Int.J.Curr.Microbiol.App.Sci (2017) *6*(10): 3488-3495

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 10 (2017) pp. 3488-3495**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.610.411

In Vitro Evaluation of Natural Keratin Based Hydrogel from Chicken Feather Waste for Controlled Drug Release

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Keywords

Chicken Feathers, Keratin Hydrogel, Drug encapsulation.

Article Info

Accepted:
28 September 2017
Available Online:
10 October 2017

A B S T R A C T

In the present study keratin was extracted from chicken feather using the reductants beta mercaptoethanol, urea and SDS. The extracted keratin was lyophilised and was used to prepare hydrogel. Prepared hydrogel was characterized using SEM and FTIR. The prepared keratin hydrogel was used for encapsulation of tetracycline. The percentage of drug encapsulation in keratin hydrogel was found to be 92.5%. The *in vitro* release of tetracycline from keratin hydrogel was carried out in PBS for 24hrs and was found to be released in a slow and sustained manner.

Introduction

Keratin is a natural protein which has its source in animal hair, feathers, nails, hooves, beaks, claws and horns. The keratin protein is a stable protein which cannot be dissolved in any organic or inorganic solvents and it cannot be cleaved by protease enzymes such as trypsin or pepsin (Goddard and Leonor, 1935).

The stable structural morphology of the keratin protein makes it highly a versatile protein. The keratin protein can be manipulated into any bioproduct including films, scaffolds, sponges, nanoparticles and hydrogel. They have the ability to self-assemble themselves to form a stable structure or morphology.

Keratin protein can be extracted from many source, one of the abundant source is chicken feathers. The chicken feathers possess 91% of keratin protein (Khosha and Ullah, 2013). The chicken feather discards are generated into the environment in tones. The chicken feathers are dumped in the lands cause environmental issues such as production of methane or greenhouse gases and pollution of the ground water sources (Staron *et al.*, 2014; Evans and Vance, 2007). It needs some effective management system.

Hydrogels are three-dimensional network structures or polymeric structure which can absorb and retain significant amount of water. Usually it is made of natural or synthetic

polymers as a structural backbone (Syed *et al.*, 2011). These structures are a group of hydrophilic domains together bonded or cross linked in an aqueous environment. Because of these properties, hydrogel are used as a biomaterial in the field of biomedical engineering and also used in the field of tissue engineering, regenerative medicine, controlled drug delivery, biomaterial science, and polymer system studies (Enas, 2015).

The researchers in the biomedical field are in search of a suitable drug delivery system which could able to deliver the drug in a controlled manner and also reach the targeted site of infection more specifically.

Keratin proteins are self-assembling proteins and they can polymerize into a complex three dimensional structures like hydrogel and cell scaffold. Hence the present study focuses on the preparation of keratin hydrogel from chicken feather and to encapsulate the hydrogel with drug for controlled drug release studies.

Materials and Methods

Pre-treatment of feathers

Chicken feathers were collected from poultry shop, Vepery, Chennai. The chicken feathers were cleaned well with water and detergent for the removal of blood stain and other impurities. Cleaned feathers were washed again with water and 70% ethanol. The cleaned feathers were dried under sunlight. After drying the feathers were cut into small pieces and stored for further use.

Extraction and estimation of keratin

About 0.6 g of pre-treated feathers was added into 25mL of aqueous solution containing 8M urea, 1.66M 2Mercaptoethanol and 0.17M Sodium dodecyl sulphate (Akira Tachibana *et al.*, 2002; 2005) and kept at 60ºC water bath for about 4 hrs. After 4 hrs of incubation, the mixture was allowed to cool and filtered using No 1 Whatman filter paper (GE health care UK limited) and was lyophilized. The keratin extracted from chicken feather was estimated by Lowry's method (1951)**.**

Preparation of keratin hydrogel

50µL of 1X phosphate buffer was added to 40mg of keratin gel powder**.** Hydrogel formation was observed by the addition of phosphate buffer. Obtained keratin hydrogel was further characterized and used for drug encapsulation.

Characterization of keratin hydrogel

Keratin hydrogel was characterized using Scanning Electron Microscope (SEM) and FTIR.

Encapsulation of hydrogel with tetracycline

0.37mg of tetracycline suspended in PBS was added to 40mg of keratin gel powder. The hydrogel was allowed to equilibrate in tetracycline solution for 24 hours. After 24 hours the drug loaded hydrogel was used for drug release studies (Das Manali and Nirada Devi, 2015; Gabriela Buhus *et al.*, 2009).

