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The Effect of Microwave Thermal Denaturation on Release Properties of Bovine Serum Albumin and Gluten Matrices

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ABSTRACT

The purpose of this study was to compare the effects of denaturation by microwave irradiation on release properties of 2 physically different proteins. Matrices were prepared from water-soluble bovine serum albumin loaded with metoclopramide and sorbed with adequate amount of moisture were thermally denatured in a microwave oven. The release profile of the rather insoluble denatured albumin matrices followed the classical Fickian diffusion profile. The release rate was dependent on the degree of denaturation, which was highly dependent on the level of moisture originally absorbed by the albuminoidal matrices and the period of exposure to microwave energy. Conversely, attempts to reduce the rate of drug release through microwave irradiation of metoclopramide-loaded matrices prepared from water-insoluble gluten were futile. The denaturation process was shown to be limited to the relatively water-soluble protein core fraction, while aggregation between neighboring gluten proteins in the matrix was not achieved even in the presence of considerable amounts of sorbed water.

KEYWORDS: microwave, denaturation, gluten, bovine serum albumin, controlled-release.

INTRODUCTION

Since the implementation of polymeric materials in the field of pharmaceutical technology, numerous attempts have been undertaken to modify their physical and chemical properties, and thus their potential applicability into various fields of drug formulation. These include approaches to cross-link polymers through covalent bonds using different techniques, generally classified into chemical and physical means. Formaldehyde, gluteraldehyde, and epichlorohydrin are among the most commonly used chemical means of cross-linking, while UV, gamma irradiations, and temperature represent physical methods of cross-linking. Temperature has been one of the most favorable methods of cross-linking because it avoids both the application of harsh chemical materials suitable for large-scale production and the diversity of equipments and methods used in their application.[1] In the present study, microwave irradiations are used to generate the temperature required for the cross-linking process.

The use of microwaves in the pharmaceutical industry is a growing interest. Research in its implementation is active, and its concept is promising for new applications. Joshi et al presented the microwave drying of aqueous tablet film coatings,[2] while Mandal evaluated the microwave drying of pharmaceutical granulations.[3] Other reports on the microwave drying of pharmaceutical powders and excipients were also described in the literature.[4,5] The microwave dielectric heating in effect is a form of energy conversion whereby internal heating results mainly through the interaction of the rapidly oscillating electromagnetic field at 2450 MHz with randomly oriented polarized molecules and ions in the material, including water, arranging them in an ordered pattern. As the field direction changes, molecules are allowed to relax and return back into their random orientation, liberating stored potential energy in form of random kinetic energy or heat. This alignment/realignment results in the rapid oscillation of the molecule and eventual volatilization owing to the heat of friction. Bound polar molecules are more difficult to volatilize because the fixed bond inhibits rotation. Application of microwave thermal energy is considered to be a mean for producing even heating throughout the sample as compared with direct oven heating, and thus could be used to create uniform cross-linking throughout the sample.[6-8]

Studies on the employment of microwave energy in the cross-linking of polymeric materials of pharmaceutical importance are generally lacking. The cross-linking of natural macromolecules with microwave energy should have the potentiality to overcome problems of toxicity resulting from remaining traces of chemical cross-linking agents and of the in vivo biodegradation products of the chemically cross-linked macromolecule. The first report to use microwave heating in the cross-linking of pharmaceutical carriers was made by Teng and Groves,[9] who produced thermally denatured protein matrices useful as controlled-release systems. Recently, microwave irradiations were used in the preparation of cross-linked gelatin microspheres and were proposed to be effective drug-delivery systems.[10] Moreover, a contemporary article discussing the development of controlled-release solid dosage forms of alginate and

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chitosan using microwave heating has been reported.[11] On the other hand, the microwave-assisted polymerization of monomeric compounds has been demonstrated for a wide range of materials. Copolymers of lactic acid and/or glycolic acid were manufactured by irradiating their corresponding solutions with 2.45 GHz microwave.[12] The microwave-assisted solid-state copolymerization of maleic anhydride and allylthiourea has also been reported.[13]

In this study, since there has been extensive investigation on the possibility of using proteinaceous materials as drug-delivery vehicles, matrices were prepared from 2 physically different proteins: water-soluble bovine serum albumin and water-insoluble gluten. Since previous work showed that most proteins sorb moisture when exposed to high relative humidities,[14] the possibility of producing protein materials denatured to various extents through combination of sorbed water, as a microwave energy absorbent and reaction medium, along with microwave heating as a means for effecting uniform cross-linking throughout the matrix is investigated. Such denatured protein materials could have future applications in the design of controlled drug delivery systems. Consequent comparison of the effect of microwave denaturation on the release properties of both proteins is performed. Metoclopramide hydrochloride, a freely water-soluble drug was selected as a model drug for the bulk work of the present research.

