CURCUMIN NANOPARTICLES, A REVIEW ON METHODS OF SYNTHESIS AND APPLICATIONS

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ABSTRACT
Design and expansion of herbal nanoparticles has become a frontier research in the Nano formulation arena. Curcumin is the main bioactive component controlled in Curcuma longa, largely employed in traditional medicine. Curcumin is the main slope of “Turmeric” a famous Indian spice and food additive. The marvellous nutritional and medicinal effects of curcumin finished it a decent alternative to some conventional drugs and food flavouring or colouring materials. Recently, beneficial properties, useful for prevention and treatment of several disorders, have been exposed for this compound. Although curcumin has revealed therapeutic efficacy against many human ailments, one of the major problems with curcumin is its poor bioavailability, which seem to primarily due to poor absorption, rapid metabolism, and rapid systematic removal. Therefore, introduction of the nanotechnology offers a solution towards amplified bioavailability of curcumin. In this review, a lot methods of preparation of curcumin nanoparticles and its applications are briefly deliberated.

KEYWORDS: Curcumin, nanoparticles, central nervous system diseases.

INTRODUCTION
Curcumin (also known as curcumin-1),[1] is the principle curcuminoid originate in Indian curry spice turmeric (Curcuma longa, Family-Zingiberaceae). The other curcuminoids current in turmeric are demethoxycurcumin (curcumin-II),[2] bisdemethoxycurcumin (curcumin-III),[3] and recently cyclocurcumin.[4] Curcumin is a potent Phyto molecule with an extensive range of biological activities.[5] Curcumin is a principal nutraceutical molecule along with other curcuminoids and it has been rummage-sale in the food and pharmaceuticals industries.[6] Its clinical applications are limited largely due to its poor solubility and quick metabolism, which results in poor bioavailability. Curcumin is well thought-out with very low solubility in water (11ng/ml) and imperative presystemic biotransformation, mainly via glucuronide and sulfate conjugation. The extreme oral dose of 8 gm/day of curcumin does not yield any toxic effects.[7] Curcumin was inaccessible for the first time in 1815, while its chemical structure was determined in 1973 by Roughley and Whiting. The melting point of curcumin is 176–177°C, and it forms red to brown-colored salts when preserved with alkalis.[8] Commercial curcumin has around 77% diferuloylmethane, 17% demethoxycurcumin, and 6% bisdemethoxycurcumin.[9] Curcumin is a natural composite, which is hydrophobic in nature. It consists of two polyphenolic rings, which are replaced by methoxy ether at the ortho position, and tautomerization of curcumin ascends in a pH-dependent condition; in neutral and acidic conditions, curcumin possesses a bis-keto form [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione]. Curcumin works as an antioxidant, anti-inflammatory, and anti-atherosclerotic. It prevents scarring, cataract, gallstone formation, liver injury, and kidney toxicity, and also indorses wound healing and muscle regeneration. Curcumin uses medicinal benefits against psoriasis, diabetes, multiple sclerosis, Alzheimer’s, HIV, septic shock, cardiovascular disease, lung fibrosis, arthritis, and inflammatory bowel disease.[10] It has been used to cure liver problems, digestive disorders, and in treatment of several skin ailments and in wound healing.[11] Antioxidant property of curcumin can be enhanced by its structural modification or by synthesis of its analogs.[12,9] Naz and coworkers.[13] reported antimicrobial activity of C. longa against changed strains of bacteria such as Bacillus subtilis, Bacillus macerans, and Bacillus licheniformis. Curcumin also comprehensive spectrum activity against insects’ pests, plant, pathogens, fungi, and weeds.[14] Many clinical trials have demonstrated that the biological action of drugs like curcumin can be
achieved by enhancing the activity of the drug. The bioavailability means the rate at which the drug molecule is being distributed and fascinated on the site of action (www.merckmanuals.com). Curcumin possesses better pharmacodynamics but unfortunate pharmacokinetics. Thera curcumin is a form of curcumin, which is highly answerable and dispersible in water. It is framed by using nanoparticulation and surface processing techniques. Human trials have publicized that Thera curcumin has the ability to cure liver problems and heart failure.

**Methods of synthesis of curcumin nanoparticles**

Various methods are being expansion for the synthesis as well as to increase the activity of curcumin nanoparticles.

