

## Inclusion complexes of Sulconazole with cyclodextrins: characterization in aqueous solution and in solid state

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## Complexe de incluziune ale sulconazolului cu ciclodextrine: caracterizarea în soluție apoasă și în stare solidă

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**Abstract:** *Aim.* Complexation between sulconazole (SULC), an imidazole derivative with in vitro antifungal and antiyeast activity, and cyclodextrins (alpha cyclodextrin ( $\alpha$ CD), beta cyclodextrin ( $\beta$ CD), gamma cyclodextrin ( $\gamma$ CD) and hydroxypropyl beta cyclodextrin (HP  $\beta$ CD)) was studied in aqueous solution and in solid states. *Material and method.* Complexation in solution was evaluated using Higuchi-Conors phase solubility studies in the solid state X Ray diffraction studies were used. *Results.* Solubility studies suggested the existence of inclusion complex between SULC and  $\alpha$ CD,  $\beta$ CD,  $\gamma$ CD and HP  $\beta$ CD. X Ray studies confirmed the formation of the inclusion complexes. *Discussion.* Solubility of SULC in solid complexes was studied by the dissolution method and it was found to be much more soluble than the uncomplexed drug.

**Keywords:** *cyclodextrin; sulconazole; inclusion complex, phase solubility*

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**Abstract:** *Scop:* Procesul de complexare a sulconazolului (SULC), derivat imidazolic cu activitate fungicidă și fungistatică cu diferite ciclodextrine (alfa ciclodextrină ( $\alpha$ CD), beta ciclodextrină ( $\beta$ CD), gama ciclodextrină ( $\gamma$ CD) și hidroxipropil beta ciclodextrină (HP  $\beta$ CD)) a fost studiat în faza lichidă și solidă utilizând diferite metode de caracterizare. *Material și metodă.* Complexarea în soluție a fost determinată utilizând metoda solubilității de fază Higuchi-Conors. Pentru caracterizarea comportamentului complexelor în faza solidă s-a utilizat difracția de raze X. *Rezultate.* Studiile de solubilitate au demonstrat formarea complexelor de incluziune dintre SULC și CD, existență demonstrată și de studiile de difracție de raze X. *Discuții.* Solubilitatea SULC în complexii de incluziune în apă a fost studiată cu ajutorul metodei de solubilitate și s-a observat creșterea solubilității în apă a sulconazolului în forma complexată comparativ cu forma liberă.

**Cuvinte cheie:** *ciclodextrina, sulconazol, complex de incluziune, solubilitate de fază*

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### Introduction

Cyclodextrins (CDs) and modified cyclodextrins act as host molecules to form inclusion complexes rather nonspecifically with a wide variety of guest molecules. Complexation of guest compounds with CDs or modified cyclodextrins can alter guest solubility, increase stability against the effects of light, heat, and oxidation, mask unwanted physiological effects, and reduce volatility (1). The most common application of CDs in the pharmaceutical industry is to enhance drug solubility, dissolution rate, and bioavailability of poorly water soluble drugs. A large variety of drugs encapsulated through noncovalent interactions into unmodified or modified CDs (especially hydroxypropyl  $\beta$ -cyclodextrin (HP  $\beta$ CD)) cavity were described. (2). In an aqueous solution, the slightly apolar cyclodextrin cavity is occupied by water molecules that are

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energetically unfavored (polar–apolar interaction), and therefore can be readily substituted by appropriate “guest molecules”, which are less polar than water. The dissolved cyclodextrin is the “host” molecule, and part of the “driving force” of the complex formation is the substitution of the high-enthalpy water molecules by an appropriate “guest” molecule (3).

Sulconazole nitrate (figure 1) is an imidazole derivative with in vitro antifungal and antiyeast activity called (+)-1-[2,4-dichloro-*b*-(*p*-chlorobenzyl)-thio]-phenethyl]imidazole mononitrate. Sulconazole nitrate is a broad-spectrum antifungal agent intended for topical application.

