# Impact of Egg White Protein on the Quality and Stability of Corn Oil-in-Water Emulsion

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**Summary:** The effect of egg albumin has been examined on the texture and stability of O/W emulsion. The corn oil was used as dispersed phase while the aqueous phase as continuous phase of the emulsion. The aqueous phase was designed with the protein contents (0.5- 4 wt. %) at pH 7. The different oil phase (10-40 wt. %) were homogenized in aqueous phase (90-60 wt. %). It was observed that the viscosity and turbidity of the emulsion were increased with the increase of protein concentration and oil phase contents. Flow profile showed that shear stress was increased with increase of shear rate but it decreased at higher shear rate (100 $s^{-1}$) in heated emulsion. On the other hand the emulsion viscosity was decreased with the increase of shear rate showing non- Newtonian behavior. This work may be useful in the formulation and physicochemical properties of food products i.e. sauces, mayonnaise etc.

**Key Words:** O/W Emulsion, Protein Microsphere, Viscosity, Reduced Fat, Stability

## Introduction

When two immiscible liquids are mixed together they form an emulsion; one liquid dispersed in the form of droplets throughout the other continuous phase [1, 2]. The dispersion process is a key technology in pharmaceutical, cosmetic and food industries during different concentrations of oil and water are mixed together to form O/W or W/O food emulsions [3-5]. However, an emulsion is the thermodynamically unstable and to make it stable is considered a primary requirement for a wide variety of considerable applications in various industries [6, 7]. Emulsions are the complex fluids that constitute the broad classes of materials. Their physical behaviour cannot be explained from the chemical constituents alone, without taking into account the characteristics and organization of the structures making up the bulk fluid. Emulsion quality relies on its quality, stability, rheology etc. which are closely related to each other [8, 9]. Emulsion instability tends to phase separation very fast, through creaming, flocculation, coalescence, Ostwald ripening, phase inversion, and rancidity [8]. Further, during packing, transportation, and storage, the system faces a number of external restrictions like shear forces, temperature, lipid oxidation, etc. These factors can enhance the emulsion instability and may lead to phase separation earlier than expected. The properties of the interfacial film will determine the stability of emulsions [10-12].

Some attempts have been made to make emulsion stable using different ingredients [10-15]. The addition of proteins/polysaccharides may increase the viscosity of aqueous phase which can lead to increase the quality and stability O/W emulsion [13, 14]. Other strategies have been developed to examine the effect of emulsification process, oil and salt on the particle size distribution, creaming/sedimentation and coalescence process with respect to coalescence time [16, 17].

Biopolymers are classified in three groups, depending on the nature of the repeating unit they are made of polysaccharides are made of sugars, proteins of amino acids and nucleic acids of nucleotides [18]. Proteins, polysaccharides and their blends are the natural biopolymers, are also surface active agents. Biopolymers may be considered as amphiphilic macromolecules that play an essential role in stabilizing food formulations (foams, emulsions and dispersions). Under specific conditions (pH, ionic strength, temperature, mixing processing such as protein-to-polysaccharide ratio), these biopolymers form gels [14, 19-21]. Different proteins improved the thermal stability and increased resistance to external treatment (high pressure) involved in food processing.

Previously the emulsification of a very simple system of oil-in-water emulsion has been carried by ultrasonification process and the impacts

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of various parameters have been investigated on the quality and stability of emulsion [22]. In the current study we studied the influence of the egg albumen on stability and texture of food emulsion. Egg albumen plays an important role in the process of dispersion in producing stable, oil-based emulsions [23, 24]. The powder Egg Albumin increases the amount of oil the stability of many food emulsions i.e. dressings, sauces, and mayonnaises [25, 26].It has remarkable bonding agents, foaming properties and forms stronger gel during heating at particular pH lead to three- dimensional structures, resistant to stretching, heat setting, bending and shearing[27]. The specific objectives of the current study are to investigate the influence of the Egg Albumin on the texture and the shelf life of O/W emulsion. The results of the current may be used to design the food products such as sauce, dressing and mayonnaises.

