Araştırma Makalesi / *Research Article*
Kimya / *Chemistry*

Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 9(1): 130-138, 2019
***Journal of the Institute of Science and Technology*, 9(1): 130-138, 2019**

DOI: 10.21597/jist.430007

ISSN: 2146-0574, eISSN: 2536-4618

Jeffamine® Core PAMAM Dendrimers as Solubility Enhancer of Carvedilol

Ali Serol ERTÜRK[1*] Mustafa Ulvi GÜRBÜZ[2] Metin TÜLÜ[2] Abdürrezzak Emin BOZDOĞAN[2]

ABSTRACT: The aim of this study was to investigate the effect of Jeffamine® core poly(amidoamine) PAMAM dendrimers (JCPDs) on the aqueous solubility of carvedilol (CAR), a Biopharmaceutical Classification System (BCS) Class II drug, and a nonselective beta-adrenegenic blocking agent with alpha 1-blocking activity. The aqueous solubility of CAR was measured in the presence of JCPDs at room temperature in phosphate-buffered saline using traditional rotating bottle technique. Results obtained from the phase solubility studies revealed that the molar aqueous solubility of CAR increased significantly with a proportional increase in the concentration of fourth-generation JCPD, P4.NH_2. Likewise, the encapsulation efficiency of JCPD, P4.NH_2 improved as its concentration increased and the highest capacity was observed to be 60.75%. Furthermore, the drug binding constant of P4.NH_2 (11177.31 ± 0.15 M^{-1}) was found to be fifty times higher than that of β-cyclodextrin (227 M^{-1}), which is the most common studied solubility enhancer excipient for CAR drug. Overall, it can be concluded that PAMAMs, used for the first time in this study as the successful solubility enhancer of CAR, might be helpful and good candidates for the development of various formulations in the future studies.

Keywords: Carvedilol, complexation, drug binding constant, PAMAM dendrimer, Jeffamine

Karvedilolun Çözünürlüğünü Arttırıcı Olarak Jeffamine® Çekirdekli PAMAM Dendrimerler

ÖZET: Bu çalışmada amacımız Jeffamine® çekirdekli PAMAM dendrimerlerin, biyofarmasötik sınıflandırma sistemi sınıf II ilacı ve alfa 1 engelleyici aktivite ile seçici olmayan bir beta-adrenerjik bloke edici ajan olan karvedilol'ün (CAR) sudaki çözünürlüğü üzerindeki etkisini araştırmaktır. CAR'ın sudaki çözünürlüğü, geleneksel döner şişe tekniği kullanılarak fosfat tamponlu tuz içinde oda sıcaklığında dendrimerler varlığında ölçüldü. Faz çözünürlük çalışmalarından elde edilen sonuçlar, CAR'ın sudaki çözünürlüğünü, dendrimer konsantrasyonuyla neredeyse orantılı olduğunu ve PAMAM dendrimerlerin mevcudiyetinde önemli ölçüde arttığını gösterdi. Bu çalışmalar gösterdi ki, PAMAM dendrimerlerin CAR'nin çözünürlüğünü arttırıcı olarak düşünülebileceğini ve çeşitli formülasyonların geliştirilmesine yardımcı olabileceğini gösterdi.

Anahtar kelimeler: Çözündürme, ilaç bağlama sabiti, karvedilol, kompleks, PAMAM dendrimer

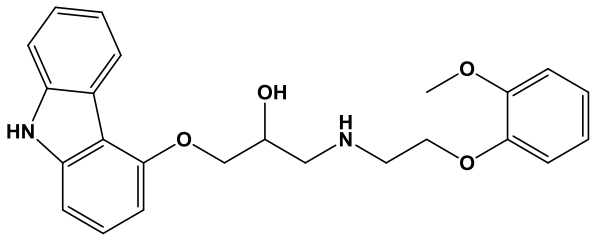
[1] Ali Serol ERTÜRK (**Orcid ID:** 0000-0001-5352-7939), Adıyaman University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Adıyaman, Turkey
[2] Mustafa Ulvi GÜRBÜZ (**Orcid ID:** 0000-0002-8684-5746), Metin TÜLÜ (**Orcid ID:** 0000-0001-9791-4922), Abdürrezzak Emin BOZDOĞAN (**Orcid ID:** 0000-0003-2102-2906), Yıldız Technical University Department of Chemistry, Istanbul, Turkey
*Sorumlu Yazar/Corresponding author: Ali Serol ERTÜRK, e-mail: aserturk@gmail.com

