



## Pharmaceutical Nanotechnology

## Bioavailability and pharmacokinetics of sorafenib suspension, nanoparticles and nanomatrix for oral administration to rat

Xue-qing Wang<sup>a</sup>, Jie-ming Fan<sup>a</sup>, Ya-ou Liu<sup>b</sup>, Bo Zhao<sup>a</sup>, Zeng-rong Jia<sup>a</sup>, Qiang Zhang<sup>a,\*</sup><sup>a</sup> State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China<sup>b</sup> Department of Pharmacy, The First Hospital of Peking University, Beijing 100034, China

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## ABSTRACT

Sorafenib is slightly absorbed in the gastrointestinal tract due to its poor solubility in water. To improve its absorption, a novel nanoparticulate formulation-nanomatrix was used in the study. The nanomatrix was a system prepared from a porous material Sylsisa<sup>®</sup> 350 and a pH sensitive polymer Eudragit<sup>®</sup>. The formulations were optimized by orthogonal design ( $L_9(3^4)$ ) and their bioavailability were evaluated in rat, comparing to pH-sensitive Eudragit nanoparticles and suspension of sorafenib. In the formulations, the ratio of sorafenib to Eudragit<sup>®</sup> S100 was found to be more important determinant of the sorafenib bioavailability than the ratio of sorafenib to Sylsisa<sup>®</sup> 350. As for the bioavailability, the  $AUC_{0-36h}$  of sorafenib nanomatrix was 13–33 times to that of sorafenib suspension, but only 16.8% to 40.8% that of Eudragit<sup>®</sup> S100 nanoparticles. This may be resulted from the different drug dispersion degree, release character and bioadhesion activity. However, because all the materials used in the nanomatrix formulation are commonly adjuvant, safe, easy to get and cheap, above all, the nanomatrix formulation can solve the stability and scaling up problems in the nanoparticles, it had potential to develop into a product in the future.

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## 1. Introduction

With the development of combinatorial chemistry, high throughput screening and cell based activity assays (Stegemann et al., 2007), more and more new chemical entities (NCE) are developed. However, up to 40% of the NCE in development have been suggested to be 'poorly water-soluble' (Porter et al., 2008). To improve the oral bioavailability of these poorly water-soluble NCE, many strategies have been applied, such as using water soluble polymer (Savolainen et al., 1998), surfactant (Ruddy et al., 1999), micronization (Rasenack et al., 2003), solid dispersion technology (Vasconcelos et al., 2007), lipid-based formulations (Douroumis and Fahr, 2006), nanocrystal technology (Junghanns et al., 2008), self-microemulsifying drug delivery system (SMEDDS) (Lee et al., 2009) and nanoparticulate technology (Merisko-Liversidge and Liversidge, 2008).

Nanoparticles are carriers ranging in size from 10 to 1000 nm, generally smaller than 200 nm. It has been widely used to improve the bioavailability of poorly water-soluble drugs (Merisko-Liversidge and Liversidge, 2008; Jaeghere et al., 2000; El-Shabouri, 2002; Wang et al., 2008). The previous study in our lab embedded cyclosporine A into a series of pH sensitive nanoparticles, and

demonstrated the effectiveness of such approach in terms of oral absorption enhancement (Wang et al., 2004, 2008; Dai et al., 2004).

Although nanoparticles are used to improve the absorption successfully, there are also some obstacles for such formulation to be commercialized. First, the stability of such a system is a challenge. Nanoparticle dispersion is a typical thermodynamically unstable system due to its large specific interfacial area. After a period of storage, particle aggregation often occurs. Mainly two important efforts have been adopted to increase the stability. One technique is lyophilization and the other is adding suspending agents. As for lyophilization, the particle size could increase during the procedure of freeze drying, which may affect the drug pharmacokinetic characteristics (Saez et al., 2000) or the lyophilized nanoparticles may aggregate after some time of storage (Chacón et al., 1999, Dai et al., 2005). In the second method, we had demonstrated the improvement in stability of nanoparticle colloid by adding some suspending agents. Although the relative bioavailability was also bioequivalent to the initial after 18 months storage at 25 °C, it was indeed decreased (nearly 20%) (Wang et al., 2006b). Furthermore, the dosage form was oral solution, which was not so convenient as tablets or capsules.

Another challenge of the nanoparticle colloid system is the possibility in scaling-up, which is an essential process for a marketed product.

Here, we introduce a novel technology, nanomatrix technology to solve these difficulties. We define the nanomatrix as a

\* Corresponding author. Tel.: +86 10 82802791; fax: +86 10 82802791.

E-mail address: [zqdodo@bjmu.edu.cn](mailto:zqdodo@bjmu.edu.cn) (Q. Zhang).

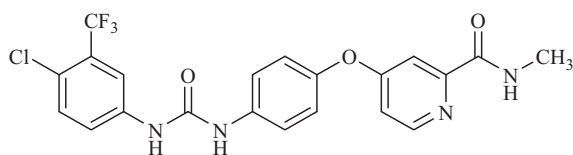


Fig. 1. Chemical structures of sorafenib.

system composed of a matrix material with nano-structure, drug and other excipient. The mesoporous silica particles of Sylsya 350 in this study, with a mean particle size of 3.9  $\mu\text{m}$  and a large number of internal pores about 21 nm, are the typical matrix material with nano-structure. And they provide large specific surface area (300  $\text{m}^2/\text{g}$ ) (Wang et al., 2006a) to support and disperse drug molecules as well as excipient, such as the pH sensitive polymer tested here. Eudragit S100 may disperse drug molecules, prohibit drug crystallization and enhance the bio-adhesion of the system in GI tract. Anyhow, a nanomatrix system seems favorable in terms of drug absorption enhancement.

