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Curcumin loaded casein submicron-sized gels as drug delivery systems

S Milenkova¹, I Manolov¹, B Pilicheva², M Nikolova³, M Marudova^{1*}

¹ Department of Physics, Faculty of Physics and Technology, University of Plovdiv "Paisii Hilendarski, 24 Tzar Asen Str., BG-4000 Plovdiv, Bulgaria

² Medical University of Plovdiv, Faculty of pharmacy, 15A Vassil Aprilov Blvd, BG-4003, Plovdiv, Bulgaria

³ Department of Biochemistry and Microbiology, Faculty of Biology, University of Plovdiv "Paisii Hilendarski, 24 Tzar Asen Str., BG-4000 Plovdiv, Bulgaria

E-mail: marudova@uni-plovdiv.net

Abstract. Hydrogels from natural polyelectrolytes possess many important features such as low toxicity, biocompatibility, biodegradability and hydrophilicity. These properties make them very suitable for applications such as immobilization and controlled release of drugs and other types of biologically active molecules. In the present study submicron-sized hydrogels made from casein by ionotropic gelation are investigated. For this purpose, two types of crosslinking agents are used at different pH conditions. In order to characterize these submicron gels, their sizes, chemical structures and thermal stability are examined by dynamic light scattering (DLS), FT-IR and Differential Scanning calorimetry (DSC) respectively. To prove their immobilization ability, active compound, namely curcumin, is immobilized in the hydrogel's structures. DPPH assay is conducted to establish the antioxidant properties of the curcumin before and after the immobilization. The loading efficiency of the nanostructures together with the curcumin release kinetics are evaluated and modelled mathematically.

1. Introduction

Over the past decades the application and modification of biodegradable polymers has attracted the attention of the scientists from all around the world. They can be used as drug delivery systems with controlled release [1] or targeted drug delivery [2], edible coatings [3], renewable sources [4], enzyme immobilization and delivery [5], and so on. Some of their main advantages are the ability to form different types of structures – layers [3], particles[6], micelles[7], gels[7]; the lack of immune response from the body [8], the lack of environmental pollution for their formulation, because they already exist in the nature [9]. Curcumin is a low molecular weight compound from the polyphenolic group. This compound possesses many beneficial properties for the human health. Amongst them are: antioxidant, anti-inflammatory, anti-cancer properties. It has been applied in the treatment of different diseases such as atherosclerosis, neurodegenerative diseases, liver fibrosis, diabetes, autoimmune diseases and so on [10]. However, there are two major limitations in the curcumin use [11]. Firstly, it is insoluble in water at acidic and neutral pH, which significantly reduces its bioavailability. Secondly, curcumin is stable at acidic pH but unstable at neutral and basic pH [12]. Therefore, the efforts of scientists are aimed at developing various strategies to stabilize curcumin and increase its solubility [13]. A promising achievement in this direction is the immobilization of curcumin in submicron hydrocolloidal systems.



Some earlier studies have shown that curcumin can hydrophobically bind with proteins including milk casein [14], β -lactoglobulin [15], and soy protein [16] which may greatly improve the solubility, stability, and bioactivities of curcumin.

Casein is a major milk protein and a vital substance in many peoples' way of living. Along with its significance as a food substance, casein can be used in the field of biomedicine and it is a promising candidate for novel drug delivery systems [7]. It can form polyelectrolyte complexes with other biopolymers, hydrogels, micelles, film coatings, beads and particles. As far as its molecules contains both carboxyl and amino groups, depending on the pH of the medium, casein can behave as both polyanion or polycation [7].

In this study we present submicron-sized gels made from casein crosslinked with two types of crosslinking agents in different pH conditions. We explore their ability to immobilize curcumin and model mathematically their releasing properties of the active compound afterwards.

2. Materials and methods

2.1. Materials

Sodium Caseinate (Casein sodium salt from bovine milk), Curcumin and Sodium Tripolyphosphate were bought from Sigma Aldrich. Calcium dichloride, ethanol and acetic acid were used with analytical grade of purity.

