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Investigation of triacetin effect on indomethacin release from poly(methyl methacrylate) microspheres: Evaluation of interactions using FT-IR and NMR spectroscopies

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ABSTRACT

The purpose of this study was to form indomethacin (IND)-loaded poly(methyl methacrylate) (PMMA) microspheres having an extended drug release profile over a period of 24 h. Microspheres were prepared by solvent evaporation method using sucrose stearate as a droplet stabilizer. When PMMA was used alone for the preparation of microspheres, only 44% of IND could be released at the end of 8 h. Triacetin was added to PMMA, as a minor phase, and the obtained microspheres showed a high yield process with recovery of 89.82% and incorporation efficiency of 102.3%. A desired release profile lasting 24 h was achieved. Differential scanning calorimetry (DSC) analysis showed that IND was found to be in an amorphous state in the microspheres. Fourier transform infrared (FT-IR) and nuclear magnetic resonance (^{1}H NMR) spectra suggested that there might be a hydrogen bond present between the IND hydroxyl group and PMMA. No interaction between triacetin and IND or PMMA as the formation of secondary bonds was observed. The release enhancement of IND from microspheres was attributed to the physical plasticization effect of triacetin on PMMA and, to some extent, the amorphous state of the drug.
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1. Introduction

The oral route of drug delivery is still the easiest and most convenient resource for drug administration, especially when repeated or routine administration is necessary. Nevertheless, there are some income limitations leading to either partial or ineffective absorption of the drugs initiating from the physicochemical properties, such as solubility and permeability of a drug and the physiological characteristics of the gastrointestinal (GI) system (Chen and Langer, 1998; Ping-Yang et al., 1998; Amidon et al., 1995). Modified-release formulation technologies present an effective means to optimize the bioavailability and resulting blood concentration-time profiles of drugs that are subject to such limitations (Charman and Charman, 2003). Modified-release products include delayed-release products, extended- (prolonged, sustained) products, and pulsatile-release products. Extended-release drug products reduce dosing frequency, and the contained drug is released for an extended period of time following peroral administration (EP 6.0, 2008; USP 30, 2007). Approaches for improving the solubility of poorly soluble drugs and fast-dissolving dosage forms might also be included in the context of modified release (Charman and Charman, 2003).

Among the modified-release dosage forms, polymeric microspheres have potential advantages of better dispersibility in the GI system, predictable GI transit time, more reproducible drug absorption, and reduced risk for gastric irritation caused by drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) (Yuksel et al., 1996; Yuksel and Beba, 2009; Akhgari et al., 2005; Charman and Charman, 2003). Synthetic biodegradable polymers including poly(lactic acid), poly(lactide-co-glycolide), polycaprolactone, etc.; natural biodegradable polymers including chitosan, albumin, alginate, and collagen; and non-biodegradable polymers such as polyacrylates and their copolymers and blends are widely employed for the preparation of microspheres. The role of the polymers is to carry drugs while modifying their release characteristics (Rastogi et al., 2007; Govender et al., 2005; Uhrich et al., 1999; Sokolsky-Papkov et al., 2007; Freiberg and Zhu, 2004).

Poly(methyl methacrylate) (PMMA) in the group of polyacrylates is a biocompatible non-biodegradable material that has been employed in industrial and medical fields. PMMA can be used alone as a matrix material or as a minor phase to improve some properties of biodegradable matrices (Gutierrez-Villarreal and Rodriguez-Velazquez, 2007; Ahlin et al., 2002; Ladron de Guevara-Fernandez et al., 2003; Elvira et al., 2004). PMMA is also used for the preparation of artificial joints, dental prostheses, implants, contact lenses, and bone cements with or without drug (Freitag et al., 2009; Ladron

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de Guevara-Fernandez et al., 2003; Virtoa et al., 2003; Sugino et al., 2008).

Indomethacin (IND) is a non-steroidal drug with anti-inflammatory, antipyretic and analgesic properties. Serious GI toxicity such as bleeding, ulceration and perforation can occur in patients treated chronically with NSAID therapy (PDR, 2005). The successful formulation of poorly water soluble drugs is one of the major problems in pharmaceutical manufacturing. IND shows low oral bioavailability due to poor dissolution of the drug in the fluids of the GI tract and is practically insoluble in water (0.02 mg/mL) (Jain, 2008). This physical property may increase the incidence of irritating side effects on the GI tract because of a prolonged contact time with the mucosa (Bajdik et al., 2004; Nokhodchi et al., 2005; Bogdanova et al., 2007). Extended-release formulations of NSAIDs have been developed in order to reduce their contraindications on the GI system (Lu et al., 2007; Gong et al., 2006, 2007).

The rationale of this study was to prepare microspheres that would have an extended-release profile of IND over a period of 24 h using PMMA. The pharmacopoeia monograph of IND (USP 30) was used to establish the best microsphere formulation. When PMMA was used alone for the preparation of microspheres, only 44% of IND could be released at the end of 8 h. Other polymers were added to PMMA as a minor phase in order to increase the dissolution rate of IND from the microspheres, as follows: a swellable polymer (Eudragit RS), a polymer soluble above pH 6 (Eudragit L), a water-soluble polymer (polyethylene glycol 4000), and a hydrophilic polymer (poly(2-hydroxypropyl methacrylate)). A plasticizer, triacetin, was also used for the same purpose. Solvent evaporation technique was applied for the preparation of IND-containing microspheres. After testing the different formulations mentioned above, the most suitable was found to be a triacetin-containing formulation to study for 24 h-release of IND. The use of triacetin in acrylic microspheres produced by solvent evaporation method is a less commonly applied approach. In the present study, we prepared and characterized the formulation containing triacetin and investigated the possible interactions between IND and PMMA-triacetin by differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT-IR), and ^{1}H nuclear magnetic resonance spectroscopy (NMR).

