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The Effect of Egg Yolk on Enhancing the Solubility of Candesartan Cilexetil

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ZAID AL-OBAIDI[1], HASANAIN SHAKIR MAHMOOD[2], ZAINAB FALAH MUSTAFA[3], AYOOB JABBAR MOHAMMED ALESAOE[4], HAJIR NABEEL SAHIB[5]

1. *MSc Pharmaceutical Analysis, University of Kerbala, College of Pharmacy, Dep. of Pharmaceutical Chemistry,*
2. *MSc Pharmaceutics, University of Kerbala, College of Pharmacy, Dep. of Pharmaceutics*
3. *4. 5. BSc Pharm, University of Kerbala, College of Pharmacy, Iraq.*

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ABSTRACT

Background: Candesartan is a selective antagonist of the angiotensin II (Ang II) type 1 (AT1) Receptor. It is administered orally as candesartan cilexetil tablets. It is insoluble in water but soluble in methanol. In this study, egg yolk (lecithin) is used as a surfactant to enhance the solubility of candesartan. **Materials and instruments:** Candesartan cilexetil, chickens' eggs and methanol were used. **Method:** 1ml of candesartan stock solution was prepared. Then, triplicates of 10mL of the following five concentrations (0.1, 0.2, 0.3, 0.4, and 0.5) % (v/v) were diluted from the stock solution. After that, the diluted samples were assayed utilizing UV instrument. **Results and discussion:** The selection of λ_{max} of the absorption wavelength of candesartan was highly dependent on the cut-off values of the solvent system. The plotted curve, of the saturated solubility of CAND with various percentages of EY, shows linearity (y= 0.0051x) and a good r-squared value (R^2=0.99972).

INTRODUCTION

Candesartan is a selective antagonist of the angiotensin II (Ang II) type 1 (AT1) receptors. The oral pro-drug candesartan cilexetil is marketed for the treatment of hypertension, chronic heart failure and left ventricular systolic dysfunction. Candesartan cilexetil works by relaxing and dilating blood vessels. Hence, blood pressure will be lowered. Additionally, candesartan cilexetil reduces the load on the heart to pump blood to all parts of the body[(1)]. Currently,irbesartan, losartan, telmisartan and valsartan are available as highly selective AT1 receptors antagonist. However, different affinities for the AT1 receptor and durations of receptor blockage were documented among these compounds in *in vitro* studies. Candesartan has a long duration of action due to its high affinity for the AT1 receptors and slow dissociation [(2)]. According to the chemical structure of candesartan cilexetil, as shown in Figure 1 [(3)], it is insoluble in water but soluble in methanol and sparingly soluble in aqueous buffers. On the other hand, it has a high permeability[(4)]. Therefore, candesartan cilexetil belongs to Class II of the Biopharmaceutics Classification System (BCS) [(5)].

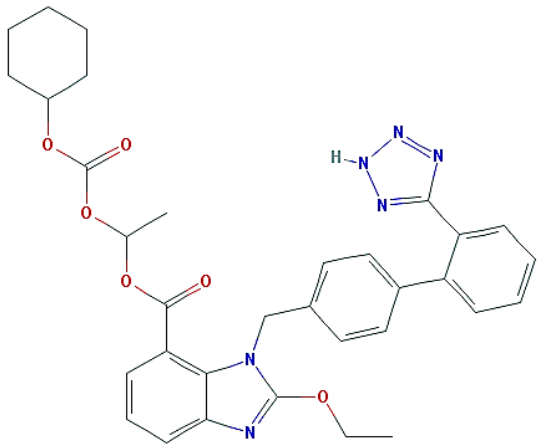

Figure 1: The chemical structure of candesartan cilexetil. Molecular Formula is $C_{33}H_{34}N_6O_6$. IUPAC name is 1-cyclohexyloxycarbonyloxyethyl 2-ethoxy-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]benzimidazole-4-carboxylate [(3)].

A drug need to be solubilised in order to reach the systemic circulation and achieve the desired pharmacological effect [(6)]. Therefore, the solubility of candesartan cilexetil was considered as the rate limiting step for its bioavailability [(7)]. The high patient compliance and ease of administration accompanied with the low cost for the production of oral medications have

encouraged the scientists to improve the aqueous solubility of these medications[8]. First, the selection of a suitable salt of the poorly soluble drug, changing the polymorphic form. Second, the reduction of particle size to less than 200nm (application of nanotechnology). Advantages of the application of nanotechnology include an increase in the dissolution rate leading to a higher drug bioavailability; the need for lesser doses and hence lower toxicity will be experienced [9]. Third, the use of excipients that function as solubilisers or transporters (including complexing agents, permeability enhancers or surfactants).

