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Effect of *N*-trimethyl chitosan enhancing the dissolution properties of the lipophilic drug cyclosporin A

Xiaoju Zhou, Yan Hu, Yanping Tian, Xianming Hu *

The State Key Laboratory of Virology, College of Pharmacy, Wuhan University, Iuojia Hill, Wuhan 430072, PR China

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ABSTRACT

The aim was to evaluate the influence of *N*-trimethyl chitosan chloride (TMC) as a carrier for solid dispersion on the dissolution of poorly water-soluble drugs. In this study, we used cyclosporin A(CyA) as a model drug and TMC as a carrier. The effect of various formulation and process variables including TMC-to-CyA mixing weight ratio, weigh molecular(M_w) of TMC and methods used to disperse CyA along with the TMC on the drug dissolution was investigated. The nature of CyA dispersed in the matrix was studied by powder X-ray diffractometry (PXRD), diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS), and dissolution rate analyses. It was proved that all solid mixtures of CyA with TMCs showed a significantly rapid dissolution rate compared to pure drug and physical mixture. The greater the TMC content the higher the drug dissolution was, up to a maximum corresponding to a polymer: drug ratio of 3:1. The lower the M_w of TMC, the more important the polymer effect was. The dissolution of CyA was remarkably improved by the solid dispersion. The drug dissolution enhancement was attributed to the decreased drug crystallinity and size and polymer wetting effect. There was no significant difference in the efficiency of improving the drug dissolution between the solid dispersions prepared by solvent dispersing and by co-grinding. It was suggested that the TMC with a lower molecular weight is a useful carrier for solid dispersion.

1. Introduction

Chitosan(CS) is the *N*-deacetylated product of the polysaccharide chitin. For its good mucoadhesive properties, excellent biocompatibility, and complete biodegradability in combination with low toxicity, CS has been widely used in pharmaceutical research and in industry as a carrier for drug delivery (Doclane & Vilivalam, 1998; Henriksen, Green, Smart, Smistad, & Karlsen, 1996; Lehr, Bouwstra, Schacht, & Junginger, 1992). It has been demonstrated to be a good vehicle for enhancing the penetration of macromolecules across the different human mucosa such as the intestinal (Borchard et al., 1996) and increasing the dissolution properties and bioavailability of a number of poorly water-soluble drugs (Shiraishi, Arahira, Imai, & Otagiri, 1990; Genta, Pavanetto, Conti, Giunchedi, & Conte, 1995; Portero, Remunan-Lopez, & Vila-Jato, 1998). However, chitosan has an apparent pKa of 5.5 and is only soluble in acidic solutions with pH values lower than 6.0. It aggregates when the bulk pH is above 6 (Kotze, Luessen, de Boer, Verhoef, & Junginger, 1998), which interferes with the biomedical application of this polymer.

In contrast, *N*-trimethyl chitosan chloride (TMC, Fig. 1), a partially quaternized chitosan derivative, has good water solubility over a wide pH range. Moreover, soluble TMC has mucoadhesive properties and excellent absorption enhancing effects even at neutral pH (Hamman, Stander, & Kotze, 2002; Thanou, Verhoef, Verheijden, & Junginger, 2000). It has been proven that TMC is a potent and safe absorption enhancer of peptide drugs. TMC opens the tight junctions between intestinal epithelial cells, to increase the paracellular transport of drugs.

Solid dispersions (SD) have been widely used to enhance the solubility, dissolution rate, and bioavailability of poorly soluble drugs (Chiou & Riegelman, 1971). With regard to carriers for SD, several water-soluble polymer carriers such as polyethylene glycol (PEG), PVP, hydroxypropylmethylcellulose (HPMC) and chitosan have been reported to improve the solubility and bioavailability of poorly water-soluble drugs (Yamashita et al., 2003). But until now, no evaluation of SD using TMC as a carrier has been performed.

