FORMULATION AND IN-VITRO EVALUATION OF ECONAZOLE NITRATE LOADED CHITOSAN NANOPARTICLES USING IONIC GELATION METHOD

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ABSTRACT:

Aim: The aim of the present study was to develop Econazole Nitrate loaded Nanoparticles and *In-vitro* evaluation of prepared nanoparticle.

Methodology: Nanoparticles were prepared using the biodegradable natural polymer Chitosan by Ionic gelation technique. The interaction between the drug and excipient was examined by DSC, FTIR, and SEM. *In vitro* release studies were conducted in pH 7.4 phosphate buffer and based on the results the ideal formulation was selected and subjected to stability studies.

Results: The purity of the drug was determined by Differential scanning calorimetry (DSC) and by Thiel's tube method, it was found to be 180 °C and 176°C respectively. FTIR studies revealed that there is no possible drug-excipient interaction. The % entrapment efficiencies ranged from 77.9 - 87.9%. The chitosan nanoparticles having particle diameter ranging approximately 233-423nm and a zeta potential -33.7 mV. SEM revealed that the nanoparticles were roughly spherical in nature. From the *In vitro* drug dissolution, it was found that the fastest release effect of nanoparticles depends on the polymer concentration.

Conclusion: In this study, chitosan nanoparticles loaded with Econazole Nitrate were prepared by Ionotropic gelation method, The *In vitro* release profile observed for these nanoparticles was characterized by an initial fast release followed by a controlled release phase**.**

Keywords: Econazole Nitrate, Nanoparticles, Chitosan, Ionic gelation method.

INTRODUCTION

Dermatophytes are the most common cause of fungal infections worldwide, Based on their host specificity, these fungi are classified into three ecological groups: geophilic, zoophilic, and anthropophilic.[1] Generally dermatrophytic infection in human are referred to as tinea infection and are consequently named with specific reference to the area of the body involved. Tinea pedis is an infection of the foot and is usually referred to as Athlete's foot. It is more common in those who wear occlusive shoes. Most common agents are T. rubrum, T. mentagrophytes. E floccosum, M persicolor. T raubitschekii and T violaceum,[2] Nanoparticles are small particles that range in size from 10 to 1000 nm. They contain Nano molecular materials in which active ingredients are dissolved, entrapped and/or encapsulated. Over the past few decades, Nano particles have gained recognition in medical field as an effective means of drug delivery and therapy.[3]

Nanoparticles have unique physicochemical properties such as, ultra small size, large surface area to mass ratio and high reactivity which are different from bulk materials of the same composition. These properties can be used to overcome some of the limitations found in traditional therapeutic and diagnostic agents Thus Nanostructure mediated drug delivery enhances drug bioavailability, improves the time release of drug molecules and enables precision drug targeting. Chitosan is of major importance in industry because it possesses excellent properties such as biocompatibility, biodegradability, non-toxicity and absorption properties. These properties make chitosan a good candidate for conventional and novel drug delivery systems. The use of controlled release system has certain advantages over the conventional dosage forms, as they can minimize side effects, prolong the efficacy of the drug release rate and can reduce the frequency of administration of the drug. Thus assuring better patient compliance.[4,5]

Ionic gelation is based on the ability of polyelectrolytes to cross link in the presence of counter ions to form hydro gels. Simply an ionic gel is produced by physical cross-linking between a polycationic and a polyanionic component. The chosen polyanion is the sodium TriPolyPhosphate (TPP). It is largely used in food industry, in detergents and biomedical applications for its non – toxicity and fast gelling ability[6,7]. Econazole nitrate 1-(2,4-dichloro-[(p-chlorobenzyloxy)phenylethyl] imidazole derivatives. It is a white (or almost white) crystalline powder which is almost odourless and belongs to BCS CLASS II. It works by slowing the growth of fungi that is responsible for fungal infection and due to its poor solubility. Econazole Nitrate is incompletely absorb after oral administration and bioavailability vary among the individuals to solve this problem Econazole Nitrate loaded Chitosan Nanoparticles are formulated using Ionic gelation method.[8]

MATERIALS AND METHODS

The Econazole Nitrate was received as a gift sample from Mahrshee Laboratories Pvt. Ltd. Bharuch, Gujarat, India. Potassium dihydrogen phosphate, sodium hydroxide and were purchased from thermo fisher scientific India Pvt. Ltd., Bangalore, India. The distilled water was produced in our research laboratory with a distillation unit.

