Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 4 [11] October 2015: 14-18

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE **OPEN ACCESS**

Enhanced Antibacterial Effects of Ciprofloxacin Enclosed In Cyclodextrin and Nano-Suspension Carrier Systems

M. J. Ansari[1], M. Althubaiti[2], M.O. Ibnouf [3], M.K. Anwer[1], M. M. Ahmed[1], F. Fatima[1], S. Jamil[1]
[1]Department of Pharmaceutics, College of Pharmacy, Prince Sattam Bin Abdul Aziz University, Al-Kharj, Saudi Arabia.
[2]Graduate Student, College of Pharmacy, Prince Sattam Bin Abdul Aziz University, Al-Kharj, Saudi Arabia.
[3]Department of Pharmaceutical Microbiology, College of Pharmacy, Prince Sattam Bin Abdul Aziz University, Al-Kharj, Saudi Arabia.
*Corresponding Author's Email:javedpharma@gmail.com

ABSTRACT
This paper presents enhanced antimicrobial activities of ciprofloxacin when it was delivered through molecular inclusion complex system or nano-suspension carrier system. Ciprofloxacin is a broad spectrum second generation fluoro-quinolone antibiotic which is effective against gram positive and gram negative bacteria. It kills bacteria by interfering with topoisomerase which stops synthesis of DNA and of protein. It is practically insoluble in water and sensitive to sunlight losing its antibacterial activity. Objective of this research is to enhance solubility of ciprofloxacin so as to achieve better efficacy. Solubility enhancement of ciprofloxacin was achieved using a classical cyclodextrin inclusion complex and a current nanotechnology approach. Inclusion complexes were prepared with beta-cyclodextrin using kneading method. Nanosuspension of ciprofloxacin was prepared by anti solvent precipitation method. Pure ciprofloxacin suspension and equivalent amount of nano-suspension and inclusion complexes were tested and compared for antibacterial activity using agar diffusion method against gram positive bacteria such as MRSA ATCC # 33591, B. subtilis ATCC # 10400, E. coli ATCC # 13706, K. pneumoniae ATCC # 13368, P. aeruginosa ATCC # 27853. Observed zone of inhibition by all treatment were statistically analyzed to achieve a valid conclusion. Statistical significance was determined by one-way analysis of variance with $p<0.05$ considered as significant. All tested treatments were found effective against all tested strains. The statistical analysis revealed that there is significant difference ($p<0.05$) between pure ciprofloxacin and its formulations such as inclusion complex and nanosuspension however there was no significant difference between the formulations.
KEYWORDS: *Ciprofloxacin, Agar diffusion method, antibacterial assay.*

Received 11.08.2015 Revised 12.09.2015 Accepted 01.10.2015

INTRODUCTION

Solubility of the drug is supposed to be the most important factor that controls the formulation of the drug as well as therapeutic efficacy of the drug [1]. The formulation development and efficient delivery of poorly water-soluble drugs has always been a challenge. It has been estimated that more than 40% of the discovered drugs are poorly water-soluble and belong to either class II or class IV [2]. Bioavailability and performance of such drugs are usually low as their absorption is dissolution rate limited. Moreover, such drugs often have an incomplete or erratic absorption profile thus highly variable bioavailability. Several techniques are available to improve the solubility of such drugs in order to improve their efficacy [3] Classical and conventional techniques to improve water-solubility include formation of solid dispersions [4], inclusion complexes with cyclodextrins [5], co-solvency [6] and co-grinding [7]. Cyclodextrins (CDs) are torus shaped cyclic oligomers consisting of 6 (α),7 (β) or 8 (γ-CD) glucose units with a-1,4-linkages with a hydrophobic cavity and a hydrophilic exterior [8]. Cyclodextrin complexation is one of the most widely used classical technologies to increase the aqueous solubility of poorly water-soluble drugs. Cyclodextrins are able to form inclusion complexes with many drugs by taking up the drug molecule or some lipophilic moiety of the molecule, into the central cavity [9-11]. These classical techniques are very simple and economical however these are associated with several demerits. Formulation as nano-suspension is an attractive and promising alternative poorly soluble drugs. Nano-suspension consists of the pure poorly water-soluble drug without any matrix material suspended in dispersion.

