was found to be optimum as intense distinct bands were observed upon destaining.

5 Formulation of destaining protocol for plant extract:

5.1 ***Variation in components of destaining solution*****:** Clear and well separated bands were observed after destaining the gel with distilled water. Also it was noted that the gel did not shrink upon destaining with distilled water which is contrary to the gel shrinkage that occurs upon conventional destaining method. Destaining solution needs to be changed atleast thrice inorder to obtain clear bands in both conventional and new destaining method.

5.2 ***Variation with time:*** Destaining of 2 hours was found to be enough to obtain a clear and distinct band with minimum background staining.

6 ***Sensitivity of Novel staining formulation:***

Upon staining the proteins with novel staining formulation and conventional method, distinct bands of comparable intensities were observed upto 0.1mg/ml. Hence comparable sensitivity was observed between both, novel staining formulation and conventional staining method

CONCLUSION:

- The method developed in the present study was found to be **comparable** to the conventional method in terms of **efficiency**.
- Up to **84% reduction in the use of organic solvents** was observed when compared with conventional staining and destaining method.
- Along with **reduced use of organic solvent**, use of **plant waste** for the preparation of the extract makes this formulation **eco friendly.**
- Also, the staining and destaining by employing this method is **completed within 7 hours** whereas the conventional method requires more than 20 hours. Hence, it is less time consuming.
- **Standardized protocol**.

Components	**Novel eco friendly staining formulation**		**Conventional CBB method**	
	Staining Solution	Destaining solution	Staining Solution	Destaining solution
Dye	Crude plant extract-5%(w/v)	Not Applicable	CBB-0.25%(w/v)	Not Applicable
Methanol	10%	-	50%	50%
Glacial acetic acid	10%	-	10%	10%
Distilled water	80%	100%	40%	40%
Time required	5 hours	2 hours	2-4 hours	overnight

Table 2: Comparison of Novel staining formulation and conventional CBB staining

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