Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading

Thermal characterisation of PEGylated mucin

Momoh MA*, Adikwu MU, Ibezim CE, Ofokansi KC, Attama AA

Department of Pharmaceutics, University Nigeria of Nsukka, Enugu State

ARTICLE INFO

Article history:
Received 17 April 2010
Received in revised form 7 May 2010
Accepted 29 May 2010
Available online 20 June 2010

Keywords:
PEGylation
Mucin
Matrices
DSC

ABSTRACT

Objective: To investigate the characteristics of PEGylated mucin and its potential usage. **Methods:** Mucin was extracted from giant African land snails and PEGylated mucin was prepared with different ratios of PEG 2000-Mwt and mucin (1 : 1, 0 : 1, 2 : 1, 1 : 3 and 3 : 1 to form batch A-E) using solvent technique. The physicochemical properties of mucin were identified and the solubility of mucin was assessed. The thermal properties of PEGylated mucin were measured by differential scanning calorimetry (DSC). **Results:** Carbohydrates, proteins and trace amounts of fats were present in snail mucin. The mucin powder was water-soluble at 30℃ and more water-soluble at 35 ℃, but not soluble; in acetone, ethanol, 0.1 M NaOH, 0.1M H_2SO_4 and 0.1 NH_4OH was water-soluble. The melting point T_m ranged from 58.58 ℃ to 61.17 ℃, crystallization temperature Tc 37.08 ℃ to 39.83 ℃, and glass transition temperature Tg 126.85 ℃ to 138.39 ℃. The variation in T_m, T_c, and T_g with the composition in the PEGylaton showed that an interaction between PEG and mucin occured. **Conclusions:** This result can serve as a basis for further evaluation of the PEGylation method and be used for drug delivery.

1. Introduction

PEGylation, generally described as the molecular attachment of polyethylene glycols (PEGs) with different molecular weights to active drug molecules or surface treatment of drug-bearing particles with PEGs, is one of the most promising and extensively studied strategies with the goal of improving the pharmacokinetic behavior of the therapeutic drugs[1]. A variety of PEGs, both linear and branched, with different molecular weights have been exploited successfully for use in this procedure in the form of reactive PEG species. Both reversible and irreversible PEG-drug conjugates have been prepared with relative advantages/disadvantages. The main pharmacokinetic outcomes of PEGylation are summarized as changes occurring in overall circulation life-span, tissue distribution pattern, and elimination pathway of the parent drug/particle[2]. Based on these favorable pharmacokinetic consequences leading to desired pharmacodynamic outcomes, a variety of proteins/peptides as well as small molecule drugs have been PEGylated and evaluated successfully. Also a number of corresponding products have been approved by the Food Drug Adminisctration (FDA) for specific clinical indications and some others are underway.

Many biopharmaceuticals suffer from performance problems due to short half lives, immunogenicity, and poor solubility and stability[3]. Clinically proven PEGylation Technology can improve performance and dosing convenience of peptides, proteins, some water soluble drugs, antibodies, oligonucleotides and many small molecules by optimizing pharmacokinetics, increasing bioavailability, and decreasing immunogenicity and dosing frequency. Nektar Advanced PEGylation Technology also can increase therapeutic efficacy by increasing drug concentration, improving biodistribution, and prolonging dwell time at the site of action[4]. As a result, therapeutic drug concentrations can be achieved with less frequent dosing and significant benefit to patients with injection and long time medication especially diabetes management treatment[5]. Hence, this study was aimed at investigating the mixing characteristics of PEGylated-mucin by differential scanning calorimeter (DSC).

2. Materials and methods

2.1. Materials and reagent

PEG Mw-2000, snail, acetone, DSC machine. All other chemical are of analytical grade. Distilled water were freshly prepared and used for the study.

2.2. Extraction of snail mucin (slime)

After procurement, the shells of the giant African land

*Corresponding author: Dr. Momoh MA, Department of Pharmaceutics, University Nigeria of Nsukka,Enugu State.
Tel : 08037784357
E-mail: jointmomoh@yahoo.com

snails were knocked open at the apex and a spirally coiled rod was inserted to remove the fleshy body from where the excretory parts were removed. The fleshy parts were then placed in 250 mL of water and washed several times until the (slime) mucin was completely washed off. These washings were pooled together in a plastic bucket, precipitated with chilled acetone and lyophilized in a lyophilizer. The greyish-brown lyophilized flakes of the snail mucin were pulverized into fine powder using a mortar and pestle and stored in an airtight container until used.

