"EXPLORING AGAROSE GEL CONCENTRATION EFFECT ON ELECTROPHORESIS OF PLASMA PROTEIN: INSIGHT FROM MURSHIDABAD"

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Abstract:

Introduction: The electrophoresis of plasma protein is a technique to analyse plasma proteins. As per this study the effect of agarose gel concentration in the process of plasma protein electrophoresis has been evaluated focusing in the population of Murshidabad, situated in eastern part of India which was not previously explored.

Methods: For conduction of this study a well establish protocol for plasma protein electrophoresis has been used. Tris-glycine buffer and amido black as a stain has been used by this method for protein bands visualisation. The manipulation of the concentration of the agarose gel has been done to observe their effect on protein migration.Agarose gel was prepared in different concentration ranging from 0.6 percent to 1 percent to understand how it impacts on protein migration rates**.**

Results: From our study there is clear correlation between agarose gel concentration and migration rate of plasma protein.Slower protein migration has been observed with higher agarose gel concentration, where as lower concentration of agarose was associated with faster protein movement.

Discussion: The relationship which has been observed that is agarose gel concentration and protein migration rates.It can be used in optimization of diagnostic and research condition. Furthermore, this study has been done in Murshidabad, Eastern India. In conclusion, we have tried to put light on the interplay between agarose gel concentration and protein migration in plasma protein electrophoresis.

Keywords: Protein Migration, Electrophoretic Conditions, Agarose Gel Concentration, Plasma Protein Electrophoresis

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DOI:-10.53555/ecb/2022.11.11.138

INTRODUCTION: As the plasma protein electrophoresis has been carried out with agarose gel electrophoresis all the discussion and literature regarding the procedure of plasma protein

electrophoresis has been reviewed to understand the process(6),(1),(2).We have performed the plasma protein electrophoresis using the standard protocol for plasma protein electrophoresis(1,2).We have used Tris-glycine buffer as buffer and amido black stain as a stain for the plasma protein electrophoresis .With different concentration of agarose gel different rate of migration indicated by the forward movement of tracking dye that is bromophenol blue observed(3).Protein electrophoresis has been a important part of diagnosis of the disorder like multiple myeloma, alpha 1 antitrypsin deficiency,liver cirrhosis etc(4), (7).Also there is study where tris-tricine and tris-glycine buffer has been used in comparison ,but we have taken Tris-glycine buffer to study the plasma protein electrophoresis (5).In the eastern part of India ,especially in Murshidabad,this kind of observational study has not been done before.

Materials and Methods: For our research, essential chemical and reagent has been arranged according to availability for the experiment. Thermofisher scientific provided us with Agarose, Amido Black 10B (lobachemi), Tris (from Srlbiochem), Bromophenol Blue from Merck life sciences private limited. We obtained glycine (from Srlbiochem) and glycerol from Merck, Methanol from Finar private limited, and Acetic acid glacial from rose chemical industries, Kolkata. Ethyl alcohol (99.9%)was procured from ChangshuHongsheng Fine Chemicals.All other reagents used were of analytical grade.For our laboratory work and electrophoresis system from Genei and used PH meter from a K-Roy balance for weight balance during our study. These materials and equipment were instrumental in ensuring the reliability and integrity of the research outcome.

DETAILS OF METHODOLOGY FOR AGAROSE GEL ELECTROPHORESIS:
Foragarose gel electrophoresis, the following protocol has been employed:

1.PREPARATION OF TRIS-GLYCINE BUFFER (PH~8.3): A solution was made by addition of 3 grams of Tris (0.025M) and 14.4 grams of Glycine (0.192M) was dissolved in one litre of distilled water.

2.FORMULATION OF SAMPLE BUFFER:
To create 10 millilitres of sample buffer, the following components were combined: 2.5 mL of Tris-Glycine buffer, 2 mL of Glycerol, and 5.5 mL of distilled water.

