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RESEARCH ARTICLE

A NOVEL METHOD FOR KERATIN DISSOLUTION AND TESTING.

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Abstract

Objective: To find a suitable method for dissolution of keratin and performing protein color reactions.

Material and method: 1 gram Keratin powder obtained from animal horn was dissolved in 100 ml, 10% freshly made KOH. Heat was applied for 1-2 minutes to warm the solution. Solutions were then cooled and 10% Acetic acid was added in equal amount. Solution was gently mixed and homogenous solution was prepared. Qualitative protein color were performed. Tests were compared with previously described method of preparing Keratin solution in 40% KOH and heat. Solutions in 5%, 10%,15%,20% and 40% KOH alone and 5%, 10%, 15% and 20% Acetic acid were also tested. 5% and 20% Acetic acid were also used with 10% KOH to see the effect of changes in Acetic Acid concentration.

Results and Observations: Solution of Keratin made in 10% KOH & 10% Acetic acid made a homogenous solution of keratin and gave positive results with all protein color reactions. Ninhydrin test, Hopkincole test, Xanthoproteic test and Millon test was negative with keratin in 40% KOH solution.

Conclusion: 10% KOH and 10% Acetic acid should be used for Keratin dissolution and its protein color reactions.

Introduction:-

Keratin has an antique history in human civilization. Man has used keratin from sheaths of horn to make drinking vessels, fur for clothing, skin of reptiles for leather used in numerous accessories, feather for bedding material and clothing, mammalian yarn for spin yarn, baleen as whalebone, tortoise shell in combs and decorative objects. Hooves have been used as slowly decaying fertilizers.[1] Keratin-rich tissues are important in the wool industry, cosmetics and dermatology.[2]Byproduct of mass-produced poultry has presented a concern for environment.[3]Keratin can provide a tough, fibrous matrix along with ability to flex in many directions without tearing. Toughness of keratinized structure can vary from flexible hair to impenetrable scales. Keratin proteins can be fabricated into films, sponges and hydrogels owing advantages of both natural and synthetic materials involved in tissue engineering and drug delivery applications. Resemblance in amino acid sequence of keratin with Extracellular matrix allows usagein development of biomaterials.[4]

Keratin has been described as difficultly soluble and morphologically inhomogenous. Variousreagents has been tried to solubilize keratin, to use it in many applications. Keratin has been classified in two homogenous families Type I and Type II Keratins.[5]

By analysis of the primary structures of these keratins and other intermediate filament proteins, Hanukoglu and Fuchs suggested a model that keratins and intermediate filaments proteins contain a central ~310 residue domain

with four segments in α-helical conformation that are separated by three short linker segments predicted to be in beta-turn conformation.[5] This model has been confirmed by the determination of the crystal structure of a helical domain of keratins.[6]

Fibrous keratin molecules supercoil to form a very stable, left-handed superhelical motif to multimerise, forming filaments consisting of multiple copies of the keratin monomer.7

The major force that keeps the coiled-coil structure is hydrophobic interactions between apolar residues along the keratins helical segments.[8] In addition to intra- and intermolecular hydrogen bonds, keratins have large amounts of the sulfur-containing amino acid cysteine, required for the disulfide bridges that confer additional strength and rigidity by permanent, thermally stable crosslinking—a role sulfur bridges also play in vulcanized rubber. Human hair is approximately 14% cysteine.[9]

Difficulties in keratin testing needs to be solved and better methods of dissolution and usage of this protein are required. We attempted to develop a new method for making homogenous solution of keratin with 10% KOH and 10% Acetic acid which can give all protein test positive.