2.3. Preparation of PEGylated-mucin

Mucin and PEG were mixed in the following ratios: 1 : 1, 0 : 1 , 2 : 1, 1 : 3 , 3 : 1 to form batch (A-E). A known quantity of mucin and polyethylene glycol were weighed and put in a separate 100 mL baker. A total of 20 mL of solvent (water) were added into each beaker and were allowed to hydrate for 72 h. The solution were later mixed together and allowed to stand for another 72 h, this was to give room for possible interactions and bond formation. The mixture was precipitated with chilled acetone. The precipitate was collected, dried, pulverized and kept in a tight container until used. This method was applied to all the batches.

2.4. Solubility profile of snail mucin

The solubility of snail mucin in several solvents was determined by dispersing 100 mg of the snail mucin in definite volume of each solvent- acetone, ethanol, water, sodium hydroxide, hydrochloric acid and ammonium hydroxide at different temperatures (27 ℃, 35 ℃, 40 ℃).

2.5. Physiochemical properties of snail mucin

The physiochemical properties of the mucin were carried out using the standard analytical method as described in our previous work[6].

2.6. DSC study

In order to characterize the thermal behavior of the polymer, DSC was performed by using a DSC821e (Mettler Toledo, Greifensee, Switzerland) equipped with a refrigerated cooling system (RCS). The system was cali brated by an indium standard. Approximately 5 mg of PEGylated mucin or polymer was weighed in aluminum pans and sealed. The calibration pan was the hermetically sealed type, and heating (tempearture rise) was 5 degrees per min from 20 to 220 degrees and then cooled back to 20 i.e. two way process. The results were expressed as the mean of two independent measures.

3. Results

The physicochemical test showed copiously present of carbohydrate, rich of protein and trace amount of fat.

The solubility test on the mucin at various temperature showed that at 30 ℃ and 35 ℃, the mucin powder were soluble, and more at 35 ℃. In acetone, ethanol, 0.1 M NaOH, 0.1M H_2SO_4 and 0.1 NH_4OH, it was not soluble at the temperature tested. Besides, Figures 1-5 showed the results of the thermographs of the various formulation and peaks, transitions temperature and crystallization state. Double peaks (exothermic and endothermic) were very obvious in the formulations that contained mucin-PEG as compare to sample B with only one peak that served as a control as it contained only mucin without PEG. This indicated the basic variation and the role of PEG in the preparation.

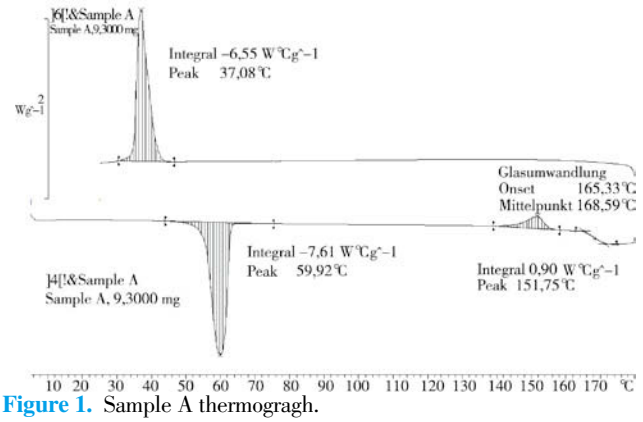

Figure 1. Sample A thermogragh.

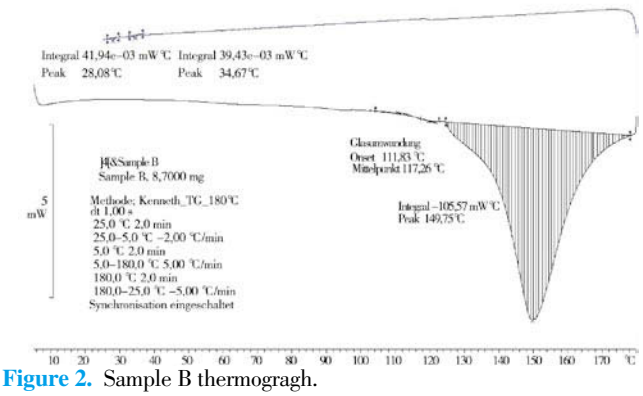

Figure 2. Sample B thermogragh.