Geliş tarihi / *Received*: 02.06.2018
Kabul tarihi / *Accepted*: 24.09.2018

INTRODUCTION

Carvedilol (CAR), (±)-1-(9H-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]-2-propanol (Fig. 1), is a nonselective β-adrenergic blocking agent with α1-blocking activity and used in the treatment of cardiovascular diseases such as hypertension, angina pectoris and cardiac insufficiency (Dollery, 1999). The empirical formula of CAR is $C_{24}H_{26}N_2O_4$ and molecular weight of 406.5 g/mol. Poor aqueous solubility of CAR (S_o≈0.02 mg/mL) is considered to be one of the most important reasons for its low bioavailability after oral administration, and also a handicap for the nasal spray and sublingual tablet formulations (Wen et al., 2004). Thus, CAR needs enhancement in solubility to improve its bioavailability.

To date, Several techniques have been used to improve the solubility and dissolution rate of CAR such as the microsphere (Wei et al., 2005), addition of surfactants, solid dispersion (Sharma and Jain, 2010; Kovačič et al., 2011). Among these techniques, complexation with cyclodextrins (Virmani et al., 2007; Hirlekar and Kadam, 2009; Pamudji et al., 2014) have been widely investigated to improve solubility and dissolution properties of CAR. However, the aqueous solubility of CAR could not be improved significantly. For this reason, ideal and new emerging candidates to improve the solubility of CAR is still a challenge and alternative approaches are necessary.



Carvedilol (CAR)

Figure 1. Molecular structure of carvedilol (CAR)

Over the last decades, the use of poly(amidoamine) PAMAM dendrimers as novel drug-delivery systems and applications, including increasing the solubility and low bioavailability with poor solubility of drugs has gained much attention. The solubility of several commercial drugs such as naproxen (Yiyun and Tongwen, 2005a), ibuprofen (Milhem et al., 2000), cis platin, resveratrol, genistein, curcumin (Abderrezak et al., 2012), furosemide (Devarakonda et al., 2007), sulfamethoxazole (Gürbüz et al., 2016), candesartan cilexetil (Ertürk et al., 2016), ketoprofen, and vitamins like nicotinic acid (Yiyun and Tongwen, 2005b) and riboflavin (Filipowicz and Wołowiec, 2011) have been increased successfully. This could be attributed to the high level of control over dendritic architecture (size, branching density, terminal groups) of dendrimers for enhanced drug solubilization.

Commercially available ethylene diamine or ammonia cored PAMAMs have been extensively studied for the solubility enhancement of poor soluble drugs and well established for a long time as above mentioned. In our recent study (Ertürk et al., 2014), we indicated the importance of polymeric cored PAMAMs as challenging dendrimers because of their wide range of applications and presented the microwave assisted synthesis of new generation JCPDs.

Jeffamine® is a polymer, which has large and unsymmetrical chains having propylene oxide repeating units. These large repeating units can retard the steric hindrance, but, enhance the reactivity and water solubility. Therefore, synthesized JCPDs using the Jeffamine polymer as a core for the synthesis of higher generation PAMAMs can be a new kind of emerging potential solubility enhancer for poor soluble drugs.

The aim of this study was to investigate whether water-soluble JCPDs could be used to increase the solubility of CAR. On the basis of this aim, the solubility of CAR at different concentrations of highest generation JCPD, $P4.NH_2$ was measured. Drug loading properties of $P4.NH_2$ was also examined by the pH behavior of hydrophobes and supported by performing experiments with the different ratio of substrate/dendrimer complexes. Moreover, stable and miscible ion pairs of CAR with dendrimers' internal basic tertiary amines were observed by UV-Vis spectroscopy in order to suggest that these dendrimer-drug systems could be facilitated as potential drug-delivery systems. Hence, the present work might be helpful for the development of various formulations.

