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Preparation and Characterization of Biopolymer Based Bioactive Mucoadhesive Films with Turmeric Extract

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Abstract: Antioxidants are the molecules that inhibit the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals leading to the chain reactions that may damage cells. Antioxidants terminate these chain reactions forming reactive oxygen species that cause many diseases. Antioxidants can be taken directly from the food sources or food supplements including antioxidants. In addition to the inhibition of oxidation reactions, some plant-derived antioxidants may also function as bioactive agents for the prevention of infections due to their antimicrobial activities. In turmeric extract, there are several natural compounds called as curcuminoids which have both antioxidant and anti-inflammatory properties.

In this study extraction of Curcumin and possibly enhancement of its bioavailability by developing film carrier systems were studied. For evaluating the optimum conditions for extraction, the effect of different solvents and solid-to-liquid ratios on extraction yield were investigated. Aqueous solutions of glycerin and ethanol were used as extraction solvents. In 70 % ethanol-water solution, optimum extraction condition for Curcumin was found as 1:30 solid-to-liquid ratio with extraction yield of 3.80 %. In 50 % glycerin-water solution, optimum extraction condition for Curcumin was found as 1:60 solid-to-liquid ratios with extraction yield of 2.04%. Kinetic studies of extraction were performed at optimum extraction conditions for both solvents. The results showed that after an hour of extraction time highest extraction yield could be reached in ethanol-water solution. However, it was observed that higher extraction yield was reached with increasing extraction time in glycerin-water solution.

Curcumin has a low bioavailability when it was taken from gastrointestinal route due to the harsh conditions such as pH. To overcome this obstacle and increase the bioavailability of Curcumin new carrier systems are needed for oral delivery.

In our study for pharmaceutical purposes, zein was used to prepare carrier films loaded with turmeric extract. Mucoadhesive properties of the films were achieved using okra gum rich in polysaccharides. The FT-IR results revealed the successful coating turmeric extract loaded films with okra gum. Then the release profiles of Curcumin from prepared films were also studied.

Keywords: Antioxidants, Curcumin, Mucoadhesive, Turmeric, Zein, Okra Gum.

1 Introduction

With the increasing interest in well-living antioxidants become indispensable. By interacting with free radicals antioxidants act as a protector of the biomolecules. Antioxidants in right dosage drastically delays or prevents oxidation of the oxidizable substrate. Curcumin is a polyphenolic compound that can be extracted from the turmeric. Turmeric consists of 2-9% curcuminoids. Curcuminoid specifies a group of compounds such as curcumin, demethoxycurcumin, bis-demethoxycurcumin and cyclic curcumin. Curcumin is the major component in all of these compounds [1]. Curcumin has been widely used both in traditional and scientific applications. Curcumin has a wide spectrum of biological actions such as anti-inflammatory, antioxidant, anticancer, antidiabetic, antiallergic, antiviral, antibacterial and antifungal activities [2]. Due to its hydrophobic nature curcumin is poorly soluble in water. However, it is soluble in organic solvents such as ethanol, methanol, acetone, glycerin, and isopropanol.

Stability has a very significant function on conservation of physiological activities of curcumin. Like any other antioxidants curcumin is also sensitive to environmental factors such as light, heat and it can be decolorized by contacting with UV light [3]. When orally taken curcumin has very low bioavailability due to harsh conditions in gastrointestinal route. To overcome this obstacle and increase the bioavailability of curcumin new carrier systems are needed for oral delivery.

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By using zein as biopolymer carrier stability of curcumin is improved. It has been reported that zein could linked and cover lipid compounds, and decreasing degradation ratio [4]. Zein is a protein which has applications in the food, pharmaceutical and biotechnology industries. Advantages of using zein are its low-cost values, commercially feasible, biodegradable, biocompatible and even applicable to the food processes. Due to lack of tryptophan and lysine in its structure zein is also alcohol soluble which makes it convenient choice as curcumin carrier [5].

