ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXII, N 3, 15 SECTIO DDD 2009

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Influence of egg albumin on vitamin E release from gelatin capsules

Wpływ albuminy z jaj kurzych na uwalnianie witaminy E z kapsułek żelatynowych

dl-α- Tocopherol acetate (VE) is a poorly water soluble antioxidant vitamin. It has been previously reported that VE following oral administration is poorly absorbed into the body from oily preparations and much better in the presence of surfactants or from emulsified vehicles. There is thus an increased interested in self-emulsifying systems where lipophilic drugs such as fat soluble vitamins may be stored in concentrated oil-surfactant solution [18].

The process of dissolution is very important for drug release from its dosage form and making it available for gastrointestinal absorption. Dissolution depends on the physiochemical properties of the drug, formulation of the dosage form, composition and viscosity of the dissolution medium and many other factors. The level of interest in the *in vitro* dissolution poorly water-soluble drugs has increased in recent years due to the need of finding a suitable dissolution media for pharmaceutical formulations that may reflect their *in vivo* performance [5]. The significance of a dissolution test is based on the fact that for a drug to be adsorbed and available to the systemic circulation, it must previously be solubilized [8].

Dissolution systems containing both proteins and surfactants are used in modern technologies and are widely found in nature. Proteins are natural high molecular weight particles having the surface activity [17]. Proteins stabilize foams and emulsions by forming a mechanical barrier at the interface that encapsulates the dispersed phase and resists random surface perturbations, droplet coalescence, or flocculation. Proteins and surfactants can interact in solution to form surfactant-protein complexes, whose properties differ from those of the pure protein differing in their surface activity [3, 14]. Protein-surfactant interaction has been of considerable interest due to its application in drug delivery, cosmetics and foods [16].

Surfactant can change the stability and rheology of protein stabilized emulsions in different ways. The properties of such emulsions are dependent on the interactions between the surfactant and protein in the bulk as well as the interface, and in many cases are determined by the formation of the surface active protein/ surfactant complexes of variable composition [4]. The interaction at the interface and in the bulk phase between bovine serum albumin (BSA) and Tween 20 was described by Nino and Patino [14]. According to them at low Tween 20 concentration, BSA reduces the surface tension to a greater extent than BSA-Tween 20 mixed system. The opposite was observed at high Tween 20 concentration above the critical micellization concentration, because the BSA molecules are displaced from the interface by the Tween 20.

The complexation due to specific interaction (hydrogen bonding) between polar groups of Tween 80 and BSA molecules was described by Zadymowa at al. [17]. At the concentration above the critical association concentration the association of nonionic surfactant–protein hydrophobic complexes leads to the formation of large particles (16–350 nm). At large both surfactants molar

ratios Tween 80 molecules are additionally bound by the nuclei of new phase. According to Derkatch at al., increase in concentration of nonionic surfactant leads to a decrease in viscosity and suppression of emulsion elasticity [4].

Imai at al., to improve solubility and absorption of dl-α-tocopherol or other poorly water soluble drugs, formulated solid dispersion with egg albumin. The solubility of vitamin E was increased 300 times in the presence of egg albumin. The dissolution rate of dl-α-tocopherol from solid dispersion with egg albumin was markedly enhanced in comparison with vitamin E alone. They noticed that egg albumin protected vitamin E against oxidation. Egg albumin shielded vitamin E from air and light, increasing storage stability [11].

Dissolution media generally used in the laboratory cannot dissolve poorly water-soluble drugs completely. To overcome solubility problems and to increase drug solubility, large amounts of dissolution media, co-solvent method and surfactants were used. *In vivo* poorly-water soluble drugs are solubilized through endogenous surfactants, e.g. bile salts, lecithin, cholesterol. The use of media containing surfactants, was proposed as suitable for solubilizing poorly water soluble drugs due to the presence of natural surfactants in the gastrointestinal tract [5, 13]. The presence of a surfactant in the aqueous media can enhance the wetting of drug particles by lowering the advancing contact angle and surface area increases causing dissolution increases [1]. To enhance dissolution of poorly water soluble compounds, the authors propose a lot of surfactants. To test dissolution behaviour of diclofenac sodium prolonged release tablets, Bertocchi at al. used simulated intestinal fluid without pancreatin containing 1% Tween 20 [2]. To develop dissolution of poorly water soluble compound CI-1041, Chen at al. used Tween 80 [3]. For nimodipine tablets dissolution he et al. used a newly developed dissolution medium of pH 4.5 acetate buffer containing 0.05% sodium dodecyl sulfate (10). The effect of types of surfactant on the solubilization and dissolution of poorly soluble acid drugs: mefenamic acid, nimesulide, ibuprofen was developed by Park and Choi. Cetyltrimethylammonium bromide as a cationic surfactant, sodium lauryl sulfate as an anionic surfactant and Tween 80 as an non-ionic surfactant were used in the study [13].