3. AGAROSE GEL PREPARATION:

- A.1% Agarose gel preparation: We have Dissolved 1 gram of agarose in 100 mL of Tris-Glycine buffer, adjusting the pH to a range of 8.3 to 8.5. The mixture has been heated until it becomes transparent.
- B.0.8 % Agarose gel preparation: We have taken 0.8 gram of agarose in 100 mL of Tris-Glycine buffer, adjusting the pH to a range of 8.3 to 8.5.Then the mixture was heated until it becomes transparent.
- C.0.6 % Agarose gel preparation: We have taken 0.6 gram of agarose and dissolve it in 100 mLofTris-Glycine buffer while carefully adjusting the pH to fall within the range of 8.3 to 8.5. The mixture was heated until it achieves transparency.

4.5% BROMOPHENOL BLUE (BPB) SOLUTION: A solution of 5% Bromophenol Blue in a 0.1M Phosphate buffer at a pH of 7.38 has been made.

5. AMIDO BLACK STAIN PREPARATION:
One gram of Amido Black was dissolved in 90 mL of ethanol, followed by the addition of 10 mL of glacial acetic acid.

6.AMIDO BLACK DESTAINING SOLUTION: Combine 7 mL of glacial acetic acid with 50 mL of methanol, then add distilled water to reach a final volume of 100 mL for the destaining solution.

EXPERIMENTAL PROCEDURE:
SAMPLE PREPARATION: A total of 20 µL of diluted plasma, prepared by mixing it in a 1:1 ratio with sample buffer and adding 2 µL of tracking dye, was used for analysis. The leftover samples have been taken from central laboratory of Murshidabad medical college and Hospital for this purpose.
TRACKING DYE: Preparation of tracking dye solution was done by combining 196 µL of sample buffer with 4 µL of 5% Bromophenol Blue dye.
ELECTROPHORETIC CONDITIONS: The electrophoresis was conducted at 50 volts until the tracking dye reached the end of the gel and the time noted. Throughout the procedure, the electrophoresis apparatus was kept on ice for good outcome.After the tracking reached 50 percent of the total slide length the time noted for each of the concentration of agarose gel.
GEL STAINING PROCEDURE:
After the electrophoresis run, the gels were immersed in already prepared amido Black stain

within a container (petri dish), and this staining process was carried out for 5 minutes at room temperature.

GEL DESTAINING PROCEDURE:

Following the staining step, the gels were subsequently transferred to a already prepared destaining solution and left overnight at room temperature for destaining properly.

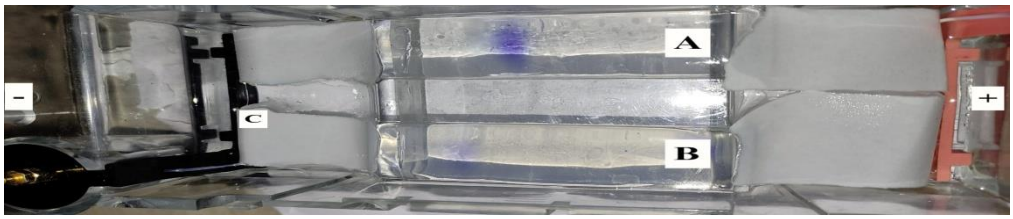

Fig 1:Showing the fast migration of tracking dye of the gel with lowest concentration (0.6 %-slide marked as "A",0.8%-slide marked as "B"),"c" stands for cathode.

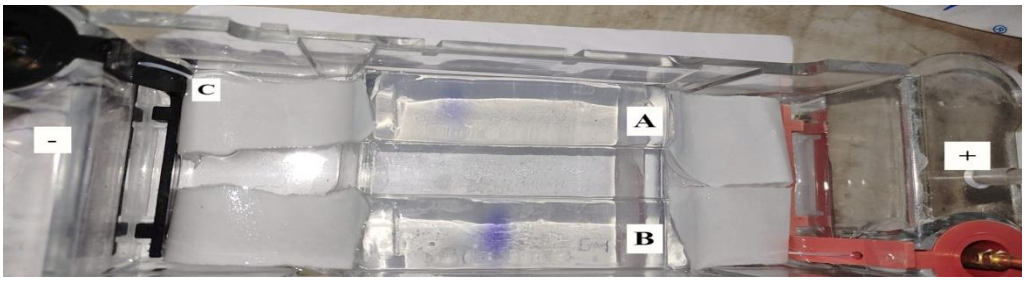

Fig 2: Showing the fast migration of the tracking dye with lowest concentration of the gel(0.8%-slide marked as "B",1%-the slide marked as "A")