2. Materials and methods

2.1. Materials

IND was kindly supplied by Nobel Ilac (Istanbul, Turkey). PMMA (M.A. 350,000), triacetin, dimethylsulfoxide (DMSO)-d_6 and potassium bromide (KBr) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Sucrose stearate (Crodesta F160) was provided by Croda GmbH (Mettelal, Germany).

2.2. Preparation of microspheres

The solvent evaporation method was used for preparing microspheres (Yuksel et al., 1996; Yuksel and Baykara, 1997). The formulation for the preparation of microspheres is tabulated in Table 1. IND and then PMMA with or without triacetin were dissolved in acetone. To this mixture, sucrose stearate was added and the mixture was agitated at 500 rpm in a water bath on a magnetic stirrer at 10 °C for 20 min. This inner phase was emulsified in liquid paraffin previously cooled to 10 °C as it was stirred at 500 rpm using an overhead stirrer (Model RZR-2000; Heidolph Elektro GmbH, Kelheim, Germany). The temperature of the resulting emulsion was increased to room temperature and the emulsion was agitated for 4 h to remove the acetone by evaporation. The solidified inner phase droplets in the form of microspheres were separated by vacuum filtration, washed with *n*-hexane and stored in a desiccator after drying in an oven at 40 °C overnight.

2.3. Characterization of microspheres

2.3.1. Entrapment efficiency and recovery

Microspheres containing theoretically 10 mg IND were accurately weighed and dissolved in chloroform. After dilution with chloroform, drug concentration was determined by UV spectrophotometry at 242.5 nm (Shimadzu UV-1202, Tokyo, Japan) ($n = 3$). Entrapment efficiency was calculated as:

$$\text{Entrapment efficiency}\,(\%) = \frac{\text{calculated drug content}}{\text{theoretical drug content}} \times 100 \qquad (1)$$

Recovery was calculated as the ratio of the amount of microspheres obtained to the solid content used for the preparation of microspheres, multiplied by 100.

2.3.2. Surface morphology and particle size analysis of microspheres

The shape and surface characteristics of microspheres were examined by a scanning electron microscope (SEM) (JSM 6400, JEOL Ltd., Tokyo, Japan). Microspheres were dusted onto double-sided carbon tape on an aluminum stub. The stubs were coated with gold using a cool sputter coater to a thickness of 400 Å. The samples were imaged using a 20-kV electron beam. Particle sizes of the microspheres were measured as volume diameter by laser diffraction technique (HELOS-SympaTec GmbH, Germany).

2.3.3. In vitro drug release studies

Drug release studies were performed in USP apparatus 1 (SOTAX AT 7smart; Sotax AG, Basel, Switzerland) (basket method) according to the USP monograph for Indomethacin Extended-Release Capsules-Test 3 (USP 30). The microspheres (containing 75 mg of IND) were filled into transparent hard gelatin capsules (number 1) and put into the baskets and placed in 750 mL of dissolution medium (pH 6.8 phosphate buffer) thermostated at 37 °C. The basket rotation rate was kept at 75 rpm. At determined intervals, the samples (5 mL) were withdrawn and replaced with an equal volume of the fresh medium. After filtering the samples through Whatman No. 42 filter paper, drug content was determined by UV spectrophotometry at 263 nm ($n = 3$). Cumulative percentages of the drug released from micromatrices were calculated.

2.4. Investigation of the interaction between IND and excipients used in microspheres

2.4.1. Preparation of dispersions

Dispersions, the formulations of which are given in Table 1, were prepared in order to comparatively elucidate the interaction between IND and other ingredients in the microspheres. The ingredients of dispersion I were dissolved in acetone and then the solvent was evaporated on a water bath thermostated at 50 °C. Dispersion II was prepared by dissolving PMMA and triacetin in acetone and adding water as a non-solvent at room temperature. The precipitate was separated. The dispersions obtained were dried in an oven at 40 °C overnight.

2.4.2. Differential scanning calorimetry (DSC)

Thermal analysis was conducted on the drug, PMMA, sucrose stearate, microspheres, and dispersions (Table 1) using a DSC (DSC-60 Shimadzu, Tokyo, Japan). Samples (5 mg) were accurately weighed into aluminum pans and then sealed. The thermograms of the samples were taken at a scanning rate of 10 °C/min over a temperature from 30 °C to 220 °C.

Table 1
Compositions and characterization of the formulations.

Ingredients	Microsphere formulations		Dispersions	
	I	II	I	II
IND	0.750 g	0.750 g	0.750 g	–
PMMA	3.000 g	2.400 g	2.400 g	2.400 g
Triacetin	–	0.600 g	0.600 g	0.600 g
Sucrose stearate	0.425 g	0.425 g	0.425 g	–
Aceton (inner phase)	50 mL	50 mL	50 mL	50 mL
Liquid paraffin (outer phase)	200 mL	200 mL	–	–
Water (non-solvent)	–	–	–	50 mL
Entrapment efficiency (%)	75.06	102.3		
Calculated drug content (mg)	7.550 ± 0.039	10.29 ± 0.048		
Theoretical drug content (mg)	10	10		
Recovery (%)	93.17	89.82		
Particle size (d) and distribution				
$d_{50\%}$ (μm)	398.3	309.8		
$d_{10\%}$ (μm)	70.41	161.3		
$d_{90\%}$ (μm)	815.9	546.5		
Span[a]	1.872	1.243		

[a] $(d_{90\%} - d_{10\%})/d_{50\%}$.

2.4.3. Fourier transform infrared (FT-IR) analysis

FT-IR is the preferred method of infrared spectroscopy. The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint, no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis including drug formulations.