The following formula is used to find the percentage of drug loaded

$$DL = \frac{W1 - W2}{W2} \times 100$$

W1-Weight of the drug loaded hydrogel
W2-Weight of the dry hydrogel
DL-Percentage of drug loaded

In vitro drug release studies

The drug loaded hydrogel was immersed separately in 10mL of distilled water. At

regular time intervals the medium was taken out and the absorbance was read at 420nm (Das Manali and Nirada Devi, 2015; Gabriela Buhus *et al.,* 2009). The absorbance values were used to determine the concentration of drug released from keratin hydrogel. The standard curve was plotted using the known concentration of the tetracycline and their respective optical density.

Results and Discussion

Estimation of keratin hydrogel

The amount of keratin extracted from chicken feather was found to be 0.58 mg/ml.

Characterization of keratin hydrogel by scanning electron microscope (SEM)

The keratin hydrogel prepared was characterised using SEM. The SEM images (Fig 1) showed the structure and morphology of the hydrogel. The images revealed the internal structure of the hydrogel. SEM revealed the presence of pores in the hydrogel and the cross linking of the keratin. Sizes of pores were uniform. The sharp peak like structures was observed in the keratin structures, which were self-assembled and crosslinked to form the pores.

FTIR spectrum of keratin extract

The keratin extract obtained showed peaks (Fig 2) between $1701cm^{-1}$, $1699cm^{-1}$ to $1600cm^{-1}$ and it showed another peaks between $1560cm^{-1}$to $1500cm^{-1}$, it Proved the presence of both amide 1 and 2 group. There were four peaks between $1656cm^{-1}$ to $1650cm^{-1}$ and four peaks between $1550cm^{-1}$ to $1541cm^{-1}$ which proved the presence of alpha helical structure. The keratin extract also showed peaks between $1635cm^{-1}$ to $1614cm^{-1}$ and peaks between $1535cm^{-1}$ to $1521cm^{-1}$. These peaks confirmed the presence of beta sheets in it. The number of peaks observed was more in the beta conformation range. Therefore the conformation contains larger distribution of beta sheets in its protein morphology.

FTIR of keratin hydrogel

The peaks obtained in the keratin gel were matched well with the keratin extract spectrum. The slight changes in the keratin gel spectrum was observed due to the cross linking of the disulphide bonds or reformation of bonds to form a polymeric hydrogel.

The hydrogel powder showed various peaks (Fig 3), which confirmed the predominant presence of beta sheets and less distribution of alpha helix. The peaks were quite elevated in the keratin extract between the region $2265cm^{-1}$ to $125cm^{-1.}$ The keratin gel didn't show any sharp peak as it was in the keratin extract in that particular range. The peaks between the range $3288cm^{-1}$ to $3309cm^{-1}$ proved the presence of the hydrogen bonded amine group. The high frequency of hydrogen bond and nitrogen group proved that the structure was more folded. Hence the more folded structures in keratin hydrogel were obtained. The comparison between keratin hydrogel and keratin extract proved that the formed product was keratin hydrogel.

Drug encapsulation

The percentage of drug encapsulated in keratin hydrogel was found to be 92.5%.

