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Research paper

A novel nanomatrix system consisted of colloidal silica and pH-sensitive polymethylacrylate improves the oral bioavailability of fenofibrate

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ABSTRACT

A novel solid particle system with a nanomatrix structure and without surfactant for the oral delivery of insoluble drugs was prepared. This used a combination of pH-sensitive polymethylacrylate and nanoporous silica, in order to improve the drug absorption using only pharmaceutical excipients and a relative simple process. The in vitro drug dissolution and in vivo oral bioavailability of this formulation, using fenofibrate as the model drug, were compared with other reference formulations such as a suspension, micronized formulation or self microemulsion drug delivery system (SMEDDS). The supersaturation stabilizing effect of different polymers was evaluated and the physicochemical characterization of the optimal formulation was conducted by SEM, TEM, surface area analysis, DSC, and XRD. The optimized formulation prepared with polymethylacrylate (Eudragit®L100-55) and silica (Sylysia®350) markedly improved the drug dissolution compared with other reference preparations and displayed a comparative oral bioavailability to the SMEDDS. Fenofibrate existed in a molecular or amorphous state in the nanomatrix, and this state was maintained for up to 1 year, without obvious changes in drug release and absorption. In conclusion, the nanomatrix formulation described here is a promising system to enhance the oral bioavailability of water-insoluble drugs.

1. Introduction

It has been estimated that about 40–70% of all new drug candidates merging from drug discovery programs exhibit low solubility in water, resulting in poor oral bioavailability due to insufficient dissolution along the gastrointestinal (GI) tract [1]. High drug lipophilicity is a great handicap to efficient oral drug delivery [2]. More and more techniques have been developed to overcome this obstacle for water-insoluble APIs, including salt formation [3], the use of prodrugs [4], lipid-based formulations [1] such as oil solutions, emulsion and lipid–drug conjugates, solid dispersions [5], cyclodextrin inclusion complexes [6]. However, there are still many limitations to these approaches [1,3,4,7]. Today, the nanotechnology-based formulations are attracting much attention.

The nanotechnology for increasing drug dissolution and absorption mainly involves two strategies. One is that the drug itself is prepared as nano-sized particles and the other is that the nanoparticles are prepared from some carrier materials, for instance the polymer. Great progress has already been made, as shown by the five marketed oral pharmaceutical products and more in clinical trials that use nanocrystal technology [2]. However, big challenges remain to both approaches, including the requirement for sophisticated equipment for industrial-scale production, high cost, lack of pharmaceutical excipients, the poor long-term stability of the nanoparticles, low drug loading capacity, and the safety of polymer and surfactants [8–10]. Therefore, there is a need to develop a stable nano-drug delivery system using only pharmaceutical excipients and a relative simple preparation process.

Fortunately, it is interesting that there are some pharmaceutical excipients which have nano-structures. Colloidal silicon dioxide (Sylysia® [11] or Aerosil® [12]), calcium silicate (Florite® [13]), and magnesium aluminum silicate (Neusilin®, Veegum®HV [14,15]) are pharmaceutical additives with either a non-ordered mesoporous network or nano-sized particles. Since 2001, mesoporous silica materials with an ordered pore network, such as MCM-41 and SBA-15 have been investigated as drug delivery system [16,17]. In these studies, it was found that adsorbing or incorporating insoluble drug into the hydrophilic silica matrix can increase the drug dissolution and bioavailability. However, there are only a few reports of the in vivo study of using porous silica as main drug carrier in oral drug delivery system [18,19].

It has been shown in previous reports that a suitable pH-sensitive polymer is also crucial for the increased absorption of insoluble drugs [20,21]. For instance, pH-dependent Eudragit®

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can be used to form a solid dispersion with an insoluble drug, and provide consistent supersaturation along the small intestine for the released drug, and ultimately improve absorption [22]. The pH-sensitive polymer can also protect the drug from being released in the stomach, where the released drug may precipitate due to insufficient absorption. Meanwhile, pH-dependent Eudragit® can produce a bio-adhesive effect in the GI tract [23].

Fenofibrate, a pro-drug of fenofibric acid, is virtually insoluble (<1 μg/ml) [24] and highly lipophilic (log P = 5.24) [25], and is used clinically to lower lipid levels. Its therapeutic efficacy has been compromised for years [26] due to its low oral bioavailability (about 30% in human [27]). Today, there are several marketed preparations of fenofibrate, such as Lipanthyl®, a micronized drug formulated in a capsule, TRICOR®, a microparticle formulation with phospholipids as surface stabilizing agents [28], and Triglide™, a nanocrystal preparation [2]. Other approaches like the use of solid dispersions [29], liposomes [30], microemulsions [31], lipid-based vehicles [32], supramolecular assemblies with block copolymers [33], and silica based formulations [34], have been reported to increase its absorption.

In the present study, nano-structured silica with a huge surface area and pH-sensitivity polymethylacrylate were used to construct surfactant-free solid particles with a nanomatrix structure, containing fenofibrate as the model drug. All the materials used are commercially available and proven to be safe as drug excipients by FDA. The in vitro drug dissolution and in vivo drug absorption have been proved, and the possible mechanism of action has been investigated.

2. Materials and methods

2.1. Materials

Fenofibrate and fenofibrate acid were obtained from Kaifeng Pharmaceutical Factory (He Nan, China); Lipanthyl® was a commercial product produced by Laboratoires Fournier S.A. (Chenove, France); As a nano-structured carrier, SiO_2 powder Aerosil®200 (a gift from Degussa, Darmstadt, Germany) and Sylysia®350 (a gift from Fuji Silysia Co., Ltd., Aichi, Japan) were used; Polymethylacrylate Eudragit®L100-55, Eudragit® L100 and Eudragit®S100 were donated by Rohm Pharma (Darmstadt, Germany); PVP-K29/32 was a gift from ISP Technologies (Wayne, NJ, USA); HPMC-E5 and HPMC-E50 were from Dow Chemical (Michigan, USA); Others include Maisine 35-1 (Gattefosse, Saint Priest, Cedex, France), Cremophor EL (BASF, Ludwigshafen, Germany), ethyl oleate and PEG 400 (Sigma, St. Louis, USA). Methanol and acetonitrile of HPLC-grade were obtained from Promptar (Elk Grove, USA). All other chemicals, such as sodium dodecyl sulfate (SDS), ethanol and inorganic salt, were of analytical grade.

