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SURPLUS THE BIOTIC RESISTANCE OF ANTIBIOTIC DRUGS WHENCONJUGATED WITH SILVER FORMING NANO-COMPOSITES

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ABSTRACT

Antibiotic resistant now a days is a cause of severe worldwide peril. To reduce the mortality along with infection rates a variety of approaches are now being familiarized. Blending of metals with antibiotics are operative against drug resistant microorganisms. Metallic nanoparticles (NPs) are reported to show antimicrobial activity. Herein, describe successful synthesis of ciprofloxacin HClloaded silver nanoparticles (AgNPs) by co-precipitation method and their incorporation in the gel base of Carbapol 940. Successful synthesis of silver nanoparticles and their conjugation with ciprofloxacin HCl was confirmed by various test (zeta poteinal, particle size measurement, silver content, drug loading and entrapment efficiency). AgNPs were small in size with particle size distribution as obtained from TEM. To determine the release mechanism of formulated gel various models such as zero-order, first-order, Higuchi and Korsmeyer-Peppas were fitted to the release data. The improvement in antimicrobial upshot of drug was seen when combined with silver nanoparticles.Synergistic and additive effects were found with activity of ciprofloxacin HCl when doped with silver nanoparticles with in a gel base against S. aurease and MRSA During evaluation of minimum inhibitory concentration (MIC) values, similar concentration of prepared gel was capable of producing antibacterial activity.

INTRODUCTION

1.1 Antimicrobial applications of silver

Silver is a soft and shiny transition metal which is acknowledged to have the highest reflectivity of all metals. Out of its many useful properties, it is well known to possess antimicrobial activity. Silver is known to be biologically active when it is dispersed into its monoatomic ionic state (Ag^{+}), when it is soluble in aqueous environments silver ions execute their terminal work by perforating holes in bacterial membranes and wreaking havoc once inside. Surface coatings incorporating silver are a common application A recently published review includes more details about the bactericidal mechanisms of silver, along with methods of silver nanoparticle preparation.

1.2 Metal nanoparticles

Metal particles are predominantly concerned in nanoscale systems as they can be synthesized and modified chemically effortlessly. Metals like gold, silver, palladium, platinum, titanium, etc can be synthesise into nanoparticles. Amongst these, gold and silver nanoparticles have been generally considered for diverse range of applications. Aimed at illustration, the antibacterial activity of metal nanoparticles such as silver colloids are strictly related to their size; that is, the smaller the silver nuclei, the higher the antibacterial activity. Thus, control on thesize and size distribution is a chief job.

This Research emphases on exploring the potential of silver (Ag) as nanoparticles for antimicrobial applications.

1.3 Silver nanoparticles

As of their small size, silver nanoparticles (AgNPs) are being extensively useful as topical wound materials and the total surface area of the nanoparticles is maximized which centrals on highest values of the activity to weight ratio. [13] The recent learning also testified the ability of silver to synergistically boost bactericidal activity of antibiotics counter to drug resistant bacteria. The key synthesis aspects of silver because it has always been used against various diseases; in the past it found use as an antiseptic and antimicrobial against Gram-positive and Gram-negative bacteria due to its low cytotoxicity This allows silver to be used in a wide range of biomedical applications such as prevention of infection, wound healing, and anti-inflammation. [15] By enhancing the surface to volume ratio, the nanoscale silver antibacterial properties can be improved [16] AgNPs are increasingly utilized in medical fields due to their peculiar properties, including antibacterial, antifungal, antiviral, anti-inflammatory, anti- cancer, and anti-angiogenic agents. They possess the ability to overawed the problematic bacterial resistance to a wide range of antimicrobial agents such as antibiotics.[17]Ag NPs shows synergy with other antibiotics and antiseptics (ceftazidime, streptomycin, kanamycin, polymyxin).

1.4 CHOICE OF DRUG

In year 1987, Ciprofloxacin was introduced into clinical practice, and registered as a vital medicine by the WHO (World Health Organization). Subsequently its introduction to clinical practice, more than 250 million patients have been treated along with vast research investigation—as reflected in more than 32,000 publications.