**Nanoprecipitation method**

Nanoprecipitation method is also standard as solvent displacement method. In this method, the wanted polymer is suspended in solvent, and curcumin is then in polymeric solution. After that, this polymeric solution is soluble in water under continuous stirring in water, which results in precipitation.

**Single emulsion method**

The single emulsion method is the conservative method of curcumin nanoparticle synthesis. In this method of synthesis, curcumin nanoparticles are ready by dispersing in solvent followed by high-speed homogenization or ultrasonication. Further solvent is disappeared by continuous magnetic stirring at room temperature or under reduced pressure. The solidified nanoparticles are ultrasonicated and composed, followed by washing with distilled water to remove additives and lyophilized to get the nanoparticles. Curcumin-loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles can also be prepared.

**Wet milling method**

Wet-milling is the system of synthesis for curcumin nanoparticles. The hydrophobic drug-like curcumin is adjourned in appropriate dispersing solvent curcumin. The obtained solution is further agitated under ultrasonication method. Distilled water is also required for the synthesis of curcumin nanoparticles. The obtained solution is then allowed to be centrifuged, and the nanoparticles are found by this method. The synthesis of curcumin nanoparticles by this technique is reported by researchers.

**Microemulsion**

Microemulsion is act as an ideal method for nanoparticle fabrication. The surfactants, which are rummage-sale in this method, are hydrophobic in nature for water and hydrophilic in nature for oil. Microemulsion is molded when a small amount of surfactant is stirred and curcumin is added in it along with oil and water. Its outcomes in the formation of a turbid solution, which generally looks like small droplets. The curcumin nanoparticles are produced by this method in order to

increase the biological activity of curcumin. Various types of surfactants are cast-off to increase the surface stabilization of curcumin nanoparticles. The process is easy and can be used for drug delivery with few energy expenditures. Microemulsion technique is pretentious by certain parameters like temperature and pH variation.

**Fessi method**

In this method of synthesis, curcumin is unstiffened in a suitable solvent under sonication condition. The solution thus found is further added in pure water along with a certain surfactant with constant stirring. The curcumin nanoparticles can be spontaneously manufactured by this method. Moorhi and coworkers have hand-me-down this method for the fabrication of curcumin nanoparticles.

**Coacervation technique**

In this method of synthesis of curcumin nanoparticles, a polymer is liquefied in organic solvent (e.g., dichloromethane, ethyl acetate, or acetonitrile), and the hydrophobic drug-like curcumin is suspended directly in the polymeric solution, and it is permissible to stir and mix properly. The nanoparticles are collected by centrifugation. It is an inexpensive way, and solvents are not used. The main drawback of the coacervation technique is that it requires a large amount of solvent.

**Chirio et al. formulated curcumin-loaded nanoparticles by using this technique.**

**Ultrasoundication**

This method is generally working for the drugs, which are less water soluble. For fusion purposes by means of this technique, curcumin is first melted in organic solvent, and the resulting solution is then added into the polyelectrolyte solution under ultrasonication condition for several intervals of time, and after that, the curcumin nanoparticles can be manufactured by this method. Zhang et al. have made the curcumin nanoparticles by using this practice of ultrasonication.

**Solid dispersion method**

In this method, matrix and hydrophobic drugs like curcumin are diverse. Matrix can be in the shapeless or in crystalline form. This method can be considered to liquify the insoluble hydrophobic drug.

**Ionic gelation method**

A hydrophobic drug such as curcumin is dissolved in the

This is an easy and simple method of nanoparticle synthesis. proper solvent, which displayed complete solubility of curcumin in it, and then this solvent is supplementary into a polymeric solution under constant stirring condition. This method be contingent on the crosslinking of the polymer along with the drug such as curcumin. Chabib and coworkers testified the synthesis of curcumin nanoparticle used chitosan as a polymer. This polymer improved the solubility and stability of the curcumin nanoparticles.
Spray drying method
In this process of synthesis, curcumin and a polymer are liquefied in the same solvent or mixture of solvents. After that, the solvent is acceptable to evaporate by hot air flow. In spray drying caused in the formation of drugs in the amorphous state, which may get partially crystallized during processing. Curcumin nano-crystals can be framed by spray drying method.

