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Formulation Development and Evaluation of Cosmeceutical Formulation Containing Keratin Derived from Human Hair

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ABSTRACT

Introduction: Keratin has widely existed in wool, hair, feathers, nails and reptile skin, which is a kind of biological characteristics of excellent and good mechanical properties of biological macromolecules. As a kind of functional material, keratin is widely used in biology, medicine, material and other aspects in recent years.

Aim: This research aims at formulation, development and evaluation of Cosmeceutical formulation using keratin derived from human hairs.

Objectives: The main objectives of this study were accomplished by following step: 1. To develop a Cosmeceutical formulation containing keratin derived from human hair. 2. To evaluate and develop formulation for its stability and efficacy. 3. Comparative evaluation of developed formulation with marketed product.

Methods Used: Extraction, purification and isolation of keratin from human hair.

Conclusion: From all the parameter studies, we concluded that keratin gel for human hair have nourishing activity with hair strength, hair shining and softness to the hair. Favourable formulation texture profile along with viscosity, pH, spreadability and nourishing activity has ensured the quality and stability of the gel as compared with different marketed formulations.

Key Words: Keratin, Formulation, Cosmeceutical formulation, Extraction, Purification, Isolation

INTRODUCTION

The term 'keratin' originally refers to the broad category of insoluble proteins that associate as intermediate filaments (Ifs) and form the bulk of cytoplasmic epithelia and epidermal appendage structures (hair, wool, horns, hooves and nails). These keratins can be classified into two distinct groups ("hard" and "soft") based on their structure, function and regulation. Keratin is a form of protein mainly found in human hair and fills up around 70-85% in human hair. Sulphur is primary component in human hair in the presence of Cysteine (24%) as total amino acid.

Keratin widely exist in wool, hair, feathers, nails and reptile skin, which is a kind of biological characteristics of excellent and good mechanical properties of biological macromolecules. As a kind of functional material, keratin is widely used in biology, medicine, material and other aspects in recent years."Hard" keratin filaments form ordered arrays and are the primary contributors to the tough structure of epidermal appendages.

AIM

The aim of this study is to explore the feasibility of transforming human hair wastes into the functional protein biomaterials. In this paper, the keratin was extracted by reduction method from discarded human hair, sodium sulphide as two disulphide bond reducing agent, sodium hydroxide as stratum corneum disrupting agent, twelve sodium dodecyl sulfate as keratin stabilizing agent. The effects of sodium hydroxide and sodium sulfite amount on the extraction yield of keratin were investigated and the reaction time was also studied. Furthermore, the extracted keratin was analyzed by fourier transform infrared spectroscopy to confirm its structure.

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Methods Used: Extraction, purification and isolation of keratin from human hair.

Research Methodology: Extraction of Keratin from Human Hair

HairsTreatment

a) The human hairs was soaked in ether for 24hours.
b) Under the sunlight wet hairs was dried.
c) Collect all the dried hairs cut in small piece and blend the hairs then keep the blend hairs in the sealed plastic bag carefully.

Dissolving of Human Hair

a) The 2L of 0.5M sodium sulfide solution in the 2L conical flask was prepared.
b) 50g of the blended human hairs was weighted and added to the sodium sulfide solution.
c) Stir the solution for 6 hours and maintained the condition of solution at30°C and pH range of10-12.
d) The solution was filtered to get the supernatant liquid.
e) Placed the supernatant liquid in a beaker and stir the solution.

Preparation of ammonium sulfate solution:

a) 700g of ammonium sulfate was dissolved in 1L deionized water.
b) The solution was stirred until all the ammonium sulfate particles are dissolved.
c) The solution was then filtered to make it particle free.

Protein precipitation:

a) The hair filtrate solution collected earlier placed in a beaker and stirred.
b) Ammonium sulfate solution was added slowly dropwise.
c) The ratio of feather filtrate solution and ammonium sulfate solution added was 1:1.
d) The supernatant precipitates are collected separately.

