Solubility and Dissolution Rate Enhancement of Ketoprofen by Nanoparticles

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Abstract

Reduction in particles size cause increase in effected surface area of drug particle. So as dissolution occurred at the surface of solute, rapid increase in dissolution rate due to large surface area. Ketoprofen belong to class II according Biopharmaceutics Classification System in which has low solubility and high permeability.the aim of study was to enhance solubility as well as dissolution rate of Ketoprofen when preparation of Ketoprofen nanoparticles by solvent evaporation method. Different formula of Ketoprofen nanoparticles were prepared using different type of polymers (pvpk15,HPMCK15, MCA4C,and CMC 30) . All of the prepared Ketoprofen nanoparticles formulas showed a particle size result within nano-range with entrapment efficiency ranged from (85.7% to 98.3 %). The average particle size of Ketoprofen nanoparticles was observed from (55 nm to 995 nm). The selected formula was investigation for DSC, PXRD, FTIR, SEM, entrapment efficiency and invitro drug release. The invitro dissolution study showed a significant ($p<0.01$) enhancement in dissolution rate of nanoparticles formula as compared to pure Ketoprofen (drug a lone) and physical mixture(drug and stabilizer), at 10 min. 75.7% of drug release from nanoparticles, while only 15.2%, 22.7% for pure ketoprofen and PM(drug: stabilizer), respectively.

***Key words**: Ketoprofen, nanoparticles, Particle size, Dissolution rate*

Introduction

Solubility play an important role in absorption of drug. The limitation in the rate of dissolution that result from low solubility lead to low in a bioavailability[1], furthermore difficulty in develop of pharmaceutical solid dosage form [2], there are many of newly discover drugs with their lipophilicity and high molecular weight and low water solubility result from emerging trends of drug design and combinatorial chemisrey [3]. 40% of the drug in the market have poor water solubility according to BCS, and 90% of drug development have characterization of poor water solubility [4]. There are many approach available to increase water solubility of drug like physical modification(modification of crystal habit, self emulsification[5], solid dispersion [6,7] solubilization by surfactant [8,9]), chemical modification (salt formation [10,11] , pH modification [12], complexation [13,14] in addition to other method (co crystallization) [15], use of co solvent, micronization [16] and nanosization). According to biopharmaceutics classification system, the relationship between invitro drug dissolution and permeability of drug are most important parameter for controlling rate and the extent of drug absorption. The classes involved: class I (high solubility and high permeability), classII (poor solubility and high permeability), class III (high solubility with poor permeability), class IV (high solubility and high permeability) [4] Nanoparticles can be define as reduction in particle size between 100-1000nm. So particles size with less than 100nm show to have a highly saturated solubility, according to Ostwald- Freundich equations [17], [18] in the other hand lead to increment in dissolution rate according to Noyes-Whiney equation [17] , [18] and improve bioavailability and reduce fed/fast effect [2] Ketoprofen is a white crystalline powder, practicle insoluble in water, belong to class II according to BCS. It's a nonsteroidal anti-inflammatory agent (NSAIA) with analgesic and antipyretic properties [19,20].

Materials and Method

Materials

Ketoprofen was purchase from Lishui Nanming Chemicals CO.Ltd from China. Polyvininypyrrolidine PVPk15 from Alpha Chemika India, hydroxypropyl methylcellulose HPMCk15 and methylcellulose (MC) A4C provide from Gromax Enterprises Corporation USA, Carboxy methylcellulose CMC30 from EMD Chemical Germany, were purchased from. Acetone was supply from Romil UK. Potassium phosphate and dihydrogen disodium hydrogen phosphate were obtained from SPINE-CHEM. Limited, and BDH Laboratory Supplies from England, respectively.

Determination saturation solubility of Ketoprofen

Saturated solubility of ketoprofen done by using shake flask method in water and 0.1NHCl (pH1.2). Conical flask contain 10 ml of water with excess amount of ketoprofen was sealed well and allow to agitate for 24 hr at 37 ^{0}C. visual notes to precipitation of ketoprofen in flask. Aliquot was allow to filtered by 45µm filtered paper, then these filtrate diluted and analyzed on UV/ visible spectrophotometer at 260nm wave length [21,22].

