International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 03 Issue 12 December 2020
DOI: 10.47191/ijcsrr/V3-i12-08, Impact Factor: 6.595
IJCSRR @ 2020
www.ijcsrr.org

Formulation Development, Characterization and *In Vitro* Antibacterial Activity Evaluation of Cefazolin Loaded Mesoporous Silica Nanoparticles

Dr. Muhammad Abuzar Raza Naqvi[1], Dr. Syeda Warda Zahra[2]

[1]Faculty of Health & Medical Science, Hamdard University, Karachi 75270, Pakistan
[2]Nishtar Medical University and Hospital, Multan 60000, Pakistan

ABSTRACT: The main aims of this manuscript are to: i) investigate the high drug loading of cefazolin and its characterization, ii), demonstrate the bioactivity of the cefazolin particles *in vitro* on Staphylococcus aureus. From our results, it is observed that the cefazolin loading into MCM-41 particles is 34 *wt* %. Furthermore, particles showed the burst release of cefazolin at pH 6.8. At higher concentration, MCM-41 particles are comparatively more cytotoxic as compared to lower concentration. Finally, cefazolin loaded particles showed higher *in vitro* antibacterial activity against Staphylococcus aureus as compared to cefazolin only.

KEYWORDS: antibiotics, cefazolin, drug delivery, mesoporous silica nanoparticles, MCM-41

1. INTRODUCTION

Infectious diseases are the second major cause of death worldwide [1]. Antibiotic treatment sometimes fails due to emergence of drug resistant strains, or due to insufficient activity or concentration of antibiotics at the site of infection. Different strategies have been adopted to improve antibiotic therapies, such as chemical modification of current antibiotics, development of new antimicrobial agents, or improvement of currently available antibiotics by new formulations [2].

Nanomaterials offer specific advantages to drug formulations, as they can function as antimicrobial agents , while also increasing therapeutic effects of current antibiotics by extended blood circulation, targeted delivery to the site of infection, and controlled release that lessens adverse side effects by minimizing nonspecific interactions and decreasing the required treatment dose [3].Cefazolin (CEF) is a cephalosporin with increased activity against most gram-positive, many gram-negative, and certain anaerobic bacteria. Cefazolin is mainly used for topical bacterial infections according to FDA with great scope for soft tissue infections preoperatively and postoperatively. It is the most prescribed drug among the patients undergoing orthopaedic surgical procedures because of the increased risk of exposure to Staphylococcus aureus especially in knee arthroplasty [4]. But the drug accounts for its repeated administration due to its shorter half-life (1−2h) as it gets rapidly eliminated from the body thus accounting for bacterial resistance. Antimicrobial agents are decided according to the type of microorganism based on the clinical practice guidelines for post-surgical infections. Cefazolin serves as the standard of care and first drug of choice according to the current practice in antimicrobial prophylaxis [5]. It has been the most widely used agent with confirmed efficacy against S. aureus as well as widely common organisms encountered in surgery such as Escherichia coli and various strains of Streptococci, Proteus mirabilis, and Klebsiella species [6].

Ideally, an antibiotic delivery system should provide sustained and controlled release to nearby tissues, eliminating the need for systemic infusions or repeated injections of the drug [7]. Localized release allows for total dose reduction and minimizes systemic toxicity and resistance, and various biodegradable and bioresorbable carriers of antibiotics for the treatment and prevention of prosthetic infections have been studied [8]. Therefore, many materials have been introduced and chemically modified to maintain extended-release properties in the past decade [9]. Simultaneously, with the development of new polymers and inorganic porous matrices [3], nanotechnology has increasingly influenced the field.

In recent decades, mesoporous silica nanoparticles (MSNs) have been one of the most studied drug carriers due to several advantageous properties, such as high surface area, large pore volume, versatile surface chemistry, ease of surface functionalization, high biocompatibility, high chemical and thermal stability, and simple synthesis methods [10]. In this work, we have synthesized, characterized and shown the release kinetics of cefazolin from nanopores of MSNs and bactericidal activity of them on *Staphylococcus aureus.*

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 03 Issue 12 December 2020
DOI: 10.47191/ijcsrr/V3-i12-08, Impact Factor: 6.595
IJCSRR @ 2020
www.ijcsrr.org

