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Preparation and *in vitro* evaluation of indomethacin loaded solid lipid microparticles

Indometazin içeren katı lipit mikropartiküllerin hazırlanması ve *in vitro* değerlendirilmesi

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Abstract

In this study, solid lipid microparticles of indomethacin have been formulated using Tween® 80, Labrasol® and Sucroester® WE15 in combination with Compritol® 888 ATO. ATR-FTIR spectroscopic studies and SEM results showed that lipid dispersions and microparticles of indomethacin were highly in amorphous form controversially in contrast to indomethacin crystal form. Particle size distribution analysis showed that indomethacin loaded lipid particles had a particle size distribution in the micron range. *In vitro* drug release studies indicated that indomethacin release is prolonged when Sucroester® WE15 used as surfactant and type of the surfactant was found to affect the dissolution rate of indomethacin.

Key words: Lipid microparticles, glyceryl behenate, sucrose ester, Labrasol®, Tween 80®.

Introduction

Solid lipid microparticles combine many advantages as drug carrier systems. The amount of drug encapsulated can vary up to 80% for lipophilic compounds and they are well tolerated in living systems because they are made from physiological or physiologically related materials (Sanna et al. 2003). The solid matrix protects loaded labile substances against degradation and they offer the possibility of controlled drug release and drug targeting (Tursilli et al. 2007). The suitability of lipid particles as a prolonged release formulation for lipophilic drugs has been demonstrated (Mühlen et al. 1998).

Solid lipid microparticles in dispersions can be obtained by melting method and they have been found as attractive alternatives to polymeric carrier systems obtained by solvent evaporation technique (Reithmeyer et al. 2001). Melting method has been agreed as a convenient technique because of the simply manufacturing process and the lack of solvent removal and recovery (Vasconcelos et al. 2007, Zajc et al. 2005). Lipid particle dispersions obtained by melting method can be transferred to powder by spray drying (Freitas and Müller 1998) or lyophilizing (Jaspart et al. 2007).

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Compritol® 888 ATO, diglyceryl behenate, a commonly known lipid based excipient with surface activity (HLB 2), is a mixture of 15% mono-, 50% di- and 35% triglycerides of behenic acid (C_{22}) (Jenning et al. 2000, Vasconcelos et al. 2007). Its melting point lies between 59.3-70.5°C (Chauan et al. 2005). Compritol® 888 ATO has been known to release the active agent in a prolonged manner and may be used in sustained release applications (Jannin et al. 2008, Kalava et al. 2005).

Indomethacin is a non-steroidal anti-inflammatory agent used in the symptomatic management of painful and inflammatory conditions (Sweetman 2005, Matthew et al. 1984, Becirevic-Lacan 1994). The adverse effects of indomethacin have been reported to be dose related, and a controlled release dosage form may reduce the severity of side effects associated with its oral administration (Biswanath and Mondal 1998).

The purpose of the present work was the preparation and characterization of solid lipid microparticles of indomethacin with Compritol® 888 ATO combined with Tween 80 (HLB 15), Labrasol (HLB 14) or Sucroester® WE15 (HLB 15). The use of polyoxylglycerides (i.e. caprylocaproyl polyoxylglyceride, Labrasol®) and polyalcoholesters of fatty acids (i.e. polysorbate 80, Tween 80) as solubilizers or bioavailability enhancers alone or in combination with lipid based excipients like Compritol® 888 ATO is a common approach (Jannin et al. 2008). Sucrose esters are surfactants with potential pharmaceutical applications because of their low toxicity, biocompatibility and biodegradability (Ayala-Bravo et al. 2003, Ullrich et al. 2008). They can be found as controlled release agents in tablets and they have evaluated as alternative surfactants in microencapsulation and microemulsion systems (Bolzinger et al. 1998, Soultani et al. 2003, Thevenin et al. 1996). Sucroester® WE15 (sucrose mono palmitate) has a low melting point (<60°C), is amphiphilic and has ability to prevent drug recrystallization during storage (Youan et al. 2003). We intend to evaluate Sucroester® WE15 (HLB 15) as an alternative surfactant to Tween 80 (HLB 15) and Labrasol (HLB 14), in stabilizing the emulsion for the preparation of indomethacin loaded lipid microparticles and investigate the effect of these surfactants on in vitro dissolution of indomethacin.