Preparation of Ketoprofen nanoparticles

Ketoprofen nanoparticles was prepared by using solvent evaporation method (solvent antisolvent precipitation method). Organic phase consist from 100mg of ketoprofen in 5ml of acetone, while aqueous phase consist from polymer in water. The results organic phase was added to aqueous phase drop by drop with using of syringe. The mixture of drug and polymer agitate on magnetic stirrer at 600 rpm. Ketoprofen water in soluble, however , precipitation of ketoprofen was occur in water with stabilizer. Different type of stabilizer with different ratio (weight :weight) of (drug :stabilizer) 1:1, 1:2, and 1:3 were used in preparation of ketoprofen nanoparticles.

Entrapment efficiency

Entrapment efficiency was done for all formulas of nanoparticles. Freshly prepared nanosuspensions (10ml) were centerifugate by ultracentrifugation at 45,000 rpm. The supernatant was taken to determinate the free drug after diluted with appropriate dilution, follow that substrate from initial amount of drug to obtain encapsulation amount and encapsulation ratio can be measure by following equation [23] :

$$\text{Entrapment efficiency} = \frac{W\,\text{initial} - W\text{free}}{W\,\text{initial}} \times 100\%$$

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Measuring of particles size

Particles size, polydispersity (IP), and specific surface area(SSA), were determined by using ABT-9000 Nano Laser Particle Size Analyzer (Angstrom Advanced Inc USA). All measurement were carried at scattering angle 90^0 at constant temperature at 25 ^{0}C.

Freeze drying of nanosuspension to obtain dried Ketoprofen nnoparticles

Freeze drying the most appropriate method to obtain dried ketoprofen nanoparticle, in which a sample of nanosuspension was putting in cell of layophillizer and keep in deep freeze -40 ^{0}C for 24hr., follow that put in layophillizer (LABCONCO) for 72hr. at condenser temperature -40 ^{0}C and pressure (0.9 mbar).

Determination drug content of lyophilized powder

Accurate amount of ketoprofen nanoparticles equivalent to (10mg) dissolve with methanol (10ml) and sonicated for 30 min. then diluted with appropriate volume of methanol and read in UV/ visible spectrophotometric to determine the amount of ketoprofen in lyophilize powder at 255.4 nm.

Fourier transforms infrared spectroscopy (FTIR)

To determine the chemical compatibility, the sample mix with KBr and applied hydraulic pressure to obtain pellets and scan at 400-4000 cm^{-1}

Deferential scanning calorimetry (DSC)

DSC was done by putting 5mg of sample in aluminum pas in METTELER DSC30 instrument, allow the temperature to elevated from 30 ^{0}C to 200 ^{0}C at heating rate (10^{0}C/min.) [24]

Powder x-ray diffraction (PXRD)

PRDX was confirm at continues scan range 2θ (10-50)^0 , in which the generator set at 30 Kv with current 40mA, [25]

Scan Electron Microscope (SEM)

To determination morphology of particles, INSPECT S50 was use in which the sample put in double- side tape carbon cover with gold.

In vitro drug release

Drug release study was curried by using apparatus type 2 paddle ,accurate weight of sample of dried ketoprofen nanoparticle equivalent to (50 mg of kp) put in hard gelatin capsule in 900 ml media of 0.1NHCl (pH1.2) at 37^{0}C ±5 at 50 rpm. 5ml sample withdrawal at specific time interval (2, 5,10,15,20,25,30,45,60 min.),then filter through 0.45 µm filter paper, the sample analyze at 260 nm by UV spectrophometr (1600 Shimadzu, Japan), fresh prepare medium was added to maintain the volume of media constant. The amount of ketoprofen was calculated by using appropriate calibration curve at specific media. Pure powder of ketoprofen and physical mixture (drug: polymer) were also used for compare.

Result and Discussion

Measuring of particles size

The average particle size for all formulas of ketoprofen nanoparticles appeared within nanosize range from 55-995nm. As show in table(1),The smallest size (55nm) for formula F2 with SSA(46.2) and PDI (0.00023), while largest one F6 (995nm) with SSA(2.04) and PDI (0.005). The lowest PDI mean the highly uniform in particle size distribution [26], all formulas have PDI less than 0.2 mean monodisperse distribution and a good physical stability [27].