Ciprofloxacin is a second-generation fluoroquinolone used to treat varioussusceptible bacterial infections. IT is, considered a benchmark when comparing new fluoroquinolones, shares with these agents a common mechanism of action: inhibition of DNA gyrase. While ciprofloxacin demonstrated a fair activity against gram-positive bacteria, it is against gram-negative organisms that it proved to be more potent than other fluoroquinolones**.** Ciprofloxacin is administrated both through intravenous and oral routes.

Ciprofloxacin and other fluoroquinolones share a common mechanism of action that begins with the trapping of gyrase and topoisomerase IV on DNA as a ternary complex. Ciprofloxacin and other fluoroquinolones are important for controlling infections of lungs, joints, bones, airways, and the urinary tract. Other chronic infections, such as infectious diarrhea, anthrax, and intra-abdominal infections are also being commonly addressed with ciprofloxacin.

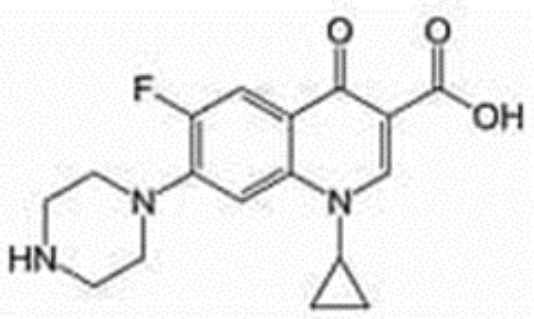

Fig. 1: Structure of Ciprofloxacin.

1.5 MECHANISM OF ACTION

Ciprofloxacin acts by trapping the type II topoisomerases, DNA gyrase and topoisomerase IV, on DNA. These enzymes solve topological problems associated with DNA biology, including chromosome replication. The ternary drug–enzyme–DNA complexes (cleaved complexes) rapidly inhibit DNA synthesis but reversibly complexes. Cell death derives from chromosome breakage, in part from the accumulation of toxic reactive oxygen species.

1.6 BACTERIAL RESISTANCE

When bacteria changes response to the used Antibiotic medicines is said to be asbacterial resistance. No humans or animals, become antibiotic-resistant in fact these bacteria may blight humans and animals, and the infections they cause are harder to treat than those caused by non-resistant bacteria. Antibiotic resistance leads to higher medical costs, prolonged hospital stays, and increased mortality.

The world urgently needs to change the way it prescribes and uses antibiotics. Even if new medicines are developed, without behaviour change, antibiotic resistance will remain a major threat. Behaviour changes must also include actions to reduce the spread of infections through vaccination, hand washing, practising safer sex, and good food hygiene.

1.7 Scope of the problem

Antibiotic resistance is intensifying to dangerously high levels in all parts of the world. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases. A growing list of infections – such as pneumonia, tuberculosis, blood poisoning, gonorrhoea, and foodborne diseases – are becoming harder, and sometimes impossible, to treat asantibiotics become less effective.

Where antibiotics can be bought for human or animal use without a prescription, the emergence and spread of resistance is made worse. Similarly, in countries without standard treatment guidelines, antibiotics are often over-prescribed by health workers and veterinarians and over-used by the public.

Without urgent action, we are heading for a post-antibiotic era, in which common infections and minor injuries can once again kill.

2 PREPARATION OF THE DOSAGE FORM

2.1 Method of synthesis of silver nanoparticles

- Ciprofloxacin hcl (drug),
- Silver nitrate ($agno_3$),
- Distilled water (H_2O),
- 1% tri-sodium citrate

Above listed substances were used for synthesis of silver nanoparticles. Nanoparticles were prepared by the reduction method by reduction of silver nitrate via co-precipitation process. Tri-sodium citrate behaves like a reducing agent for the procedure. All the solutions of reacting materials were prepared in distilled water.

In typical procedure 50 ml of $AgNO_3$ (0.001 M) was heated until boiled. 5ml of 1% trisodium was added drop by drop citrate to above said solution. Until change of colour was detected (pale yellow) solutions were mixed vigorously andheated. There after solution was removed from the heating element and stirred until cooled to room temperature. A calculated amount of ciprofloxacin was liquified in distilled water and was added to the above mixture during thesynthesis of silver nanoparticles.