Materials and Methods

Materials and Equipment

The following materials were used: wheat gluten (Produits Pour Laboratoires, RHONE-POULENC, Decines, France), bovine serum albumin (PAA Laboratories GmbH, Linz, Austria), metoclopramide hydrochloride (Biochemical and Synthetic Products Ltd, Hyderabad, India). The following substances and solvents were available through the Jordanian Pharmaceutical Manufacturing Co (JPM, Naur, Jordan) and were of analytical grade: sulfuric acid, sodium hexane sulfonate, acetonitrile, methanol, and glacial acetic acid. The following equipment was used: microwave oven (model EM-X670, 2450 MHz, with maximum power output 1000 W and oven cavity dimensions of 550 mm × 317 mm × 457 mm height × width × depth; Sanyo, Singapore), single-punch tableting machine (Manesty F3, Merseyside, UK), dissolution apparatus (Erweka, Heusenstamm, Germany), UV spectrophotometer (Cary-Varian, Palo Alto, CA), compatibility and stability ovens (Carbolite, Derbyshire, UK), high-performance liquid chromatography (HPLC, SP Thermoquest 1000, single wavelength, Canton, MA), Hermle Z 320 centrifuge (Wehingen, Germany), Schleuniger tablet hardness tester 6D (Schleuniger, Switzerland), and SAS Statistical Analysis System, Version 4.0 (SAS Institute Inc, Cary, NC).

Methods

Stability Studies

Metoclopramide hydrochloride (0.1 g) was accurately weighed and placed in small vials. The vials were placed in a microwave oven and heated for 4 and 5 minutes at a microwave power output of 600 W. Heating was stopped every 30 seconds to allow sufficient cooling of the central turntable and vials. The process was run in duplicate. The microwave-exposed metoclopramide was then dissolved in 20 mL of methanol and diluted to 100 mL with mobile phase. Dilution was then performed by taking 10 mL of the resulting solution and adding sufficient quantity of mobile phase to produce a final volume of 100 mL. Nonexposed standards were prepared using the same method. HPLC analysis was performed by injecting 20 μL aliquots of the standards and microwave-treated samples into HPLC system using Spherisorb ODS1 (5 μm, 250- × 4.6-mm column calibrated at a flow rate of 1.8 mL/min and wavelength of 265 nm, Waters, Milford, MA). The mobile phase was prepared by dissolving adequate amount of sodium hexane sulfonate in a mixture of acetonitrile and water (60:40) to produce a final concentration of 0.01M. The resulting solution was adjusted to pH 4 by glacial acetic acid.

Compatibility Studies

Compatibility studies were performed by mixing 0.1 g of metoclopramide hydrochloride with an equivalent amount of each protein (gluten and albumin). The mixtures were placed in small vials and stored in duplicate for 10 days at conditions of room temperature/0% relative humidity (RH), 50ºC/75% RH, and 65ºC/0% RH. The vials were then removed and contents assayed. Methanol (20 mL) was added and then diluted to 100 mL with mobile phase. The solution was placed in a centrifuge for 5 minutes at 2750 rpm. The supernatant (5 mL) was taken and diluted to 50 mL with mobile phase. Placebo was prepared following the same method and HPLC analysis was carried out according to the same procedure cited under the stability study.

Preparation of Protein Matrices

Homogenous mixtures of each protein, bovine serum albumin and gluten, with metoclopramide hydrochloride were prepared at a ratio of 4:1. The powdered mixtures were placed at 150-mg quantities into a 7-mm shallow concave punch. The mixtures were directly compressed by a single-punch tableting machine maintained at a hardness of 25 kN.