Cyclosporin A (CyA), a highly lipophilic cyclic undecapeptide, is commonly used as immunosuppressant to prevent allograft rejection in various organ transplantation such as kidney, liver, heart, lung and pancreas (Matzke & Luke, 1988). However, in spite of the great therapeutic interest of this drug, the bioavailability after oral dosing is low (10–60%) with a higher variability (Lindholm, Henricsson, Lind, & Dahlqvist, 1988; Ptachcinski, Venkataramanan, & Burckart, 1986). It is known that the absolute bioavailability of cyclosporin A is low due to the poor absorption which is related

* Corresponding author. Tel.: +86 27 68753532; fax: +86 27 68754629.
E-mail address: xmhu@whu.edu.cn (X. Hu).

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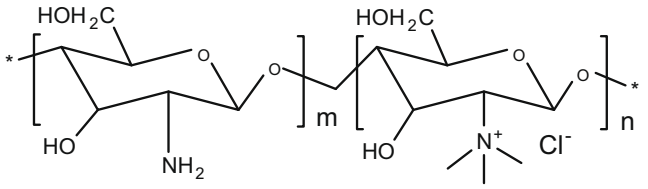

Fig. 1. The structure of *N*-trimethyl chitosan chloride (TMC).

to the relatively high molecular weight, very high lipophilicity (Taylor et al., 1993) and poor solubility in aqueous fluids (Ismailos, Reppas, Dressman, & Macheras, 1991).

Taking all this into account, this study was carried out to investigate the feasibility of TMC as the carrier of solid dispersion to enhance the dissolution of CyA, which is a model of poorly water-soluble drugs. The effect of various formulation and process variables including TMC-to-CyA mixing weight ratio, type of TMC and methods used to disperse CyA along with the TMC on the drug dissolution was investigated. The physical state of CyA was investigated by using X-ray diffraction analysis and Fourier transform IR technique.

2. Materials and methods

2.1. Materials

Chitosans (CS: Degree of deacetylated was >90%, and molecular weights were 200 and 600 kDa) were purchased from Zhejiang Aoxing Biotechnology Co., Ltd. Cyclosporin A (lot number: 050803) was obtained from Fujian Kerui Pharmaceutical Co., Ltd. Methyl iodide,1-methyl-2-pyrrolidinone and other reagent were products of Wuhan Shenshi Chemicals and instruments Co., Ltd.

2.2. Synthesis of TMC

TMC with a degree of quaternization of 60% was synthesized by using two-steps synthesis described in the literature (Sieval et al., 1998). Two TMCs obtained from chitosans of different M_w (200 kDa, Low-M_w grade; 600 kDa, High-M_w grade) were called TMCL and TMCH respectively. They were characterized by ^{1}H NMR. The products were measured in D_2O at 80 °C, using a 300 MHz spectrometer (Mercury Vx-300 Varian). The degree of quaternization (DQ) of the synthesized TMC polymers were calculated with the following equation (Hamman, Stander, & Kotze, 2002):

$$\mathrm{DQ}(\%) = \left[\left(\int \mathrm{TM} \Big/ \int \mathrm{H}\right) \times (1/9)\right] \times 100$$

where $\int$TM is the integral of the chemical shift of the hydrogens of the trimethyl amino group at 3.3 ppm, and $\int$H is the integral of the H-1 peaks between 4.7 and 5.7 ppm, related to hydrogen atoms bound to carbon 1 of the chitosan molecule, which is taken as the reference signal.