2. EXPERIMENTAL SECTION

2.1. Materials

Cetyltrimethylammonium bromide (CTAB, 99%), tetraethyl orthosilicate (TEOS, reagent grade 98%), (3-Aminopropyl) triethoxysilane (APTES, 99%), 3-(trihydroxy silyl) propyl methyl phosphonate (THMP, 90%), dimethyl sulfoxide (DMSO), Toluene (ACS), Acetone (analytical grade), hydrochloric acid (32%) Acetonitrile (HPLC grade), Trifluoro acetic acid were purchased from MERCK, Frenchs Forest, Australia. Cefazolin Sodium was purchased from Sigma-Aldrich, Australia. All aqueous solutions were prepared using DI water (18 MW) from Millipore Milli-Q system.

2.2. Synthesis of MCM-41

MCM-41 particles were prepared using a previously published protocol with slight modification [11]. Briefly, CTAB (1 g) was dissolved in 480 mL of deionised water to which 3.5 mL of NaOH (2 M) was added, and the temperature raised to 80℃. TEOS (6.7 mL) was slowly added to this mixture and stirred for 2 h at 80℃. The sample suspension was then vacuum filtered and washed with deionised water. The filtrate was dried overnight at room temperature followed by calcination at 550℃ for 5 h in a muffle furnace at a temperature ramp rate of 5 ℃/min for up-ramp and 10℃/min for down-ramp.

2.3. Loading of cefazolin into MCM-41 using immersion method

To obtain 30 % mass loading, CEF (35 mg) and pristine or functionalized MCM-41 (65 mg) were added in 5 mL of water for the preparation of nanoparticles. The solution was magnetically stirred at 4 ℃ for 24 h. Drug loaded particles were freeze-dried overnight and stored at 4 ℃.

2.4. Characterization of nanoparticles

TEM images of MSNs were acquired by Hitachi 7700 (Hitachi, Japan) electron microscope at acceleration voltage of 100 kV. Nitrogen adsorption -Brunauer-Emmett-Teller (N_2-BET) (Tristar, Micromeritics-II, Norcross, GA, USA) was operated to measure the pore size, volume, and surface area of MSNs. The particle size and surface charge were calculated in water (solvent) by dynamic light scattering (DLS) and zeta potential measurements (Malvern, Nano-ZS, ATA Scientific, Taren Point, Australia). Thermogravimetric analysis (TGA) (Mettler Toledo, TGA/DSC 2, columbus, OH, USA) was performed with a heating rate of 10 ℃/min in an airy ambiance. The HPLC analytical (Waters 2695 Separations Module, Waters Corporation, Milford, MA, USA) method for CEF was developed based on with slight modifications. Briefly, the system consisted of 0.05 M KH_2PO_4: acetonitrile (90:10, *v*/*v*) pH 5.0 as the mobile phase at an isocratic flow rate of 1.5 mL/min, and detection wavelength 254 nm [12]. Chromatographic separations were performed on Phenomenex® C8 RP-HPLC column (250 mm × 4.6 mm, 5 µ). Standard curve was plotted with n = 3.

2.5. *In vitro* release study

The CEF-MCM with 500 µg equivalent cefazoline were dispersed in 5 mL of the united states pharmacopoeia (USP) specified simulated gastric fluid test solution without enzymes (pH 1.2) and simulated intestinal fluid test solution without enzymes USP (pH 6.8) and stirred at 150 rpm and 37. Aliquots (200 µL) were taken at 0.08, 0.5, 1, 2, 4, 6, 8, and 24 h and centrifuged at 12000 rpm for 3 min. Equal volume of fresh buffers (pH 1.2 and 6.8) were added to rinse the pellet and put back the suspension to the total volume of release buffer. Samples were analyzed using the established HPLC method explained in the previous section. All experiments were performed in triplicate.