Male Sprague–Dawley (SD) rats weighing 190–210 g were provided by Animal Institute of Peking University Health Science Center (Beijing, China). All care and handling of animals were performed with the approval of Institutional Authority for Laboratory Animal Care.

2.2. Preparation of the nanomatrix system and other reference formulations

The nanomatrix of fenofibrate with colloidal silica and Eudragit® was prepared using the rotary evaporation method. Briefly, 300 mg (or 600 mg, or 0 mg) of the silica was suspended in 20 ml ethanol solution containing 100 mg fenofibrate and ultrasonicated for 30 min. Three hundred milligram (or 0 mg, or 600 mg) of the Eudragit® dissolved in 20 ml ethanol solution. The two liquids were mixed for 1 h under magnetic stirring and then the mixture was introduced into a flask and the solvent was removed under reduced pressure in a rotary evaporator at 40 °C. The powder obtained was micronized by grinding in a mortar and the resultant mass was passed through a 100 mesh sieve.

The physical mixture of fenofibrate, silica and Eudragit® was also prepared by thorough mixing. The commercial product Lipanthyl® is a capsule containing micronized fenofibrate produced by Laboratoires Fournier S.A. A self-microemulsified drug delivery system (SMEDDS) of fenofibrate has also been formulated, and this consists of 50 mg fenofibrate, 200 mg Maisine 35-1, 150 mg ethyl oleate, 600 mg Cremopher EL, and 200 mg PEG 400. Briefly, fenofibrate was placed in a glass vial, and then, oil, surfactant, and cosurfactant were added. These components were mixed by stirring and heated at 40 °C until the fenofibrate dissolved completely. The mixture was stored at room temperature until use. The particle size was measured by the Malvern Zetasizer Nano-ZS laser particle size analyzer (Malvern Instruments Ltd., UK). Fenofibrate crude drug suspension was prepared by suspending the crude drug powder in a 0.5% HPMC solution under magnetic stirring for 1 h.

2.3. HPLC analysis of fenofibrate in vitro

The HPLC system consisted of a SHIMADZU LC-20AT pump, an auto sampler SIL-20A, and a UV detector SPD-20A set at 288 nm. The separation of fenofibrate was performed on a 250 × 4.6 mm BDS Hypersil C18 column (Thermo Electron Corporation, USA) at 35 °C, eluting with water and methanol (80:20%, v/v) at a flow rate of 1.0 ml/min. The calibration curve was liner with a correlation coefficient of 0.9999 over the range of 0.1–50 μg/ml. The within-day and between-day coefficients of variation did not exceed 4%. The limit of detection (LOD) value for fenofibrate was 15 ng/ml, and the limit of quantitation (LOQ) value was 50 ng/ml, respectively. The accuracy of the method was verified with recovery values of 98–102%.

2.4. In vitro dissolution study

Dissolution tests were performed with a dissolution apparatus (RC-6A, Precise Apparatus of Tianjin University Co., Ltd., China) using the paddle method according to the Chinese Pharmacopoeia (2005 edition). The nanomatrix system (equivalent to 3.5 mg of fenofibrate) was placed in 200 ml PBS solution (0.01 M, pH 7.3 ± 0.1) containing 0.3% (w/v) sodium dodecyl sulfate (SDS) at 37 °C and 50 rpm. An aliquot of 1.5 ml release media was withdrawn at intervals of 5, 10, 20, 30, 45, 60 min, and then replaced by 1.5 ml of fresh dissolution fluid. Each sample was passed through a 0.45 μm syringe filter and determined by HPLC (see Section 2.3). The measurements were performed in duplicate and averages are reported here.

2.5. In vivo bioavailability study

2.5.1. Animals and dosing

Male SD rats (body weight 200 ± 10 g) were fasted over night. Each group consisted of five animals and received one of the formulations. The oral dose of fenofibrate was 33 mg/kg. Blood samples were taken at time points of 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 30, and 36 h. In the comparison study between the freshly prepared and 1-year storage formulation, the oral dose was 10 mg/kg, each group consisted of three animals and samples were collected at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h.

2.5.2. Plasma processing and HPLC analysis

Plasma processing and HPLC analysis were performed according to literatures with a little modification [35,36]. An aliquot of 100 μl plasma and 400 μl methanol was transferred to EP tubes and

vortexed for 1 min, followed by the centrifugation at 12,000 rpm for 10 min at 10 °C. An aliquot of 20 μl supernatant was injected into the HPLC system and the fenofibric acid was detected. The HPLC system and separation conditions were the same as those described above, except that the mobile phase was changed to 0.02 M phosphoric acid and acetonitrile (40:60, v/v). Calibration samples were prepared as described above with the exception that instead of 400 μl methanol, 380 μl methanol and 20 μl fenofibric acid dissolved in methanol were added to the blank plasma. Two standard curves were obtained, with a correlation coefficient of 0.9998 over the concentration range of 0.20–4.93 μg/ml and 0.9999 over 4.93–157.63 μg/ml, respectively. LOD and LOQ were 0.005 and 0.020 μg/ml, respectively. The within-day and between-day relative standard deviation did not exceed 5% for the same batch of reagents. The accuracy of the method was verified with recovery values of 96–103%.

2.5.3. Data analysis

Standard non-compartmental pharmacokinetic parameters (±SD) were calculated using the pharmacokinetic program WinNonlin (Pharsight Corporation, Mountain View, CA). Statistical comparisons between groups were made using MS Excel by the Student's *t*-test for independent groups assuming equal variances within each group.