2.3. Preparation of drug: TMC solid mixtures

Drug:polymer solid mixtures were prepared by incorporating CyA within TMC in varying weight ratios using different methods: (i) physical mixtures of drug and polymer were obtained by simply blending with a spatula followed by sieving the previously sieved (<100 μm) TMCL and CyA in various weight ratios (polymer:drug ratio = 1:1, 3:1 and 6:1); (ii) co-ground mixtures of two different TMCS (TMCL and TMCH) and CyA in a polymer:drug ratio of 3:1 were obtained by co-grinding for 20 min in a ceramic mortar (mortar grinder RM200, Restch Inc., China). and sieving through a 100 μm mesh sieve; (iii) co-ground mixtures of TMCL and CyA in various weight ratios (polymer:drug = 1:1, 3:1 and 6:1, w:w) were obtained as described above; (iv) solid dispersions in a poly-

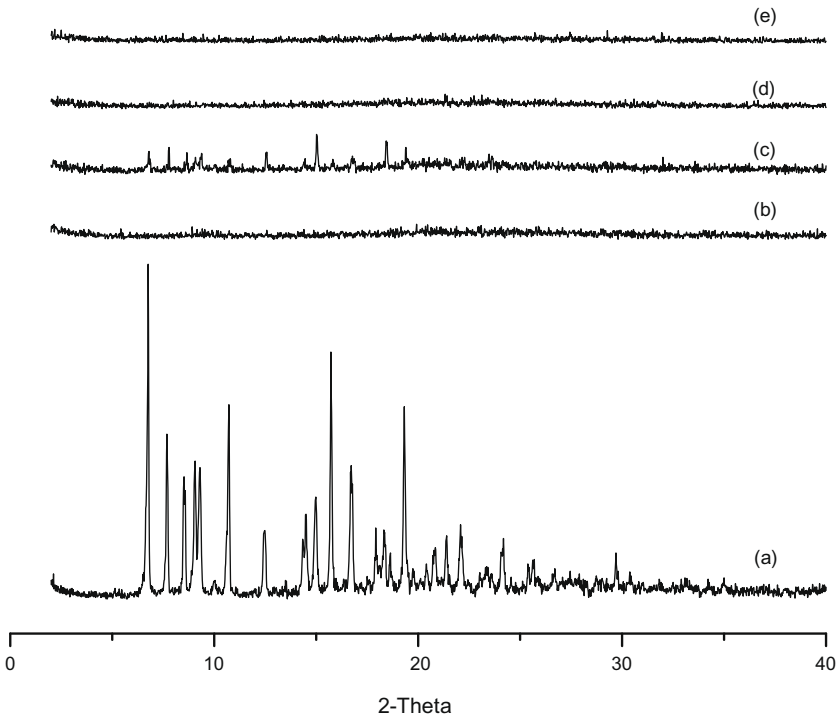

Fig. 2. X-ray powder diffraction patterns of (a) pure CyA; (b) neat TMCL; (c) physical mixture of CyA and TMCL (1:3); (d) co-ground mixture of CyA and TMCL (1:3); (e) solid dispersion of CyA (25% w/w) in TMCL (1:3).

mer:drug weight ratio of 3:1, were obtained by a solvent dispersing:drying technique. Polymer solutions (10%, w:w) were prepared by dissolving TMCH in distilled water at room temperature. The CyA was dissolved in a small volume of ethanol (ethanol:water, 1:4) and then added to the TMCH aqueous solution. The solvents of the drug-containing polymer solutions were removed under reduced pressure using a rotavapor at 50 °C. The solid mixture obtained was then crushed, pulverized and sieved through a 100 mm mesh sieve. All samples were stored in a desiccator (containing silica gel) at room temperature until the assay.

2.4. Powder X-ray diffractometry (PXRD) studies

PXRD patterns of each of the pure ingredients, physical mixtures, co-ground mixtures and solid dispersions containing varying proportions of CyA in the TMC were recorded using an X-ray diffractometer (Bruker AXS Co. Ltd., Germany) with Ni filtered CuKα line as the source of radiation. The scan was from 2° to 40° 2θ with a step size of 0.02° and the residence time of 1.2 s.