The purpose of this work was to investigate the possible use of natural polymer such as egg albumin as a solubilizing/wetting agent to improve VE dissolution.

EXPERIMENTAL DESIGN

Material and methods. Vitamin E was in the form of soft gelatin commercial capsules, each capsule containing 100 mg of oily dl-α-tocopherol acetate. Egg albumin was purchased from Ubichem Ltd., England and Tween 20 was received from POCH, Gliwice, Poland. Phosphate buffer pH=6.8 was prepared according to FP VII using potassium dihydroxyphosphate and sodium hydroxide purchased from POCH, Gliwice, Poland [7].

Dissolution study was performed with a FP VII paddle apparatus [7]. The dissolution medium was constituted by 500 ml of 0.75, 1.5, 3.0% (w/v) egg albumin in phosphate buffer pH=6.8 or 0.75, 1.5, 3.0% (w/v) egg albumin in phosphate buffer pH=6.8 with 1% (w/v) Tween 20 added, thermostatted at 37±0.5°C and stirred at 90 rpm. Samples were withdrawn at 15, 30, 45, 60, 75, 90,105 and 120 min after dissolution process had started and analyzed fluorometrically on Shimadzu RF-5301 PC spectrofluorometer according to method described by Taylor S.L, Lamden M.P., Tappel A.L. Wavelength settings for detection were 280 nm for excitation and 310 nm for emission [15]. An equal volume of fresh dissolution medium, maintained at the same temperature, was added after withdrawing each sample to maintain the volume. Each time dissolution medium viscosity was measured using Höeppler viscosimeter according to FP VI [6].

Kinetics of drug release. To describe the kinetics of drug release form vitamin E capsules, Higuchi's model was used [12, 19].

$$Mt^* = K_0\left(\sqrt{t} - \sqrt{T_D}\right)$$

Mt^* – amount of drug released in time t , K_0 – release rate constant, $\sqrt{t}$ – square root of time, $\sqrt{T_D}$ – square root of dissolution lag time.

The amount of drug released in time t (Mt*) was calculated using Higuchi equation, the release rate constant (K_0) was calculated from the slope of the individual measured amount of drug released in time t (Mt) – square root time ($\sqrt{t}$) curves. The area under the dissolution curve (AUDC) was calculated by the trapezoidal rule using time (t) and the amount of drug released in time t (Mt) or the amount of drug released in time t calculated from Higuchi equation (Mt*) – AUDC*. AUDC percentage deviation for sampling time points from ideal Higuchi model was presented in Tables 1,2. Maximum deviation was 16.89%, minimum deviation was 0.04%. As shown, the release profiles of vitamin E capsule fit Higuchi model, so the relation of the amount of drug released during 120 min. and dissolution medium viscosity was compared using the area under the dissolution curve (AUDC*) for ideal Higuchi release profile [9].

RESULTS AND DISCUSSION

Table 3 shows kinetic parameters obtained after *in vitro* dissolution study of vitamin E capsules. Table 4 shows the relation between AUDC* and dissolution medium viscosity. Figure 1 shows dissolution profiles of vitamin E capsules in media of pH 6.8 phosphate buffer containing 0.75, 1.5, 3.0% albumin, and Figure 2 shows dissolution profiles of vitamin E capsules in the same media with 1% Tween 20 added. The amount of drug released in time 120 min (Mt*), the area under dissolution curve (AUDC*) and release rate constant (K_0) were the lowest for dissolution medium containing 0.75% albumin and these parameters were increased with albumin concentration. AUDC* was increased 1.47-times with albumin concentration from 0.75% to 1.5% and AUDC* was increased 2.17-times with albumin concentration from 0.75% to 3%. A similar relation can be observed in dissolution media with albumin and Tween 20, where the amount of the drug released in time 120 min (Mt*), the area under dissolution curve (AUDC*) and release rate constant (K_0) were the lowest for dissolution medium containing 0.75% albumin with 1% Tween 20. AUDC* was increased 1.15-times with albumin concentration from 0.75% to 1.5%. With albumin concentration increasing from 0.75% to 3% AUDC* was increased 1.62-times. Mt*, AUDC* and K_0 values were significantly higher for dissolution media with albumin and Tween 20 suggested that vitamin E released easier and faster in the presence of albumin and nonionic surfactant. AUDC* was 1.57 times higher for dissolution medium containing 3% albumin with 1% Tween 20 added than the medium without Tween 20.