2.6. Surface area and pore size analysis

The nitrogen adsorption–desorption isotherms were recorded at 77 K using an ASAP2010 rapid surface area and pore size analyzer (Micromeritics Co., USA). All samples were degassed at 70 °C under vacuum for 12 h prior to analysis. The specific surface area and the pore size were determined according to the multiple-point Brunauer–Emmett–Teller (BET) method [37] and the Barrett–Joyner–Halenda (BJH) method [38] by using the nitrogen desorption branches of the isotherms. The pore volume was computed with the BET model.

2.7. Scanning electron microscopy and transmission electron microscopy

Scanning electron microscopy (SEM) was carried out with a NOVA NANOSEM 430 instrument (FEI, USA). The samples were dispersed in ethanol (or water for Nanomatrix-1), then deposited on a silica plate and gold-plated prior to imaging. Transmission electron micrographs (TEM) were obtained using a Tecnai F30 electron microscopy (FEI, USA) operated at 300 kV. Before examination, the sample were dispersed in ethanol (or water for Nanomatrix-1) and deposited on a copper grid.

2.8. Differential scanning calorimetry

The powders (weight 2–6 mg) were analyzed in open aluminum sample pans, using a DSC Q100 V9.8 Build 296 calorimeter (Thermal Analysis Co., USA), and the samples were heated from 30 to 140 °C at 10 °C min^{-1}.

2.9. Powder X-ray diffraction

The crystallinity of each formulation was analyzed using the D/MAX 2000 X-ray diffractometer (Rigaku Co., Japan) equipped with a Cu Kα radiation source at a 40 kV voltage and 40 mA current. Diffraction patterns were obtained over the range of 5–60° using a 5°/min scan speed.

2.10. Stability study

The optimal Nanomatrix-1 was sealed in EP tubes and stored at room temperature for 1 year, then crystallinity, drug release, and absorption studies were carried out.

2.11. Supersaturation stabilizing effect of different polymers [39]

Different types of polymers, including Eudragit®L100-55, Eudragit®L100, Eudragit®S100, HPMC-E5, HPMC-E50, and PVP-K29, were dissolved in PBS (pH 7.4) at a concentration of 0.12%. Fenofibrate–DMSO solution with a high concentration (100 mg/ml) was prepared first, and then 100 μl of this solution was added to 100 ml of the polymer solution under agitation. A PBS (pH 7.4) without any polymer was used as a reference. After 5, 30, 60, 120 min, 2 ml samples were immediately removed and passed through a 0.2 μm PTFE membrane. The samples were immediately diluted in a 1:1 ratio with ethanol, mixed and transferred to 1.5 ml vials for analysis. The measurements were performed in duplicate and averages are reported here.

3. Results and discussion

3.1. The preparation of nanomatrix and the reference formulations

The fenofibrate-loaded nanomatrix with different combinations of silica and Eudragit® was obtained as listed in Table 1. The preparation process was found to be easy and simple except that the Nanomatrix-6 was rather sticky and hard to handle, possibly due to the absence of a dispersion effect from the colloidal silica. An illustration diagram of the fenofibrate existing in the nanomatrix of Sylysia®350 and Eudragit® is given in Fig. 1.

The physical mixture was prepared with the same composition of Nanomatrix-1. The droplet size of the self-made fenofibrate SMEDDS after emulsification in water was around 30 nm and there was no precipitation in the resultant emulsion within 8 h as shown by visual inspection. Meanwhile, the crude fenofibrate (the mean particle size was around 35 μm) suspended in 0.5% HPMC solution was also formulated as a control for the pharmacokinetic study. All the formulations had the same drug content.

3.2. In vitro dissolution study

Fig. 2A illustrates the release of fenofibrate from different formulations in PBS solution. There was a clear increasing sequence observed in terms of the dissolution rate: crude fenofibrate powder < Lipanthyl® < Nanomatrix-5 < Nanomatrix-6 < Nanomatrix-1 and Nanomatrix-1 after 1 year of storage. As expected, the crude drug displayed the slowest dissolution under the same conditions, followed by the commercial product with micronized fenofibrate, suggesting that micronization had a favorable effect on drug release. When the drug was associated with Sylysia®350, the release increased further, which explained the contribution of the high surface area of the silica particles to the higher drug dissolution. On the other hand, the behavior of Nanomatrix-6 demonstrated that the solid dispersion of fenofibrate in Eudragit®L100-55 efficiently promoted the in vitro drug release. However, the combination of both colloidal silica and Eudragit®L100-55 led to the highest dissolution rate of fenofibrate, which confirmed the effect of nanomatrix composition on the dissolution enhancement. After 1 year of storage at room temperature, Nanomatrix-1 remained almost the same drug release behavior, confirming the physical stability of the nanomatrix formulation.

In order to achieve the optimal composition, we compared the in vitro drug release of nanomatrix formulations with different

Table 1
Main fenofibrate formulations prepared for the studies.

Code	Fenofibrate (F)	Sylysia®350 (S350)	Aerosil®200 (A200)	Eudragit® L100-55 (EL100-55)	Eudragit® L100 (EL100)	Eudragit® S100 (ES100)
Nanomatrix-1	100 mg	300 mg	–	300 mg	–	–
Nanomatrix-2	100 mg	300 mg	–	–	300 mg	–
Nanomatrix-3	100 mg	300 mg	–	–	–	300 mg
Nanomatrix-4	100 mg	–	300 mg	300 mg	–	–
Nanomatrix-5	100 mg	600 mg	–	–	–	–
Nanomatrix-6	100 mg	–	–	600 mg	–	–
Physical mixture	100 mg	300 mg	–	300 mg	–	–