## Experimental

### *Materials*

Corn oil purchased from a local supermarket is used as a liquid oil to prepare the dispersed phase of O/W emulsions. Egg Albumin is used as emulsifying and gelling agent for the stability and texture of the emulsion. It consists of 10% protein, 0.5 % carbohydrates, 0.6 % ash and 88.5 % water. Sodium Phosphate and Sodium chloride (E Merck, Germany) were used to control the pH and ionic strength of the aqueous phase. The water used for the preparation of emulsion was freshly double distilled and de-ionized; its conductance is 5-10μS. All the chemicals used were of analytical grade.

### *Preparation of Sample*

Sodium Chloride (100 mM) and phosphate buffer (10mM) were used to prepare aqueous phase and were mixed in de-ionized water. Different of Egg Albumin was prepared by dissolving them into the aqueous salt solution. The oil phase was prepared by using corn oil.

### *Preparation of emulsion*

Oil in-water emulsions were prepared by homogenizing aqueous phase with oil phase at the room temperature. Albumin (egg/oil/water) emulsions were prepared by homogenizing oil phase and aqueous phase having egg albumin at ambient temperature (25°C). An emulsion was prepared using a high-speed blender (Kenwood HB711M Blender Tokyo, Japan) for 50 sec. The aqueous phase was dispersed gradually into the oil phase under agitation with a magnetic stirrer and then blended together using blender (500 rpm) for 50 second. After homogenization, the emulsions were cooled to room temperature (25°C). Then, the emulsion containing water droplets with albumin egg inside was separated into two portions: (i) one portion was kept at ambient temperature; (ii) the other portion was heat-treated at 90 °C (Fig. 1). All emulsions were then analysed.

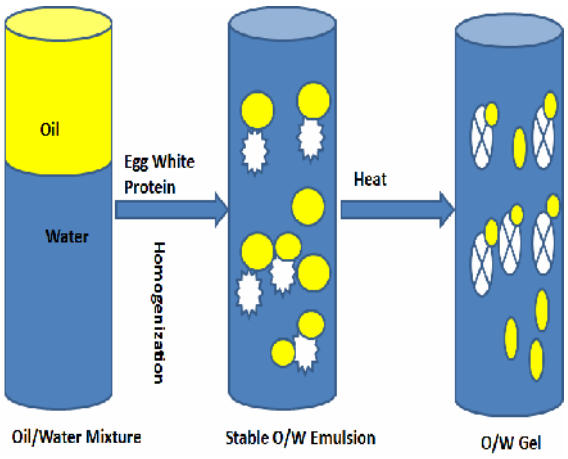



Fig. 1: Schematic diagram showing the formation of O/W Emulsion gel using controlled aggregation of Egg White Protein.

### *Stability of Prepared Emulsions*

In order to examine the stability of prepared emulsion, the prepared emulsions were stored in clean air tight vessels and studied as a function of storage time. The samples for analysis were taken from the stored emulsions, using pipette and sucking it slowly so that the shear forces employed in this case should remain at minimum and not to affect the samples. It was also considered that the rest of the stored emulsions should not be disturbed or shaken during the process. The samples were investigated as a function of coalescence/ storage time till the both layers were separated from each other.

### *Characterization of Emulsion*

The prepared emulsions were subjected to different techniques, including optical microscopy and viscometry to characterize the emulsion and to investigate the coalescence.

### *Microscopic Measurement*

After the homogenization the emulsion was subjected to microscopic measurements to get the

microstructure of the droplets. For the purpose an optical microscope fitted with high performance computer controlled digital camera (CCD) was used. Emulsions were gently stirred to form a homogenous mixture without forming air bubbles. A small aliquot of these emulsions was then transferred to a glass microscope slide covered with a glass cover slip. The cover slip was fixed to the slide using nail polish to avoid evaporation.