Water Sorption Studies

The average weight of 10 tablets was calculated. Tablets were individually stored for 1 week in desiccators maintained at RH of 19%, 58%, and 94% generated by applying

Table 1. High-performance Liquid Chromatography Analysis for Stability and Compatibility of Metoclopramide Hydrochloride*

Sample	Condition	% Assay	% RSD
M	4 minutes microwave	99.6%	0.90%
M	5 minutes microwave	96.7%	0.60%
M	50°C/75% RH	97.4%	1.40%
M	65°C/0% RH	99.0%	2.60%
M + A	Room temperature	96.7%	0.40%
M + G	Room temperature	98.2%	1.60%
M + A	50°C/75% RH	99.2%	0.14%
M + G	50°C/75% RH	98.8%	0.20%
M + A	65°C/0% RH	98.4%	0.06%
M + G	65°C/0% RH	95.6%	1.80%

*M indicates metoclopramide hydrochloride; A, albumin; G, gluten; RSD, relative standard deviation; and RH, relative humidity.

different concentrations of H_2SO_4 aqueous solutions. Matrices were removed from desiccators and average weight determined. They were then placed on a small watch glass and denatured by microwave heating. The average weight of the 10 tablets was calculated at the following time points during the course of drying: 0, 0.5, 1, 1.5, 2, 3, 4, and 5 minutes.

Denaturation of the Protein Matrices

Matrices removed from desiccators were placed on a small watch glass and denatured by microwave heating. Heating was carried out at a total power output of 600 W and stopped every 30 seconds to allow sufficient cooling of the matrices, watch glass, and central turntable. The hardness of nonexposed gluten matrices compared with those exposed to microwave irradiations was measured using a hardness tester.

Dissolution Studies

Dissolution was carried out according to *United States Pharmacopeia (USP)* specified apparatus 2. The stirring rate and temperature were adjusted to 75 ± 1 rpm and 37°C ± 0.5°C, respectively. Water (600 mL) was employed as a dissolution medium. All dissolution trials were performed in triplicate, whereby 5-mL samples were obtained at specified times and replaced by fresh media. Samples were taken up to 8 hours, while the system was kept running up to 12 hours. At the end of each dissolution run, any remaining matrices were crushed, and the total amount of drug calculated. The absorbance of metoclopramide hydrochloride was measured at a wavelength of 272 nm. Microsoft Excel, Version 2000, was used to generate equations of the respective model.

Statistical Data Analysis

Statistical data analysis was performed using Student's t test with $P < .05$ as the minimal level of significance.

Results and Discussion

Results for the stability and compatibility of metoclopramide for the conditions of the present research are shown in Table 1. Results indicate that metoclopramide hydrochloride is adequately stable under the microwave conditions chosen for the present study and is compatible with bovine serum albumin and gluten, the proteinaceous materials that comprise the bulk structure of the prepared matrices.

The percentage weight increase for albuminoidal matrices and gluten matrices after storage for 1 week in desiccators maintained at various RH is provided in Table 2. It is evident that matrices stored at 94% RH had the most prominent increase in weight due to water sorption. The corresponding percentage weight loss due to water evaporation

Table 2. The Average Percentage Weight Increase for Bovine Serum Albumin and Gluten Matrices After Storage for 1 Week in Desiccators Maintained at Relative Humidities of 19%, 58%, and 94%*

Sample	19% RH	58% RH	94% RH
Albumin compacts (mean ± SD, n = 10)	6.87 ± 0.34	9.69 ± 0.48	22.27 ± 1.11
Gluten compacts (mean ± SD, n = 10)	……	……	18.87 ± 0.94

*RH indicates relative humidity.

after microwave drying was determined using the following simple expression:

$$\%\ weight\ loss = \frac{W_o - W_t}{W_o} \times 100\%, \qquad (1)$$

whereby W_o is the initial weight before microwave drying and W_t is the weight after an exposing period of time t. The time-dependent weight loss for albumin compacts maintained at 19%, 58%, and 94% RH for 1 week and dried by microwave heating is shown in Figure 1. It is evident that higher degrees of weight loss occur as the initial level of sorbed moisture increases. It is believed that weight loss is a result of water evaporation rather than direct interaction with the proteinaceous material, since the large size and dipole moment of proteins prohibit rotation under microwave field.[15] The figure also indicates that water evaporation takes place over 2 distinct stages. Most of the sorbed water evades the system rapidly during the first minute, which characterizes the first stage, while the second stage reflects the slow evaporation of water traces left behind and entrapped within the matrix. Since thermal energy resulting from microwave exposure and required for cross-linking is a direct function of the initial level of water in the system, the degree of cross-linking is considered to be directly proportional to the level of sorbed moisture. It is thus theorized that most of the cross-linking process occurs during the first stage during which most of water evaporation takes place.