The drug used in the study was sorafenib. Sorafenib (Fig. 1) is a small molecule that inhibits tumor-cell proliferation and tumor angiogenesis and increases the rate of apoptosis in a wide range of tumor models (Wilhelm et al., 2004; Chang et al., 2007). It has been approved by the FDA for the treatment of patients with advanced renal cell carcinoma in 2005 and unsecretable hepato-carcinoma in 2006. However, sorafenib is poorly soluble in water and its solubility was smaller than the quantitative limit (25  $\text{ng}/\text{ml}$  in HPLC method) in our previous test in deionized water. To improve its solubility, sorafenib tosylate is used and prepared into tablets (Nexavar, Bayer HealthCare Pharmaceuticals-Onyx Pharmaceuticals). Practically, sorafenib tosylate is also insoluble in aqueous media (PCT, 2008), and its solubility is only 60  $\mu\text{g}/\text{ml}$  in water at pH 6. Because of its poor water solubility, sorafenib tosylate is slightly absorbed in the gastrointestinal tract and exhibits a large interindividual variability in pharmacokinetics (Blanchet et al., 2009). Up to now, there are only a few studies on the absorption improvement of sorafenib (PCT, 2008; Liu et al., 2011).

In the present study, sorafenib suspension, nanoparticle colloids and nanomatrix were prepared and their oral absorption was evaluated.

## 2. Materials and methods

### 2.1. Materials

Sorafenib was purchased from Wish pharmaceutical Co., Ltd (China). Internal standard megestrol acetate was from Qingdao Ruige Co., Ltd (China). Methanol and acetonitrile were the products of Merck (Germany). Eudragit L100-55, Eudragit L100 and Eudragit S100 were from Evonik (Germany). Sylsya 350 was the gift from Fuji Chem. (Japan). All other chemicals were of analytical grade. Sprague-Dawley (SD) rats were obtained from Animals Center of Peking University Health Science Center. The animal experiment was adhered to the principles of care and use of laboratory animals and was approved by the Institutional Animal Care and Use Committee of Peking University Health Science Center.

### 2.2. Preparation of sorafenib and sorafenib tosylate suspension

Sorafenib suspension and sorafenib tosylate suspension were prepared by dispersing sorafenib or sorafenib tosylate in 0.9% saline containing 4  $\text{mg}/\text{ml}$  starch (final concentration of sorafenib was 10  $\text{mg}/\text{ml}$ ) through ultrasonication for 3 min.

### 2.3. Preparation of sorafenib nanoparticle colloids

Three types of Eudragit (Eudragit L100-55, Eudragit L100 and Eudragit S100) nanoparticles used in this study were prepared by solvent displacement method. Briefly, 37.5 ml ethanol containing 9 mg sorafenib and 187.5 mg Eudragit was injected as soon as possible into 93.8 ml stirring water containing 2  $\text{mg}/\text{ml}$  Pluornic F<sub>68</sub> with a 7# needle for bone marrow puncture. Afterward, the mixture was stirred for another 15 min and evaporated to about 30 ml in a 60 °C water bath. The particle size was determined by dynamic light scattering (Malvern Zetasizer Nano-ZS, Malven Instruments, Malven, UK).

### 2.4. Preparation of sorafenib nanomatrix

The sorafenib nanomatrix was prepared as follows: first, 100 mg sorafenib and prescribed Eudragit S100 were dissolved in 40 ml ethanol. Then prescribed Sylsya 350 was dispersed into the solution under stirring. After ultrasonication for 20 min, the dispersed system was transferred to rotary evaporation to remove the ethanol. The solid product was collected, milled and sieved through 100 mesh. The sieved powder was used for the bioavailability studies.

Totally nine sorafenib nanomatrix formulations were prepared for optimization, which was conducted by an orthogonal design ( $L_9(3^4)$ , Table 1). The factors include the ratio of sorafenib to Eudragit S100 and the ratio of sorafenib to Sylsya 350. The evaluation index is the area under the concentration–time curve ( $\text{AUC}_{0-36\text{h}}$ ) of different formulations in bioavailability studies.

### 2.5. Bioavailability studies

The formulations used in this study include: (1) sorafenib suspension and sorafenib tosylate suspension; (2) sorafenib Eudragit nanoparticle colloids; (3) sorafenib nanomatrix formulations. Healthy male Sprague-Dawley rats (weighing 160–190 g) were used and they were fasted overnight with free access to water.

The experiments were done by three times. First, 10 rats were divided into two groups at random (5 each group). One group was administered with a single dose of sorafenib suspension at 100  $\text{mg}/\text{kg}$ . The other group was given sorafenib tosylate suspension at a single dose 137  $\text{mg}/\text{kg}$  (including sorafenib 100  $\text{mg}/\text{kg}$ ). Second, 18 rats were divided into three groups at random (6 each group). Each group was administered with a single dose of sorafenib Eudragit nanoparticle colloids at 3  $\text{mg}/\text{kg}$ , respectively. Third, 45 rats were divided into nine groups at random (5 each group). Each group was administered with a single dose of sorafenib nanomatrix at 15  $\text{mg}/\text{kg}$ , respectively.

After administration of different formulations to rats by gavage, blood samples were collected from orbital venous into the heparinized tubes at preset time points of 0.5, 1, 2, 4, 8, 12, 24 and 36 h. Blood samples were centrifuged at 3000 rpm for 10 min and the resultant plasma were transferred into capped tubes and stored at  $-20\text{ }^\circ\text{C}$  until analysis.