2.6. Cell viability assay

MCM-41particls were tested for their potential cytotoxic effect on RAW 264.7 macrophages. RAW 264.7 macrophages was cultured in high glucose DMEM medium, containing 100 µg/mL streptomycin sulfate and 100 units/mL penicillin, 10% (v/v) FBS, and 4 mM L-glutamine. The cells were grown at 37 °C in a humidified incubator with 5% CO_2. RAW 264.7 macrophages (2×10^4 cells/well) were seeded into 96-well plates and grown for 24 h. The weighed mass of MCM-41 (25, 50, 100, 250, 500, and 1000 µg/mL) were incubated for 24 h. Cells with medium only were used as controls for each plate. After incubation, 25 µL/well of MTT reagent was added for a further 4 h at 37 °C. The cell culture medium was then aspirated, and the formazan crystals were dissolved by adding 100 µL/well DMSO. The absorbance signal of formazan was measured at 595 nm using a microplate reader.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 03 Issue 12 December 2020
DOI: 10.47191/ijcsrr/V3-i12-08, Impact Factor: 6.595
IJCSRR @ 2020
www.ijcsrr.org

2.7. In vitro antibacterial assay

2.7.1. Broth microdilution method

This method was used to determine the minimum inhibitory concentration (MIC_{90}) of antimicrobial agent that inhibits visible growth of a microorganism such as *S. aureus* (ATCC 29213 and clinical strain 22) according to the procedure established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [13, 14]. Briefly, serial two-fold dilutions of antibiotic samples such as CEF and CEF nanoparticles (concentrations of MER in nanoparticles is equivalent ranged from 0.0313 mg/L to 16 mg /L) were prepared in CAMHB (cation adjusted Mueller Hinton Broth) and aliquoted (100 µL) into flat-bottom microtiter plates. McFarland 0.5 standardized inoculum suspension (1×10^6 CFU/mL) prepared in sterile distilled water was added into cation adjusted Mueller Hinton Broth (CAMHB) to give rise to approximately 1×10^6 CFU/mL. Then 100 µL of inoculum suspension prepared in CAMHB was added to the drug samples containing microtiter plates to give rise to approximately 5×10^5 CFU/mL. After that, inoculated plates were incubated at 37 °C for 16 to 20 h and MIC values were determined spectrophotometrically at 620 nm. This experiment for the determination of MIC values was repeated three times.

2.8. Statistical analysis

All experiments were done in triplicate unless otherwise stated. Datasets were analysed using one-way ANOVA and post-hoc Tukey's test where applicable.

3. RESULTS AND DISCUSSION

3.1. Characterization of the nano-systems

The synthesised particles were first characterised using transmission electron microscopy (TEM) to assess the success of the synthesis. As shown in **Figure 1**, the MCM-41 particles were mostly spherical in shape with a slightly rough outer surface, and hexagonal pore arrangement. Furthermore, **Figure 2 and Figure 3** show dynamic light scattering data in water including particle size and surface potential of these particles before and after CEF loading, respectively. The hydrodynamic size of MCM-41 is 263 nm, which increased to 345 nm after CFE loading (CEF-MCM-41).. The MCM-41 and CEFMCM-41 particles had negative zeta potentials of −17.2 and −17.7 mV, respectively. We next performed Nitrogen (N_2) sorption isotherm analysis, pore size distribution analysis, and BET surface area plots on MCM-41 and their drug loaded particles. N_2 adsorption-desorption analysis demonstrated that all particles displayed typical IUPAC type-IV isotherms, indicating the mesoporous nature of the silica samples (**Figure 4**). The particles also displayed a steep capillary condensation step at a relative pressure (P/Po) range of 0.2–0.4, characteristic of MCM-41 type mesoporous materials (**Figure 4**). BET surface area plots demonstrated that the pristine MCM-41 had a surface area of 970 m^2/g which was reduced upon drug loading with CEF to 342 m^2/g (Figure 3a). Similarly, the pore size of MCM-41 (2.2 nm) was reduced to 1.7 nm (CEF-MCM). Finally, pore volume of MCM-41 (0.90 cm^3/g) was reduced to 0.78 cm^3/g for CEF-MCM. These changes in physical properties induced by drug loading of the nanoparticles are consistent with previous reports [10, 15].

Drug loading was also analysed using Fourier transform infrared (FTIR) spectroscopy. As shown in **Figure 5**, CEF shows a sharp intense peak at 1757 cm^{-1} (which clearly shows presence of carbonyl group), which confirms the carboxylic group in CEF. Some of the other notable peaks seen in cefazolin containing samples include that at 1647 cm^{-1} and 1593 cm^{-1} indicating C=N stretching and the presence of amide [16]. For MCM-41, a peak at 3430 cm^{-1} is related to the stretching vibration of -OH groups (Si-OH), while other characteristic peaks at 1068 cm^{-1} and 802 cm^{-1} are assigned to Si–O and Si–O–Si vibrations of silanol groups [17]. However in case of CEF-MCM-41, the characteristic peak of CEF at 1768 cm^{-1} can be seen with lower intensity , which confirm the loading of CEF in MCM-41.

