Sigma J Eng & Nat Sci 35 (1), 2017, 47-58

Sigma Journal of Engineering and Natural Sciences
Sigma Mühendislik ve Fen Bilimleri Dergisi

Research Article / Araştırma Makalesi

CURCUMIN, INCREASING ITS WATER SOLUBILITY BY ULTRASOUND AND PEG

Huceste ÇATALGİL GİZ*[1], Sevim İŞÇİ[1], Fatemeh BAHADORİ[2], Zeynep KALAYCIOĞLU[1], Barbaros AKKURT[1]

[1]*ITU Fen-Edebiyat Fakultesi, Maslak-ISTANBUL*
[2]*Department of Pharmacognosy/Phytochemistry, Faculty of Pharmacy, Bezmialem Vakıf University, ISTANBUL*

Received/Geliş: 18.12.2016 Revised/Düzeltme: 30.12.2016 Accepted/Kabul: 23.01.2017

ABSTRACT

Water solubility of curcumin is increased by PEG in ultrasound medium. Different PEG-curcumin complexes were prepared with and without using ultrasound at 25^{0}C. Curcumin concentrations of complexes were determined by UV. The surface charges of PEG-curcumin complexes were investigated by zeta measurements. Critical aggregate concentrations were determined from both UV and zeta potential measurements. Complexes prepared by ultrasound were found to be more compact and homogenous in both size and charge distribution and they also carried more curcumin. Antioxidant capacity of the complexes, determined by DPPH and FRAP methods, were higher than the capacity of curcumin itself.
Keywords: Curcumin, solubility, ultrasound, complex formation, polyethylene glycol.

ULTRASES VE PEG KULLANILARAK KÜRKÜMİNİN SUDAKİ ÇÖZÜNÜRLÜĞÜNÜN ARTTIRILMASI

ÖZ

Kürküminin sudaki çözünürlüğü PEG ile ultrases ortamında arttırıldı. Farklı PEG-kürkümin kompleksleri ultrases ortamında ve ultrases kullanmayarak 25^{o}C da hazırlandı. Komplekslerdeki kürkümin miktarı UV çalışması ile ölçüldü. PEG-Kürkümin komplekslerinin yüzey yükleri ölçüldü. Kritik agregat konsantrasyonu hem UV hem de zeta potansiyel ölçümlerinden belirlendi. Ultases kullanılarak hazırlanan komplekslerin daha homojen yapıda oldukları ve daha fazla kürkümin taşıdıkları görüldü . Komplekslerin antioksidan kapasiteleri DPPH ve FRAP metodları ile tayin edildi ve her iki metodun sonuçları da kürküminin kendi başına verdiği değerden yüksek çıktı.
Anahtar Sözcükler: Kürkümin, çözünürlük, ultrases, kompleks oluşumu, polietilen glikol.

* Corresponding Author/Sorumlu Yazar: e-mail/e-ileti: catalgil@itu.edu.tr, tel: (212) 285 32 79

1. INTRODUCTION

Curcumin (diferuloylmethane) is a major component of the yellow spice turmeric, derived from the rhizomes of curcuma longa and is commonly used as a flavoring and coloring agent in foods. Curcumin has also been reported to show potential in terms of antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic activities [1-6].

In rodent experiments, enzymatic effects in anti-inflammatory and anticancer activities of curcumin were shown by Piper [1]. Chemopreventive effects in skin, stomach and colon carcinogenesis were investigated by Rao and Kawamori [2-3]. Oral toxicity and the effect against human cancers has been investigated in clinical pilot studies [4]. The effects in preventing and treating human cancers were shown by Aggarwal [5]. Mode of action of curcumin on carcinogenesis, gene expression mechanisms and drug metabolism of curcumin and its properties has been investigated by Duvoix [6].

Curcumin is highly hydrophobic. Its insolubility in water is the main obstacle which hinders its usage for production of useful drugs. In order to increase its applicability in food and drug preparations its solubility in water has to be increased.