*Viscosity Measurement*

The viscosity of emulsion was measured, using DV_E Viscometer, Brookfield, Germany. The equipments were carefully washed with deionized water and dried before taking measurements. A fixed amount of water and emulsion was taken in the bulb of Viscometer and then analysed.

**Results and Discussion**

*Impact of Protein Content on Emulsion Quality*

This study is based on the impact of egg albumin on the texture and stability of O/W emulsion. Initially we investigated the influence of different albumin egg contents on the turbidity and viscosity of emulsion (Fig. 2). It was observed from the Fig. that both the viscosity and turbidity were increased with the increase of Egg Albumin concentration (0.5- 4 wt. %). It is due to the increase of protein- protein interactions and binding of protein with water and oil droplets which could lead to make the emulsion more viscous and turbid. In order to get some proofs for instrumental measurement some visual observation was also obtained from the macrostructure containing test tubes (data not shown).A fixed amount of different emulsions having various contents of protein was taken and their macrostructure were taken. It was observed from the test tubes that the materials were become more viscous as the concentration of protein was increased. These optical observations supported the instrumental viscosity and turbidity analysis, with the samples were becoming more viscous and turbid with increasing of protein contents. The influence of 3 wt. % of Egg Albumin on emulsion containing different oil phase contents has investigated (Fig. 3). The Fig. showed that with increasing of oil contents in the presence of egg white protein increased the droplet-droplet interaction which enhanced the viscosity and turbidity as well as improve the emulsion quality.

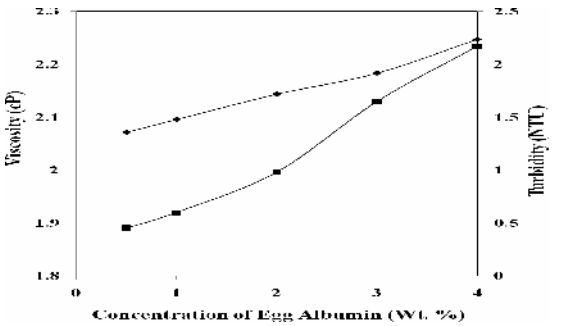



Fig. 2: Viscosity and Absorbance of 20% Oil/ Water Emulsions as a function of different concentration of Egg Albumin. The oil phase contained of 20 Wt. % corn oil while the aqueous phase consisted of 0.5- 4 Wt. % Albumin Egg (pH 7, 100mM NaCl).

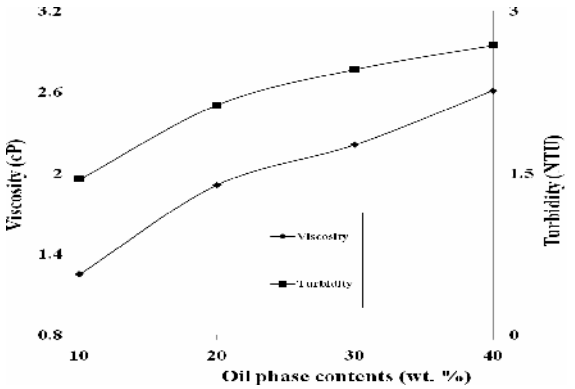



Fig. 3: Viscosity and Absorbance of Oil/ Water Emulsions as a function of different concentration of oil phase. The aqueous phase consisted of Egg 3 Wt. % Albumin Egg (pH 7, 100mM NaCl).

