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Formulation and characterization of oil/water emulsions stabilized by biopolymer complexes

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Abstract

Emulsions are thermodynamically unstable colloidal systems consisting of two immiscible liquids. To overcome the use of potentially irritating synthetic surfactants, this study evaluates the stabilization of simple oil/water emulsions using natural biopolymers, specifically proteins and polysaccharides. Emulsions were formulated using olive oil and mixtures of chitosan, starch, and mycological peptone. The influence of pH (2, 3, and 4) on the formation of biopolymer complexes was investigated. Macroscopic and microscopic characterizations revealed that the emulsion stabilized by the chitosan/protein complex at pH 4 exhibited the best physical stability. This stability was manifested by the absence of phase separation, sedimentation, and coalescence. UV-Visible spectrophotometry analyses showed a maximum absorbance at this pH, indicating maximal interaction between the macromolecules. Finally, infrared spectroscopy confirmed the formation of physical interactions between the protein and the polysaccharide, leading to a perfectly homogeneous phase. The chitosan/protein emulsion at pH 4 demonstrated superior stability, maintaining a homogeneous structure without phase separation, unlike the unstable chitosan/starch emulsions. FTIR analysis revealed strong interactions between chitosan and protein, evidenced by changes in the O–H, C–H, and C=O bands, whereas the unstable emulsions showed no significant spectral changes, indicating a lack of intermolecular interactions.
Keywords: *Biopolymers, Chitosan, Emulsion, Protein, Stability*

I. Introduction

Emulsions are constantly present in our daily life and are encountered in numerous fields, including the pharmaceutical, cosmetic, food, paint, agrochemical, and petroleum industries [1]. In the pharmaceutical industry, applications are numerous, such as parenteral emulsions, topical emulsions, and oral or ophthalmic emulsions [2]. The term emulsion refers to a colloidal system comprising at least two immiscible liquids (usually water and oil), where one liquid is dispersed as small droplets in a continuous phase constituted by the other liquid, in a more or less stable form. Droplet size varies depending on conditions, from 0.1 to a few tens of micrometers [1].

Emulsions are thermodynamically unstable systems, and their stability is ensured by the presence of emulsifiers or stabilizers. To overcome the use of potentially irritating synthetic surfactants, natural biopolymers such as proteins and polysaccharides have gained interest as effective emulsifiers and stabilizers due to their surface activity and gelling properties [3]. Many proteins can act as emulsifiers because of their ability to adsorb at the oil-water interface. Some polysaccharides also possess surface activity and are capable of stabilizing emulsions by adsorbing at the interface between the two liquids [4].

Furthermore, the complexation between polysaccharides and proteins, often driven by electrostatic interactions between oppositely charged macromolecules, can lead to improved emulsion stability. The formation of soluble or insoluble complexes depends on pH and ionic strength [5]. These biopolymer complexes not only enhance physical stability against coalescence and flocculation but also offer controlled release and encapsulation benefits for bioactive compounds [6]. Recent advances have demonstrated their potential in designing clean-label food products and biocompatible pharmaceutical formulations [7]. According to et al [8] Biopolymer-based nanocarriers, such as nanoparticles, nanoemulsions, nanoliposomes, micelles, and nanofibers, effectively encapsulate phenolic compounds like quercetin, curcumin, catechin, and resveratrol, enhancing their solubility, stability, and biological effectiveness for targeted therapeutic and nutraceutical applications.
This study focuses on the stabilization of simple oil-in-water emulsions using biopolymer complexes. The specific objectives are to formulate emulsions with olive oil as the active lipophilic phase and mixtures of chitosan (cationic polysaccharide), starch (neutral polysaccharide), and mycological peptone (protein), and to investigate the influence of pH (2, 3, and 4) on the formation and stability of

these biopolymer complexes. The novelty of this study lies in the first-time association of mycological peptone with chitosan to develop a stable emulsion through enhanced intermolecular interactions. Physicochemical characterizations, including macroscopic and microscopic observations, UV-Visible spectrophotometry to predict zones of maximal interaction, and infrared spectroscopy to identify molecular interactions, are performed to evaluate emulsion stability.

