Preparation and Evaluation of Etodolac Loaded Copper Nanoparticles

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Abstract:
Rheumatoid arthritis is most prevalent chronic inflammatory joint disease. Inflammation is biological response of homeostasis of the damaged tissue. At this fundamental stage, it is a tissue-destroying method involving the recruitment into the disturbed tissue of blood-derived product such as a plasma protein, fluid and leucocytes. Inflammation is prevalent pathogenesis of numerous chronic diseases, involving cardiovascular and intestinal illness, diabetes, arthritis and cancer. Pain is generally occurring arthritis pain is elicited by activation of afferent sensory nerve fibers (C-fibers) in inflamed despite regression of inflammatory signs. Rheumatoid arthritis is chronic damaged in synovial membrane that's lead to synovitis and damaged of joint architecture resulting in pulmonary, ocular, vascular and other organs structure affect by inflammatory process. The aim of this research is to increase the solubility and effectiveness of water insoluble Etodolac. The goal of this work is to develop etodolac conjugate copper nanoparticles and enhance its solubility by lessen the size to nanometer scale. Preformulation studies were carried out. Total nine batches were prepared by using copper sulphate as precursor and hydrazine hydrate as reducing agent. FTIR spectroscopy indicates that there is no formation of any other compound. Copper nanoparticles were synthesized with chemical reduction method. After preparation of copper nanoparticles were subjected to various evolution parameters including % drug release, SEM, Particle size analysis, FTIR and Solubility.

Keywords: *copper nanoparticles, rheumatoid arthritis, inflammation, Etodolac*.

Introduction:

Rheumatoid arthritis is most prevalent chronic inflammatory joint disease. Inflammation is immune system's reaction to damaging stimuli like infections, poisonous substances, and damaged cells, and it is utilized in healing progression to eliminate and irritate damaging stimuli. It also specifies mechanisms that are critical to safety. Swelling is a typical symptom of chronic conditions, having cardiovascular and gastrointestinal disorders, diabetes, arthritis, and cancer. [1]. The name "arthritis" is derived from blending of Grek and Latin words. The Greek word "arthros" means "joint," while the Latin word "itis" means "inflammation". As a result, arthritis is commonly thought to be disease caused by inflamed joints. Nearly forty million adult peoples and 300,000 childrens in United State of America alone are suffering. When bone folds over comparable segments to keep functional flexibility, its referred to joint. The ligaments play like elastic strip, helping in maintain to bones in similar side at all times. Cartilage tissue protects outward of bones to prevent direct rubbing, allowing the limbs to move freely without generating friction pains or bone degradation. Synovial fluid is released by synovial membrane cells that are positioned in joint cavity with the ligaments and fills the cavity inside the joints. The pain in this case of arthritis is mostly caused by defective joints. There are many reasons to cause the disease; A) possible cartilage injury, B) synovial fluid shortage, C) autoimmune attack, D) infections [2]. Osteoarthritis (OA) is a condition that affects the joints (diarthroidal joints) that move the limbs. The syndrome is characterized by cartilage deterioration and reactive bone growth at the articular edges. The affected joints experience pain and stiffness as result of degeneration and creation of new bone. OA affect diarthroidal joint, however it is most typically located at hand, hip, and knee joints [3]. rheumatoid arthritis is chronic and including swelling, systemic autoimmune disease with no known cause and no treatment. Its characterized by swelling of joints, which leads to cartilage and bone degradation [4].

In Today's world, metallic material are produced and by using chemical functional group for updated it and then they are capable to bind with antibodies and ligand so it can be exhibit various use in targeted delivery of drug and bio technology. Nanoparticles used in biotechnology have particle sizes ranging from 10 to 500 nanometer, rarely excel 700 nm. This nano size of particles are capable to communicate to biomolecules when facade of cell showing nano sized particles on it. In same ways that can decode and assigned to various of biochemical and physiochemical property of these cells. In similar way, its possible use in drug delivery systems and noninvasive imaging offered benefits over traditional pharmaceutical agent. Targeting systems that are more particular are meant to identify certain cells like carcinogenic cells. This can achieved by linking of nano sized particle with ligand and drugs which shows particular activity for aimed cells. Furthermore, nano sized particles give stage for many duplicates of a curative material to be attached to it, resulting in a higher concentration of therapeutic and diagnostic compounds at the diseased location [5]. copper is well known for its antimicrobial activity and has been used as a healing element in wounds, in skin remodulation, and in anti-inflammatory therapies [6].