*Flow Profile and Viscosity Analysis*

In this section the stress sweep (Shear stress vs. Shear rate) and viscosity of emulsion as a function of shear rate has been investigated (Fig. 4 & 5) and droplet contents on flow behaviour at constant shear rate ($10s^{-1}$) have been examined (Fig. 6). The stress sweep measurement was used to describe the strong and weak gel in O/W emulsion. The Fig. 4a showed that the shear stress increases linearly with the increase of shear rate in unheated emulsion. It was also observed that the shear stress increased with the increase of oil phase concentration i.e. 10 wt. % emulsion shows lower stress than the higher concentration (30 wt. % and 40 wt. %). It was found that heated emulsion has higher shear stress value than the unheated emulsions (Fig. 5a). The shear stress first increases with increasing of shear rate but at higher shear rate it was decreased. It might be due to the fact

that at low shear rate the material exhibit solid- like but it changed from solid- like to liquid- like at higher shear rate [28]. Heating caused a tremendous changed in shear stress i.e. the material showed pseudo plastic behaviour due to the interaction of protein molecules in aqueous phase [27].The heated O/W emulsions with higher dispersed oil phase contents (20 to 40 wt. %) exhibited non-ideal plastic behavior. These modifications are divided into three categorized (i) low applied shear rate (<10 $s^{-1}$): very low flow was measured below the 10 $s^{-1}$ because the applied disruptive forces are not very strong to overcome the attractive forces (ii) Intermediate rate (10-30 $s^{-1}$): the shear stress was increased with the increase of shear rate due to the stronger disruptive forces to overcome the attractive forces of egg albumin microsphere and enable to flow; (iii) At high shear rate (60- 100$s^{-1)}$ the shear stress decreased because the internal structure of the protein is broken (Fig. 5a). Similarly the viscosity of unheated emulsions was decreased with increasing of shear rate showing non- Newtonian behaviour (Fig. 4b). The viscosity was increased with the increase of oil phase contents and 40 wt. % has higher viscosity than 10 wt. % which could be due to the more droplet-droplet interaction in higher concentration. An ideal Newtonian fluid shows that shear stress is related to the shear rate; the viscosity independent from shear rate [28] and explained by the following equation.

$$\tau = \eta \times \frac{d\gamma}{dt} \qquad (1)$$

Here τ, γ, t, η are the shear stress, shear rate, time, and is the shear viscosity of the system. As compared to unheated emulsions, the heated system showed shear thinning model; the viscosity was decreased with the increase of shear rate (Fig. 5b). The viscosity of both unheated and heated emulsions was strongly dependent on droplet concentration, with the apparent viscosity increasing with the increase of oil phase contents (Fig. 6). Thermal treatment (90 °C, 30 min) of emulsions containing egg albumin protein in continuous phase led a pronounced change in the flow behavior of the materials (Scheme 1). The 10 wt. % unheated emulsions showed little resistance to flow and had relatively low apparent viscosity. However, the viscosity of the 10 wt% heated emulsions was appreciably higher than that of the equivalent unheated emulsions (**Fig. 6**), suggesting that the volume of the dispersion medium was increased due to thermal denaturing of protein in the system. Due to heating the structural change occurred in the emulsion because of protein aggregation microspheres leading to phase separation. The physicochemical properties of egg albumin show that it composed of globular protein called ovalbumin which having free sulfhydryl groups [29]. When the egg albumin protein is heated the ovalbumin protein in aqueous solution change may due to some important reasons (i) unfolding of protein in aqueous solution; (ii) disruption of secondary and tertiary structures on the heating; (iii) Hydrophobic interaction denatured forms of protein; (iv)Aggregation of unfolded protein lead to the formation of three- dimensional network throughout the aqueous phase and in the whole O/W emulsion due the droplet interact ion with the other [29, 30, 34]. The optical observations showed that unheated emulsions have liquid- like properties while heated emulsions have some gel- like properties (Scheme 1). The thermal processing of protein in emulsion could explain the formation of heat- induced gel is the most important functional properties and it results from external exposure of sulfhydryl groups [29]. Some supporting information about the role of 3 wt. % egg albumin and the thermal gelation of the 40 wt. % O/W emulsions microstructures were obtained using regular optical microscopy. It was examined from the microstructure that isolated oil droplets dispersed throughout the oil phase, while after heating these emulsion (90 $^{o}$C, 30 minutes) clusters of aqueous phase were seen in O/W emulsion. Optical microstructure of unheated emulsions showed that the dispersed phase droplets were interacting with each other (Fig. 7a). After heating, the aqueous phase containing albumin egg spread interact with the droplet of oil phase lead to the formation of cluster of droplets in whole system (Fig. 7b). The optical analysis therefore provide some support the hypothesis that the egg albumin protein unfolded, aggregated in whole O/W emulsions and after heating form three-dimensional network that lead to some solid-like properties.