2.2. Methods

2.2.1. Submicron-particles formulation. The particles were formed on the base of casein hydrogel. In this study 1% and 2% solutions of casein dissolved in distilled water were used. Their pH was adjusted to pH=3 or to pH=8.5 using 3M HCl and 1M NaOH respectively. The solutions were stirred (500–1000 rpm) on a magnetic stirrer, and an ethanolic solution of curcumin (0.2%) was added dropwise and stirred for 30 min. Then varying volumes of the crosslinking agents (5 % w/v solution in distilled water) were added dropwise to this mixture to achieve mass ratios casein:crosslinker of 3:1, 5:1, and 10:1. In our case the casein hydrogel was crosslinked at the low pH with NaTPP and at the high pH with CaCl₂. The mixtures were left to form the gels for 2 hours stirring on a magnetic stirrer. After that the crosslinked casein gel particles were centrifuged at 15 000 rpm for 30 minutes to precipitate and washed with distilled water for another 15 minutes.

2.2.2. Dynamic Light Scattering. This technique was applied in order to examine the average sizes of the gel particles. Dynamic light scattering equipment NANOTRAC WAVE Particle Size, Zeta Potential and Molecular Weight Analyzer (Microtrac) was used for these measurements.

2.2.3. Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy. The characterization of the interactions between the active compounds in the gel samples was carried out using ATR technique of FT-IR spectroscopy (Perkin Elmer Spectrum 100) in the spectral range of 4000–650 cm⁻¹ with a resolution of 4 cm⁻¹.

2.2.4. Yield of the preparative process. In order to quantify the amount of casein transformed into gel particles, after the centrifugation and washing, the particles were freeze dried at -50°C and 10 Pa pressure for 72 h. Then the yield of the gelation process was estimated as the ratio between the dry mass of the particles and the total dry mass in the formulation:

$$\text{Yield (\%)} = \frac{\text{dry mass of the particles}}{\text{total dry mass in the formulation}} \cdot 100 \quad (1)$$

2.2.5. Encapsulation efficiency. The loading efficiency of all the types of particles that we have crosslinked was evaluated. The concentration of the curcumin which was put in the solutions was kept the same (2 mg/ml). The resulting systems were centrifuged at 15 000 rpm for 30 minutes and the absorbance of the supernatant was measured at 432 nm. The concentration of the left curcumin in the supernatant was calculated based on previously prepared calibration curve. The loading efficiency was calculated using the equation below:

$$E (\%) = \frac{(C_0 - C)}{C_0} \cdot 100, \quad (2)$$

where: C_0 – the total amount of curcumin, C – the non-loaded amount of curcumin.

2.2.6. Differential Scanning Calorimetry. The thermal stability and the phase state of the used compounds before and after the immobilization were examined using DSC 204F1 Phoenix (Netzsch Gerätebau GmbH, Germany) based on the heat flux principle and cooled with an intracooler. An indium standard ($T_m=156.6^\circ\text{C}$, $\Delta H_m=28.5 \text{ J/g}$) was used for the heat flow and the temperature calibration. All of the samples were hermetically sealed in aluminum sample pans and an empty pan, identical to the sample one, was used as reference. The measurements were conducted under argon atmosphere at a heating rate of $10^\circ\text{C}/\text{min}$.

2.2.7. Evaluation of the drug-release kinetics and mathematical modeling of the process. The curcumin release from the particles was investigated by the diffusion method. A weighted amount of particles (equivalent to 3.68 mg of curcumin) was suspended in 1 ml of saliva buffer (pH=7.4). Then, this solution was placed in dialysis membrane. Then each bag was placed in saliva buffer (pH = 7.4) medium in 20 ml volume containing 150 μl of TWEEN 20 at a temperature of 37°C and a shaking speed of 50 rpm. Samples of 2 ml were taken at selected time intervals (15 min, 30 min, 45 min, 60 min, 90 min, 120 min, 150 min, 180 min, 210 min, 240 min and 300 min) for spectroscopic analysis at 432 nm. An equivalent amount of buffer was added back to the release medium after each taking of sample. To predict drug release kinetics, the data obtained from the release studies were applied to various kinetic models such as zero order, first order, Higuchi and Korsmeyer-Peppas [17].