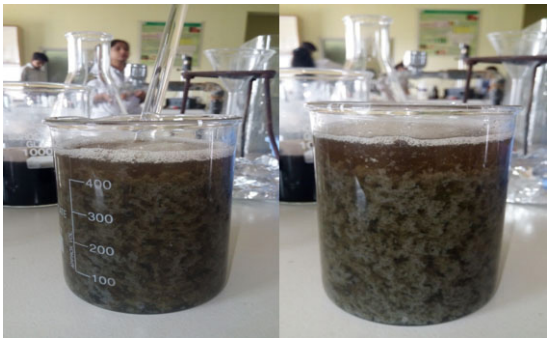


Figure 1: Keratin Formation.

Protein purification:

a) The solid particles collected are added into 100ml of ethanol and maintain pH 12 with 0.1N NaOH solution and stirred. Place this solution on mechanical shaker overnight.
b) Filter the solution to get supernatant liquid and solid particles.
c) Use 0.5N NaOH sodium hydroxide solution to dissolve the solid particles.
d) Centrifuge the solution at 10000 rpm at 5 minutes. Collect the liquid and discard the solids.
e) The liquid sample was dried and collect the keratin crystals.

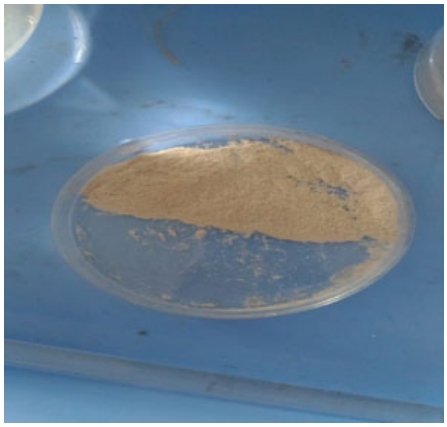


Figure 2: Extracted Keratin Crystals.

Characterization of extracted Keratin:-

1. Protein content
2. UV spectroscopy
3. Fourier transform infraredspectroscopy(FTIR)
4. Thin layer chromatography(TLC)

Determination of Protein Content

Total Protein Content:

Digestion of organic matter with sulfuric acid in the presence of a catalyst, Rendering the reaction product alkaline then distillation and titration of the liberated ammonia, Calculation of the nitrogen content, Multiplication of the result by the conventional factor 6.38 to obtain the crude protein content.

Digestion of sample:

On calibrated weighing balance, weighed accurately 500 mg of test sample on butter paper. Put it in Kjeldahl digestion flask. Added the mixture of 9 gm sodium sulphate and 1 gm copper sulphate in Kjeldahl digestionflask. Added 23-25 ml concentrated sulphuric acid (98%) in Kjeldahl flask and digest the sample on gas burner up to the color changes to green. After digestion, cool the digestion and then cool under running tap water. Added carefully slowly small quantity of water init. Measured 50 ml distilled water + 50 ml 0.1 N HCl in 500 ml titration Flask. Added 2 to 4 drops of methyl red indicator.

Distillation:

Arranged the Kjeldahl's distillation assembly. Joint should be properly fitted with plaster of Paris so that there should not be any leakage of vapors. Keep the titration flask below the condensing coil. The receiver of the coil is dip in the solution. Now added the cooled digested solution in distillation flask. Add 120 mlof 40% caustic solution. Add 400 ml distilled water. Make the complete volume 600 ml by adding distilled water. Stopped the addition and close the cork of addition funnel. Fill the addition funnel with water.

Started heating by switching ON heating mental and collect the distillate in titration flask up to 400ml. Titrated the condensed distillate with 0.1 N NaOH solutions, until the color changes pink to faint yellow as endpoint. Perform blank titration with 50 ml 0.1 N HCL solution and 50 ml distilled water. Add few drop of methyl red indicator. Titrate it with 0.1N NaOH solution until the colour changes pink to faint yellow as endpoint. Calculate exact normality of 0.1N NaOH solution. Calculate the percentage of nitrogen content by following formula.