In order to improve the carrier system okra gum was used as plasticizer. Plasticizers increase the flexibility of the film. Selection of plasticizer is a very crucial point. Suitability of plasticizer with the polymer and the chosen solvent are important parameters for plasticizer selection. Plasticizers affect the mechanical properties of the film. In recent years okra gum is commonly used in the pharmaceutical industries as thickeners, water retention agents, emulsion stabilizers, suspending agents, binders and film former [6]. Okra gum contains random coil polysaccharides. When it is extracted in water, these polysaccharides can produce highly viscous solution with a slimy appearance which gives the mucoadhesive properties of okra gum as well.
In this project hibiscus esculentus (okra) is used as a plasticizer. The binding and mucoadhesive property of Okra gum, which plays an important role in the formulation of sustained release drug delivery system, was investigated.

2 Experimental

2.1 Materials

Standard Curcumin was purchased from Sigma-Aldrich. For the extraction of curcumin turmeric was used. Solvents used for the extraction were ethanol and glycerin purchased from Sigma-Aldrich and Dalan Chemistry respectively. Okra gum was obtained from okra.

2.2 Preparation of Turmeric Extract

The turmeric extract was prepared with different solvents and different solid-to-liquid ratios in order to optimize the extraction conditions. The solvents used in extraction were 70 % aqueous ethanol and 50 % aqueous glycerin solutions. Standard Curcumin was used to obtain calibration curve and Curcumin amount was determined by measuring the absorbance values of the liquid extract with a spectrophotometer (Thermo Scientific Genesys 10S UV-Vis) at 427 nm.

2.3 Extraction of Okra Gum

Okra gum extraction was done as explained in the literature [7]. Dried and ground okra was stirred in distilled water for four-hour at 50 ºC. After the extraction solution was filtered acetone was added and okra gum was obtained.

2.4 Preparation of Turmeric Extract Loaded Zein Films

Zein was used as a film forming agent. Due to the high solubility of zein in ethanol; the solution was prepared in aqueous ethanol (80% v/v) with 5% (w/v) zein. After the zein solution was prepared, it was mixed with turmeric extract. Prepared films were dried and coated with extracted okra gum.

2.5 Film Coating

After the turmeric extract-loaded zein films were prepared in order to provide better interaction, isoelectric points of zein and okra were determined. From the literature survey, isoelectric points of zein and okra gum were determined as 6.2 and 5.1 respectively. The pH of okra gum was adjusted to these isoelectric points. After the prepared films were dried, they were immersed in okra gum solution and left for drying. In order to determine the effect of coating some films were immersed multiple times in okra gum solution.

2.6 FT-IR Analysis

FT-IR analysis was used to characterize the prepared films. The spectra of the samples were recorded in the 4000 - 650 cm-1 region at room temperature. The obtained spectrum was investigated in order to confirm the successful coating of zein films by using okra gum solution.

2.7 Kinetic Release Studies of the Okra Gum Coated Turmeric Extract Loaded Zein Films

For the kinetic release studies of the prepared films isotonic pH solution was used. The prepared films were immersed into the solution and every half an hour a sample was taken from the solution. The collected samples were analyzed by using UV-Spectrophotometer.

3 Results and Discussion

3.1 Preparation of Turmeric Extract

Parameters for the preparation of turmeric extract were the types of extraction solvents and solid-to-liquid ratios. The

obtained results for extraction yields in both ethanol and glycerin solutions were given in Table 1.

Table 1. Extraction yield values for the first extraction (Sample no 1-3: in aqueous ethanol solution with the solid-liquid ratios of 1:10, 30, 50; Sample no 4-6; in aqueous glycerin solutions with the solid-liquid ratios of 1:10, 30, 50)

Sample no	Extracted Curcumin amount (mg)	Turmeric powder used in extraction (mg)	Yield (%)
1	42.8	3000	1.43
2	38.0	1000	3.80
3	21.7	600	3.62
4	5.0	3000	0.17
5	0.3	1000	0.03
6	0.2	600	0.03

After the first extraction obtained yield results were compared. Extraction yields were higher in aqueous ethanol solution rather than aqueous glycerin solution as extraction solvent. For further investigations, successive extractions were performed with both aqueous ethanol and glycerin solutions. With the second extraction, the extraction yields in aqueous ethanol solution were almost the same. On the other hand, extraction yields in aqueous glycerin solution increased. Results were given in Table 2.

