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ROLE OF ACETONITRILE (DIELECTRIC CONSTANT 36) IN ELUTING PRIMERS BY GEL FILTRATION CHROMATOGRAPHY: A METHOD FOR PURIFICATION OF OLIGONUCLEOTIDES.

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ABSTRACT

Introduction: Acetonitrile(ACN) is completely miscible with water and its high dielectric constant and dipole moment makes it suitable solvent for many inorganic and organic compounds. The solvent has a medium polarity and a weak hydrogen bond acceptor. The objective of this study is to know how different dilutions of ACN(Acetonitrile) will affect the concentration and purity of primers eluted through the column. **Method:** Trityl off synthesized B-actin primers in triplicates were eluted through the size exclusion columns which is prewashed with the wash buffer. Different dilutions of ACN were prepared with with a polar solvent to look at the concentration and purity of eluted primers. **Results:** 50% ACN eluted primers were giving an average concentration of 591ng/ul and purity of 1.8(260/280) than that of 100% and 20% CAN eluted primer with an average concentration of 297ng/ul and 189ng/ul and purity of 1.72 and 1.5 respectively. **Conclusion:** Trityl off primers were eluted in higher concentration and purity in 50% ACN as the elution buffer through the gel filtration columns.

KEYWORDS: Gel Filtration Chromatography.Trityl off, Acetonitrile.

INTRODUCTION

Acetonitrile is completely miscible with water and its high dielectric constant and dipole moment makes it suitable solvent for many organic and inorganic compounds. The solvent has medium polarity and a weak hydrogen bond acceptor. ACN is an aprotic solvent because unlike of protic solvents (H+) protons upon dissociation.

AIM

The objective of this study is to know how different dilutions of ACN will affect the concentration and purity of the primers eluted through the column.

MATERIALS AND METHODS

Trityl off synthesized β actin primers, forward (5'GGACTTCGAGCAAGAGATGG3') synthesized in Mermade 12 [Figure 1] deprotected with Ammonium hydroxide reagent 28.0-30.0%(Sigma Aldrich 215-647-6) from the Controlled pore glass. The protecting group were cleaved as per the instructions from the Bioautomation. These protecting groups were then purified from the solution using Bio pure N10 Gel filtration columns (emp Biotek Centripure colums) upto 0.5ml sample volume. [Figure :2]

Figure:1.

Figure:2

The column is stabilized by removing the cap from the top of Centripure N5 column. The excess fluid is allowed to drain via gravity into waste reservoir. The equilibration buffer was made in 3 different concentrations for the specific application and the same has been used for the elution steps. The chosen buffer was Acetonitrile in three concentrations such as 50%,20% and 100%. Each column for the purification is equilibrated with the specific buffer by allowing the buffer to enter the gel bed completely and continue elution until approximately 7ml of buffer has been eluted. The sample (primer forward) trityl off 5' OH group without the di methoxy trityl on the nucleoside (The trityl [Figure: 3] group enhances the hydrophobicity of the primer and need to be cleaved by an acid) and the other protecting groups for the bases G (dmf protected) [Figure 4], C (Ac protected) and A (bz protected) were allowed to enter the gel bed completely. It is studied that the variation in dilution of Acetonitrile in water results in noticeable changes of polarity of mixtures which have observable changes in separation characteristic of sample molecules when mixed with water in the same ratio, the acetonitrile based solution generally has higher strength.