Methods used to increase its solubility include; solid dispersion techniques in polyethylene glycol (PEG) 4000 and 6000 [7], in poly vinylpyrrolidone [8], and cellulose matrices [9]. In the work by Song group curcumin was solubilized by food emulsifiers and deposited in alginate beads [10]. Conjugation onto alginate beads requires chemical synthesis [11]. Increasing the solubility by using cyclodextrin was preferred by many groups [12-19]. Other techniques for improving its solubility include using rubusoside [20], betacasein [21], soyprotein [22], hyaluronic acid [23], and micellization of curcumin in cationic, anionic and non-ionic surfactant solutions [24] and solubilization by biocompatible natural polymers like alginate [25]. Chemical conjugation of curcumin on water soluble polymers is another method to improve the water solubility [26].

The non-toxicity, biodegradability and biocompatibility of PEG makes it suitable for various biomedical applications [27]. All valuable effects of curcumin depend on its solubility in blood stream, in other words its water solubility

In this work, the water solubility of curcumin is increased. Highly water soluble PEG is used to provide a curcumin-soluble organic medium and ultrasound (US) is used for efficient mixing for PEG-curcumin complex formation. Curcumin-PEG complexes were also prepared without using US for comparison. Curcumin existence and concentrations in complexes were determined by IR and UV measurements. Zeta potential measurements and size measurements were performed for the complexes. Critical aggregate concentrations (CAC)'s were determined from UV and zeta potential measurements. Antioxidant capacity of PEG-curcumin complexes were determined by the DPPH assay and the ferric-reducing antioxidant powers (FRAP) procedure.

2. EXPERIMENTAL

2.1. Materials and Methods

PEG200, PEG600, PEG2000 and Curcumin are Merck products. 2,2 diphenyl-1-picrylhydrazyl, 2,4,6-tripyridyl-s-triazine and $FeCl_3.6H_2O$ were from Sigma Chemical Co (Steinheim, Germany). Sodium acetate trihydrate, methanol, acetic acid, hydrochloric acid and $FeSO_4.7H_2O$ were from Merck (Darmstadt, Germany).

2.2. Complex Preparation

Different concentrations of 10 g, PEG solutions were prepared, (PEG concentrations (weight percentages, g/g in water) are given in Table1) and 20 mg of curcumin was added to each of

them. One group of samples was prepared by sonicating in an ultrasound bath for 2 hours at 25°C (Bandelin Sonorex RK, 80W, 35kHz). At this power and frequency, chain scission limit is approximately 100000 ±20000 [28] and significant molecular weight reduction is not expected for PEG chains, however sonication is expected to result in more homogenous size and charge distributions. The other group was prepared by magnetic stirring for 2 hours on a magnetic stirrer. At the end of 2 hours each group was centrifuged for 1 hour at 6000rpm and the dissolved part was decanted and the amount of dissolved curcumin determined by UV spectroscopy. Neither alcohol nor any other organic chemical were used during preparations. Complexes form clear solutions with water at all dilutions and were observed for one year and found to be remain stable in refrigerator. For identification purposes, complexes prepared in ultrasound bath will be named as "sonicated" and complexes prepared by mixing on a magnetic stirrer will be named as "mixed" in the rest of the paper. Surface charges and sizes of complexes were measured by Malvern zetasizer and nanosizer instruments.

2.3. FTIR Measurements

FTIR spectrum of sonicated complexes are given in Figure 1a and b, for PEG200-curcumin and for PEG600-curcumin samples.

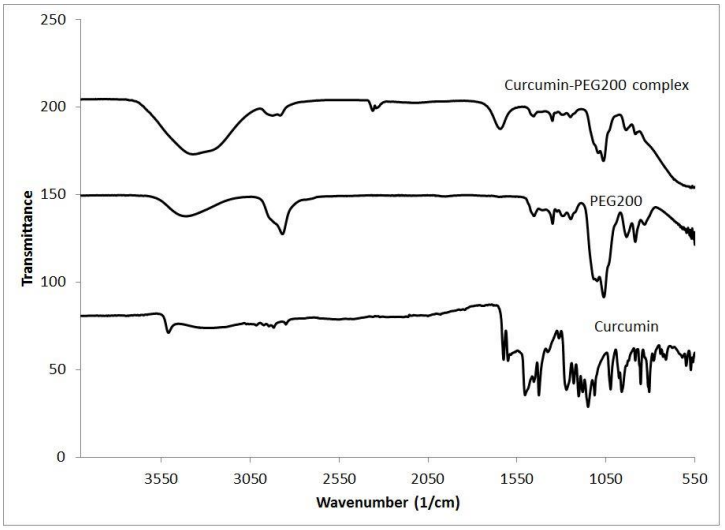

Figure 1a. IR Spectra of Curcumin, PEG200, and PEG200-Curcumin complexes

A detailed study on the vibrational spectra of curcumin has been reported by Kolev et al [29]. The FTIR spectrum of curcumin is shown in Figure's 1a and 1b as a first spectrum from the bottom.