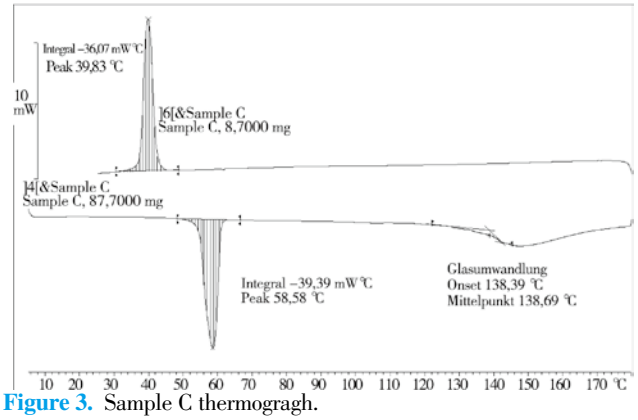

Figure 3. Sample C thermogragh.

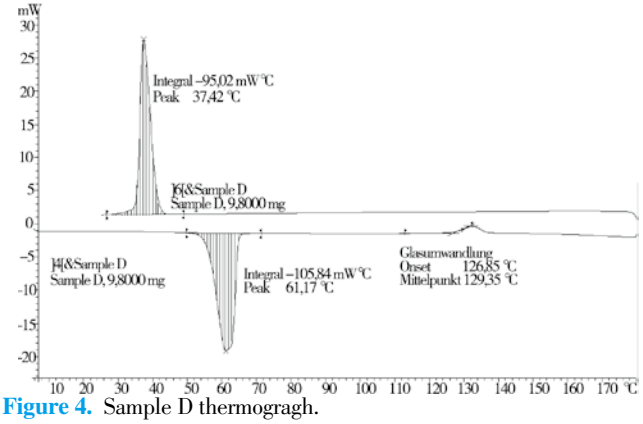

Figure 4. Sample D thermogragh.

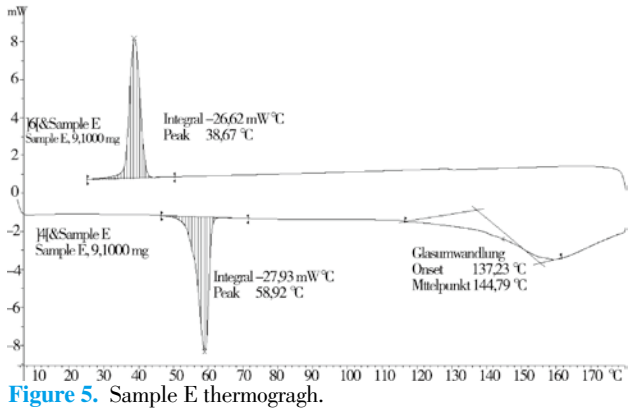

Figure 5. Sample E thermogragh.

4. Discussion

Results of some physiochemical tests performed on the snail mucin showed that carbohydrates, proteins and trace amounts of fats. In both wet and dry states, the extracted mucin was light-brownish in colour, tasteless with a pleasant meaty odour. Snail mucin when dispersed in water gave a slight viscous dispersion. This is unlike gelatin – a typical purified animal protein that swells in cold water with a resultant colloidal solution when heated. The snail mucin is not soluble in ethanol, acetone, 0.1 M sodium hydroxide, ammonium hydroxide and sulphuric acid. Research have shown that snail mucin contained both soluble and insoluble fraction, the soluble fraction gave the brownish colour as observed in this study[7].

The various formulations show that there are different peak formations and the melting point was also different. The DSC was used to detect evidence of good PEGylation and molecular bond interaction between mucin and poly(ethylene glycol-2000) for possible drug delivery. The DSC curves show an initial characteristics endothermic peak which represent a melting point of the polymer, and show a closed temperature to the glass transition temperature of A-E respectively. The initial endothermic curve in all the batches with exception of mucin alone (sample B) were very sharp with narrow width. The T_m range from 58.58 ℃ to 61.17 ℃ only sample B gave high temperature value of 149.75℃, this is an indication that there are sign of impurity or the substance is partially crystallised as shown in the size of width of the peak. The crystallization temperature T_c ranged from 37.08 ℃ to 39.86 ℃ in all the sample. And the variation were not significant at $P>0.05$. The mucin (sample B) alone shows a double peaks. All samples, except sample B, show a very good exothermic sharp peak curve which represents the crystallization phase. It indicates a transitional change in the phase of the polymer. The curve in all the polymer matrices showed that both the PEG and mucin have an interaction, although weakly in nature because of some of the batches that show a small second peak as in sample B. This further suggests that the hydrogen-bonding interactions are responsible for the binding during PEGylation as shown in peak A, C, D and E. The weaker mucin interaction in the PEGylation exercise, however, allow for PEG coated surface to adhere to surface-bound mucin in the presence of soluble mucin when they are combined. Such characteristics are particularly desirable for oral drugs delivery carriers[7].