2.2 Preparation of the Carbopol gel base

Carbopol-940 forms very good consistency transparent gel at low concentration. 2% Carbopol gel base was prepared by dispersing 2 g Carbopol- 940 in 86 ml hot distilled water. 0.6g of propyl paraben was dissolved in ethanol.

0.3 g of methyl paraben was dissolved in 10 ml of propylene glycol. The mixturewas stirred until thickening occurred and then neutralized by the drop wise addition of 50% (w/w) triethanolamine to maintain pH 6-7.

2.3 Incorporation of nanoparticles in the gel base

The nanoparticle formulation containing drug was slowly added in Carbopol-940 gel base and mixed by using a mechanical stirrer for 5 min.

Composition of The Formulated Gel

Table 1: Composition of Formulated Gel.

Ingredients	Concentration
Carbopol 940	2%
Propylene glycol	10%
Methyl paraben	0.3%
Propyl paraben	0.6%
Triethanolamine	q. s.

+

API	Quantity
Ciprofloxacin HCl loaded AgNPs	**100 mg**

3 EVALUATION OF NANOPARTICLE GEL

3.1 Measurement of pH of the nanoparticle gel

1gram of ciprofloxacin nanoparticle gel was mixed in 100 ml distilled water with homogenizer. Then the electrode was immersed in the prepared gel solution and readings were recorded from digital pH meter in triplicate and average value was calculated.

3.2 Viscosity study

Viscosity measurements were done on Brookfield viscometer by selectingsuitable spindle number and rpm. 50 g of preparation was kept in 50 ml beaker which was set till spindle groove was dipped and rpm was set and dial reading was measured after three minutes. From the reading obtained, viscosity was calculated by using factor. The procedure was repeated three times and observations are recorded as mean. [113]

3.3 Spreadability

It is the term expressed to denote the extent of area to which gel readily spreads on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value. Spreadability is expressed interms of time in seconds taken by two slides to slip off from gel and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability. It is calculated by using the formula:

$$S = \frac{M \cdot L}{T}$$

Where

M: wt. tied to upper slide

L: length of glass slides

T: time taken to separate the slides

0.1g of nanoparticle gel was pressed between two slides (divided into squares of 5 mm sides) and left for about 5 minutes where no more spreading was expected. Diameters of spreaded circles were measured in cm and were taken as comparative values for spreadability. The standardized weight tied on the upper slide was 125 g. The results obtained are average of three determinations. [114]

3.4 Extrudability study

The extrudability of nanoparticle gel was determined by filling nanoparticle gel in the collapsible tubes. The extrudability of the nanoparticle geldetermined in terms of weight in grams required to extrude a 0.5 cm ribbon of gel in 10 second.

3.5 Content Uniformity

50mg of nanoparticle loaded gel was accurately weighed and dissolved 50ml of in methanol. The drug content was determined by diluting the resulting solution for 10 times with methanol and measuring the absorbance at 278nm using UV spectrophotometer. The Avg was taken out of ten reading and used fordrug content. [115]

3.6 Percentage yield

The empty container was weighed in which the nanoparticle gel formulation was stored then again, the container was weighed with nanoparticle gel formulation. Then subtracted the empty container weighed with the containerwith gel formulation then it gives the practical yield. Then the percentage yield was calculated by the formula.

$$Percentage\ yield = \frac{\text{Practical yield} \quad \text{x } 100}{\text{Theoretical yield}}$$

3.7 Silver content determination

Gel pieces of known weight were placed in glass bottles containing 5 ml of aqua regia (HCl: HNO_3 3:1); the bottles were sealed and stored at room temperature until the gels were completely dissolved. The silver ion content in the dissolved acid solution was determined by inductively coupled plasma– atomic emission spectroscopy (ICP–AES) analysis against the Primar 28 elementstandard. [116]

3.8 IN VITRO RELEASE STUDIES

3.8.1 Skin permeation studies

Franz diffusion cell was used for permeation studies. Study was conducted using a rat skin. 50 ml of PBS 7.4 was taken in receptor compartment and was continuously stirred at 75 rpm through a magnetic stirrer and equilibrated at 37^{O} C. The prepared rat skin was mounted facing stratum corneum upward into the donor compartment. 1 g of nanoparticle gel formulation was taken in donor compartment and covered with parafilm to avoid any evaporation process. 5 ml sample was withdrawn through the sampling port at regular intervals and each sample is replaced with equal volume of fresh dissolution medium. Then the samples are analyzed for drug content by using phosphate buffer as blank with UV-Visible double beam spectrophotometer at 278 nm. Similar study was performed with pure ciprofloxacin.