2.2.8. Antioxidant assay. To establish the antioxidant properties of the curcumin before and after its immobilization in the casein particles, DPPH assay was conducted. 1ml of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was added to 1 ml of the samples and left for 30 minutes to react. At the end of the time the absorbance at 517 nm was measured. The amount of the scavenged radicals was calculated by the equation:

$$\text{Inhibited radicals (\%)} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \cdot 100, \quad (3)$$

where: A_{blank} – the absorbance of the control sample, A_{sample} – the absorbance of the sample containing curcumin. The measurements were repeated 3 times for each sample. Their scavenging ability is presented as percentage of inhibition.

3. Results and discussion

3.1. Formation and characterization of CAS submicron particles

The curcumin loaded casein submicron particles were successfully synthesized by the ionic gelation method at pH 3.0 and 8.5 using NaTPP and CaCl_2 as the cross-linking agents. These are less toxic than other cross-linkers such as glutaraldehyde which can cause antigenicity. The composition and some main characteristics of the particles are presented in Table 1. The sizes of the particles formed at low pH are in micron range and vary between 1.85 μm and 5.84 μm . Smaller particles formed at higher

casein concentration and lower NaTPP concentration. Similar results were reported by other authors [18]. The CAS particles formed at pH 8.5 are nano-sized and the smallest one (the average diameter is 160 nm) were synthesized from 2% CAS solution and CAS:CaCl₂ ratio 10:1. The gelling efficiency is higher at low pH and fell sharply at lower concentration of the crosslinker. For all tested particles the curcumin loading efficiency is practically 100%. This result could be correlated with the low solubility of curcumin in water and the hydrophobic interactions with CAS.

Table 1. Composition and characteristics of the formulated ionically crosslinked curcumin-loaded casein (CAS) particles.

Formula	Variables			Particle size, µm	Particle yield, %	Curcumin loading efficiency, %
	CAS amount, %	pH	CAS:NaTPP ratio			
F1	1	3.0	3:1	5.84±0.47	71	99.9
F2	1	3.0	5:1	4.01±0.47	50	99.9
F3	1	3.0	10:1	4.07±0.48	47	99.9
F4	2	3.0	3:1	2.67±0.31	70	99.9
F5	2	3.0	5:1	1.85±0.52	54	99.9
F6	2	3.0	10:1	2.57±0.19	40	99.9
			CAS:CaCl ₂ ratio			
F7	1	8.5	3:1	-		-
F8	1	8.5	5:1	0.63±0.05	32	99.8
F9	1	8.5	10:1	0.53±0.06	26	99.8
F10	2	8.5	3:1	-		-
F11	2	8.5	5:1	0.51±0.12	31	99.8
F12	2	8.5	10:1	0.16±0.02	11	99.8

3.2. ATR-FTIR analysis

ATR-FTIR analysis was used to establish the interactions occurring during the gelling and of curcumin loading processes. The characteristic spectra of casein, sodium tripolyphosphate, calcium dichloride and crosslinked casein gels are presented in figure 1 and figure 2. The characteristic peaks of casein are at 2929 cm⁻¹ (symmetric -CH₂ stretch), 2873 cm⁻¹ (asymmetric -CH₂ stretch), 1634 cm⁻¹ (NHCO stretch) and the interval between 1300 cm⁻¹ and 1000 cm⁻¹ for the carboxyl groups [19]. NaTPP is characterized with P=O stretch in the interval between 1200 cm⁻¹ and 1050 cm⁻¹ and a P-O-P stretch at 887 cm⁻¹ [20]. The ionotropic gelation process is confirmed by the shift of the amide and the P=O groups. The electrostatic interactions between the casein's carboxylate ion and Ca²⁺ are confirmed by the shift and the change in the shape of the carboxylic group's peak (figure 2).

Curcumin has characteristic bands at 1601 cm⁻¹ (C=C), 1505 cm⁻¹ and 1427 cm⁻¹ (C-C), 1273 cm⁻¹ (C-O) and 1203 cm⁻¹ (C-O-C) [21]. The presence of curcumin in the casein-NaTPP and casein-CaCl₂ gels can be confirmed by the existence of its characteristic peaks at 1505 cm⁻¹ (C-C stretch) in the spectrum of the loaded complex (figure 3 and figure 4).

