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The effects of surfactants on the solubility and dissolution profiles of a poorly water-soluble basic drug, carvedilol

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This study investigated the most suitable surfactant medium for the dissolution testing of a poorly soluble basic drug, namely, carvedilol reflecting the *in vivo* behavior. Sodium lauryl sulfate (SLS), hexadecyltrimethylammonium bromide (CTAB) and polysorbate 80 were used as anionic, cationic and nonionic surfactants, respectively. Saturation solubilities of carvedilol were determined in the presence of SLS, CTAB and polysorbate 80 (0.5, 1 and 2% (w/v)) at pH 1.2 and 6.8. Dissolution behaviors of the commercial tablets were studied using USP apparatus II in pH 1.2, 4.5 and 6.8 buffers and pH 6.8 dissolution media with 0.5% (w/v) SLS, polysorbate 80 and CTAB. Polysorbate 80 enhanced the solubility of carvedilol irrespective of pH, while SLS and CTAB exhibited larger solubilization effect than polysorbate 80 depending on pH and the ionic nature of the surfactant. Based on *in vitro* dissolution profile similarity, pH 6.8 dissolution medium with 0.5% (w/v) polysorbate 80 was found to be the most biorelevant medium, which probably reflects the bioequivalence of test products to the reference product of carvedilol.

1. Introduction

Since solubility and permeability interactions and their impact on intestinal drug absorption were most prominently described by the Biopharmaceutics Classification System (BCS), it has continued to attract strong interest from pharmaceutical industry and drug regulatory authorities worldwide (Amidon et al. 1995; Shah and Amidon 2014). BCS is a scientific framework for classifying drugs into four groups according to their aqueous solubility and intestinal permeability: class I (high solubility – high permeability), class II (low solubility – high permeability), class III (high solubility – low permeability) and class IV (low solubility – low permeability) (Amidon et al. 1995). Currently, the US Food and Drug Administration (FDA) has promoted the BCS as a scientific approach to permit a waiver of *in vivo* bioequivalance (BE) testing for immediate release (IR) solid dosage forms for Class I drugs based on *in vitro* dissolution of a drug product, while the 2010 European Medicine Agency (EMA) BE Guideline has further extended its discussion of biowaivers to Class III drugs with very rapid dissolution (FDA Guidance 2000; EMA Guideline 2010). Current FDA and EMA regulations do not allow biowaiving for BCS Class II drugs, however World Health Organization (WHO) considers biowaiving of certain weak acids belonging to BCS Class II (diclofenac, ibuprofen and ketoprofen) which are exhibiting poor solubility at acidic pH but high solubility at pH 6.8 (Chuasuwan et al. 2009; Potthast et al. 2005; Shohin et al. 2012). For BCS Class II drugs, absorption is rate limited by *in vivo* dissolution, and possible *in vitro-in vivo* correlation (IVIVC) can be established to predict *in vivo* performance of a BCS Class II drug product (Yu et al. 2002). However, it is clear that more research is needed to provide a mechanistic understanding of the correlation between *in vitro* dissolution and *in vivo* performance for BCS Class II drugs at the present time (Shah and Amidon 2014; Tubic-Grozdanis et al. 2008). Therefore, it is a challenge to develop an appropriate *in vitro* dissolution test for BCS Class II drug products for quality control (QC) purposes and drug product development, as well as for the establishment of IVIVC or biorelevance. For weakly acidic and basic Class II drugs, the pH and composition of the dissolution medium are of great impact on the dissolution process. In such cases, surfactants may be added to dissolution media to enhance drug solubility and dissolution and provide sink conditions due to the physiological relevance of the synthetic surfactants (Amidon et al. 1995; Park and Choi 2006; Sheng et al. 2006; Shah et al. 1989).

The aim of the present study was to investigate the effects of medium pH and different types of surfactants (anionic, cationic and nonionic) on the solubility of carvedilol and the dissolution of its products and identify the most suitable surfactant medium for the dissolution testing reflecting *in vivo* dissolution of the drug. Being a BCS II weak base (Wu and Benet 2005), carvedilol (1-(9H-carbazol-4-yloxy)-3-[2-(2-methoxyphenoxy)ethylamino]propan-2-ol) was chosen as the model drug for the present study. Carvedilol is a nonselective β-blocking agent with α1-adrenergic antagonist activity (Fig. 1). It has been clinically used in the treatment of mild to moderate congestive heart failure and hypertension (Packer et al. 1996; Nägele et al. 2000; Rizos and Elisaf 2014). Carvedilol is rapidly and extensively absorbed with peak plasma concentration occurring 1 to 2 h after the administration and elimination half-life of 4 to 7 h (Morgan 1994). However, it is extensively metabolized resulting in approximately 24% absolute bioavailability (Neugebauer et al. 1987).