MATERIALS AND METHODS

Materials

Jeffamine® T-403 Mn 440 was purchased from Aldrich. Methyl acrylate, ethylenediamine, n-butanol, were purchased from Merck. All other chemicals are analytical grade and used without further purification. Carvedilol (CAR) was kindly supplied from DEVA Holding, Turkey. All solutions were prepared with 18.2 MΩ Millipore Milli-Q deionized. Dendrimer solutions were stored at 4 ^{o}C. Unless otherwise stated all chemicals were in analytical grade and used without further purification. Liquid-phase polymer-based retention (LPR) ultrafiltration membranes, Amicon 8000 Stirred Cell and dialysis membranes having the molecular cut of size (MWCO) 500, 1000, 3000 Da were supplied from Millipore. Glass pH electrode was calibrated with Merck pH 4.0, 7.0, 11.0 buffer solutions.

Instruments

The CEM Focused Microwave™ Synthesis System, Model Discover (CEM Corporation, North Carolina, USA) with a continuous microwave power delivery system with operator selectable power output from 0-300 watts (± 30 watts) programmable in 1-watt increments, infrared temperature control system programmable from 25- 250 ^{o}C, and 5-125 mL vessel capacity was used as microwave reactor during the synthesis of PAMAMs. pH of the phosphate buffered saline (PBS) and dendrimer solutions were measured and adjusted with IoLine ultra precise glass electrode with iodine/iodide reference system.

The IR spectra (4000–400 cm^{-1}, resolution 4 cm^{-1}) were recorded with a Perkin Elmer Spectrum One (Serial No: C68739) in ATR. NMR spectra were recorded on a Bruker Avance 400 MHz Spectrometer. Thermo Scientific Flash EA 2000 Series (Organic Elemental Analyzer) CHN/S was used for the determination of main organics. GPC analyses were performed on a Viscotek TDA302, with a column set Tosoh TSK G3000PWxl, and with buffer (PBS) as eluent.

Synthesis of JCPDs

Synthesis of PAMAMs, with Jeffamine® T-403 core, were accomplished by following the procedure reported in our recent studies (Ertürk et al., 2014; Erturk et al., 2015). The synthesis involves two consecutive reactions, which are Micheal addition and amidation reaction, respectively. Micheal addition of excess methyl acrylate (2.5 M eq. per terminal amine) to Jeffamine core in methanol gives the ester terminated half-generation dendrimers,

Pn.5.OCH_3. The successive amidation reaction of ester terminated PAMAMs (PAMAM.OCH_3) with excess ethylene diamine (EDA) (10 M eq. of EDA per ester branched half-generation) in methanol under appropriate microwave irradiation produces amine-terminated full generation PAMAMs (PAMAM.NH_2) referred to Pn.NH_2. Repetition of Micheal addition and amidation reactions gives next higher generations. By repeating the Micheal addition and amidation reactions, we synthesized both ester terminated (P0.5.OCH_3-P3.5.OCH_3) and amine terminated (P1.NH_2-P4.NH_2) PAMAMs (Figure 1). Purifications of both PAMAM.OCH_3 and PAMAM.NH_2 were performed by using liquid phase polymer retention technique (LPR). While resulting pure PAMAM-OCH_3 dendrimers were water insoluble, PAMAM-NH_2 dendrimers were water-soluble. Thus, highest generation P4.NH_2 was used in the solubilization studies of CAR (Figure 1).

P4.NH_2

Product is yellowish gel. Elemental analysis $C_{264}H_{533}N_{99}O_{54}$: Found: C, 54.12; H, 9.21; N, 23.38. Calc.: C, 53.21; H, 9.02; N, 23.27%. ATR-IR ν_{max}/cm^{-1} 3281(NH), 1639(HNC=O), 1548(HNC=O). ^{1}H-NMR δH(400 MHz; DMSO) 2.21 (96H, t, NCH_2CH_2CONH), 2.66 (96H, t, $CONHCH_2CH_2NH_2$), 3.06 (96H, t, $CONHCH_2CH_2NH_2$), 2.56 (96H, t, NCH_2CH_2CONH), 7.94 (48H, br s, NCH_2CH_2CONH). ^{13}C-NMR δC(400 MHz; DMSO) 171.56 (NCH_2CH_2CONH), 48.54 (NCH_2CH_2CONH), 42.04 ($CONHCH_2CH_2NH_2$), 41.21 ($CONHCH_2CH_2NH_2$), 35.90 (NCH_2CH_2CONH).

