Drug Development and Industrial Pharmacy

http://informahealthcare.com/ddi
ISSN: 0363-9045 (print), 1520-5762 (electronic)

Drug Dev Ind Pharm, Early Online: 1–5
 DOI: 10.3109/03639045.2014.950271

informa
healthcare

RESEARCH ARTICLE

Preparation and evaluation of SEDDS of simvastatin by *in vivo*, *in vitro* and *ex vivo* technique

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Abstract

The objective of this work was to formulate a Self Emulsifying Drug Delivery System (SEDDS) of simvastatin, a poorly soluble drug and to evaluate by *in vivo*, *in vitro* and *ex vivo* techniques. Oils and surfactants were screened out depending upon their solubilizing capacity. Among all of the solvents, Capryol 90 showed good solubilizing capacity. It dissolved 105 mg/ml of simvastatin. Tween-80 also showed good solubilizing capacity which was 117 mg/ml. The two excipients were used to prepare simvastatin SEDDS. Formulations were initially checked for the color, clarity and sedimentation. The SEDDS formulations were transparent and clear. Formulation F2 containing 7:3 (*m/m)* mixture of Capryol 90/Tween-80 produced smallest micro-emulsion with particles size of 0.074 μm and drug release was higher than other formulation (102% within 20 min). *Ex vivo* study of the SEDDS formulation was evaluated using guinea pig intestinal sac. Drug diffused from F2 formulation was significantly higher than pure drug ($p < 0.001$). *In vivo* study of SEDDS was performed in albino mice using plasma cholesterol level as a pharmacodynamic marker parameter. The test formulation (F2) appeared remarkable reduction in plasma cholesterol level, after oral administration which showed that SEDDS may be an effective technique for the oral administration of simvastatin.

Keywords

Capryol 90, micro-emulsion, oral administration, SEDDS, simvastatin, Tween-80

History

Received 12 May 2014
Revised 22 July 2014
Accepted 26 July 2014
Published online 20 August 2014

Introduction

Nearly 40% of all new drug candidates show poor aqueous solubility and the oral delivery of such candidates is very difficult because of their low bioavailability and low effective concentration in biofluids[1]. Furthermore, oral bioavailability of numerous drugs is obstructed owing to their high lipophilicity[2,3]. According to the Noyes–Whitney equation, the dissolution rate of solid drug particles in solution is inversely proportional to the solution concentration and the particles' radii[4]. Thus, by immensely reducing particle size, solubility can be markedly improved. Several strategies are exploited to increase the dissolution rate of drugs by decreasing the particle size, with the use of surfactants, lipids, permeation enhancers, micronization, salt formation, cyclodextrins and nanoparticles[5]. Majority of these approaches have their drawbacks because of the specialized equipment needed for the manufacturing with prolonged processing time and regulatory difficulties. Lipid based formulation approaches, particularly the self emulsifying drug delivery system (SEDDS), are well known approach for the delivery of hydrophobic drugs[6], which are integrated with poor water solubility and low oral bioavailability[7].

SEDDS are thermodynamically stable solution consisting of drug, oil, surfactant or co-surfactant which forms oil in water emulsion when diluted with water under stirring, ranging in size from ~100 nm (SEDDS) to <50 nm for self-microemulsifying drug delivery system (SMEDDS)[8]. Selection of a suitable self-emulsifying formulation depends upon the assessment of the solubility of the drug in various components and the droplet size distribution of the resultant emulsion following self-emulsification[9]. The potential of SEDDS for improving the bioavailability of poorly soluble drugs has been evident for at least a decade[10]. SEDDS technology was employed to increase the dissolution rate for different poorly water soluble drugs such as atorvastatin[11], Cinnarizine[12] and phyllanthin[13].

Simvastatin, a crystalline compound, is practically insoluble in water[14] and is a hypolipidemic drug which has attracted considerable attention because of their potential to prevent cardiovascular disease by retarding the accelerated atheroscelosis in hyperlipidemic individuals. It is a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (hydroxymethylglutaryl CoA reductases). Simvastatin is a substrate for CYP3A4. So after oral administration, simvastatin is metabolized to its b-dihydroxy, where it inhibits the rate-limiting step in cholesterol biosynthesis[15,16]. Being a Class II drug, it often shows dissolution rate-limited oral absorption and high variability in

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pharmacological effects. Therefore, development in its dissolution rate and solubility may lead to enhancement in bioavailability.