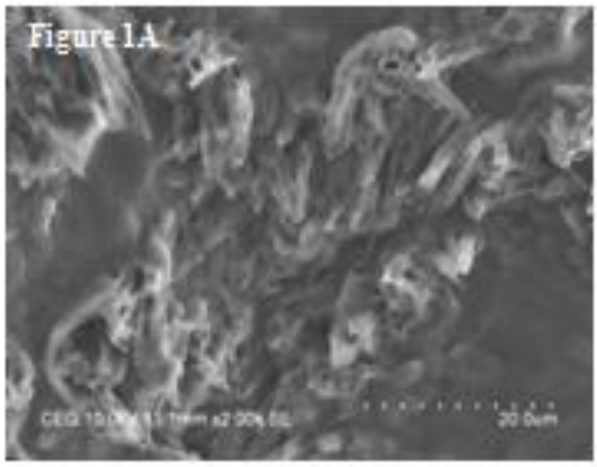
In-vitro drug release studies

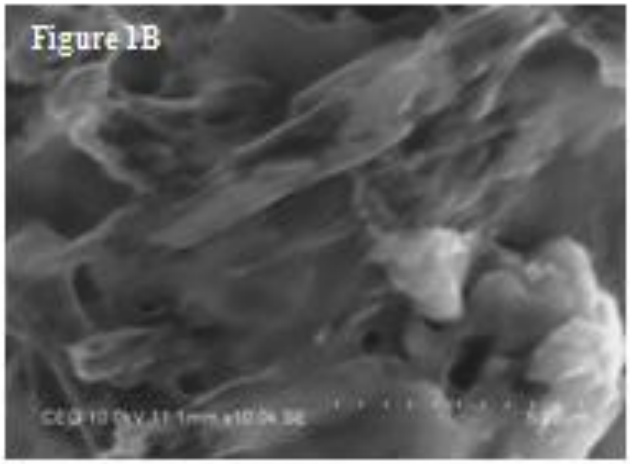
From the standard graph of the tetracycline, the concentrations of the drug released at different time interval was determined.

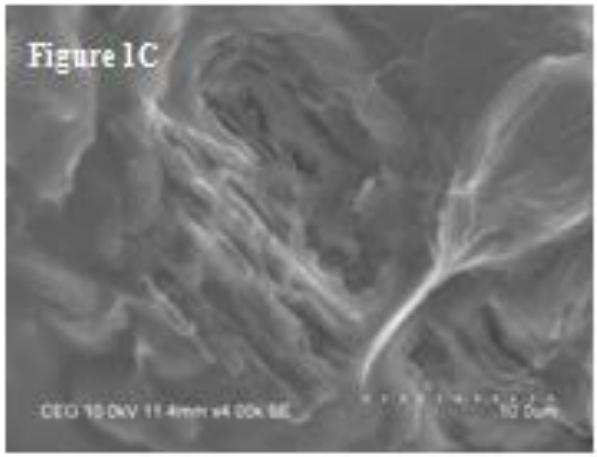
The concentration of the drug released per hour was very minimal and the release was

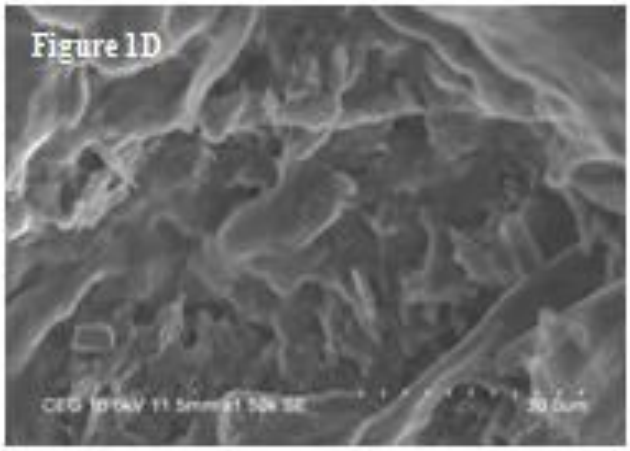
carried out in a controlled manner. The drug release was constant throughout and hence this hydrogel was proved to be an efficient drug delivery system. The constant change in concentration of drug released is shown in fig 4.