Phase Solubility Studies

Phase solubility experiments were carried out as per the method described by Higuchi and Connors (Higuchi and Connors, 1965). 1.87 x 10^{-2}, 3.73 x 10^{-2}, 5.59 x 10^{-2} and 7.46 x 10^{-2} mM P4.NH_2 dendrimer solutions were prepared. The dendrimer solutions were diluted to 5.0 mL with pH ~7.0 PBS and final pH of the solutions were adjusted to pH 7.0 by the dropwise addition of 0.01-0.1 M NaOH and HCl solutions, and transferred to sealed dark-brown glass vessels. Excess amount of CAR (20.0 mg) was added to each vessel. Resulting suspensions were shaken with orbital shaker in an incubator at 24 ± 0.1 ^oC for 72 hours.

After equilibrium reached, the insoluble excess amount of CAR was removed from solutions by using 0.45 μm cellulose acetate filter. The concentration of CAR was determined spectroscopically by using UV-Vis spectrophotometer in the wavelength ranges of 200-350 nm. In lower concentrations of CAR, λ_{max} absorbans band was observed at 241 nm instead of 249 nm. Hence, UV measurements were taken at 241 nm (n=4).

Drug Binding Constant of CAR

The apparent stability constant K_{CAR} was calculated from the phase solubility diagram according to the following Higuchi and Conners equation (1) (Higuchi and Connors, 1965):

$$K_{CAR} = \frac{\alpha}{S_0(1-\alpha)} \quad (1)$$

where, S_o is the solubility of CAR in the absence of P4.NH_2 at room temperature and α is the slope of the phase solubility diagram. Encapsulation efficiency of P4.NH_2 (P4.NH_2EE %) was calculated according to equation. 2.

$$(P4.NH_2)EE\ \% = \left(\frac{\text{Loaded CAR} - S_0}{S_0}\right) \times 100 \quad (2)$$

RESULTS AND DISCUSSION

Preparation of Jeffamine® Core PAMAM Dendrimers

The JCPDs were synthesized via divergent approach (Figure 1) and characterized with ^{1}H NMR, ^{13}C NMR, FTIR-ATR, and GPC and the results were in good agreement with the literature (Ertürk et al., 2014; Erturk et al., 2015). The prepared dendrimers were stored in methanolic solution and stored at ± 4°C. Some characteristics and the characterization data of JCPD, P4.NH_2 evaluated in this work for the solubility studies was listed in Table 1.