Figure

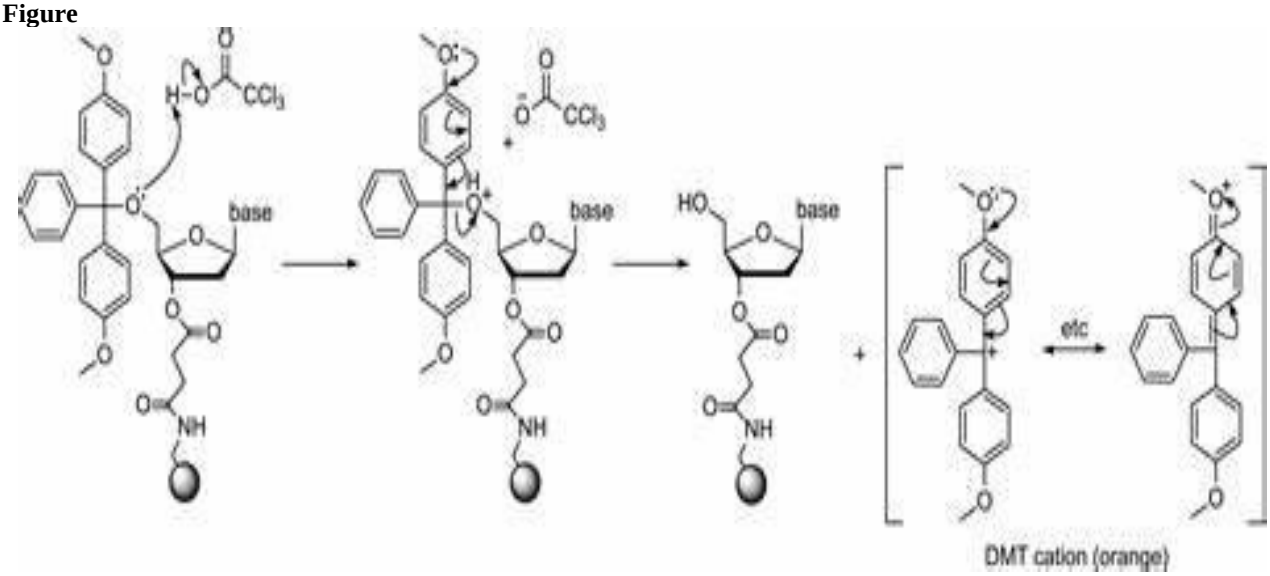

Figure:3.

Dimethoxytrityl electron shift.

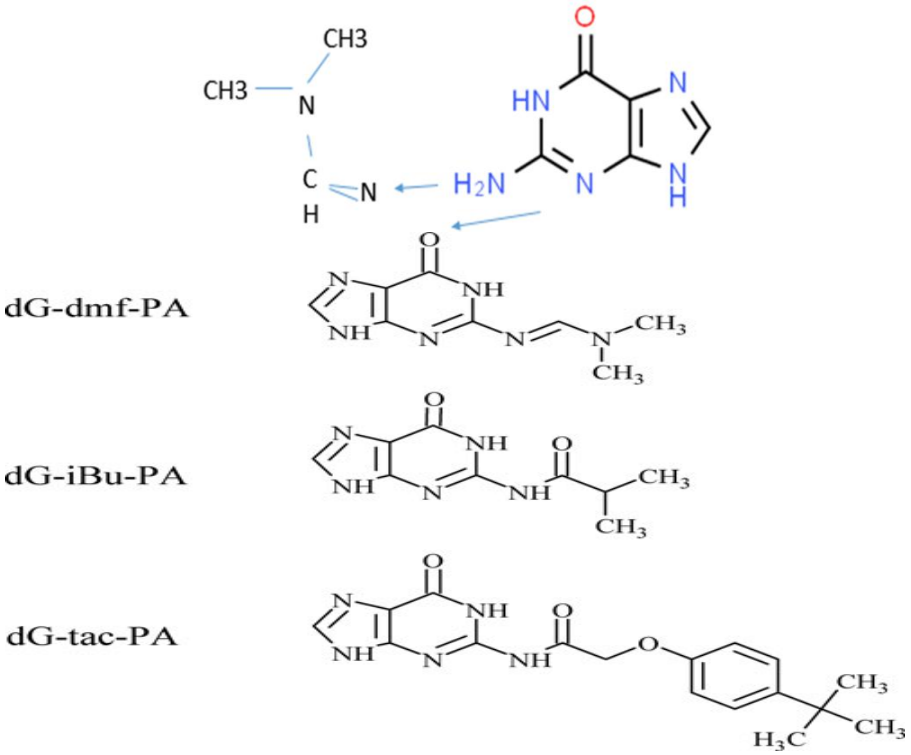

Figure 4: Protected Guanine groups.

RESULTS

50% Acetonitrile diluted primers were giving an average concentration of 591ng/ul and a purity of 1.8(260/280). Remarkably than that of 100% and 20% ACN eluted primer with an average concentration of 297ng/ul and 189ng/ul and purity of 1.72 and 1.5 respectively. (Table:1)

Concentration of Acetonitrile(%)	Primer Concentreation(ng/ul)
100%	297
50%	591
20%	189