Method of preparation

Ionic gelation technique

Chitosan nanoparticles were formulated by ionic cross linking of chitosan solution with TPP anions. Chitosan was dissolved in aqueous solution of acetic acid (0.25, v/v) at different concentrations such as 1.0, 2.0, 3.0, 4.0 mg/ml. Under magnetic stirring at room temperature, 5 ml of 0.84% (w/v) TPP aqueous solution was added dropwise using syringe needle into 10 ml chitosan solution containing 10mg of Econazole Nitrate. pH was adjusted to 6.0 by adding 0.1 M NaOH. The stirring was carried about 30 min. The prepared nanoparticles suspensions were centrifuged at 12000 × g for 30 min using C24 centrifuge. The formation of the particles was a result of the interaction between the negative groups of the TPP and the positively charged amino groups of chitosan (Ionic gelation) [9-10] (Table 1)

Characterization of prepared nanoparticles

Fourier transform infra-red spectroscopy (FT-IR) Analysis The FT-IR spectra of pure Econazole nitrate, chitosan and nanoparticles loaded with Econazole Nitrate were recorded using Shimadzu IR spectrophotometer, Model 840, Japan, to check drug polymer interaction and stability of drug.[11]

Entrapment efficiency

The content of the drug was determined by its effectiveness. The temporary suspension of nanoparticles was centrifuged at about 15000 rpm for 35- 40 minutes under 25˚C temperature control of free drug separation in the supernatant. The drug concentration in the supernatant was evaluated by using a visible UV Spectrophotometer at 271 nm after preparation for appropriate mixing. The effectiveness of the formulation (%EE) was determined using the equation below.[12]

$$\%EE = \frac{Total\ amount\ of\ drug - Amount\ of\ drug\ in\ supernatant}{Total\ amount\ of\ drug} \times 100$$

Surface morphology study

Scanning electron microscopy (SEM) of the prepared nanoparticles was carried out to examine the particle size and surface morphology. The nanoparticles were arranged on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument. The photographs were taken using a Jeol scanning electron microscope under magnification of 7500–20000 ×.

Particle size distribution

The particle size distribution of the nanoparticles was determined by photon correlation spectroscopy (PCS, Coulter Counter model N4 MD, Coulter Counter Co. USA). The nanoparticle dispersions were added to the sample dispersion unit containing stirrer and stirred to reduce the aggregation between the nanoparticles. The average volume-mean particle size was calculated after performing the experiment in triplicate.

Zeta potential

The Zeta-potential of drug loaded nanoparticles were measured by Zeta sizer (Malvern Zetasizer 3000HS, UK). To determine the zeta potential, nanoparticles samples were diluted with KCl (0.1 mM) and placed in electrophoretic cell where an electrical field of 15.2 V/cm was applied. Each sample was analysed in triplicate.[13]

In vitro release studies

In vitro release studies were carried out by using dialysis tubes with an artificial membrane. The prepared Econazole nitrate nanoparticles were re-dispersed in 5 ml of phosphate buffer pH 6.4 and subjected to dialysis by immersing the dialysis tube to the receptor compartment containing 150 ml of phosphate buffer pH 6.4. The medium in the receptor was agitated continuously using a magnetic stirrer and the temperature was maintained at 37 ± 1˚C. 5ml sample of receptor compartment was taken at various intervals of time over a period of 24 h and each time 5 ml fresh buffer was replaced. The amount of drug released was determined spectrometric ally at 271 nm.[14]

Kinetic modelling

In order to understand the kinetic and mechanism of drug release, the result of in vitro drug release study of nanoparticles was fitted with various kinetic equation like zero order (cumulative% release vs. time), first order (log% drug remaining vs. time), Higuchi's model (cumulative% drug release vs. square root of time), Peppas plot (log of cumulative% drug release vs. log time). R2 (coefficient of correlation) and k (release rate constant) values were calculated for the linear curve obtained by regression analysis of the above plots.[15]