% Total Nitrogen = (B - S) X 1.4 X 2 X Exact normality of 0.1N NaOH solution.

Where,

B = Blank Titration reading

S = Sample Titrationreading

1.4 = Constant factor for nitrogen

UV spectroscopy

a) Determination of λmax:

Set the spectrophotometer wavelength to 200 nm and with a cuvette containing distilled water to set the instrument reference level. Place the cuvette containing the prepared dilution in the sample compartment record the absorbance. Repeat this step at wavelength increment of 200 nm up to 400 nm and record absorbance at each wavelength setting. Plot the results as absorbance against wavelength. From the graph note the wavelength of maximum absorbance for this solution.

b) Calibration curve of Keratin:

When the UV spectrum of 10mg keratin in 100ml of 0.5 N NaOH to obtained working standard of 100 µg/ml. Aliquots of 0.2 to 1ml from the stock solution representing 2 to 10 µg/ml of drug concentration were transferred to 10 ml volumetric flask and the volume was adjusted up to mark with 0.5 N NaOH and prepared different µg solution was scanned at 400nm to 200nm maximum absorbance was observed.

Fourier Transform Infrared Spectroscopy (FTIR):-

The FTIR spectra of keratin were studied. Above sample were mixed with KBr, The chemical component of Keratin was determined using FTIR spectroscopy to detect the presence of various chemical groups. Shimadzu FTIR (IR affinity 1 Model, Japan) was used for chemical characterization of drug in 4000 and 700 cm-1 wave number range.

Thin Layer Chromatography (TLC):-

Material:

a) Adsorbent: - TLC silica gel 60 F254 Aluminium sheet 20 × 20 cm.
b) Developing agent: - Butanol: Acetic acid: water (6.5: 3.5:1).

Procedure:-

Dissolved 0.1gm keratin in 10ml 0.5 N NaOH. The solvent system that was Butanol: Acetic acid: water. Mobile phase chamber was placed for activation for 1hr. On3 silica plates, small drops was placed and kept in chamber. After solvent run, then dried the plate. After that, placed the plate in iodine chamber for 2 min. Then observed the band of drug. And calculate the Rf value.

Formulation development of cosmeceutical gel:-

Optimization of Hydrated Carbopol 934:-

In-process quality control (IPQC) is a crucial phase in the formulation of keratin protein wound healing gel. 1% gel were prepared by 1gm of Carbopol 934 was added in 100ml distilled water. Hydrolyzed for 24 hours.

Trial formulation of Keratin gel:-

Procedure:-

1. Take Keratincrystal.
2. Triturate with 3 ml of Cremophor RH 40 as a solubiliser in mortar and pestle.
3. Then added 50 gm of hydrated Carbopol934.
4. Added mehylparaben and propyl paraben as a preservative.
5. Added triethanolamine for gel formation and maintain the pH.

Table 1: Trial formulation of Keratin gel

Sr. No.	Ingredient	F1 (50 gm)	F2 (50 gm)	F3 (50 gm)
1	Carbopol 934	50 gm	50 gm	50 gm
2	Keratin	3 gm	2 gm	1 gm
3	Cremophor RH 40	3 ml	3 ml	3 ml
4	Methyl paraben	0.01 gm	0.01 gm	0.01 gm
5	Propyl paraben	0.01 gm	0.01 gm	0.01 gm
6	Triethanolamine	Q. S	Q. S	Q. S

Optimized formulation of 2% Keratin gel

Procedure:

1. Take 1gm of keratin crystal.
2. Triturate with 3ml of Cremophor RH 40 as a solubiliser in mortar and pestle.
3. Then added 50gm of hydrated carbopol934.
4. Added methyl paraben and propyl paraben as a preservative.
5. Added triethanolamine for gel formation and maintain the pH.