The peak at 1626 cm^{-1} has a mixed C= C and C= O character. Another band at 1601 cm^{-1} is attributed to the symmetric aromatic ring stretching vibrations C = C ring. The 1508 cm^{-1} peak is assigned to the C = O.

PEG200 and PEG600 spectra are shown in the middle in both figures. Here CH bending is attributed to the peaks at 1454cm^{-1} 1351cm^{-1} in PEG 200 and 1464cm^{-1} 1345 cm^{-1} in PEG600.

The spectra of the PEG200-curcumin and PEG600-Curcumin complexes are shown as the top spectra in Figure 1a and 1b. Peaks at 1637 cm^{-1} in PEG200-curcumin and 1640cm^{-1} in PEG600-

curcumin attributed to mixed C=C and C=O character and small shoulder at PEG600-curcumin at 1507cm^{-1} is attributed to C=O stretching as a part of curcumin structure.

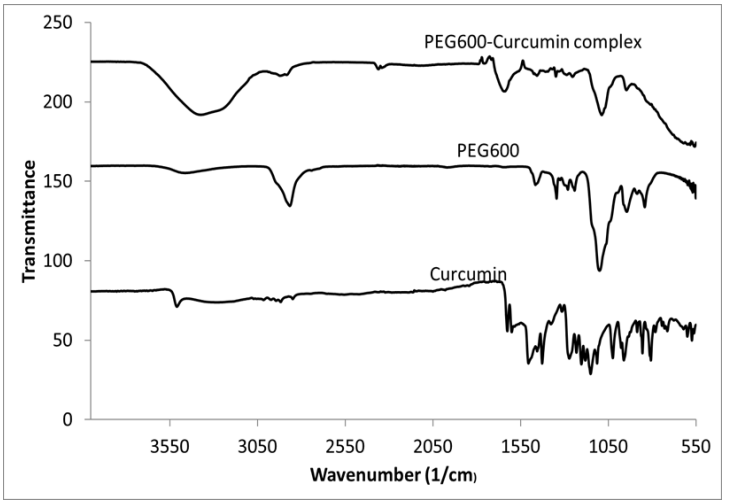

Figure 1b. IR Spectra of Curcumin, PEG600, and PEG600-Curcumin complexes

2.4. Antioxidant Capacity Determination

1. DPPH free radical scavenging assay

The free radical-scavenging activity of PEG-curcumin complexes were determined by the DPPH assay described by Blois [30] with slight modification. 20 mg/L DPPH solution was prepared in methanol. 1980 µl of this DPPH solution was added to 20 µL sample solution. Thirty minutes later the absorbance at 515nm was measured at Shimadzu UV-1800 spectrophotometer. Inhibition of free radical DPPH in percent (I %) was calculated by using the following equation where A_{sample} is the absorbance of the samples and $A_{control}$ is the absorbance of the water, since the complexes were in water.

$$\text{Percentage inhibition (I \%)} = [(A_{control}-A_{sample})/A_{control}] \times 100 \quad (1)$$

2. FRAP Assay

The ferric-reducing antioxidant powers (FRAP) of complexes were determined, following the method of Benzie and Strain [31]. The FRAP reagent was prepared containing 1:1:10 ratio of 10 mmol/L 2,4,6-tripyridyl-s tri-azine (TPTZ) solution in 40 mmol/L HCl, 20 mmol/L $FeCl_3$ and 0.3 mol/L acetate buffer at pH 3.6, and warmed up to 37 °C, for 10 min prior to use. The mixture which containing 100 µl sample, 100 µl deionized water, and 1.8 ml FRAP reagent incubated at 37 °C for 10 min. The absorbance of the mixture at 593 nm was measured by a Shimadzu UV-1800 spectrophotometer. Results were expressed as mM Fe (II)/g curcumin. The calibration equation for FeSO4.7H2O was y = 1.0474x-0.1174, (R^2= 0.998).