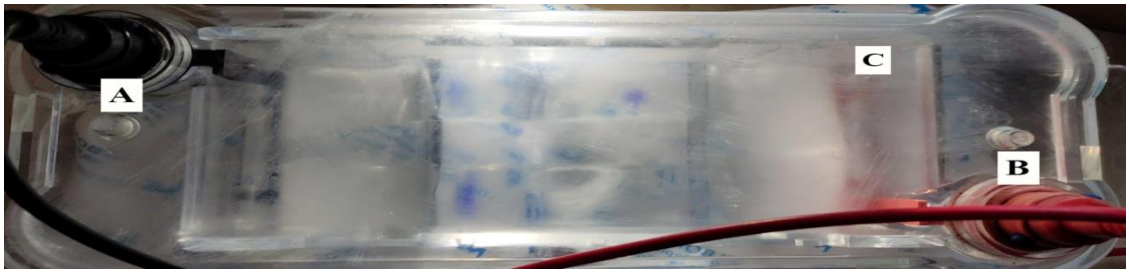

Fig 3: Picture clicked from above clearly demonstrate the whole apparatus has been kept on ice pack during the procedure.

Fig 4:"A"-Agarose powder,"B"-power pack for electrophoresis,"C"-electrophoresis instrument kept on ice pack and connected to the power pack during the procedure

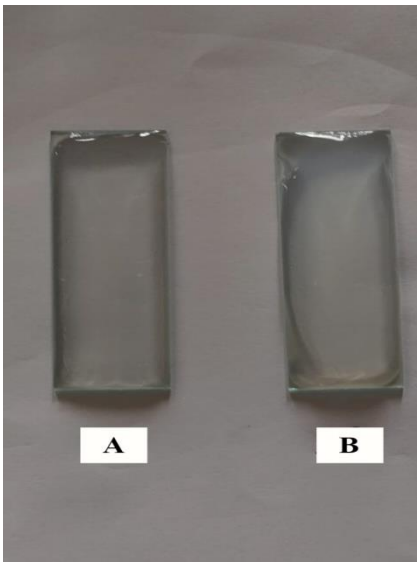

Fig 5: Showing the prepared slide A:0.6 percent,B:0.8percent

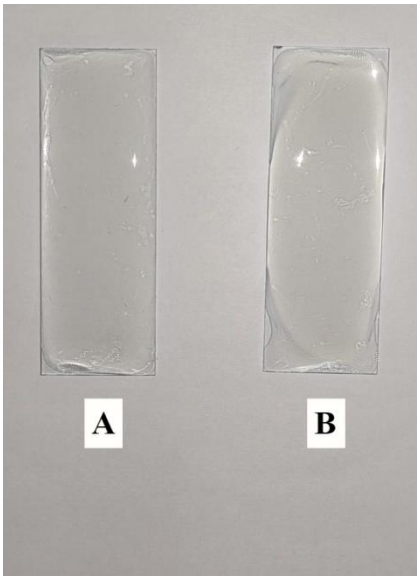

Fig 6: Showing the prepared slide A:0.8 percent,B:1percent

RESULTS

: Observations were made regarding the staining of gels. In the 5-minute stained gel, discernible protein bands were readily visible.

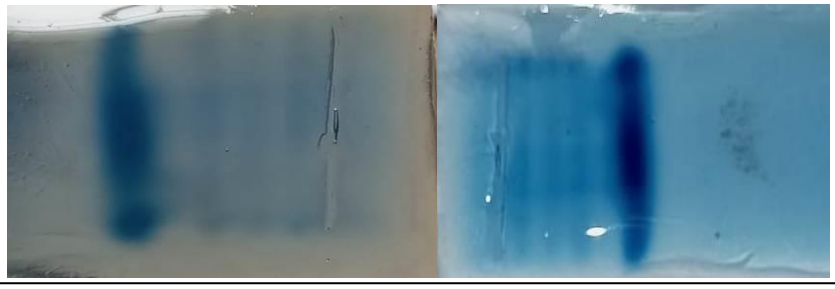

Fig 7: Image Showing Different Electrophoretic Bands Of Plasma Protein Electrophoresis After Destaing Overnight