2.5. Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS)

The Fourier-transformed infrared (FTIR) spectra of samples were obtained, after appropriate background subtraction, using an FTIR spectrometer (Series II Magna 750, Nicollet Instrument Corp., Madison, WI) equipped with a deuterated triglycine sulfate (DTGS) detector, a diffuse reflectance accessory and a data station. About 1–2 mg of the sample was mixed with dry potassium bromide and the sample was scanned from 400 to 4000 cm^{-1}.

2.6. Solubility measurements

Solubility measurements were carried out by adding either pure CyA , or co-ground mixtures, or physical mixtures containing varying proportions of CyA and TMCL (polymer:drug ratio = 1:1, 3:1 and 6:1), each containing 40 mg of CyA, to 50 ml of distilled water. The suspensions were magnetically stirred at 37 °C in a dark room for 24 h, at the end of which samples were withdrawn and filtered through 0.45 μm membrane filters. The concentration of CyA in this phase was determined by HPLC. All experiments were performed in triplicate.

2.7. Dissolution studies

The dissolution of CyA alone, CyA from the physical mixture, CyA from the co-ground mixtures and CyA from the solid dispersions were determined using Apparatus No. 2 of the CP XC (2005). The dissolution medium maintained at 37 °C. Because CyA is not completely soluble in water even at low concentrations, a better solvent, such as ethanol, needs to be added to the dissolution medium to maintain sink conditions. In order to provide a more discriminating dissolution medium, the dissolution medium was consisted of distilled water containing ethanol (20% v/v). Samples equivalent to 20 mg of CyA was added to 1000 ml of dissolution medium in a 1000 ml cylindrical beaker. A two-blade stirrer centrally placed 20 cm from the bottom of the beaker provided stirring at 100 rpm. At suitable time intervals, 2.0 ml samples were withdrawn at definite intervals. The same volume of preheated dissolution medium was infused into the medium after each sample was taken in order to maintain a constant volume of the dissolution medium throughout the test. The samples were filtered (pore size 0.45 μm) and the CyA content was determined by HPLC. All experiments were carried out in triplicate and the results presented are the mean values of the three experiments.

2.8. HPLC analysis

The chromatographic system consisted of a Shimadzu LC – 6A solvent delivery pump equipped with a 20 μl loop and rheodyne sample injector and SPD-6AV UV–visible detector. The column used was Elite C18 analytical column (ODS-2 Hypesil, 5 μm, 250