3. RESULTS AND DISCUSSION

The amount of curcumin in sonicated and mixed complexes, are given in Table 1. Amount of curcumin in complexes increased as the PEG % and PEG molecular weight are increased, and sonicated complexes carried more curcumin in all cases. The most efficient results are obtained with PEG2000 and solubilized curcumin in sonicated complexes are three times more than mixed

ones. In the literature, curcumin solubility of 8.4×10^{-8} M ($3.09\ 10^{-5}$ mg/ml) was obtained in phosphate buffer solution containing 10.0% PEG 400.[27] In this work, curcumin solubility obtained in sonicated PEG200-Curcumin, PEG600-Curcumin and PEG2000-Curcumin samples are, 0.0071mg/ml, 0.066mg/ml and 0.038mg/ml respectively. In the case of mixed samples, solubility values obtained are 0.0069mg/ml, 0.011mg/ml and 0.018mg/ml respectively and higher than the literature results in all systems. One possible reason for the low curcumin solubility value in the above work is the phosphate salt used.[27] Available curcumin junctions on the PEG chains had saturated by salt and curcumin solubility is decreased in PEG containing phosphate buffer solutions.

Table 1. Amount of curcumin in sonicated and mixed PEG-Curcumin complexes

	Sonicated	**Mixed**
%PEG in water	(mg.cur)/(g solution)	(mg.cur)/(g solution)
	PEG200-Curcumin complexes	
10	0.0071±0.0006	0.0069±0.0006
20	0.0130±0.005	0.0397±0.0376
30	0.0497±0.001	0.0676±0.001
40	0.3830±0.003	0.2275±0.2260
50	0.7430±0.022	0.6409±0.0178
	PEG600-curcumin complexes	
10	0.0081±0.0585	0.0093±0.0023
20	0.0219±0.002	0.0223±0.0015
30	0.0546±0.012	0.0519±0.0156
40	0.2646±0.012	0.2236±0.0951
	PEG2000-curcumin complexes	
1	0.00663±0.00073	0.00243±0.00007
2	0.01167±0.00317	0.0029±0.0003
5	0.01833±0.00363	0.00755±0.00024
10	0.0378±0.0013	0.01785±0.00045
20	0.12395±0.01645	0.03235±0.00125
30	0.2506±0.0206	0.0855±0.0045

Curcumin concentrations obtained from UV results in sonicated and mixed PEG2000-Curcumin complexes are shown in Figure 2. As can be seen from the figure, up to a certain PEG percentage, complexes carry only a little amount of curcumin. After this point (approximately 7 % PEG, for sonicated and 15 % PEG for mixed complexes the curcumin concentration increase is rapid. The intersection of the two regimes is identified as the CAC for the PEG2000-curcumin system. To avoid repetition only PEG2000-curcumin system is shown as an example. PEG

required to reach critical aggregate concentration (CAC) was determined as %30, 25%, %7 for sonicated PEG200, PEG600 and PEG2000-curcumin complexes and as %35, %25, %15 for mixed PEG200, PEG600, PEG2000-curcumin complexes respectively.

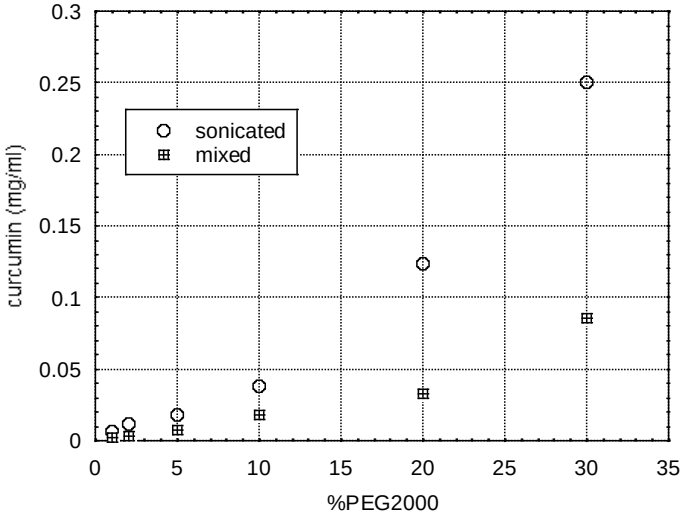

Figure 2. CAC determination in sonicated and mixed PEG2000-Curcumin complexes.