Other strategies which were applied to increase the aqueous solubility of oral medications involve chemical modifications such as hydrophilic groups' substitution, prodrug preparation and co-crystallisation or solvate formation [10]. According to [11], co-solvency is achieved when water miscible solvents are added to solutes with poor water solubility. Polyethylene glycol with a molecular weight of 400 (PEG400) is frequently used as a co-solvent with poorly soluble drugs such as candesartan cilexetil[12][13][14]. Moreover, B-cyclodextrin was utilized to enhance the solubility of candesartan[15]. The presence of hydrogen bond donor and/or acceptor groups, as well as small hydrocarbon regions in the structure of co-solvents, could reduce the interfacial tension between the aqueous solution and the hydrophobic solute [16]. The solubility study of candesartan cilexetil indicated that its water solubility was enhanced from 0.012mg/mL to 0.6mg/mL through the addition of 5% PEG400 [4]. Microemulsion technique is used to improve solubilization capacity of poorly water-soluble drugs. Microemulsions are colloidal systems with thermodynamical stability which are formed by mixing oil and surfactant[17]. In addition to their use in liposome mediated drug delivery systems, surfactants are used in membrane biochemistry as molecular tools. Egg yolk was used successfully in enhancing the solubility of Telmisartan[18]. In this study egg yolk (lecithin) was used as surfactant to enhance solubility of candesartan cilexetil. An egg consists of approximately 27% yolk, 64% albumen and 9% shell. Egg yolk contains numerous vital nutrients and preservative substances, due to its original role as an embryonic chamber. The major constituents of the yolk are proteins (16%) and lipids (32%) presented mainly in the form of lipoproteins. It also contains carbohydrates (1.0%), mostly oligosaccharides, which are bound to proteins and minerals (1.0%) [19]. Yolk is a complex system composed of particles suspended in yellow fluid named plasma, which contains proteins. The main type of particles are granules with vitellogeninas the main precursor of egg yolk proteins.

Its production takes place in hen's liver and increases with sexual maturation due to oestrogen secretion.

During egg formulation, vitellogenin is enzymatically cleaved into fragments termed lipovitellin I (120 kDa), phosvitin (44 kDa), and lipovitellin II (32 kDa), located in yolk granules, and a 40 kDa glycoprotein, YGP40, located in yolk plasma [20][21][22]. Apart from egg yolk phosvitin and phosvitin-derived peptides of protein, hydrolysates of lecithin. Lecithin is one of the most common natural phospholipids that is mainly composed by three compounds, PhosphatidylCholine (PC), PhosphatidylEthanolamine (PE) and PhosphatidylInositol (PI) [23]. The wide application of lecithin in the pharmaceutical production and industry is attributed to its valuable physicochemical, biological and physiological property [24]. As displayed in Figure 2[25], a balance between the hydrophobic and hydrophilic parts in the formula of lecithin is originated from the various phospholipids in its structure [26]. Therefore, lecithin works as an effective and stable emulsifier.

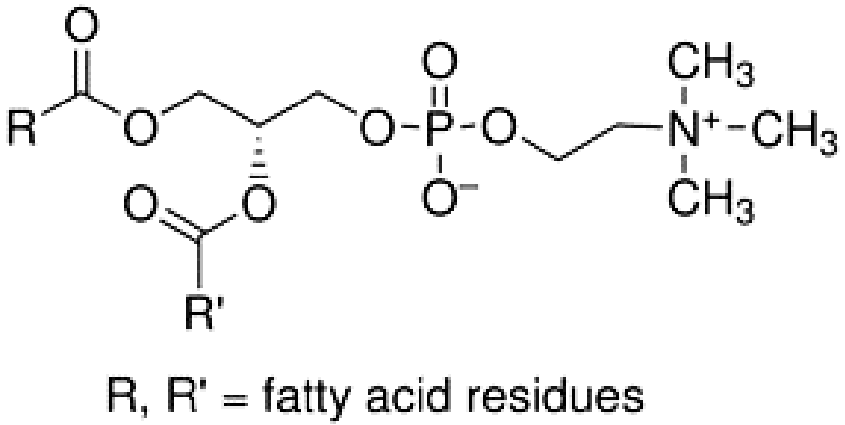

Figure 2: Chemical structure of lecithin [25].

MATERIALS AND METHODS

This study was performed in September 2015 at the University of Kerbala, College of Pharmacy, Kerbala City, Iraq.

Materials:

Candesartan cilexetil-USP (Batch No: PP/CT/006 F12) was purchased from ProvizerPharma (www.provizerpharma.com, Gujarat, India). Chicken eggs were purchased from local store of

Alkafeel (http://www.alkafeel.com/), Kerbala, Iraq. Qualitative filter paper was purchased from Fushun Civil Administration Filter Paper Factory-Qualitative filter paper 80g/㎡ Medium 102 type, Φ12.5cm (http://www.fsmzlz.com, Fushun, China).