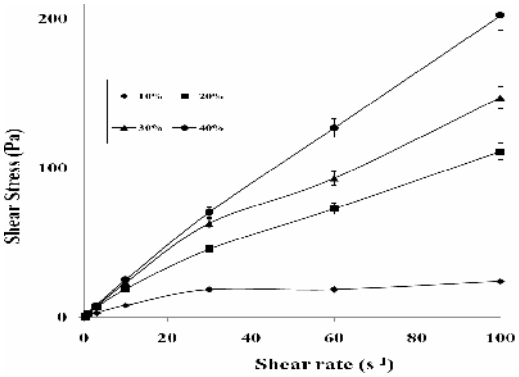



Fig. 4a: Flow Profile (Shear stress vs. shear rate) of unheated Oil/ Water Emulsions. The oil and aqueous phases were homogenized for 50 seconds while the aqueous phase consisted of 3 Wt. % Egg Albumin (pH 7, 100mM NaCl).

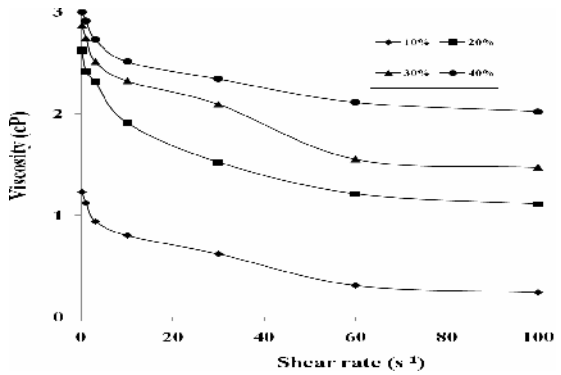



Fig. 4b: Viscosity vs. shear rate of unheated Oil/ Water Emulsions. The oil and aqueous phases were homogenized for 50 seconds while the aqueous phase consisted of 3 Wt. % Egg Albumin (pH 7, 100mM NaCl)

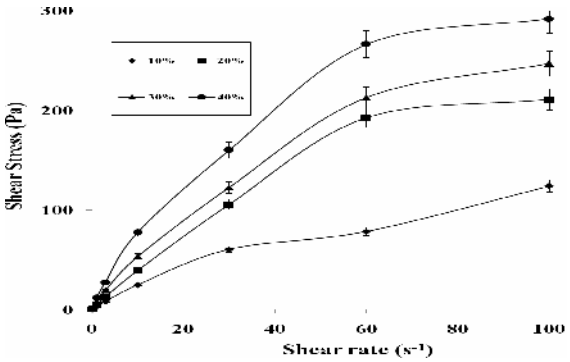



Fig. 5a: Flow Profile (Shear stress vs. shear rate) of heated Oil/ Water Emulsions. The oil and aqueous phases were homogenized for 50 seconds while the aqueous phase consisted of 3 Wt. % Egg Albumin (pH 7, 100mM NaCl)

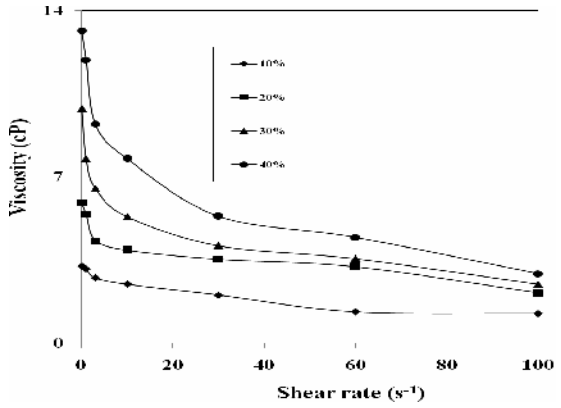



Fig. 5b: Viscosity vs. shear rate of heated Oil/ Water Emulsions. The oil and aqueous phases were homogenized for 50 seconds while the aqueous phase consisted of 3 Wt. % Egg Albumin (pH 7, 100mM NaCl)