### 2.6. HPLC assay of sorafenib in plasma

The concentration of sorafenib in the plasma was determined by HPLC. The Shimadzu LC-10A HPLC analysis system equipped with an ultraviolet detector (SPD-10A) was used. Chromatographic separation was achieved on a reversed phase C18 column (250  $\text{mm} \times 4.6\text{ mm}$ , 5  $\mu\text{m}$ , Phenomenex, USA) maintained at 40 °C with a mobile phase consisted of acetonitrile and water phase (70:30, v/v) at a flow rate of 1.0  $\text{ml}/\text{min}$ . The water phase contained triethylamine (20  $\text{ml}/1000\text{ ml}$ ) besides distilled water, and

**Table 1**  
Results of orthogonal experiment and direct-viewing analysis for the optimization of nanomatrix formulations.

No.	Factors		Formulations Sorafenib/Sylsya 350/Eudragit S100	AUC <sub>0–36h</sub> (ng/ml h)	
	A	B		Mean	SD
1	1	1	1:1:1	25669.6	2924.7
2	1	3	1:1:3	27142.6	4072.5
3	1	5	1:1:5	27256.8	2761.4
4	3	1	1:3:1	15175.4	1414.9
5	3	3	1:3:3	29751.8	4336.2
6	3	5	1:3:5	30543.1	1282.7
7	5	1	1:5:1	15489.5	2114.7
8	5	3	1:5:3	28923.8	2448.8
9	5	5	1:5:5	12567.6	2017.7
K1	80069.0	56334.5			
K2	75470.3	85818.2			
K3	56980.9	70367.5			
k1	26689.7	18778.2			
k2	25156.8	28606.1			
k3	18993.6	23455.8			
R	7696.0	9827.9			
Order	2	1			
Level	A1	B2			
Optimal formulation	1:1	1:3	1:1:3		

A: Sorafenib/Sylsya 350; B: Sorafenib/Eudragit S100.

was adjusted to pH 5.4 by phosphoric acid. The absorbance of the eluent was monitored at 265 nm.

For the analysis of sorafenib in plasma, the frozen plasma was thawed prior to the protein precipitation. An aliquot of 15  $\mu$ l megestrol acetate (internal standard, 80  $\mu$ g/ml) was added to 90  $\mu$ l of plasma. After mixing for 10 s, 270  $\mu$ l of acetonitrile was added to precipitate proteins. The tubes were vortex-mixed for 20 s, and centrifuged at 10 000 rpm for 10 min. The supernatant was transferred into a glass tube. Each sample of 20  $\mu$ l was injected into the chromatographic system.

### 2.7. Sorafenib release from nanomatrix

Sorafenib release tests from nanomatrix 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 or sorafenib suspension (equivalent to 0.1 mg of sorafenib) was placed in 200 ml 30% isopropyl alcohol solution (pH 6.8) at 37 °C and 100 rpm. An aliquot of 2 ml release media was withdrawn at intervals of 2, 5, 10, 20 min and then replaced by 2 ml of fresh release fluid. Each sample was passed through a 0.45  $\mu$ m syringe filter and determined by HPLC (see Section 2.6). The measurements were performed in triplicate and averages are reported here.

### 2.8. Data analysis

The pharmacokinetic parameters were obtained by using a pharmacokinetic software DAS (DAS 2.1.1 by the Clinical Trial Center of Shanghai University of Traditional Chinese Medicine, Shanghai, China). The area under the concentration–time curve (AUC<sub>0–36h</sub>) was estimated according to the trapezoidal rule. The maximal blood concentration ( $C_{max}$ ) and the time to reach  $C_{max}$  ( $T_{max}$ ) were obtained directly by observation. The differences between pharmacokinetic parameters in various test groups were estimated by the student *t* test.

## 3. Results and discussion

Sorafenib suspension, sorafenib tosylate suspension, sorafenib Eudragit nanoparticle colloids and sorafenib nanomatrix were prepared in the study. The particle sizes of the three Eudragit

nanoparticle formulations prepared from Eudragit L100-55, Eudragit L100 and Eudragit S100 were 81.8, 64.2 and 64.6 nm, respectively. And the final sieved powder of the nanomatrix was about 150  $\mu$ m.

The bioavailability of sorafenib and sorafenib tosylate was compared first. The mean sorafenib concentrations in the plasma after oral administration of a single dose of sorafenib suspension or sorafenib tosylate suspension to rats are depicted in Fig. 2A. The concentration–time data of the two preparations were best fitted to one-compartment-model with a weight of 1, and the relevant pharmacokinetic parameters such as  $C_{max}$ ,  $T_{max}$ , AUC<sub>0–36h</sub>, elimination constant (*K*), half time of elimination ( $t_{1/2}$ ), the absorption constant from gastrointestinal to compartment ( $K_a$ ), half time of absorption ( $t_{1/2a}$ ), mean residence time (MRT<sub>0–36h</sub>) and relative bioavailability ( $F_r$ ) are given in Table 2.

The results showed that the  $K_a$  of sorafenib suspension and sorafenib tosylate suspension were  $0.309 \pm 0.155$  h and  $0.317 \pm 0.116$  h, respectively. The tosylate salt was absorbed a little faster than sorafenib suspension, but without significant difference ( $p=0.929$ ). The  $C_{max}$  of sorafenib tosylate suspension ( $453.7 \pm 33.2$  ng/ml) was higher than that of sorafenib suspension ( $338.0 \pm 63.3$  ng/ml,  $p=0.011$ ). When the AUC<sub>0–36h</sub> was considered, the AUC<sub>0–36h</sub> of sorafenib tosylate suspension ( $7327.4 \pm 1443.1$  ng/ml h) was higher than that of sorafenib suspension ( $6118.2 \pm 1508.3$  ng/ml h,  $p=0.215$ ), about 20% increased. But, when the result compared with the intravenous formulation, it was rather poor (lower than 4.5% of intravenous microemulsion, data not shown).