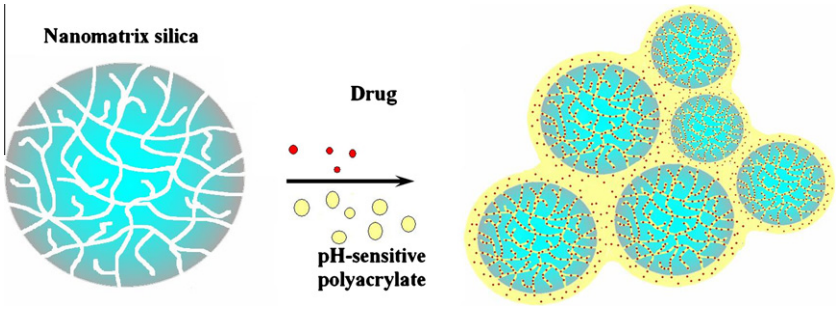

Fig. 1. Schematic diagram of nanomatrix structure of fenofibrate, pH-sensitive polymethylacrylate, and mesoporous silica. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

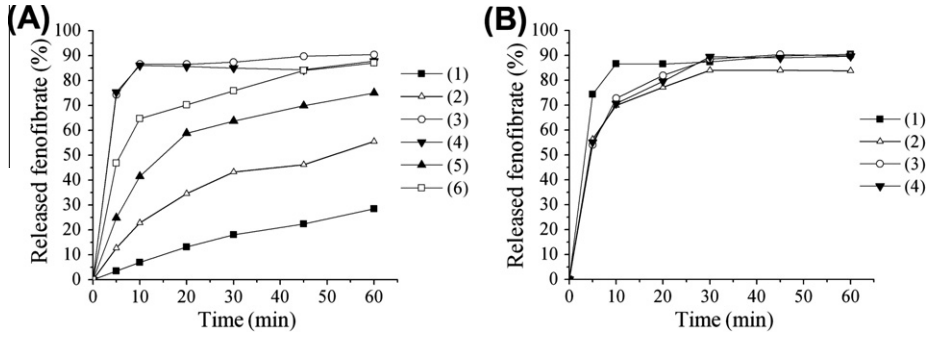

Fig. 2. Dissolution of fenofibrate from different formulations in PBS solution (0.01 M, pH7.3 ± 0.1) containing SDS (0.3%, w/v) at 37 °C. (A): (1) crude fenofibrate powder, (2) a capsule of micronized fenofibrate (Lipanthyl®), (3) Nanomatrix-1, (4) Nanomatrix-1 after 1 year of storage, (5) Nanomatrix-5, (6) Nanomatrix-6; (B): (1) Nanomatrix-1, (2) Nanomatrix-2, (3) Nanomatrix-3, (4) Nanomatrix-4.

kinds of colloidal silica and pH-sensitive polymethylacrylate, and the results are presented in Fig. 2B. It was found that the dissolution rate decreased slightly when Sylysia®350 was changed to Aerosil®200, or Eudragit®L100-55 to Eudragit®L100 or S100. The difference among the three polymethylacrylates might be related to their dissolution pH, and that between Sylysia®350 and Aerosil®200 might be connected with their properties, such as their surface area. However, the best choice to date seems to be Nanomatrix-1, although this needs to be proved by in vivo studies. In the dissolution study, 0.3% SDS was added to the release medium in order to meet the requirement for the sink condition.

The polymethylacrylates, Eudragit®L100-55, L100, and S100, used in this study, dissolve at about pH 5.5, 6.0, and 7.4, respectively, and insoluble in acidic mediums. They are the typical enteric (or pH-sensitive) materials used for enteric coatings in pharmaceutical industry for many years. In other words, their pH-sensitivity has been well proved. As a matter of fact, our group has tested the drug release from the nanoparticles made of these polymethylacrylates in different pH mediums before [20]. So, we did not emphasize on their pH-sensitivity in this study, but the effect of these polymers on the drug dissolution in vitro and the drug absorption in vivo.

3.3. In vivo absorption study

The plasma drug concentration–time profiles of fenofibrate after oral administration of various formulations to SD rats was shown in Fig. 3 and the related pharmacokinetic parameters are summarized in Table 2.

As seen in Fig. 3A, the drug absorption of Nanomatrix-1 was compared with that of other reference formulations, Lipanthyl®-a capsule of micronized fenofibrate, a SMEDDS formulation, and the crude fenofibrate suspension in 0.5% HPMC solution. The oral bioavailability of the drug suspension, the micronized drug, SMEDDS and Nanomatrix-1 increased steadily as shown by the AUC_{0-36h} values, although these formulations had a similar MRT in rats. The relative bioavailability of Nanomatrix-1 was 535%, 256%, and 113% in comparison with fenofibrate suspension, Lipanthyl®, and SMEDDS, respectively. The formulation of SMEDDS exhibited the shortest T_{max} (1 h) and highest C_{max} (111.5 μg/ml) among all the tested preparations, while its drug level also decreased faster from the peak than the others. It is well known that SMEDDS, with a high level of powerful surfactants and nano-sized droplets after emulsification in water, can substantially facilitate the absorption of the insoluble drugs, making it very suitable for