The objective of this present study was to develop a self-emulsifying drug delivery system (SEDDS) for simvastatin, where different proportions of oils and surfactant systems were used for oral administration. Isotropic systems were evaluated for the quality of emulsion produced, mean droplet size and *in vitro* drug diffusion. Optimized formulation was further evaluated for its *in vivo* performance in albino mice.

SEDDS of simvastatin can be evaluated by *ex vivo* permeability study as intestinal permeability which appeared to be one essential part in the prediction of oral bioavailability[17]. Varieties of methods for evaluating the intestinal permeability of a given drug have been developed and reviewed[18]. Isolated intestinal sacs of different animal species including rabbit, dog, pig, rat and monkey can be used in permeability studies[19]. Kitaoka et al.[20] performed absorption studies of theanine by using the guinea pig intestinal sac. Storkholm et al.[21] also used the guinea pig intestinal sac to carry out the effect of partial chronic intestinal obstruction on motility, morphology and collagen content in guinea pig small intestine. In this study, we also used the guinea pig intestine segment for permeability studies of simvastatin SEDDS based on the assumption that membrane permeability of drug is not species-dependant, since the composition of plasma membrane of intestinal epithelial cells are similar across the species.

Pharmacodynamic marker parameters are frequently used to evaluate the performance of different classes of drugs. In this study, *in vivo* performance of simvastatin SEDDS was evaluated in albino mice using plasma cholesterol level as pharmacodynamic marker parameters.

Materials and methods

Materials

Simvastatin was a generous gift from Incepta Pharmaceutical Ltd, Bangladesh. Labrafac PG, Lauroglycol 90, Labrafac Lipophile WL 1349, Labrafil M 1944 CS, Peceol, Lauroglycol FCC, Maizine 35-1, Plurol Oleique CC 497, Labrasol, Transcutol HP, Capryol PGMC and Capryol 90 were kind gifts from Gatoforese, France. Tween-80, Span 20 and Tween-20 were purchased from JHD Chemicals Ltd., India. Other reagents were of analytical-reagent grade and purchased from the local market. Water was deionized and double distilled. For *ex vivo* study, EasyMate® I (Plasma Cholesterol Measuring Instrument, Jhunan Township, Taiwan) was purchased from the local market. All other chemicals and reagents were of analytical grade and used as received.

Solubility analysis

Apparent solubilities of simvastatin were determined according to the modified method of Patel and Vavia[22] in different oils, surfactants and co-surfactants at ambient temperature for the selection of appropriate oil and surfactant. About 0.5 ml of each of vehicles was taken to different cap tube, where 10 mg of simvastatin was added initially. The amount of simvastatin was increased in different vehicles gradually and finally 100 mg drug was added in each vehicle. After sealing, the mixtures were heated at $100 \pm 20\,^{\circ}C$ in a water bath shaker to facilitate the solubilization. Samples were then analyzed for determination of solubility by HPLC.

Formulation of simvastatin SEDDS

Solubility of simvastatin SEDDS formulations was done in different excipients to develop the SEDDS formulation. Here, Capryol 90 (105 mg/ml) and Tween-80 (117 mg/ml) showed good solubilizing capacity. Accurately weighed, 10 mg of simvastatin was taken in a glass vial, then 900 mg of Capryol 90 and 100 mg of Tween-80 (Table 1) were added. Then the components were mixed by gentle vortexing and sonicating in water bath until simvastatin was properly dissolved. Other three SEDDS formulation were developed using same oil and surfactant at different ratio. The prepared formulations were preserved at ambient conditions until further use.

Table 1. Formulation of simvastatin SEDDS.

Name	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)
Simvastatin (active drug)	10	10	10	10
Capryol 90 (oil)	900	700	500	300
Tween-80 (surfactant)	100	300	500	700

Visual examination of the physical properties of simvastatin SEDDS

Simvastatin SEDDS formulations were visually examined for clarity, homogeneity, viscosity and color. Normal visual examination was performed to observe the color of the formulations. Screw-cap vials containing SEDDS Formulations were kept standing against light to examine the clarity. Presence or absence of precipitation was also noticed to assess the homogeneity of the prepared formulations. Flowability of the SEDDS Formulations was also checked to determine their viscosity. The SEDDS Formulations were than kept in optimum room temperature for 1 month. After 1 month, those properties of the SEDDS formulations were again examined to determine the stability of the formulation of simvastatin SEDDS.