Stability studies

The stability study was carried out using the batch F-1. Formulation F-1 was divided into 3 sets of samples and stored at 4˚C in refrigerator, room temperature (37˚C), 45 ± 2˚C/75% RH in humidity control ovens. After 60 d drug content of all samples were determined by the method as in drug content. In vitro release study of formulation F-1 was also carried out after 60 d of storage.[16]

RESULTS AND DISCUSSION

Physicochemical characterization of nanoparticles

Nanoparticles were formed spontaneously upon incorporating TPP solution into the chitosan solution under magnetic stirring. Chitosan Nanoparticles are obtained by ionic gelation, a simple process where particles are formed through electrostatic interactions between the positively charged chitosan chains and polyanions employed as cross linkers. The FTIR spectrum shows that there were no significant changes in the chemical integrity of drug and also indicates that the polymer and drug are compatible with each other. Nanoparticles prepared by ionic gelation technique were found to be discrete and through

SEM analysis (Fig. 1), where the mean size distribution were found to be 233 nm and 423 nm. Since the particle size is less than 1000 nm, this drug delivery system can be used for parenteral formulations, drugs administered by such routes will achieve direct systemic delivery, thereby avoiding first pass hepatic metabolism. The drug entrapment efficiency of nanoparticles prepared by ionic gelation technique containing drug: polymer in various ratios of 1:1, 1:2, 1:3, 1:4 was found to be 87.92%, 85.03%,81.62%, 77.95%. (Table 1) and thus, there was a steady increase in the entrapment efficiency by decreasing the polymer concentration in the formulation. The high entrapment efficiency is likely due to electrostatic interactions between the drug and the polymer. The Zeta potential of ideal formulation was found to be -33.7 mV.

Table 1: Formulation and Physicochemical characterization of Econazole nitrate nanoparticles.

Sl. no	Code	Drug: Carrier ratio	Entrapment efficiency (%)	Particle size (nm)
1	F1	1:1	87.92	233
2	F2	1:2	85.03	279
3	F3	1:3	81.62	345
4	F4	1:4	77.95	423

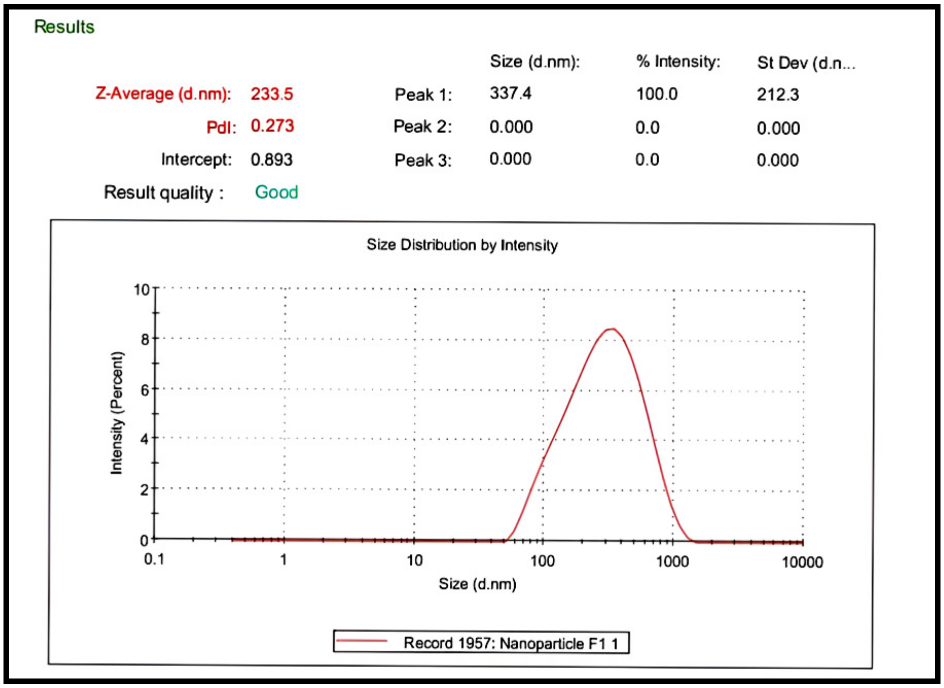

Fig 1: Particle size Distribution of F1 formulation.