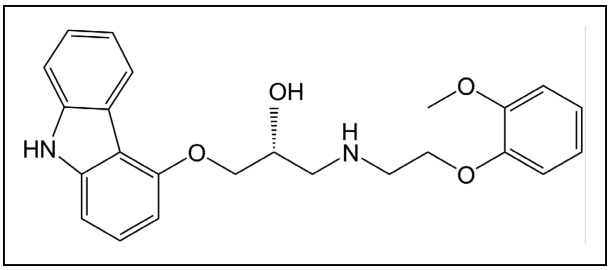

Fig. 1: Chemical structure of carvedilol.

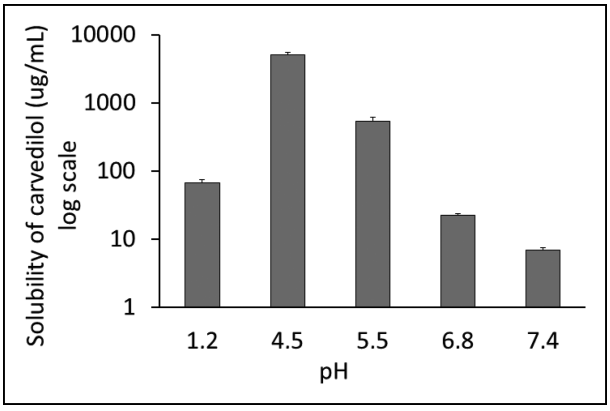

Fig. 2: Solubility of carvedilol (μg/mL) in buffers of different pHs at 37 °C (n = 3).

In the present study, sodium lauryl sulfate (SLS), hexadecyltrimethylammonium bromide (CTAB) and polysorbate 80 were used as the anionic, cationic and nonionic surfactants, respectively. The solubility of carvedilol in different buffers and surfactant media within the physiological pH range was measured. *In vitro* dissolution tests were performed in various media to compare the dissolution profiles of the bioequivalent products of carvedilol.

2. Investigations and results

2.1. pH dependent solubility

Saturation solubility values of carvedilol at different pH values are presented in Fig. 2. Calcd. dose numbers (D_O) and relative sink conditions (C_S/C_D) in the buffers of different pHs are presented in Table 1. Solubility of carvedilol increased significantly as pH increased from 1.2 to 4.5, and it decreased as pH further increased, giving low solubility values at pH 6.8 and 7.4. The solubility values of carvedilol determined in pH 4.5 and 5.5 acetate buffers were 75 and 8 fold higher than that in pH 1.2 hydrochloric acid buffer at 37 °C, respectively.

Table 1: Dose numbers (D_O) and relative sink conditions (C_S/C_D) of carvedilol in buffers

Medium	D_O[a]	C_S[b]/C_D[c]
pH 1.2 hydrochloric acid buffer	1.5	2.42
pH 4.5 acetate buffer	0.02	181
pH 5.5 acetate buffer	0.19	19.3
pH 6.8 phosphate buffer	4.4	0.815
pH 7.4 phosphate buffer	14	0.252

[a]dose number; [b]saturation solubility of carvedilol; [c]theoretical concentration of drug assuming complete dissolution of 25 mg carvedilol tablet in 900 mL dissolution medium.

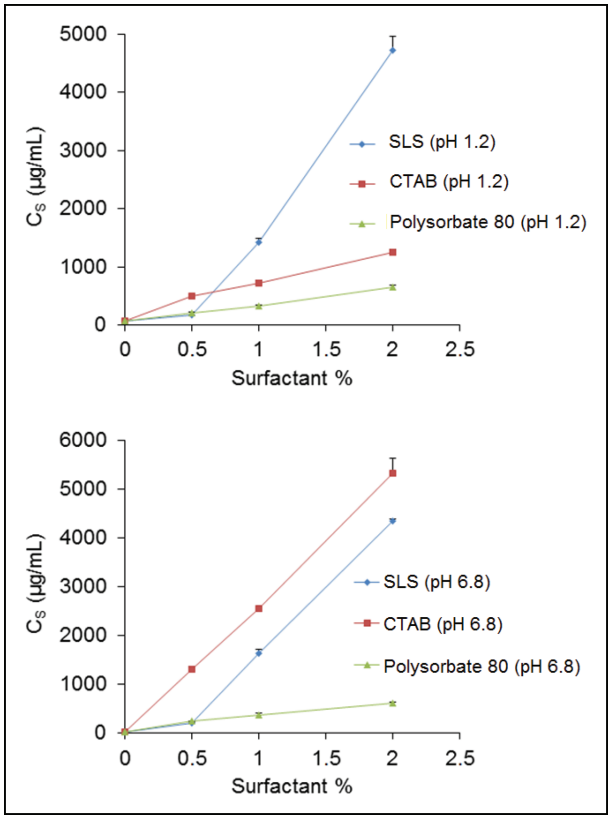

Fig. 3: Effects of surfactants (SLS, CTAB and polysorbate 80) on saturation solubility (C_S) of carvedilol in pH 1.2 hydrochloric acid and pH 6.8 phosphate buffers (n = 3).