Nanosuspensions are submicron colloidal dispersions of nano-sized drug particles stabilized by surfactants. The formulations consist of water, drug, and one or more generally regarded as safe excipients [12-13]. Both cyclodextrin complexation [14, 15] and nanosuspensions [16-19] are known to improve antimicrobial activities of the enclosed drugs. Ciprofloxacin is a broad spectrum second generation fluoro-quinolone antibiotic which is effective against gram positive and gram negative bacteria. It kills bacteria by interfering with topoisomerase which stops synthesis of DNA and of protein [20]. It is practically insoluble in water and sensitive to sunlight losing its antibacterial activity. Objective of this research is enhancement of solubility of ciprofloxacin so as to achieve better antimicrobial activity profiles.

MATERIAL AND METHODS

Ciprofloxacin base was obtained as gift from Riyadh Pharma. (Riyadh, Saudi Arabia). β-cyclodextrin and Pluronic F68 were purchased from Sigma-Aldrich (St Louis, MA, USA). Other solvent like ethanol, methanol, dicloromethane, sodium chloride, disodium hydrogen phosphate, sodium hydroxide were of analytical grades.

PREPARATION OF INCLUSION COMPLEX

One mole of ciprofloxacin was kneaded for 30 minutes in mortar with one mole of beta cyclodextrin in 50:50 mixtures of water and ethanol. Mixture was then dried at 60°C in hot air oven.

PREPARATION OF NANO-SUSPENSION

Accurately weighed amount of ciprofloxacin was dissolved in 5 ml of dichloromethane by using ultra-sonicator (Bandelin, Germany). This drug solution is then injected into anti-solvent (22.5 ml water) in the presence of stabilizer/surfactant (0.1%-0.5% Pluronic F68). Rapid addition of solution to such anti-solvent (generally water) leads to rapid super-saturation of drug in the solution, and formation of ultrafine amorphous or crystalline drug. Ultrasonic energy was employed for preparing a stable suspension with minimum particle size.

COMPARISON OF ANTIBACTERIAL ACTIVITY

Prepared inclusion complex and nano suspensions were evaluated for in vitro antimicrobial activity against some gram positive and gram negative bacteria using well diffusion medium [21]. Muller Hinton Agar Medium was prepared by dissolving 33.9 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1L of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121 for 15 minutes °C. The molten sterile medium was then poured onto petriplates. Petriplates containing 20ml Muller Hinton medium were seeded with culture of bacterial strains to be tested. All microorganisms were obtained from the American Type Culture Collection (ATCC). Two gram positive (Methicillin-resistant Staphylococcus aureus ATCC 33591, *Baccilus subtilis* ATCC 10400), and three gram negative bacterial species (*Escherichia coli* ATCC 13706, *Klebsiella pneumoniae* ATCC 13368, *Pseudomonas aeruginosa* ATCC 27853) were included in the test. Wells were cut and 100 µl of the pure ciprofloxacin suspension and equivalent amount of nano-suspension or inclusion complexes were added into wells. Pure ciprofloxacin suspension was used as positive control. Beta cyclodextrin and surfactant solutions were used as negative control. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

STATISTICAL ANALYSIS:

Observed zone of inhibition by all treatment were statistically analyzed to achieve a valid conclusion. Statistical significance was determined by one-way analysis of variance with $p<0.05$ considered as significant.

RESULTS AND DISCUSSION

The study was conducted to compare the antibacterial activities of ciprofloxacin suspension with that of its beta cyclodextrin complexes and its nanosuspension using agar diffusion test. Agar diffusion tests are often used as qualitative methods to determine whether a bacterium is resistant, intermediately resistant or susceptible [21]. During incubation the antimicrobial agent diffuses into the agar and inhibits growth of the bacteria if susceptible. Well standardized methods are essential for all kinds of susceptibility testing, since the methods are highly sensitive to variations in several factors, such as size of inoculums, contents and acidity of the growth medium, time and temperature of incubation. The agar diffusion methods are also strongly influenced by factors, such as agar depth, diffusion rate of the antimicrobial agent and growth rate of the specific bacteria [21]. Diffusion method is cheap compared to most MIC-determination methods. Moreover, diffusion method can be used for determination of MIC values provided the necessary reference curves for conversion of inhibition zones into MIC values are available [22]. Nevertheless, the goal the study was to make a qualitative comparison between different treatments such as pure ciprofloxacin and inclusion products. The results of the study in terms of