Emulsion polymerization method
This is a fast and readily scalable method used for curcumin nanoparticle synthesis. The organic and continuous phase are two types of emulsion techniques, which can be used for the synthesis of curcumin nanoparticles. A surfactant is mixed in pure water by ultrasonication, then curcumin is dissolved in organic solvent and finally, the solution is added to the surfactant. Moorthi and coworkers have conveyed the synthesis of curcumin nanoparticles by using this method, and piperine was used along with curcumin to increase the biological activity of the synthesized curcumin nanoparticles.

Antisolvent precipitation method
Antisolvent precipitation is the technique of synthesis of a poorly water-soluble drug. In this method of synthesis, curcumin is mixed in organic solvent shadowed by the addition of this solution to the deionized water under constant stirring. Hence, the curcumin nanoparticles can be produced by this method. Kakarn and coworkers used this process for synthesis of curcumin nanoparticles. The advantage of this method of synthesis is that it is a suitable technique for the combination of the poorly soluble curcumin nanoparticles.

Solvent evaporation method
The solvent evaporation system includes two major steps: 1) preparation of a solution entailing of polymer and drug-like curcumin (2) and evaporation of dispersing solvent secondhand for dissolving curcumin. It results in the creation of solid mass. The emulsion molded is then converted into a nanoparticle suspension by evaporation of the solvent. The advantage of this scheme is that low temperature is required for the evaporation of the solvent, and thermal deposition can be prevented.

The disadvantages are: (i) the reagents occur in the method are quite expensive, (ii) selection of the proper solvent is somehow tough, and evaporation of the organic solvent is time-consuming course. PLGA-loaded curcumin nanoparticles were created by this technique.

Thin film hydration method
In this method, curcumin and the surfactants are acceptable to blend in organic solvent under sonication condition. The solvent is bearable to diffuse under pressure, and after that, distilled water is further in sonication condition, and the obtained nanosuspension is then centrifuged to obtain curcumin nanoparticles. Moorthi et al. demonstrated curcumin nanoparticles synthesis by this method of synthesis, and they used piperine along with curcumin.

Different applications of curcumin and nanocurcumin
Anti-inflammatory Activity
In ancient Indian medicine, turmeric has been castoff as an anti-inflammatory agent. Rocha et al. associated the anti-inflammatory activity of normal curcumin and nanocurcumin in rat. The inhibitory effect exposed by nanocurcumin at dose 50 mg/kg was similar to that of normal curcumin at dose 400 mg/kg which demonstrated the improved anti-inflammatory activity of nanocurcumin. Curcumin compressed exosomes were studied for their potency in lipopolysaccharide-induced septic shock mouse model. In that experiment, curcumin sent by exosome demonstrated more stability, target specificity and they were found in high concentrations in blood.

Anticancer Activity: In 1987, Kuttan and coworkers, for the first time, conveyed on the anticancerous activity...
of curcumin on humans. They executed clinical trials on 62 patients with external cancerous lesions. It was initiate from the study that curcumin is effective and remarkable in the case of itching, lesion size, and pain.\textsuperscript{[46]}

Topical curcumin was start to produce symptomatic relief as evidenced by reductions in smell. Curcumin, either alone or in combination with other agents, acts as a potential agent against the different types of cancer such as colorectal cancer, pancreatic cancer, breast cancer, prostate cancer, multiple myeloma, lung cancer, oral cancer, and head and neck squamous cell carcinoma (HNSCC).\textsuperscript{[47]}

A] Prostate cancer
Prostate cancer is a disease which arrangement in the prostate gland of the male reproductive system. Gradually, it may occur to other parts of the body like bones and lymph nodes.\textsuperscript{[48]} Curcumin-laden poly (lactic-co-glycolic acid) (PLGA) nanoparticles furnished by Yallapu et al.,\textsuperscript{[49]} substantiated the anticancer activity of curcumin nanoparticles against prostate cancer. The \textit{in vitro} studies of curcumin-loaded PLGA nanospheres in prostate cancer cell lines unveiled sustained delivery of curcumin for a long period of time and increased rate of intracellular uptake of nanospheres.\textsuperscript{[50]}

B] Breast cancer
Breast cancer is the most mutual and frequently analyzed cancer affecting women worldwide. Somasundaram et al.,\textsuperscript{[51]} stated a significant inhibition of tumor regression in a xenograft mouse model of human breast cancer. Lvov et al.,\textsuperscript{[52]} cast-off gelatin layer glazed nanoparticles to deliver polyphenols effectively to breast cancer sites. The combination of curcumin-condensed nanoparticles with electroporation performance in MCF-7 human breast cancer cells portrayed better anticancer activity.\textsuperscript{[53]}