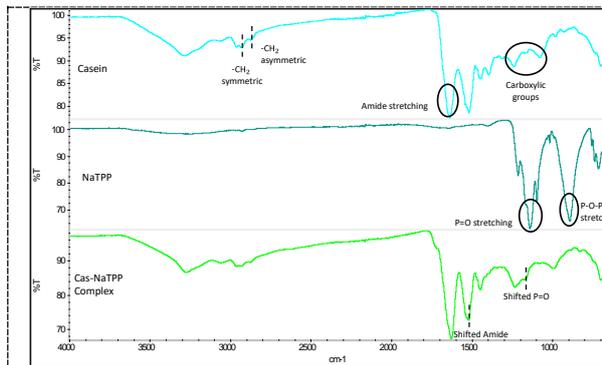


Figure 1. ATR-FTIR spectra of Casein, NaTPP and crosslinked with NaTPP casein gel

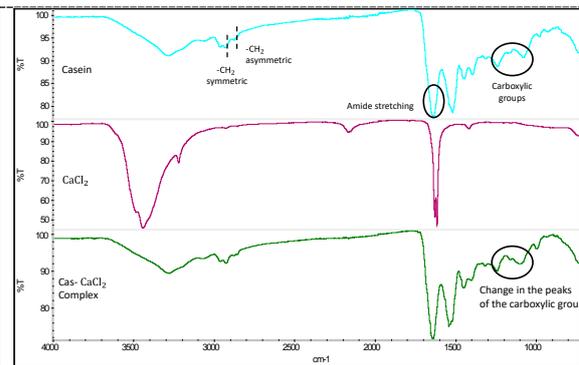


Figure 2. ATR-FTIR Spectra of Casein, CaCl₂ and crosslinked with CaCl₂ casein gel

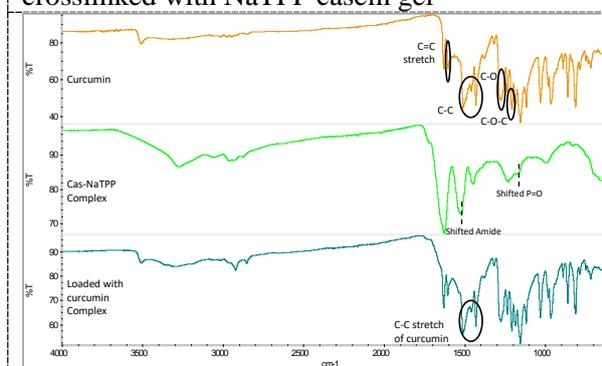


Figure 3. ATR-FTIR Spectra of Curcumin, Cas-NaTPP gels before and after the loading of curcumin

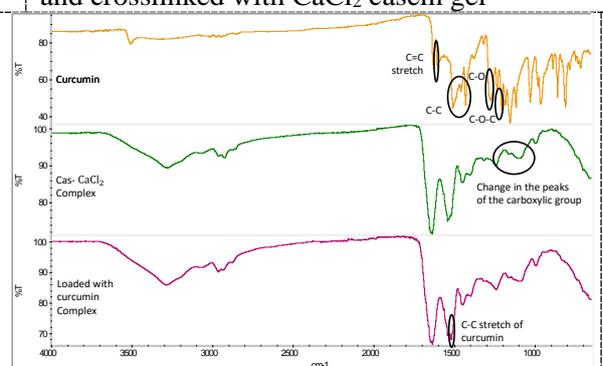


Figure 4. ATR-FTIR Spectra of Curcumin, Cas-CaCl₂ gels before and after the loading of curcumin

3.3. Thermal stability and phase state of loaded curcumin

The thermal stability of the submicron gel particles and the phase state of loaded curcumin were estimated by the method of differential scanning calorimetry (DSC). The resulting thermograms are presented in figure 5.