TGA was performed to determine the % drug loading. As shown in **Figure 6**, 34 % CEF was loaded into MCM-41 nanoparticles.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 03 Issue 12 December 2020
DOI: 10.47191/ijcsrr/V3-i12-08, Impact Factor: 6.595
IJCSRR @ 2020
www.ijcsrr.org

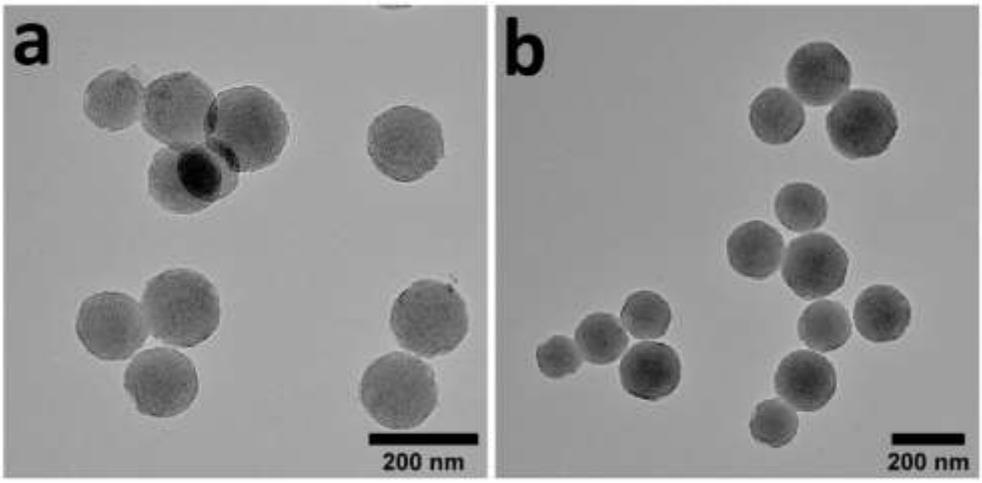

Figure 1. Transmission electron microscope (TEM) images. TEM images of particles; **(a)** MCM-41 and **(b)** CEF-MCM

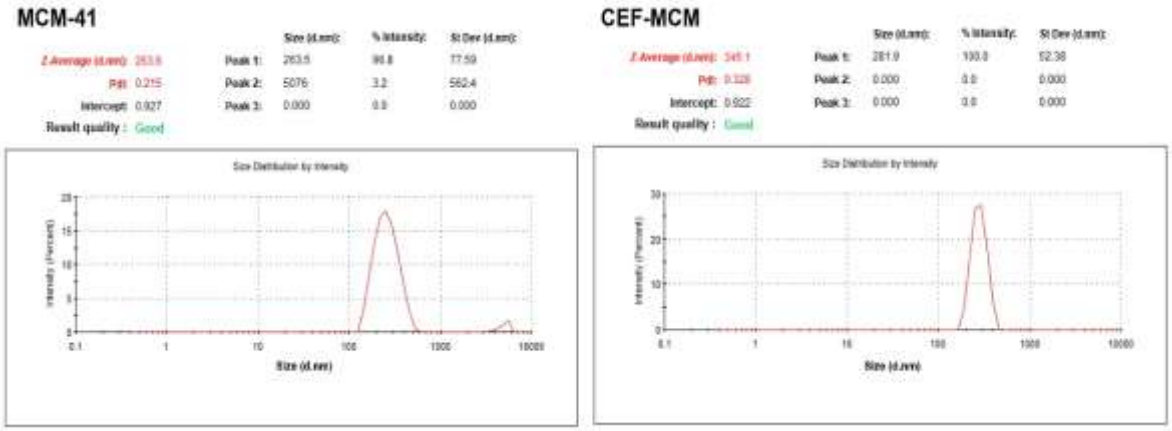

Figure 2. Dynamic light scattering (DLS) data. Hydrodynamic size of particles; MCM-41 and CEF-MCM