inhibition potency measured as zone diameters in mm are given in table 1. The diffusion method described in this protocol is in accordance with the international recommendations given by the National Committee for Clinical Laboratory Standards (NCCLS). The NCCLS describes how to perform the tests and sets international guidelines for interpretation of the results [23]. The comparison of the results with the NLCCS Control limits for monitoring inhibitory zone diameters (mm) shows that all the results fall within the acceptance range. Photograph of the plates with the zone of inhibition of different treatments against the tested bacterial strains is given in figure 1. Zone of inhibition of different treatments is plotted against the tested bacterial strains as given in figure 2. All tested treatments were found effective against all tested strains; however all of them were not similar in their antibacterial activities except in case of activity against *K. pneomoniae*. The statistical analysis revealed that there is significant difference between pure ciprofloxacin and its formulations such as inclusion complex and nanosuspension however there was no significant difference between the formulations. It may be due the fact that both cyclodextrins inclusion and nano-suspension technology have improved the solubility of the ciprofloxacin leading to better diffusion as compared to pure ciprofloxacin and this is in accordance with available reports [14-19].

Table 1: Zone of inhibition of different treatments and strains tested.

STRAINS	SAMPLE	CP	CPBCD	CPNS
MRSA	1	32.0	37.0	42.0
ATCC #	2	31.0	38.0	41.0
33591	3	32.0	38.0	42.0
	MEAN	31.7	37.7	41.7
	SD	0.6	0.6	0.6
B. SUBT	1	27.0	38.0	36.0
ATCC #	2	25.0	39.0	37.0
10400	3	25.0	38.0	39.0
	MEAN	25.7	38.3	37.3
	SD	1.2	0.6	1.5
E COLI	1	28.0	38.0	38.0
ATCC #	2	31.0	37.0	39.0
13706	3	32.0	39.0	39.0
	MEAN	30.3	38.0	38.7
	SD	2.1	1.0	0.6
P.AERU	1	33.0	39.0	38.0
ATCC #	2	34.0	39.0	38.0
27853	3	33.0	38.0	39.0
	MEAN	33.3	38.7	38.3
	SD	0.6	0.6	0.6
K. PNEU	1	31.0	30.0	32.0
ATCC #	2	31.0	31.0	31.0
13368	3	32.0	32.0	32.0
	MEAN	31.3	31.0	31.7
	SD	0.6	1.0	0.6

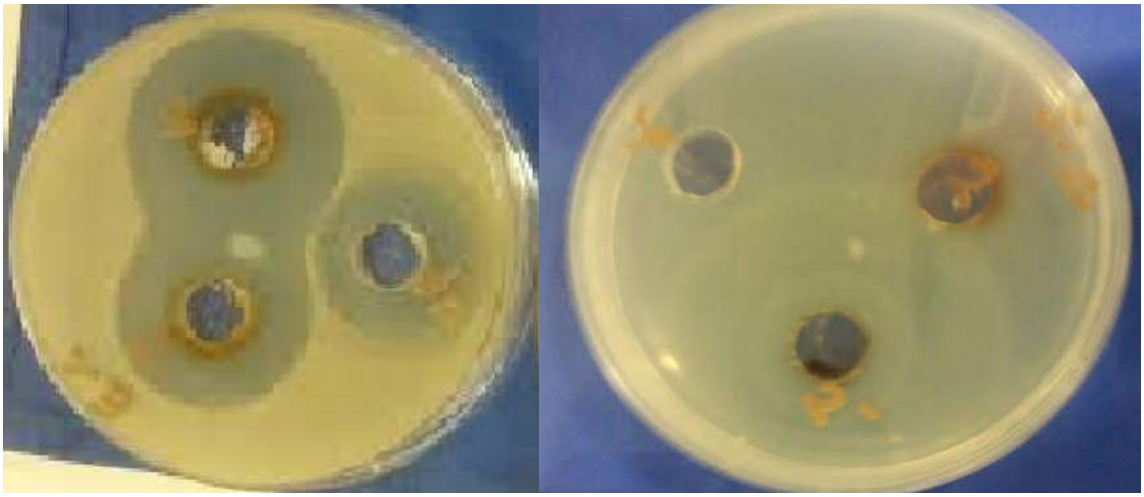

Fig.1: Petridishes showing zone of inhibition of different treatments.