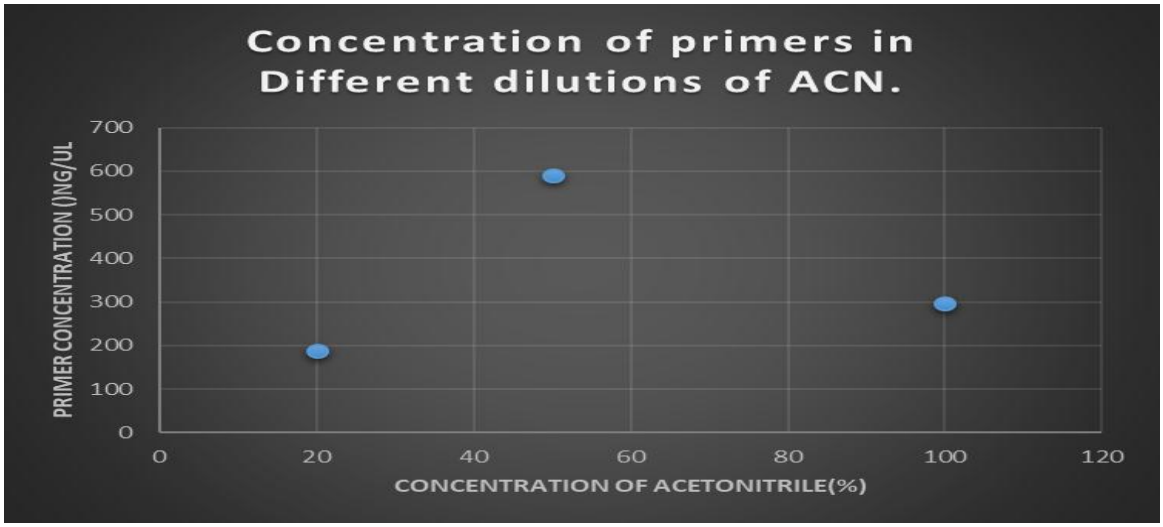

Figure 9: Yield of Primer.

Concentration of Acetonitrile(%)	Purity of primers(OD)
100%	1.7
50%	1.8
20%	1.5

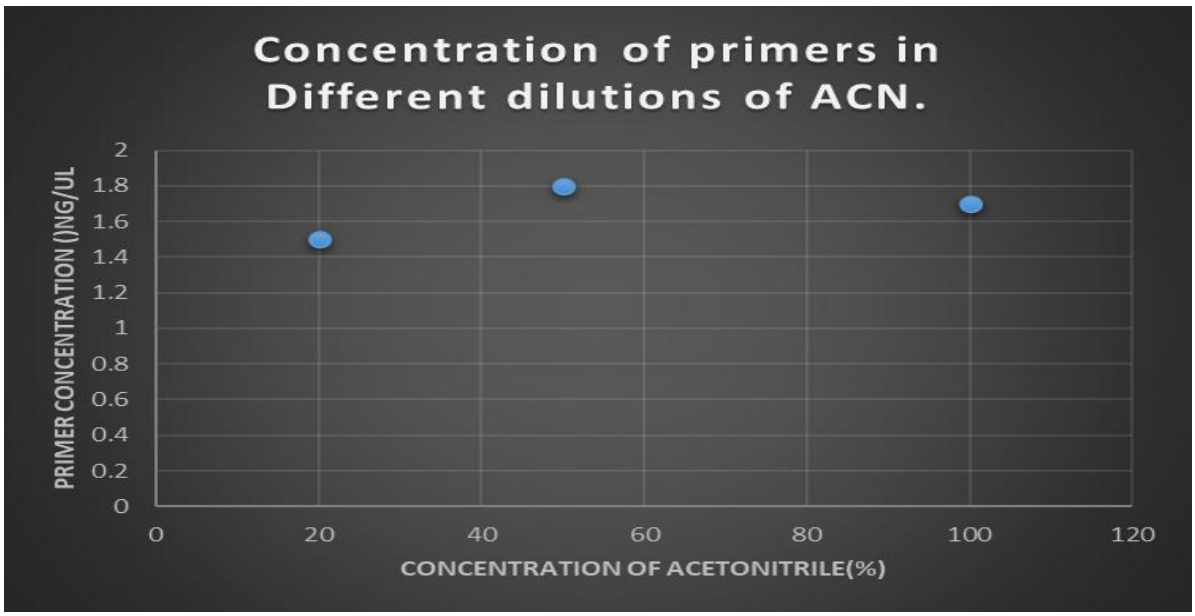

Figure 10: Purity of Primers.

CONCLUSION

Trityl off primers were eluted in higher concentration and purity in 50% ACN (dielectric constant 36) as the elution buffer through the gel filtration columns and the significant increase in the yield and purity of primers were studied and standardized using 50% ACN.

ACKNOWLEDGEMENT

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