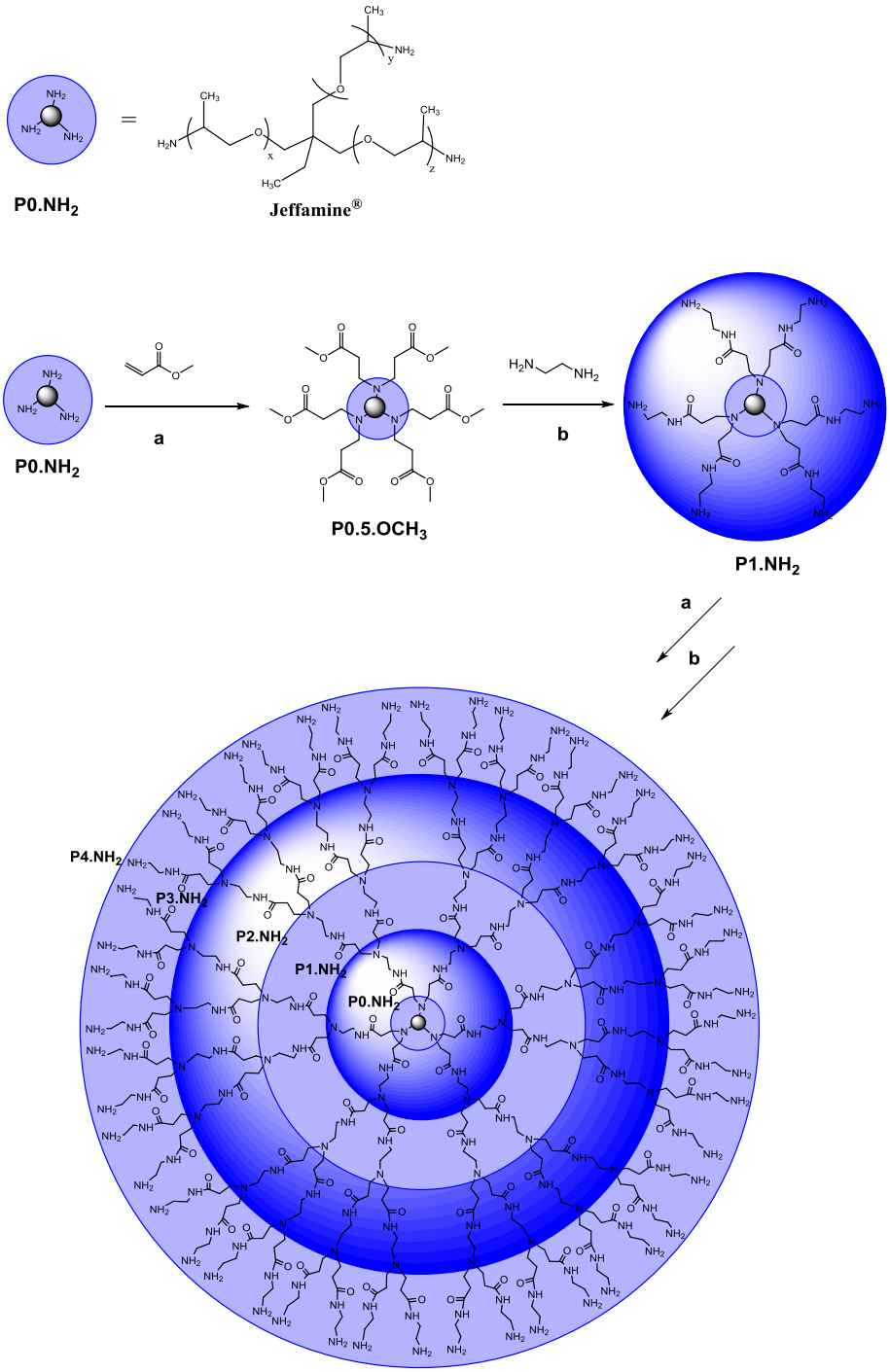


Figure 1. Synthesis and structure development of JCPD, P4.NH_2, (a) Micheal addition step and (b) Amidation step (Ertürk et al., 2014)

Table 1. Selected physico-chemical properties of JCPD, P4.NH_2 [a]

Generation	Mw	Mn(SEC)	MW(SEC)	PDI	Number of tertiary amines	Number of primary amines	Number of total amino groups
P4.NH_2	10700	9200	9600	1.04	45	48	183

[a] Mw: theoretical molecular weight (g/mol), Mn (SEC): nominal molecular weight measured by size exclusion chromatography; Mw (SEC): molecular weight measured by size exclusion chromatography; PDI: polydispersity index (Ertürk et al., 2014)

Effect of Dendrimer Concentration on The Aqueous Solubility of CAR

Fig. 2 shows the phase solubility diagram of P4.NH_2. Investigation of the change in the molar solubility of CAR as a function of increasing PAMAM (P4.NH_2) dendrimer concentration reveals that there is a good linear correlation (R^2 = 0.9937) between the solubility of CAR and the dendrimer concentration. The solubility of CAR increases as the dendrimer concentration increases.

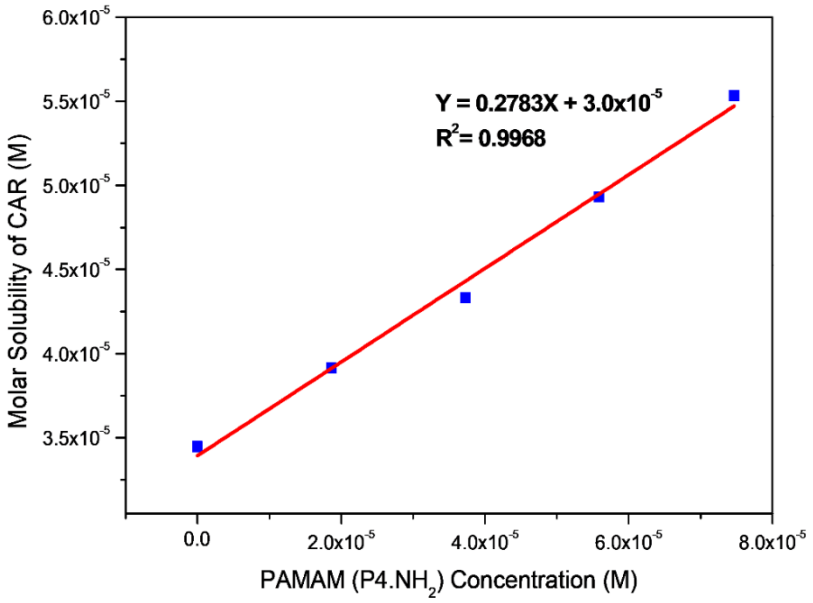


Figure 2. Phase solubility diagram of JCPD, P4.NH_2. Change in molar solubility of CAR as a function of increasing PAMAM (P4.NH_2) dendrimer concentration.