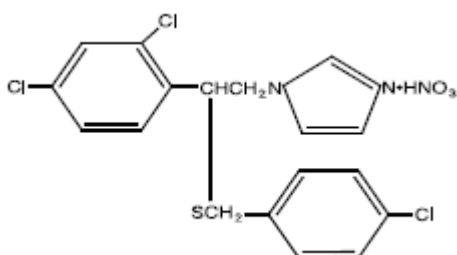


Figure n<sup>o</sup>. 1 sulconazole nitrate

Sulconazole nitrate is a white to off-white crystalline powder with a molecular weight of 460.77. It is freely soluble in pyridine, slightly soluble in ethanol, acetone, and chloroform; and very slightly soluble in water (1.9 mg/mL). It has a melting point of about 130°C.

Sulconazole is used to treat skin infections such as athlete's foot, jock itch, and ringworm. Like all azole antifungals, it inhibits the fungal cytochrome P-450 3-A dependent enzyme 14-alpha demethylase, thereby interrupting the synthesis of ergosterol. Inhibition of this critical enzyme in the ergosterol synthesis pathway leads to the depletion of ergosterol in the cell membrane and accumulation of toxic intermediate sterols, causing increased membrane permeability and inhibition of fungal growth (4). Azole antifungals can also inhibit many mammalian cytochrome P450-dependent enzymes involved in hormone synthesis or drug metabolism (5). Therefore, azole antifungals are particularly susceptible to clinically-significant drug interactions with other medications metabolized through the P450 pathway (6).

Sulconazole nitrate has a broad-spectrum antifungal activity that inhibits the in vitro growth of the common pathogenic dermatophytes including *Trichophyton rubrum*, *T. mentagrophytes*,

*Epidermophyton floccosum* and *Microsporum canis*. It also inhibits (in vitro) the organism responsible for *Pytiritiasis versicolor*, *Malassezia furfur*. Sulconazole nitrate has also been shown to be active in vitro against *Candida albicans* and certain gram positive bacteria.

A modified Draize test showed no allergic contact dermatitis and a phototoxicity study showed no phototoxic or photoallergic reaction to sulconazole nitrate cream. Maximization tests with sulconazole nitrate cream showed no evidence of contact sensitization or irritation (4). There were no systemic effects and only some cutaneous adverse sulconazole may cause side effects including: itching, rash, burning, irritation or stinging, redness.

There are no adequate and well controlled studies in pregnant women. Sulconazole nitrate should be used during pregnancy only if clearly needed. Sulconazole nitrate has been shown to be embryotoxic, it was not teratogenic in rats or rabbits. Sulconazole nitrate given orally to rats resulted in prolonged gestation and dystocia. Several females died during the prenatal period, most likely due to labor complications (7).

Complexation of sulconazole with cyclodextrin offers the possibility to improve the aqueous solubility of sulconazole without modification of its original structure. This may allow a homogeneous delivery system of sulconazole increasing its bioavailability. We synthesized  $\alpha$ -cyclodextrin-sulconazole nitrate ( $\alpha$ CD-SULC),  $\beta$ -cyclodextrin-sulconazole nitrate ( $\beta$ CD-SULC),  $\gamma$ -cyclodextrin-sulconazole nitrate ( $\gamma$ CD-SULC) and hydroxypropyl  $\beta$ -cyclodextrin-sulconazole nitrate (HP  $\beta$ CD-SULC) inclusion complexes, in order to make it more available for the yeast metabolism, and to reduce consequently the dosage, the treatment period and the gravity of all possible side effects.

## Materials and Methods

Sulconazole nitrate (SULC) (Fluka) it was used as given.  $\alpha$ CD,  $\beta$ CD,  $\gamma$ CD and HP  $\beta$ CD were obtained from Cyclolab. Double distilled water was used throughout the study.

### Solubility studies

Solubility studies were carried out according to the Higuchi and Connors method (8).  $\alpha$ CD,  $\beta$ CD,  $\gamma$ CD or HP  $\beta$ CD solutions of different concentrations (0,33 – 24,6 10<sup>-4</sup>M) were added to a supersaturated solution of SULC and shaken at room temperature (22 ± 1°C) for 24 hours. After reaching equilibrium, the solutions were filtered.

The absorbance of solutions containing different mole fraction of the drug and  $\beta$ CD or HP  $\beta$ CD was measured by UV at 190 nm and the concentration of SULC in the each solution was determined with reference to a suitably constructed standard curve.