After the first extraction, due to the lower yield extraction values a new set of solid-to-liquid ratios were used. Results were given in Table 3.

After the first extraction 1:5 solid-to-liquid ratio was eliminated due to high viscosity. With the other solid-liquid ratios extractions were continued. And the collected data were given in Table 4.

Table 2. Extraction yield values for the second extraction (Sample no 4-6: in aqueous glycerin solutions with solid-liquid ratios of 1:10, 30, and 50)

Sample no	Extracted Curcumin amount (mg)	Turmeric powder used in extraction (mg)	Yield (%)
4	20.2	7120	0.28
5	4.0	2330	0.17
6	3.8	1610	0.23

Table 3. Extraction yield values obtained at different solid-to-liquid ratios in aqueous glycerin solution for 21-hour extraction time

Solid-liquid ratio	Extracted Curcumin amount (mg)	Turmeric powder used in extraction (mg)	Yield (%)
1:5	42.6	6000	0.71
1:30	19.7	1000	1.97
1:60	10.2	500	2.04
1:80	7.0	375	1.86
1:100	3.4	300	1.13

Table 4. Extraction yield values obtained at different solid-to-liquid ratios in aqueous glycerin solution for 48-hour extraction time

Solid-liquid ratio	Extracted Curcumin amount (mg)	Turmeric powder used in extraction (mg)	Yield (%)
1:60	16.9	500	3.38
1:80	4.0	375	1.07
1:100	5.4	300	1.80

After the extractions, optimum solid-to-liquid ratios for aqueous solutions of ethanol and glycerin were determined as 1:30, 1:50 respectively. After the optimum solid-to-liquid ratios were determined, extraction kinetics studies for both aqueous solutions of ethanol and glycerin were done. The data obtained for aqueous ethanol solution for 1:30 solid-to-liquid ratio was given in Figure 1. As seen from the

Figure 1 extraction in ethanol solution reaches its highest curcumin amount within an hour. The data obtained for glycerin solution for 1:60 solid-to-liquid ratios was given in

Figure 2. As seen from the Figure 2 when results were compared extraction in aqueous glycerin solution take longer to reach its highest curcumin amount than that of

aqueous ethanol solution due to the different polarity of the extraction solvents.

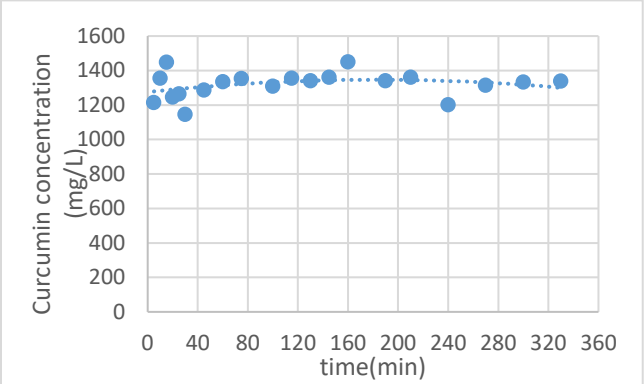

Figure 1. Extraction kinetics of turmeric in aqueous ethanol solution at solid-to-liquid of 1:30.