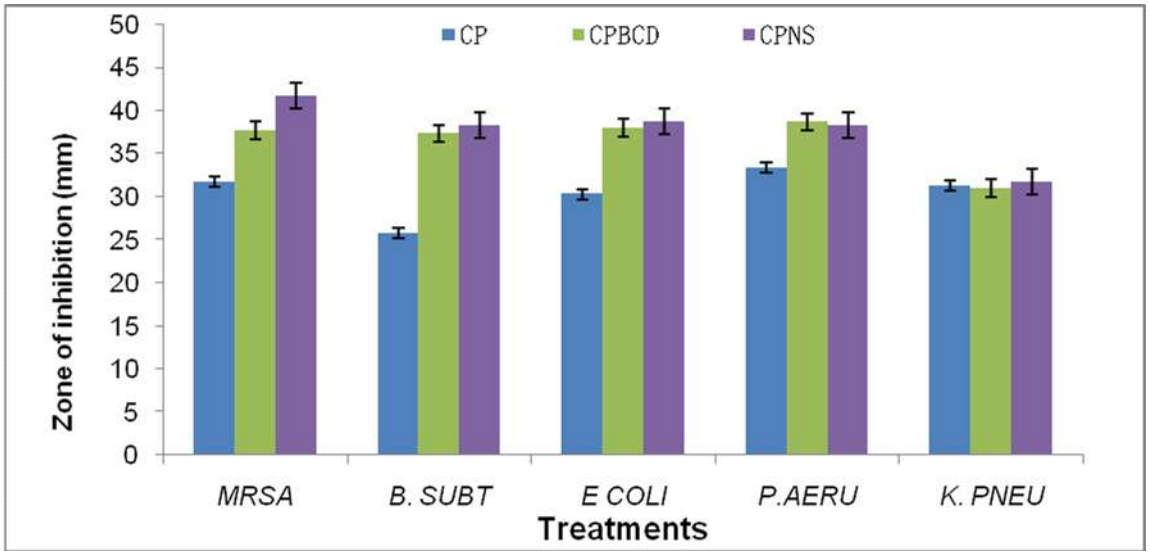

Fig.2: Histograms showing mean zone of inhibition (n=3, ± SD) of different treatments. CP: Pure ciprofloxacin; CPBCD: Ciprofloxacin and beta cyclodextrins inclusion complex and CPNS: ciprofloxacin nanosuspension.

CONCLUSION

Antibacterial activities of different formulations of ciprofloxacin were investigated by agar diffusion method. Formulations demonstrated better antibacterial activities as compared to pure ciprofloxacin which is due to the fact that these drug delivery systems enhance the efficacy of drugs by improving drug properties like solubility and permeability.

ACKNOWLEDGEMENT

Author is grateful to the Dean, College of Pharmacy, for providing opportunity, resources, instrumentation and facility to carryout and complete this research project and also to the colleagues for their help and support.

REFERENCES

1. Loftsson, T. & Brewster ME. (2010). Pharmaceutical applications of cyclodextrins: basic science and product development. J Pharm Pharmacol., 62(11):1607-21.
2. Vemula, V.R., Lagishetty, V. & Lingala, S. (2010). Solubility enhancement techniques. Int. J. Pharm.Sci.Rev. Res., 5: 41-51.
3. Lipinski, C.A. [2000]. Drug-like properties and the causes of poor solubility and poor permeability. J Pharmacol Toxicol Methods., 44(1):235-49.
4. Chiou, W.L.& Riegelman, S [1970]. Oral absorption of griseofulvin in dogs: increased absorption via solid dispersion in polyethylene glycol 6000. J Pharm Sci., 59(7):937-42.