Etodolac is a kind of nonsteroidal anti-inflammatory drug that inhibits enzyme cyclooxygenase-2. Etodolac is routinely used to cure to peoples having rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis in several countries. Because of its great anti-inflammatory and analgesic actions, it also relieves ache caused by minor surgery and some other forms of pain. When compared to different other nonsteroidal anti-inflammatory drugs, etodolac has a positive safety profile, with low frequency of major gastrointestinal events such as bleeding, ulceration, and perforation [7]. Ibuprofen, naproxen, indomethacin, nabumetone and various drugs belong to this class. If the pain, fever, and swelling are mild to moderate then Etodolac will be utilized to control it. They operate by lowering amounts of prostaglandins, which are chemicals cause pain, heat and soreness when inflammation occurs. Etodolac affects to enzyme that produces prostaglandins which exhibit reduced prostaglandin levels. Etodolac is utilized to cure osteoarthritis, rheumatoid arthritis and juvenile rheumatoid arthritis swelling and pain. It's also utilized to treat soft tissue ailments including tendinitis and bursitis and menstrual cramps [8]. Dissolution rate is a stage in absorption process for medicines with high membrane permeability but very poor water solubility, depending on biopharmaceutical categorization system (class II drugs). Etodolac is not soluble in water (75 μg/mL) and is belonging to class II drugs. In healthy adults, etodoalc is very good absorbed, with maximum plasma concentrations reaching within one to two hours. With therapeutic dosages, the area under plasma concentration-time curve of racemic Etodolac rises linearly. Etodolac's plasma elimination half-life is between 6 and 8 hours [9].

Materials and method:
Copper sulphate pentahydrate and hydrazine hydrate were purchased from Research Lab Fine Chem Industries, Islampur . Etodolac was received as a gift sample from IPCA lab Mumbai. Ethanol (HPLC grade) was purchased from Research Lab Fine Chem Industries, Islampur. All other solvents are used in the study were of analytical grade and used as received.

Method:

Preparation of copper nano sized particles:
Nano sized particles of copper are prepared via chemical reduction method. In this were copper sulphate pentahydate is precursor salt for copper nano particles and hydrazine hydrate is powerful reducing agent is reduce to copper sulphate pentahydrate. The hydrazine hydrate is reducing to copper salt and form copper ions. Copper sulphate pentahydrate is dissolved in distilled water and heat at 80 OC then stirred at 700 rpm for 15 min. After the stirring of copper sulphate solution. Prepared the aqueous solution of hydrazine hydrate and added drop wise into copper sulphate solution the color is changed from lite blue color to ocher color. This solution is stirred for 24 hr at 700 rpm. Then copper nano sized particles are separated by using centrifuger at 300rpm. The prepared copper nano particles washed with ethanol and collected.
Formulationtable:

Preparation of etodolac loaded copper nanoparticles:

The 100 mg pure etodolac is dissolved in 5ml ethanol in beaker and added 100mg copper nano sized particles in this beaker. The ethanolic solution of etodolac drug and copper nano sized particles are stirred at 500rpm by magnetic stirrer for 24 hr. The prepared etodolac conjugate copper nano sized particles are collected.

Characterization and evaluation:

1. scanning electron microscopy:
SEM test carried out for change in morphology of Etodolac conjugate copper nanoparticles image were performed with ZEISS, image clearly exhibit in different figure. At 3 KV, SEM picture taken with 10 KX magnification. Sample shows different mutual orientation.