To improve its bioavailability, sorafenib or sorafenib tosylate was loaded into Eudragit nanoparticles. Eudragit has been successfully used in nanoparticle preparation (Dai et al., 2004; Wang et al., 2004). The encapsulation efficiency of sorafenib in the Eudragit nanoparticles was higher than that of sorafenib tosylate (data not shown). The reason might be that sorafenib was more hydrophobic than sorafenib tosylate. So sorafenib was selected for the further nanoparticle study.

The mean sorafenib concentrations in the plasma after oral administration of a single dose of sorafenib nanoparticles to rats are shown in Fig. 2B and the relevant pharmacokinetic parameters are listed in Table 2. From the results we could see that at the dosage 3 mg/kg, the  $T_{max}$  of the three nanoparticle formulations were all 4 h, smaller than that of sorafenib suspension

**Table 2**  
Pharmacokinetic parameters of sorafenib after oral administration of sorafenib suspension, sorafenib tosylate suspension, sorafenib Eudragit nanoparticles to rat (mean  $\pm$  SD,  $n = 5-6$ ).

Parameters	Unit	Sorafenib (100 mg/kg)		Sorafenib tosylate (137 mg/kg)		Eudragit L100-55 NP (3 mg/kg)		Eudragit L100 NP (3 mg/kg)		Eudragit S100 NP (3 mg/kg)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
$C_{max}$	ng/ml	338.0	63.3	453.7	33.2	703.3	63.0	816.4	43.5	812.9	94.6
$T_{max}$	h	8.0	0.0	6.4	2.2	4.0	0.0	4.0	0.0	4.0	0.0
$AUC_{0-36h}$	ng/ml h	6118.2	1508.3	7327.4	1443.1	10188.1	398.1	13475.2	239.9	14953.7	1190.6
$K$	1/h	0.088	0.029	0.112	0.041	0.108	0.016	0.065	0.004	0.072	0.007
$t_{1/2}$	h	8.9	4.0	7.2	3.7	6.5	0.9	10.8	0.6	9.6	0.9
$K_a$	1/h	0.309	0.155	0.317	0.116	0.480	0.074	0.992	0.140	0.415	0.102
$t_{1/2a}$	h	2.9	1.8	2.4	0.9	1.5	0.2	0.7	0.1	1.8	0.4
$MRT_{0-36h}$	h	13.2	1.4	11.8	1.7	10.8	0.5	12.4	0.4	12.6	0.4
$F_r$ (%)		100		119.8		5551		7342		8147	

$F_r$  (%): The relative bioavailability of sorafenib tosylate suspension (ST) or sorafenib Eudragit nanoparticles (SNP) to sorafenib suspension (SS), which was calculated using the following equation:  $F_r(\%) = (AUC_{(0-36h)}^{ST \text{ or } SNP} / X_0^{ST \text{ or } SNP}) / (AUC_{(0-36h)}^{SS} / X_0^{SS}) \times 100$ .

(8 h). The  $C_{max}$  of sorafenib Eudragit L100-55, Eudragit L100 and Eudragit S100 nanoparticles were  $703.3 \pm 63.0$ ,  $816.4 \pm 43.5$  and  $812.9 \pm 94.6$  ng/ml, which were 69, 80 and 80 times that of sorafenib suspension at the same dose, respectively. The  $AUC_{0-36h}$  of sorafenib Eudragit L100-55, Eudragit L100 and Eudragit S100 nanoparticles were  $10188.1 \pm 398.1$ ,  $13475.2 \pm 239.9$  and  $14953.7 \pm 1190.6$  ng/ml h, representing 55, 73 and 81 times that of sorafenib suspension at the same dose, respectively. That is to

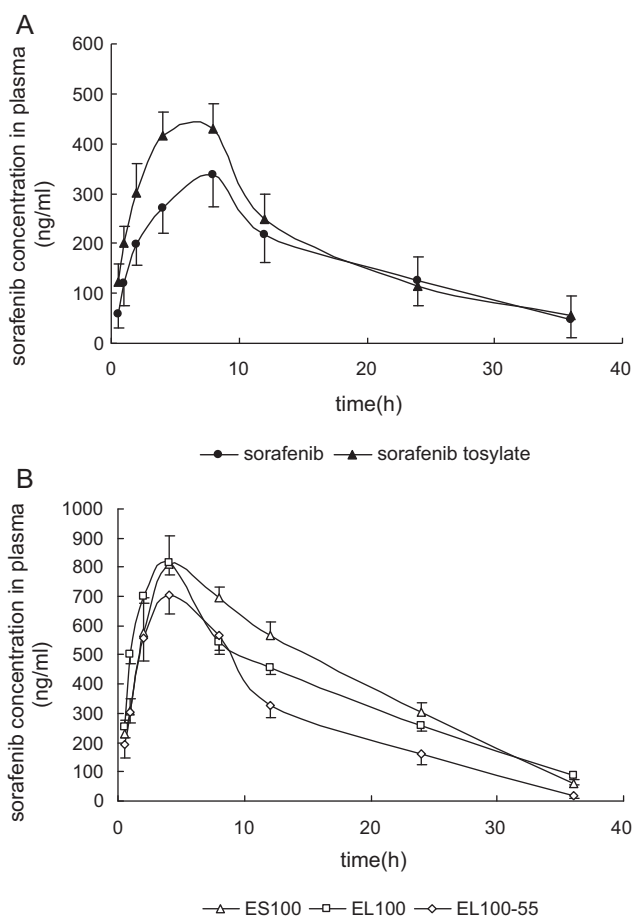
say, the three nanoparticle formulations increased the absorption of sorafenib quite effective (more than 50 times), no matter which type Eudragit was used. The reasons might be: (1) the amorphous or molecularly dispersed state of drug within the polymeric matrices (Wang et al., 2008), (2) supersaturated condition of drug in the intestinal lumen by the use of pH-dependant carrier polymers (Miller et al., 2008; Janssens et al., 2010), (3) the good bioadhesion activity of nanoparticle formulations to GI mucosa and their site-specific behaves, etc. (Wang et al., 2008).