2.2. Effects of surfactants on solubility

The effects of surfactants (SLS, CTAB and polysorbate 80) on the saturation solubility of carvedilol at pH 1.2 and 6.8 are presented in Fig. 3. The solubility of carvedilol increased gradually with the increased concentrations of all surfactants ($r^2 = 0.928$-0.999). SLS and CTAB exhibited larger solubilization effect than polysorbate 80 at both pH 1.2 and 6.8. 0.5-2% (w/v) SLS enhanced the solubility of carvedilol 2.5-70 and 8.9-192 fold at pH 1.2 and 6.8, respectively. Upon addition of 0.5-2% (w/v) CTAB, a dramatic increase in the solubility of carvedilol (58-235 fold) was observed at pH 6.8, while the effect at pH 1.2 was much smaller than that at pH 6.8 (7.3-18 fold). The highest concentration of polysorbate 80 (2% (w/v)) enhanced the drug solubility only 10 and 27 fold at pH 1.2 and 6.8, respectively. In the presence of the same polysorbate 80 concentrations, solubility values of carvedilol were similar at pH 1.2 and 6.8. Relative sink conditions of carvedilol in the presence of different concentrations of surfactants at pH 1.2 and 6.8 are summarized in Table 2. The ratio of saturation solubility to drug concentration calculated by dividing the dose by 900 mL dissolution medium (C_S/C_D) represents the closeness to the sink condition. C_S/C_D values greater than three are considered to provide sink condition (USP 2007). Therefore, 0.5% (w/v) surfactant (SLS, CTAB or polysorbate 80) was used in dissolution medium (pH 6.8) for the dissolution of 25 mg carvedilol tablets.

2.3. Surfactant mediated dissolution

Based on the C_S/C_D values (2.42 and 0.815 at pHs 1.2 and 6.8, respectively), sink conditions were not met at pH 1.2 and 6.8 media. However, it is recommended to perform the dissolution of carvedilol tablets using apparatus II (paddle) in 900 mL pH 1.2 simulated gastric fluid (SGF) without enzyme at 50 rpm (FDA

Table 2: Relative sink conditions (C_S/C_D) of carvedilol in surfactant media

	$C_S{}^a/C_D{}^b$								
		SLS[c] concentration (% w/v)			CTAB[d] concentration (% w/v)			Polysorbate 80[e] concentration (% w/v)	
pH		0.5	1	2	0.5	1	2	0.5	1	2
1.2	2.42	5.94	50.9	170	17.6	26.1	44.7	7.46	11.7	23.6
6.8	0.815	7.23	58.6	156	46.9	91.3	191	8.51	13.2	21.9

[a]saturation solubility of carvedilol; [b]theoretical concentration of drug assuming complete dissolution of 25 mg carvedilol tablet in 900 mL dissolution medium; [c]sodium lauryl sulfate; [d]hexadecyltrimethyl ammonium bromide; [e]polyoxyethylenes orbitan monooleate.

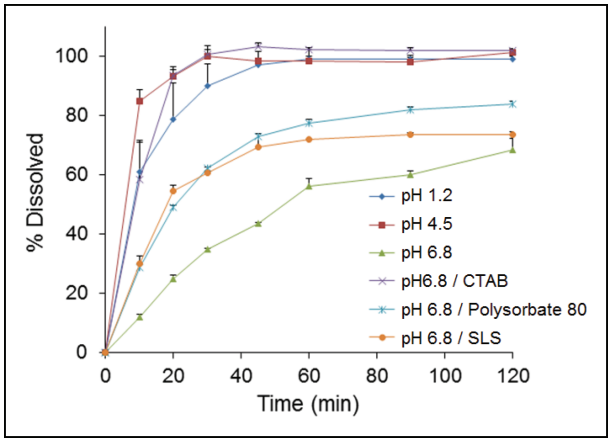

Fig. 4: Dissolution profiles of reference tablet of carvedilol in pH 1.2 hydrochloric acid, pH 4.5 acetate and pH 6.8 phosphate buffers, as well as in 0.5% (w/v) surfactant (SLS, CTAB or polysorbate 80) containing pH 6.8 media (n = 3).

Drug Databases). Dissolution profiles of the reference product in pH 1.2 hydrochloric acid, pH 4.5 acetate and pH 6.8 phosphate buffers and the effects of the surfactants on the dissolution profiles are presented in Fig. 4. Dissolution of reference formulation was rapid in pH 1.2 hydrochloric acid and pH 4.5 acetate buffers (>85% in 30 and 15 mins, respectively). In pH 6.8 buffer, the percentage of drug dissolved from reference product was only 68 and 79% within 2 and 6 h, respectively (Fig. 4). This is related to the low C_S/C_D ratio at pH 6.8 (0.815).

Polysorbate 80 and SLS appreciably enhanced the solubility of carvedilol, however in the presence of polysorbate 80 or SLS, dissolution rate and extents of the reference were significantly lower than those in the presence of CTAB at pH 6.8. Dissolution of reference product increased up to 84 and 73% in 2 h, when 0.5% (w/v) polysorbate 80 and SLS were added to pH 6.8 dissolution medium, respectively. Dissolution rate was the highest in the presence of 0.5% (w/v) CTAB with complete dissolution within 30 min (Fig. 4).