Zeta potential measurements of sonicated and mixed PEG2000 complexes are shown in Figure 3. All complexes are negatively charged at low PEG concentrations and converged towards zero as the PEG concentration increases. Zero charge is seen to occur when the polymer fully covers the particle surface at CAC. Sonication results in better dispersion of particles and surface covering occurs faster and at a lower concentration. The CAC, indicated by zero potential, occurred at approximately 12% PEG2000 in sonicated and 17% PEG in mixed samples. All zeta results are shown in Table 2 and CAC concentrations from zeta potential results are found as 30, 30, 12 in sonicated complexes and 40, 30, 17 in mixed complexes for PEG200, PEG600 and PEG2000-curcumin complexes respectively. The approximate coincidence of the "zero zeta potential" concentration with the point of regime change supports identification of this point as the CAC. As the PEG molecular weight increases required amount of PEG to reach the CAC decreased. Less PEG was needed to reach CAC in sonicated samples due to more homogenous mixing.

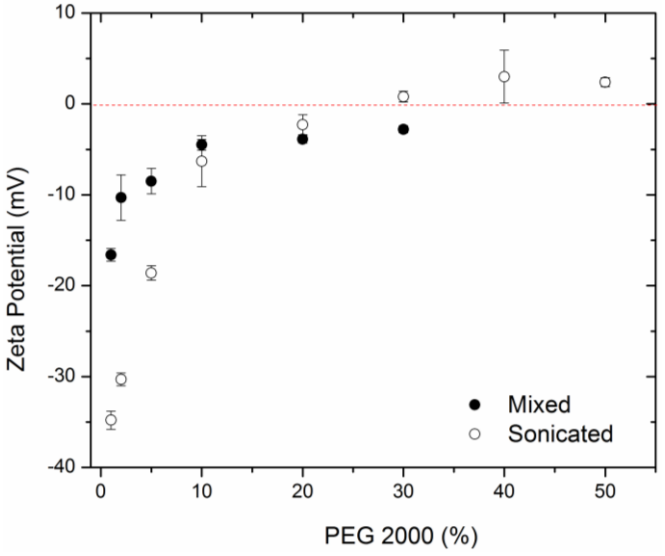

Figure 3. CAC determination from zeta potential measurements for sonicated and mixed PEG2000 complexes.

Table 2. Zeta potential of sonicated and mixed PEG-curcumin complexes

		Sonicated	**Mixed**
	% PEG in water	Zeta Potential	Zeta potential
PEG200-Curcumin			
	10	-0.8±1.2	-13.8±0.6
	20	-4.9±2.4	-9.0±0.7
	30	+5.2±4.9	-3.1±5.6
	40	-0.9±3.0	-1.0±2.4
	50	+0.8±0.8	+3.0±2.2
PEG600-Curcumin	10	-14.1±1.1	-8.3±2.4
	20	-4.9±0.9	2.5±9.9
	30	0.6±1.3	0.1±1.2
	40	1.3±0.4	1.6±0.3
PEG2000-Curcumin	1	-34.8±1.0	-16.6±0.6
	2	-30.3±0.7	-10.3±5.0
	5	-18.6±0.8	-8.5±2.3
	10	-6.3±2.8	-4.5±0.5
	20	-2.3±1.1	-3.9±0.2
	30	1.0±0.4	-2.8±0.3
	40	3.1±2.9	
	50	2.4± 0.5	

Schematic representations of complexes are shown in Figure 4, below critical aggregate concentration (CAC) (a) and above CAC (b). Below CAC, complexes could carry very little amount of curcumin, and above CAC curcumin concentration increased rapidly.

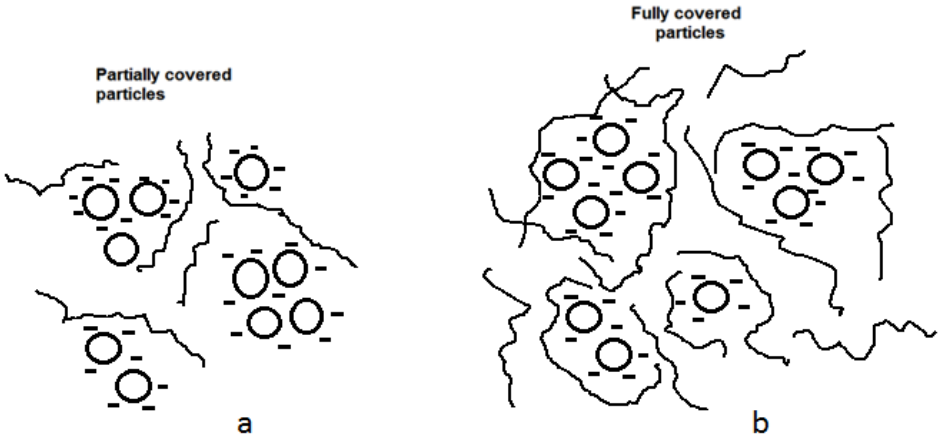

Figure 4. A graphical representation of PEG-Curcumin complexes, before (a) and after (b) CAC is reached.