5. Frank, S.G. & Cho, M.J.(1978). Phase solubility analysis and PMR study of complexing behavior of dinoprostone with beta-cyclodextrin in water. J Pharm Sci.,67(12):1665-8.
6. Rubino, J.T. & Yalkowsky, S.H.(1987). Cosolvency and cosolvent polarity. Pharm Res., 4(3):220-30.
7. Otsuka, M. & Matsuda, Y, (1994). Dissolution behavior of phenytoin-bile salt complexes prepared by co-grinding. Chem Pharm Bull, 42(11):2382-4.
8. Bender, M.L. & Komiyama, M. (1978). Cyclodextrin chemistry. Berlin:Springer; 124.
9. Bender, H. (1986). Production, characterization and application of cyclodextrins. Adv Biotech Proc., 6: 31-6.45.
10. Blanchard, J. & Stefan, P. (1999). Some important considerations in the cyclodextrins. Pharm Res, 1796-1798.
11. Rajewski, R.A. & Stella V.J. (1996). Pharmaceutical applications of cyclodextrins, in vivo drug delivery, J. Pharm.Sci., 85, 1142-1169.
12. Patravale, V.B., Date, A.A. & Kulkarni, R.M. (2004). Nanosuspension: a promising drug delivery strategy. J. Pharm. Phamaco., 56(7), 827-840.
13. Müller, R., Jacobs, C. & Kayser. O. (2001). Nanosuspensions as particulate drug formulations in therapy. Rationale for development and what we can expect for the future. Adv Drug Deliv Rev, 47(1):3-19.
14. Denadai, A.M., Teixeirak K.I., Santorok M.M., Pimentak A.M., Cortésk M.E.& Sinisterrak, R.D. (2007). Supramolecular self-assembly of beta-cyclodextrin: an effective carrier of the antimicrobial agent chlorhexidine. Carbohydr Res., 342(15):2286-96.
15. Aleem, O., Kuchekar, B., Pore, Y. & Late S. (2008). Effect of beta-cyclodextrin and hydroxypropyl beta-cyclodextrin complexation on physicochemical properties and antimicrobial activity of cefdinir. J Pharm Biomed Anal., 47(3):535-40.
16. Guo, J.J., Yue, P.F., Lv, J.L., Han, J., Fu, S.S., Jin, S.X., Jin, S.Y. & Yuan H.L.(2013). Development and in vivo/in vitro evaluation of novel herpetrione nanosuspension. Int J Pharm., 441(1-2):227-33.
17. Rabinow, B., Kipp, J., Papadopoulos, P., Wong, J., Glosson, J., Gass, J., Sun, C.S., Wielgos, T., White, R., Cook, C., Barker, K. & Wood, K. (2007). Itraconazole IV nanosuspension enhances efficacy through altered pharmacokinetics in the rat. Int J Pharm., 339(1-2):251-60.
18. Kayser, O., Olbrich, C., Yardley, V., Kiderlen, A.F. & Croft, S.L. (2003). Formulation of amphotericin B as nanosuspension for oral administration. Int J Pharm., 254(1):73-5.
19. Peters, K., Leitzke, S., Diederichs, J.E., Borner, K., Hahn, H., Müller, R.H. & Ehlers, S. (2000). Preparation of a clofazimine nanosuspension for intravenous use and evaluation of its therapeutic efficacy in murine Mycobacterium avium infection. J Antimicrob Chemother., 45(1):77-83.
20. Pommier, Y.Z, Leo, E., Zhang, H. & Marchand, C. (2010). DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. Chem. Biol, 17: 421-33.
21. Bauer, A.W., Kirby, W.M.M, Sherris. J.C. & Turk, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol, 45:493-6.
22. Korgenski, E.K. & Daly, J.A. (1998). Evaluation of the BIOMIC video reader for determining interpretive categories of isolates on the basis of disk diffusion susceptibility results. J Clin Microbiol, 36:302-4.
23. Clinical and Laboratory Standards Institute (2009). Performance standards for antimicrobial susceptibility testing. Nineteenth informational supplement M100-S19. Wayne, PA: Clinical and Laboratory Standards Institute.

CITATION OF THIS ARTICLE

M. J. Ansari, M. Althubaiti, M.O. Ibnouf , M.K. Anwer, M. M. Ahmed, F. Fatima, S. Jamil.Enhanced Antibacterial Effects of Ciprofloxacin Enclosed in Cyclodextrin and Nano-Suspension Carrier Systems. Bull. Env. Pharmacol. Life Sci., Vol 4 [11] October 2015: 14-18