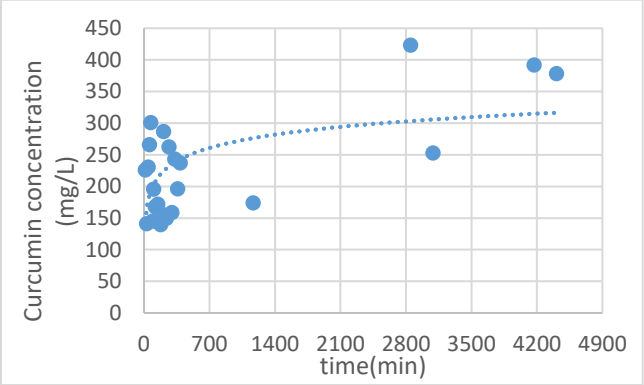

Figure 2. Extraction kinetics of turmeric in aqueous glycerin solution at solid-to-liquid of 1:60.

3.2 Extraction of Okra Gum

After following the extraction steps extraction yield of okra gum was determined. Mucilage (okra gum) yield was found as 20.2%.

3.3 Characterization of Prepared Turmeric Extract

Characterization of turmeric extract was done by FT-IR analysis. In order to characterize the turmeric extract samples, FT-IR spectra of both standard curcumin and turmeric extract were obtained and given in Figure 3.
The bands at 878, 1087 and 1385 cm-1 were attributed to the bending vibrations of the C-H bond of alkene groups. An intense band at 1651 cm-1 assigned to the vibration of the carbonyl bond (C=O). Presumably, these observed groups O-H stretching in phenols, C-H stretching of alkanes and C=O stretch of carbonyl groups comprise Curcumin the bioactive substance of C. longa which is mainly consist of –C-H stretching of alkanes and bonded O-H [8].

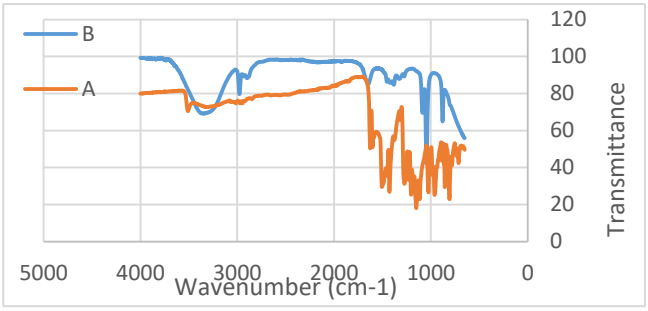

Figure 3. FT-IR spectra of standard curcumin (A) and turmeric extract (B).

3.4 Characterization of Extracted Okra Gum

Characterization of extracted okra gum was done by FT-IR analysis. FT-IR spectra of extracted okra gum was obtained and given in Figure 4.

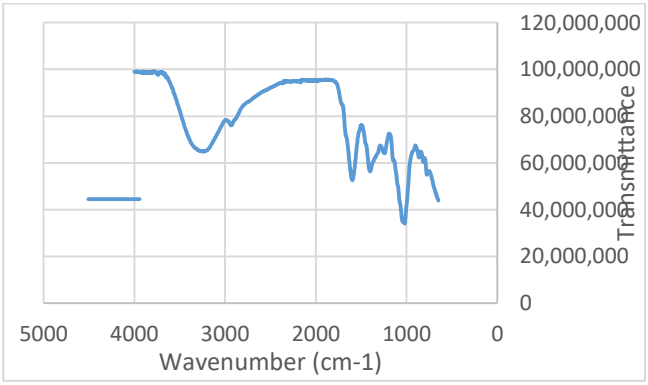

Figure 4. FT-IR spectrum of extracted okra gum.

The focus was on the functional group that forms the protein, such as NH, CO and CH. Strong band at 3628 cm-1 indicated the presence of OH stretch in the solid samples. O–H groups are able to bind with water molecules and produce bound moisture to the polymer components. The existence of O–H groups represents the hydrophilic characteristic that is present in the polysaccharide. Peaks at 3247 indicated N-H bend and stretch, respectively. A peak at 1419 and 3058 cm-1 were due to the C–H stretching frequency in Okra solid. And the peak at 1731 cm-1 was due to C=O functional group. In Okra gum powder, the peaks for NH, CO, OH, and CH were clearly observed [9].

