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Fabrication of metal incorporated polymer composite: An excellent antibacterial agent

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ABSTRACT

US Food and Drug Administration (FDA) allowed for direct addition of castor oil for human consumption as food and most recently FDA approved castor oil as over-the-counter (OTC) for laxative drug. The present article highlights the green route phosphorylation of castor oil (COL) via condensation polymerization. Further, the incorporation of metal ions Cu (II) and Zn (II) into the polymer matrix have been carried out at elevated temperature using catalyst p-toluene sulphonic acid (PTSA). The modification of the said material has been confirmed by FT-IR, UV–VIS, and 1H and 31P-NMR spectroscopy. Further, the in vitro antibacterial activities of the metal incorporated-COL has been performed by standard methods against B. cereus (MCC2243) (gram-positive) and E. coli (MCC2412) (gram-negative) bacteria. The results revealed that the incorporation of metal ions into the polymer matrix increases the antibacterial activity largely. This may be governed by the electrostatic interaction between metal ions and microbes, also the generation of free active oxygen hinders the normal activity of bacteria. These results suggest that the synthesized material may act a potential candidate for low cost, environment friendly antibacterial agents and may find their application in clinical fields. Herein we are also proposing mechanism of antibacterial activity.

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1. Introduction

Polymers have made revolutionary changes in the daily lives need and accessories, polymers are successfully converted into clothes, electronic gadgets and even sophisticated medical/surgical instruments such as personal protective equipment (PPE) which made urgent headlines in the present Covid-19 pandemic. U.S. department of labor under occupational safety and health administration guidelines (OSHA 3151–12R) recommended chemical-resistant gloves are made with different kinds of rubber: natural, butyl, neoprene, nitrile and fluorocarbon (viton); or various kinds of plastic: polyvinyl chloride (PVC), polyvinyl alcohol and polyethylene. Most polymers are based on petrochemicals, which is largely environmental concern considering the rate of global warming. The utter need for polymers to meet the requirements of sustainable development is necessary to the scientific society for better human friendly development. Biomedical applications of polymers especially synthetic polymers in clinical medicine have drawn a new era of polymeric medicine [1,2]. Vegetables oil based polymers meets many of the 12 Principles of Green Engineering of sustainable development [3]. The rise of antibacterial resistance (AMR) has destabilized the mechanism and functions of present bactericidal & bacteriostatic drugs. Overuse of traditional antibacterial drugs have made bacteria highly drug resistant with grave consequences. A recent report by WHO in 2019, on AMR estimated about 10 million deaths every year by 2050 (greater than cancer), which could return to dark age of medicine [4]. In order to counter AMR, adequate researches have been made to obtain antibacterial property without bacterial resistant. The trend of antibacterial drug resistance has made traditional and over exposed antibacterial futile. The sustainable and distinctive source for antibacterial could make potential antibacterial productive. The grown interest in extracted materials from natural products for
antimicrobial discovery is due to their high degree of stereochemo-
istry with low toxicity and upright sustainability. A wide range of
procedures have been applied to meet the green route synthesis
[5]. Castor oil (COL) has drawn extraordinary research interest due
to their idiosyncratic physicochemical properties and ability to get
modified into polymers by acyclic triene metathesis (ATMET) poly-
merizations [6], ring-opening metathesis polymerization (ROMP)
[7], polyamides [8], polyurethane [9], high solids polyurethane [10],
diacene diene metathesis (ADMET) polymerization [11], polyamides
[8], polyurethane [9], high solids polyurethane [10], alkyl-Castor
Oil-Epoxy Merizations to procedures [12]. COL has also been modified into polyurethane, epoxide, polyester, and metal incorporation via double bond modification, con-
densation and polymerization [6,15]. COL was also transformed into
to rigid polyurethane, waterborne polyurethane, thermoplas-
tic polyurethane and linear & branched acetal polymers via ac-
etal metathesis polymerization are few [17–20]. Literature survey
widely revealed that the incorporation of metals in the fabricated
compounds and polymer can enhance the antibacterial proper-
ties. Literature survey further revealed that metals especially transi-
tion metals ions like Silver [Ag(I)], Platinum [Pt (II)], Copper [Cu
(II)], and Zinc [Zn (II)] etc. are some bioactive metals proclivity to influence the biological activity in the ligands, macromolecule,
monomer and their environment [21]. Among them, Cu (II) and
Zn (II) are two prominent metal ions with antibacterial, antifu-
gal, and catalytic influences [22,23]. Researchers have made plant
extract based metal composite with enhanced biological activity
[24–26]. The degree of variation in the structures due to modifica-
tions and their ability to release metal ions in the pathogen active
sites, apart from electrons movement have given a big go for metal
composite as a potential antibacterial with little bacterial defance.
It is then expected that the incorporation of Cu (II) & Zn (II) with modified-COL could have the desirable antibacterial functions
compared to virgin COL. COL is a very potential sustainable material for
drug discovery and development due to its traditional practice as
medicine. The transfers of electrons between the synthesized ma-
terials with the bacterial surface have suggested their potentiality
as an antimicrobial agent [27–34]. The transfer of electrons plays
most critical step of bacterial respiration; the supply of energy for
growth, proliferation and maintenance of cell of bacteria is carried
out on cell membrane via electron transfer [35–37]. Irregularities of
electron transfer in bacteria results the production of reactive oxygen
species (ROS), which subsequently inhibit the growth of
bacteria [37]. Charging the surface of bacteria can also inhibit the
growth both in positive, negative surfaces and can facilitate the
antibacterial efficiency. For instance, the efficiency of chitosan as
an antibacterial for gram negative bacteria on polymers has been
reported in many advance literature [38–41]. Although COL based
polymers with antibacterial activity is reported but without es-
tablished mechanism [42], but the scope of metal ion incorporated
modified COL for potential antibacterial is yet to be investigated
and propagated.