Comparison of dissolution profiles of the reference and test products in buffers with different pH values, as well as in the surfactant media are presented in Fig. 5. Table 3 summarizes the calcd. f_2 values for test tablets in different media with/without surfactant. 85% of the labeled amount was dissolved within 30 min for all test products in pH 1.2 hydrochloric acid buffer, and all test products showed dissolution profile similarity to the reference product at pH 1.2 (Fig. 5a). Test products, except test B showed dissolution profile similarity to reference at pH 4.5. 85% of drug was dissolved from all test products within 10-30 min at pH 4.5 (Fig. 5b). In pH 6.8 phosphate buffer, drug dissolved from test products were in the range of 52-66 and 72-77% in 2 and 6 h, respectively (Fig. 5c). Comparison of the dissolution profiles of test products with that of reference demonstrated that only test A and test E exhibited profile similarity to reference in pH 6.8 dissolution medium.

Dissolution rates and extents of test tablets significantly increased when 0.5% (w/v) CTAB was added to dissolution medium at pH 6.8 (Fig. 5e). Dissolution of all test products was completed within 1 to 2 h. However, the dissolution profiles of test D and test E were different from that of reference in 0.5% (w/v) CTAB containing medium due to the slow dissolution rates of these products in this medium, while the other products have f_2 values greater than 50. In 0.5% (w/v) SLS containing medium, test A and test D exhibited dissolution profile similarity to reference. However, drug dissolved from all test and reference products was in the range of 57-76% in 0.5% (w/v) SLS containing medium in 2 h. Significant decrease in the dissolution extents of test and reference products in SLS containing medium compared to the corresponding pH 6.8 medium with/without other surfactants indicated a possible interaction of carvedilol or any excipients in the formulations with SLS (Fig. 5d). In 0.5% (w/v) polysorbate 80 containing medium, the rates and extents of dissolution were less than those in 0.5% (w/v) CTAB containing medium for all test and reference products. However, compared to SLS containing medium, the rates and extents of dissolution were higher in polysorbate 80 containing medium for reference and all test products, except test D. Test D displayed a similar dissolution rate and extent in both media. The number of *in vitro* dissolution profiles similarity of test products to reference product increased when 0.5% (w/v) polysorbate 80 was added to pH 6.8 dissolution medium. Reference and all test products except test D dissolved > 80% within 45 min to 3 h in polysorbate 80 containing medium. Only test D exhibited different dissolution profile from reference ($f_2 = 47.5$) due to the slow rate and less extent of dissolution for this product in polysorbate 80 medium (73% in 3 h) (Fig. 5f). Moreover, dissolution of test D was also slower, compared to the reference and other test products in 0.5% (w/v) CTAB containing pH 6.8 medium.

3. Discussion

It is well known that careful choice of dissolution medium is required to perform the dissolution studies for the dosage forms of poorly water soluble drugs. When the pH and volume adjustments, without neglecting the aspects of physiological relevance, are not able to provide sufficient drug solubility in dissolution medium, the addition of a surfactant should be considered. Synthetic surfactants are the first choice to increase the solubility of a poorly water soluble drug and mimic *in vivo* solubilization and sink conditions due to continuous intestinal absorption.

In the present study, the effects of various types of surfactants on the solubility of carvedilol and dissolution profiles of innovator and generic products were investigated. Carvedilol was selected as the model drug on the basis of its lipophilic characteristics and poor solubility in water (0.01 mg/mL) (Benet et al. 2011). Furhermore, six multisource bioequivalent products are available on the Turkish market to make a comparison.