Table 2: Optimized formulation of 2% Keratin gel

Sr. No.	Ingredient	For 50 gm
1	Carbopol 934	10 gm (1% w/v)
2	Keratin	0.1 gm
3	Cremophor RH 40	3 ml
5	Methyl paraben	0.01 gm
6	Propyl paraben	0.01 gm
7	Triethanolamine	Q. S

Characterization of formulation:-

1. pH
2. Viscosity
3. Spreadability
4. Extrudability
5. Stability

pH Evaluation:

A definite amount of gel base (1gm) was weighed. The pH of base was recorded using Digital pH Meter (GLOBAL-pH DPH-507).

Viscosity determination:-

The viscosity of the bases was measured using Brookfield Viscometer (DV-E 1 model). The measurement was carried out at 25 ± 1°C, 0.5rpm speed using spindle no. 64 in triplicate.

Spreadability:

The parallel-plate method is the most widely used method for determining and quantifying the spreadability of semisolid preparations by using a parallel-plate extensometer based on the sliding-plates design. The advantages of the method are simplicity and relative lack of expense. Spread ability of cream was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method spread ability was measured on the basis of slip and drag characteristics of gel. An excess of gel (about 1 g`) under study was placed on this fixed slide. The gel was then sandwiched between this slide. To expel air 1 kg weight was placed on the top of the two slides for 5 minutes and to provide a uniform film of the gel. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 gm. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5cm be noted. A shorter interval Indicate better spread ability.

The following equation was used for the purpose:

$$S = m \times \frac{l}{t}$$

Where,

S is the spreadability of the gel formulation,

m is the weight (g) tied on the upper plate,ā

l is the length (cm) of the glass plates,

t is the time taken (s) for the plates to slide the entire length.

Extrudability:

Extrudability is defined as the weight in grams required for extruding 0.5 cm long ribbon of formulation in 10 sec. The gel formulation was filled in aluminum tubes and sealed by crimping to the end. The tubes were placed between two slides and were clamped. 500g weight was placed over the slides and then the cap was removed. The length of the ribbon of the formulation that came out in 10 sec was recorded.

Stability studies of formulations:-

This study was carried out on three types of formulations (F1, F2, F3) by using REMI SC-6 plus stability chamber according to ICH guideline. The stability conditions of 30±2oC / 60±5% RH for 30 days. The physical stability of gel formulations were observed periodically. All three types of formulations were evaluated after one month for the pH, viscosity, TLC, spreadability, extrudability.

RESULT AND DISCUSSION

Characterization of Keratin:

Protein Content:

Blank Titration Reading	:	58.8	ml.
Sample Titration Reading	:	11.1	ml.
Weight of test sample	:	500 mg	

Exact Normality of 0.1 Nm NaOH Solutions: 0.1020N

% of Total Nitrogen : (Blank TR - Sample TR) X

1.4X2 X Exact Normality of 0.1NaOH Solution

% of Total Nitrogen	: (58.8 -11.1) X 1.4X2 X 0.1020
% of Total Nitrogen	: 13.62%w/w.

Protein Content	: Total Nitrogen X 6.38(Protein Factor)
Protein Content	: 13.62 X 6.38

Protein Content: 86.89%w/w.

The protein content was determined by Kjeldahl method. 86.89%w/w protein content was obtained from 500mg Keratin sample.

Thin layer chromatography (TLC) for Keratin:-

Figure 3: TLC plate of Keratin.

Table 3: Result of TLC

Sr. No.	Sample	Developing Solvent	Ratio (ml)	Rf Value	
				Obtained value	Reference value
1.	Keratin	Butanol: acetic acid: water	6.5: 3.5: 1	0.5	0.45

The Rf value of Keratin extract was found to be 0.5 which compared with standard Rf value of Keratin (0.45). The proposed method comprises of mobile phase Butanol: acetic acid: water (6.5: 3.5: 1 v/v) gave a sharp and well defined peak for extracted Keratin.