Among the three nanoparticle formulations, their  $AUC_{0-36h}$  was ranked as Eudragit L100-55 < Eudragit L100 < Eudragit S100, all with significant difference. This might be due to the different pH sensitivity and lipophilic character of these polymers. The dissolution pH of Eudragit L100-55, L100 and S100 were 5.5, 6 and 6.8, respectively. Because the Eudragit S100 nanoparticles exhibited the largest relative bioavailability relative to sorafenib suspension (8147%), Eudragit S100 was selected for the following nanomatrix study.

The nanomatrix system used here was composed of Sylsya 350 and Eudragit S100. Orthogonal design ( $L_9(3^4)$ ) was used to optimize the nanomatrix formulation. And the orthogonal analysis results are shown in Table 1. The table shows that the factors include the ratio of sorafenib to Sylsya 350 (A) and the ratio of sorafenib to Eudragit S100 (B).  $AUC_{0-36h}$  was the objective function value.  $K$  was the sums of  $AUC_{0-36h}$  for the two factors under different levels.  $K_1$ ,  $K_2$  and  $K_3$  were the sums of  $AUC_{0-36h}$  under lever 1, lever 2 and lever 3, respectively. The value of  $k$  was the averages of  $AUC_{0-36h}$  for the two factors under different levels. The difference between the highest and the lowest among  $k_1$ ,  $k_2$ , and  $k_3$  was defined by the symbol "R". The higher the R, the greater the effects on the sorafenib bioavailability. As seen from Table 1, we found that R of the two factors was ranked as RB > RA ( $9827.9 > 7696.0$ ), which indicated the order of the two factors' effect on sorafenib bioavailability B > A. That is to say, the ratio of sorafenib to Eudragit S100 was more important determinant of the sorafenib bioavailability than the ratio of sorafenib to Sylsya 350.

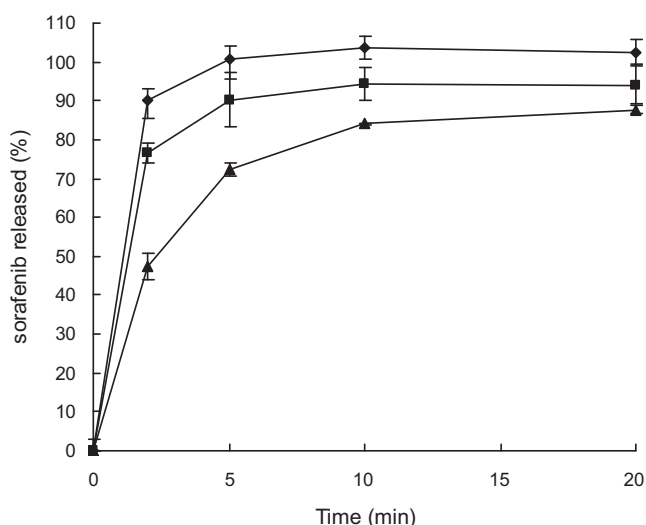
The value of  $k$  was used to determine the optimal level in the two factors; level with maximum  $k$  value was the optimal level. The individual levels within each factor were ranked as: A:  $1 > 2 > 3$  ( $26689.7 > 25156.8 > 18993.6$ ); B:  $2 > 3 > 1$  ( $28606.1 > 23455.8 > 18778.2$ ). Based on the optimized results of orthogonal design, the optimum formulation should be A1B2, that means the ratio of sorafenib, Sylsya 350, and Eudragit S100 was 1:1:3.

From our sorafenib bioavailability results in Table 1, we could see that the predicted best formulation (1:1:3) was not same as the experimental best formulation (1:3:5). The differences between



**Fig. 2.** The curves of sorafenib concentration in plasma vs time after different formulations were orally administrated to rat. (A) Sorafenib suspension (●) and sorafenib tosylate suspension (▲) at a dose 100 mg/kg ( $n = 5$ , mean  $\pm$  SD). (B) Sorafenib Eudragit L100-55 (◇), Eudragit L100 (□) and Eudragit S100 (△) nanoparticles at a dose 3 mg/kg ( $n = 6$ , mean  $\pm$  SD).





**Fig. 3.** Sorafenib release from nanomatrix or suspension formulations in 30% isopropyl alcohol solution (pH 6.8) ( $n=3$ , mean  $\pm$  SD). Nanomatrix 1:1:3 (◆); nanomatrix 1:3:5 (■); suspension (▲).

the two formulations were estimated by the  $t$  test. There was no significant difference in  $AUC_{0-36h}$  between the two formulations ( $p=0.107$ ).