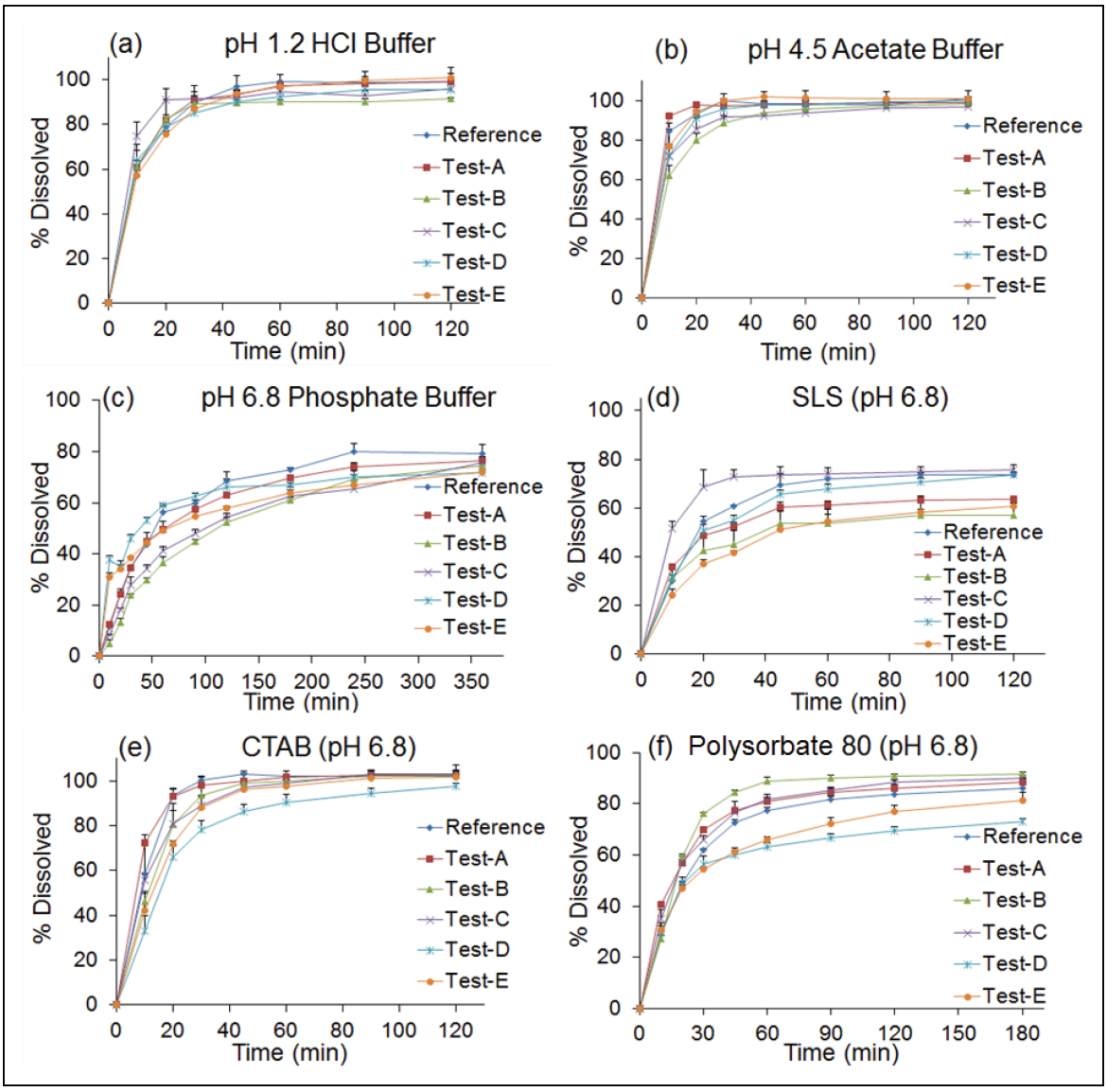

Fig. 5: Comparison of dissolution profiles of reference and test tablets of carvedilol in pH 1.2 hydrochloric acid, pH 4.5 acetate and pH 6.8 phosphate buffers, as well as in 0.5% (w/v) surfactant (SLS, CTAB or polysorbate 80) containing pH 6.8 media (n = 3).

Carvedilol is a weak base with a molecular weight 406.5 g/mol, a pKa of 7.8 and log P of 4.19 (Benet et al. 2011; Loftsson et al. 2008). As the degree of ionization increases, solubility of carvedilol is expected to increase. Thus, having an ionizable group carvedilol demonstrated a pH dependent solubility with decreased solubility at high pH values. However, a significant increase in solubility of carvedilol was observed in pH 4.5 and 5.5 acetate buffers, compared to pH 1.2 hydrochloric acid buffer in the present study. A similar result was reported elsewhere, where an addition of acetic acid resulted in a significant

Table 3: Similarity factor (f_2) for carvedilol test tablets in buffers with/without surfactant

	f_2[a] value					
Drug product	**pH 1.2 hydrochloric acid buffer**	**pH 4.5 acetate buffer**	**pH 6.8 phosphate buffer**	**pH 6.8 phosphate buffer with 0.5 % w/v SLS[b]**	**pH 6.8 phosphate buffer with 0.5 % w/v CTAB[c]**	**pH 6.8 phosphate buffer with 0.5 % w/v Polysorbate 80[d]**
Test A	78.2 Similar	63.2 Similar	70.6 Similar	52.9 Similar	54.0 Similar	59.7 Similar
Test B	68.8 Similar	42.0 Different	44.5 Different	41.7 Different	50.4 Similar	51.0 Similar
Test C	50.6 Similar	50.1 Similar	48.8 Different	47.8 Different	51.8 Similar	64.0 Similar
Test D	67.0 Similar	56.0 Similar	47.9 Different	72.4 Similar	33.5 Different	47.5 Different
Test E	73.3 Similar	66.2 Similar	50.3 Similar	40.1 Different	41.0 Different	57.3 Similar

[a]similarity factor; [b]sodium lauryl sulfate; [c]hexadecyltrimethylammonium bromide; [d]polyoxyethylene sorbitan monooleate.

increase in the aqueous solubility of carvedilol due to the formation of carvedilol acetate salt, which had over 400 times higher solubility than the hydrochloride (Loftsson et al. 2008), since different pH-adjusting acids may have significant effects on the aqueous solubility of weak bases (Loftsson et al. 2008).