Effect type and concentration of polymer

Different type of polymer were used in preparation of ketoprofen nanoparticle (PVPk15, HPMCk15, MCA4C and CMC30) at drug: polymer ratio(1:1, 1:2,and 1:3) for formulas (F1-F12) with particle size range from 55nm-995nm, as show in table(1). The results show the F2 (kp +PVPk15 at 1:2) with smallest average particle size 55 nm and uniform distribution of particles (PDI 0.00023), so its selected as the best formula. PVPk15 its nonionic surfactant with steric stabilization in which that stabilizer adsorb on the surface of particles through anchor segment in which highly interact with dispersed particle and act as wetting agent because has ampiphlic moiety in which extend in bulk medium [28]. HPMC also show a nanosize range steric stabilization [29] in which poses alky moiety, however have a good affinity toward hydrophobic part of ketoprofen.

To study effect concentration of polymers used on the size of nanoparticles, three ratio were taken for drug: stabilizer (1:1, 1:2, and 1:3), the results show for formulas (F1,F2) when PVPK15 used, increase concentration of PVPK15 lead to decrease particle size, while F3 increase particle size was noted as increase concentration of PVPK15, this mean F2 at ratio(1:2) drug: stabilizer was optimum concentration of PVPK15 used to obtain small particle size. While for formulas (F4-F6) increase concentration of HPMC K15 cause increase in particle size due to increase stabilizer concentration cause increment in osmotic pressure and cause particle to aggregated to each other and increase particle size [30].

Table (1) ketoprofen nanoparticles, size, PDI and SSA

Formula no.	Particle size	PDI	SSA
F1	99.7	0.008	24.61
F2	55	0.00023	46.2
F3	92	0.0012	22.95
F4	561	0.015	3.71
F5	795	0.008	2.64
F6	995	0.005	2.04
F7	629	0.015	3.66
F8	788	0.022	2.34
F9	887	0.0021	2.12
F10	625	0.017	3.45
F11	319	0.012	6.93
F12	295	0.011	7.59

Entrapment efficiency

Entrapment efficiency explain the ratio of a mass of

drug that present in nanoparticles to the mass of drug that present initially. Entrapping efficiency for ketoprofen nanoparticles was ranged from 85.7%-98.3%. The results explain a suitability of polymers that used and antisolvent technique in the preparation of ketoprofen nanoparticles.

Determination drug content of lyophilized powder

The drug content of the selected formula F2 was 98.87%, which mean a suitability of antisolvent precipitation method in preparation of ketoprofen nanoparticles.

Saturated solubility

Saturated solubility of pure ketoprofen and best formula F2 were determinate by flask shake method in water and in 0.1NHCl (pH1.2), the results appeared increase in saturated solubility approximately 7 fold as compare with pure ketoprofen. Saturated solubility of pure ketoprofen was 0. 11mg/ml and 0.09 ml in water and pH1.2, respectively. In other hand, saturated solubility for ketoprofen nanoparticles was 0.75 mg\ml, 0.23 mg\ml in water and pH1.2 respectively. The increase in saturated solubility result from reduction in particle size cause increase in surface area[32], furthermore, cause enhancement in the hydrophilicity that responsible about increment in the saturated solubility [31]

FTIR

FTIR spectrum for ketoprofen and lyophilize powder of F2(best formula) were determination by using (FTIR spectroscopy, shimadzu, Japan). FTIR – spectrum for pure ketoprofen show characteristic absorption at 1697.38 cm^{-1} and 1654.92cm^{-1} for (C=O streching) vibration of both of ketone functional group and carboxylic group, respectively. (C=C) streching of phenyl group at 1448.54 cm^{-1} and 1595.13 cm^{-1}, [33] as show in figure (1). FTIR-spectrum for lyophilized powder of ketoprofen nanoparticles (F2), were appeared that all principle peaks of drug and polymer were present which mean no interaction occur between drug and polymer.