Total No Of Electrophoresis(No+Time)	Concentration Of Agarose Gel(Percent)	Time To Reach End Of The Slide Of The Slide (Miniutes)
A1	1 %	220
A2	1 %	218
A3	1 %	223
A4	1 %	220
A5	1 %	225
B1	0.8 %	192
B2	0.8 %	190
B3	0.8 %	187
B4	0.8 %	191
B5	0.8 %	193
C1	0.6%	160
C2	0.6%	158
C3	0.6%	161
C4	0.6%	157
C5	0.6%	163

STATISTICAL TEST:
ONE WAY ANOVA SHOWING THE SIGNIFICANCE

TIME	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9424.933	2	4712.467	756.011	.000
Within Groups	74.800	12	6.233		
Total	9499.733	14			

We have used Spss version 20(Statistical Package for Social Sciences, version 20), According to conventional criteria p value of less than 0.001 has been taken as highly statistically significant and less than 0.05 has been taken as statistically significant.

Multiple Comparisons

Dependent Variable: TIME
Tukey HSD

(I) AGAROSECONCENTRATION	(J) AGAROSECONCENTRATION	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.6PERCENT	0.8PERCENT	-30.80000*	1.57903	.000	-35.0126	-26.5874
	1.0PERCENT	-61.40000*	1.57903	.000	-65.6126	-57.1874
0.8PERCENT	0.6PERCENT	30.80000*	1.57903	.000	26.5874	35.0126
	1.0PERCENT	-30.60000*	1.57903	.000	-34.8126	-26.3874
1.0PERCENT	0.6PERCENT	61.40000*	1.57903	.000	57.1874	65.6126
	0.8PERCENT	30.60000*	1.57903	.000	26.3874	34.8126

*. The mean difference is significant at the 0.05 level.

DISCUSSION: The plasma protein electrophoresis represents significant information in context of plasma protein electrophoresis in Murshidabad,situated at eastern part of India. These methods are well-established and have been used in many studies. The observed variations in migration rates of plasma proteins in response to different agarose gel concentrations indicates that the density of the gel matrix affects the ease with which proteins can move through it. Understanding this relationship of agarose gel concentration and its impact on plasma protein

electrophoresis may lead to insights into the electrophoresis of plasma protein.
The relationship between protein migration and agarose gel electrophoresis is the focus of our study.The plasma which has been used in this study taken from leftover sample in the central

laboratory has been analysed in the laboratory of Biochemistry department shown that it has a total protein value of 6.2 gm/dl and albumin level 3.6 gm/dl.We have performed total 15 experiment of electrophoresis including 5 of each 0.6 percent, 0.8percent and 1 percent. Total time recorded each of the experiment and using ANOVAs test with the help of the software statistical package for social sciences (Spss version 20). From the statistical analysis it is clear that our test is statistically significant.

INFERENCE: According to the above analysis it can be concluded that the time difference to reach the gel to the entire slide in a various concentration of agarose is statistically significant. So there is a clear cut connection between agarose gel and rate of migration of protein during the electrophoresis procedure. With higher concentration of the agarose gel there is a decrease of rate of protein migration and with lower concentration vice versa. This phenomenon can be explained by the density of gel matrix as it serves as a molecular sieve and thus higher concentration result into a denser network.So it can be concluded that maintaining the consistent agarose gel concentration is very much necessary for quality purpose.

LIMITATION OF THE STUDY: Sample size and variation in the laboratory methods may disturb the robustness of the study, also for a generalised acceptance a multicentric study and further research using different sample size to be done for more detailed information.Also a potential diagnosis may be established by further more detailed research which is also a limitation of this the study.

CONFLICTS OF INTEREST: None

FINANCIAL DISCLOSURE: None

ACKNOLEDGEMENT:
Dr.kausik Bandyopadhyay, Dr. Md. HefjurRahaman, Dr. Debojyoti Bhattacharjee, and all other staffs including medical lab technicians of Department of Biochemistry, Murshidabad Medical College and Hospital, West Bengal.

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