2. Drug Entrapment Efficiency:
After separating the copper nano sized particles by centrifugation, supernatant solution taken for quantification of drug in it. Accurately 1 mL of supernatant solution was diluted with phosphate buffer and absorbance was measured to calculate concentration of unentrapped drug. The entrapment efficiency of copper nanoparticles could be determined from the formula.
Drug entrapment efficiency = Total amount of drug – drug in supernatant solution / Total amount of drug ✕100

3. % Drug release:
It is done by using dissolution type apparatus type 1 containing the basket. An accurately weighed quantity of etodolac conjugate nanoparticles (equivalent to 100 mg drug) was filed in hard gelatin capsules and placed in basket, which was immersed in 900 ml phosphate buffer having pH 7.4. The temperature of media maintains to stable at 37 °C ± 0.2 °C and stirred at sped of 75 rpm. At specific time intervals (1, 2, 3, 4, 5, 6, 7, 8 h) 1 ml of samples were remove from bowl and volume was substituted with equal amount of fresh warm dissolution medium. The collected samples were filtered and analyzed at 227 nm, using UV-visible spectro photometer against the phosphate buffer having pH 7.4 as blank.

4. Particle size analysis:
Using particle size analyser (Horiba scientific SZ100), the vesicle size of Etodolac conjugate copper nano sized particles calculated. For particle size calculation the sample was diluted with double distilled water. Particle size calculation depends on number of particles and form of solution containing ion.

5. FTIR:
To determine and interpret functional group in drug and excipient. FTIR of prepared Etodolac conjugate copper nanoparticles was studied by directly taking the sample on ATR disk and proceed for next step to monitoring data which was shows the peaks on particular wavelengths. After taking of graphs peaks was found and take result for it. The IR peaks were analyzed using a JASCO 4600 spectrometer from Japan. Data was evaluated through standard values based on results.

Result and Discussion :

1 scanning electron microscopy:
The external morphology of etodolac conjugate copper nano particles as shown in figure no. 1 indicates presence of round oval shape and etodolac loaded at surface of copper nano sized particles.