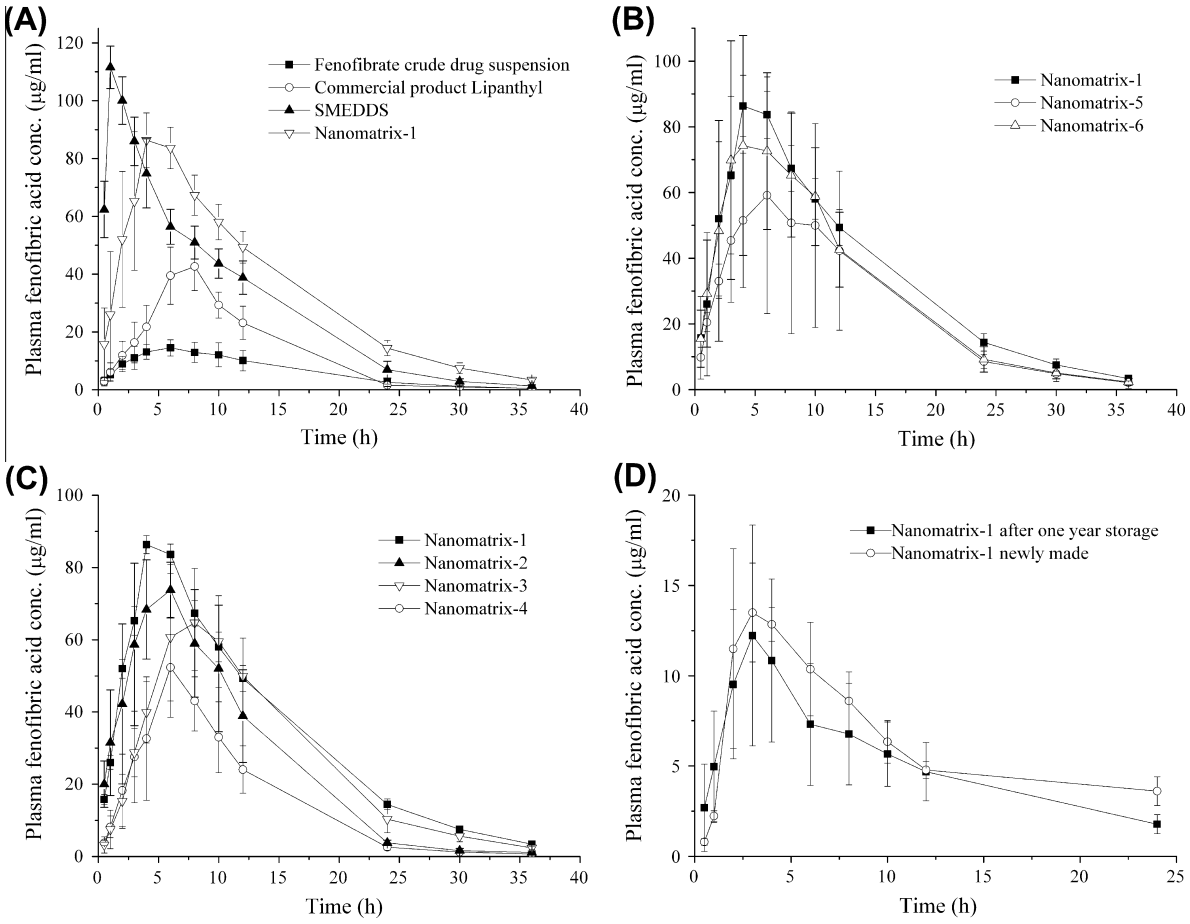

Fig. 3. Average plasma drug concentration–time profiles of fenofibrate following oral administration of different formulations to SD rats. The oral dose in Fig. 3A–C was 33 mg/kg, and the oral dose in Fig. 3D was 10 mg/kg. Data are presented as means ± SD (n = 5 in Fig. 3A, 3B, 3C and n = 3 in Fig. 3D).

Table 2
Pharmacokinetic parameters of fenofibrate formulations in SD rat (dose 33 mg/kg, n = 5, means ± SD).

Formulation	T_{max} (h)	C_{max} (μg/ml)	AUC_{0-36} (h·μg/mL)	MRT_{0-36} (h)
Crude drug	6.8 ± 2.3	15.2 ± 3.7 [b]	226.9 ± 37.6 [b]	10.9 ± 1.4
Lipanthyl®	6.8 ± 1.1 [b]	47.5 ± 5.9 [b]	473.5 ± 59.9 [b]	9.4 ± 0.5 [a]
SMEDDS	1.0 ± 0.0 [b]	111.5 ± 7.3 [b]	1070.5 ± 120.8	8.2 ± 0.5 [b]
Nanomatrix-1	4.4 ± 1.5	90.2 ± 6.4	1214.3 ± 70.2	10.8 ± 0.7
Nanomatrix-2	5.0 ± 1.4	75.3 ± 9.2	926.0 ± 217.6 [a]	8.7 ± 0.4 [b]
Nanomatrix-3	8.4 ± 1.7 [b]	65.3 ± 15.0 [a]	964.1 ± 187.4 [a]	11.4 ± 0.7
Nanomatrix-4	6.4 ± 0.9	53.5 ± 13.6 [b]	560.7 ± 120.2 [b]	9.2 ± 0.6 [a]
Nanomatrix-5	5.4 ± 1.3	60.5 ± 26.1 [a]	903.9 ± 344.0	10.7 ± 0.9
Nanomatrix-6	5.6 ± 3.3	85.9 ± 20.8	1071.1 ± 236.8	10.0 ± 0.9

[a] $p \leqslant 0.05$ vs. Nanomatrix-1.
[b] $p \leqslant 0.01$ vs. Nanomatrix-1.

drug absorption. Here, Nanomatrix-1 had comparative oral bioavailability with SMEDDS and also has advantages in terms of the absence of surfactant and a solid state.

Fig. 3B shows the contribution of two excipients, Sylysia®350 and Eudragit®L100-55, to the drug absorption. It is clear from Table 2 that Nanomatrix-6 (only Eudragit®L100-55) had a higher AUC_{0-36h} value than Nanomatrix-5 (only Sylysia®350), while Nanomatrix-1 (with two excipients) had a higher AUC_{0-36h} than either of them. If we compare these data with those of the references in Fig. 3A, it is clear that Nanomatrix-5 and Nanomatrix-6 exhibit a higher drug absorption than the suspension and the micronized powder. Especially for the formulation prepared with only Eudragit®L100-55, the AUC_{0-36h} had no significant difference when compared with Nanomatrix-1, which means Eudragit® here played an important role in improving the drug absorption. It had been reported that Eudragit® can serve as a stabilizing agent for supersaturating state of drugs and have the potential for prolonging drug absorption presumably by extending the release of drug from the polymeric matrix along the small intestine [22,40]. However, when only Eudragit® was used in the formulation, the finally products become rather sticky and hard to handle. In the literature, silica was reported to serve as an anti-adhesion agent [41]. In our composite, the silica serves not only as an anti-adhesion agent but also a matrix with nano-sized pores. In vivo results demonstrate that the combination of Sylysia®350 and Eudragit® reduces the variability of fenofibrate absorption. All these data support the beneficial effects of Sylysia®350 and Eudragit®L100-55 with regard to absorption enhancement.