Materials and method:-

This experiment was conducted in Department of Biochemistry, PGIMS, Rohtak. Animal horn, sheep wool and human hair were selected for keratin analysis. Horn was finely powdered. Ten percent Potassium Hydroxide and 10 percent Acetic acid solution were prepared. 1 gram of powdered horn was added to 100 ml of 10% KOH to make 1% solution. The solution was labelled as 'A'. Similarly 1% solution of wool and human hair were made and labelled solution 'B' and 'C'. Solution 'A' was heated for 1-2 minutes while rest two were heated to boil in round bottomed flask. After cooling these solution, 10% acetic acid, 100 ml was added to all and PH was recorded with PH strip which was 6-6.5 (8.9). Homogenous solutions were obtained in A, B and C flasks which underwent protein colour reactions. We selected Ninhydrin test, Biuret test, Xanthoproteic test, Sakaguchi test, Millon's test, Hopkincole's test and lead acetate test as protein color qualitative tests. We also analyzed 5% and 20% Acetic acid concentration with 10% KOH to find a suitable combination of reagents for keratin dissolution. To check whether KOH and Acetic acid separately can be equally effective we also made similar solutions with 5, 10,15,20,40% KOH and 5,10,15,20% Acetic acid. pH of these solutions was recorded. (Figure 1) Previously described method for keratin preparation i.e. 40% KOH was also compared.

Results and observations:-

Best homogenous solution was found to be that made from 10% KOH and 10% AA.(figure 1)Solution A, B and C gave all 7 test positive. Solutions made with various concentrations of KOH alone did not gave Ninhydrin test and Millon's test. Weak positive reaction in Hopkincole test was given in 5, 10, 15% KOH and rest tests were positive. Ninhydrin test was also not given by increasing concentration of Acetic acid alone wherebrown ring was found in Hopkincole's test and Rest tests were positive. 10% KOH and 5% Acetic acid, 10% KOH & 15% AA and 10% KOH & 20% AA combinations were tried but keratin dissolution was lesser than 10% KOH & 10% AA combination. Results are depicted in table 1 and 2. Colour reactions are shown in figures 2and 3.

Discussion:-

Keratin is an unique tough protein and it dissolves with difficulty in solution and gives protein color reactions poorly. So this experiment was done to find a suitable method for making keratin solution easily which can give protein color reaction positive.

Keratin is not a single molecule, it is rather a complex mixture of proteins, like Keratins, KFAPs and enzymes of epithelium.[10] Keratins are present in epithelial cells and are characterized by unique biochemical features.[11,12]One of which is resistance to digestion by the proteases pepsin or trypsin and insolubility in dilute acids, alkalis, water and organic solvents. [13,11]Keratins are soluble in solutions with denaturing agents like urea and insoluble in aqueous salt solutions.[11]In aqueous solution, keratins may reassemble intermediate filaments.[11,12] Keratins are classified on the basis of their molecular structure, physicochemical characteristics, the epithelial cells producing them and the epithelial type containing the keratin-producing cells.[11] Various reducing agents used for extracting Keratin from various tissues are thioglycollate, dithiothreitol or mercaptoethanol, which cleave disulfide bonds.[14,15,11]Nature of these solvents indicates that breaking hydrogen bonds is also required between individual protein chains in order to dissolve them. In humans, type I keratins have a pI of 4.9–5.4, whereas type II keratins have a pI of 6.5–8.5.[16]

In bovines, type I keratins have a pI< 5.6 and type II keratins have a pI> 6.0.[17] Keratins that are specific to hair, nail or wool have a pI of 4.7–5.4[18]. The pI of keratins can be altered due to post-translational modifications of their primary structure.[16]

Intra- and intermolecular disulfide bonds are formed in keratins by connecting two sulfhydryl residues of two amino acids (such as two cysteines) enzymatically via the enzyme sulfhydryl oxidase.[19]

Previous methods describe dissolving keratin in 40% KOH and 10% KOH with heat. These methods do not completely dissolve keratin.[20] Moreover by this method keratin fails to give Ninhydrin test, Xanthoproteic test, Millon's test and Hopkincole's test. Modification suggested by some authors for Ninhydrin test is adding few drops of 1% Acetic acid neutralize and then boilingis done with Ninhydrin.[20] Some textbook writes biuret test as negative for insoluble proteins like Keratin.[21]

In the proposed method Keratins are reduced by 10% KOH and thenlittle heat application and cooling is followed by oxidation by 10% Acetic acid which renders keratin solubilized in solution. Other possibility of providing an optimum pH in which keratin solubilizes.. This method makes keratin homogenous solution in few minutes. Other advantages of this method is that it utilizes easily available lab reagents and it is cheaper than other agents mentioned above. Chromatography for keratin can also be done with this homogenization method.

Exact mechanism for this quick dissolution of Keratin in 10% KOH followed by 10% Acetic acid should be explored by further studies.