According to the current guidelines related to the BCS biowaivers, carvedilol is classified as a poorly soluble drug based on the solubility of highest dose strength (25 mg) in 250 mL of aqueous media over the pH range of 1.0-7.5 (or 1.0-6.8 according to EMA) at 37 °C (FDA Guidance 2000; EMA Guideline 2010). The FDA high solubility criterion is also considered to be conservative due to the volume and pH range defined (Shah and Amidon 2014; Yu et al. 2002). In the present study, dose numbers of carvedilol were found to be greater than 1 at pH 1.2, 6.8 and 7.4 (1.5, 4.4 and 14, respectively) although 85% of the labeled amount of carvedilol was dissolved within 30 min at pH 1.2 and 4.5, indicating that the dissolution is likely to be complete in the stomach and then entering the intestine, carvedilol is likely to be present in a supersaturated state before precipitating in the higher pH of the small intestine. During this supersaturation state, drugs can be well absorbed across the intestinal mucosa (Bevernage et al. 2013). Thus, completely dissolving in the stomach (pH 1.4-2.1 (Dressman et al. 1998)) and becoming supersaturated in the upper intestine (pH 4.4-6.6 and pH 5.1-6.2 in fasted and fed states, respectively (Dressman et al. 1998)), carvedilol can be rapidly and extensively absorbed from the upper intestine which seems to be its main absorption site.

It is clear that the key determinant for *in vivo* bioavailability is the solubility in the intestine which is affected by pH and/or surfactants in the absorption site (Yu et al. 2002). This suggests a potential to define an intermediate solubility class for drugs that are soluble either in the intestine or in the stomach (Yu et al. 2002; Polli et al. 2004; Yazdanian et al. 2004). In a gastrointestinal (GI) simulation study, Tubic-Grozdanis et al. demonstrated that BCS II weak acids and bases may be eligible for biowaivers if the dose dissolves completely before reaching the middle jejunum (Tubic-Grozdanis et al. 2008). Carvedilol has recently been subclassifed as BCS Class IIb weak base according to the lately suggested BCS subclassification based on drug biopharmaceutical properties to set an *in vivo* predictive dissolution methodology (Tsume et al. 2014). Pharmacokinetic data indicate that carvedilol is rapidly absorbed after oral dose with a bioavailability of approximately 24% due to a first-pass effect and demonstrates dose-linear behavior in the dose of 25-50 mg. Carvedilol is metabolized extensively in humans. 16% of carvedilol is excreted in urine in the form of metabolite, while 60% of the dose is excreted via bile into the feces (Neugebauer et al. 1987; Schaefer et al. 1998). Fraction of dose absorbed was reported to be 65% in humans (Varma et al. 2012). The extent of absorption and peak plasma concentrations are not affected by food with an insignificant delay in time to reach peak plasma concentrations (Louis et al. 1987). However, potential biowaiver extention definitely requires careful and extensive research and case-by-case approach at the present time.

In the present study, significantly enhanced solubility of carvedilol was observed due to the micellar solubilization at surfactant concentrations above the critical micelle concentration (CMC) of each surfactant (1.1, 1.0 and 0.050 mM for SLS, CTAB and polysorbate 80, respectively (Balakrishnan et al. 2004)) in pH 1.2 and 6.8 media. However, polysorbate 80 exhibited lower solubilization performance than the cationic and anionic surfactants, CTAB and SLS, respectively. Polysorbate 80 enhanced the solubility of carvedilol to the same extent at pH 1.2 and 6.8, while in the presence of SLS and CTAB, the extent of solubility enhancement due to incorporation of carvedilol into micelles depends on the pKa of the drug and ionic nature of the surfactant. Additionally, dissolution rates of carvedilol from reference and test products were found to be slower in the presence of 0.5% (w/v) polysorbate 80 compared to 0.5% (w/v) CTAB. In another study, where low soluble griseofulvin was used as a model compound to assess the effects of various types of surfactants (SLS, CTAB, polysorbate 80 and Cremophor EL) on the intrinsic dissolution rate of griseofulvin, it was shown that the low solubilization effect of polysorbate 80 and Cremophor EL was further attenuated by their two fold lower micelle diffusivities, and these surfactants minimally enhanced the dissolution of griseofulvin (Balakrishnan et al. 2004). Thus, the slower dissolution rates of carvedilol from reference and test products can be attributed to lower diffusivity of drug-micelle complex due to the large molecular weight of polysorbate 80 (1310 g/mol) (Balakrishnan et al. 2004). SLS largely increased the solubility of carvedilol at concentrations above CMC in both pH 1.2 and 6.8 media, however, increase in solubility seems to be less below CMC, compared to the concentrations above CMC of SLS (Fig. 3). Carvedilol (pKa: 7.8) is completely ionized at pH 1.2 due to the protonation of secondary amine, the unionized fraction of carvedilol is nearly zero up to pH 5.5-6 and gradually increases as pH increases to give the sigmoidal profile ($\sim$ pH 10). Therefore, below CMC of SLS, desolubilization may occur due to the formation of an insoluble salt between cationic carvedilol and negatively charged anionic surfactant, SLS. As the concentration of anionic surfactant increased, the solubility increased, since the complex/salt was miscellary solubilized. Similar results were also reported on the formation of insoluble complex/salt between the cationic active ingredients and anionic surfactant, SLS resulting in decreased solubility and low dissolution rate below CMC (Jain et al. 2004; Bhattachar et al. 2011). Cationic surfactant, CTAB increased the solubility of carvedilol 3-4 times more at pH 6.8 compared to pH 1.2, probably due to the repulsion between similar charges of carvedilol and cationic surfactant at low pH.