Sonication process produced well dispersed particles with a homogeneous distribution.

Particle sizes of complexes are shown in Table 3. Before CAC sonicated and mixed complexes gave two or three different size distribution. In sonicated complexes after CAC one distribution showed up however in mixed samples size distribution still carried two humps. Particle sizes increased as PEG molecular weight is increased and PEG % increased.

Antioxidant capacity of complexes were determined by two different methods and results are given in Table 4. Following the same procedures % DPPH inhibition value of pure curcumin is 25±3.80 and the ferric-reducing antioxidant powers (FRAP) of pure curcumin is found as 1926± 58.95. As can be seen from the table all complexes have higher values than these. Antioxidant capacity of complexes increased as the amount of curcumin in the complex increased. Especially in PEG2000 after CAC has passed antioxidant capacity showed remarkable increase.

Tablo 3. Particle Size of sonicated and mixed PEG-Curcumin complexes

	% PEG	Particle diameter(nm)	%	Width (nm)
		Sonicated		
PEG200-Curcumin	20	94.4 271.8	33.3 66.7	11.3 97.4
	40	82.5	100	26.8
PEG600-Curcumin	20	92.1 151.7	68.1 31.9	13.0 19.4
	40	88.6	100	26.7
PEG2000-Curcumin	2	541	99.9	193
	2	439	100	209
	5	688	99.7	271
	5	627	99.8	214
	30	2042	100	690
	30	2146	100	939
		Mixed		
PEG200-Curcumin	20	1.2 433.9 5219	50 47.5 2.5	0.2 85.8 723.3
	40	81.7	100	28.8
PEG600-Curcumin	20	148.1	100	61.7
	40	70.9 363.1	99.9 0.1	18.0 110.0
PEG2000-Curcumin	2	425	100	116
	2	379 87.2	36 63	132 17
	2	468 103	39 61	143 25
	5	579 158	38 62	194 37
	5	500 128	50 50	144 25
	30	1701	100	1024
	30	1899	100	1007
	30	1596	100	961

Table 4. Antioxidant capacities of PEG-Curcumin complexes

	%PEG in water	**DPPH (% inhibition)**	**FRAP (mM Fe(II)/g curcumin)**
		Sonicated	
PEG200-Curcumin	20	45.47±3.019	4059±52
	40	61.13±5.258	6814±85
PEG600-Curcumin	20	47.16±3.874	4230±138
	40	66.88±5.698	18813±225
PEG2000-Curcumin	1	41.85±1.055	5628±72.4
	5	42.36±1.736	7441±85.7
	10	44.33±2.625	14709±146
	20	53.96±3.592	18162±125
		Mixed	
PEG200-Curcumin	20	42.35±2.658	2079±48.3
	40	57.74±4.215	9026±126
PEG600-Curcumin	20	43.77±3.756	1832±145
	40	86.95±5.698	5527±256
PEG2000-Curcumin	1	37.36±1.018	1226±25.19
	5	39.43±1.387	2604±28.87
	10	46.13±1.528	4863±53.45
	20	46.69±2.034	5025±46.81

4. CONCLUSION

The CAC was observed to decrease with increasing PEG molecular weight. In all experiments it is seen that sonicated samples have narrower zeta potential distributions, indicating that the US mixing is much better and the sonicated samples are more homogenous compared to the mixed examples. Antioxidant capacity of complexes were higher than pure curcumin itself in all cases; especially above the CAC. Complexes gave clear solutions at all dilutions with water. Another promising result; the complexes were found to be stable at all dilutions with water. No precipitation or phase separation was observed in either sonicated or mixed samples as long as they were observed (for more than a year).