In Fig. 3C, the drug concentration–time profiles of different nanomatrix formulations are compared. According to the results, the combination of Sylysia®350 and Eudragit®L100-55 showed the best drug absorption, which is in good agreement with the in vitro release test. It can be seen in Fig. 3C that the type of colloidal silica had a significant impact on drug absorption. When Sylysia®350 was changed to Aerosil®200, the AUC_{0-36h} value was reduced by more than half while the type of Eudragit® had less

of an effect. The nano-porous silica Sylysia®350 has stronger potency to promote drug absorption. Eudragit®L100-55, L100, and S100 produced a different decreasing C_{max} likely related to their different dissolution pH values (5.5, 6.0, and 7.4, respectively). The formulation prepared with Eudragit®L100-55 displayed higher bioavailability compared to Eudragit®L100 and S100 formulation, with p value was 0.038 and 0.043, respectively. There is no significant difference between the formulation prepared by Eudragit® L100 and S100.

Fig. 3D shows that the oral bioavailability of Nanomatrix-1 after 1 year of storage was similar to that of the freshly prepared form. This finding was also consistent with the in vitro release study. The good storage stability is very important since this is usually a big problem for nano-sized drug delivery systems. Possibly due to the huge surface area of the colloidal silica and the solid dispersion effect of Eudragit®L100-55, the drug molecules could be maintained in a molecular or amorphous state for a relatively long time.

In general, the in vivo studies demonstrated that Nanomatrix-1, with the combination of Sylysia®350 and Eudragit®L100-55, produced the highest oral bioavailability for fenofibrate in rats and also reduced the variability of absorption. So, it was used in the following investigation of the absorption mechanism.

3.4. Surface area and pore size analysis

The key parameters including the surface area, pore volume, and pore diameter of colloidal silica and Nanomatrix-1 derived from the nitrogen adsorption experiments are listed in Table 3. The pore size distribution curves are also presented (Fig. 4) in order to show more clearly the difference among the three samples.

As seen in Table 3, all materials tested, especially the two kinds of colloidal silica, had a small pore volume but a huge surface area. Clearly, sylysia®350 had a larger surface area and pore volume than Aerosil®200, and the BJH desorption cumulative surface area of the pores for Sylysia®350 is almost twofold that of Aerosil®200. This observation is very important since it fully explains the higher dissolution rate and the higher oral bioavailability of the nanomatrix formulation containing Sylysia®350 (Figs. 2B and 3C), as well as the significance of the huge surface area for drug absorption.

As we know, Aerosil®200 is a non-porous silica material with an average particle size of 12 nm (data of the company), and Sylysia®350 is a porous silica material. As shown in the pore volume vs. pore diameter curves (Fig. 4), Sylysia®350 had a great pore volume and a corresponding pore diameter around 18 nm. While Aerosil®200 did have a small pore volume which may be related to the pores of the aggregates of the primary particles. Table 3 gives more details about the nano-pores of Sylysia®350, the optimized silica material in this study, including a big pore surface area of 341.5 m^2/g and a great pore volume of 1.5 cm^3/g, as well as an average pore diameter of 17.8 nm. It was also found that Nanomatrix-1 (Sylysia®350 with drug and Eudragit) still had a pore diameter in nano-range as shown in Table 3. Therefore, the test here demonstrated that Sylysia®350 and Nanomatrix-1 possessed the nano-structure (nano-pores).

Additionally, from Sylysia®350 to Nanomatrix-1, it was observed that the pore diameter decreased from 17.8 nm to 13.1 nm, the pore surface area from 341.5 m^2/g to 58.6 m^2/g, and the pore volume from 1.5 cm^3/g to 0.2 cm^3/g, respectively (Table 3). All these data revealed that there might be some drug and Eudragit located inside of the pores of the silica particles, besides some adsorption on the outside surface. Considering the preparation process of Nanomatrix-1, it seems reasonable to obtain such results. Anyhow, all these support the illustration about the structure of the nanomatrix system as shown in Fig. 1.

Table 3
The related properties of colloidal silica and Nanomatrix-1.

Samples	Sylysia®350	Aerosil®200	Nanomatrix-1
BET Surface Area (m^2/g)	261.4	192.1	48.4
BJH Desorption Cumulative Surface Area of Pores(m^2/g) [a]	341.5	179.6	58.6
BJH Desorption Cumulative Pore Volume of Pores (cm^3/g) [a]	1.5	0.4	0.2
BET Average Pore Diameter (nm, 4 V/A)	24.2	10.2	18.1
BJH Desorption Average Pore Diameter (nm, 4 V/A)	17.8	8.9	13.1

[a] pores with diameter between 1.7 and 50.0 nm.

3.5. Scanning electron microscopy and transmission electron microscopy

The structural characteristics of Aerosil®200, Sylysia®350, and the Nanomatrix-1 are clearly visible in their SEM and TEM images, as shown in Fig. 5. As seen from the SEM images, Aerosil®200 (Fig. 5a) was composed of small particles much less than 1 μm, while the Sylysia®350 (Fig. 5b) was made up of more compacted micron-sized particles. The morphology of Nanomatrix-1, formulated with Sylysia®350, Eudragit® and drug, was basically the same as that of Sylysia®350 (Fig. 5c). The TEM images show the more delicate structures of the materials tested (Fig. 5d–f). It was clear that the Aerosil®200 was small nanoparticles with a visible boundary and the particle size was about 10 nm (Fig. 5d). A sponge-like mesoporous structure with irregular pores can be seen in Sylysia®350 (Fig. 5e). Compared with that of Sylysia®350, the black area increased in the TEM image of Nanomatrix-1 (Fig. 5f) under the same test conditions, suggesting the adsorption of the drug and Eudragit® on the particles. It is important that the sponge-like mesoporous structure in Nanomatrix-1 is retained, which may account for the enhanced drug release and absorption. In general, TEM images confirmed again the findings in above studies on surface area and pore size.