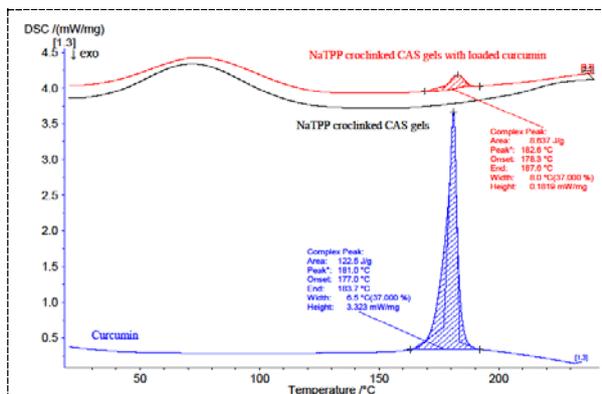


Figure 5a. DSC thermograms of non-loaded and curcumin loaded CAS gels crosslinked with NaTPP.

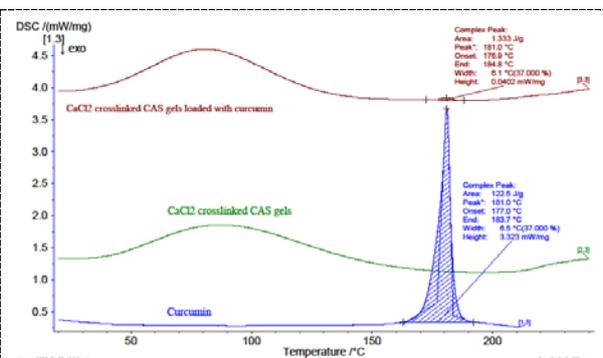


Figure 5b. DSC thermograms of non-loaded and curcumin loaded CAS gels crosslinked with CaCl₂.

The native state of curcumin is crystalline solid with melting point 181°C and enthalpy of fusion 122.5 J/g. In loaded state, curcumin crystals melt in a wider temperature range, which is due to

heterogenous crystal with different size that occur under unfavorable crystallization conditions. Based on the enthalpy of fusion it can be estimate that about 35% of the curcumin loaded in the NaTPP crosslinked casein gels is in crystal phase. The crystal curcumin amount is much lower (about 5.4%) when it is loaded in CaCl_2 crosslinked casein gels. The change of curcumin phase from crystal to predominantly amorphous during the loading in casein gels is a prerequisite for increased bioavailability.

3.4. *In vitro* release and release kinetic study

The cumulative release of curcumin in PBS medium at pH 7.4 and 37°C from 0 min to 360 min is shown in figure 6 and figure 7. The cumulative curcumin release was around 0.2% in the first 6 h, demonstrating that it presented no burst release of surface adhered drugs. Hence, curcumin is well encapsulated in the casein gels. The burst release is undesired because of the toxic effect and reduced delivery efficiency [22]. Similar release behaviour was reported by other researcher [23] where the period for total curcumin release was 28 days. The prolonged period of curcumin release could be due to the hydrophobic properties of the drug and its difficult dissolution in water media. On the other hand, casein is hydrophilic polymer, the gel particles swell in the PBS buffer, resulting in hindering the diffusion of curcumin into the medium and drug release was controlled effectively. This hypothesis is confirmed by the result presented in figure 6. The increase of crosslinker concentration leads to delayed release. A similar finding has been observed by Baimark and Srisuwan [24] who reported that the amount of drug release dropped when the concentration of Ca^{2+} cross-linker in alginate gels rose from 5% to 10% due to harder swelling of alginate network.

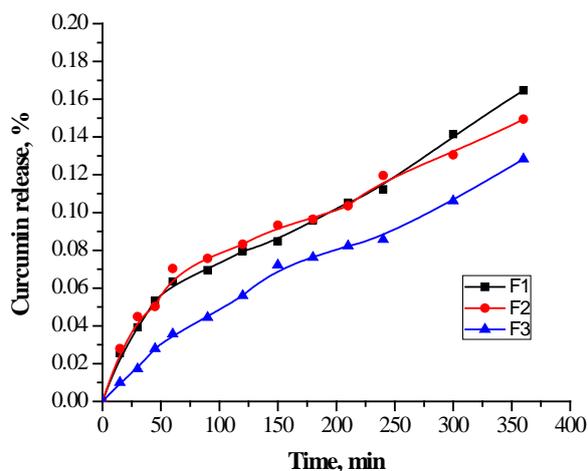


Figure 6. Curcumin release at pH 3, CAS concentration 1% and different concentrations of NaTPP