REFERENCES / KAYNAKLAR

[1] Piper, J.T., Singhal, S.S., Salameh, M., Torman, R.T., Awasthi, Y.C., Awasthi, S., "Mechanisms of anticarcinogenic properties of curcumin: the effect of curcumin on glutathione linked detoxification enzymes in rat liver". Int. J. Biochem. Cell. Biol. 30, 445-456, 1998.

[2] Rao, C.V., Rivenson, A., Simi, B., Reddy, B.S., "Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound". Cancer Res. 55, 259-266, 1995.

[3] Kawamori, T., Lubet, R., Steele, V.E., Kelloff, G.J., Kaskey, R.B., Rao, C.V., Reddy, B.S., "Chemopreventive effect of curcumin, a naturally occurring antiinflammatory agent, during the promotion/progression stages of colon cancer". Cancer Res. 59, 597–601, 1999.

[4] Cheng, A.L., Hsu, C.H., Lin, J.K., Hsu, M.M., Ho, Y.F., Shen, T.S., Ko, J.Y., Lin, J.T., Lin, B.R., Ming-Shiang, W., Yu, H.S., Jee, S.H., Chen, G.S., Chen, T.M., Chen, C.A., Lai, M.K., Pu, Y.S., Pan, M.H., Wang, Y.J., Tsai, C.C., Hsieh, C.Y., "Phase I clinical

trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions". Anticancer Res. 21, 2895–2900, 2001.

[5] Aggarwal, B.B., Kumar, A., Bharti, A.C., "Anticancer potential of curcumin, preclinical and clinical studies". Anticancer Res. 23, 363–398, 2003.

[6] Duvoix, A., Blasius, R., Delhalle, S., Schnekenburger, M., Morceau, F., Henry, E., Dicato, M., Diederich, M., "Chemopreventive and therapeutic effects of curcumin", Cancer Letters. 223, 181–190, 2005.

[7] Modasiya, M.K., Patel, V.M., "Studies on solubility of curcumin", Int J of Pharm Life Sci (IJPLS), 3,1490-1497, 2012.

[8] Kaewnopparat, N., Kaewnopparat, S., Jangwang, A., Maneenaun, D., Chuchome, T., Panichayupakaranant, P., "Increased Solubility, Dissolution and Physico chemical Studies of Curcumin-Polyvinylpyrrolidone K-30 Solid Dispersions", World Academy of Science, Engineering and Technology, 2009, 31, 225-230.

[9] Li, B., Konecke, S., Wegiel, L.A., Taylor, L. S., Edgar, K. J., "Both solubility and chemical stability of curcumin are enhanced by solid dispersion in cellulose derivative matrices", Carbohydrate Polymers, 98, 1108-1116, 2013.

[10] Song, S., Wang, Z., Qian, Y., Zhang, L., Luo, E., "The Release Rate of Curcumin from Calcium Alginate Beads Regulated by Food Emulsifiers", J. Agric. Food Chem. 60, 4388−4395, 2012.

[11] Dey, S., Sreenivasan, K., "Conjugation of curcumin onto alginate enhances aqueous solubility and stability of curcumin", Carbohydrate Polymers, 99, 499-507, 2014.

[12] Singh, R., Tønnesen, H.H., Vogensen, S.B., Loftsson, T., Masson, M., "Studies of curcumin and curcuminoids. XXXVI. The stoichiometry and complexation constants of cyclodextrin complexes as determined by the phase-solubility method and UV–Vis titration", J. Incl. Phenom. Macrocycl. Chem,b66, 335–348, 2010.

[13] Mohan, P.R.K., Sreelakshmi, G., Muraleedharan, C.V., Joseph, R., "Water soluble complexes of curcumin with cyclodextrins: Characterization by FT-Raman spectroscopy", Vibrational Spectroscopy, 62, 77– 84, 2012.

[14] Syed, H.K., Peh, K.K., "Comparative Curcumin Solubility Enhancement Study of beta-Cyclodextrin (beta CD) and its Derivative Hydroxypropyl-beta-Cyclodextrin (HP beta CD)", Latin American Journal of Pharmacy, 32, 52-59, 2013.

[15] Yadav, V.R., Suresh, S., Devi, K., "Effect of Cyclodextrin Complexation of Curcumin on its Solubility and Antiangiogenic and Anti-inflammatory Activity in Rat Colitis Model", AAPS Pharm. Sci. Tech.10, 752-762, 2009.