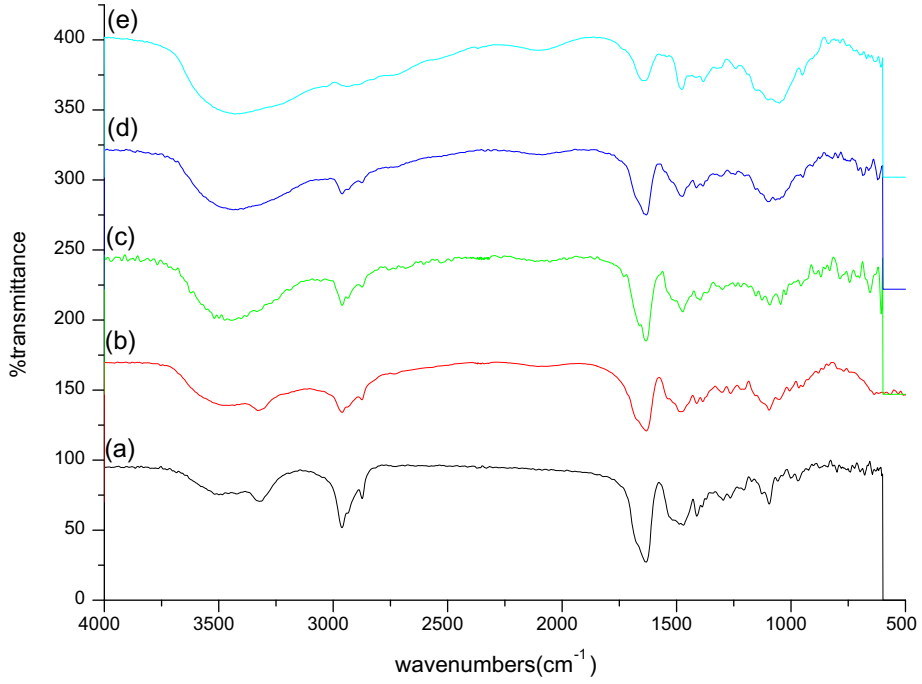

Fig. 3. FTIR spectra of (a) pure CyA; (b) physical mixture of CyA and TMCL (1:3); (c) co-ground mixture of CyA and TMCL (1:3); (d) solid dispersion consisting of CyA and TMCL (1:3); (e) neat TMCL.

× 4.6 mm). The mobile phase consisted of methanol/water (85:15, v/v), and the flow rate was 1.0 ml/min. The eluate was monitored at 220 nm and the column temperature was maintained at 50 °C.

3. Results and discussion

3.1. Solid-state characterization

The PXRD patterns of pure CyA, neat TMCL, Co-ground mixture of CyA and TMCL in a polymer:drug ratio of 3:1 and the solid dispersion of CyA in TMCL are shown in Fig. 2. The powder X-ray diffractometry of pure CyA showed numerous distinctive peaks that indicated a high crystallinity, while TMCL exhibited amorphous pattern. The PXRD pattern of the physical mixture exhibited all the characteristic diffraction peaks of crystalline CyA, but of lower intensity. However, these crystalline peaks almost disappeared in the co-ground mixture and solid dispersion. This study revealed that CyA became amorphous in the co-ground mixture and solid dispersion. The loss in crystallinity was expected a fast dissolution and a higher bioavailability of this water-insoluble drug.

In order to further ascertain whether CyA undergoes a polymorphic change during the preparation of the Co-ground mixtures and to test for possible intermolecular interactions between CyA and the constituents of the dispersion matrix, DRIFTS was used (Fig. 3). CyA powder exhibited sharp peaks in the FTIR spectrum indicating its crystalline nature. The TMCL exhibited relatively broad peaks due to the large molecular sizes of the polymer and their amorphous nature. In the FTIR spectra of CyA (Fig. 3a), absorption bands of N—H stretching vibration at 3320 cm^{-1}, C=O stretching vibrations at 1634 cm^{-1} were observed, These bands were also observed for the physical mixture of CyA and TMC with the same absorbance (Fig. 3b). From these results, it was confirmed that there is no interaction between CyA and TMC in the physical mixture. In contrast, the absorption band due to the N—H stretching vibration of CyA at 3320 cm^{-1} were disappeared in co-ground mixture and solid dispersion (Figs. 3c and 3d), whereas the other stretching vibrations of CyA were not affected. These results suggest that the N-H functional groups of CyA are interacted with the functional group of TMC in the co-ground mixtures and solid dispersions.

Fig. 4 showed the powder X-ray diffraction patterns of the CyA/TMCL systems in different ratios. In the CyA/TMCL co-ground systems, the diffraction peaks decreased with an increase in the TMCL

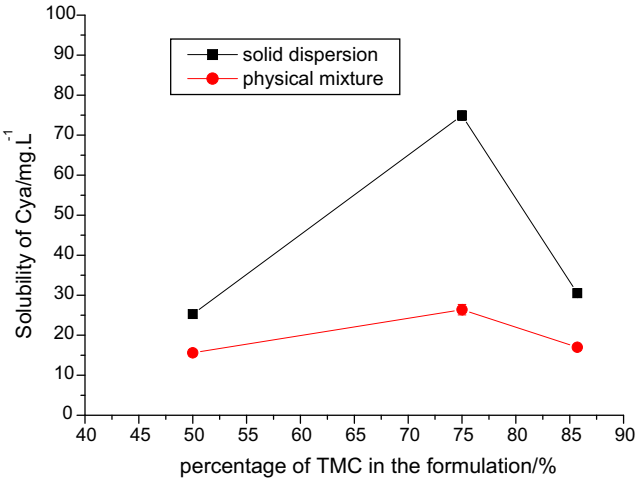

Fig. 5. Solubility of CyA from physical mixtures of CyA and TMCL and CyA from Co-ground mixtures consisting of CyA and TMCL in distilled water at 37 °C; data shown the mean ± *SD*, *n* = 3.