The dissolution of albumin matrices maintained at 19%, 58%, and 94% RH and microwaved for 1, 3, and 5 minutes compared with nontreated matrices is shown in Figures 2, 3, and 4. The drug release data were fitted into Peppas equation as follows:

$$M_t/M_\infty = Kt^n, \qquad (2)$$

where M_t/M_∞ is the fractional release of the drug; t is the release time; K is a constant incorporating structural and geometric characteristics of the controlled-release device; and n is the diffusional exponent indicative of the mechanism of drug release.[16] The data were fitted up to 60% of drug released. The values of n, K, and the coefficients of determination r^2 obtained for both proteins, bovine serum albumin and gluten, are shown in Table 3.

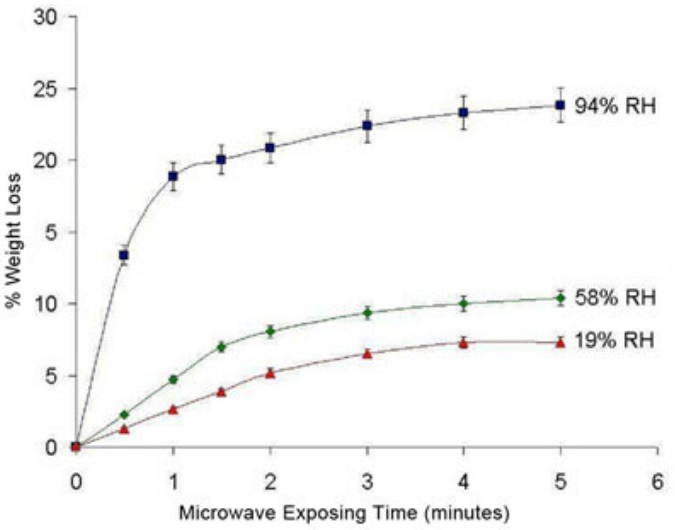

Figure 1. Mean weight loss for albumin matrices at various RH, n = 10.

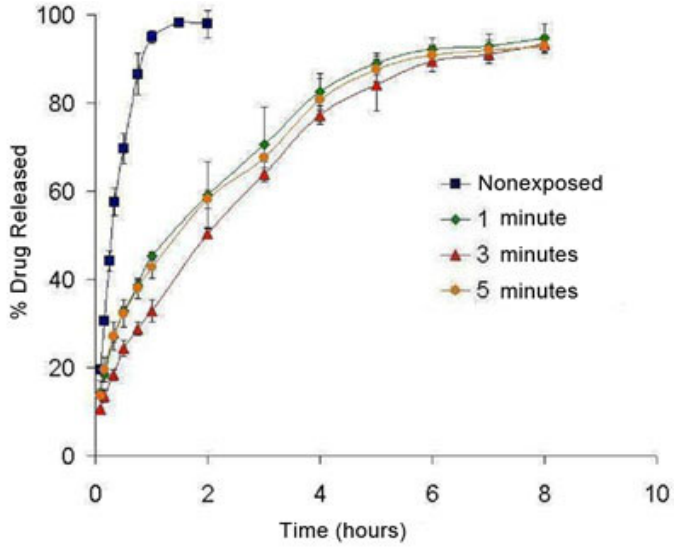

Figure 2. The dissolution profile for albumin matrices conditioned at 94% relative humidity for 1 week and microwaved for 1, 3, and 5 minutes versus nonexposed matrices.

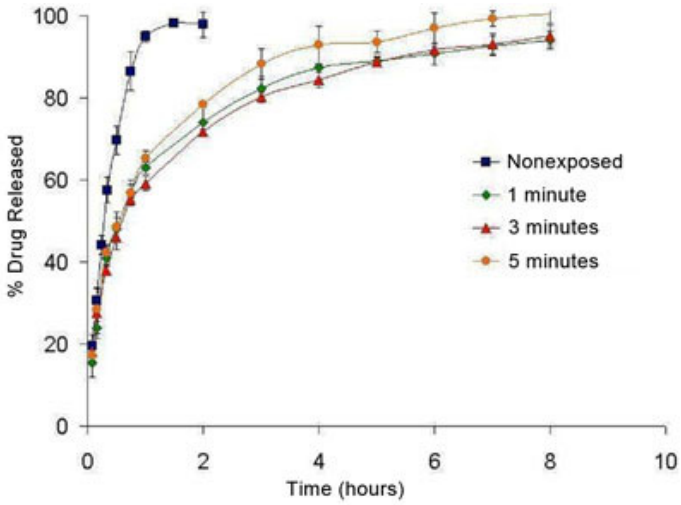

Figure 3. The dissolution profile for albumin matrices conditioned at 58% relative humidity for 1 week and microwaved for 1, 3, and 5 minutes versus nonexposed matrices.