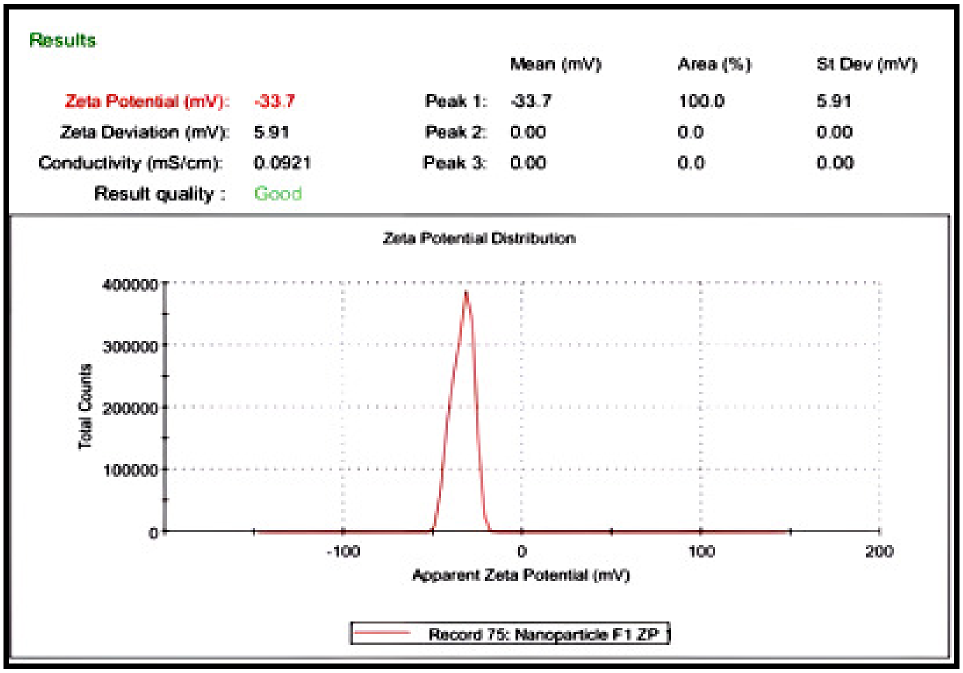

Fig 2: Zeta potential of F1 formulation.

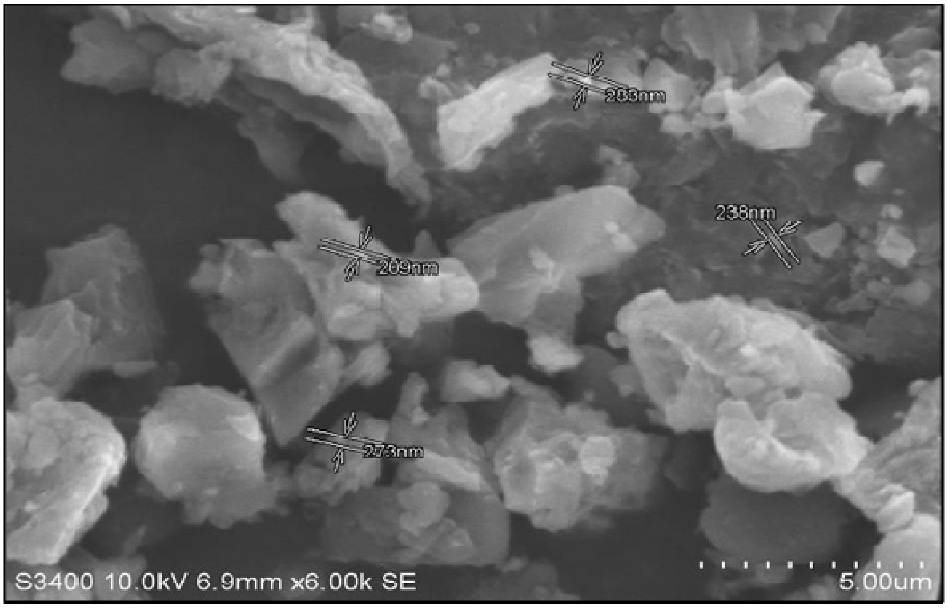

Fig 3: SEM of F1formulation

In vitro release of nanoparticles

Cumulative percentage drug release for F4, F3, F2 and F1 after 24 h were found to be 69.29%, 73.22%, 76.77%, and 80.70% respectively (Fig.4). It has been concluded that if we increase the concentration of polymer the release of drug decreases. An initial, burst release suggests that some drug was localized on the surface of the Nanoparticles.