IND, PMMA, sucrose stearate, microspheres, and dispersions (Table 1) were milled with KBr to form a very fine powder. This powder was then compressed into a thin pellet for analysis. The measurements were attempted over the range of 400–4000 cm^{-1}. FT-IR spectra were recorded on a FT-IR spectrometer (Jasco, Model 420, Japan). By monitoring the peak frequencies and places of certain bands, all possible chemical changes and interactions were determined.

2.4.4. ^1H Nuclear magnetic resonance (NMR) analysis

NMR spectroscopy is one of the principal techniques used to obtain physical, chemical, electronic and structural information about molecules due to the chemical shift http://en.wikipedia.org/wiki/Zeeman_effect on the resonant frequencies of the nuclei present in the sample. Thus, NMR is a powerful tool for the identification of drug molecules and quantitative applications valuable to pharmaceutical analysis.

IND, PMMA, sucrose stearate, microspheres, and dispersions samples were prepared using deuterated DMSO. TMS (tetramethyl silane) was used as internal standard for each sample. ^1H NMR spectra were measured over the range of 0–14 ppm with a Varian 400 MHz instrument (Agilent, Avondale, USA). From the obtained spectra, peak integrals and places of certain signals, all possible chemical changes and interactions were determined.

3. Results and discussion

3.1. Characterization of microspheres

3.1.1. Entrapment efficiency and recovery

Solvent evaporation method is the most widely used method for the preparation of microspheres. It is feasible to obtain the microspheres with uniform size and shape, which have high values of recovery and drug entrapment efficiency (Yuksel et al., 1996; Yuksel and Baykara, 1997). The method depends on the formation of an emulsion system initially and then the evaporation of the solvent used in the inner phase. Considering the solubility of the drug and polymer, there are principally two systems from which to choose: water-in-oil (w/o) or oil-in-water (o/w) (Huang and Ghebre-Sellassie, 1989; Jalil and Nixon, 1989). In the present study, w/o system was chosen since IND and PMMA are soluble in acetone, a water-miscible solvent. Recoveries of the microspheres were high, ranging from 90% to 93% (Table 1). The drug entrapment efficiency of microspheres containing PMMA alone (formulation I) was 75.06%, while it was 102.3% in the microspheres containing a plasticizer, triacetin (formulation II), as shown in Table 1.

Plasticizers are important ingredients in the formulation of polymers for film and implant preparation, coatings and hot-melt extrusion. They increase the flexibility and workability of the polymers and degradability of the biodegradable polymers (Zhu et al., 2006; Gutierrez-Villarreal and Rodriguez-Velazquez, 2007; Pietro et al., 2006). While a solid polymer dissolves in a solvent, plasticizers weaken the intermolecular forces between the polymer macromolecules and cause the polymer relaxation, hence increasing the free volume in the polymer matrix (Gutierrez-Villarreal and Rodriguez-Velazquez, 2007). After IND was dissolved in acetone, PMMA and triacetin were added to this solution. The increasing drug entrapment efficiency in the microspheres of formulation II might be attributed to the fact that the drug molecules and triacetin together with the solvent are easily diffused into the polymer matrix in the presence of a plasticizer.

3.1.2. Surface morphology and particle size analysis of microspheres

The mean particle size (d_{50}) of the microspheres prepared from formulation I was 398.3 μm and the value of span was 1.872. The value of span shows the width of distribution. In the presence of triacetin, the mean particle size of the microspheres was 309.8 and the value of span was 1.243, indicating a more uniform particle size distribution when compared to that of the microspheres prepared from PMMA alone (Table 1).

The shape and surface characteristics of microspheres prepared from formulation II are shown in Fig. 1. Polymeric fibers in lengths of about 100 μm surrounded the surfaces of microspheres and numerous cracks were observed in the structure of micromatrices. It has been thought that polymer chains of PMMA solidify in the form of polymeric fibers during solvent evaporation by stirring, since triacetin increased the mobility of individual polymer chains.

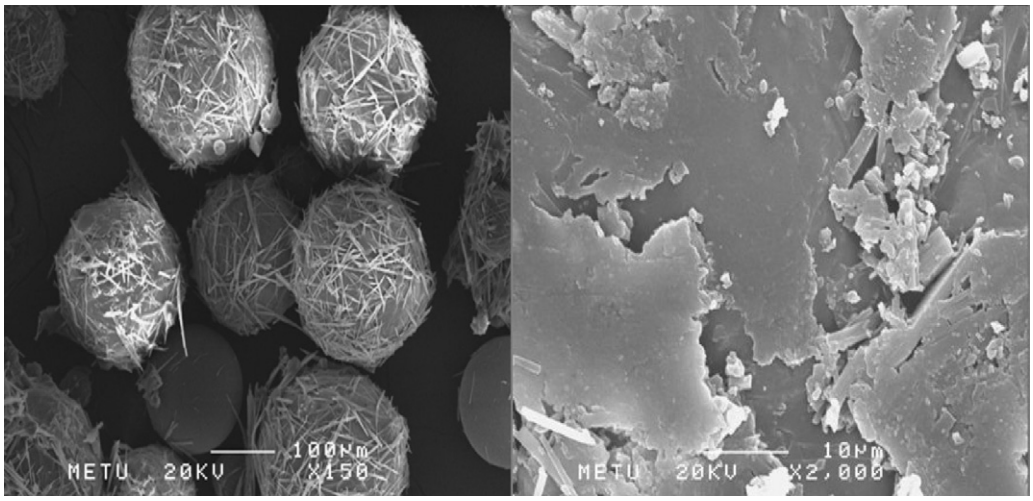

Fig. 1. Scanning electron micrographs of microspheres prepared from formulation II (left: magnified 150×; right: magnified 2000×).