3.5 Characterization of Okra Gum Coated Zein Films

FT-IR analyses were performed to confirm the efficient coating of zein films with okra gum. The results are given in Figure 5. Figure 5 shows that okra gum coated zein films matched the okra gum peaks indicating uniform coating.

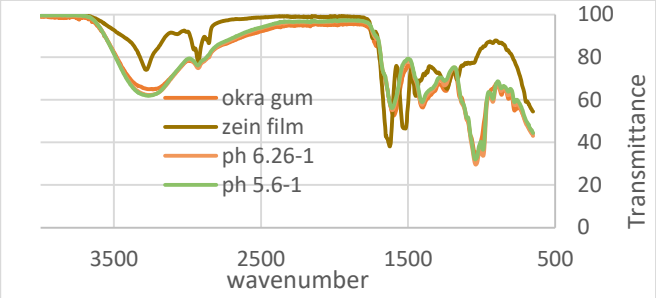

Figure 5. FT-IR spectra of okra gum coated zein films.

3.6 Kinetic Release Study of Okra Gum Coated Prepared Films

The okra gum solution was prepared at two different pH values. One was the pH 5.6 which is adjusted to the isoelectric points of zein and okra gum. The other pH value was 6.26 which is the natural pH of okra gum. By adjusting the pH to the isoelectric points, it was aimed to have stronger interactions between okra gum and zein biopolymer molecules during coating process of films. The coating of turmeric extract loaded zein films with okra gum did not affect the release behavior significantly. However sustained release of curcumin from films having mucoadhesive properties was achieved. The results are given in Figure 6. This result was expected since okra gum was readily soluble in water there was no extra mass transfer resistance for curcumin to diffuse out.

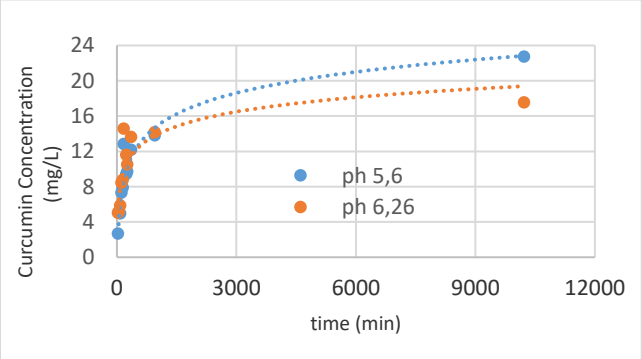

Figure 6. Release kinetics of curcumin from okra gum coated zein films at different pH values.

The second parameter investigated for the effects on the release profile was the number of times of okra coating done on the turmeric extract-loaded zein films. For this purpose, the prepared films were immersed once and twice to the okra gum solution and the release kinetics data are given in Figure 7.

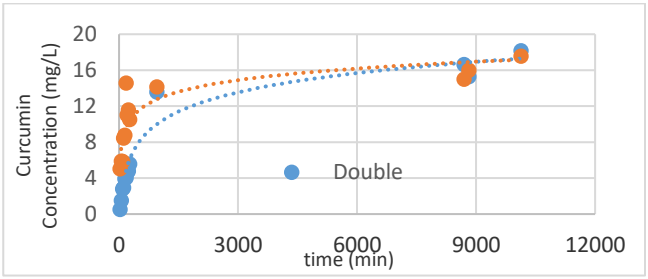

Figure 7. Release kinetics of curcumin from zein films single and double coated with okra gum.

As expected increase in the coating number did not cause slower release of the curcumin. This could be attributed to the solubility of okra gum in water. On the other hand, double coating with okra gum resulted in good enough sticky or mucoadhesive film surface.

4 Conclusions

In this study biopolymeric (zein) film carrier system was successfully prepared and turmeric extract containing curcumin was loaded into these prepared films to preserve the stability of bioactive natural compound, curcumin. Further, the prepared films allowed us to achieve a sustained release of curcumin. The coating of the turmeric extract loaded zein films with okra gum made these films to have mucoadhesive property.

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