The relation between AUDC* and the concentration of egg albumin shows that AUDC* was increased with the concentration of egg albumin more in dissolution media without Tween 20. Generally, AUDC* and the amount of drug released were increased with the concentration of egg albumin and dissolution time in the presence and absence of Tween 20. The enhanced dissolution rate of vitamin E may have been due to the increased solubility of vitamin E through complexation with egg albumin. The dissolution rate was also enhanced by improvement of wettability of vitamin E [11].

Viscosity of dissolution media was increased with albumin concentration. The viscosity of 3% albumin with 1% Tween 20 dissolution medium –1.201 mPa·s was slightly lower than medium without

Tween 20-1.211 mPa·s. That may suggest that complexation occurs due to a specific interaction between polar groups of Tween 20 and egg albumin molecules.

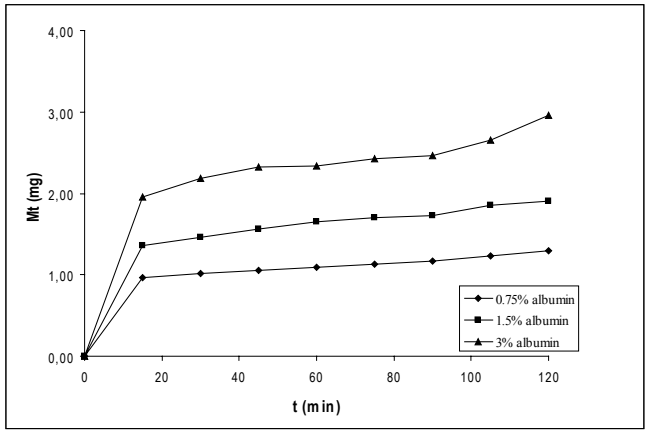

Fig. 1. Dissolution profiles of vitamin E capsules in media of pH=6.8 phosphate buffer containing 0.75, 1.5, 3.0% albumin

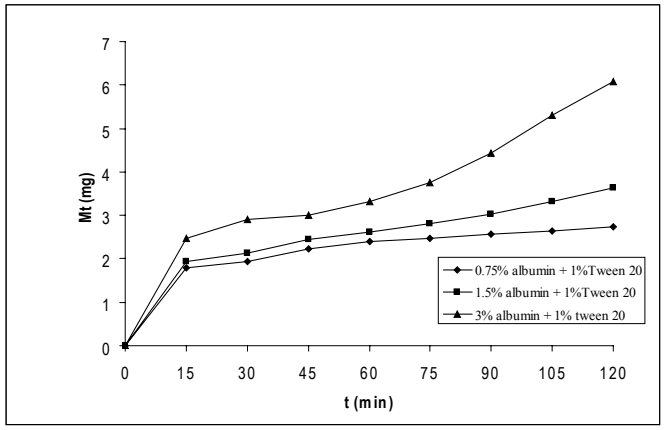

Fig. 2 Dissolution profiles of vitamin E capsules in media of pH=6.8 phosphate buffer containing 0.75, 1.5, 3.0% albumin with 1% Tween 20 added

Surfactant can indirectly interact with protein by competing in the process of adsorption at oil/water interface or they can also directly interact with proteins by binding to them, which may lead to changes in protein conformation.

To conclude, we can observe that total dissolution rate of vitamin E in media of pH 6.8 phosphate buffer containing albumin was increased with surfactant concentration and dissolution time. A similar relation can be observed for dissolution media of pH 6.8 phosphate buffer containing albumin and 1% Tween 20. The dissolution rate of vitamin E was increased with the concentration of albumin and dissolution time. Viscosity was increased with the concentration of albumin for both of media. The total amount of drug released was highest for dissolution media with albumin and 1% Tween 20 showing the beneficial influence of Tween 20 on dissolution rate of vitamin E.