3.1.3. In vitro drug release studies

In solvent evaporation method, a small percentage of droplet stabilizers (or dispersing agents) are used to overcome the droplet coalescent during the solvent evaporation from the inner phase of the emulsion. In w/o emulsion systems, the substances used as dispersing agents include metallic soaps (magnesium stearate, aluminum tristearate), sorbitan fatty esters (Spans, Arlacels), sucrose fatty esters, and polyoxyethylene fatty ethers (Brijs) (see Yuksel and Baykara, 1997). In the present study, sucrose stearate was chosen as a dispersing agent in order to prevent incompatibility between the carboxylic acid group on the IND molecule and metallic stearates, and any possible negative effect on the dissolution rate of IND. It has been reported that sucrose stearate might increase the dissolution rate of the drugs due to its high hydrophilic–lipophilic balance value (HLB = 15) (Ntawukulilyayo et al., 1993).

In vitro release tests of the microspheres were performed according to the USP monograph for Indomethacin Extended-Release Capsules-Test 3 (USP 30). Orally administered IND is especially retained in the small intestines, and thus pH 6.8 phosphate buffer is used as dissolution medium. Additionally, the drug has a pK_a value of 4.5 and is dissolved at higher pH values due to ionization of its carboxylic acid group (Akhgari et al., 2005; Nokhodchi et al., 2005).

IND release from the microspheres of formulation I was about 44% at the end of 8 h. The release profile was biphasic. An initial rapid drug release (burst effect) from the surfaces of the microspheres was followed by a slower drug release phase (Fig. 2). According to the USP monograph, the lower limit value of IND release at the 6th hour is 65%; thus, the release test of the formulation I was not further examined. PMMA-based microparticles have been reported to show an incomplete drug release due, possibly, to the hydrophobic property of PMMA and a lower diffusivity of the drug through this polymer (Streubel et al., 2003).

The release of IND from the microspheres could be surmounted by addition of a plasticizer to formulation II, as seen in Fig. 2. The release profile was biphasic. About 31% of IND was initially released within 30 min but the release in the second phase was faster than that of the microspheres prepared from formulation I, and 72.91% of IND was released at the end of 8 h. The released amount of IND reached 85.85% at the end of 24 h. This value meets the limit given in the USP monograph of IND. The release of the drug is understood to be controlled by diffusion through the polymeric micromatrices rather than through the water-filled channels and pores due to the low water solubility of IND (Zhu et al., 2006). Triacetin decreases the glass transition temperature of PMMA and increases the mobility of polymer chains, and thus the free volume available for diffusion. In this case, the diffusion of the drug within the polymer is facilitated as stated by Streubel et al. (2003). This finding may indicate that the drug is dissolved in PMMA containing triacetin, and the further investigation of the microspheres prepared from formulation II is given below.

3.2. Investigation of the interaction between IND and excipients used in microspheres

3.2.1. Differential scanning calorimetry (DSC)

DSC analysis was conducted to examine the thermal behavior of microsphere formulation II. Fig. 3 shows the DSC data of formulation II and dispersions, and their ingredients are given in Table 1. A sharp endothermic peak corresponding to the melting of crystalline IND was seen at 161.7 °C (Fujii et al., 2005). The melting peak of sucrose stearate was located at 53 °C. The transition peak at 129.5 °C in the thermogram of PMMA was attributed to the glass transition temperature (T_g) of polymer. Triacetin is a liquid with a negative Tg value of −68 °C and thus, a broad peak only occurred at 152.3 °C, corresponding to the flash point of triacetin.

Dispersion II was prepared to determine any possible interaction between PMMA and triacetin. In the thermogram of dispersion II, the flash point of triacetin was shifted to a higher temperature, 160.1 °C, while the glass transition peak of PMMA disappeared due

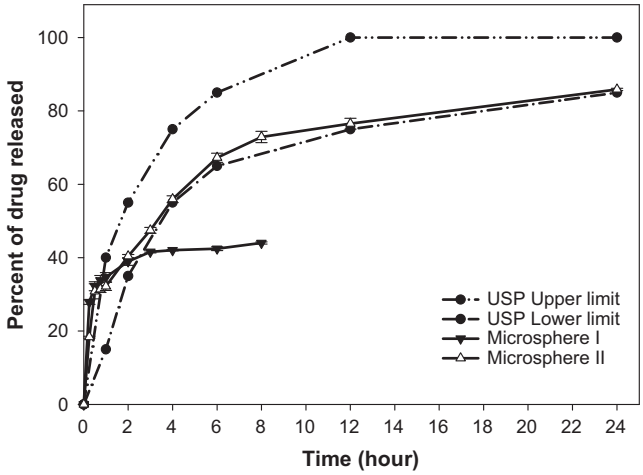

Fig. 2. Release profiles of IND from the microspheres.