The apparent stability constant was calculated from initial straight portion of the phase solubility diagram using the Eq. 1 (8).

$$K1:1 = \text{slope}/\text{So} (1-\text{slope}) \quad (1)$$

where: **So** is the solubility of SULC in the absence of CD<sub>s</sub>

**slope** is the slope of the experimental phase solubility diagram for  $\alpha$ CD-SULC,  $\beta$ CD-SULC,  $\gamma$ CD and HP  $\beta$ CD-SULC.

#### Preparation of the solid complex

The inclusion complexes (C<sub>s</sub>) were prepared by freeze drying method. An aqueous solution containing SULC and  $\alpha$ CD,  $\beta$ CD,  $\gamma$ CD or HP  $\beta$ CD in a 1:1 molar ratio was frozen by immersion in liquid nitrogen and freeze-dried in a Martin Christ, ALPHA 1-2LD Freeze-Dryer. The aqueous solutions were obtained by dissolving  $4,34 \times 10^{-4}$  mol SULC and  $4,34 \times 10^{-4}$  mol cyclodextrin in 25 ml distilled water and stirring it at room temperature for 48 h.

Preparation of the physical mixture (Ph.M) was performed by mixing the powders in a 1:1 molar ratio in a ceramic mortar.

X-ray diffraction (XRD) XRD patterns were obtained on a Bruker AXS D8 advance RX diffractometer.

UV visible spectroscopy studies were performed on a Specord 200 Analytik Jena 200 spectrophotometer. Stirred samples were maintained at 23°C for 1 hour before the experiment

in order to ensure the attainment of thermal equilibrium.

## Results and discussion

### Phase solubility Studies

The solubility of SULC in water is very low,  $1,9 \text{ mg}\cdot\text{mL}^{-1}$  at 25°C, as described in literature (7). In figure 2 it is shown the solubility curve obtained for SULC in presence of  $\alpha$ CD,  $\beta$ CD,  $\gamma$ CD and HP  $\beta$ CD in distilled water.

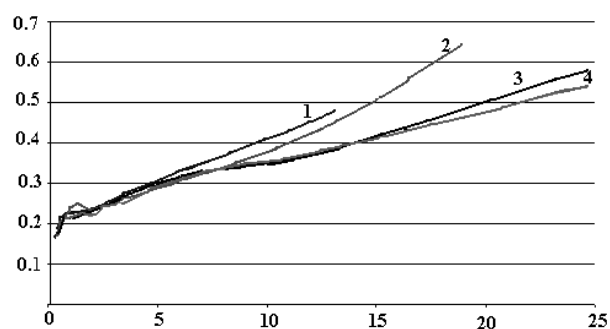


Figure n°. 2 Phase solubility curves, 1- $\alpha$ CD-SULC, 2- $\beta$ CD-SULC, 3-  $\gamma$ CD-SULC, 4-HP  $\beta$ CD-SULC.

As it can be seen, SULC solubility in water presents a linear growth, the resulting linear curve can be classified, in general, as an AL type (linear positive isotherm), as described in literature [8]. Since the slope of the four diagrams are less than 1 (0,1966 for  $\alpha$ CD-SULC, 0.1562 for  $\beta$ CD-SULC, 0.2216 for  $\gamma$ CD-SULC and 0.1413 for HP  $\beta$ CD-SULC) was assumed that the stoichiometry of each complex is 1:1 according to Higuchi and Connors (8).

Table 1

The K1:1 values obtained for the inclusion complexes

Complex	$\alpha$ -CD-Sulc	$\beta$ -CD-Sulc	$\gamma$ -CD-Sulc	HP $\beta$ -CD-Sulc
K1:1	85, 47 M <sup>-1</sup>	74, 30 M <sup>-1</sup>	30, 45 M <sup>-1</sup>	113, 87 M <sup>-1</sup>

The apparent solubility constant, **K1:1**, of each complex was calculated from the correspondent curve of Figure 2 according to Eq. 1 and the values obtained for the analyzed inclusion complexes are presented in Table 1.

#### Powder X-Ray Diffraction

X Ray powder, diffraction patterns of pure SULC and  $\beta$ CD, and corresponding solid inclusion complexes with CD, are shown in Figure 3.