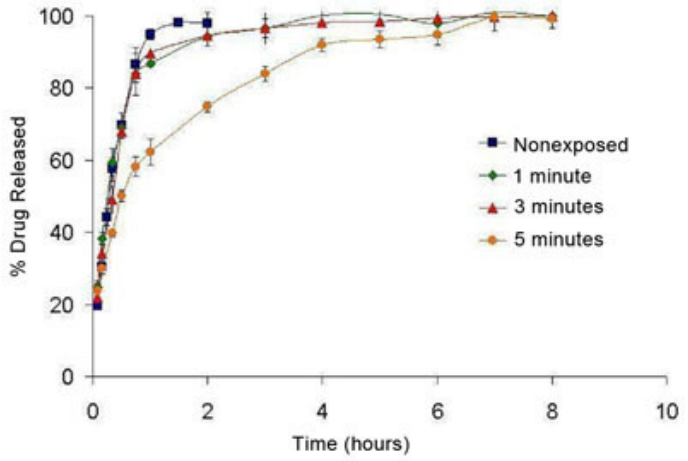

Figure 4. The dissolution profile for albumin matrices conditioned at 19% relative humidity for 1 week and microwaved for 1, 3, and 5 minutes versus nonexposed matrices.

The release profiles for the microwave-treated albumin matrices maintained at RH of 94% as compared with nonexposed matrices indicate massive cross-linking within the proteinaceous matrix, which is attributed to the high level of initial moisture content and denaturation of the albuminoidal protein (Figure 2). While rapid disintegration characterized the nonexposed albumin matrices, the microwave-treated ones remained intact throughout the whole period of dissolution, and a significant decline in the rate of drug release was observed. The main mechanism governing the release of metoclopramide from albumin matrices heated for 1, 3, and 5 minutes was shown to follow the classical Fickian diffusion mode (Table 3). Since most of water evaporation occurs during the first minute, insignificant differences in the rate of drug release were observed between the various microwave treatments. The slight deviation of the diffusional exponent *n* from 0.5 is attributed to the formation of a limited number of pores in the system, which increases the rate of drug release and reduces the value of the diffusional exponent.[16]

Since the degree of cross-linking is a direct function of the initial level of sorbed moisture and the period of microwave heating,[9] the rate of drug release from albumin compacts maintained at an RH of 58% and microwaved for 1, 3, and 5 minutes is expected to be faster than those conditioned at 94% RH and treated similarly (see Figure 3). The main mechanism governing the release of metoclopramide from these matrices is Fickian diffusion indicated by diffusional exponent *n* (Table 3). Insignificant differences were detected between the various microwave treatments. The slight deviation in the release exponent *n* from 0.5 is mainly owing to the formation of pores as discussed previously. These pores increase in number as the microwave-exposing period increases. They are mainly attributed to water evaporation during the first phase; however, water molecules entrapped in the matrix and evaporated during the second phase contribute to the formation of this porous structure. It is believed that these entrapped water molecules achieve high temperatures before finally evading the matrix through pore formation. Their effect is most imminent in systems that have been minimally cross-linked and thus fail to hold the matrix against the force generated by the rapidly evaporating water molecules.

Alternatively, bovine serum albumin compacts maintained at an RH of 19% contain minimal amount of sorbed water to produce adequate cross-linking in the proteinaceous system when microwaved. Since cross-linking is minimal, the release profile for those treated for 1 and 3 minutes showed an insignificant difference from that of the nonexposed matrices (Figure 4). However, the release profile for matrices exposed for 5 minutes showed a significant decline in the rate of drug release, particularly during the late stage of the drug-release profile. This finding is believed to be the result of slight cross-linking in the system caused by the amount of water evaporated during the second stage. The diffusional exponent *n* (Table 3) gives evidence regarding this second stage of water evaporation. The release exponent