C] Pancreatic cancer
Pancreatic cancer is one of the most shared cancers, and the fourth chief cause of cancer-related mortality. Bisht et al., synthesized curcumin-loaded polymeric nanoparticles by means of the co-polymers N-isopropylacrylamide, N-vinyl-2-pyrrolidone and poly (ethylene glycol) monacrylate. It performs as a potential agent to prevent the tumor growth in xenograft models of human pancreatic cancer. The therapeutic effectiveness of nanocurcumin was occurred by cell viability and clonogenic assays.\textsuperscript{[54]} Curcumin-loaded magnetic nanoparticles significantly stationery the growth of human pancreatic cancer cells (HPAF-II and Panc-1) in xenograft mouse model. This formulation exposed higher stability with increased bioavailability and biodistribution when equated with normal curcumin.\textsuperscript{[55]} Nanoparticle-Encapsulated Curcumin (NanoCurc) prevents Tumor Growth and Metastases by binding to NF-xB hence remedial the DNA-binding capacity of cellular components.\textsuperscript{[56]}

D] Ovarian cancer
Ovarian cancer embraces different types of cancer depending on the cells from which they form. The major difficulty in giving advanced ovarian cancer is chemoradiotherapy resistance. But the curcumin nanoparticles (conjugated with Monoclonal antibody) grow the site specificity and sensitivity of the chemoradiotherapy resistance of ovarian cancer cells (Yallapu et al.).\textsuperscript{[57]} Upon pre-treatment of test subjects with curcumin, it dramatically prevents proliferation and clonogenic potential of cisplatin resistant cells (A2780CP) in the occurrence of low levels of cisplatin or radiation. Curcumin has been originating to completely inhibit the effect of C-reactive protein (CRP) which has a propensity to harm the vascular endothelial cells.\textsuperscript{[58]}

E] Cervical cancer
Cervical cancer is one of the most joint and fatal cancers among women worldwide and is linked with persistent Human Papillomavirus (HPV) infection. Nano-CUR effectively prevents cell growth, encourages apoptosis, and seizures the cell cycle in cervical cancer cell lines. The in vitro antitumor activity of curcumin in HPV accompanying cells has been reputable.\textsuperscript{[59]} Curcumin modifies the in vitro expression and function of P-gp in multidrug-resistant human KB-VI cells.\textsuperscript{[60,61]} The effect of curcumin in HPV-associated cells was initiate to mix the down-regulation of viral oncogenes, NF-jB and AP-1.\textsuperscript{[60,62]}

Antimalarial activity
Malaria is begun by parasites and passed by female Anopheles mosquitoes. The \textit{in vivo} studies of curcumin-loaded hydrogel nanoparticles stated by Dandekar et al.,\textsuperscript{[63]} showing antimalarial activity. The toxicity studies evidenced the oral safety and cytotoxic effects of the nano formulations. Curcumin-loaded chitosan nanoparticles preserved the mice infected with \textit{Plasmodium yoelli} by disruptive the hemozoin synthesis.\textsuperscript{[64]}

Antimicrobial activity
Micro-organisms show a major role in instigating numerous infections to humans. Traditionally, turmeric has been secondhand as an antimicrobial agent. Curcumin nanoparticles were castoff as they are recognized to possess superior antimicrobial activity than the normal curcumin. Bhawanet al., described the antibacterial and antifungal activities of nanocurcumin arranged by wet-milling technique. The nanocurcumin was more water in the soluble of any surfactants and highly lively against \textit{Staphylococcus aureus}, \textit{Bacillus subtilis}, \textit{Escherichia coli}, \textit{Pseudomonas aeruginosa}, \textit{Penicillium notatum} and \textit{Aspergillus Niger}. The nanocurcumin formulation was more volatile against Gram-positive bacteria than the Gram-negative bacteria.\textsuperscript{[65]} In another study, curcumin-condensed nanoparticles reserved the growth of methicillin-resistant \textit{S. aureus} and \textit{P. aeruginosa} and heightened the wound-healing activity in an \textit{in vivo} murine wound model.\textsuperscript{[66]}