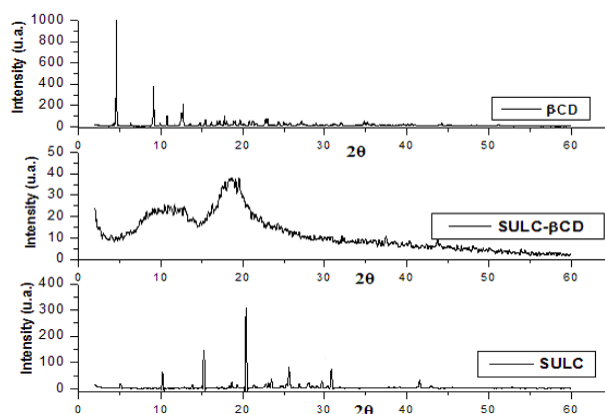


Figure n<sup>o</sup>. 3 X-Ray Diffraction Patterns of: SULC,  $\beta$ CD and  $\beta$ CD-SULC.

In the X-ray diffractograms of SULC and  $\beta$ CD powder, sharp diffraction peaks are present, indicating their crystalline state. By contrast the X-ray diffraction patterns of  $\beta$ CD-SULC system were characterized only by large diffraction peaks, in which it is no longer possible to distinguish the

characteristic crystallinity peaks of pure drug or  $\beta$ CD. These results indicate that SULC is no longer present as a crystalline material, and its CD solid complexes exist in the amorphous state.

The interplanar distances for the investigated samples are presented in Table 2.

Table 2

Sample code	<i>Interplanar distances calculated from X ray pattern</i> d (Å)
$\beta$ CD	19.19 13.88 8.90 8.20 6.90 5.77 5.48 5.43 4.97 4.64 4.50 4.14 3.85 3.57 3.44 3.27 3.07 2.25
SULC	8.56 4.34 2.91 2.40 2.20 2.11 1.77 1.70 1.63 1.55 1.50 1.16
$\beta$ CD-SULC	8.56 5.74 4.70 4.32 3.45 2.87
$\alpha$ -CD	10.001, 8.45, 6.72, 6.3, 5.91, 5.74, 5.16, 4.84, 4.69, 4.37, 4.05, 3.7, 3.38, 3.34, 3.03, 2.94, 2.9, 2.78, 2.65, 2.43, 2.36, 2.19, 2.07.
SULC- $\alpha$ -CD	8.59, 6.34, 5.77, 5.72, 4.67, 4.32, 3.46, 2.99, 2.86.
$\gamma$ -CD	17.98, 9.89, 8.132, 7.053, 4.865, 4.686, 4.173, 3.85, 3.304.
SULC- $\gamma$ CD	8.61, 5.74, 5.16, 5.13, 4.701, 4.604, 4.31, 4.07, 3.84, 3.56, 3.49, 3.30, 3.16, 2.99, 2.89.
HP $\beta$ CD	7.77 4.94 4.74 4.52 2.06
HP $\beta$ CD-SULC	16.68 7.29 4.64 4.32 3.47 2.43

The data related to  $\beta$ CD (see Figure 3 and Table 2), being compared with the data published in Refs. (9, 10), prove the same structure of  $\beta$ -CD as previously reported. The data related to SULC can be seen from Figure 3 and Table 2 show also a crystalline structure. As can be seen from Figure 3 and Table 1,  $\beta$ CD-SULC complex has different (individual) structure, showing almost complete amorphization of the drug and CD. We could recognize only one diffusion reflex (d = 8.56 Å from SULC). The formation of an amorphous state proves that the drug was dispersed in a molecular state with CD (11).

The same behavior can be observed analyzing  $\alpha$ CD-SULC inclusion complexes. The X-ray diffraction pattern of the inclusion compound differed considerably from that of the drug or of the  $\alpha$ CD alone (Figure 4).