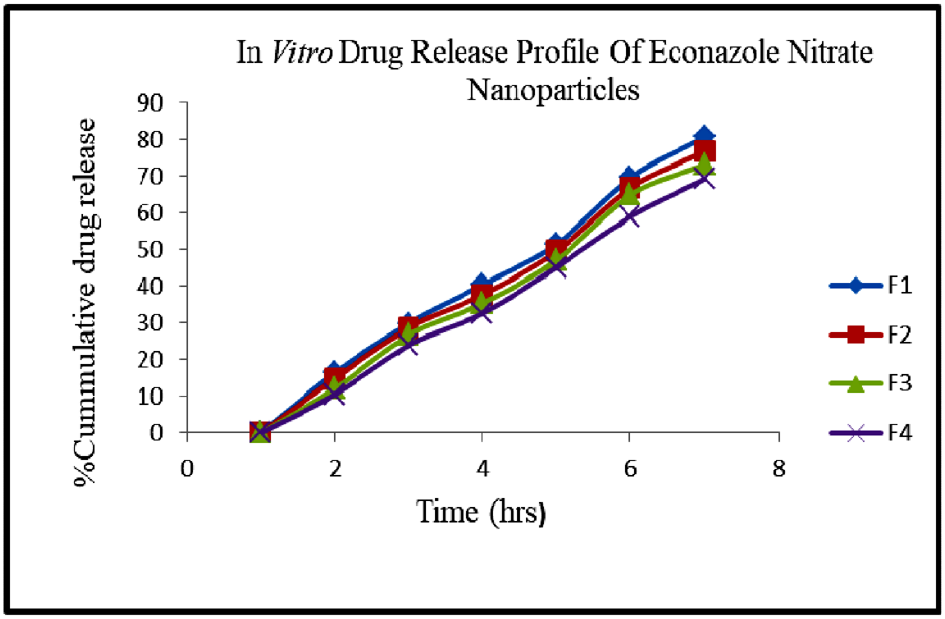

Fig 4: Comparative *In vitro* drug release profile of F1-F4 Formulation

Kinetic studies

In order to describe the release kinetics of all four formulations the corresponding dissolution data were fitted in various kinetic dissolution models like zero order, first order, and Higuchi, respectively (Table 2). As indicated by higher R2 (Coefficient of correlation) values, the drug release from all formulations follows Zero order release and Higuchi model. Since it was confirmed as Higuchi model, the release mechanism was swelling and diffusion controlled. The Peppas model is widely used to confirm whether the release mechanism is Fickian diffusion, non-Fickian diffusion or zero order. 'n' (release exponent of Korsemeyer-Peppas model) value could be used to characterize different release mechanisms. The 'n' values were found to be more than 0.62, which indicates that the super case II transport mechanism where drug release occurs by swelling and relaxation of polymer chains. Stability studies The results of drug content of ideal formulation F-1 after 60 d of stability testing at different storage conditions were shown in Fig. 5. In vitro release profiles for the same formulation stored at different storage conditions were also showed in Fig. 6.