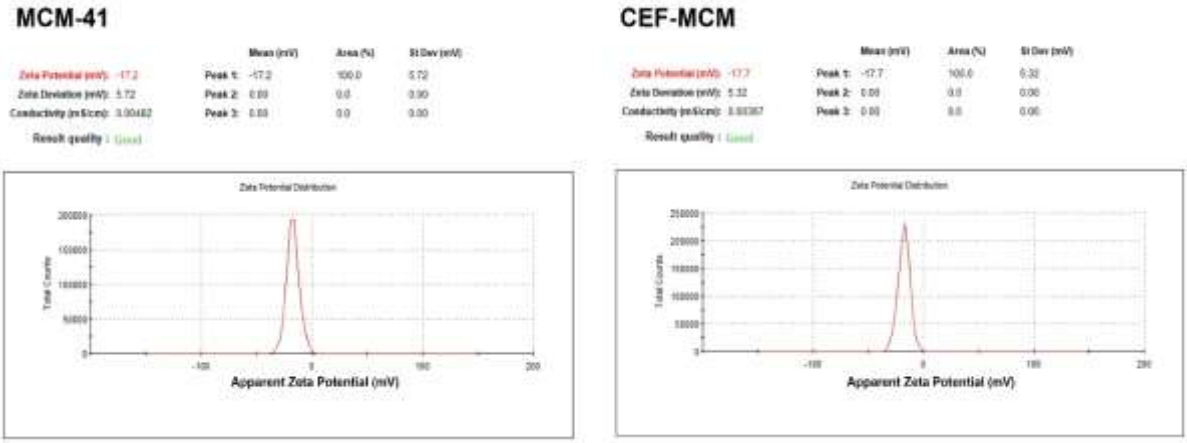

Figure 3. Dynamic light scattering (DLS) data. Zeta potential of particles; MCM-41 and CEF-MCM

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 03 Issue 12 December 2020
DOI: 10.47191/ijcsrr/V3-i12-08, Impact Factor: 6.595
IJCSRR @ 2020
www.ijcsrr.org

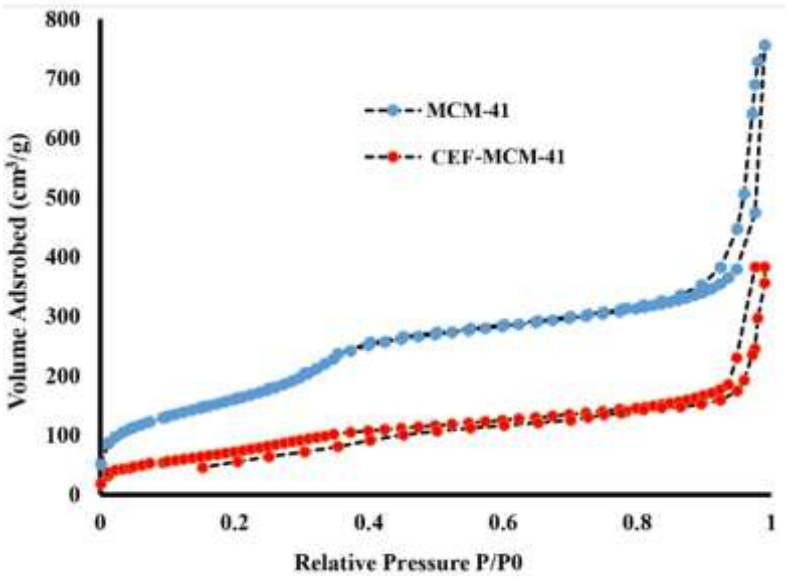

Figure 4. N_2-BET plot forMCM-41 and CRF-MCM-41. The particles exhibit IUPAC-type IV isotherm and retain the porous architecture after drug loading