Determination of droplet size of simvastatin SEDDS

Laser Diffraction Technology of MALVERN (Mastersizer, 2000) was used to determine the droplet size of SEDDS formulation. About 0.5 ml SEDDS formulations were diluted with 50 ml distilled water. Droplet size distribution was done with the help of the droplet size distribution pattern. Effect of drug loading and dilution medium on droplet size of SEDDS was also studied for F2 formulation.

Release study of simvastatin SEDDS

In vitro *release study*

For *in vitro* study, USP Type I (Basket type) dissolution apparatus (Electro Lab, India) was used. SEDDS formulations equivalent to 10 mg of simvastatin and only 10 mg of simvastatin drug were filled in a hard gelatin capsule shell (size #0) and were undertaken for dissolution study. Distilled water was used as dissolution medium that was maintained at $37 \pm 0.5\,^{\circ}C$ and stirred at 100 rpm. Dissolution samples were withdrawn at predetermined time intervals. Each time 10 ml of the dissolution sample was withdrawn with a calibrated plastic disposable syringe and the media were replenished with distilled water and then filtered with 0.45 μm syringe filter (Microswit, Hannover, Germany) and were analyzed for drug content by RP-HPLC (Shimadzu, Japan).

Simvastatin SEDDS permeability through guinea pig intestinal sac

Ex vivo permeability study was carried out by using guinea pig intestinal sacs. Guinea pig was sacrificed and the duodenal part of the small intestine was isolated. The tissue was then washed with distilled water to remove the mucous and lumen content and then put them into the oxygen chamber. About 3 cm long sacs

were prepared by tying up the two end of the sac with cotton thread. SEDDS formulation(F2) equivalent to 10 mg simvastatin were then inserted inside a 3-cm sac and for comparison intestinal sac containing 10 mg pure drug with 0.5 ml methanol, 10 mg pure drug with 1 ml water, 10 mg of pure drug were also included. The sacs were then put into different dissolution media. The samples were withdrawn at a predetermined time intervals. Each time 10 ml of the sample was withdrawn with a calibrated plastic disposable syringe and media were replenished with fresh medium. Then, the samples were filtered and amount of simvastatin permeated through the intestinal membrane was analyzed spectrophotometrically by a UV–VIS spectrophotometer (UV–mini 1240, Shimadzu Cord, Kyoto, Japan).

In vivo *study*

SEDDS formulation of simvastatin (F2) and pure drug were undertaken for *in vivo* study in mice. Each of the mice was administered 50 µg of coconut oil orally daily in the morning using micro pipette to get their cholesterol level high. Blood samples were collected from mice tails and and the plasma cholesterol level was measured using EasyMate® I blood cholesterol meter. After 14 days, 100 l of SEDDS formulation equivalent to 10 mg simvastatin was administered to one group of mice orally. For comparison, pure drug of simvastatin was administered to another group of mice. Additionally another group of mice were studied for controlled treatment.

Result and discussion

Development of HPLC method for the analysis of simvastatin

To develop and validate HPLC method, a Shimadzu (Japan) HPLC system was used which consist of a CMB-20 Alite system controller, two LC-20AT pumps, SIL-20A autosampler and CTO-10ASVP column oven. SPD-20A UV–VIS detector (Shimadzu, Japan) was used to achieve ultraviolet detection. The drug analysis data were acquired and processed using LC solution (Version 1.2, Shimadzu, Japan) software running under Windows XP on a Pentium PC. Separation of the drug was achieved from C18 column (5 µ, 4.6 mm × 150 mm, Waters, Milford, MA) at 35 °C temperature with a mobile phase consisting of methanol: water (ratio:70:30) at a flow rate of 1.5 ml/min and detection wavelength of 242 nm. The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision and robustness. The system is found suitable in respect to retention time (%RSD 0.165) and mean theoretical plate count (%RSD 0.768). The absence of additional peaks of excipients interfering with the target peak in the chromatogram indicates the selectivity and specificity of the method. The linearity of the method was determined at different concentration levels (1–20 µg/ml, $r^2 = 0.997$). For accuracy testing, six replicate runs of samples containing excipients and drug were performed. The % recovery value, which was 100.14 ± 0.57%, indicated the accuracy of the method. Precision was determined by repeatability (intra-day) and intermediate precision (inter-day) and assessed by %RSD (Table 2). Finally robustness test was performed by changing the flow rate, column temperature and mobile phase composition and no marked changes in the chromatograms were observed, which exhibited that the method was robust.