Table 1. AUDC percentage deviation from Higuchi model for dissolution media containing 0.75, 1.5, 3.0% albumin

t (min)	$\sqrt{t}$	AUDC 0.75% of albumin	AUDC* 0.75% of albumin	Absolute difference of AUCDs	% deviation from Higuchi model	AUDC 1.5% of albumin	AUDC* 1.5% of albumin	Absolute difference of AUCDs	% deviation from Higuchi model	AUDC 3.0% of albumin	AUDC* 3.0% of albumin	Absolute difference of AUCDs	% deviation from Higuchi model
15	3.9	7.28	6.86	0.41	5.99	10.20	10.03	0.17	1.65	14.63	15.20	0.58	3.81
30	5.5	14.93	14.33	0.60	4.16	21.15	21.01	0.14	0.68	30.98	31.40	0.42	1.34
45	6.7	15.53	15.39	0.13	0.86	22.65	22.67	0.02	0.07	33.75	33.14	0.61	1.84
60	7.7	16.05	16.24	0.19	1.19	24.08	23.99	0.08	0.34	34.95	34.53	0.42	1.20
75	8.7	16.65	16.97	0.32	1.91	25.13	25.14	0.01	0.04	35.70	35.74	0.04	0.10
90	9.5	17.25	17.63	0.38	2.14	25.73	26.15	0.43	1.64	36.60	37.05	0.45	1.22
105	10.2	18.00	18.22	0.22	1.22	26.93	27.08	0.16	0.58	38.40	39.77	1.37	3.44
120	11.0	18.90	18.77	0.13	0.68	28.20	27.94	0.26	0.93	42.15	43.55	1.40	3.22

AUDC – area under dissolution curve calculated according to sampling time points of dissolution study (mg min), AUDC* – area under the dissolution curve for ideal Higuchi release profile calculated according to sampling time points of dissolution study (mg min)

Table 2. AUDC percentage deviation from Higuchi model for dissolution medium containing 0.75, 1.5, 3.0% albumin with 1% Tween 20

t (min)	$\sqrt{t}$	AUDC 0.75% of albumin	AUDC* 0.75% of albumin	Absolute difference of AUCDs	% deviation from Higuchi model	AUDC 1.5% of albumin	AUDC* 1.5% of albumin	Absolute difference of AUCDs	% deviation from Higuchi model	AUDC 3.0% of albumin	AUDC* 3.0% of albumin	Absolute difference of AUCDs	% deviation from Higuchi model
15	3.9	13.43	13.62	0.19	1.40	14.55	12.45	2.10	16.89	18.53	17.54	0.99	5.62
30	5.5	27.98	28.82	0.84	2.93	30.53	28.08	2.44	8.69	40.28	38.47	1.80	4.69
45	6.7	31.20	31.63	0.43	1.35	34.28	33.72	0.56	1.65	44.25	44.47	0.22	0.49
60	7.7	34.65	33.87	0.78	2.30	37.88	38.23	0.35	0.92	47.48	49.27	1.79	3.64
75	8.7	36.53	35.80	0.72	2.01	40.65	42.11	1.46	3.46	53.10	53.40	0.30	0.55
90	9.5	37.80	37.53	0.27	0.72	43.73	45.57	1.84	4.04	61.35	61.55	0.20	0.33
105	10.2	39.00	39.10	0.10	0.25	47.55	48.72	1.17	2.40	72.98	73.61	0.63	0.86
120	11.0	40.28	40.55	0.28	0.68	52.20	51.64	0.56	1.09	85.35	84.87	0.48	0.57

AUDC – area under dissolution curve calculated according to sampling time points of dissolution study (mg min), AUDC* – area under the dissolution curve for ideal Higuchi release profile calculated according to sampling time points of dissolution study (mg min)

Table 3. The amount of vit. E (Mt) released *in vitro* as a function of experimental time (n = 6)