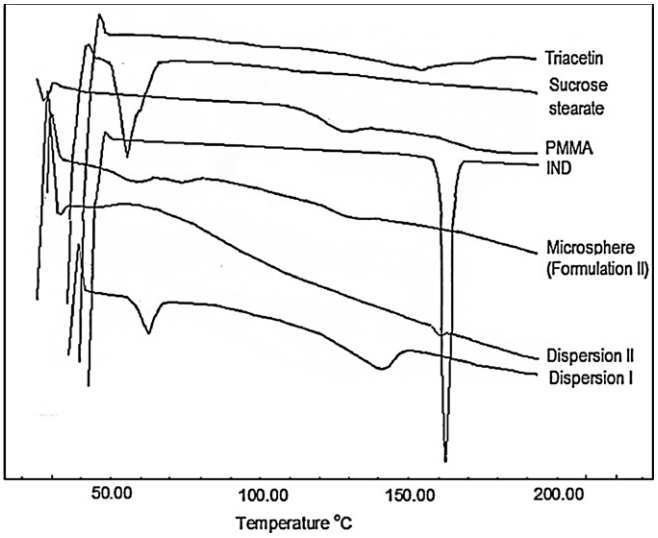

Fig. 3. DSC thermograms of the ingredients in the formulations, microspheres (formulation II), and dispersions (scanning rate: 10 °C/min between 30 °C and 220 °C).

to the effect of the plasticizer, triacetin. The increasing flash point of triacetin might have resulted from the placement of the molecules of triacetin into the PMMA macromolecules. Dispersion I is a physical mixture containing all ingredients used for the preparation of microspheres of formulation I. The peak at 54.5 °C corresponded to the melting point of crystalline sucrose stearate. The peak for the flash point of triacetin was depressed to a lower temperature, 142.7 °C, because of the presence of other ingredients, IND and sucrose stearate. The crystalline melting peak of IND was absent.

In the thermogram of microspheres, a peak related to the melting of sucrose stearate, a shoulder next to it at 70.50 °C, and a small broad peak at 135.5 °C, possibly showing a further depressed flash point of triacetin, were observed. The decreasing flash point of triacetin in the presence of IND and sucrose stearate indicated that IND and sucrose stearate could also behave as a plasticizer and cause the displacement of triacetin molecules into the PMMA macromolecules (Blasi et al., 2007). The peak at 70.50 °C possibly corresponded to the T_g of PMMA. This transition was shifted from 129.5 °C to 70.50 °C due to the plasticizer, triacetin, and to some extent, IND and sucrose stearate. These substances reduced the

Table 2
FT-IR results of dispersion I and microspheres prepared from formulation II.

FT-IR results (KBr disk, cm^{-1})			
	Acid C=O	Ketone C=O	OH
Indomethacine	1715	1691	3420
Dispersion I			
Indomethacine	1750	1692	Not observed
Microspheres (formulation II)			
Indomethacine	1750	1692	Not observed

secondary intermolecular forces between the polymer chains by separating them, and hence, the mobility of the chains increased. A sharp endothermic peak confirming crystalline drug did not occur. The results of DSC analysis revealed that IND was in an amorphous state and most likely to be dissolved in the PMMA matrix forming a solid solution, i.e. the drug molecules are uniformly dispersed between the macromolecular chains of the polymer within microspheres (formulation II) and dispersion I.

3.2.2. *Fourier transform infrared (FT-IR) analysis*

Dispersion I: The FT-IR spectrum showed that there might be a hydrogen bond interaction between IND COOH and PMMA (Table 2, Figs. 4 and 5). The acidic carbonyl absorption band of IND was found to have a slightly higher (1750 cm^{-1}) wavelength than the one present in the IND pure spectrum (1715 cm^{-1}). This could be due to the result of the hydrogen bond. The wavelength of ketonic carbonyl of IND was found in exactly the same place (1691 cm^{-1}) in IND and physical mixture spectra. PMMA bands were present at expected wavelengths. This clearly indicates the absence of any interaction between ketonic C=O of IND and PMMA in the physical mixture. Investigation of the stretch band of OH group that is observed in 3400 cm^{-1} of IND was found to have disappeared or to be very broad in the dispersion spectrum. This finding also shows an interaction of the OH group of IND with PMMA.

Microspheres of formulation II: The FT-IR spectra of the microsphere formulation of IND were similar to the physical mixture spectrum (Table 2, Figs. 4 and 5). The main observation was found as shifting the acid C=O starching band of IND at 1715 cm^{-1} to 1750 cm^{-1}, which clearly shows an interaction between IND acidic C=O and PMMA. This shift or new peak appearance may be due to the hydrogen bond formation, which causes shifting of the starching band of C=O to higher wavelengths.

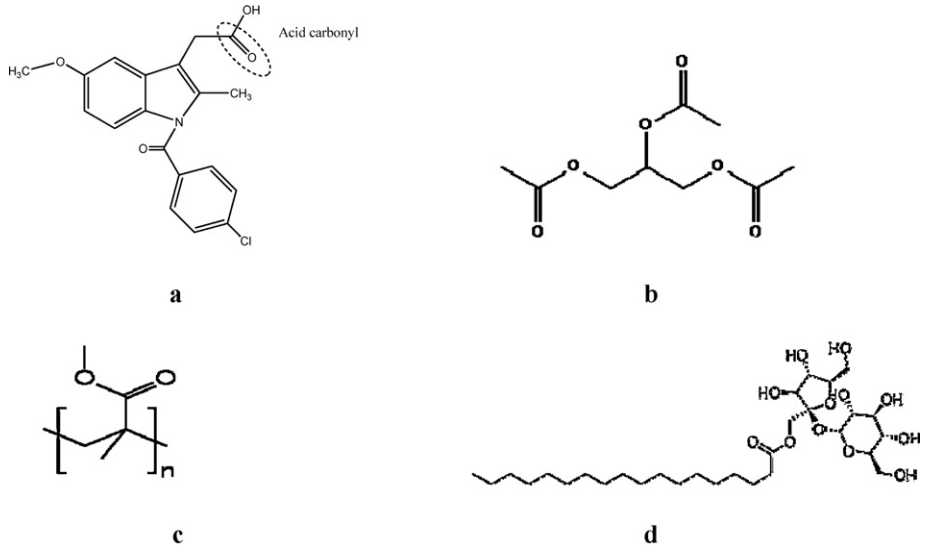

Fig. 4. Chemical formula of (a) indomethacin, (b) triacetin, (c) PMMA and (d) sucrose stearate.