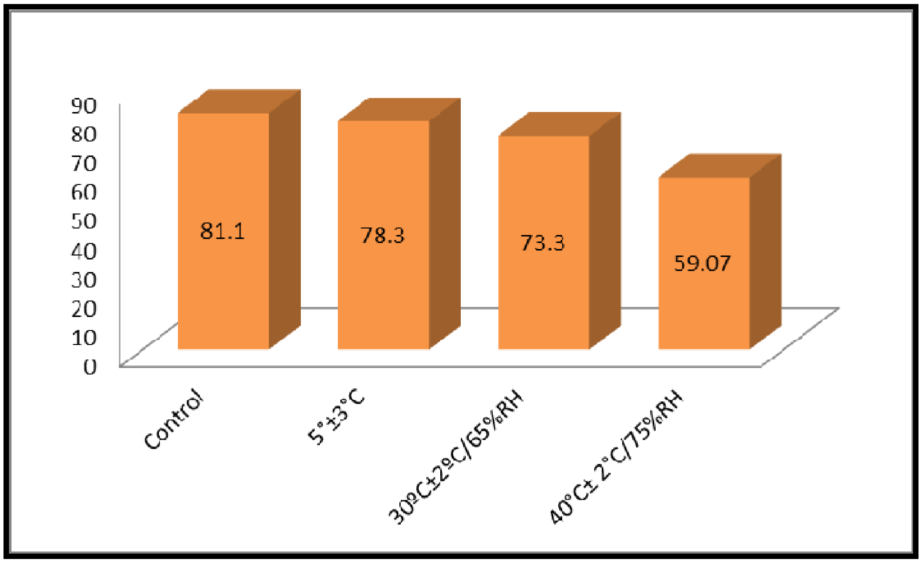

Fig 5: Stability study: comparison of drug content of formulation F-1 at 5˚C, room Temperature and 40 ± 2˚C/ 75% RH.

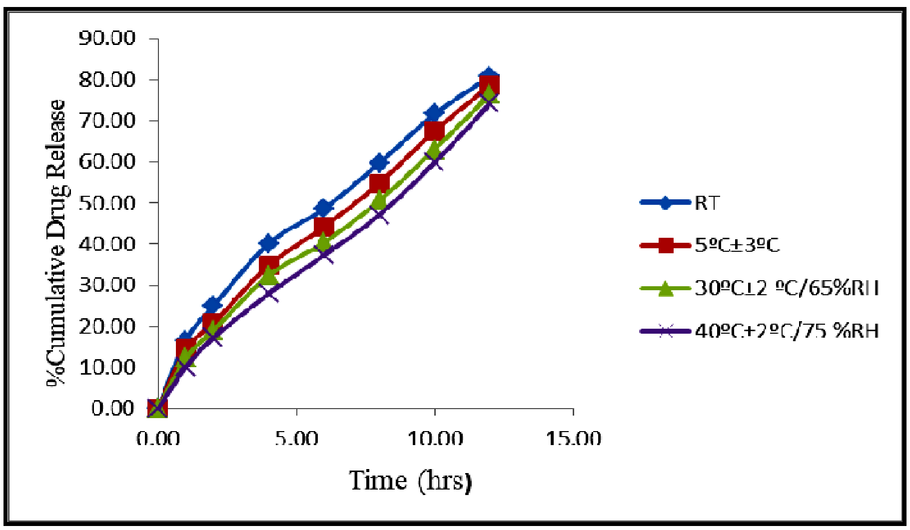

Fig 6 : Stability study: comparison of in vitro drug release profile for Formulation F-1 at 4˚C, room temperature (32˚C) and 45 ± 2˚C/75% RH after two months storage.

Table 2: Correlation coefficients according to different kinetic equations (F1).

Time (hrs)	Log T	SQRT	% CDR	Log	% Drug remaining	Log % drug remaining
0	0	0	0	0	100	2
1	0	1	16.535	1.218	83.464	1.921
2	0.301	1.414	29.921	1.475	70.078	1.845
4	0.477	2.342	40.551	1.608	59.448	1.774
6	0.602	2.449	51.574	1.712	48.425	1.685
8	0.778	2.828	69.685	1.843	30.314	1.481
12	0.903	3.464	80.708	1.906	19.291	1.285

Table 3: Data for the different kinetics model for F1 formulation.

Formulation	F1
Cumulative drug release	80.70(%)
Zero order (r2)	0.9776
First order (r2)	0.9421
Peppas plot (r2)	0.9865
'n' values	0.62

On comparing this data with the previous data of F-1, it was observed that there was a slight decrease in drug content when the formulation was stored at 5˚C and Room temperature, but there was significant decrease in drug content when the formulation was stored at 45 ± 2˚C/75% RH because at higher temperature, there might be chance for drug degradation that decreased the drug release.

CONCLUSION

Based on drug content, drug entrapment efficiency, particle size morphology, zeta potential and in vitro release, formulation F-1 was selected as an optimum formulation. Stability studies were carried out for the selected formulation F-1. The stability studies showed that maximum drug content and closest in vitro release to previous data was found for F-1 stored at 4˚C and room temperature. Thus, nanoparticles of Econazole nitrate (F-1) with core: coat ratio 1:1 was found to be spherical, discrete and free flowing and able to prolong the drug release.

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