Solubility analysis

Solubility of simvastatin in various components (oil and surfactant) was studied for the selection of oil & surfactants. Among all of the vehicles, Tween-80 and Capryol 90 showed maximum solubility for simvastatin which was 117 and 105 mg/ml, respectively (Figure 1). These two excipients (Capryol 90 and Tween-80) were used to prepare simvastatin SEDDS.

Table 2. Accuracy and precision result of HPLC validation method.

Validation parameters	Simvastatin
Accuracy	
% Recovery	100.14 ± 0.57
% RSD	0.57
Precision (% RSD)	
Repeatability	0.55
Ruggedness	0.58

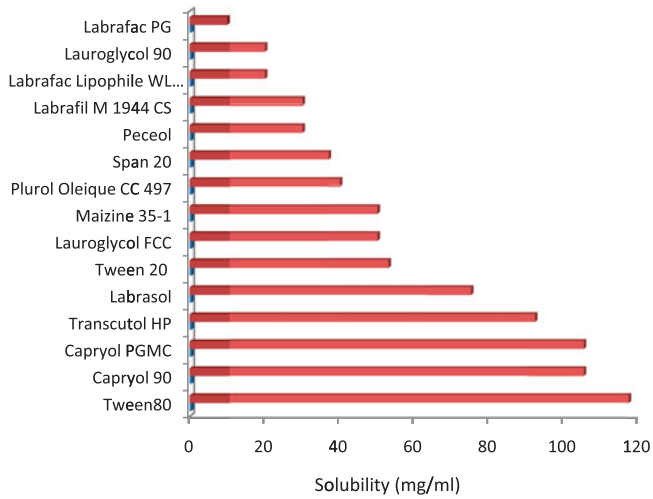

Figure 1. Solubility profile of simvastatin in various vehicles.

Visual evaluation of the physical properties of simvastatin SEDDS

Simvastatin SEDDS formulations were evaluated for different physical properties after preparation and after 1 month from preparation (Table 3).

Determination of droplet size of simvastatin SEDDS

Laser Diffraction Technology of Malvern (Mastersizer, 2000) was used to determine the droplet size of SEDDS formulation. Particle size distribution was characterized with the help of droplet size distribution of 10% particles – *d* (0.1), droplet size distribution of 50% droplet – *d* (0.5) and droplet size distribution of the 90% particles – *d* (0.9). Particle size range of the SEDDS formulation of system I, II, III and IV was in the range of 0.074–16.448 µm.

Formulation F2 showed the smallest droplets which were 0.074 µm and formulation F4 showed the largest droplets which were 16.448 µm. For formulation F1, 90% droplet was within 6.997 µm, 50% droplet was within 3.508 µm and 10% droplet was within 1.789 µm. In this formulation, 90% Capryol 90 and 10% Tween-80 were used. For formulation F2, 90% particle range is 0.212 µm, 50% particle range is 0.122 µm and 10% particle range is 0.074 µm. In this formulation, 70% Capryol 90 and 30% Tween-80 were used which produced a very clear emulsion with droplet size <1 µm when water was added with agitation. For formulation F3, 90% particle range is 12.142 µm, 50% particle range is 4.226 µm and 10% particle range is 2.002 µm. In this formulation, 50% Capryol 90 and 50% Tween-80 were used. For formulation F4, 90% particle range is 16.448 µm, 50% particle range is 7.510 µm and 10% particle range is 3.027 µm. In this formulation, we used higher concentration of Tween-80 (70%).

Table 3. Physical properties of simvastatin SEDDS formulation after preparation and after 1 month from preparation.