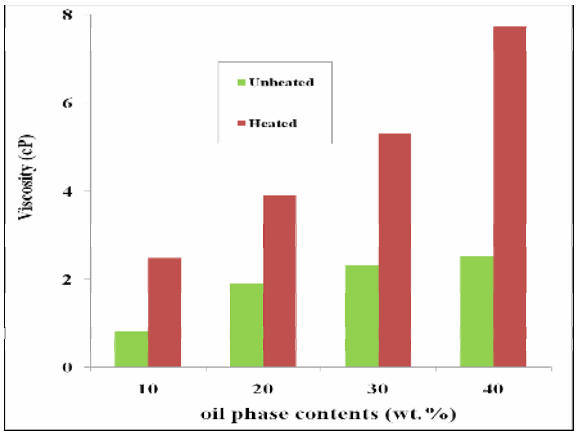
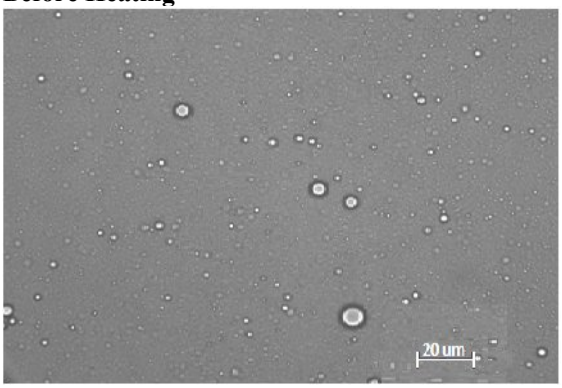



Fig. 6: Viscosity of unheated and heated O/W emulsions at constant shear rate (10 $s^{-1}$) with different oil phase contents (10 to 40%).The oil and aqueous phases were homogenized for 50 seconds while the aqueous phase consisted of 3 Wt. % Egg Albumin (pH 7, 100mM NaCl).

**Before Heating**




**After Heating**

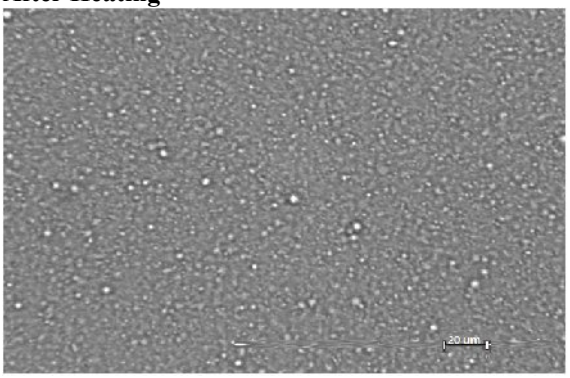



Fig. 7: Microstructures of 40 wt % O/W emulsions before and after heating taken by optical microscopy. The aqueous phase of the emulsions contained 3 wt% Egg White Protein and these emulsions were either heated at 90 °C for 30 min or maintained at ambient temperature. The scale bars are 20 micrometers in length.