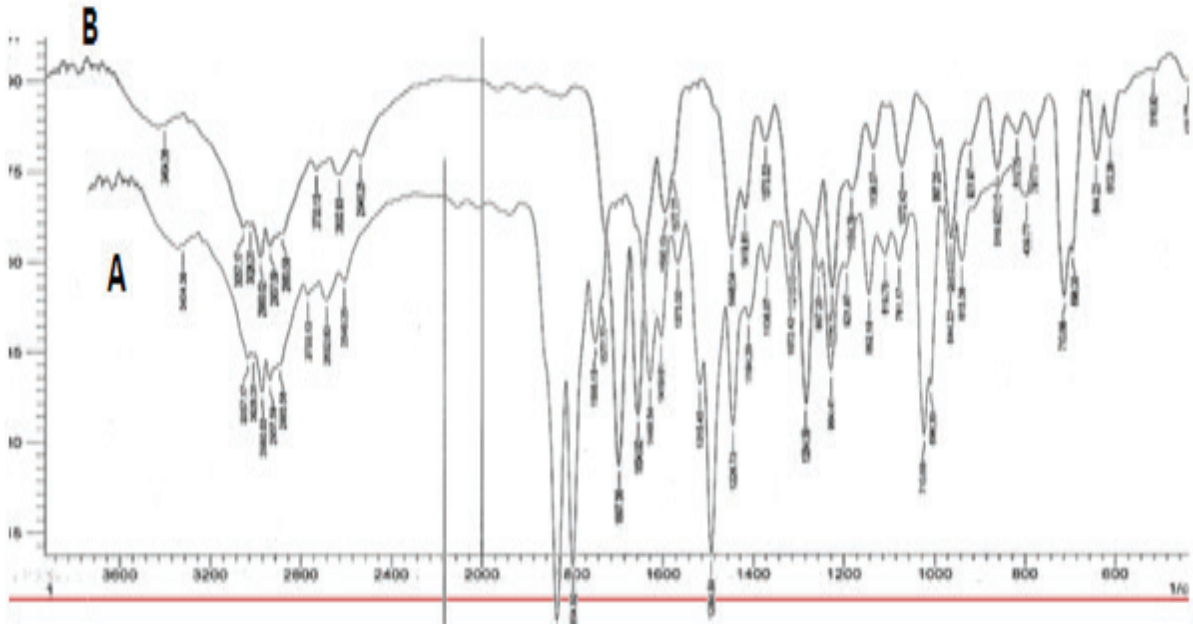

Figure (1). FTIR for (A)pure Ketoprofen, (B) ketoprofen nanoparticles

Deferential scanning calorimetry DSC

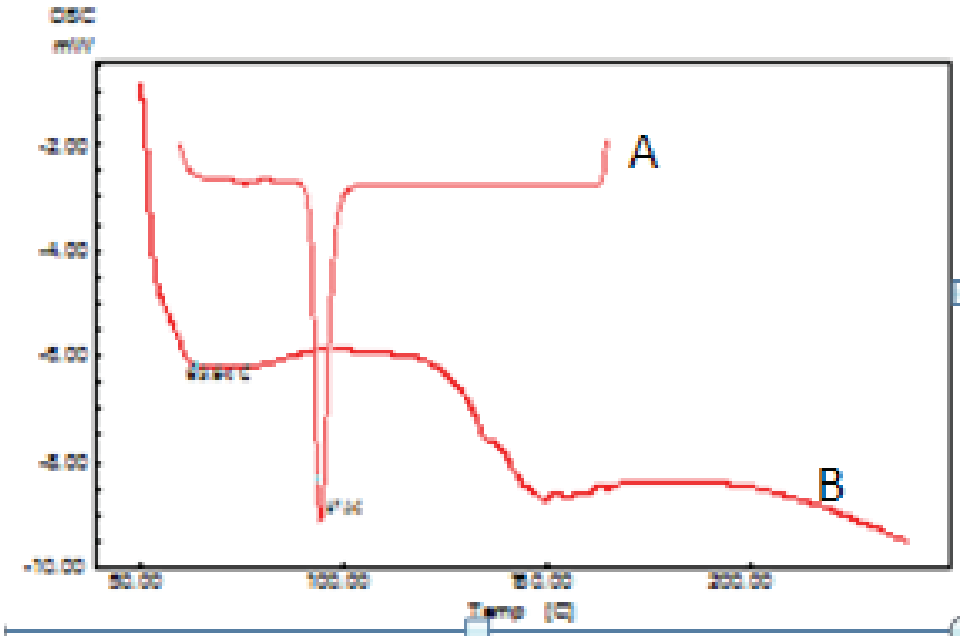

Figure (2). DSC for (A)pure ketoprofen, (B) ketoprofen nanopartiles

The thermal behavior for both pure ketoprofen and ketoprofen nanoparticles F2 was determined by DSC, also to determinate compatibility between drug and polymer. Figure (2) show a single sharp endothermic peak for pure ketoprofen at 97.2 ^{0}C which it's a melting point of ketoprofen, while for ketoprofen nanoparticles showed abroad endothermic peak below 70^{0}C which mean converted of ketoprofen from crystal to amorphous state [24].