	After preparation			After 1 month from preparation		
Formulation	Color	Clarity	Precipitation	Color	Clarity	Precipitation
F1	Slight yellowish	Clear	No	Slight yellowish	Clear	No
F2	Slight yellowish	Clear	No	Slight yellowish	Clear	No
F3	Slight yellowish	Clear	No	Slight yellowish	Clear	No
F4	Slight yellowish	Clear	No	Slight yellowish	Clear	No

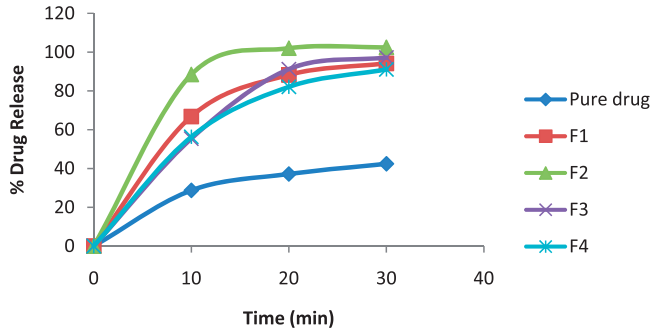

Figure 2. Percent drug release from four SEDDS formulation and pure drug.

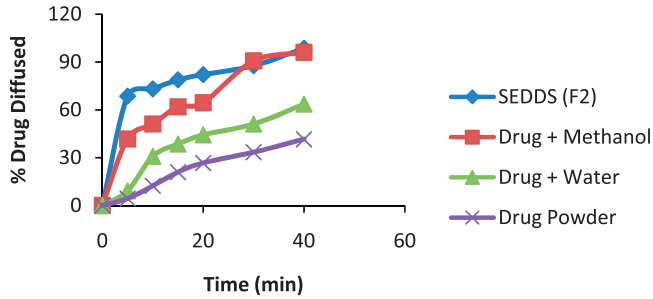

Figure 3. Simvastatin permeability through guinea pig intestine sac.

Formulation F1, F3 and F4 produced larger droplet size than F2 formulations. So we can say that only formulation F2 containing 70% Capryol 90 and 30% Tween-80 produced SEDDS with lower droplet size.

The droplet size of the four formulations were also determined after 3- and 6-month storage and there was no significant differences in the droplet size due to the storage (% RSD = 0.458%). Effect of dilution medium (water, phosphate buffer P^H 6.8) on the droplet size was found insignificant ($p > 0.05$). On the other hand effect of drug loading (0.99% and 1.5%) on the droplet size was found significant ($p < 0.001$).

In vitro release study

F2 formulation containing 7:3 (m/m) mixture of Capryol 90/ Tween-80 showed higher *in vitro* drug release than other formulations (Figure 2). Within 20 min 102% drug was released from F2 formulation ($n = 6$). This may be due to the small droplet size produced in the emulsion.

Drug release from F1 formulation was lower than formulation F2. Within 30 min, 94% drug was released. This is due to the higher droplet size. So the drug release rate was found to depend on the droplet size of emulsion. Droplet size depended dissolution has also been reported earlier[23]. Drug release from Formulation F3 was higher than formulation F1 but less than formulation F2. Within 30 min, 97% drug was released. For formulation F4, 91% drug was released within 30 min.

Dissolution profile of active drug of simvastatin was also studied for comparison with SEDDS formulations, where only 42% drug was released in 30 min. So from this study, it was concluded that the drug release from simvastatin SEDDS formulations was higher than the active drug. Statistical analysis for percent drug release with in 30 min was done by ANOVA ($p < 0.05$). Dissolution profiles of different SEDDS formulation and pure drug, were also compared by employing model independent approach of difference factor (f1), similarity factor (f2) and % dissolution efficiency (% DE). The results were confirmed by Bonferroni's multiple comparison as a *post hoc* test. Percent drug release from different SEDDS formulations were statistically significant ($p < 0.001$).