254 nm 366 nm Day light

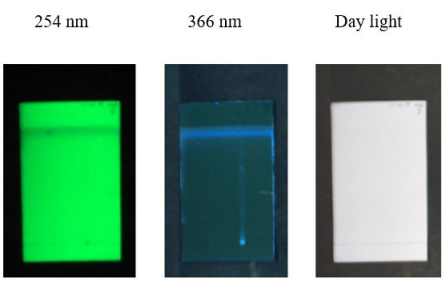


Figure 4: Photos documentation of Keratin extract and Keratin gel.

UV Spectroscopy:-

Graph of absorbance Vs. concentration was plotted and found to be linear over the range of 2 to 10 µg/ml, indicating its compliance with Beers and Lamberts law.

Keratin

Table 4: Graded absorbance of Keratin at λ_{max} 280nm

Concentration (µg)	Absorbance
2	0.056
4	0.108
6	0.172
8	0.224
10	0.280

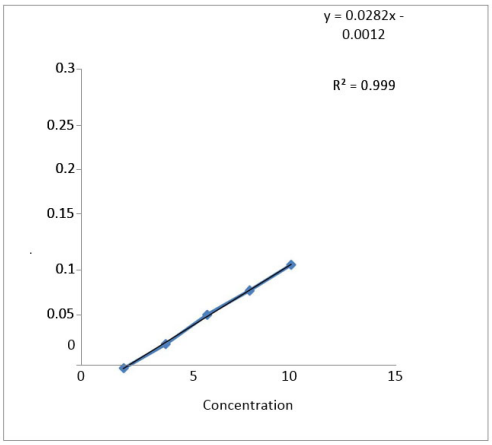


Figure 5: Standard calibration curve of Keratin.

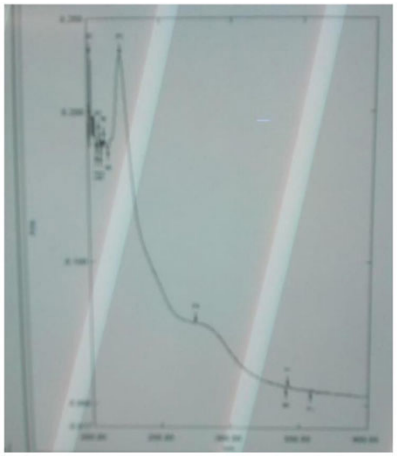


Figure 6: Spectra of Keratin.

Table 5: Detection of λ_{max} and correlation coefficient

Sr. No.	Parameter	Drug (Keratin)
1	Detection of wavelength (λ_{max})	0.280
2	Regression equation	Y=0.00282x-0.012
3	Correlation coefficient	R^2= 0.999

The standard curve followed the Beer- Lambert's law in the concentration range 2 to 10 µl/ ml with R^2 = 0.999 and the λmax were obtained 0.280nm.

1.1.4. Fourier Transform Infrared Spectroscopy study (FTIR):-

Keratin Chemical structure:

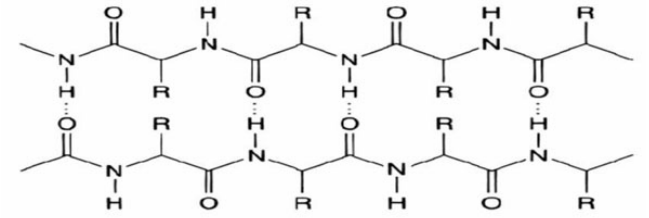
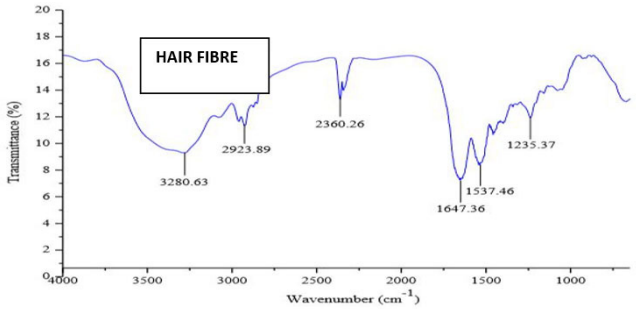