Similarly, the in vitro studies of curcumin-loaded chitosan tripolyphosphate nanoparticles on mouse skin stifled the growth of S. aureus and P. aeruginosa.\cite{67}

**Anti-HIV activity**

Human immunodeficiency virus (HIV) outbreaks the immune system by finishing CD4+ T cells. The progressive failure of the immunity finally centrals to attained immunodeficiency syndrome (AIDS). The CD4+ T cells are a type of white blood cells that defend the body from infections. The antiretroviral drug overturns the virus, but the complete removal was not yet achieved. Hence, it is necessary to discovery an alternative therapy to extravagance this fatal condition. Curcumin covering apo transferrin nanoparticles display effective inhibition of HIV-1 replication in vitro. The nanocurcumin down controls gag gene expression as a result prevents the synthesis of proviral genes. However, the cytotoxicity of the cell is fewer and the nanoparticle effectivity is reliant on the cellular up take mediated by transferrin receptor.\cite{68}

**Antioxidant property**

Researchers have calculated the antioxidant property of curcumin and its results desmethoxycurcumin and bisdemethoxycurcumin.\cite{69} Curcumin displays a higher superoxide-scavenging activity associated to desmethoxycurcumin and bisdemethoxycurcumin.\cite{70} Research bare that curcumin averts the oxidation of hemoglobin at a concentration of 0.08 mm. The lower level of oxidation persuaded by nitrate can be inhibited by diacetyl curcumin.\cite{71} Curcumin is an effective candidate for hunting of the reactive oxygen and nitrogen species. Reactive oxygen species are responsible for producing numerous pathogenic diseases due to which antioxidants are receiving impetus as one of the therapeutics that are valuable in the treatment of many diseases. Antioxidants like curcumin are real against Parkinson’s disease and various autoimmune diseases. The major drawback of antioxidants is that they show low absorption and low bioavailability. Researchers are annoying to mature antioxidant-loaded nanoparticulate carriers like solid lipid nanoparticles or liposomes. Some of the successful instances of summarizing drugs and other active ingredients are vitamin E, coenzyme Q 10, vitamin A, curcumin, lucopen, silymarin, and superoxide dismutase.\cite{72}

**Parkinson's Disease**

Parkinson’s disease is a disorder that moves the central nervous system. It is triggered by the abnormal accumulation of aggregated a-synuclein (aS) which ensues due to genetic mutations and exposure to neurotoxins and intracellular reactive oxygen species (ROS) that disturbs the mitochondria that leads to mitochondrial disfunction. It is initiate that curcumin can lessen aS-induced cytotoxicity as long as with the advantage that curcumin can cross the blood barrier system in neuron degeneration. In Parkinson’s disease curcumin abridged ROS levels that has been generated by oligomeric a-synuclein.\cite{73,74}

**Alzheimer’s Disease**

Alzheimer’s disease (AD) is a liberal neurodegenerative disorder that happens all over the world. It is a common type of dementia linked with memory loss and gradual death of brain cells. AD is regarded as by the incidence of extracellular deposition of collective amyloid-β (AB) peptide and intraneuronal accumulation of hyper phosphorylated Tau protein and activation of caspase pathway.\cite{75} Curcumin suppressed oxidative tissue injury and condensed amyloid-β deposit in mice. Nps-Cur could be a auspicious drug delivery strategy to protect neurons against oxidative harm in Alzheimer’s disease.\cite{76} Nanocurcumin preserved mice presented better cue memory in the contextual fear conditioning test and greater occupied memory in the radial arm maze test.\cite{77} PLGA-coated curcumin nanoparticles in coupling with Tet-1 peptide possess anti-amyloid and antioxidant property and it can be rummage-sale as a potential drug for giving AD.\cite{78} Thus, employing nanocurcumin showed to be a better therapeutic approach for the treatment of AD.