It is clear that *in vivo* dissolution of poorly soluble drugs can be further complicated by the formulation factor. To further investigate the effect of formulation properties on dissolution characteristics of carvedilol, five generic products on the market, which are essentially bioequivalent to the reference product were tested *in vitro*, using different dissolution media and compared with the reference product. Since there is no difference between *in vivo* performance of the reference and test products, it would be a challenge to achieve similar dissolution profiles for these formulations of carvedilol. In this respect, the present study demonstrated that the dissolution rates and extents of carvedilol test and reference products are significantly dependent on the type of surfactant used in the dissolution medium and the product itself. Dissolution rates and extents of carvedilol products increased most in the presence of cationic surfactant, CTAB. However, it was stated that in the case of the fact that dissolution is rate limiting to drug absorption, a medium in which 100% drug release is achieved within the duration of the test may be unsuitable for the prediction of *in vivo* performance (Galia et al. 1998). Anionic surfactant, SLS was found to be sensitive to the formulation differences between the products of carvedilol, suggesting a potential interaction between SLS and some excipients of test and reference formulations. Moreover, this may be due to the fact that negatively charged SLS is able to form a less soluble complex with the cationic drug, resulting in a noticeable decrease in the dissolution rate of carvedilol (Jain et al. 2004). From a formulation point of view, the nature of the drug formulation is of great impact on the dissolution process. Therefore, in the presence of such interactions with formulation components or drug itself, *in vitro* results may not be meaningful. Based on *in vitro* dissolution profile similarity, two out of five test products (test A and E) exhibited dissolution profiles similar to that of reference product in three media: pH 1.2, 4.5 and 6.8 buffers.

It increased to three out of five test products (test A, C and E) in the presence of polysorbate 80 in pH 6.8 dissolution medium. This study demonstrated that, nonionic surfactant, polysorbate 80 in pH 6.8 dissolution medium most successfully reflected the bioequivalence of test products (four out of five: test A, B, C and E) to the reference product of carvedilol. Therefore, polysorbate 80 containing medium can be considered as the most biorelevant medium among the other surfactant media for the dissolution of carvedilol products.

Over the past two decades, biorelevant media containing bile salts (sodium taurocholate) and phospholipids (lecithin) which simulate the fasted (FaSSIF) and fed states (FeSSIF) in the small intestine and updated compositions have been developed and widely used for *in vivo* predictivity (Galia et al. 1998; Dressman and Reppas 2000; Klein 2010). The increase in solubility of carvedilol in biorelevant media (55.9 ± 1.0 and 305 ± 2 μg/mL in FaSSIF (pH 6.5) and FeSSIF (pH 5.0), respectively) resulting in a shift from BCS Class II to Class I in the classification of carvedilol in FeSSIF, but not in FaSSIF was reported (Fagerberg et al. 2010). It was also found that only 1.2 and 1.3 fold higher solubility was observed for carvedilol in FaSSIF and FeSSIF media in comparison to the corresponding blank buffers, respectively (Fagerberg et al. 2010). This result indicates that lecithin (0.75 and 3.75 mM in FaSSIF and FeSSIF, respectively) and sodium taurocholate (3 and 15 mM in FaSSIF and FeSSIF, respectively) in biorelevant media do not have much effects on the solubility of carvedilol, while pH plays a significant role. Interestingly in a recent study, some combinations of bile salt, sodium oleate and lecithin have been found to reduce the solubility carvedilol (Khadra et al. 2015). Futhermore, acetic acid present in FeSSIF medium (pH 5.0) clearly improves the solubility of carvedilol. Therefore, using complex and expensive FaSSIF and FeSSIF media may be questioned for the dissolution studies of carvedilol. Although *in vivo* intestinal dissolution is more complex than *in vitro* dissolution and has time dependent characteristics due to the physiology of the GI tract, as well as the physicochemical characteristics of the drug compound and formulation related factors, surfactant mediated *in vitro* dissolution may reflect the *in vivo* behavior of poorly soluble drugs. The usefulness of conventional surfactant (SLS and polysorbate 80) media as surrogates for FaSSIF to enhance the dissolution of BCS Class II drugs (danazol, spironolactone and a phase I drug molecule) and predict *in vivo* drug absorption have been evaluated by Lehto et al., and the use of *in vivo* prognostic amounts of synthetic surfactants in dissolution testing was found to be beneficial for predicting the drug absorption (Lehto et al. 2011). In conclusion, the present study clearly demonstrated the need for careful choice of surfactant media to perform the dissolution studies of poorly soluble drugs. A well designed and validated test medium containing a favorable surfactant can be used as an appropriate QC or surrogate test, as well as for IVIVCs. It is clear that the drug-surfactant interactions are specific, and *in vivo* prognostic amounts of synthetic surfactants should be adjusted for each drug specifically. In the case of carvedilol, polysorbate 80 containing pH 6.8 phosphate buffer seems to be physiologically meaningful in forecasting the *in vivo* performance of IR solid dosage forms of carvedilol.