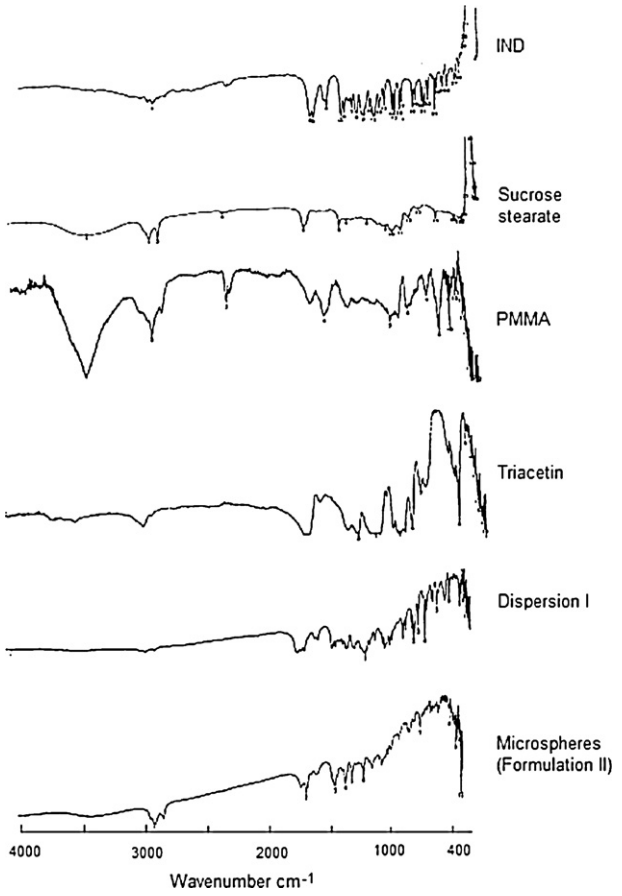

Fig. 5. FT-IR spectra of the ingredients in the formulations, microspheres (formulation II), and dispersion I (400–4000 cm^{-1}; KBr discs).

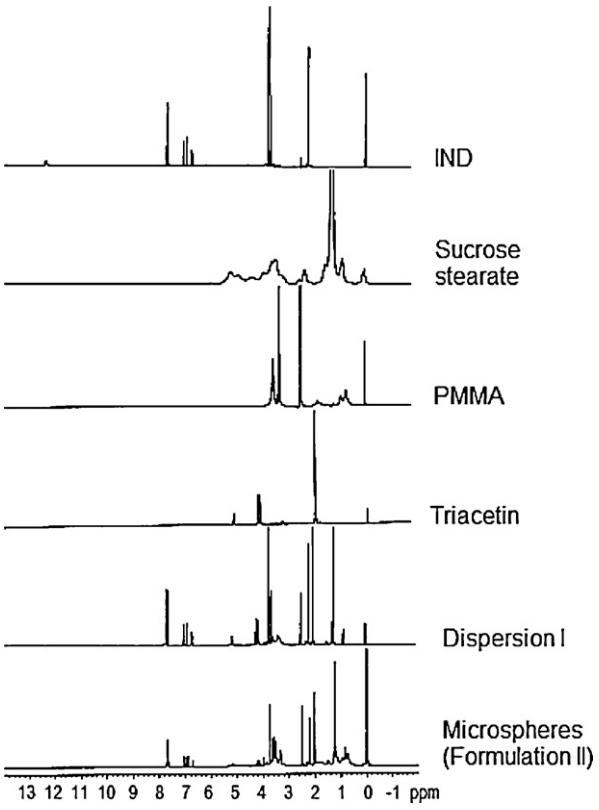

Fig. 6. ^{1}H NMR spectra of the ingredients in the formulations, microspheres (formulation II), and dispersion I (0–14 ppm; in DMSO).

The microspheres and dispersion I spectra showed a peak at 1692 cm^{-1} and this was attributed to ketonic IND C=O stretching band that was observed in pure IND IR spectrum at 1691 cm^{-1}. These bands showed that the ketonic C=O group of IND did not take a role in causing any interaction with PMMA. The OH stretching band of IND was found as a very broad (almost flat) band. This may be explained by a hydrogen bonding between IND OH and PMMA. One of the most identifiable peaks in an FT-IR spectrum is the broad OH absorption. However, in most cases, the peak may not have a characteristic shape. OH may be hydrogen bonded to a slightly different extent and FT-IR peak appears broadened. Furthermore, the greater the masses of molecules, the lower the FT-IR frequency at which the bond will absorb. For this reason, ^{1}H NMR was used to confirm the results of FT-IR.

3.2.3. ^{1}H Nuclear magnetic resonance (NMR) analysis

Investigation of the ^{1}H NMR spectrum of dispersion I and microsphere formulation (II) of IND (Table 3, Fig. 6) showed that the OH signal of IND that was present at 12.4 ppm was not observed. Although it is possible to not observe the OH signal in certain cases, one possible explanation could be the interaction of the OH group

Table 3
^{1}H NMR results of dispersion I and microspheres prepared from formulation II.