Fig.1A, B, C, D and E SEM images of keratin hydrogel









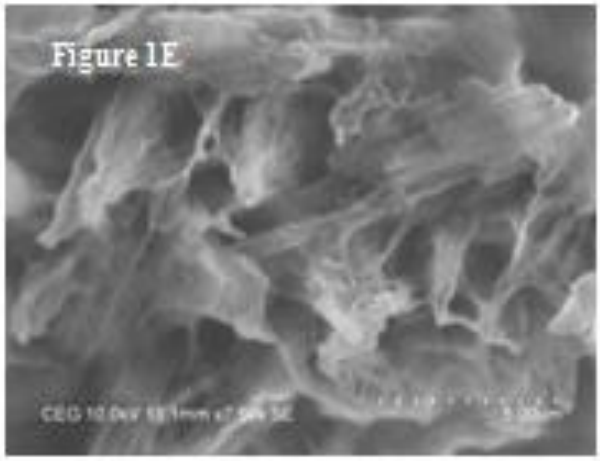


Fig.2 FTIR spectrum of keratin hydrogel

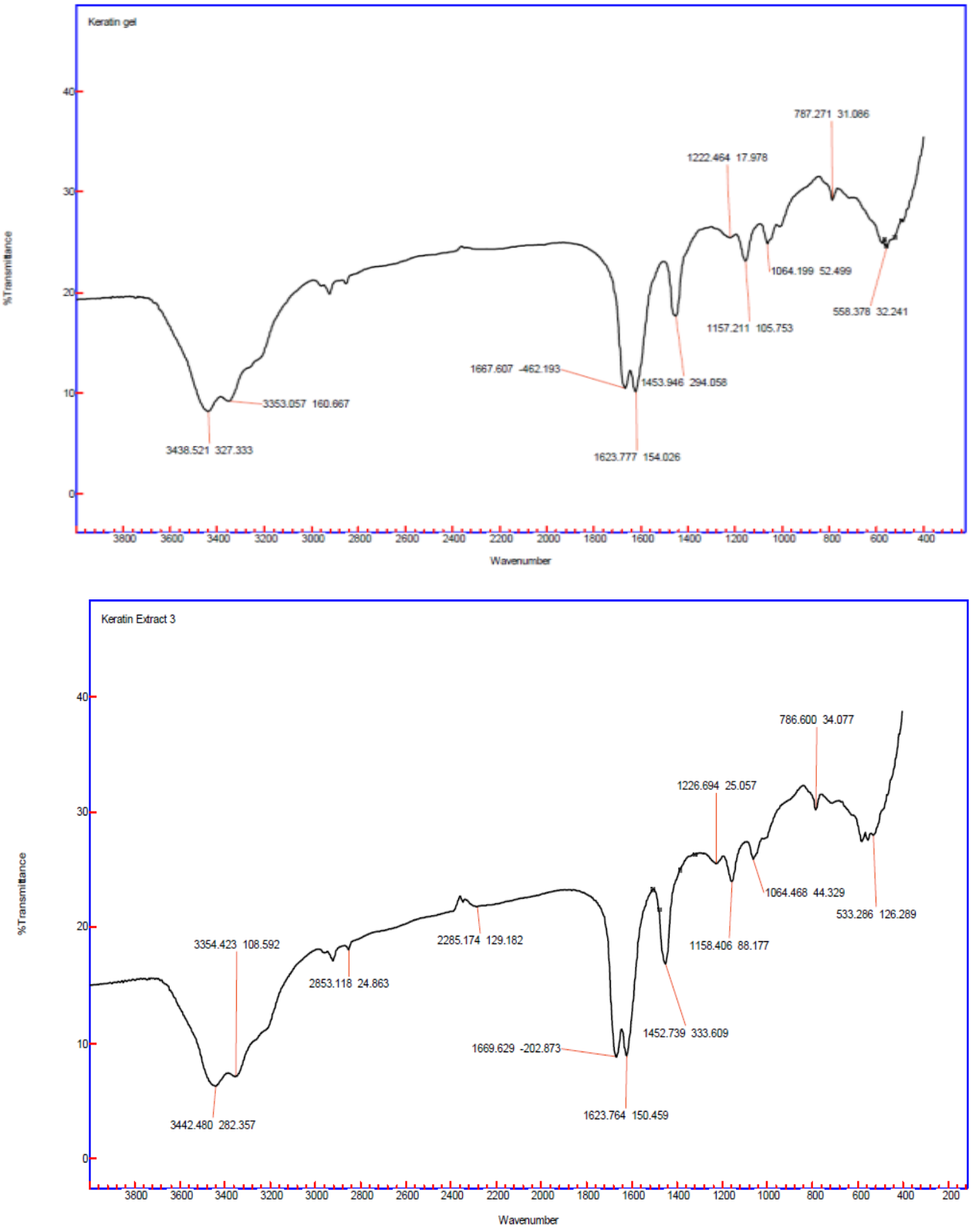
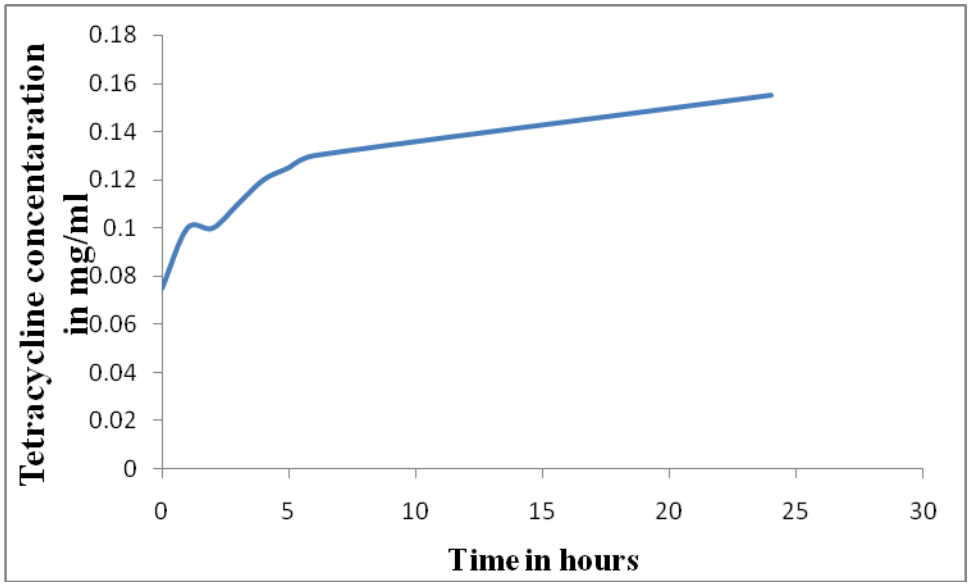