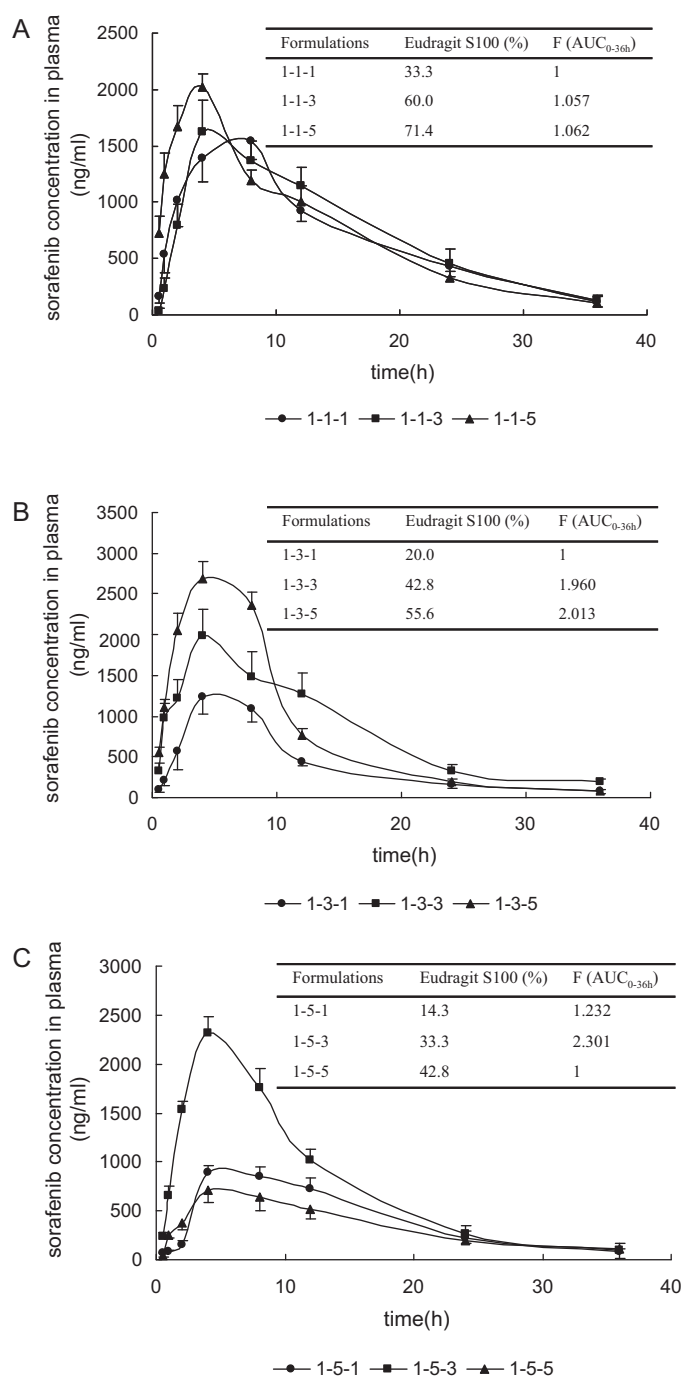
The *in vitro* release of sorafenib from the two nanomatrix systems (1:1:3 and 1:3:5) was tested here, comparing to sorafenib suspension. Because sorafenib is poorly soluble in most mediums, we selected 30% isopropyl alcohol solution as the release medium. As indicated in Fig. 3, the release profiles of the two nanomatrix formulations were similar, and both of them released quickly than sorafenib suspension.

Now, we analyze the nine nanomatrix formulations carefully. Fig. 4 compares the effect of the amount of Eudragit S100 on the absorption at three Sylysia 350 levels. Fig. 4A shows that at the level of sorafenib to Sylysia 350 1:1, when the amount of Eudragit S100 increased from 33.3% to 60% to 71.4%, the  $AUC_{0-36h}$  changed little, without significant difference ( $p>0.05$ ). Fig. 4B shows that at the level of sorafenib to Sylysia 350 1:3, when the amount of Eudragit S100 was 20.0%, the  $AUC_{0-36h}$  was much smaller than that at 42.8% and 55.6% (about half times,  $p<0.01$ ). Fig. 4C depicts that at the level of sorafenib to Sylysia 350 1:5, when the amount of Eudragit S100 was 42.8%, the  $AUC_{0-36h}$  was the smallest. When the amount of Eudragit S100 was 33.3%, the  $AUC_{0-36h}$  was the largest.

Fig. 5 compares the effect of the amount of Sylysia 350 on the absorption at three Eudragit S100 levels. Fig. 5A shows that at the level of sorafenib to Eudragit S100 1:1, when the amount of Sylysia 350 increased from 33.3% to 60%, the  $AUC_{0-36h}$  decreased about 40.9% ( $p<0.01$ ). When the amounts of Sylysia 350 were 60.0% and 71.4%, the  $AUC_{0-36h}$  were similar ( $p=0.82$ ). Fig. 5B depicts that at the level of sorafenib to Eudragit S100 1:3, when the amount of Sylysia 350 increased from 20.0% to 42.8% to 55.6%, the  $AUC_{0-36h}$  were similar ( $p>0.05$ ). Fig. 5C illustrates that at the level of sorafenib to Eudragit S100 1:5, when the amount of Sylysia 350 was 42.8%, the  $AUC_{0-36h}$  was the smallest. When the amount of Sylysia 350 was 33.3%, the  $AUC_{0-36h}$  was the largest.