[16] Tonnesen, H.H., Masson, M., Loftsson, T. "Studies of curcumin and curcuminoids. XXVII. Cyclodextrin complexation: solubility, chemical and photochemical stability". International Journal of Pharmaceutics,244, 127-135, 2002.

[17] Mangolim, C.S., Moriwaki, C., Nogueira, A.C., Sato, F., Baesso, M.L., Neto, A.M., Matioli, G. "Curcumin-β-cyclodextrin inclusion complex: stability, solubility, characterisation by FT-IR, FT-Raman, X-ray diffraction and photoacoustic spectroscopy, and food application". Food Chemistry, 153, 361, 2014.

[18] Jantarat, C., Sirathanarun, P., Ratanapongsai, S., Watcharakan, P., Sunyapong S., Wadu, A. "Curcumin-Hydroxypropyl-beta-Cyclodextrin Inclusion Complex Preparation Methods: Effect of Common Solvent Evaporation, Freeze Drying, and pH Shift on Solubility and Stability of Curcumin", Tropical Journal of Pharmaceutical Research, 13, 1215-1223, 2014.

[19] Popat, A., Karmakar, S., Jambhrunkar, S., Yu, C., "Curcumin-cyclodextrin encapsulated chitosan nanoconjugates with enhanced solubility and cell cytotoxicity", Colloids And Surfaces B-Biointerfaces,117, 520-527, 2014.

[20] Zhang, F., Koh, G.Y., Jeansonne, D.P., Hollingsworth, J., Russo, P.S., Vicente, G., Stout, R.W., Liu, Z. "A Novel Solubility-Enhanced Curcumin Formulation Showing

Stability and Maintenance of Anticancer Activity", Journal of Pharmaceutical Sciences,100, 2778–2789, 2011.

[21] Esmaili, M., Ghaffari, S.M., Moosavi-Movahedi, Z., Atri, M. S., Sharifizadeh, A., Farhadi, M. "Beta casein-micelle as a nano vehicle for solubility enhancement of curcumin; food industry application", LWT - Food Science and Technology,44, 2166-2172, 2011.

[22] Tapal, A., Tiku, P.K., "Complexation of curcumin with soy protein isolate and its implications on solubility and stability of curcumin", Food Chemistry, 130, 960–965, 2012.

[23] Manju, S., Sreenivasan, K. "Conjugation of curcumin onto hyaluronic acid enhances its aqueous solubility and stability", Journal of Colloid and Interface Science, 359, 318–325, 2011.

[24] Patra, D., Barakat, C., "Unique role of ionic liquid [bmin][BF4] during curcumin–surfactant association and micellization of cationic, anionic and non-ionic surfactant solutions", Spectrochimica Acta, Part A,79, 1823–1828, 2011.

[25] Tonnesen, H.H. "Solubility and stability of curcumin in solutions containing alginate and other viscosity modifying macromolecules - Studies of curcumin and curcuminoids XXX", Pharmazie, 61, 696-700, 2006.

[26] Li, J., Wang, Y., Yang, C., Wang, P., Oelschlager, D.K., Zheng, Y., Tian, D.A., Grizzle, W.E., Buchsbaum, D.J., Wan, M. "Polyethylene glycosylated curcumin conjugate inhibits pancreatic cancer cell growth through inactivation of Jab1". Molecular Pharmacology,76, 81-90, 2009.

[27] Haukvik, T., Bruzell, E., Kristensen, S., Tønnesen, H.H. "Photokilling of bacteria by curcumin in selected polyethylene glycol 400 (PEG 400) preparations Studies on curcumin and curcuminoids, XLI", Pharmazie,65, 600–606, 2010.

[28] Akyuz, A., Catalgil-Giz, H., Giz, A.T. "Kinetics of ultrasonic polymer degradation: Comparison of theoretical models with on-line data", Macromol. Chem. & Physics, 209, 801-809, 2008.

[29] Kolev, T.M., Velcheva, E., Stamboliyska, B.A., Spiteller, M. "DFT and experimental studies of the structure and vibrational spectra of curcumin", Int. J. Quantum Chem.102, 1069–1079, 2005.

[30] Blois, M.S., "Antioxidant determinations by the use of a stable free radical", Nature,181: 1199- 1200, 1958.

[31] Benzie I.F.F., Strain J.J. "Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay". Anal Biochem., 239:70-76, 1996.