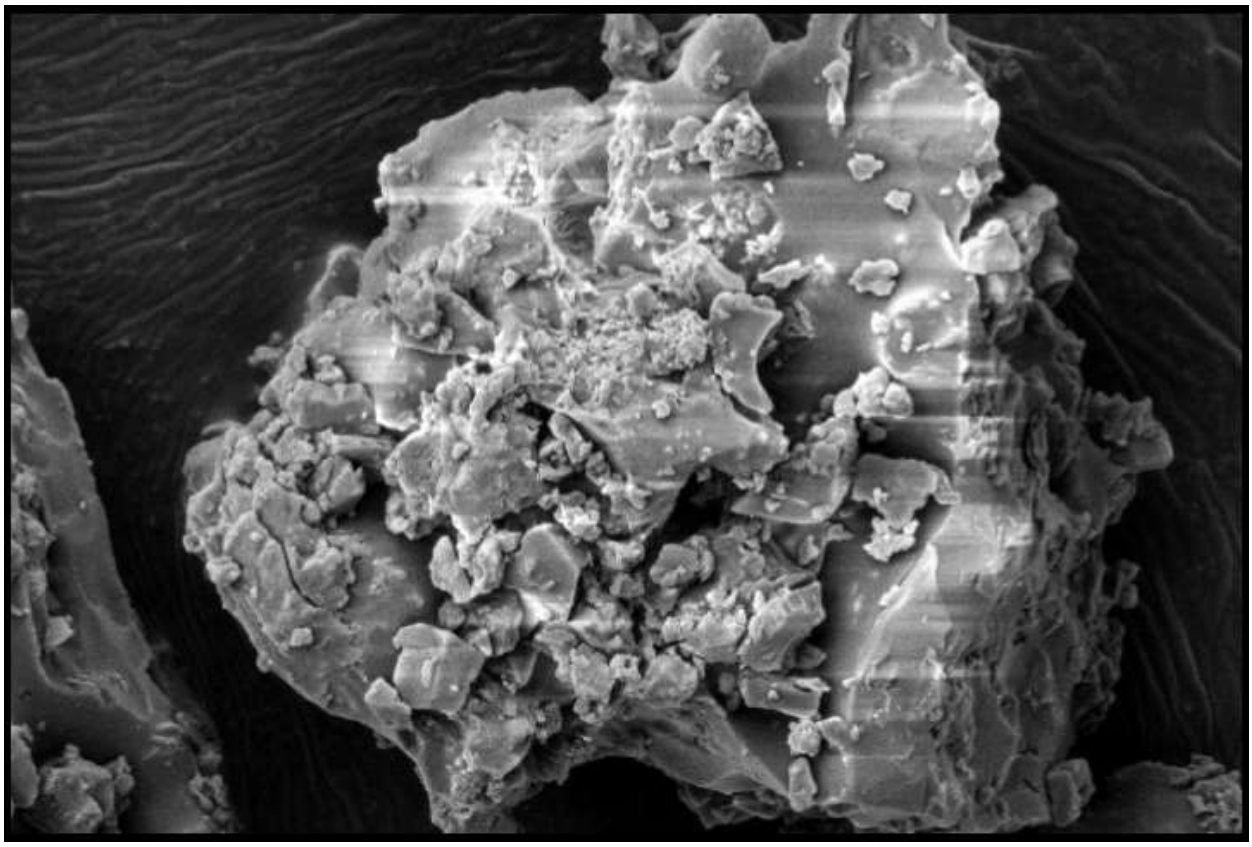

Figure no.1: Etodolac loaded copper nanoparticles

2. Drug Entrapment Efficiency:
Percentage of entrapment efficiency of all formulations ranges between 91% to 95.9% and their variation with change in concentration of excipients. Formulation no. 4 shows maximum entrapment efficiency i.e. 95.9

Table No. 1: Drug entrapment efficiency

Sr.no.	Formulation no.	Drug entrapment efficiency (%)
1	F1	91
2	F2	91.8
3	F3	94.7
4	F4	95.9
5	F5	94.2
6	F6	93.5
7	F7	90.7
8	F8	92.4
9	F9	93

3. % Drug release:

Table no. 3 shows Etodolac conjugated copper nanoparticles displayed release of drug over period of 8 hrs. At end of 8 hrs maximum drug release was achieved by formulation 4 compared to slower drug release observed in other formulation. This could due to higher drug loading, optimum entrapment efficiency and normal particle size.

Table no.2: % drug release of Etodolac from formulation for different time interval

TIME	F1	F2	F3	F4	F5	F6	F7	F8	F9
0 Hr	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1 Hr	**7.2**	**20.7**	**18**	**19.80**	**16.2**	**21.6**	**9**	**17.10**	**21.60**
2 Hr	**9**	**27**	**27.92**	**25.22**	**23.41**	**29.72**	**19.81**	**21.61**	**34.22**
3 Hr	**12.6**	**36**	**36.05**	**35.15**	**31.54**	**36.95**	**31.53**	**36.94**	**50.46**
4 Hr	**19.83**	**61.29**	**42.39**	**57.68**	**45.97**	**49.59**	**44.16**	**43.28**	**58.61**
5 Hr	**36.95**	**66.74**	**55.03**	**61.25**	**61.33**	**57.75**	**50.51**	**52.33**	**67.68**
6 Hr	**50.49**	**79.41**	**72.19**	**74.02**	**73.09**	**72.21**	**64.97**	**69.59**	**80.35**
7 Hr	**68.55**	**84.90**	**74.07**	**86.70**	**83..07**	**83.99**	**79.44**	**82.16**	**89.44**
8 Hr	**82.12**	**93.09**	**91.26**	**96.69**	**94.87**	**94.89**	**92.13**	**93.95**	**94.94**

4. Particle size analysis:

Table no. 4 display size distribution of particles of etodolac conjugate copper nano particles. The subsequent particle size study by revealed maximum sensitivity in distribution of size of etodolac loaded copper nano sized particles. The usual size of particles of prepared F1-F9 batches was obtained between 32.1- 52.6 nm. The particle size of optimized batch was found to be 32.1.