Powder x-ray diffraction (PXRD)

PXRD was the most appropriate method to determine the crystallite of drug molecules. PXRD for pure ketoprofen appeared strong several peaks which refer to crystalline molecules of ketoprofen as show in figure (3)

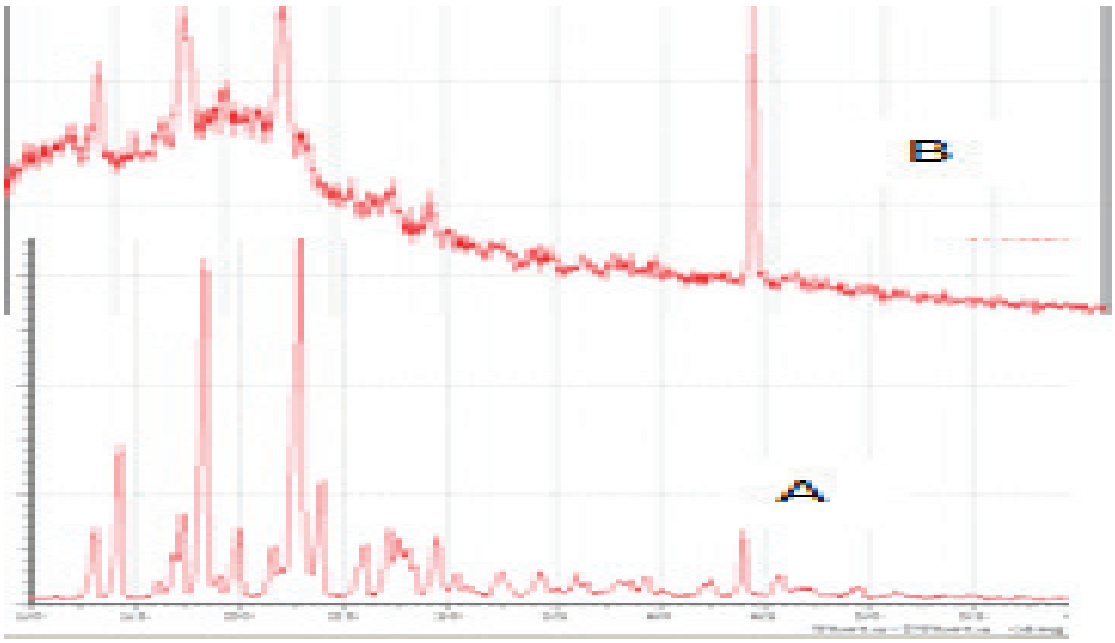

Figure (3). PXRD for (A)pure ketoprofen, (B) ketoprofen nanoparticles

Scan electron microscope SEM

SEM saw used to determine the morphology and topograghy of nanoparticles. Figure (4) for pure ketoprofen show regular crystalline shape with large size, where as for ketoprofen nanoparticle F2 show spherical shape with no aggregation of particles.