3.6. Differential scanning calorimetry

The DSC thermographs of various formulations are shown in Fig. 6A. For the crude fenofibrate, a sharp endothermic peak occurred at 82.2 °C. In the physical mixture of drug and excipients, with the same composition as in Nanomatrix-1, a melting peak of 80.2 °C was observed, although the peak was markedly reduced because of the dilution effect, indicating that the drug was still in crystalline form. In addition, there were no endothermic peaks found in the DSC curves of the other test groups. In addition, there were no endothermic peaks found in the DSC curves of the other test groups. For colloidal silica and Eudragit®, this was likely due to their amorphous states. As for the Nanomatrix-1, this indicated that fenofibrate was highly dispersed in the nanomatrix formulation in a molecular or amorphous state. Even after 1 year of storage, there was still no crystalline peak observed, which was consistent with drug release and oral bioavailability, confirming again the good physical stability of the nanomatrix formulation. This might be attributed to the nanomatrix composition in terms of crystallization inhibition due to the adsorption effect of colloidal silica and the dispersion effect of Eudragit®.

3.7. Powder X-ray diffraction

Fig. 6B displayed the XRD patterns of different formulations investigated. Generally, the XRD analysis agreed well with the DSC results. The raw fenofibrate showed obvious diffraction peaks

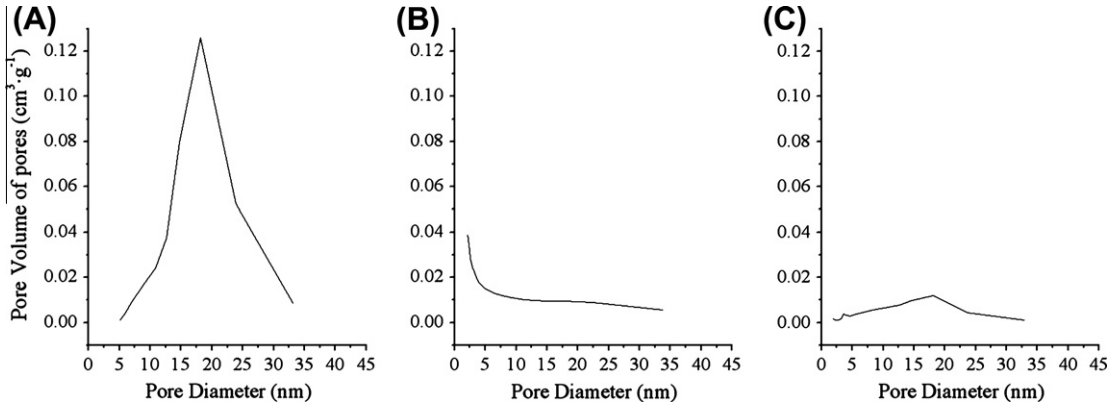

Fig. 4. The pore size distribution curves deprived from desorption isotherms. (A): Sylysia®350; (B): Aerosil®200; (C): Nanomatrix-1.

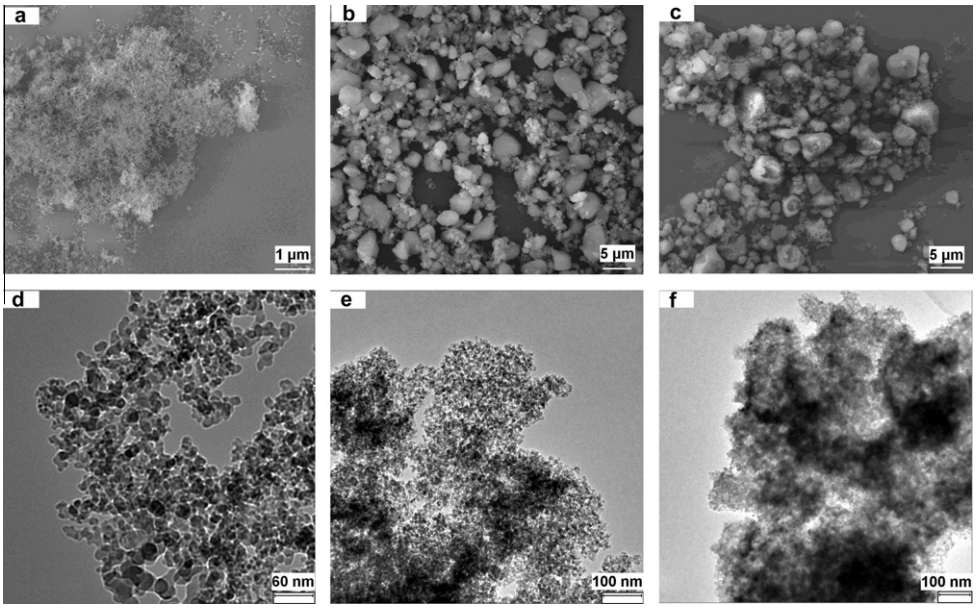

Fig. 5. SEM images of Aerosil®200(a), Sylysia®350(b), and the Nanomatrix-1(c); TEM images of Aerosil®200(d), Sylysia®350(e), and the Nanomatrix-1(f).