Table 3. Exponent (n), Constant (*K*), and Coefficient of Determination (r^2) According to $M_t/M_\alpha = Kt^n$*

Protein type and equilibrating conditions in the desiccator	Microwave exposure time (minutes)	n	K	r^2
Albumin/94% RH	1	0.4620	0.3506	0.9971
Albumin/94% RH	3	0.5079	0.3426	0.9925
Albumin/94% RH	5	0.4509	0.3614	0.9982
Albumin/58% RH	1	0.5754	0.3547	0.9817
Albumin/58% RH	3	0.4757	0.3503	0.9920
Albumin/58% RH	5	0.5227	0.3491	0.9811
Albumin/19% RH	5	0.4054	0.4710	0.9935
Gluten/nontreated	0	0.5392	0.2174	0.9932
Gluten/94% RH	1	0.5071	0.2263	0.9909
Gluten/94% RH	3	0.5151	0.2224	0.9986
Gluten/94% RH	5	0.5533	0.2279	0.9961

*RH indicates relative humidity.

n equal to 0.4054 indicates that minimal amount of water entrapped in the inner core of the matrix and responsible for the slight degree of cross-linking observed, achieves high temperatures before finally evading the system, creating a porous structure in the relatively minimally cross-linked matrix. While disintegration of compacts maintained at an RH of 19% and microwaved for 1 and 3 minutes occurred rapidly and completely, compacts microwaved for 5 minutes initially disintegrated, which explains the initial rapid boost in the drug-release profile; however, disintegration failed to proceed and a spherical insoluble compact remained in the system. This compact is believed to be responsible for the decline in the rate of drug release seen afterwards, which agrees with what has been mentioned regarding the late evaporation of water after achieving a substantial level of cross-linking in the matrix sufficient to strengthen the system and prevent disintegration.

The influence of sorbed water on the extent of denaturation and consequently on the release profile is depicted in Figure 5. A significant decline in the rate of drug release was observed with increasing humidity and under the same period of microwave exposure. From the previous discussion, it is believed that water-insoluble denatured protein materials suitable for the design of controlled drug delivery systems could be developed under specific conditions of RH and microwave exposure.

Attempt to develop the results obtained with bovine serum albumin through use of gluten was then investigated. Gluten is a water-insoluble protein, which associates in water through hydrophobic bonding.[17] The use of gluten as a pharmaceutical excipient has been prohibited due to the possibility of eliciting a range of sensitization reactions, widely known as gluten intolerance, in some individuals.

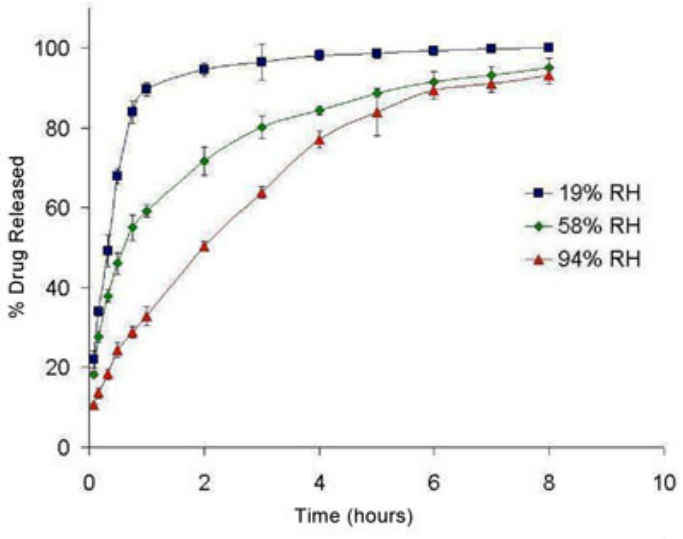

Figure 5. The dissolution profile for albumin matrices maintained at various RH and microwaved for 3 minutes.