Instruments:

The instruments employed in the study are documented below in Table 1.

Table 1: Illustrates the instrument that have been employed in the study.

No	Instrument	Manufacturer	Model	Country
1	Micropipette	Slamed	N/A	Germany
2	Four-Digits Sensitive Balance	WagiElektroniczne	Radwag	Poland
3	Hettichzentrifugen	Tuttlingen	D-78532	Germany
4	Sonicator-ultrasonic cleaner	Copley scientific	SRI	UK
5	UV-Visible Spectrophotometer	Sco Tech	SPUV-26	Germany
6	Agitator	Memmert	SV 1422	Germany

Methods:

Part 1: Calibration curve preparation:

A stock solution of candesartan (CAND) was prepared by dissolving exactly 10mg in 10mL of methanol then 1mL of this stock was diluted up to 10mL to get 100µg/mL solution. The resultant solution was utilised to prepare different concentrations of candesartan (0, 4, 8, 12, 16, 20, and 24) µg/mL. The UV-absorption of the prepared concentrations were observed and the calibration curve was plotted.

Part 2: Samples preparations:

10-Egg yolks were elicited from chickens' eggs and carefully isolated from egg whites. These egg yolks were homogenised and immediately utilised in preparing egg yolk solutions. One litre of freshly prepared 1% (v/v) egg yolk solution was used to prepare triplicates of 10mL of each of the five following concentrations (0.1, 0.2, 0.3, 0.4, and 0.5) % (v/v). Then an excess of candesartan cilexetil powder was inserted in each of the fifteen test tubes. The tubes were submitted for sonication for one hour. Afterwards, these fifteen tubes were centrifuged at 1000

rpm for 10 minutes. The resultant samples, i.e. (0.1, 0.2, 0.3, 0.4, and 0.5) % v/v, were filtered and diluted with distilled water to get 50mL of 0.01% (v/v) EY. Then the diluted samples were assayed utilising UV instrument.

RESULTS

A.CAND calibration curve:

The CAND/EY solution was scanned to observe the λ_{max} which found to be 258nm as shown in Figure 3, while the calibration curve was successfully plotted as shown in Figure 4.

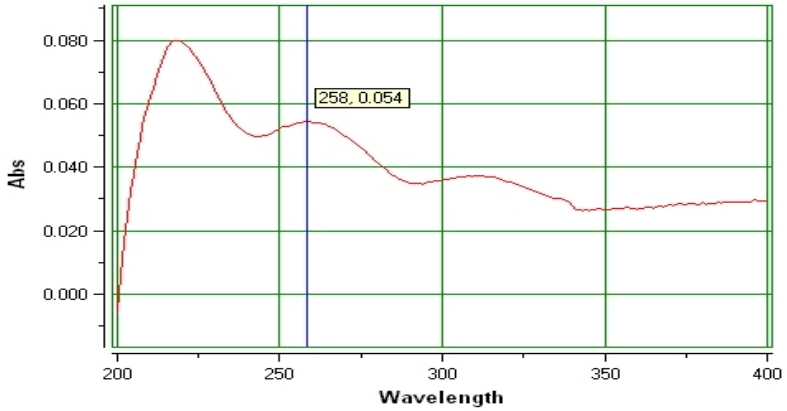

Figure 3: Shows the selected λ for CAND absorption at UV-instrument. The observed wavelength was found to be 258nm.