4. Experimental

4.1. Materials

Carvedilol was kindly supplied from Drogsan Pharmaceuticals (Ankara, Turkey). Sodium lauryl sulfate (SLS), hexadecyltrimethylammonium bromide (CTAB), sodium chloride, sodium acetate, potassium dihydrogen phosphate, sodium dihydrogen phosphate and sodium hydroxide were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Polyoxyethylene sorbitan monooleate (polysorbate 80) was from Merck Schuchardt OHG (Hohenbrunn, Germany). Six commercial IR tablets of 25 mg carvedilol were purchased from the local market in Turkey. The following commercial IR tablets of carvedilol were tested: reference, batch no: A023386; test A, batch no: 2055004A; test B, batch no:1304U816; test C, batch no: D0837; test D, batch no: DD2060; test E, batch no:13E03201A. All other chemicals were of analytical reagent grade.

4.2. Methods

4.2.1. Saturation solubility measurements

Saturation solubility of carvedilol was determined in pH 1.2 HCl/NaCl buffer, pH 4.5 acetate buffer (22 mM), pH 5.5 acetate buffer (44 mM) and pH 6.8 and 7.4 phosphate buffers (50 mM). For solubility determination, an excess amount of carvedilol (50-100 mg) was placed into each dissolution medium in a 10 mL volumetric flask and stirred with a magnetic bar at 37 °C for 24 h. Saturated solutions were filtered using a 0.45 μm syringe filter (Chromafil® CA45/25, Macherey-Nagel GmbH & Co.KG, Düren, Germany). Drug concentrations were measured by UV spectrophotometry (UV-1700, Shimadzu Corp., Japan). In addition, studies with surfactant were identically performed, but contained either SLS, CTAB or polysorbate 80 (0.5, 1 or 2% (w/v)) in pH 1.2 HCl/NaCl and pH 6.8 phosphate buffers. All solubility experiments were carried out in triplicate. All data are presented as mean ± standard deviation (SD).

4.2.2. Dissolution study

Dissolution tests were carried out using a Varian VK 7000 dissolution apparatus (Varian, Inc., North Carolina, US). The commercial tablets were placed in 900 mL of dissolution media at 37 °C using USP dissolution apparatus II (paddle) with the paddle rotating at 50 rpm. pH 1.2 HCl/NaCl, pH 4.5 acetate (22 mM) and pH 6.8 phosphate buffers (50 mM) were used as the dissolution media. To evaluate the effects of surfactants on drug dissolution, 0.5% (w/v) SLS, CTAB or polysorbate 80 dissolved in pH 6.8 phosphate buffer was used as the dissolution medium. An aliquot of medium was withdrawn at predetermined time intervals, and an equivalent amount of fresh medium was added. Withdrawn samples were filtered using a 0.45 μm syringe filter (Chromafil®CA45/25, Macherey-Nagel GmbH & Co.KG, Düren, Germany), and analyzed spectrophotometrically. All dissolution experiments were carried out in triplicate and mean cumulative percentages of drug dissolved from the tablets were plotted against time.

4.2.3. Assay

UV absorbance of the samples after appropriate dilution with the corresponding dissolution medium was determined at 241 nm by UV spectrophotometry (UV-1700, Shimadzu Corp., Japan). The samples with polysorbate 80 were analyzed at 331 nm. The amount of carvedilol was calculated using the respective calibration curves.

4.2.4. Data analysis

Dose number (D_O) defined as the mass divided by an uptake volume of 250 mL and solubility of drug was calculated from Eq. (1) for buffers with/without surfactant (Amidon et al. 1995).

$$D_o = M_o / C_s V_o \quad (1)$$

where M_O is the highest dose of drug administered, V_O is the initial gastric volume (250 mL), C_S is the saturation solubility.

f_2 similarity test was used to determine similarity of drug products and compare dissolution profiles of reference and test products in different dissolution media with/without surfactant (FDA Guidance 1997). Dissolution profile comparison was performed under identical conditions. Similarity factor (f_2) was calculated according to Eq. (2):

$$f_2 = 50 \log \frac{100}{\sqrt{1 + \frac{1}{n}\sum_{t-1}^{n}(R_t - T_t)^2}} \quad (2)$$

where R_t and T_t are the cumulative percentage dissolved at time point t for the reference and test products, respectively, and n is the number of sampling points.

The obtained f_2 values greater than 50 indicate similarity of two profiles under the assumption of maximum allowable coefficient of variance of 20% at the earlier time points and 10% at other time points. The number of sample points was limited to not more than one, once reference and test products reach 85% dissolution.

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