II. Material and methods

II.1 Materials

Olive oil was selected as the dispersed (lipophilic) phase. Its main physicochemical properties include a dynamic viscosity of 0.1 Pa·s at 20°C and insolubility in water. The stabilizing agents tested included polysaccharides such as chitosan (cationic, molecular weight 100,000-300,000, soluble in acidic media), commercial starch and synthesized starch, as well as a protein (mycological peptone, a yellowish powder soluble in water, pH 5-7 at 2% w/v). The surfactants PEG 600 (hydrophilic) and Span 80 (lipophilic) were used during emulsification. Hydrochloric acid (1N HCl) was used for pH adjustment, and distilled water served as the aqueous phase.

II.2 Preparation of polymeric solutions

Biopolymer mixtures were prepared by combining 50 mL of a 0.2% chitosan solution with 40 mL of 0.2% starch or protein, supplemented with the active ingredient (olive oil) and the homogenization speed was 10,000 rpm. The temperature of the mixture was maintained at 50 °C for 30 minutes. The pH of these solutions was then adjusted to target values of 2, 3, or 4 by adding hydrochloric acid (1N HCl). Three mixtures were prepared: Mixture 1 (chitosan + commercial starch), Mixture 2 (chitosan + mycological peptone), and Mixture 3 (chitosan + synthesized starch).

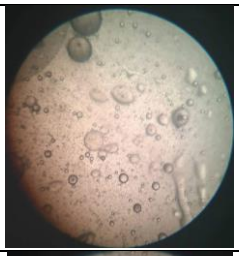
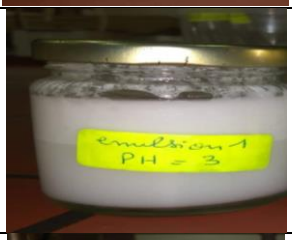
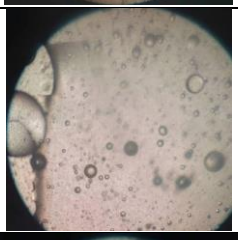
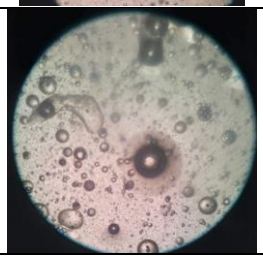
II.3 Emulsification

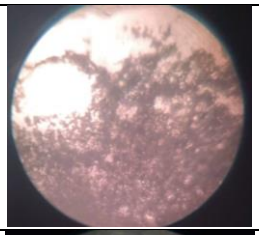
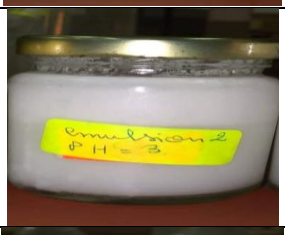
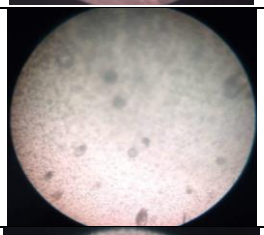
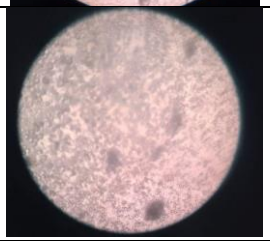
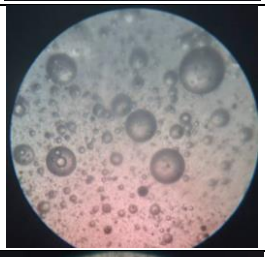
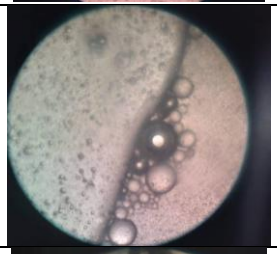
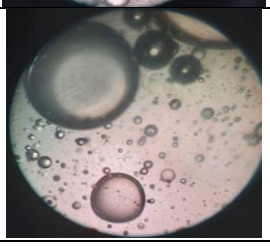
The aqueous phase (containing the hydrophilic surfactant PEG) was mixed with the oily phase (containing Span 80 and olive oil). Final homogenization was carried out using a mixer under strong agitation for 5 minutes to reduce droplet size.