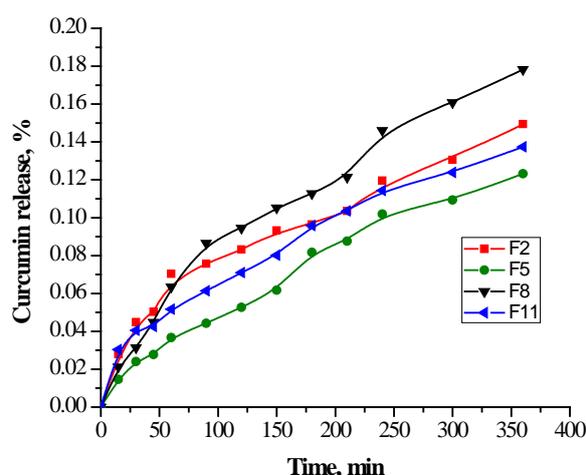


Figure 7. Curcumin release at CAS:crosslinker ratio 5:1 and different pH and CAS concentrations

Increasing the concentration of the casein solution from which the submicron gel particles were formed from 1% to 2% results in a delay in the curcumin release – figure 7, F2 and F7. A possible reason for the observed behavior is the much larger particle size obtained under these conditions. Generally, the release of a drug from large-sized structures is slower due to the relatively smaller surface contacting the release medium [25]. Similar results were observed for curcumin release from chitosan nanoparticles [26] and from cellulose beads [27]. The rate of curcumin release greatly depends on the type of crosslinker (figure 7, F2 and F8). Curcumin releases faster from CAS gels, crosslinked with CaCl_2 . This effect is probably due to different gel structures – the NaTPP crosslinks the CAS molecules via formation of covalent bond, while the interactions between CaCl_2 and CAS are electrostatic. In this case the gels are more unstable and prone to swelling, which facilitates the drug release.

The obtained results demonstrated that the curcumin release profile could be controlled by the concentration of casein and crosslinker and by the type of the crosslinker. Based on the drug release results, the drug release kinetics was evaluated by fitting the release results into different mathematical models including zero-order, first order, Higuchi and Korsmeyer-Peppas models. The correlation coefficients (R^2) were used to determine the accuracy and prediction ability of these models. The best fitting was found for Korsmeyer-Peppas model with the correlation coefficient $R^2 = 0.99$, followed by the Higuchi model with the correlation coefficient $R^2 = 0.97$, first-order model with the correlation coefficient $R^2 = 0.96$. The zero-order model appeared not to be the suitable model to present the curcumin release kinetic due to its $R^2 = 0.84$. The exponent n in the Korsmeyer-Peppas model was used to characterize the release mechanism of curcumin. It can be seen that the average n value was 0.61, showing the anomalous (non-Fickian) diffusion mechanism which includes both diffusion-controlled release and swelling controlled release [28].

3.5. Antioxidant activity

The scavenging ability of immobilized and native curcumin was established. The results are shown in percentage of scavenged radicals with standard deviations after 30 minutes in Table 2. The results are in good correspondence with previously tested systems [11,29]. The main conclusion is that curcumin is being stabilized after immobilization and its scavenging abilities are being improved.

Table 2. Results from the DPPH assay presented in %

Type of sample	Pure curcumin	Immobilized curcumin at pH=3	Immobilized curcumin at pH=8.5
Scavenged radicals (%)	79.7%±0.83	85.4%±4.13	90%±0.36

4. Conclusion

Curcumin-loaded casein gels crosslinked with NaTPP and CaCl_2 were investigated in this study. The average size of the gel particles varied from 160 nm to a couple of microns, depending on the type of crosslinker and casein:crosslinker ratio. The loading of curcumin into the nanospheres did not lead to chemical interactions between chitosan and curcumin. The percentage of the loaded curcumin was practically 100%. The loaded curcumin was predominantly amorphous, which increased its bioavailability. The curcumin release is very slow and the release kinetics is described by Korsmeyer-Peppas model. The release is governed by anomalous (non-Fickian) diffusion mechanism which includes both diffusion-controlled release and swelling controlled release.

