DETERMINATION OF HYDROPHILE-LIPOPHILE BALANCE (HLB) OF BOVINE MUCIN FOR POSSIBLE EMULSIFYING PROPERTIES

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ABSTRACT

The Hydrophile-Lipophile balance (HLB) of bovine mucin was evaluated. Mucin was processed from the small intestine of freshly slaughtered cow via precipitation with chilled acetone, air-drying and pulverization. Series of emulsion were formed with bovine mucin and paraffin oil, in varying ratios, the most stable emulsion with the least creaming level was found to be mucin-oil ratio of 1:9, after a period of 7 days and the HLB value of mucin calculated according to standard methods. The HLB value of mucin was 8.4. These HLB value fell within the range of 8 – 18, that is characteristics of oil-in-water (o/w) emulgents.

Keywords: Hydrophile-Lipophile balance, Bovine mucin, Emulsifying properties

INTRODUCTION

The type of emulsion produced, oil-in-water (o/w) or water-in-oil (w/o), depend primarily on the property of the emulsifying agent. The balance between the hydrophilic and lipophillic moieties of surface-active molecules has been used as the basis for a more rational means of selecting and classifying agents than the empirical methods traditionally used (Pharmaceutical Codex, 1979). This characteristic is referred to as the Hydrophile-Lipophile Balance (HLB); and in the system, each emulsifying agents is assigned a number between 1 and 20. (Graffin, 1954) define HLB of a surfactant as the moles of the hydrophilic group divided by 5. In the case of non-ionic substances, the number is calculated from the hydrocarbon chain length and the number of polar groupings. In this study mucin was evaluated for possible emulsifying property.

Mucin or mucus glycoproteins are a family of polydisperse molecules, which carry out multiple tasks at mucosal surface throughout the body. They contribute to the mucus gel barrier and are part of the dynamic, interactive mucosal defensive system (Corfield *et al.,* 2002). Mucus is very high molecular weight, carbohydrate rich protein with up to 80 % *O*-glycosidically linked carbohydrate. Several studies carried out on mucus glycoproteins from many organs have suggested that these macromolecules consist of subunits held together by interchain disulphide bonds and further stabilized by non-covalent interaction. The end result of multiple interconnections is an extended and random network, which imparts to mucus secretions their characteristic property of viscoelasticity. Evidence that S-S bond play an important structural role has been provided by demonstrations that thiol-group reagents decrease the viscosity and increase the solubility of the native mucus secretions (Forstner *et al.,* 1979) and in some cases decrease the molecular weight of purified mucin. Researchers observed that the sedimentation coefficient of a large porcine gastric mucin decreased from 335 mol. wt. 2.3 x 10^6 to 145 mol. wt. 25 x 10^5 after treatment with 0.2 M of 2- mercaptoethanol (Carrigan and Bates, 1973). Somewhat similar but less dramatic effects have been observed after reduction of bronchial mucus glycoproteins (Forstner *et al.,* 1979).

MATERIALS AND METHODS

Extraction of Bovine Mucin: The small intestines of freshly slaughtered cow were obtained from the Nsukka abattoir and dissected starting from the beginning of the jejunum to the ileocaecal sphincter. The intestines, sectioned into short lengths, were flushed through with chilled saline, and the mucosal surface was exposed by longitudinal dissection. By using a microscope slide, the mucus layer was gently scraped off in to chilled saline. The mucus was precipitated using chilled acetone and dried. The resultant flakes were pulverized using end runner mill' machine and stored in an air-tight container until used.

Test for Sugars: Three drops of freshly prepared Felling's solution A and B were added to 1 mg of 1 % w/v aqueous dispersion of bovine mucin and then heated in a boiling water bath for 5 min and observed.

Test for Carbohydrates

a. Reduction test: Two drops of 1 % iodine solution was added to 1 ml of the (1 % w/v) of bovine mucin and then observed for blue-black colouration.

b. Molisch's test: This reaction is a general test for the presence of carbohydrate and other organic compounds that could form furfuradehyde or hydroxymethyl furfuradehyde in the presence of conc. sulphuric acid. For the test, two drops of αnaphthol solution was added to 2 ml of the bovine mucin dispersion and mixed thoroughly. Then 1ml of conc. sulphuric acid was gently poured down the side of the tube and observed.

c. Tollen's reagent test: The silver ions in a solution containing silver ammonia complex are reduced to metallic silver. By aldehydes, reducing sugars, polyhydroxyl phenols, formic acid and other reducing agents. Tollen's reagent prepared as 1 ml of 5 % silver nitrate solution was treated with a few drops of 5 % sodium hydroxide solution. A volume of aqueous ammonia just enough to redissolve the precipitate was added to 3 drops of the bovine mucin dispersion and the mixture warmed in a boiling water bath for a few minutes. The colour of the precipitate formed was observed.

Test for Proteins

a. Biuret test: Two drops of water and 1 ml of dilute sodium hydroxide were added to 20 mg of bovine mucin. Two drops of 1 % copper sulphate solution was added and the solutions shaken thoroughly after each drop and observed.

b. Xanthoproteic reaction: Two drops of concentrate nitric acid were carefully added to 2 % dispersion of bovine mucin. A white precipitate was formed, which turns yellow on heating. The contents of the test tube were cooled and three drops of ammonia solution added and the precipitate observed.

Test for Fixed Oils: A drop of the acetone extract was placed on a filter paper. The solvent was allowed to evaporate and the filter paper observed carefully.