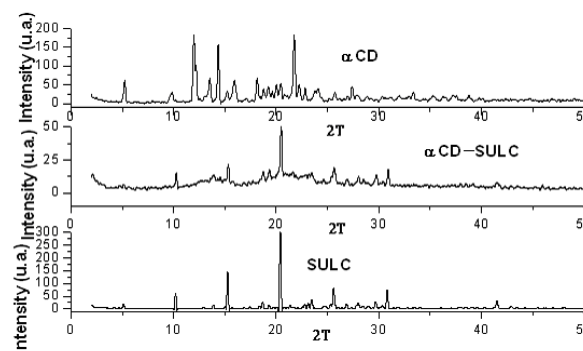


Figure n<sup>o</sup>. 4 X-Ray Diffraction Patterns of: SULC,  $\alpha$ CD and  $\alpha$ CD-SULC

The diffractogram of the complex showed peaks of diminished intensity suggesting almost complete amorphisation of the drug (Figure 4, Table 2). The diffractogram of inclusion complex shows a different crystalline state which is similar to that of the case described anterior.

In case of  $\gamma$ CD-SULC inclusion complexes diffractogram we can observe that the inclusion complex is more crystalline than the initial components, confirming that the inclusion complex formation takes place but the inclusion efficiency is reduced comparing with all the inclusion complexes described in this paper (Figure 5, Table 2).

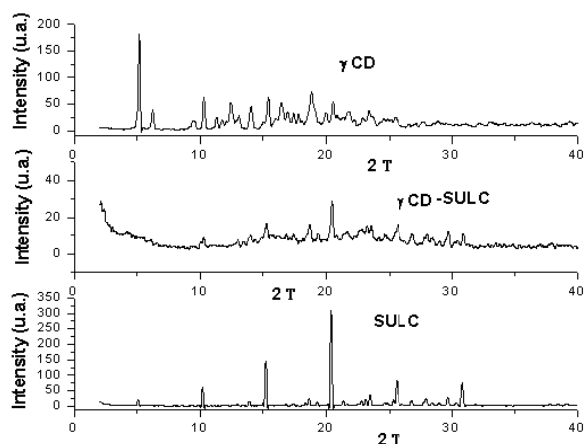


Figure n°. 5 X-Ray Diffraction Patterns of: SULC,  $\gamma$ CD and  $\gamma$ CD-SULC

The amorphization behavior is obvious also when analyzing the diffractograms obtained for HP  $\beta$ CD-SULC and for the cyclodextrin and pure antifungal drug (Figure 6, Table 2).

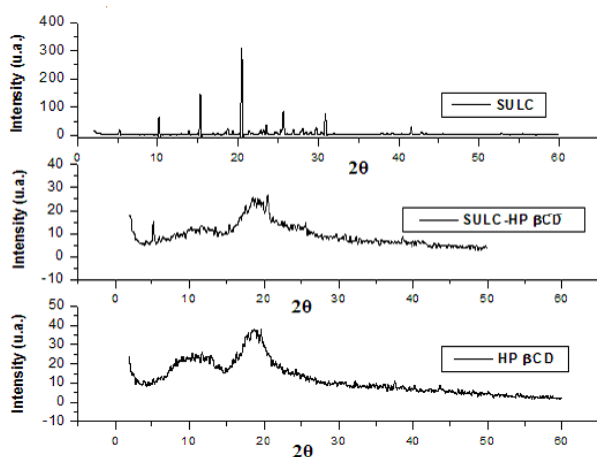


Figure n°. 6 X-Ray Diffraction Patterns of: SULC, HP  $\beta$ -CD and HP  $\beta$ -CD-SULC

A significant increase in dissolution rate can be observed by analyzing Higuchi phase solubility diagrams, which can be explained due to the formation of the complex. We can conclude that  $\gamma$ CD will increase SULC solubility in water more than  $\alpha$ CD,  $\beta$ CD and HP  $\beta$ CD (Figure 7).