t (min)	Dissolution media (% albumin in phosphate buffer pH 6.8)																							
	0.75%				1.5%				3.0%				0.75% with 1% Tween 20				1.5% with 1% Tween 20				3.0% with 1% Tween 20			
	Mt	±SD %	Mt*	K_0	Mt	±SD %	Mt*	K_0	Mt	±SD %	Mt*	K_0	Mt	±SD %	Mt*	K_0	Mt	±SD %	Mt*	K_0	Mt	±SD %	Mt*	K_0
15	0.97	0.1	0.92		1.36	0.1	1.34		1.95	0.13	2.03		1.79	0.29	1.82		1.94	0.13	1.66		2.47	0.21	2.34	
30	1.02	0.12	1.00		1.46	0.15	1.46		2.18	0.14	2.16		1.94	0.1	2.03		2.13	0.12	2.08		2.9	0.13	2.79	
45	1.05	0.1	1.06		1.56	0.14	1.56		2.32	0.23	2.26		2.22	0.25	2.19		2.44	0.33	2.41		3.0	0.28	3.14	0.282
60	1.09	0.11	1.11		1.65	0.05	1.64		2.34	0.24	2.34	0.082	2.4	0.21	2.33		2.61	0.24	2.69		3.33	0.51	3.43	
75	1.13	0.07	1.15	0,05	1.70	0.1	1.71	0.078	2.42	0.21	2.42		2.47	0.2	2.45	0.132	2.81	0.17	2.93	0.265	3.75	0.20	3.69	
90	1.17	0.1	1.20		1.73	0.12	1.78		2.46	0.17	2.52		2.57	0.21	2.56		3.02	0.16	3.15		4.43	0.26	4.52	
105	1.23	0.12	1.23		1.86	0.18	1.84		2.66	0.27	2.78	0.344	2.63	0.16	2.66		3.32	0.3	3.35		5.3	0.50	5.30	1.023
120	1.29	0.11	1.27		1.90	0.17	1.89		2.96	0.31	3.03		2.74	0.24	2.75		3.64	0.25	3.54		6.08	0.79	6.02	
*AUDC**	124.43				184.01				270.38				260.92				300.51				423.17			

Mt – amount of drug released in time t (mg) , Mt* – amount of drug released in time t calculated from Higuchi equation (mg) K_0 – release rate constant (mg $min^{-0.5}$), ±SD – standard deviation, AUDC* – area under the dissolution curve for ideal Higuchi release profile (mg min)

Table 4. Relation between AUDC* and dissolution media viscosity

Dissolution medium (% of albumin solution in phosphate buffer pH 6,8)	AUDC* (mg min)	Dissolution medium Viscosity (mPa·s)
0.75%	124.43	1.042
1.5%	184.01	1.076
3.0%	270.38	1.211
0.75% with 1% Tween 20	260.92	1.077
1.5% with 1% Tween 20	300.51	1.107
3% with 1% Tween 20	423.17	1.201

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SUMMARY

The aim of this work was to investigate the influence of egg albumin on release profiles of dl-α-tocopherol acetate (VE) capsules. The dissolution medium was constituted by pH 6.8 phosphate buffer containing 0.75, 1.5, 3% (w/v) egg albumin in the presence and absence of 1% (w/v) Tween 20. The dissolution behaviour was fit to Higuchi kinetic model and described by Higuchi equation. The area under dissolution curve (AUDC*) was calculated and compared with the concentration of albumin and dissolution time. The dissolution rate of vitamin E was increased with the concentration of albumin. Viscosity increased with the concentration of albumin for both of media. The influence of nonionic surfactant (Tween 20) on the properties of albumin solution and possible complexation as a result of protein–surfactant interaction was also discussed. The total amount of drug released was the highest for dissolution medium with 3% (w/v) albumin and 1% (w/v) Tween 20, showing beneficial influence of Tween 20 on dissolution rate of vitamin E.

STRESZCZENIE

Tematem pracy było zbadanie wpływu lepkości cieczy biorczych składających się z buforu fosforanowego o pH 6,8, zawierających 0,75, 1,5, 3% (w/v) albuminy jaja kurzego z dodatkiem lub bez dodatku 1% (w/v) Tween 20, na profile uwalniania dl-α- octanu tokoferolu z żelatynowych kapsułek. Do opisu uwalniania witaminy E zastosowano kinetyczny model Higuchiego. W celu zbadania wpływu lepkości cieczy biorczych na ilość uwolnionej substancji obliczono pole pod krzywą stężeniowo–czasową (AUDC*) i porównano ze stężeniem albuminy w cieczach biorczych. Stwierdzono, że ilość uwolnionej witaminy E wzrasta wraz ze wzrostem stężenia albuminy i czasem uwalniania. Całkowita ilość uwolnionej witaminy E była największa dla cieczy biorczej zawierającej 3% (w/v) albuminę z dodatkiem 1% (w/v) Tweenu 20, wskazując na pozytywny wpływ dodatku tego emulgatora na uwalnianie witaminy E. W pracy poruszono także temat wpływu niejonowego emulgatora – Tweenu 20 na właściwości roztworów zawierających albuminę oraz możliwość powstawania związków kompleksowych w rezultacie oddziaływań białko – emulgator.