Solubility Profile of Bovine Mucin: The solubility of bovine mucin in several solvents was determined by dispersing 100 mg of the bovine mucin in definite volume of each solvent- acetone, ethanol, water, sodium hydroxide, hydrochloric acid and ammonium hydroxide at different temperature.

Preparation of the Emulsion: Oil-in-water (o/w) emulsions were prepared using liquid paraffin as the oily phase and various combinations of Tween 65 and bovine mucin powder. The proper quantity of the gum was weighted and distributed in the oil phase contained in stainless steel cup. The calculated quantity of Tween 65 was added to the oil as an aqueous dilution, and the total volume of 120 ml of the product was made by the addition of distilled water. The final emulsion contained 30 % liquid paraffin. Emulsification was effected by mixing the above mixture for 5 min with a Silverson homogenizer (L.2R 2900, Erweka, England).

Determinations of HLB Value: The HLB of the bovine mucin was determined according to the method described by Graffin and modified by some group (Graffin, 1954). Series of emulsions were prepared with varying ratios of Tween 65 and the mucin powder. The percentage of the total emulsifying agent (mucin and Tween 65) was fixed at 1 % in all cases. The first series of emulsions contained emulsifier's blends of ratios ranging from 9:1 to 1:9 respectively. The (HLB) of the mucin is then calculated using the equation below. HLB = R – (H x S) / N; Where R = "required HLB" of liquid paraffin, H = HLB of Tween 65 used, S = is the percent of Tween 65 expressed as a decimal fraction and N = is the percent of mucin powder expressed as a decimal fraction.

RESULTS AND DISCUSSION

Physicochemical Properties of Bovine Mucin: Physiochemical tests performed on the bovine mucin showed that carbohydrates, proteins and trace amounts of fats were present (Table 1). In both wet and dry states, the mucin is light- brownish in colour, almost tasteless and has a pleasant meaty odour.

Bovine mucin when dispersed in water gives 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. Bovine mucin disperses with little difficulty in cold water. The bovine mucin is not soluble in the ethanol and acetone but showed some level of solubility in 0.1 M sodium hydroxide, ammonium hydroxide and sulphuric acid. It showed a greater level of solubility in dimethylsulphoxide (DMSO) especially at elevated temperature (Table 2).

Table 1: Physiochemical properties of bovine mucin

Test	Observation	Inference
carbohydrate	+++++	Present
protein	++ + +	Present
fats	+	Trace amount

Key: *+ Present in trace amount; + + + + copiously present*

Table 2: Solubility profile of bovine mucin

Temp (°C)	Acetone	Ethanol	0.1M NaOH	0.1M H_2SO_4	0.1M NH_4OH	DMSO
	Solvents					
25	-	-	-	-	-	+
30	-	-	-	-	-	+
35	-	-	-	-	-	+

Key: - *Quite insoluble, + slightly soluble*

Table 3: Organoleptics properties of bovine mucin and its emulsion

Parameter	Organoleptics properties of bovine mucin	Organoleptics properties of mucin emulsion
Colour	Light brown	White
Odour	Characteristics	Odourless
Texture	Very fine	Pourable

Properties of Bovine Mucin Emulsion: The most stable emulsions in the series were determined, by evaluating primarily on the basis of degree of creaming. The heights of the separated layers (in cm) were recorded and the percentage calculated. In almost every case, the best emulsion of a given series showed a lesser degree of creaming than a poorer one in the series (Table 3) (Udeala and Uwaga, 1981).

Table 4: Determination of HLB value of bovine mucin

S/N	Mucin: Tween 60	Level of Creaming (cm)	% Creaming
1	1:9	28.00	23.32
2	2:8	24.14	20.10
3	3:7	52.22	43.50
4	4:6	54.26	45.20
5	5:5	70.00	58.31
6	6:4	55.00	45.82
7	7:3	63.64	53.02
8	8:2	62.00	51.65
9	9:1	63.90	53.23

% creaming = *Level of creaming (cm) / initial volume x 100.* ***Note:*** *The initial volume is taken as 100 cm*

Series of emulsion were formed with bovine mucin and paraffin oil, in varying ratios, the most stable emulsion with the least creaming level was found to be mucin-oil ratio of 1:9, and the HLB was calculated to be 8.4. The HLB value 8.4 (Table 4) is within the acceptable range of 8 -16 which are best fitted for oil/water emulsifying agents (Adikwu *et al.,* 1992). The organoleptics properties show that bovine mucin exhibited good emulsifying properties as exemplify by the odourless nature of the emulsion and easy pourability. Ideally emulsion should exhibit the rheological properties of plasticity and easily re-disperse with gentle shear stress and good pourability (Aulton *et al.,* 1998). Oily substances can be emulsified by mucin and this could be a possible mechanism whereby drugs are absorbed through the villi of the small intestine in animal and human (Guess, 1981). The pharmaceutical importance for determining the HLB of a material or substance intended to be used as a pharmaceutical emulgent is based on the fact that physically stable emulsions are best achieved by a condensed layer of emulgent at the oil/water interface and that the complex interfacial films formed by a blend of an oil-soluble emulsifying agent with a water soluble one produces a satisfactory emulsion (Corfield *et al.,* 1973). This result revealed that bovine mucin had emulsifying properties as evidenced by its ability to form good and elegant emulsions, and therefore can be used as an excipient in pharmaceutical preparation if properly harnessed.

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