II.4 Physico-chemical characterizations

- Macroscopic and microscopic appearance: Visual evaluation of homogeneity (phase separation, sedimentation) and observations under a light microscope (x40 magnification; scale bar = 100 µm) to identify instabilities such as coalescence or flocculation.
- UV-Visible spectrophotometry: Absorbance measurement between 200 and 500 nm to evaluate turbidity and predict zones of maximal interaction between macromolecules. Measurements were performed using a PerkinElmer Lambda 25 spectrophotometer.
- Infrared (IR) spectroscopy: Determination of the effect of pH on chemical bonds and interactions between macromolecules, using a PerkinElmer FT-IR spectrometer.

Table 1: Macroscopic and microscopic appearance of 9 emulsions of different pH

Biopolymer blends (9 tests)	9 emulsions PH=2 ; 3 ; 4	Macroscopic aspect	Observation	Microscopic aspect	Observation
Blend (chitosan and commercial starch)	Emulsion 1 pH=2		A phase separation		Presence of small globules
	Emulsion 1 pH=3		A phase separation		Presence of globules of different sizes: small, medium, and large; therefore, no homogeneity of the globules.
	Emulsion 1 pH=4		A phase separation		Presence of globules of different sizes: small, medium, and large; therefore, no homogeneity of the globules.

Blend (chitosan and mycological peptone)	Emulsion 2 pH=2		Homogeneous blend		The size of the globules is homogeneous.
	Emulsion 2 pH=3		Homogeneous blend		The size of the globules is homogeneous.
	Emulsion 2 pH=4		Homogeneous blend		The size of the globules is homogeneous.
Blend (chitosan and synthesized starch)	Emulsion 3 pH=2		A phase separation		Presence of globules of different sizes: small, medium, and large; therefore, no homogeneity of the globules.
	Emulsion 3 pH=3		A phase separation		Presence of globules of different sizes: small, medium, and large; therefore, no homogeneity of the globules.
	Emulsion 3 pH=4		A phase separation		Presence of globules of different sizes: small, medium, and large; therefore, no homogeneity of the globules.

III. Results and discussion

III.1 Macroscopic and microscopic characterization

The visual examination in table 1 revealed that emulsions 1 (chitosan/commercial starch) and 3 (chitosan/synthesized starch) exhibited phase separation (sedimentation) for all studied pH values (2, 3, and 4). This opaque appearance indicates low solubility and instability of the polysaccharide complex, likely due to weak electrostatic interactions leading to liquid-liquid or liquid-solid phase separation [9]. Conversely, emulsion 2 (chitosan/protein) showed a perfectly homogeneous mixture at pH 2, 3, and 4.

Microscopic analysis confirmed that emulsion 2 formulated at pH 4 provided the best long-term physical stability. The globules had a homogeneous size, with no signs of flocculation or coalescence, unlike the other samples which destabilized quickly. Emulsion 2 at pH 4 remained stable for four weeks, whereas emulsions 1 and 3 destabilized within one day. This stability is attributed to optimal electrostatic interactions between positively charged chitosan and negatively charged protein at pH 4 (below the isoelectric point of the protein) [10].

Our results agree with previous reports on chitosan–protein complexes, where enhanced emulsifying properties under

acidic conditions were attributed to strong intermolecular interactions and smaller particle sizes. Likewise, the chitosan–mycological peptone emulsion exhibited excellent stability at pH 4, supporting the hypothesis that complex formation promotes homogeneous dispersion and prevents phase separation, while introducing mycological peptone as a novel protein component for stabilizing chitosan-based emulsions [11].

III.2 UV-Visible spectrophotometry

The UV-Visible graph in figure 1 of the sediments highlighted an absorbance peak at pH 4 for biopolymer mixture 2 (protein/chitosan). This maximal value reflects increased turbidity, demonstrating that electrostatic interactions between the two macromolecules are at their maximum under these pH conditions [12]. The absorbance values for the individual biopolymers in Table 2 confirmed their intrinsic optical properties.

Table 2: The absorbances of the different biopolymers

biopolymers	Absorbance	Wavelength (cm^{-1})
Commercial starch	2.505	283
Synthesized starch	2.505	280
Protein	2.505	282
Chitosan	2.505	270.5

III.3 Infrared (IR) spectroscopy

The IR spectra of the stable emulsion (chitosan/protein) showed (figure 2) a decrease in the O-H absorption band and an increase in the intensity of the C-H and C=O bands. The glycosidic C–O–C stretching vibration of starch (1020-1080 cm^{-1}) showed slight changes in intensity after complex formation, suggesting interactions between polysaccharide hydroxyl groups and chitosan [13]. These variations prove the presence of branching and strong physical interactions between the protein and chitosan chains, which explains the homogeneity of the obtained phase. For unstable emulsions, the intensity of the bands remained unchanged due to the absence of attractive interactions between the polysaccharides [14].