Figure 7: FTIR spectra of reference Keratin.

a) Reference Keratin FTIR



b) Extracted Keratin FTIR

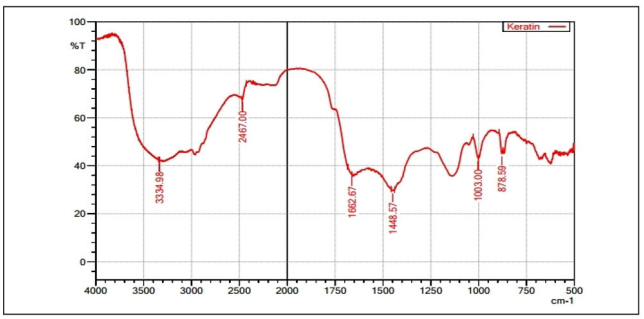


Figure 8: FTIR spectra of Extracted Keratin.

Table 6: FTIR interpretation of reference Keratin and Extracted Keratin

Peaks (cm-1) (Reference Keratin)	Peaks (cm-1) (Extracted Keratin)	Characteristics of functional group	Frequency range (cm-1)
3280.63	3334.98	C-H stretching	3100-3350
2963.89	2467.00	C-H stretching	2850-2970
1537.46	1662.67	N-H stretching	1550-1600
1647.36	1448.57	C=O stretching	1440-1685

Referring to the molecular structure of Keratin following functional group are observed C-H stretching, N-H stretching and there refractive frequency ranges were found to be 2850-2970, 3310-3100 and 1550-1600. From the observation it was calculated that the sample was pure.

Evaluation of Keratin Gel:-

Physical parameters:

Determination of pH, viscosity, extrudability, spreadability:

Table 7: Results of physical evaluation

Sr. No.	Formulation	pH	Spreadability	Viscosity (Cps)	Extrudability
1	A1	6.72	3.25 ± 0.04	17280	++
2	A2	6.92	2.96 ± 0.02	17460	+
3	A3	6.86	3.81 ± 0.01	17690	++
4	A4	7.55	4.18 ± 0.02	17740	+++
5	A5	7.50	4.10 ± 0.03	17690	+++

The formulation A4 shows the pH within the range of standard pH range (7.50 ± 0.05) of the gel and the other formulations shows lower pH. The results of pH measurement are indicated in table 7.2. The spreadability results were calculated in the unit of time among the various gel formulations. All the formulation has shown better spreadability. The formulation A4 shows the good spreadability. The results of spreadability are revealed in table 7.2. The viscosity of all formulations was determined using Brookfield viscometer. The results indicated that formulations were found uniform in consistency. The formulation A4 shows good viscosity.

Stability study

Table 8: Data of optimize formulation after stability study

Sr. No.	Formulation	pH Mean±SD		Viscosity (cPs) Mean±SD		Spreadability (gm.cm/sec) Mean±SD	
		0 Days	90 Days	0 Days	90 days	0 Days	90 Days
1	Keratin gel	7.55±0.15	7.55±0.15	17740±0.5	18010±0.15	4.18 ± 0.02	4.4±0.85

The stability studies were carried out on optimized formulation at 30±2oC temperature and 65±5% RH for 90 days. The formulation showed good stability with no remarkable change in pH, viscosity and spreadability test profile.

CONCLUSION

Safe and effective keratin hair gel formulation of combined was successfully developed. The present investigation revealed that fabrication of keratin showed good cosmetic properties. The developed formulations have good spreadability with neutral pH. The major active components needed for cosmetic activity were identified using TLC, UV, FTIR techniques. Formulation also retained good stability condition over a period of 90days. From all the parameter studies, we are concluded that keratin gel for human hair have nourishing activity with hair strength, hair shining and softness to the hair. Favorable formulation texture profile along with viscosity, pH, spreadability and nourishing activity has ensured the quality and stability of the gel as compared with different marketed formulations.

Acknowledgement: None

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