From the aspect of host-guest molecule interactions, inner cavities of PAMAMs can be hosted for the guest molecules like drug active ingredient CAR. When the dendrimer drug complexation occurs via covalent binding, it is expected to observe a new complexation band in the UV-Vis spectrum resulting from the electronic transitions between the dendrimer and drug. However, it can be clearly seen in Fig. 3 that no such kind of a complexation peak exists. In Fig. 3, UV spectrum revealed a hypsochromic rise occurred with increasing dendrimer concentration. This could be driven from the hydrogen bond formation between the inner cavity of P4.NH_2 and CAR, and indicates the inclusion complex formation by encapsulation of CAR inside the JCPD, P4.NH_2 (Fig. 4). In this case, it could be concluded that the solubility of CAR increases as the dendrimer concentration increases depending on the dendrimer drug inclusion complex formation. This formation could be supported by the phase solubility

diagram in Fig. 2 and seen from the increase in the absorbance of CAR in Fig. 3.

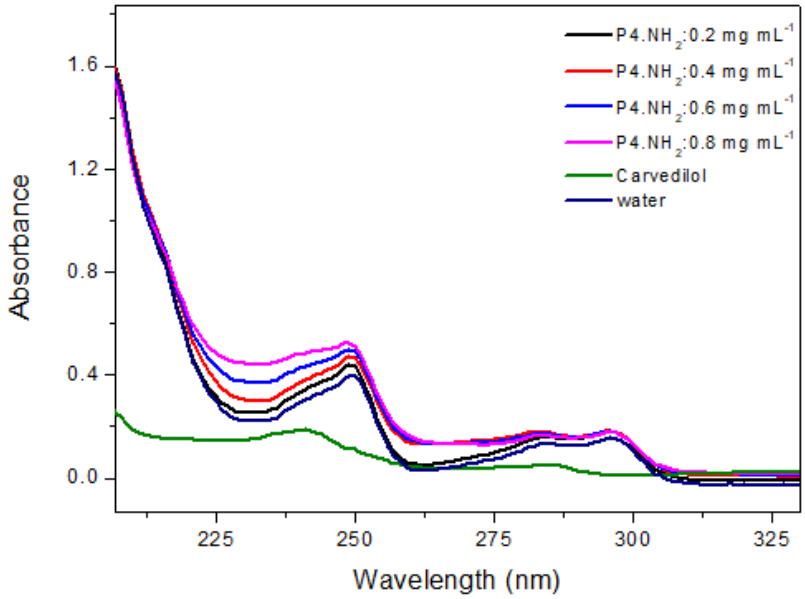
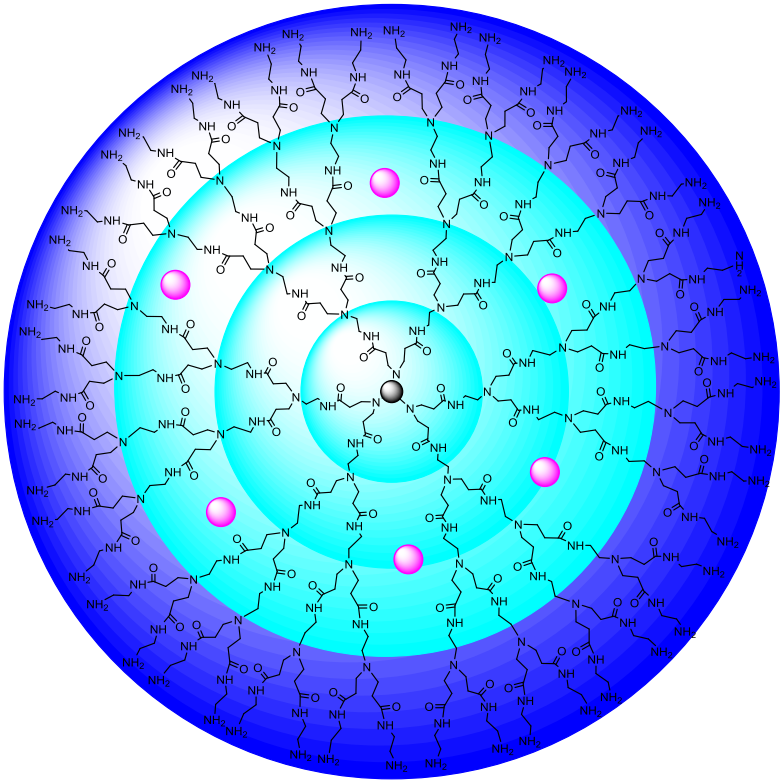
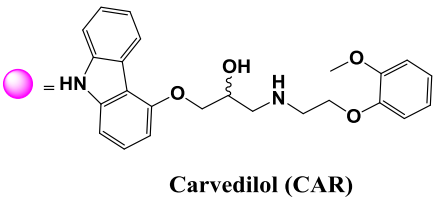