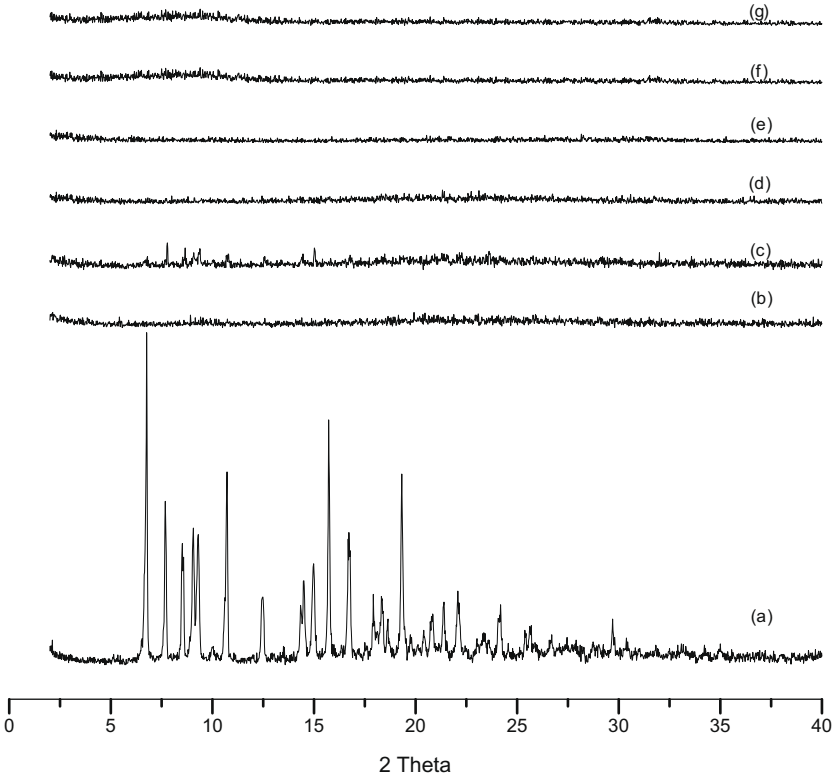

Fig. 4. Powder X-ray diffraction patterns of the CyA/TMC systems. (a) CyA; (b) TMCL; (c) CyA/TMCL = 1/1 co-ground mixture; (d) CyA/TMCL = 1/3 co-ground mixture; (e) CyA/TMCL = 1/6 co-ground mixture; (f) CyA/TMCH = 1/3 co-ground mixture; (g) TMCH.