	–CH–	$–CH_2–$	$–CH_3$	$–OCH_3$	Ar–H	OH
^{1}H NMR results (DMSO-d_6, δ, ppm)						
IND	–	3.67, s	2.21, s	3.76, s	6.72, dd 6.93, d 7.05, d 7.66, dd	12.4, broad s
Triacetin	5.16, m	4.11–4.16, dd				
		4.21–4.25, dd	–	2.03, s	–	–
PMMA	–	–	–	3.56, sharp s	–	–
Sucrose stearate		1.23, broad s				
Dispersion I						
IND	–	3.66, s	2.21, s	3.76, s	6.71, dd 6.92, d 7.04, d 7.66, dd	Not observed
Triacetin	5.15, m	4.11–4.15, dd				
		4.20–4.24, dd	–	2.03, s	–	–
PMMA	–	–	–	3.57, broad s	–	–
Sucrose stearate		1.24, broad s				
Microspheres (formulation II)						
IND	–	3.65, s	2.21, s	3.75, s	6.72, dd 6.93, d 7.03, d 7.66, dd	Not observed
Triacetin	5.15, m	4.11–4.15, dd	–	2.03, s	–	–
		4.20–4.24, dd				
PMMA	–	–	–	3.57, broad s	–	–
Sucrose stearate		1.23, broad s				

s: singlet; m: multiplet; d: doublet; dd: double doublet.

with the other chemicals in the physical mixture and microsphere formulation. For H-bonding to an electronegative acceptor atom such as oxygen, there might be a change in the chemical shift of the H-bonded hydrogen nucleus to higher frequencies (downfield shift) or the hydrogen signal appears broadened (difficult to observe). In the spectrum of the physical mixture and microsphere formulation of IND, non-observation of the OH signal may be explained by a hydrogen bonding between the IND OH and PMMA.

Amorphous forms of the drugs are metastable since these forms may crystallize after long term standing. This transition from metastable amorphous form to stable crystalline form can cause the dramatic changes in the dissolution behavior of drugs. Therefore, formation a hydrogen bond between the drug and the polymer may prevent the transition between the forms.

4. Conclusion

The extended-release microspheres of IND, which would offer the possibility of once-a-day administration to patients, were successfully produced by a w/o solvent evaporation method using PMMA as a micromatrix material. Triacetin, which was used in the internal phase, modified the release of IND according to the intended release profile. The results of DSC have shown that IND was found to be in an amorphous state in the PMMA microspheres. FT-IR and ^{1}H NMR spectra have suggested that there might be a hydrogen bond present between IND OH and PMMA. Hence, the amorphous form of IND could be stabilized as a molecular dispersion contributing to the release of the drug from polymeric micromatrix (Gong et al., 2006). The plasticization effect of triacetin on PMMA increased the diffusivity of IND from PMMA. However, this effect was not dependent on the formation of secondary bonds between triacetin and PMMA as determined by ^{1}H NMR and FT-IR analysis. This result indicates that the triacetin molecules physically separate the PMMA chains by locating between them.

As a result, the extended-release microspheres of IND, which would offer the possibility of once-a-day administration to patients, were successfully produced by a w/o solvent evaporation method using PMMA as a micromatrix material. The microspheres can be filled into hard gelatin capsules as an oral dosage form which will not affect the established release behavior of IND from microspheres. Additionally, the microspheres would present the advantages of multiparticulate systems.

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References

Ahlin, P., Kristl, J., Kristl, A., Vrecer, F., 2002. Investigation of polymeric nanoparticles as carriers of enalaprilat for oral administration. Int. J. Pharm. 239, 113–120.

Akhgari, A., Afrasiabi Garekani, H., Sadeghi, F., Azimaie, M., 2005. Statistical optimization of indomethacin pellets coated with pH-dependent methacrylic polymers for possible colonic drug delivery. Int. J. Pharm. 305, 22–30.

Amidon, G.L., Lennernäs, H., Shah, V.P., Crison, J.R., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12, 413–420.

Bajdik, J., Pintye-Hódi, K., Palaninšek, O., Tüske, Z., Tasic, L., Regdon Jr., G., Srčič, S., Erös, I., 2004. Surface treatment of indomethacin agglomerates with Eudragit. Drug Dev. Ind. Pharm. 30, 381–388.

Blasi, P., Schoubben, A., Giovagnoli, S., Perioli, L., Ricci, M., Rossi, C., 2007. Ketoprofen poly(lactide-co-glycolide) physical interaction. AAPS PharmSciTech, 8, Article 37.

Bogdanova, S., Bontcheva, E., Avramova, N., 2007. Phase characterization of indomethacin in adsorbates onto hydroxyethylcellulose. Drug Dev. Ind. Pharm. 33, 900–906.

Charman, S.A., Charman, W.N., 2003. Oral modified-release delivery systems. In: Rathbone, M.J., Hadgraft, J., Roberts, M.S. (Eds.), Modified-release Drug Delivery Technology. Marcel Dekker, Inc., New York, pp. 1–10.

Chen, H., Langer, R., 1998. Oral particulate delivery: status and future trends. Adv. Drug Del. Rev. 34, 339–350.

Elvira, C., Fanovich, A., Fernandez, M., Fraile, J., Roman, J.S., Domingo, C., 2004. Evaluation of drug delivery characteristics of microspheres of PMMA–PCL–cholesterol obtained by supercritical-CO_2 impregnation and by dissolution–evaporation techniques. J. Control. Rel. 99, 231–240.

EP 6.0, 2008. European Pharmacopoeia 6.0, vol. 1. Druckerei C.H. Beck, Nördlingen, p. 718.

Freiberg, S., Zhu, X.X., 2004. Polymer microspheres for controlled drug release. Int. J. Pharm. 282, 1–18.

Freitag, C.P.F., Kruel, C.R.P., Duarte, M.E.S., Sanches, P.R.E., Thomé, P.R.O., Fornari, F., Driemeier, D., Teixeira, F., Mollerke, R.O., Callegar-Jacques, S.M., Barros, S.G.S., 2009. Endoscopic implantation of polymethylmethacrylate augments the gastroesophageal antireflux barrier: a short-term study in a porcine model. Surg. Endosc. 23, 1272–1278.

Fujii, M., Okada, H., Shibata, Y., Teramachi, H., Kondoh, M., Watanabe, Y., 2005. Preparation, characterization, and tableting of a solid dispersion of indomethacin with crospovidone. Int. J. Pharm. 293, 145–153.