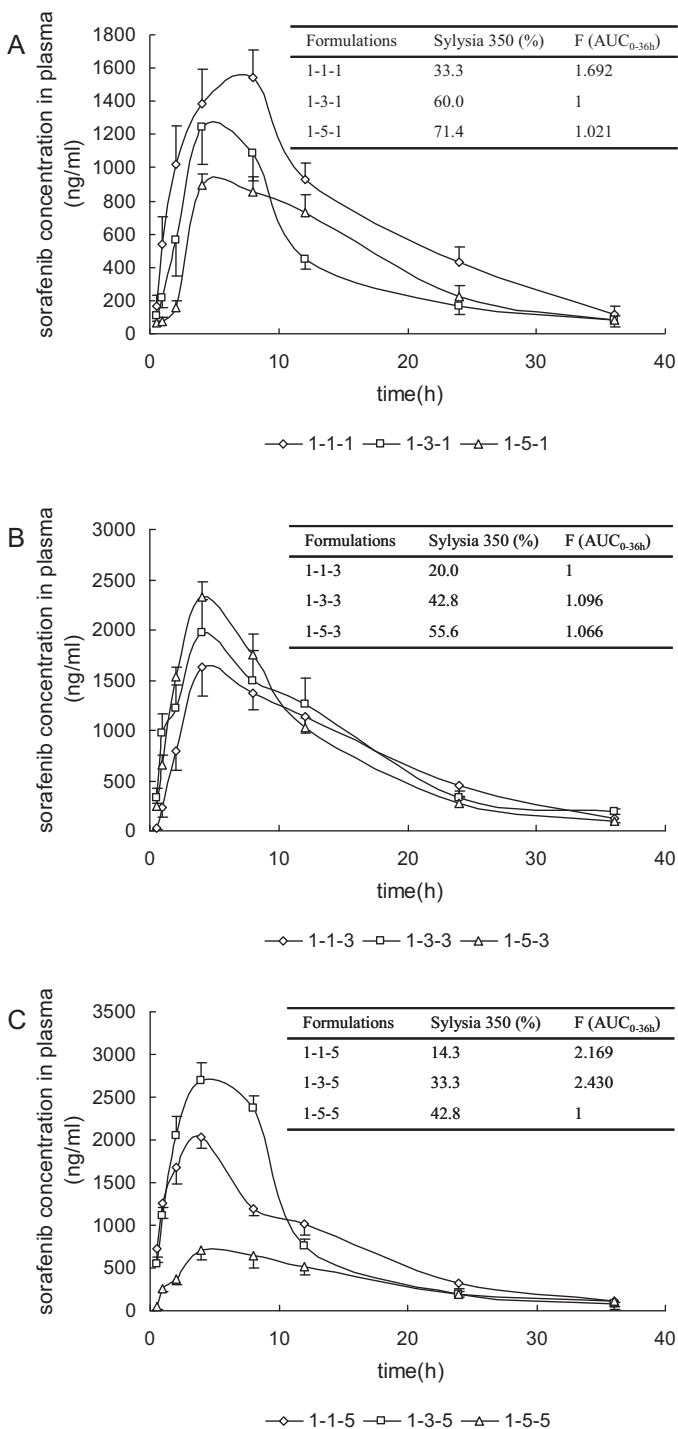
The  $AUC_{0-36h}$  of the nine formulations related to the different levels of Sylysia 350 and Eudragit S100 is summarized in Fig. 6. The area indicated by the arrow had large  $AUC_{0-36h}$ .

From all these results, we could draw a conclusion that both the ratio of Sylysia 350 and Eudragit S100 influenced the bioavailability and they had co-effects on the  $AUC_{0-36h}$ . In the nanomatrix formulations, too high ratio of Sylysia 350 or too low ratio of Eudragit S100 had poor bioavailability.



**Fig. 4.** The effects of the Eudragit S100 amount on the sorafenib concentration in plasma vs time after different nanomatrix formulations were orally administered to rat at a dose 15 mg/kg ( $n=5$ , mean  $\pm$  SD). (A) The ratio of sorafenib, Sylysia 350 and Eudragit S100 was 1:1:1 (●), 1:1:3 (■) and 1:1:5 (▲). (B) The ratio of sorafenib, Sylysia 350 and Eudragit S100 was 1:3:1 (●), 1:3:3 (■) and 1:3:5 (▲). (C) The ratio of sorafenib, Sylysia 350 and Eudragit S100 was 1:5:1 (●), 1:5:3 (■) and 1:5:5 (▲).

Sylysia 350 is fine porous silica particles and researchers (Wang et al., 2006a; Takeuchi et al., 2005) have used it to improve the dissolution property of some poorly water soluble drugs. In this system, porous silica played an important role to control the amorphous state of the adsorbed or embedded drugs. But Sylysia 350 has poor bioadhesion activity to GI mucosa. When Eudragit S100 was introduced into the formulation, the bioadhesion activity was improved. But, when the content of Eudragit S100 was too high, the dispersion of sorafenib decreased. So, the balance of Sylysia 350



**Fig. 5.** The effects of the Sylysia 350 amount on the sorafenib concentration in plasma vs time after different nanomatrix formulations were orally administered to rat at a dose 15 mg/kg ( $n=5$ , mean  $\pm$  SD). (A) The ratio of sorafenib, Sylysia 350 and Eudragit S100 was 1:1:1 ( $\diamond$ ), 1:3:1 ( $\square$ ) and 1:5:1 ( $\triangle$ ). (B) The ratio of sorafenib, Sylysia 350 and Eudragit S100 was 1:1:3 ( $\diamond$ ), 1:3:3 ( $\square$ ) and 1:5:3 ( $\triangle$ ). (C) The ratio of sorafenib, Sylysia 350 and Eudragit S100 was 1:1:5 ( $\diamond$ ), 1:3:5 ( $\square$ ) and 1:5:5 ( $\triangle$ ).

and Eudragit S100 was very important. In another word, the balance of dispersion and bioadhesion was quite important in the formulation.

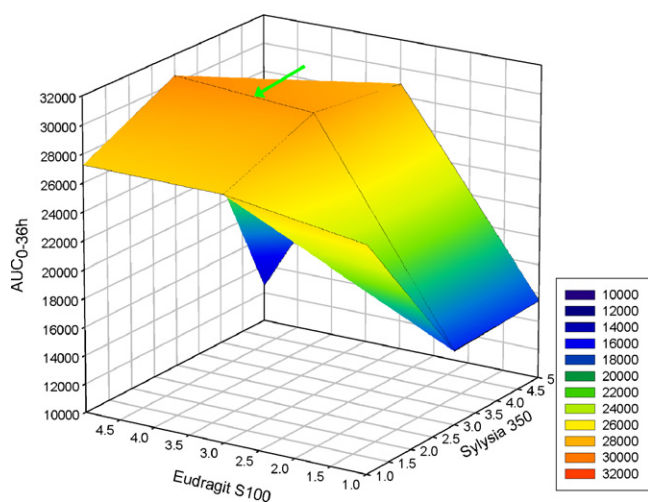
The relative bioavailability ( $F_{r1}$ ) of sorafenib nanomatrix to sorafenib suspension is shown in Table 3. It was shown that the nanomatrix improved the sorafenib absorption from 13 to 33 times. But as for the relative bioavailability ( $F_{r2}$ ) of sorafenib nanomatrix

**Table 3** Pharmacokinetic parameters of sorafenib after oral administration of different sorafenib nanomatrix to rat (mean  $\pm$  SD,  $n=5$ ).