Materials and Methods

Materials

Indomethacin was donated by Nobel Pharmaceutical Company (Turkey). Glyceryl Behenate (Compritol® 888 ATO), Labrasol® and Sucroester® WE15 were kind gifts of Gattefosse (France). Tween® 80 was purchased from Sigma. All other chemicals used were of analytical grade.

Preparation of indoemethacin loaded solid lipid microparticles

Firstly solid lipid dispersions loaded with indomethacin were prepared by melting method (Jenning et al. 2000). Glyceryl behenate was brought to 80°C to allow its melting and the dissolution of indomethacin in the melted lipid excipient. Each surfactant (Tween® 80 (1%, 1.5%), Labrasol® (1%, 1.5%) or Sucroester® WE15 (3%, 5%) and water heated together at the same temperature. The hot lipid phase was dispersed in the hot aqueous surfactant solution under high shear mixing using Ultra Turrax (CATX620, Germany) at 20500 rpm for 1.5 min. The produced hot pre-emulsion (o/w) was ultrasonified using a Bersonic (Germany) at 70°C (Castelli et al. 2005). In order to prevent recrystallization during homogenization, production temperature was kept at least 5°C above the lipid melting point. The resulted emulsion was cooled down to room temperature.

These solid lipid dispersions loaded with indomethacin were lyophilized using a freeze dryer (Leybold Hereaeus Lyovac GT2, Germany) after they were frozen at -25°C one night and water free solid lipid microparticles of indomethacin were obtained (Jaspart et al. 2007). The method of preparation and indomethacin:Compritol® 888 ATO ratio (1:20) kept constant for all formulations in order to investigate the effects of surfactant type and amount on release properties (Table 1).

Characterization of indomethacin loaded solid lipid dispersions and solid lipid microparticles

Attenuated total reflactance fourier transformed infrared (ATR-FTIR) spectroscopic analysis

The FTIR spectra of indomethacin, Compritol® 888 ATO, indomethacin:Compritol® 888 ATO (1:1) physical mixture and indomethacin loaded solid lipid dispersions were recorded using ATR with a Perkin Elmer Spectrum 100 FTIR spectrometer (UK) equipped with a ZnSe crystal in the 4000-650 cm^{-1} range.

Table 1. Compositon of indomethacin loaded solid lipid microparticles.

Formulation Code	Surfactant (%)
F1	Tween® 80 1%
F2	Tween® 80 1.5%
F3	Labrasol® 1%
F4	Labrasol® 1.5%
F5	Sucroester® WE15 3%
F6	Sucroester® WE15 5%

The sample (several mg) was placed on the middle on the sample stage and force applied by the top of the arm of the sample stage. After obtaining peaks with reasonable intensity, the spectra acquired were the result of averaging 4 scans at $4cm^{-1}$ resolution.

Scanning electron microscopic (SEM) analysis

Electron micrographs of indomethacin loaded solid lipid dispersions were obtained using a scanning electron microscope (Cambridge Instruments Stereoscan 90B, UK) to study the surface morphology of lipid dispersions and particles.

Particle size analysis

Particle size distribution of indomethacin loaded solid lipid dispersions and solid lipid microparticles were determined by laser diffractometry using a Mastersizer (Malvern Instruments, Malvern, UK). The mean particle size (d_{10}, d_{50} and d_{90}) and the polydispersity index (SPAN) were calculated automatically using the software provided.