Table no. 3: Particle size from F1 to F9 formulation

Formulation batch	Particle size(nm)
F1	**52.6**
F2	**38.2**
F3	**41.5**
F4	**32.1**
F5	**35.4**
F6	**35.8**
F7	**40.4**
F8	**37.1**
F9	**34.2**

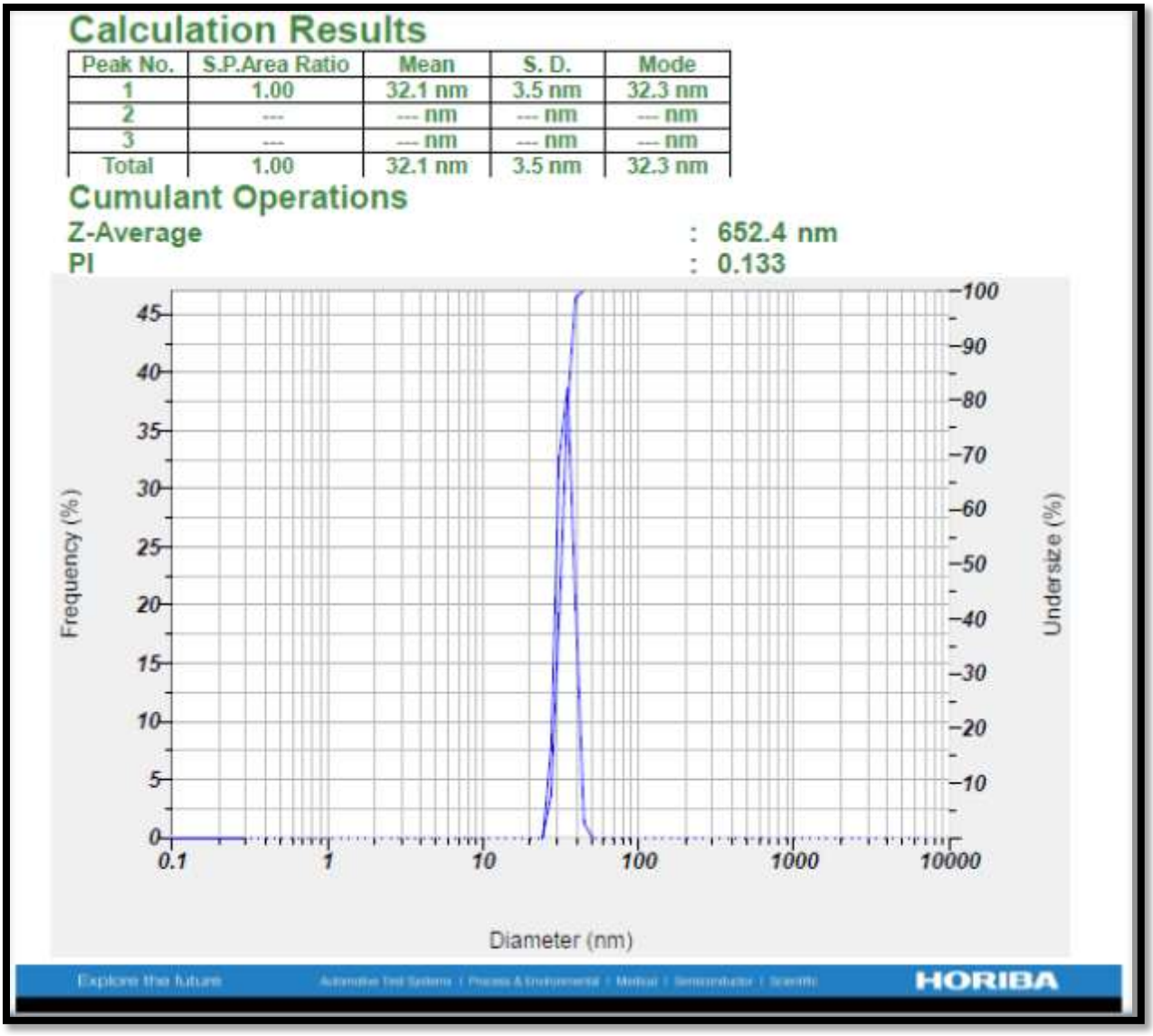

Figure no. 2: Particle size analysis of optimized batch 4

5. FTIR:

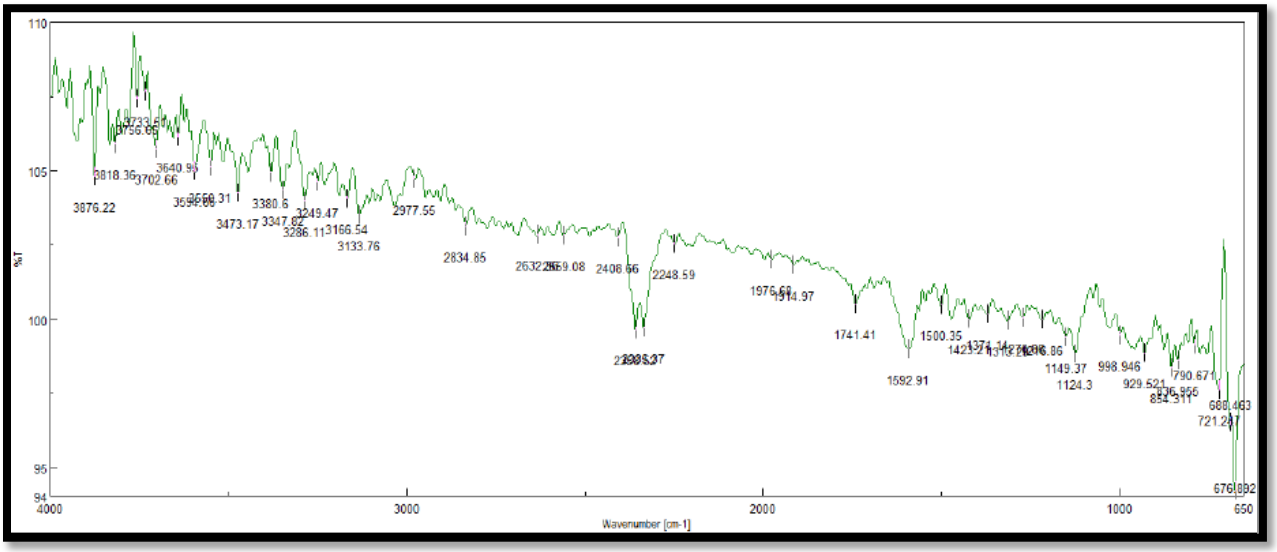

Fig No. 3: FTIR Spectra of prepared etodolac conjugate copper nanoparticles

Peak no.	Peak position	Functional group
1	**3756.65**	**O-H**
2	**3380.6**	**N-H**
3	**2977.55**	**C-H**
4	**1914**	**C=O**
5	**1500.35**	**C-C, C-C-H**
6	**1149.37**	**S=O**
7	**998.946**	**CH_2**
8	**721.217**	**CH_3**

Table 4: Interpretation of IR spectra of Etodolac conjugate copper nanoparticles

Summary and conclusion :

Based on preformulation studies drug and excipients were characterised for different tests like organoleptic properties, UV and FTIR spectroscopic study. Drug and excipient compatibility study were carried out. From obtained results drug and excipients were found to compatible to each other. There was not formation of any new compound. The drug loaded copper nanoparticles was prepared via chemical reduction method. From obtained results of all copper nanoparticles batches it is concluded that optimum drug: excipient concentration is required.

The prepared copper nanoparticles batches were characterized for different parameters like particle size, drug entrapment efficiency, % Drug release, of prepared nano sized particles. From different batches of copper nanoparticle formulation no. 4 shows maximum drug release. The drug release of prepared copper nanoparticles was carried out by using dissolution testing apparatus. The drug release was found to be 96.69%. From this copper nanoparticles, batch F4 was optimized. Particle size in batch F4 was found to be 32.1 nm. The optimized batch further evaluated for Scanning electron microscopy. Surface morphology of drug and excipient complex (copper nanoparticles) was carried out by using Scanning electron microscopy. In in vitro drug release study, drug release rate was found be depends upon optimum drug, excipient ratio and particle size of prepared copper nanoparticles. The formulation of copper nano sized particles of batch F4 show maximum drug release from copper nanoparticles within 8 hr. From all result, formulation of copper nano sized particles batch F4 optimized formulation having % drug release and particle size 32.1 nm. An optimized formulation of copper nanoparticles of batch F4 was well acceptable and palatable with better absorption and stability.

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