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References

- [1] Neffe A, Wischke C, Racheva M and Lendlein A 2013 *Exp. Rev. Med. Dev.* **10**(6) 813-833
- [2] Singh R and Lillard Jr J 2009 *Expl. Mol. Path.* **86**(3) 215-223
- [3] Valverde J, Valero D, Martínez-Romero D, Guillén F, Castillo S and Serrano M 2005 *J. Agricul. Food Chem.* **53**(20) 7807-7813
- [4] Kaplan D 1998 *Biopolymers from renewable resources* (Berlin: Springer) pp. 1-29
- [5] Bilal M and Iqbal H 2019 *Int. J. Biol. Macromol.* **130** 462-482
- [6] Hu K, Huang X, Gao Y, Huang X, Xiao H and McClements D 2015 *Food Chem.* **182** 275-281
- [7] Elzoghby A, El-Fotoh W and Elgindy N 2011 *J. Control. Rel.* **153**(3) 206-216
- [8] Huhtala A, Pohjonen T, Salminen L, Salminen A, Kaarniranta K and Uusitalo H 2007 *J. Biomed. Mat. Res. A* **83**(2) 407-413
- [9] Rao M, Bharathi P and Akila R 2014 *Sci. Rev. Chem. Commun.* **4**(2) 61-68

- [10] Menon V and Sudheer A 2007 *The molecular targets and therapeutic uses of curcumin in health and disease* (Boston: Springer) pp. 105-125
- [11] Wu Y and Wang X 2017 *Int. J. Food Prop.* **20**(12) 3295-3307
- [12] Lee W, Loo C, Bebawy M, Luk F, Mason R and Rohanzadeh R 2013 *Curr. Neuropharm.* **11**(4) 338-378
- [13] Stohs S, Chen O, Ray S, Ji J, Bucci L and Preuss H 2020 *Molecules* **25**(6) 1397
- [14] Benzaria A, Maresca M, Taieb N, Dumay E 2013 *Food Chem.* **138** 2327–2337
- [15] Sneharani A, Karakkat J, Singh S, Rao A 2010 *J. Agricul. and Food Chem.* **58** 11130–11139
- [16] Tapal A and Tiku P 2012 *Food Chem.* **130** 960–965
- [17] Nair R, Morris A, Billa N and Leong C 2019 *AAPS PharmSciTech.* **20**(2) 69
- [18] Elzoghby A, Helmy M, Samy W and Elgindy N 2013 *Int. J Nanomed.* **8** 1721
- [19] Siročić, A, Krehula L, Katančić Z and Hrnjak-Murgić Z 2016 *Chem. Biochem. Eng. Quart.* **30**(4) 501-509
- [20] Gurses M, Erkey C, Kizilel S and Uzun A 2018 *Talanta* **176** 8-16
- [21] Tsai W, Yu K, Huang Y and Lee C 2018 *Int. J. Nanomed.* **13** 903
- [22] Uyen N, Hamid Z, Tram N and Ahmad N 2020 *Int. J. Biolog. Macromol.* **153** 1035-1046
- [23] Uyen N, Hamid Z and Ahmad N 2020 *J. Drug Delivery Sci. Techn.* 101796
- [24] Baimark Y and Srisuwan Y 2014 *Adv. Powder Techn.* **25**(5), 1541-1546
- [25] Thwala L 2012 *Preparation and characterization of alginate-chitosan nanoparticles as a drug delivery system for lipophilic compounds* (Doctoral dissertation, University of Johannesburg),
- [26] Milenkova S, Pilicheva B, Tsoneva S and Marudova M 2020 *Bulg. Chem. Com.* **52**(A) 134-140
- [27] Voon L, Pang S and Chin S 2017 *Int. J. Polymer Sci.* **2017** 258176
- [28] Dehghan-Baniani D, Zahedifar P, Bagheri R and Solouk A 2019 *Starch-Stärke* **71**(5-6) 1800305
- [29] Huang X, Huang X, Gong Y, Xiao H, McClements D and Hu K 2016 *Food Res. Int.* **87** 1-9