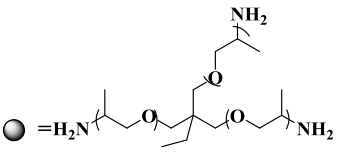
Figure 3. Increase in the absorbance of CAR with the added concentration of JCPD, P4.NH_2. λ_{max} = 241 nm instead of 249 nm in low concentrations of CAR.



P4.NH_2-CAR inclusion complex



Carvedilol (CAR)



Jeffamine® T-403

Figure 4. Representative illustration of the JCPD, P4.NH_2-CAR inclusion complex

Drug Binding Constants

In the region where the linearity is observed in the phase solubility diagram, a linear regression analysis was performed, and the following equation 3 was obtained for JCPD, P4.NH_2-CAR system.

$$y= 0.2783x + 0.00003 \quad (3)$$

In the eqn. 3, x represents the molar concentration of P4.NH_2 and y is the molar concentration of CAR. The correlation constant is 0.9937. Since the slope of the phase solubility diagram is less than 1, the stoichiometry of these complexes was assumed to be 1:1, A_L type (linear) (Higuchi and Connors, 1965). Thus, the apparent stability constant K_{CAR} can be calculated from the straight-line portion of the phase solubility diagram (Higuchi and Connors, 1965) according to the equation 1.

In eqn. 3, S_o = 3.45 x 10^{-5} M is the experimentally obtained solubility of CAR in water and α = 0.2783 (Filipowicz and Wołowiec, 2011). By using these values, the apparent stability constant (drug binding constant) K_{CAR} was calculated as 11177.31± 0.15 M^{-1}. This indicates that the CAR binding ability of P4.NH_2 is approximately fifty times higher in comparison with that of most widely studied β-cyclodextrin drug for CAR, 227 M^{-1} (Hirlekar and Kadam, 2009). This means that the solubility of CAR was increased 50 folds in the presence of PAMAMs.

Encapsulation Efficiency of Dendrimer-Carvedilol Inclusion Complexes

Dendrimer encapsulation efficiencies for the increasing 1.87 x 10^{-2}, 3.73 x 10^{-2}, 5.59 x 10^{-2} and 7.46 x 10^{-2} mM dendrimer concentrations were calculated as 13.37%, 25.87%, 43.02%, and 60.75% from the eqn. 2. Obtained results revealed that percent encapsulation efficiency of P4.NH_2 increased as the amount of P4.NH_2 increased. Hence, it could be concluded from these results that JCPDs can be used as drug-delivery systems or carriers for low bioavailable small hydrophobic acidic molecules.

CONCLUSION

CAR has a restricted bioavailability because of its lower solubility. Low bioavailability and limited solubility of CAR in water are the main problem to be overcome in future drug formulization of it. CAR binding and solubility enhancement abilities of JCPD, P4.NH_2, were investigated. It was observed from the phase solubility studies that the solubility of CAR increased proportionally with the increasing amount of added P4.NH_2 dendrimer concentrations. Likewise, CAR binding ability of P4.NH_2 was observed to be fifty times higher compared to be most widely studied complexation technique with studied β-cyclodextrin. Consequently, JCPD, P4.NH_2 significantly increased the water solubility of CAR. The solubility improvement was dependent on the concentration of used JCPD. Thus, limited water solubility of CAR could be overcome by the formation of inclusion complexes with JCPDs, which could be proposed as a new kind of drug carrier. Hence, the present work might be helpful for the development of various PAMAM-CAR drug formulations.