Fig.3 *In vitro* release of tetracycline from keratin hydrogel



Here in our study keratin protein is used for the hydrogel preparation. The keratin obtained is more stable and can remain stable in extreme pH. It has the ability to re-crosslink the cysteine groups by oxidative coupling. The biomaterials formed from keratin are stable and can survive for months (Silva *et al.,* 2014).

The keratin protein is extracted from the source using different sulfhydryl reductants. The sulfhydryl reductants like Dithiotreitol, tris (2-carboxyethyl) phosphine are reported by Elise Burmeister Getz *et al.,* (1999). The keratin from chicken feathers can be extracted by employing reducing agents such as potassium cyanide, sodium sulphide and ammonium thioglycolate. Arun Gupta *et al.,* (2012) has compared the keratin extracted from these three reducing agents to determine the efficient reducing agent that could possibly yield a high amount of keratin protein from chicken feathers.

However the most widely used reductant is beta mercaptoethanol. Mercaptoethanol effectively breaks disulphide bonds in protein and inhibits the oxidation of free sulfhydryl residues thereby maintaining the protein activity (Elise Burmeister Getz *et al.,* 1999). In this study three reducing agents like beta mercaptoethanol, urea and sodium dodecyl sulphate were used in extraction for the effective yield (Akira Tachibana *et al.,* 2002; 2005). The Sodium Dodecyl Sulphate is an anionic surfactant that can able to break the non-covalent bonds and protein linkages. Urea is also considered as protein denaturant that reduces the non-covalent bonds (Elise Burmeister Getz *et al.,* 1999).

Akira Tachibana *et al.,* has reported this method of reduction for reducing wool. However in the present study, these reducing agents were used for chicken feathers (Akira Tachibana *et al.,* 2002; 2005).

The three major types of preparation of hydrogel are Chemical crosslinking, Physical crosslinking and radiation crosslinking (Syed *et al.,* 2011). So far the keratin hydrogel preparation is done by all the three methods. Cardamone *et al.,* (2013) prepared a wool based keratin hydrogel by oxidation and reduction hydrolysis method where a number of oxidising and reducing agents are used to

prepare a keratin hydrogel. Mira Park *et al.*, (2013; 2015) has prepared both wool based and human hair based keratin hydrogel by irradiating electron beam at different ranges and also studied the stability of the protein gel. Though the hydrogel is stable the method of preparation involves electron beam for the crosslinking of the gel.

Rabiatul Adawiyah Binti Zaukifli (2012) has prepared keratin hydrogel by adding poly vinyl chloride to the extract using freeze thaw method. Silva *et al.,* (2014) has prepared the keratin gel using physical pressures such as pressure driven extrusion and sonochemical method. Sujuan Pan *et al.*, (2015) has designed a hydrogel by graft polymerisation technique using methacrylic acid, N N bis acralamide and agar.

However in this study, the gel like structure is formed during the extraction process itself. The disulphide bridges were broken and formed a gel like structure. The gel structures or hydrogel was lyophilized into powders and they are restructured into a gel by adding phosphate buffer saline. This method of hydrogel preparation is simple, cost effective and efficient. The other authors have also used Phosphate buffer saline for hydrogel preparation but the method of extraction is by partial oxidation and reduction and the source is from human hair (Lauren *et al.,* 2014).

The keratin hydrogel prepared by using chicken feather waste was found to be suitable for encapsulation of drugs and also to release drug in a controlled manner.

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How to cite this article:

Sakthi Chandini, D., M. Charulatha, R. Legadevi and Meignanalakshmi, S. 2017. *In Vitro* Evaluation of Natural Keratin Based Hydrogel from Chicken Feather Waste for Controlled Drug Release. *Int.J.Curr.Microbiol.App.Sci.* 6(10): 3488-3495.
doi: https://doi.org/10.20546/ijcmas.2017.610.411