Figure 1: Homogenous solution of keratin prepared with 10% KOH and 10% AA

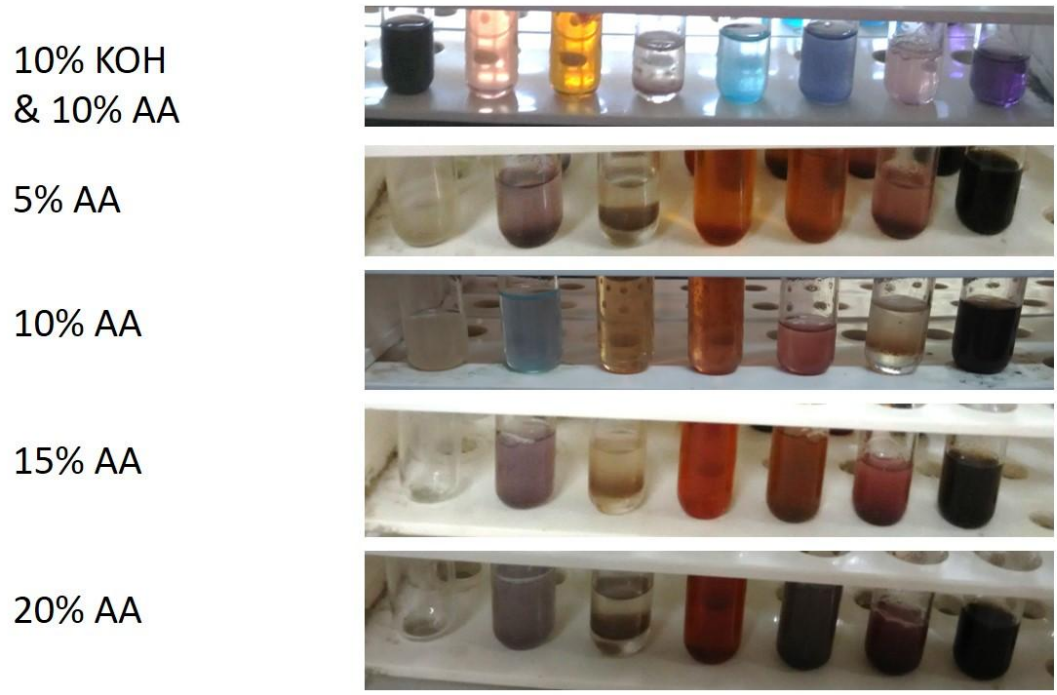

Figure 2: 10% KOH & 10% AA combination compared with increasing concentration of AA alone

Figure 3: 10% KOH & 10% AA compared with increasing concentration of KOH alone

Keratin colour reactions:-

Table 1: color reactions of different proportions of Acetic acid and Potassium Hydroxide.

	10% KOH + 5% Acetic acid (pH 12.69)	**10% KOH + 10% Acetic acid (pH 6.5)**	**10% KOH + 20% Acetic acid (pH 4.76)**
Ninhydrin	-	+	-
Biuret	+	+	+
Xanthoprotic	+	+	+
Sakaguchi	+	+	+
Hopkincole	+	+	+
Millon	+	+	+
Lead acetate	+	+	+

Table 2: color reactions given by different concentrations of KOH and Acetic acid separately

Test name	5% AA pH=3.0	10% AA pH=2.88	15% AA pH=2.79	20% AA pH=2.73	5% KOH pH=12.7	10% KOH pH=13	15% KOH pH=13.2	20% KOH pH=13.3	40% KOH pH=13.6
Ninhydrin	-	-	-	-	-	-	-	-	-
Biuret	+	-	-	-	+	+	+	+	+
Xanthoprotic	+	-	+	++	+	+	+	+	-
Sakaguchi	+	+ on adding 2ml sodium hypobromite	+	Reddish black solution	+	+	+	+	+
Hopkincole	Brown ring	Brown ring	Brown ring	Brown ring	+	-	+	-	-
Millon	+	+	+	+++	+	-	-	-	-
Lead acetate	+	+	+	+	+	+	+	+	+

Conclusion:-

Keratin dissolution methods are required for better applications of Keratins and using 10% KOH and 10% Acetic acid for this can be a good way for this as this is quite cheap, fast and easymethod.

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