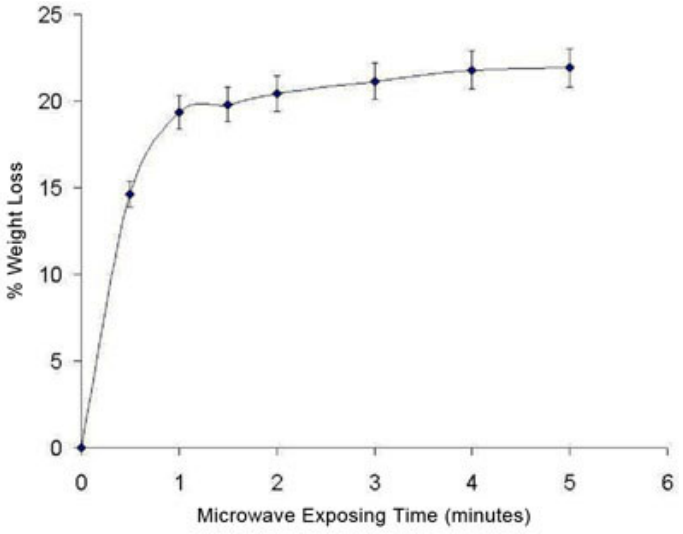

Figure 6. The dissolution profile for gluten matrices conditioned at 94% relative humidity for 1 week and microwaved for 1, 3, and 5 minutes versus nonexposed matrices.

Nevertheless, transdermal patches made of gluten for the controlled release of nifidipine have been reported.[18] For the present work, gluten is used for its hydrophobic properties.

Water-insoluble gluten matrices do sustain drug release as implied through the release profile of nonexposed matrices (Figure 6). Fitting the release profile into Equation 2 suggests that the main mechanism governing the release of metoclopramide from gluten matrices is Fickian diffusion. This creates the cornerstone for future use of gluten in the production of sustained-release matrices. From the previous discussion, it is anticipated that control over the rate of drug release from gluten matrices could be achieved through uniform cross-linking of moistened matrices in a microwave oven. Unexpectedly, the microwave-treated matrices produced conflicting results. Although matrices removed from the desiccator were moistened to a great level as shown in Table 2 and Figure 7, microwave treatment generally failed to reduce the rate of drug release. On the contrary, matrices denatured for 5 minutes showed a significant rise in rate of release after 3 hours of dissolution, which is attributed to minimal pore formation as previously mentioned. Alternatively, hardness test measurements indicated a steep increase in total tablet hardness after microwave exposure, which gives evidence that cross-linking has occurred to an appreciable extent. Tablet hardness rose from 1.2 ± 0.276 kPa for nonexposed matrices to 7.3 ± 1.623 kPa, 8.3 ± 1.534 kPa, and 11.3 ± 1.682 kPa for 1, 3, and 5 minutes microwave-treated matrices, respectively. It is believed that microwave cross-linking was restricted to the inner hydrophilic fraction of the gluten proteins. The external hydrophobic parts were not involved in cross-linking and thus neighboring gluten proteins failed to aggregate. This led to an increase in total hardness with almost no effect on the rate of drug release.

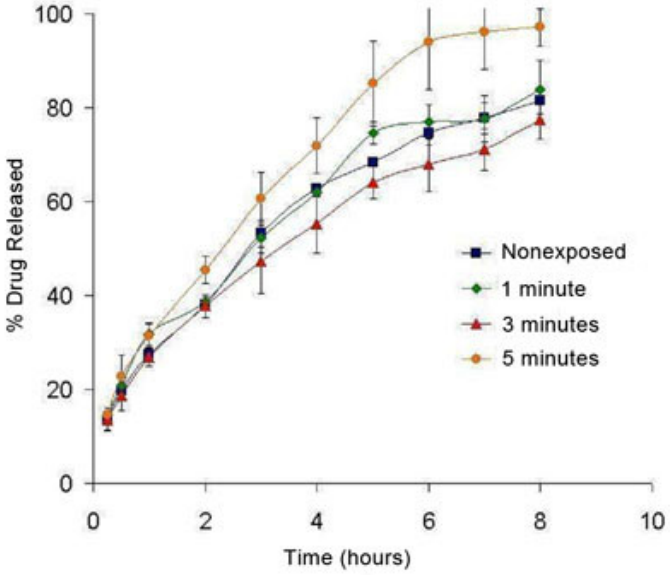

Figure 7. Mean weight loss for gluten matrices at 94% RH, n = 10.

CONCLUSION

In conclusion, the influence of microwave irradiations on protein systems sorbed with moisture depends on the physical properties of the protein itself, which are largely determined by the structural configuration and arrangement of the peptide groups in the protein. Results generated through the scope of this study should be further developed to investigate the influence of other factors such as naturally released enzymes on the feasibility of using microwave-denatured proteins in the design of controlled drug delivery devices.

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