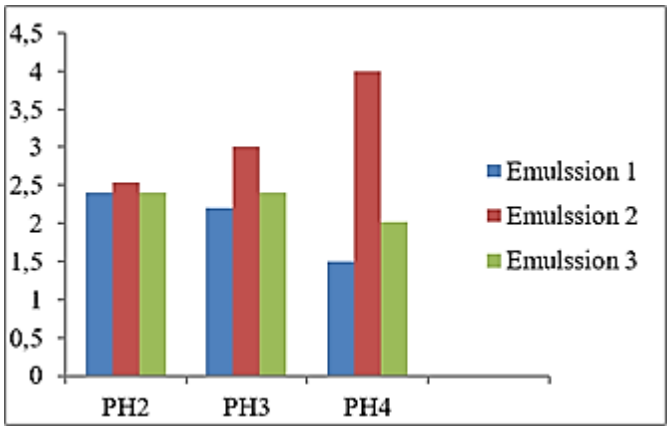


Figure 1: UV-visible graph of all the emulsions

IV. Conclusions

This study highlighted the crucial role of biopolymer complexes as stabilizers for simple oil/water emulsions. The combination of chitosan and mycological peptone at a pH of 4 resulted in the most physically stable emulsion. Microscopic characterizations, coupled with spectroscopic analyses (UV-Visible and IR), confirmed the absence of coalescence and the presence of strong structural interactions between the macromolecules. The choice of a common vegetable oil (olive oil) did not alter the stability, thus offering very promising perspectives for applications in the field of galenic pharmacy.

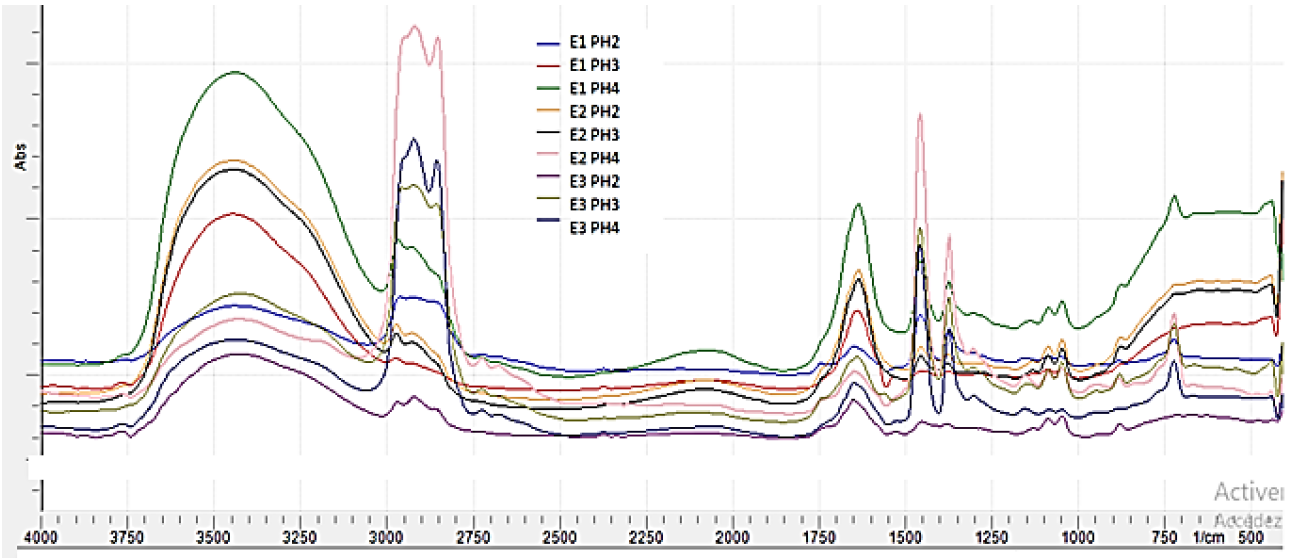


Figure 2: Infrared spectrum of all the emulsions

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