Determination of indomethacin in vitro release from solid lipid microparticles

The in vitro drug release from solid lipid microparticle formulations and pure indomethacin was studied using the rotating basket method described in the USP 33 / NF 28, Method I (Erweka Dissolution Apparatus, Germany). Basket rotational speed was held at 100 rpm. The dissolution medium was phosphate buffer, pH 7.2 thermostated at 37±0.5°C. Samples were withdrawn at predetermined time intervals, and indomethacin content of each sample was analyzed spectrophotometrically at 318 nm (Perkin Elmer UV Spectrometer, UK). All experiments were carried out in triplicate and mean values were calculated.

Results and Discussion

Indomethacin loaded solid lipid dispersions were developed through the melt dispersion technique using Compritol® 888 ATO as lipid material and different concentrations of Tween®

80, Labrasol® or Sucroester® WE15 as the emulsifier. The conversion of the lipid dispersion into a dry product can be achieved by lyophilization process (Jaspart et al. 2007). In our study lipid dispersion formulations were freeze dried and solid lipid microparticles loaded with indomethacin were obtained as white-yellowish powders.

We investigated the characteristics of the indomethacin loaded solid lipid dispersions firstly to elucidate the mechanism involved in dissolution behavior of solid lipid microparticles obtained from the dispersions.

Particle size distribution analysis performed by Malvern MasterSizer showed that indomethacin loaded solid lipid dispersion formulations had a particle size distribution in the micron range (Table 2). We achieved the smallest particle size distribution and span with indomethacin dispersions which were stabilized by Sucroester® WE15 3% and 5%, respectively (F5 and F6). Freeze drying process leads to an increase in particle size in the entire solid lipid microparticle formulations.

To elucidate molecular level dispersion of indomethacin in Compritol® 888 ATO solid dispersions, ATR-FTIR spectroscopy is used. This method is a standard and sensitive technique for drug characterization (Bahl and Bogner 2006, Qi et al. 2008). Figure 1 shows ATR-FTIR spectra of lipidic excipient, physical mixture of lipid and drug, solid lipid dispersions F1, F3, F5 and pure drug.

Table 2. Particle size distribution and span of indomethacin loaded solid lipid dispersions and solid lipid microparticles.

Code	Indomethacin loaded solid lipid dispersions				Indomethacin loaded solid lipid microparticles			
	d_{10} (µm)	d_{50} (µm)	d_{90} (µm)	span	d_{10} (µm)	d_{50} (µm)	d_{90} (µm)	span
F1	3,25	15,17	50,04	3,08	29,49	117,74	285,93	2,18
F2	2,86	18,75	58,85	2,99	33,88	138,16	374,56	2,47
F3	8,00	42,94	244,50	6,97	23,32	151,09	514,16	4,41
F4	8,97	34,26	151,60	4,16	22,35	110,45	265,37	2,38
F5	1,02	9,62	29,34	2,94	15,93	87,03	229,06	1,99
F6	1,07	6,11	17,66	2,72	11,16	86,65	243,11	2,93

The spectrum of physical mixture was equivalent to the spectrum of crystalline drug with sharp vibrational bands indicating crystallinity. This finding showed that no interaction occurred with a simple physical mixing of drug and the lipid carrier. In the region 1727-1710 cm^{-1} the absorbance of the dimer peak of indomethacin was decreased in F3 (solid lipid dispersion containing Labrasol 1%) whereas it was absent in formulations F1 (solid lipid dispersion containing Tween® 80, 1%) and F5 (solid lipid dispersion containing Sucroester® WE15, 3%). The absence of this dimer peak suggests the formation of a completely amorphous form of indomethacin (Bahl and Bogner 2006). Therefore molecular level dispersion of indomethacin in Compritol® 888 ATO matrice and the transformation of crystalline indomethacin into an amorphous indomethacin were confirmed by ATR-FTIR spectroscopy.

The surface morphology of indomethacin loaded solid lipid dispersions were examined by SEM analysis. Figure 2 show selected SEM images of representative samples which provide

the evidence of solid lipid dispersion formation and are in accordance to the results obtained from ATR-FTIR spectroscopy.