Gong, A., Darr, J.A., Rehman, I.U., 2006. Supercritical fluid assisted impregnation of indomethacin into chitosan thermosets for controlled release applications. Int. J. Pharm. 315, 93–98.

Gong, A., Rehman, I.U., Darr, J.A., 2007. Synthesis of poly(sebacic anhydride)-indomethacin controlled release composites via supercritical carbon dioxide assisted impregnation. Int. J. Pharm. 338, 191–197.

Govender, S., Pillay, V., Chetty, D.J., Essack, S.Y., Dangor, C.M., Govender, T., 2005. Optimisation and characterisation of bioadhesive controlled release tetracycline microspheres. Int. J. Pharm. 306, 24–40.

Gutierrez-Villarreal, M.H., Rodriguez-Velazquez, J., 2007. The effect of citrate esters as plasticizers on the thermal and mechanical properties of poly(methyl methacrylate). J. Appl. Poly. Sci. 105, 2370–2375.

Huang, H.-P., Ghebre-Sellassie, I., 1989. Preparation of microspheres of water-soluble pharmaceuticals. J. Microencapsulation 2, 219–225.

Jain, A.K., 2008. Solubilization of indomethacin using hydrotropes for aqueous injection. Eur. J. Pharm. Biopharm. 68, 701–714.

Jalil, R., Nixon, J.R., 1989. Microencapsulation using poly (L-lactic acid) I: microcapsule properties affected by the preparative technique. J. Microencapsulation 6, 473–484.

Ladron de Guevara-Fernandez, S., Ragel, C.V., Vallet-Regi, 2003. Bioactive glass-polymer materials for controlled release of ibuprofen. Biomaterials 24, 4037–4043.

Lu, B., Wen, R., Yang, H., He, Y., 2007. Sustained-release tablets of indomethacin-loaded microcapsules: preparation, in vitro and in vivo characterization. Int. J. Pharm. 333, 87–94.

Nokhodchi, A., Javadzadeh, Y., Siahi-Shadbad, M.R., Barzegar-Jalali, M., 2005. The effect of type and concentration of vehicles on the dissolution rate of a poorly soluble drug (indomethacin) from liquisolid compacts. J. Pharm. Pharm. Sci. 8, 18–25.

Ntawukulilyayo, J.D., Bouckaert, S., Remon, J.P., 1993. Enhancement of dissolution rate of nifedipine using sucrose ester coprecipitates. Int. J. Pharm. 93, 209–214.

PDR, 2005. Physician Desk Reference, 59th edition. Thomson PDR, Montvale, pp. 2065–2068.

Pietro, L., Silva, D.R.M., Alberto-Rincon, M.C., Duek, E.A.R., 2006. The influence of triethylcitrate on the biological properties of poly (L-lactic-co-glycolic acid) membranes. J. Mater. Sci. Mater. Med. 17, 849–857.

Ping-Yang, Y., Harma, E., Philip, L.S., 1998. Physiological considerations in the design of particulate dosage forms for oral vaccine delivery. Adv. Drug Del. 34, 123–133.

Rastogi, R., Sultana, Y., Aqil, M., Ali, A., Kumar, S., Chuttani, K., Mishra, A.K., 2007. Alginate microspheres of isoniazid for oral sustained drug delivery. Int. J. Pharm. 334, 71–77.

Sokolsky-Papkov, M., Agashi, K., Olaye, A., Shakesheff, K., Domb, A.J., 2007. Polymer carriers for drug delivery in tissue engineering. Adv. Drug Del. Rev. 59, 187–206.

Streubel, A., Siepmann, J., Bodmeier, R., 2003. Multiple unit gastroretentive drug delivery systems: a new preparation method for low density microparticles. J. Microencapsulation 20, 329–347.

Sugino, A., Miyazaki, T., Kawachi, G., Kikuta, K., Ohtsuki, C., 2008. Relationship between apatite-forming ability and mechanical properties of bioactive PMMA-based bone cement modified with calcium salts and alkoxysilane. J. Mater. Sci. Mater. Med. 19, 1399–1405.

Uhrich, K.E., Cannizzaro, S.M., Langer, R.S., Shakesheff, K.M., 1999. Polymeric systems for controlled drug release. Chem. Rev. 99, 3181–3198.

USP 30, 2007. The United States Pharmacopoeia 30. The United States Pharmacopoeial Convention. Mack Printing Co, Easton, pp. 630, 2351–2352.

Virtoa, M.R., Frutosa, P., Torrado, S., Frutos, G., 2003. Gentamicin release from modified acrylic bone cements with lactose and hydroxypropylmethyl cellulose. Biomaterials 24, 79–87.

Yuksel, N., Baykara, T., 1997. Preparation of polymeric microspheres by the solvent evaporation method using sucrose stearate as a droplet stabilizer. J. Microencapsulation 14, 725–733.
Yuksel, N., Beba, L., 2009. Preparation and optimization of superabsorbent hydrogel micromatrices based on poly(acrylic acid), partly sodium salt-g-poly(ethylene oxide) for modified release of indomethacin. Drug Dev. Ind. Pharm. 35, 756–767.
Yuksel, N., Tincer, T., Baykara, T., 1996. Interaction between nicardipine hydrochloride and polymeric microspheres for a controlled release system interaction between nicardipine hydrochloride and polymeric microspheres for a controlled release system. Int. J. Pharm. 140, 145–154.
Zhu, Y., Mehta, K.A., McGinity, J.W., 2006. Influence of plasticizer level on the drug release from sustained release film coated and hot-melt extruded dosage forms. Pharm. Dev. Technol. 11, 285–294.