3.8.2 Franz diffusion cell

According to FDA (Food and Drug Administration) regulations, it is an ideal tool for quality control of topical preparations. The gel is placed in the donor chamber of Franz diffusion cell fitted with a rat skin. The gel is then dialyzed against PBS 7.4 as dissolution medium at room temperature and stirred throughout the study at 75 rpm employing a magnetic stirrer. The samples are withdrawn from the dissolution medium at suitable intervals and analyzed for drug content in UV-Visible double beam spectrophotometer at 278 nm. Sink condition is maintained by replacing the same amount of fresh sample against the sampling.

4. RESULTS AND DISCUSSION

4.1 Determination of pH of gel base and nanoparticle gel

The pH value of prepared formulation was found in the range of 6.5, which are supposed to be suitable to pass up threat of nuisance on application to skin.

4.2 Viscosity

The viscosity of prepared silver nanoparticle gel was found to be 672.0327 cps. Which was measured by Brookfield viscometer, then the viscosity was found to be reliant on polymeric content of formulation.

4.3 Spreadability

The spreadability of the formulated ciprofloxacin HCL loaded silver nanoparticle gel is 14.3 g.cm^2/sec. The findings of spreadability depicted that formulated gel get easily spread on applying small amount of shear. Which indicating that spreadability of drug loaded silver nanoparticle gel was good.

4.4 Extrudability study

The extrudability of nanoparticle gel was found to be positive.

4.5 Percentage yield

The Percentage yield of nanoparticle gel was found to be 86.51%.

4.6 Content uniformity

The content uniformity of prepared gel was found to be 87.65%.

4.7 Silver content determination

Silver Content of nanoparticles.

Table 2: Silver Content of nanoparticles.

Sr. No	Sample	Ag Content measuredby ICP-AES System	Unit (ppm)
1	Silver gel	28.7	ppm
2.	Silver gel	31.6	ppm
3.	Silver gel	30.1	ppm

Average= 30.13±1.18 ppm

The silver content was determined in triplet and the average was found to be 30.13±1.18 ppm.

4.8 *In vitro* release study

In vitro release study was performed to determine amount of drug released atdifferent interval of time.

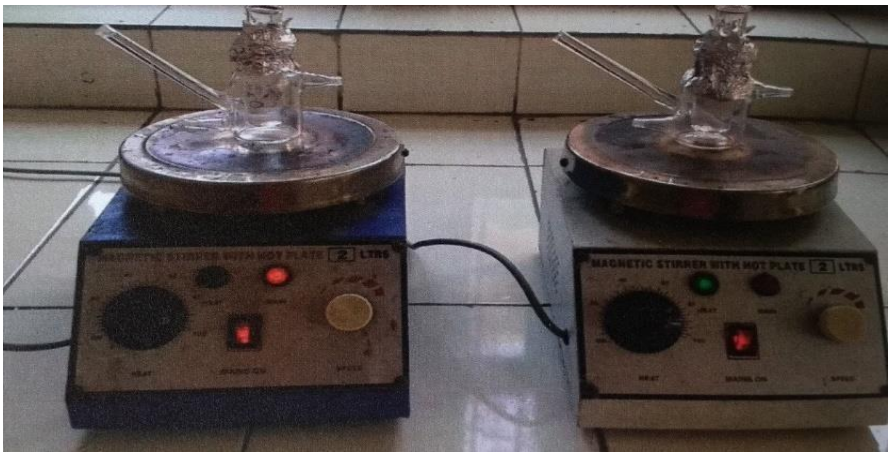

Fig. 2: Franz diffusion cell study.