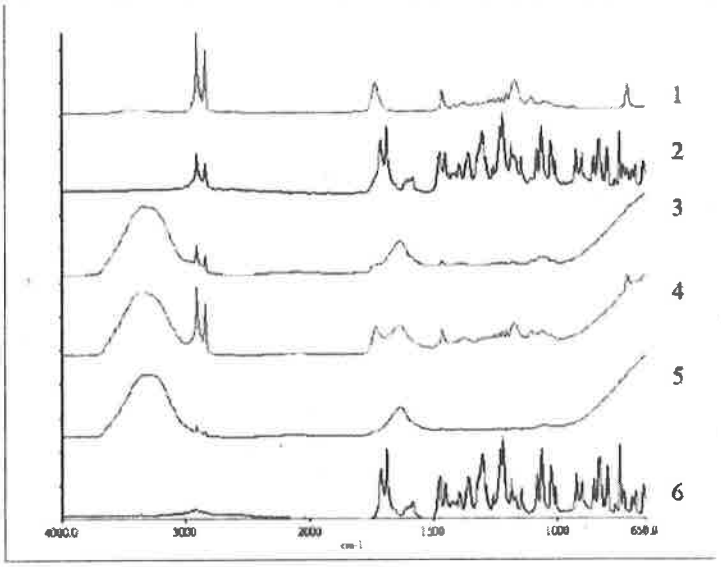

Figure 1. ATR-FTIR Spectra of Compritol® 888 ATO (1), Compritol® 888 ATO: indomethacin, Physical Mixture (2), solid lipid dispersion formulation F1 (3), F3 (4), F5 (5) and pure indomethacin (6).

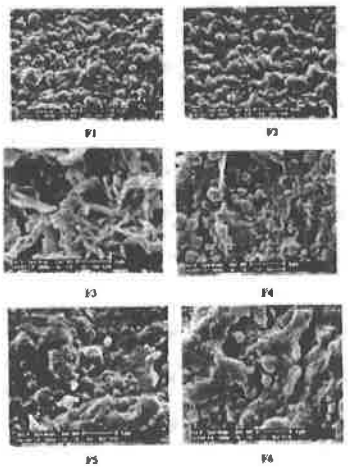

Figure 2. SEM images of indomethacin loaded solid lipid dispersion formulations F1-F6.

In vitro release profiles of indomethacin loaded lipid microparticles and pure indomethacin is depicted in Figure 3. In phosphate buffer, pH 7.2, indomethacin alone resulted in 98% dissolution in 90 min. Solid lipid microparticles obtained from the solid lipid dispersions prepared with the surfactants Tween® 80 and Labrasol® (F1-F4) showed burst drug release and the dissolution rate was higher than the release rate of formulations including Sucroester® WE15 as surfactant (F5 and F6).

In one study, the obtained burst release with tetracaine and etomidate loaded solid lipid particles was attributed to the large surface area, low viscosity in the matrix and release from the outer surface region of the particles (Mühlen et al. 1998). In our study, the observed initial

fast dissolution for microparticles was attributed to the surface indomethacin at the periphery of the microparticles whereas the following slower release rate can be attributed to entrapment of indomethacin in the solid lipid matrix. The difference in drug crystallinity may be responsible for the difference in the drug dissolution rate of different types of solid lipid microparticles. The surface active character of the lipidic excipient used also may lead to rapid dissolution of crystallized indomethacin, especially on or near to the surface. This data is in accordance with the literature (Huang et al. 2006, Tursilli et al. 2007).