Parameters	Unit	Sorafenib/Sylysia 350/Eudragit S100		1-1-1		1-1-3		1-1-5		1-3-1		1-3-3		1-3-5		1-5-1		1-5-3		1-5-5	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
$C_{max}$	ng/ml	1558.4	159.9	1633.8	274.5	2025.6	115.2	1283.2	154.3	1978.3	328.9	2703.9	180.9	909.4	57.8	2322.8	160.8	726.7	120.7		
$T_{max}$	h	7.2	1.8	4.8	1.8	4.0	0.0	5.6	2.2	4.0	0.0	4.8	1.8	5.6	2.2	4.0	0.0	4.8	1.8		
$AUC_{0-36h}$	ng/ml h	25669.6	2924.7	27142.6	4072.5	27256.8	2761.4	15175.4	1414.9	29751.8	4336.2	30543.1	1282.7	15489.5	2114.7	28923.8	2448.8	12567.6	2017.9		
$K$	1/h	0.103	0.030	0.080	0.017	0.098	0.012	0.198	0.062	0.097	0.017	0.240	0.014	0.080	0.019	0.154	0.036	0.084	0.021		
$t_{1/2}$	h	7.2	1.9	9.0	1.9	7.1	0.9	3.8	1.4	7.3	1.2	2.9	0.2	9.2	2.7	4.7	1.0	8.7	2.4		
$K_a$	1/h	0.363	0.155	1.074	0.893	0.726	0.065	0.398	0.134	0.442	0.150	0.267	0.015	1.545	1.965	0.423	0.111	0.429	0.260		
$t_{1/2\alpha}$	h	2.3	1.1	1.2	0.9	1.0	0.09	1.9	0.5	1.7	0.5	2.6	0.1	0.9	0.6	1.8	0.6	1.9	0.7		
$MRT_{0-36h}$	h	12.0	0.3	12.3	0.5	10.3	0.7	10.5	1.2	11.1	0.7	8.5	0.4	12.3	0.8	9.8	0.8	12.5	1.0		
$F_{r1}$ (%)		2797		2958		2970		1654		3242		3328		1688		3152		1369			
$F_{r2}$ (%)		34.3		36.3		36.4		20.3		39.8		40.8		20.7		38.7		16.8			

$F_{r1}$  (%): The relative bioavailability of sorafenib nanomatrix (SNM) to sorafenib suspension (SS), which was calculated using the following equation:  $F_{r1} (\%) = (AUC_{(0-36h) SNM} / X_0 SNM) / (AUC_{(0-36h) SS} / X_0 SS) \times 100$ .

$F_{r2}$  (%): The relative bioavailability of sorafenib nanomatrix (SNM) to sorafenib Eudragit nanoparticles (SNP), which was calculated using the following equation:  $F_{r2} (\%) = (AUC_{(0-36h) SNM} / X_0 SNM) / (AUC_{(0-36h) SNP} / X_0 SNP) \times 100$ .



**Fig. 6.** Relationship of  $AUC_{0-36h}$  to the amount of Sylysia 350 and Eudragit S100 in the nine orthogonal designed formulations. Sylysia 350 and Eudragit S100 were all at three levels: 1, 3, 5.

to sorafenib Eudragit S100 nanoparticles (see Table 3), the absorption of sorafenib in nanomatrix was not so good as that in Eudragit S100 nanoparticles (only from 16.8% to 40.8%). The reasons may be as follows: (1) compared to nanoparticles, the dispersion degree was decreased. The size of nanoparticles was only 64.6 nm. While the nanomatrix was milled and sieved through 100 mesh, the particles were about 150  $\mu\text{m}$ . (2) The release character maybe changed because of the adsorbed or the embedded phenomenon. The drug release process may be composed of two steps: First, Eudragit S100 dissolved, a small part of sorafenib released; second, with the dissolution of Eudragit S100, Sylysia 350 exposed. Sorafenib embedded in the pores released. It was complex than the dissolution process in the Eudragit nanoparticles. (3) The bioadhesion activity decreased because of the Eudragit S100 amount decreased in the formulation.

Compared to sorafenib suspension, nanomatrix improved the absorption of sorafenib. While compared to sorafenib nanoparticles, nanomatrix decreased the absorption. We think it is valuable for the further study because, it can solve the problems of the nanoparticles, stability and scaling up. Meanwhile, it can enhance the absorption of sorafenib quite effectively (13–33 times that of sorafenib suspension). Above all, all the materials are commonly adjuvant, safe, easy to get and cheap. So, the nanomatrix formulation has the potential to be developed into a product in the future. As for the mechanisms of the nanomatrix to improve the absorption and the stability of the system, more studies would be carried out.

#### 4. Conclusion

In summary, we prepared sorafenib suspension, sorafenib tosylate suspension, sorafenib Eudragit nanoparticle colloids and sorafenib nanomatrix and compared their bioavailability. Sorafenib Eudragit nanoparticles could improve the relative bioavailability more than 50 times, while sorafenib nanomatrix could improve 13–33 times, compared to sorafenib suspension. Because the nanomatrix formulation may solve the problems of nanoparticle stability and scaling up, it has the potential to be developed into a product in the future.

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