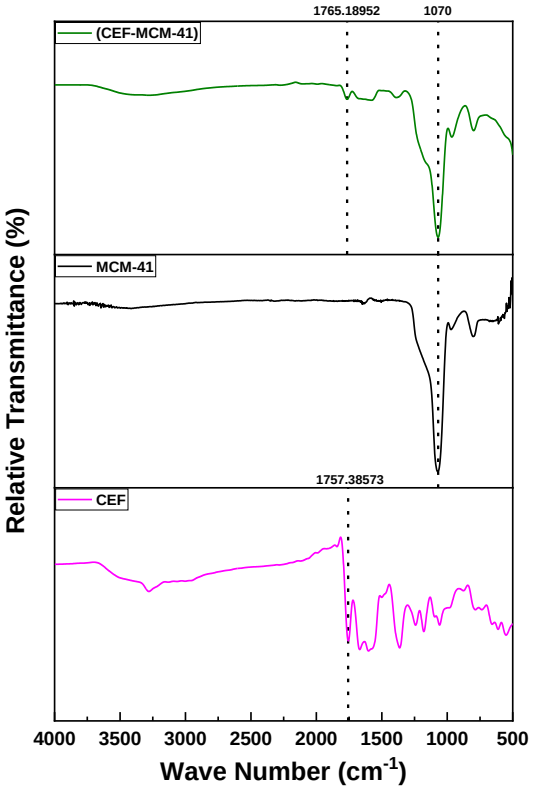

Figure 5: (a) Fourier Transform Infrared (FTIR) spectroscopy of CEF, MCM-41 and CEF-MCM-41 particles for range 500 cm^{-1} to 4000 cm^{-1}.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 03 Issue 12 December 2020
DOI: 10.47191/ijcsrr/V3-i12-08, Impact Factor: 6.595
IJCSRR @ 2020
www.ijcsrr.org

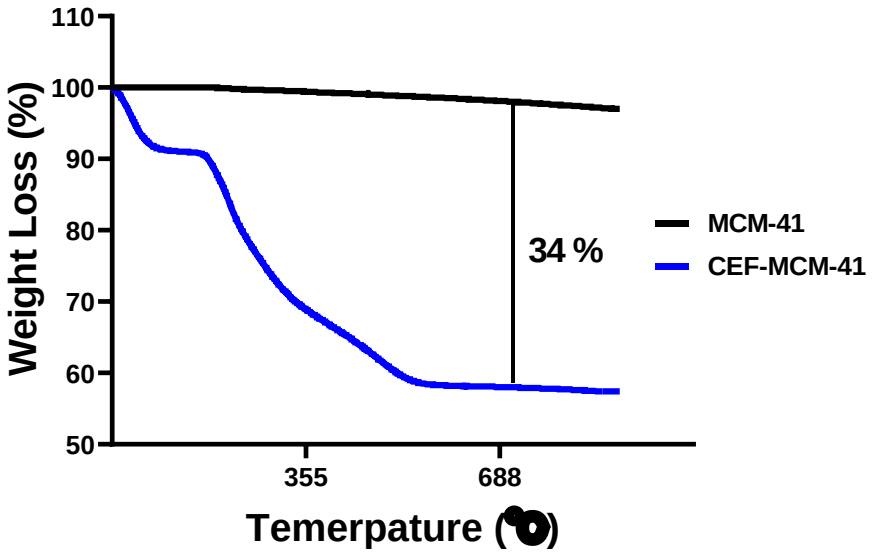

Figure 6. TGA data for drug loading

3.2. In Vitro Release Study

As seen from **Figure 7**, CEF-MCM released over 70% (burst release) of CEF in pH 6.8 in the first 30 min. After that, there was no significant increase in the drug release and total amount of drug release in 8 h was 76%.