In all the batches evaluated using DSC curve, glass transition temperature ranges from 126.85 ℃ to 38.39 ℃ except mucin with a low T_g of 111 ℃. It gave an indication that there was possible bond formation between the mucin and the PEG (Mw 2000) which would automatically give birth to new polymer. Studies show that there is obvious hydrogen bond interaction between the mucin and the PEG-2000[8]. Researchers have shown that PEGylation has the potential to increase treatment distances to more than a centimetre, which may be sufficient to prevent the recurrence of human brain tumours. Since polyethylene glycol is known for its biocompatibility and its ability to solubilise hydrophobic molecules in water, it is believed that PEG-drug conjugates show great potential for improving the brain distribution of drug released from controlled-release polymers[9].

In a related study PEGylation has already been used to improve the delivery of interferons in the treatment of hepatitis and drugs to support blood function in patients undergoing cancer chemotherapy, and is being evaluated for use with a number of experimental therapies[10]. The comparative high T_g obtained for mucin as compared with mucin-PEG is an indication of new polymer formation. Mucin is a glycoprotein made up of N-glycosidic linkages of peptide chains and also a carbohydrate moiety. The functional groups of amino acid back bone of the peptide chain may bind to a large functional group of the PEG via some weak forces of interaction like hydrogen and van der Waal forces to create a large polymer PEGylation with less flexible structure. The high chain rigidity thus requires a high temperature to cause the second order transitional change in the polymer thus resulting in high transition temperature. The second endothermic peak is a melting peak with characteristic onset approximately equal to that of mucin. This result can serve as a basis for further evaluation of the PEGylation method and use as drug delivery. The finding in this study can serve as a building block for further evaluation of the PEGylation technology and it possible use in the pharmaceutical industries for target site drug delivery.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1]Fee CJ. Size-exclusion reaction chromatography (SERC): A new technique for protein PEGylation. *Biotechnol & Bioeng* 2003; **82**(2): 200-6.
[2]Fee CJ, Van Alstine JM. PEG-proteins: Reaction engineering and separation issues. *Chem Engineering Sci* 2006; **61**(3): 924-39.
[3]Matsushima A, Hiroto M, Nishimura H, Ishii A, Ueno T, Inada Y, et al. Pegylation of proteins and bioactive substances for medical and technical applications. *Progr Polymer Sci* 1998; **23**(7): 1233-71.
[4]Morar AS, Schrimsher JL, Charez MD. PEGylation of proteins: A structural approach. *Biopharm International* 2006; **19**(4): 34.
[5]Abuchowski A, McCoy JR, Palczuk NC, van Es T, Davis FF. Effect of covalent attachment of polyethylene glycol on immunogenicity and circulating life of bovine liver catalase. *Biol Chem* 1997; **252**(11): 3582-6.
[6]Momoh MA, Adikwu MU. Evaluation of oral insulin delivery using bovine mucin in non-diabetic rats. *J Pharm Res* 2008; **7**: 5-9.
[7]Sandberg T, Carlsson J, Ott MK. Mucin coatings suppress neutrophil adhesion to a polymeric model biomaterial. *Microscopy Res Techn* 2007; **70** (10): 864-8.
[8]Cooper A. Thermodynamic analysis of biomolecular interactions. *Curr Opin Chem Biol* 1999; **3**: 557-63.
[9]Cooper A. Microcalorimetry of protein-DNA interactions. In: TraversA, Mbuckle M. *DNA-protein interactions*. Oxford: Oxford University Press; 2000, p. 125-39.
[10]Cooper MA, Nutley A. Wadood, differential scanning microcalorimetry. In: HardingSE, Chowdhry BZ. *Protein-ligand interactions: hydrodynamics and calorimetry*. Oxford: Oxford University Press; 2000, p. 287-318.