Simvastatin SEDDS permeability through guinea pig intestinal sac

Ex vivo permeability of simvastatin SEDDS was also studied through guinea pig intestinal sac in addition to conventional *in vitro* testing of SEDDS formulations. Drug diffusion studies using pretreated cellulose dialysis tubing have been well documented in the literature[24]. Tukker[19] described different methods for drug diffusion study by using the intestines of several animal species. In this study we used the guinea pig intestinal sac ($n = 6$). Drug permeability from formulation F2 was higher than the drug dissolved in methanol. Formulation F2 released 99% drug within 40 min, whereas drug solution in methanol released 96% drug within 40 min. This is due to the lower particle size of the emulsion produced by the formulation. Drug permeability from the drug dispersed in water was 64% within 40 min. On the other hand, pure simvastatin showed 42% diffusion within 40 min (Figure 3). Statistical analysis for the permeability data were done by ANOVA ($p < 0.05$). The results were confirmed by Bonferroni's multiple comparison as a *post hoc* test. Drug diffused from different samples were statistically significant ($p < 0.001$).

In vivo study

Pharmacodynamic marker parameters are often used to evaluate the *in vivo* performance of different classes of drugs[25]. Simvastatin is a cholesterol lowering drug and it decreases the plasma cholesterol level. Hence plasma cholesterol level was used as a basis for the comparison of *in vivo* performance of SEDDS of simvastatin. Plasma cholesterol level was found to increase sharply in the case of coconut oil administration. Administration of pure simvastatin decreased plasma cholesterol level slowly after the initial increase of the cholesterol level. But when we administered the SEDDS (F2) formulation, the plasma cholesterol level decreased rapidly. Initially after feeding the coconut oil, the cholesterol level in test mice were found between 117 and 120 mg/dl. After administering SEDDS formulation to each of the mice, the cholesterol level was reduced to 46–54 mg/dl in 5 days, whereas for pure drug, the cholesterol level was reduced to 60–63 mg/dl. Plasma cholesterol level data for SEDDS formulation and pure drug were analyzed by unpaired *t*-test ($p < 0.05$) and it

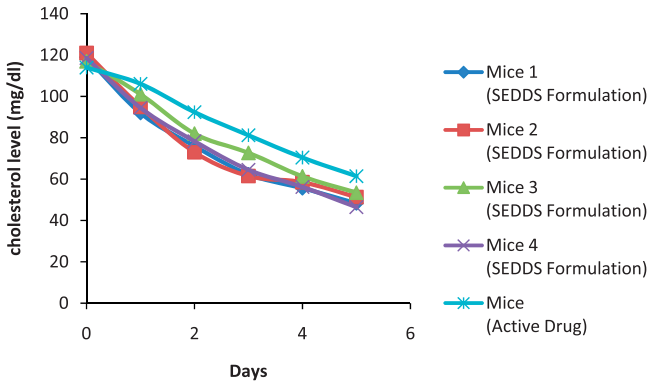

Figure 4. Effect of SEDDS and active drug on cholesterol level of albino mice.

was found that Plasma CH levels were significantly lower in case of SEDDS formulation in compared to pure drug ($p < 0.001$). From these analyses, it can be concluded that SEDDS of simvastatin are more effective than pure simvastatin to lower plasma cholesterol level ($p < 0.001$). This is due to the higher absorption of the drug from SEDDS formulation (Figure 4).

Conclusion

This study illustrated the potential of simvastatin self-emulsifying drug delivery systems (SEDDS) for oral administration. Optimized SEDDS formulations consisting of Capryol 90, Tween-80 and simvastatin were successfully developed with good stability, good emulsification efficiency, small microemulsion particles (0.074 μm) and ultimately an increased dissolution rate of poorly water soluble drug simvastatin. About 2- to 3-fold increased in simvastatin dissolution rate was obtained with the development SEDDS formulation while compared with the dissolution of pure simvastatin. The prepared SEDDS formulation showed good permeability when they were checked for permeability testing through guinea pig sac. The prepared SEDDS formulation was also checked for *in vivo* study in albino mice, where it reduced its cholesterol level rapidly within 5 days. Thus, this study confirmed that the SEDDS formulation can be used as a possible alternative to traditional oral formulations of simvastatin for improved bioavailability.

Acknowledgements

We acknowledge the equipment and material support from Incepta Pharmaceutical Ltd, Bangladesh.

Declaration of interest

The authors report no conflicts of interest. We acknowledge the support of our home institution.

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