*Stability of Emulsion*

The fresh prepared emulsion was stored at constant temperature and their stability was investigated as a function of storage time. The Fig. 8 shows that viscosity decreases with increasing of storage time in all protein contents. It was also observed that the viscosity decreased more with storage time in low protein containing emulsion as compared to higher contents. It is because an interaction between protein- water and lipid- water was not strong which cause the instability of emulsion. Along with these there are some other forces such as Brownian, gravitational and electrostatic forces which cause instability in emulsion [31-33]. The electrostatic effect has considerable effect on the viscosity of the emulsion and can be explained by the following equation:

$$\eta_{ap} = \left[1 - \frac{3\,\Lambda\zeta^2}{2\,(1+H)\,(K[a])^2}\right]^{-1} \eta_B \qquad (2)$$

where $\eta_{ap}$, $\eta$, K, $\Lambda$ and H are the apparent viscosity, actual viscosity, double layer thickness and factor depending upon the nature of dispersion medium and its conductance and H is given below.

$$H = \frac{4}{ka}\sinh^2\left(\frac{\zeta}{4}\right)\left[1 + \Lambda\right] \qquad (3)$$

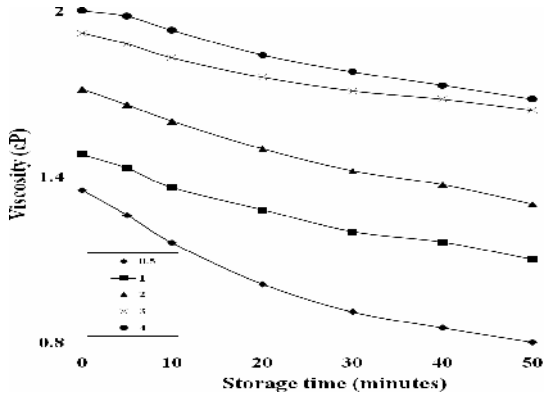



Fig. 8: Viscosity of O/W emulsions as a function of storage time. These emulsion were prepared with different contents of Egg Albumin (0.5-4 wt. %) at pH 7

The influence of a fixed concentration (3 wt. %) of egg albumin on emulsion stability has examined (containing different contents of dispersed phase). The fig. shows that the viscosity decreased with increasing of storage time (Fig. 9). It was also observed from the Fig. that more change in the viscosity has occurred in the emulsion having low oil phase contents than the higher concentration. The difference is due to the weak interaction of oil- water in emulsion containing 3 wt. % of protein in these emulsions. Further, the major component of egg albumin is ovalbumin which has more contribution as occupied more space in concentrated emulsion. These results are also proved by the macrostructure having different oil phase contents but constant egg albumin (data not shown). Overall, it was concluded that the emulsion stability increases with increasing of protein and oil phase contents.

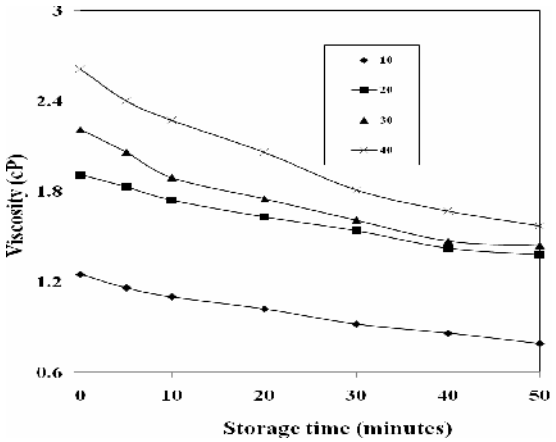



Fig. 9: Figure indicates that the viscosity of O/W emulsions is a function of storage time. These emulsions were prepared with 3 % wt. of Egg Albumin at pH 7

**Conclusion**

We have fabricated the O/W emulsion with different protein and oil phase contents. The emulsion quality and stability increased with the increase of protein contents. The flow profile data showed that at higher concentration of protein and heating the emulsions led to more viscous O/W emulsion and having pseudo- plastic behaviour. These viscous materials are formed above the thermal denaturation temperature which might be due to the unfolding of protein and led to gelation in whole emulsion. The data of this work may be useful in the formulation of O/W emulsions products such as sauces, mayonnaise and salad dressing. Moreover, further research is needed to test in real commercial products.

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