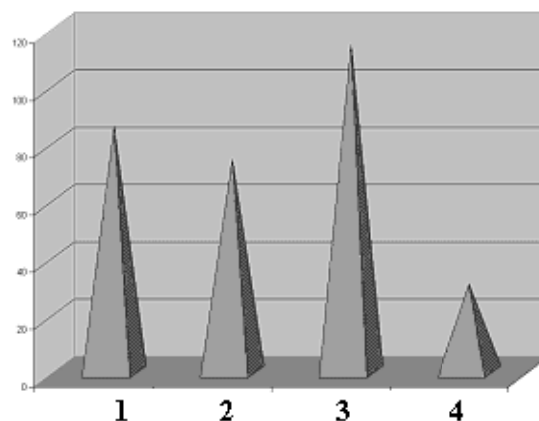


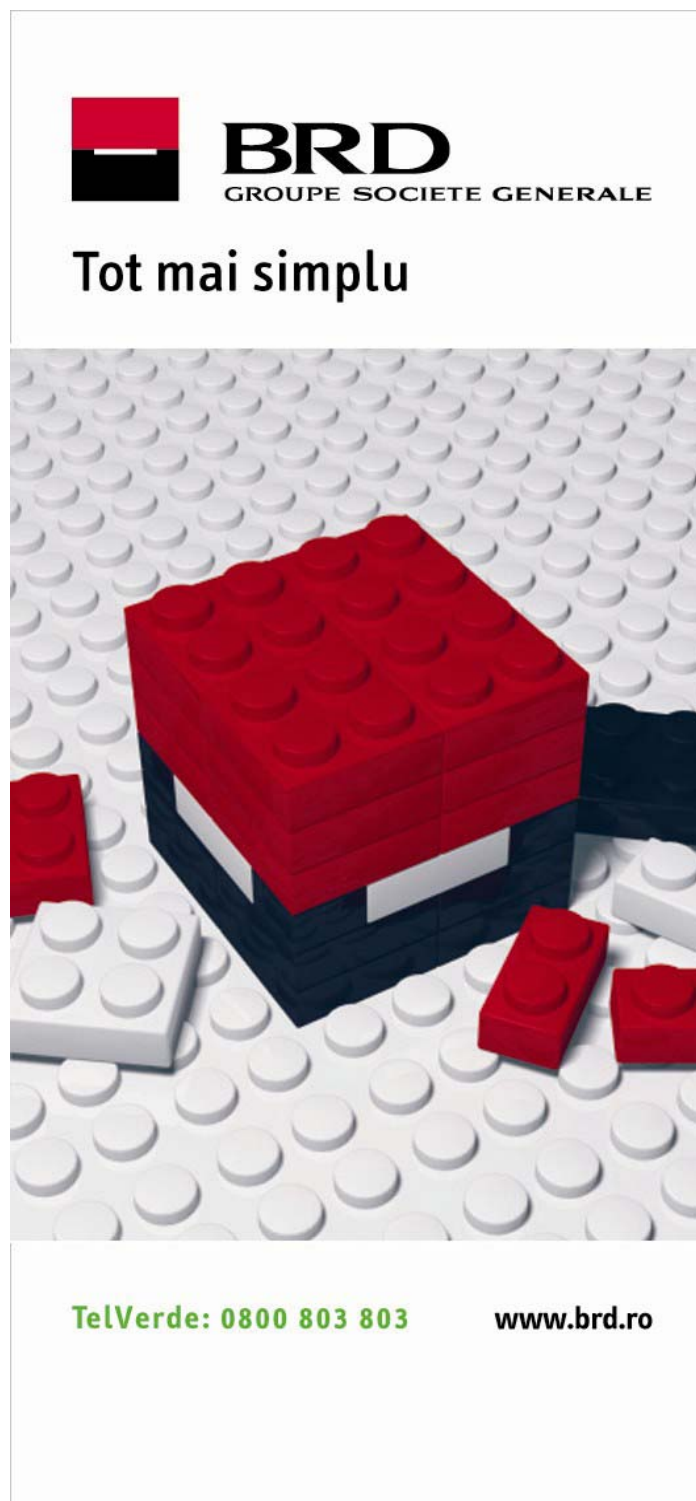
Figure n°. 7 The values of the K 1:1 obtained from the Phase Solubility Method, 1- $\alpha$ CD-SULC, 2- $\beta$ CD-SULC, 3-  $\gamma$ CD-SULC, 4-HP  $\beta$ CD-SULC.

Inclusion complexes of SULC and  $\alpha$ CD,  $\beta$ CD,  $\gamma$ CD and HP  $\beta$ CD were prepared by freeze-drying method in a molar ratio 1:1. This was confirmed by phase solubility and X Ray diffraction studies. Complexation by inclusion increases SULC solubility and dissolution.

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