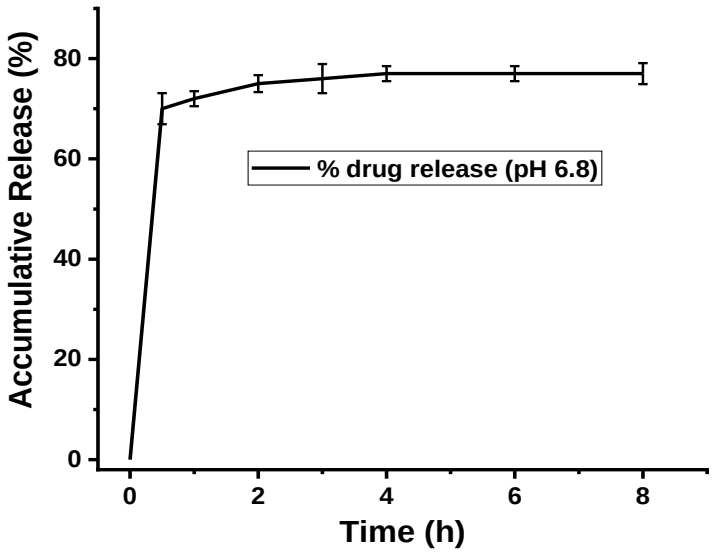

Figure 7. *In vitro* release of CEF loaded MCM-41 particles

3.3. In Vitro cell viability Study

The toxicity of the MCM-41 nanoparticles toward RAW 264.7 macrophages was evaluated using the MTT assay. The results (**Figure 8**) revealed that toxicity of MCM-41 particles at 100 µg/mL was less than 20 % (81 % of cells were viable). However, the toxicity of particles was enhanced at high concentration i.e. cell viability was 51 % at 1000 µg/mL concentration of MCM-41.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 03 Issue 12 December 2020
DOI: 10.47191/ijcsrr/V3-i12-08, Impact Factor: 6.595
IJCSRR @ 2020
www.ijcsrr.org

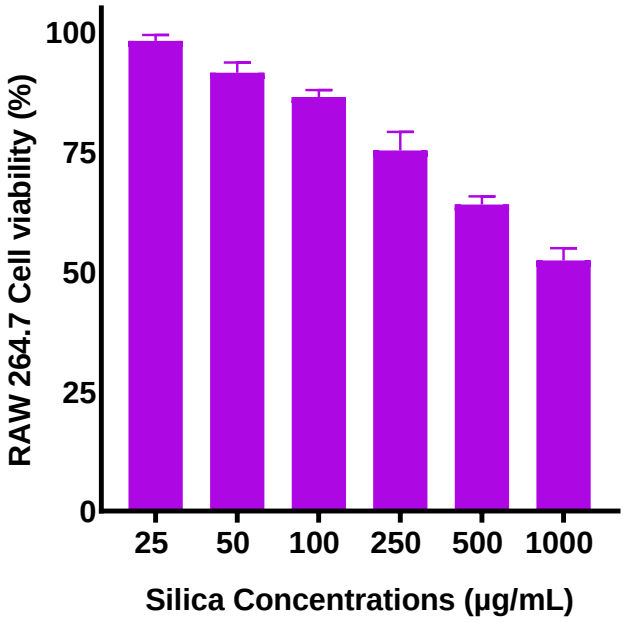

Figure 8. *In vitro* cell viability of CEF loaded MCM-41 particles

3.4. In vitro antibacterial activity of CEF-MCM

The minimum inhibitory concentration (MIC_{90}) of CEF-loaded nanoparticles was investigated using broth micro-dilution followed by EUCAST guidelines. We used gram-positive (S. aureus) bacteria (both ATCC and clinical strain) to determine the antibacterial effect of MPN formulations. As summarized in **Table 1**, MIC value for CEF-MCM particles is half as compared to free drug (CEF). Therefore, our nanoparticles showed the potential to significantly decrease the MIC value of the Pure CEF.

Table 1: Values of MIC (mg/L) of CEF and CEF-MCM against ATCC and clinical isolates *S. aureus*

Formulations	***S. aureus* (mg/L)**	
	ATCC	**clinical strain 22**
CEF	0.125	0.0625
CEF-MCM	0.0625	0.0312

CONCLUSION

In summary, we demonstrated that encapsulation of the cefazolin within MSNs represents an effective strategy for improving its physicochemical properties. MCM-41 nanoparticles showed good drug loading (34 %). Furthermore, drug loaded silica showed improved anti-bacterial activity compared to free drug against both ATCC and clinical strains of S. aureus. Our results demonstrate that mesoporous silica-based carriers have the potential to improve the antibacterial activity of cefazolin.

REFERENCES

1. J.-C. Zeng, Y.-R. Zeng, J. Li, W.-J. Feng, J.-L. Chen, P.-C. Ye, Traumatology, Advances on treatment of periprosthetic infection and antibiotic delivery system after total hip arthroplasty, China Journal of Orthopaedics, 33 (2020) 1022-1026.
2. A.J. Huh, Y.J. Kwon, "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era, Journal of controlled release, 156 (2011) 128-145.
3. A. Raza, F.B. Sime, P.J. Cabot, F. Maqbool, J.A. Roberts, J.R. Falconer, Solid nanoparticles for oral antimicrobial drug delivery: A review, Drug Discovery Today, 24 (2019) 858-866.
4. W. Tang, K. Chiu, T. Ng, W. Yau, P. Ching, W. Seto, Efficacy of a single dose of cefazolin as a prophylactic antibiotic in primary arthroplasty, The Journal of arthroplasty, 18 (2003) 714-718.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 03 Issue 12 December 2020
DOI: 10.47191/ijcsrr/V3-i12-08, Impact Factor: 6.595
IJCSRR @ 2020
www.ijcsrr.org