**Chronic Obstructive Pulmonary Disease**

COPD is occurred by bacterial and viral infections and also due to smoking. Reactive oxygen species production an important role in beginning inflammation through strain kinases and redox sensitive transcription factors such as nuclear factor (NF)-κB and activator protein. Activation of (NF)-κB growths acetylation and prevents deacetylation activity which primes to inflammatory gene expression and weakened glucocorticoid sensitivity. The polyphenols existing in curcumin show a role in supervisory the activation of NF-κB and thus it can be secondhand in lung epithelial cells to switch the expression of inflammatory gene.\cite{79}

**Heart Failure**

Heart failure is generally generated by the growth in thickness of cardiac muscle (hypertrophy). Hypertrophy is assisted with diastolic, systolic dysfunction of the heart and stimulates hypertrophy-responsive transcriptional factors. The activation of transcriptional factors is arbitrated through acetylation by histone deacetylase and intrinsic histone acetyl transferase (HAT), p300.\cite{80,81} Curcumin, a natural therapeutic agent for heart diseases hold a HAT inhibitory activity. Shimatsu et al.\cite{82} evidenced the effect of curcumin in two different heart failure models in vivo: one model was hypertensive heart disease in salt-sensitive Dahl (DS) rats, and the other model was MI in rats. The result illustrations that subdued activity of HAT by curcumin disallowed the heart failure in both models. NF-κB factor is also complicated in cardiomyocyte hypertrophy. So, curcumin which has already been known to constrain NF-κB can also be used in preventing myocarditis.
Applications of nano-curcumin

1) Applications of nano-curcumin in ALS
Mesenchymal stromal cells (MSCs) have unlocked a new horizon in enhancement of CNS repair. MSCs are bright to migrate to the injured tissues and parade the potential to anned the BBB. (83,84) Actually, the importance of MSCs in the treatment of CNS diseases such as ALS was also established through attractive neural protection and swapping dead motor neurons of the spinal cord in a study skilful by Tripodo et al. (85)

2) Applications of nano-curcumin in AD
The main pathological features of AD comprise aggregation of extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs) and hyper-phosphorylated tau protein. Although, the current diverse stories enumeration β-starchlike deposit, tauopathy, oxidative trauma, calcium overload, cholinergic, and glutamatergic neurotransmission alterations. β-amyloid cascade and tauopathy are still the most widely acknowledged central factors triggering and/or hastening the AD pathogenesis. (86) Several experimental reports proved the potential effects of curcumin in AD treatment by adaptable multiple pathways such as drop of inflammation, activation of neurogenesis and Aβ inhibition. (87,88)

3) Application of nano-curcumin in MS
MS is a chronic inflammatory autoimmune disease of the CNS which is considered by neurodegenerative processes. (89) Two major aspects of MS are acute inflammation that is accompanying with demyelination and axonal loss. At the present time, most of the strategies in treatment of MS have intensive on preventing inflammation in the CNS. (90) It has been informed that nano-curcumin has great potential for treatment of MS. (91)

4) Application of nano-curcumin in PD
Oxidative stress theaters a key role in the pathology of PD including several degeneration reactions such as nitric oxide and mitochondrial toxicity. (92,93) It has been established that nano-curcumin can significantly decrease the oxidative stress and apoptosis in the brain of PD files. (94) Similarly, encapsulation of curcumin in alginate nanoparticles heightened the neuroprotection through plummeting of oxidative stress and brain cell death in a transgenic Drosophila PD model. (95)

5) Application of nano-curcumin in epilepsy
Epilepsy is a chronic neurological disorder considered by recurrent motiveless seizures which distresses 1% of the world’s population. (96) Several lines of evidence displayed the pro missing anticonvulsant effect of curcumin. (97,98)

CONCLUSION
It can be concluded that curcumin is a natural therapeutic agent with many versatile activities such as anti-inflammatory, antioxidant, anticancer, and antimicrobial activities. Studies have revealed that novel delivery strategies including those of nanoparticles, liposomes, and defined phospholipid complexes can be used to enhance the activity of curcumin. The curcumin nanoparticles can be synthesized by various methods and can be used for the treatment of different types of cancer. Curcumin nanoparticles can also be used for the treatment of Alzheimer’s disease. Recent studies showed that curcumin-loaded microemulsion, formulated cream, and nanogel can also be used as one of the modern and novel tools for drug delivery. Thus, nanocurcumin can be used as a potential therapeutical agent against broad spectrums microorganisms.

ACKNOWLEDGEMENT
The author thanks all supporting staff, Department of Pharmaceutics, Shivlingeshwar College of Pharmacy, Almala, tq. Ausa, dist. Latur, (M.S.), India. For their kind discussion regarding the topic and guidance to the author.

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