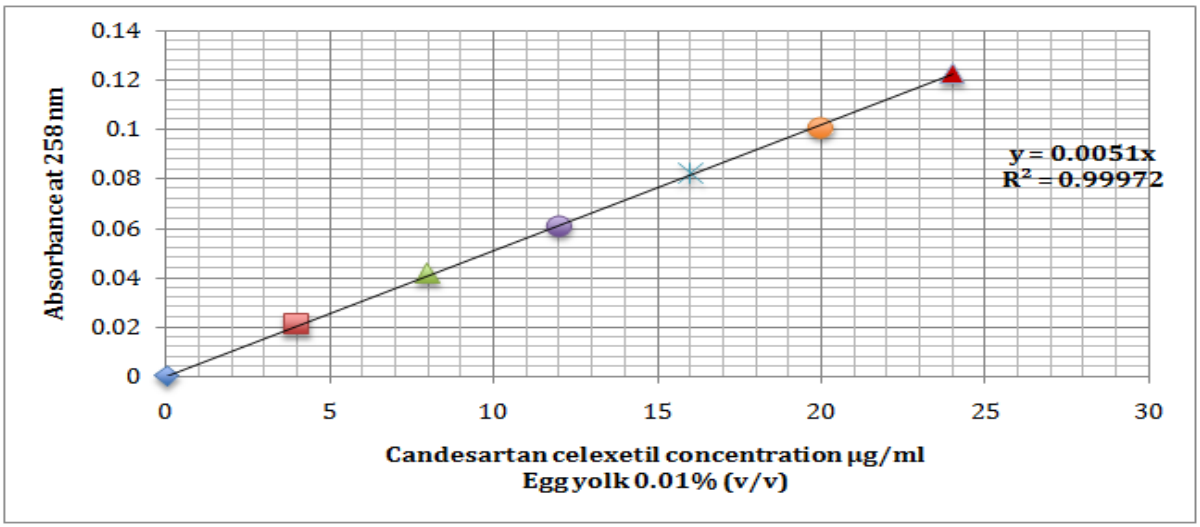

Figure 4: Reveals the calibration curve of CAND in 0.01% (v/v) EY. The R-squared equals to 0.99972 while the slope equals to 0.0051.

B. Observation of saturated solubility CAND:

The saturated solubility was successfully calculated for each EY percentage. This was after the observation of the absorbance of each diluted sample as explained in Table 2.

Table 2: shows the saturated solubility of CAND related to specific percentage of EY.
*Mean ± S.D., n=3, p= 0.00269

EY% v/v	Dilution factor to get 0.01% EY	Observed absorption*	CAND conc. µg/ml y = 0.0051x	(CAND conc. µg/ml) x (Dilution factor)
0.1	10	0.146 ± 0.03	28.63	286.27
0.2	20	0.143 ± 0.04	27.97	559.48
0.3	30	0.143 ± 0.01	28.04	841.18
0.4	40	0.142 ± 0.008	27.78	1111.11
0.5	50	0.142 ± 0.009	27.91	1395.42

Moreover, by the employment of the data in Table 2, the observed saturated solubility of CAND was smoothly plotted against the EY% as shown in Figure 5.

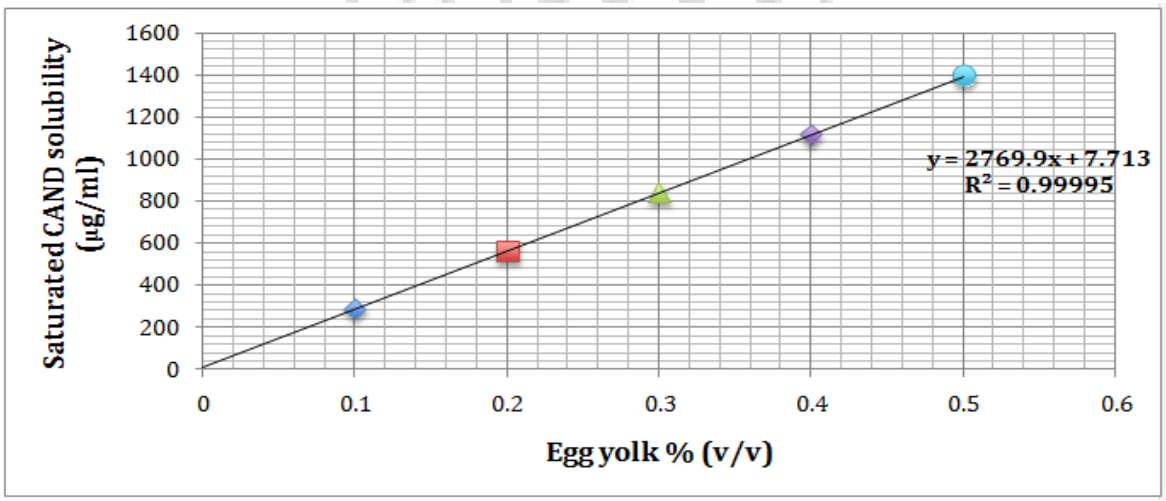

Figure 5: shows linear relationship of the saturated solubility of CAND with various percentages of EY. The slope was 2769.9, the intercept 7.713 and an excellent R^2 = 0.99995.

DISCUSSION

Candesartan is soluble in methanol. Therefore, methanol is used to prepare the solutions for the calculation of the calibration curve. The selection of λ_{max} of the absorption wavelength of candesartan was dependent on the cut-off values of the solvent system. As clearly shown in Figure 3, two λ_{max} peaks were observed at 220nm and 258nm. However, the 258nm is widely accepted as the λ_{max} for candesartan to be far from solvent effect. The 220nm wavelength was excluded as it lies within the methanol cut-off solvent value which is 205nm. The calibration curve was plotted depending on the areas of the peaks assigned for each injected concentration. The curve shows linearity (y= 0.0051x) and a good r-squared value (R^2=0.99972) as shown in Figure 4.

During calculating the saturated solubility of candesartan, each sample was diluted, as shown in Table 2, because the UV apparatus does not measure absorbance higher than 1 accurately. Table 2 shows that the saturated solubility increased with the increased concentration of added egg yolk.

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