5. D.W. Bratzler, P.M. Houck, Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project, Clinical Infectious Diseases, (2004) 1706-1715.
6. D.W. Bratzler, E.P. Dellinger, K.M. Olsen, T.M. Perl, P.G. Auwaerter, M.K. Bolon, D.N. Fish, L.M. Napolitano, R.G. Sawyer, D. Slain, Clinical practice guidelines for antimicrobial prophylaxis in surgery, Surgical infections, 14 (2013) 73-156.
7. Y. Suzuki, M. Tanihara, Y. Nishimura, K. Suzuki, Y. Kakimaru, Y. Shimizu, A new drug delivery system with controlled release of antibiotic only in the presence of infection, ournal of Biomedical Materials Research, 42 (1998) 112-116.
8. M. El-Husseiny, S. Patel, R. MacFarlane, F. Haddad, Biodegradable antibiotic delivery systems, The Journal of bone joint surgery., 93 (2011) 151-157.
9. E.M. Hetrick, M.H. Schoenfisch, Reducing implant-related infections: active release strategies, Chemical Society Reviews, 35 (2006) 780-789.
10. M. Ghaferi, M. Koohi Moftakhari Esfahani, A. Raza, S. Al Harthi, H. Ebrahimi Shahmabadi, S.E. Alavi, Mesoporous silica nanoparticles: synthesis methods and their therapeutic use-recent advances, Journal of Drug Targeting, (2020) 1-67.
11. P.L. Abbaraju, A. kumar Meka, S. Jambhrunkar, J. Zhang, C. Xu, A. Popat, C. Yu, Floating tablets from mesoporous silica nanoparticles, Journal of Materials Chemistry B, 2 (2014) 8298-8302.
12. D. Liang, D. Chow, C. White, Applications, High-performance liquid chromatographic assay of cefazolin in rat tissues, Journal of Chromatography B: Biomedical Sciences, 656 (1994) 460-465.
13. W.-D. Liu, M.-C. Shih, Y.-C. Chuang, J.-T. Wang, W.-H. Sheng, Comparative efficacy of doripenem versus meropenem for hospital-acquired and ventilator-associated pneumonia, Journal of Microbiology, Immunology and Infection, 52 (2019) 788-795.
14. M. Balouiri, M. Sadiki, S.K. Ibnsouda, Methods for in vitro evaluating antimicrobial activity: A review, Journal of pharmaceutical analysis, 6 (2016) 71-79.
15. A.H. Ibrahim, J.-H. Smått, N.P. Govardhanam, H.M. Ibrahim, H.R. Ismael, M.I. Afouna, A.M. Samy, J.M. Rosenholm, Formulation and optimization of drug-loaded mesoporous silica nanoparticle-based tablets to improve the dissolution rate of the poorly water-soluble drug silymarin, European Journal of Pharmaceutical Sciences, 142 (2020) 105103.
16. V.P. Mantripragada, A. Jayasuriya, Effect of dual delivery of antibiotics (vancomycin and cefazolin) and BMP-7 from chitosan microparticles on Staphylococcus epidermidis and pre-osteoblasts in vitro, Materials Science Engineering: C, 67 (2016) 409-417.
17. A. Pourjavadi, Z.M. Tehrani, Mesoporous silica nanoparticles (MCM-41) coated PEGylated chitosan as a pH-responsive nanocarrier for triggered release of erythromycin, International Journal of Polymeric Materials and Polymeric Biomaterials, 63 (2014) 692-697.

Cite this Article: Dr. Muhammad Abuzar Raza Naqvi, Dr. Syeda Warda Zahra (2020). Formulation Development, Characterization and In Vitro Antibacterial Activity Evaluation of Cefazolin Loaded Mesoporous Silica Nanoparticles. International Journal of Current Science Research and Review, 3(12), 256-263