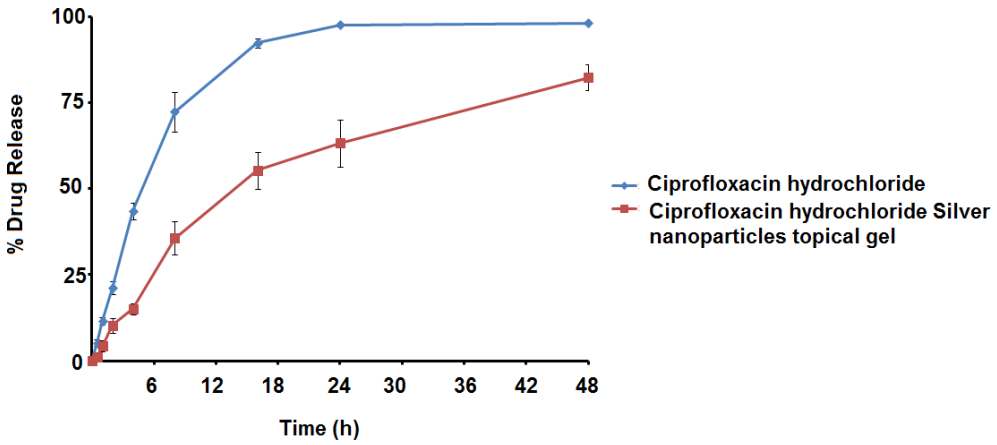

FIG. 3: *In-Vitro* Drug Release Comparison of Pure Drug andPrepare Gel.

Table 3: *In-Vitro* Drug Release Comparison of Pure Drug AndPrepare Gel.

Time	Ciprofloxacin % cumulativeDrug Release	SD	Ciprofloxacin HClSilver gel % cumulative Drug Release	SD
0	0	0	0	0
0.5	5.23	0.9	1.2	0.6
1	11.6	1.2	4.3	1.6
2	21.2	1.9	10.3	2.1
4	43.5	2.3	15.1	1.5
8	72.3	5.8	35.7	4.9
16	92.3	1.3	55.3	5.4

4.9 Antimicrobial activity

The prepared gel was interduce for bacterial activity against S. aureus and MRSA at different concentration by using cup plate method and obtained data reflects the following:

Table 4: Zone of inhibition against Staphylococcus aureus

Concentration	Ciprofloxacin HCl	Ciprofloxacin HCl+Ag NPs
2.5	3.6±0.5	5.1±0.2
5	5.4±0.3	7.6±0.3
10	7.9±0.6	13.4±0.4
20	9.6±0.7	13.8±0.4
40	10.8±0.5	13.9±0.2
80	10.9±0.2	13.5±0.3
160	10.7±0.5	13.7±0.5
MIC	40	10

Table 5: Zone of inhibition of prepared gel against MRSA.

Concentration	Ciprofloxacin HCl	Ciprofloxacin HCl+Ag NPs
2.5	1.8±0.2	3.1±0.1
5	2.6±0.5	4.5±0.7
10	3.5±0.2	6.6±0.5
20	5.8±0.5	11.4±0.3
40	7.6±0.5	11.6±0.5
80	9.2±0.4	11.8±0.2
160	9.6±0.3	11.1±0.5
MIC	80	20

It is concluded that prepared silver nanoparticle gel possesses potency of the antibacterial activity.

5 CONCLUSIONS

The above present work on the preparation of topical Silver nanoparticle gel containing ciprofloxacin hydrochloride is an attempt to utilize the potential of Ag NPs as a carrier to increase the activity of Ciprofloxacin hydrochloride. So, we developed and evaluate the Ag NPs containing Ciprofloxacin HCl to obtained the optimized formulation with increased antibacterial activity.

On the basic of the preceding findings, we can conclude the followings

- Ciprofloxacin hydrochloride was successfully incorporated into the Silver nano-particles by co-prepetition method and were evaluated for conformation of the same.
- Silver content was determined by using ICP-AES System.
- The activity of pure ciprofloxacin was compared with Prepared gel CIP loaded silver nanoparticle gel exhibited enhanced bactericidal activity against *S. aureus* and MRSA.

A concentration of 20 μg/mL of prepared gel was a benchmark concentration during evaluation of MIC values.

Which concluded that Using Silver surplus the activity of the drug as similar concentration of antibiotic was incapable of producing antibacterial activity.

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