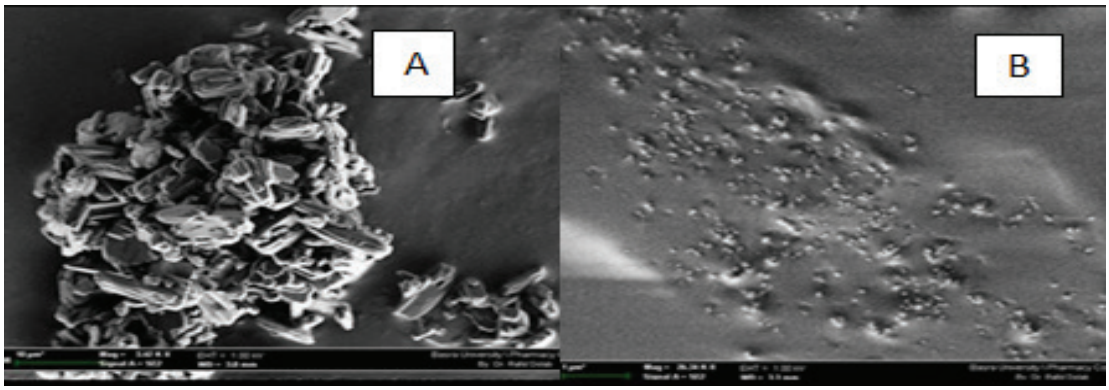

Figure (4) SEM for (A)pure ketoprofen, (B)ketoprofen nanoparticle

In vitro drug release

In vitro drug release was determined for pure ketoprofen , PM of (drug+polymer) and for nanoparticles of ketoprofen (F2). The results show, at (10 min) about 75.7% of release of ketoprofen from nanoparticle, as contract 15.2%, 22.7% for pure ketoprofen and PM (drug+polymer), respectively, as shown in figure(5). However, nanoparticles of ketoprofen appear an increase in dissolution rate as compare with pure ketoprofen and PM(drug:polymer), reduce in particle size lead to enhance surface area and cause an increase in dissolution rate.

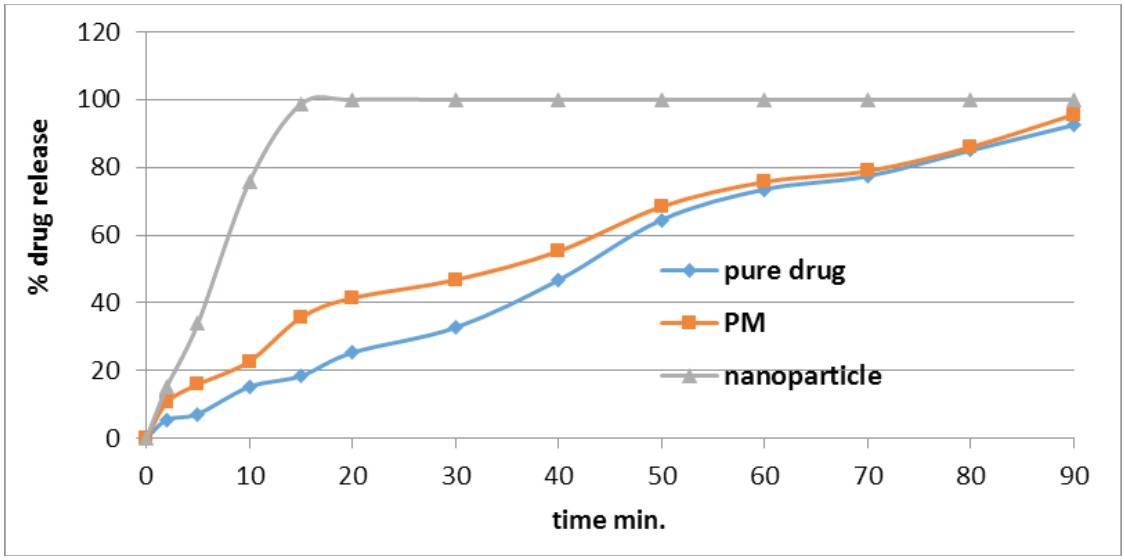

Figure (5). Drug release of prepared Ketoprofen nanoparticles(F2)compared with PM and pure Ketoprofen at 0.1NHCl(PH1.2) at 37C^0±5

Conclusion

This research demonstrate that Ketoprofen nanoparticles with size (55)nm were successfully preparation with PVPk15 by solvent antisolvent evaporation method. FTIR appear there is no chemical interaction between drug and polymer used.DSC show a good compatibility between drug and polymer and converted ketoprofen from crystal to amorphous state, these results also confirm by PXRD. SEM image show a reduction in particles size, spherical shape and no sign of aggregation. The reduction in particle size lead to increase in surface area and enhance dissolution rate ,

furthermore increase bioavailability.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Faculty of Pharmacy and all experiments were carried out in accordance with approved guidelines.

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