KAYNAKLAR

Abderrezak A, Bourassa P, Mandeville JS, Sedaghat-Herati R, Tajmir-Riahi HA, 2012. Dendrimers bind antioxidant polyphenols and cisplatin drug. PloS one, 7 (3): e33102.

Devarakonda B, Otto DP, Judefeind A, Hill RA, de Villiers MM, 2007. Effect of pH on the solubility and release of furosemide from polyamidoamine (PAMAM) dendrimer complexes. International Journal of Pharmaceutics, 345 (1–2): 142-153.

Dollery C, 1999. Therapeutic drugs+CD. Elsevier Science Health Science Division.

Erturk AS, Gurbuz MU, Tulu M, Bozdogan AE, 2015. Water-soluble TRIS-terminated PAMAM dendrimers: microwave-assisted synthesis, characterization and Cu(ii) intradendrimer complexes. RSC Advances, 5 (74): 60581-60595.

Ertürk AS, Gürbüz MU, Tülü M, 2016. The effect of PAMAM dendrimer concentration, generation size and surface functional group on the aqueous solubility of candesartan cilexetil. Pharmaceutical Development and Technology, 22 (1): 111-121.

Ertürk AS, Tülü M, Bozdoğan AE, Parali T, 2014. Microwave assisted synthesis of Jeffamine cored PAMAM dendrimers. European Polymer Journal, 52: 218-226.

Filipowicz A, Wołowiec S, 2011. Solubility and in vitro transdermal diffusion of riboflavin assisted by PAMAM dendrimers. International Journal of Pharmaceutics, 408 (1–2): 152-156.

Gürbüz MU, Ertürk AS, Tülü M, 2016. Synthesis of surface modified TREN cored PAMAM dendrimers and their effects on the solubility of sulfamethoxazole (SMZ) as an analogue antibiotic drug. Pharmaceutical Development and Technology, 22 (5): 678-689.

Higuchi T, Connors KA, 1965. Phase-solubility techniques. Advances in Analytical Chemistry and Instrumentation, 4: 117-212.

Hirlekar R, Kadam V, 2009. Preparation and characterization of inclusion complexes of carvedilol with methyl-β-cyclodextrin. J Incl Phenom Macrocycl Chem, 63 (3-4): 219-224.

Kovačič B, Vrečer F, Planinšek O, 2011. Solid dispersions of carvedilol with porous silica. Chemical and Pharmaceutical Bulletin, 59 (4): 427-433.

Milhem OM, Myles C, McKeown NB, Attwood D, D'Emanuele A, 2000. Polyamidoamine Starburst® dendrimers as solubility enhancers. International Journal of Pharmaceutics, 197 (1–2): 239-241.

Pamudji JS, Mauludin R, Lestari VA, 2014. Improvement of carvedilol dissolution rate through formation of inclusion complex with β-cyclodextrin. International Journal of Pharmacy and Pharmaceutical Sciences 6(4): 2-7.

Sharma A, Jain C, 2010. Preparation and characterization of solid dispersions of carvedilol with PVP K30. Research in Pharmaceutical Sciences, 5 (1): 49.

Virmani T, Parvez N, Yadav S, Pathak K, 2007. Solid Inclusion Complexes of Class II Imidazole Derivative With β–Cyclodextrin. Continental Journal of Pharmaceutical Sciences, 1: 1-8.

Wei L, Sun P, Nie S, Pan W, 2005. Preparation and Evaluation of SEDDS and SMEDDS Containing Carvedilol. Drug Development and Industrial Pharmacy, 31 (8): 785-794.

Wen X, Tan F, Jing Z, Liu Z, 2004. Preparation and study the 1:2 inclusion complex of carvedilol with β-cyclodextrin. Journal of Pharmaceutical and Biomedical Analysis, 34 (3): 517-523.

Yiyun C, Tongwen X, 2005a. Dendrimers as potential drug carriers. Part I. Solubilization of non-steroidal anti-inflammatory drugs in the presence of polyamidoamine dendrimers. European journal of medicinal chemistry, 40 (11): 1188-1192.

Yiyun C, Tongwen X, 2005b. Solubility of nicotinic acid in polyamidoamine dendrimer solutions. European journal of medicinal chemistry, 40 (12): 1384-1389.