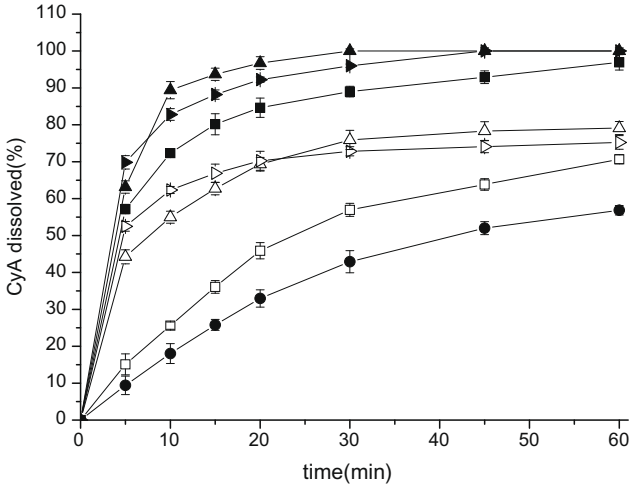

Fig. 6. Effect of the TMCm:CyA mixing ration on CyA dissolution from the solid dispersions, physical mixture and CyA powder alone. Key: (●) pure CyA; (□) 1:1, (△) 1:3, (▷) 1:6 CS60:CyA physical mixture; (■) 1:1, (▲) 1:3, (▶) 1:6 CS60:CyA solid dispersion; data shown are the mean ± *SD*, *n* = 3.

content, and a diffraction peak was almost disappeared at the mixing ratio of 1:3 and 1:6(CyA: TMCL).

In the co-ground mixtures of CyA and TMC with different M_w in a polymer:drug ratio of 3:1, no diffraction peak was observed (Fig. 4), which indicated CyA existed in the form of amorphous or solid solution state.

3.2. Solubility and dissolution studies

The solubility of CyA from the co-ground mixtures and from the physical mixture of CyA and TMCL in various weight ratios (polymer:drug ratio = 1:1, 3:1 and 6:1) in distilled water at 37 °C was shown in Fig. 5. The results showed that the solubility of CyA from co-ground mixtures was significantly higher than that from physical mixtures ($P < 0.01$). The solubility of CyA was highest for the 3:1 mixture among the three weight ratios of polymer and drug, which was in accordance with the results later observed in the dissolution profiles. The higher solubility of CyA from co-ground mixtures may due to the enhancement of the surface area and the high dispersed state of the drug in the system except the wetting effect and solubilizing effect of the carrier.

The in vitro dissolution profiles of CyA from the co-ground mixtures and from the physical mixture of CyA and TMCL in various weight ratios (polymer:drug ratio = 1:1, 3:1 and 6:1) was shown in Fig. 6. The effect of the polymer:drug mixing ratio on the percentage of CyA dissolved was compared using the 15% release time T_{15} and 63.2% release time T_d (Table 1). The physical mixtures and the co-ground mixtures exhibited significantly faster dissolution rates than the pure drug. Meanwhile, the co-grinding of CyA with TMCL markedly enhanced the dissolution rate of the drug compared with that of the physical mixtures for all polymer:drug ratios. The greater the TMCL content the higher the drug dissolution was, up to a maximum corresponding to a polymer:drug ratio of 3:1.

Fig. 7a dispicted the dissolution profile of CyA from its co-ground mixtures with the different molecular weight TMCs (TMCL and TMCH). Fig. 7b dispicted the dissolution profile of CyA from the co-ground mixtures and solid dispersion respectively. The T_{15} and T_d was shown in Table 1. The results showed both TMCs led to an enhancement of the CyA dissolution rate compared to that of the pure drug; The lower the *M*w, the faster was the drug dissolution. This behavior was predictable taking into account the relationship between M_w and viscosity of polymer solution. The slower release of TMCH was attributed to the higher viscosity of the polymer, which influences its dissolution rate. The method used to disperse drug within carriers is a main factor affecting the enhancement of drug dissolution. Therefore, the effect of the procedures of preparing TMC:CyA solid mixtures on the dissolution of CyA was investigated. It exhibited no significant differences among the co-ground

Table 1
In vitro dissolution parameters of the samples.