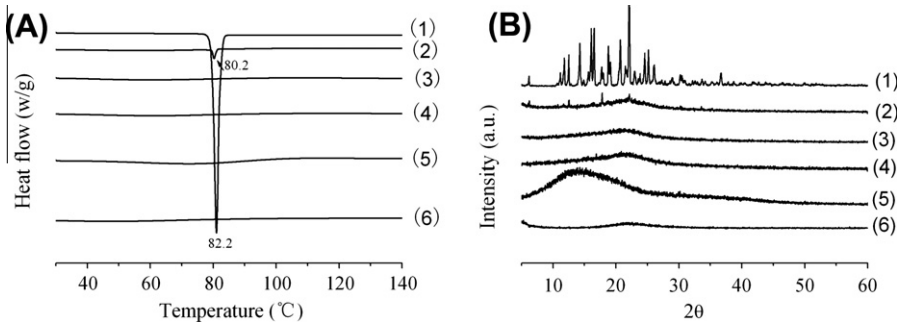

Fig. 6. DSC (A) and XRD (B) profiles of different formulations. (1): fenofibrate, (2): physical mixture of fenofibrate, Sylysia®350, and Eudragit®L100-55 (1:3:3), (3): Nanomatrix-1, (4): Nanomatrix-1 after 1 year storage, (5): Eudragit®L100-55, (6): Sylysia®350.

due to its crystalline structure, and the physical mixture of drug and excipients exhibited some small peaks, while the other test groups showed no peaks in the XRD. This further confirmed the amorphous nature of the colloidal silica and Eudragit®L100-55, the absence of drug crystals in Nanomatrix-1, and no peaks even after 1 year of storage. According to previous reports, when a drug is loaded onto the silica material, the amorphous drug tends to change into crystalline form after storage [34,42]. However, it was proved here that the addition of both Eudragit® and silica prevented the drug changing into the crystalline state and maintained the long-term stability, suggesting that the combination of silica and Eudragit® might be necessary.

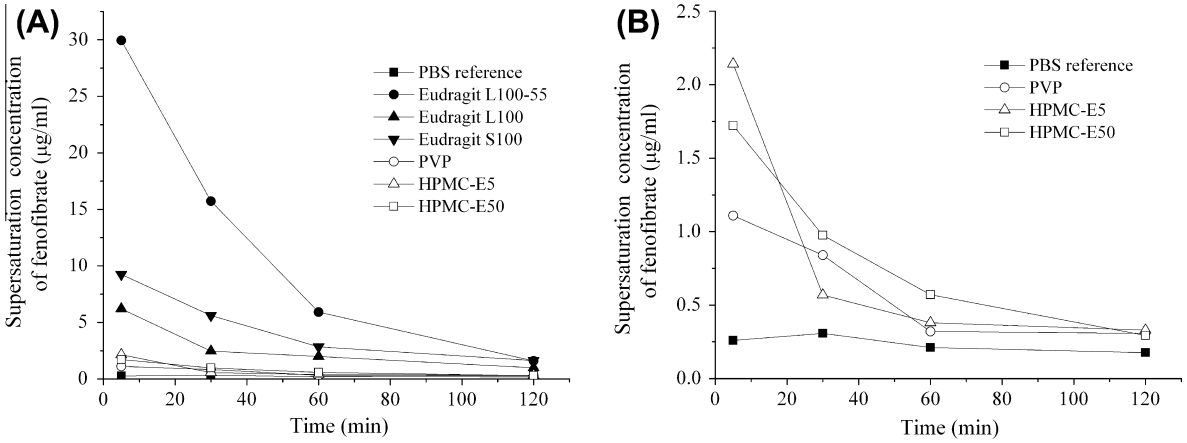

Fig. 7. Concentration-enhancing effect of different polymers on fenofibrate, the polymer concentration was 0.12%, the weight ratio of the fenofibrate to polymer was 10 mg: 60 mg. (Fig. 7B is a partial magnification of Fig. 7A).

3.8. Supersaturation stabilizing effect of different polymers

By definition, supersaturation stabilizing polymers provide increased levels of drug in solution in excess of the normal equilibrium solubility through either physical and/or chemical interactions with drug molecules that inhibit precipitation [40]. Fig. 7 showed the supersaturation stabilizing effects of six different polymers. Drug in PBS solution without any polymer precipitated very quickly. Addition of polymers resulted in an increased drug concentration in solution. It was clear that Eudragit® displayed better stabilizing effect than PVP and HPMC, while Eudragit®L100-55 was the best, which is in good agreement with previous observations involving drug release and absorption, and further confirmed our formulation optimization. It has been reported recently that some concentration-enhancing polymers can significantly improve drug bioavailability [22,43,44,39], and Eudragit® was one of concentration-enhancing agents used in these studies. Polymers may inhibit nucleation or crystal growth by adsorption on the crystal interface thereby blocking crystal growth [39].

4. Conclusion

Based on the preparation process and the above observations, it appears that the solid dispersion of fenofibrate with Eudragit® (1:3) is adsorbed on the huge surface area of mesoporous silica (1:3:3), and forms a nanomatrix structure in which the drug is present in a molecular or amorphous state. Possibly due to the great adsorption effect from the huge surface area of mesoporous silica and the good solid dispersion effect from the hydrophilic Eudragit®, the state of the insoluble drug and, accordingly the drug behaviors in vitro and in vivo, could be maintained for quite a long time. When the nanomatrix system is exposed in release medium or the GI tract, the huge surface area of mesoporous silica facilitates the drug release. In addition, the pH-sensitive Eudragit® dissolves in the small intestine, the main absorption site of drug, and acts as a supersaturation stabilizing agents, leading to a higher local drug concentration. All these factors ensure high drug bioavailability.

In conclusion, we prepared a novel nanomatrix system for oral administration of insoluble fenofibrate, in a simple and convenient way, using the pH-sensitive polymethylacrylate and nano-porous silica already used in pharmaceutical processes. It was demonstrated in our study that this system improved both the drug dissolution and the drug absorption. The different specification of polymethylacrylate and mesoporous silica had a clear effect on the in vitro and in vivo behavior of the nanomatrix formulation, and the best result was obtained with Eudragit®L100-55 and Sylysia®350. The optimized nanomatrix system produced a higher drug absorption when compared with fenofibrate SMEDDS. It was proved that the drug is present in a molecular or amorphous state in the nanomatrix, and this can be maintained for 1 year. There is a good correlation between drug release, oral bioavailability and other in vitro observations. The improvement may be due to the huge surface area of the mesoporous silica, as well as the pH-sensitivity, solid dispersion effect, and supersaturation stabilizing effect of polymethyacrylate.

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