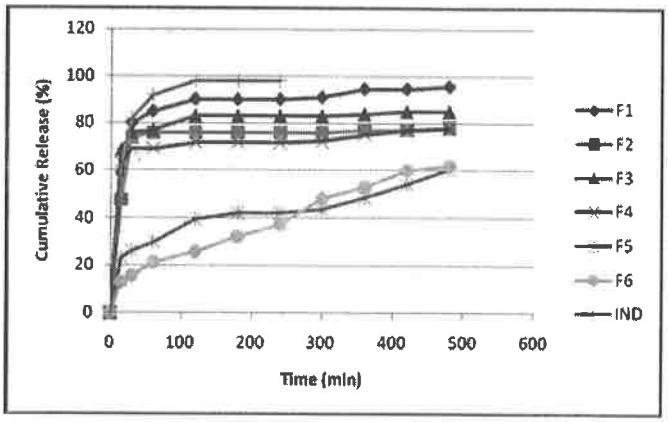

Figure 3. *In vitro* dissolution profiles of indomethacin loaded solid lipid microparticle formulations and indomethacin alone.

The time required for 50% indomethacin release from solid lipid particles was less than 20 minutes for formulations F1-F4, whereas it was 350 min and 300 min for F5 and F6.

The obtained sustained release profile with solid lipid microparticles including SucroEster® WE15 as surfactant (F5 and F6) when compared to other formulations containing Tween® 80 and Labrasol® (F1-F4) can be attributed to completely entrapment of indomethacin in the solid lipid matrix. As can be seen in Figure 4, a linear relationship was obtained when the cumulative amounts of indomethacin released from formulations stabilized by SucroEster (F5 and F6) were plotted against the square root of time (r^2=0.962 and r^2=0.976 for F5 and F6, respectively).

This observation indicated that the drug released from these lipid microparticles followed diffusion mechanism described by the Higuchi model, where the rate controlling step is the process of diffusion through formulation (Higuchi 1963).

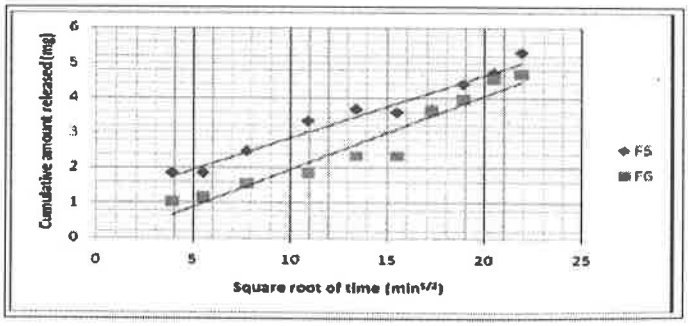

Figure 4. *In vitro* release profiles of formulations stabilized by Sucroester WE15 3% and 5% (F5 and F6) as a function of the square root of time.

Conclusion

Indomethacin loaded solid lipid microparticles were successfully prepared through the simple melting procedure. The in vitro drug release studies revealed that indomethacin release is prolonged when Sucroester® WE15 used as surfactant in combination with Compritol® 888 ATO as lipid matrix material. Our findings suggest that Sucroester® WE15 may be used as an alternative surfactant in the development of lipid microparticles for controlled oral delivery of indomethacin. It can also be concluded that drug release can be sustained with this approach and may lead to the avoidance of frequent drug administration.

Özet

Bu çalışmada Tween® 80, Labrasol® ve Sucroester® WE15 in Compritol® 888 ATO ile kombinasyonları kullanılarak, indometazinin katı lipit mikropartikülleri hazırlanmıştır. Partikül büyüklüğü dağılımı analizi indomethacin yüklü partiküllerin boyutunun mikron seviyesinde olduğunu göstermiştir. ATR-FTIR ve SEM sonuçları indomethacin lipit dispersiyonları ve mikropartiküllerinin amorf şekilde olduklarına işaret etmiştir. *In vitro* dissolüsyon çalışmaları Sucroester® WE15'in yüzey etkin madde olarak kullanıldığı formüllerden etkin madde salımının uzadığını ve kullanılan yüzey etkin maddeye bağlı olarak indomethacin dissolüsyon oranın değiştiğini göstermiştir.

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