Sample	$T_{0.5}$(min)	T_d(min)
CyA	41.76	63.46
CyA/TMCL Co-ground mixture = 1:1	3.29	6.5
CyA/TMCL Co-ground mixture = 1:3	1.48	2.82
CyA/TMCL Co-ground mixture = 1:6	2.49	4.33
CyA/TMCH Co-ground mixture = 1:3	1.52	3.73
CyA/TMCL solid dispersion = 1:3	1.25	2.29
CyA/TMCL physical mixture = 1:3	6.7	16.08

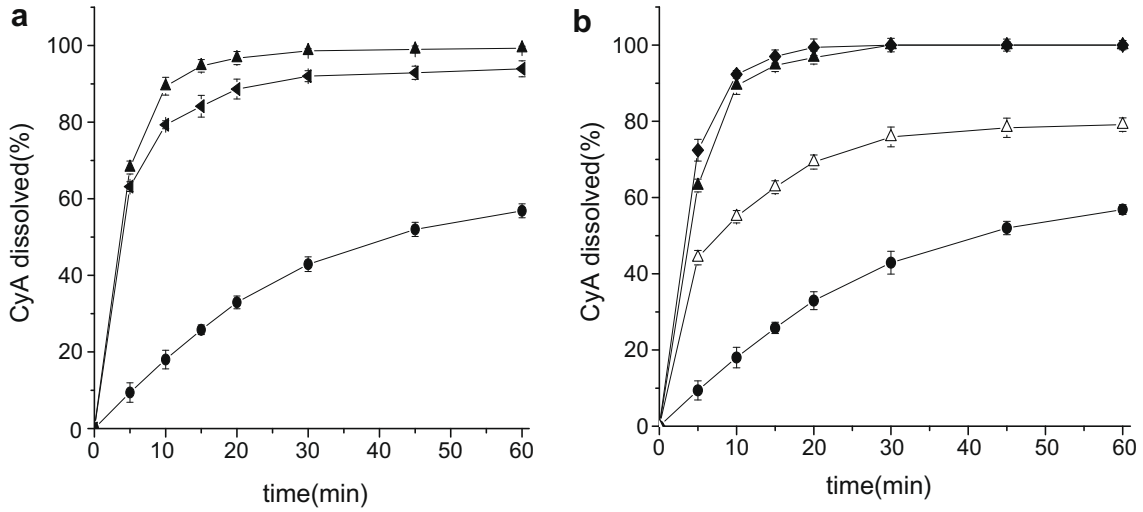

Fig. 7. (a) Effect of the type of TMC on CyA dissolution from polymer:drug(3:1) co-ground mixtures. Key: (●) pure CyA; (▲) TMCL; (◀) TMCH. (b) Effect of the method used to prepare the polymer:drug (3:1) solid mixtures on the dissolution of CyA. Key: (●) pure CyA; (△) physical mixture; (▲) co-ground mixture; (♦) solid dispersion (solvent dispersion method); data shown are the mean ± *SD*, *n* = 3.

mixtures and solid dispersion (Fig 7b), which was explained by the powder X-ray diffraction patterns (Fig. 1). Both co-grinding method and solvent dispersing method could cause CyA became amorphous and increased the solubility of CyA.

The initial increase in the dissolution rate of CyA when co-grinding or solvent dispersing with TMC is probably attributable to the following factors: (1) the reduction of the drug particle size to molecular level, (2) the solubilizing effect on the drug by the water-soluble carrier, and (3)the enhancement of the wettability and dispersibility of the drug by the carrier material (Craig, 2001; Shargel, 1993; Swarbrick, 1990).

4. Conclusions

Based on the above discussed data, it was concluded that TMC strongly affected the dissolution of the poorly water-soluble drug CyA and the low-*M*w TMC(TMCL) seemed to be the most promising polymer for the development as the carrier of solid disperion. It was proved that all solid mixtures of CyA with TMCs showed a remarkably rapid dissolution rate compared to pure drug and physical mixture. The enhanced dissolution of CyA from the solid mixtures could be mainly attributed to amorphization of the drug (as was demonstrated by X-ray diffractometry); however, a contribution role of improved wettability, reduced aggregation and increased surface area can not be discarded for some polymer:drug ratios. The simply co-grinding of CyA along with TMCL in a 3:1 TMCL:CyA ratio, which leads to solid mixtures exhibiting a significantly improved dissolution profile without using organic solvents or high temperatures, appears to be the easier and most convenient method.

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