Herein, we highlight the phosphorylation of castor oil (COL) and
the incorporation metal ions, Cu (II) and Zn (II) into the poly-
mer matrix with enhanced antibacterial resistance and mecha-
nism of antibacterial nature for the same. The formulation, struc-
ture and modification of the synthesized polymer has been con-
ﬁrmed by FT-IR, UV–VIS, and 1H and 31P-NMR spectroscopy. More-
over, the synthesized metal incorporated polymeric materials have
been explored for in vitro antibacterial activities against B.cereus
(MCC2243) (gram-positive) and E. coli (MCC2412) (gram-negative)
bacteria. The results obtained from these in vitro antibacterial
studies have suggested that the synthesized metal incorporated
polymeric materials may act as a potential antibacterial. The mod-
ified materials based on the line of sustainability that made them
convenient for exploring antibacterial application. The mechanism
of antibacterial action of metal incorporated polymeric material is
also proposed.

2. Materials and method

Castor oil (14030006) (refractive index: 1.470–1.490, hydroxyl
value: 155–167, iodine value: 80–90, saponification value: 175–
185) and phosphoric acid (1004) were purchased from S.D Fine
Chem Ltd, Mumbai. P-toluensulfonic acid (PTSA) (6101), sodium
chloride (5234), copper (II) acete monohydrate (165397), diethyl
ether (100926), ethanol (702), zinc (II) acete dihydrate (2724192),
were purchased from Fisher Scientiﬁc, New Delhi. Bacillus cereus
(MCC2243) and E. coli (MCC2412) were purchased for antibacterial
activities. All reagents and other chemicals were used without any
modiﬁcation and further puriﬁcations.

2.1. Synthesis of polyol (COP)

Castor oil and phosphoric acid in a 1: 2 Molar ratios were
poured into a three necked round bottom flask with a nitrogen
inlet tube, a mercury thermometer, a condenser and a 5 MLH
magnetic stirrer. (0.00016 g) PTSA was added for catalysis. Phos-
phoric acid was added in fractions and temperature maintained
between 130 and 140 °C at 500 rpm. The reaction was moni-
tored by measuring acid value and taking FT-IR spectra at regu-
lar intervals. When positive acid value and desired FT-IR spectrum
was recorded, reaction was stopped. The reaction was cooled and
washed with 50 ml diethyl ether followed by 15% brine solution.
The washed solution was kept for overnight and separated. The
lower layer was decanted, and upper oily layer named as COP was
kept for further modifications. Before metal ions incorporation the
COP was passed through sodium sulfate and dried again.

2.2. Synthesis of copper and zinc incorporated polymer (CPC)
and (CPZ)

2 g dried COP was taken in two clean reaction chambers in the
inert environment and 0.045 M Copper (II) Acetate monohydrate in
fractions namely 0.03, 0.005, 0.008 and 0.002 M were added into
the reaction mixture (A) at 1/2 h interval at 150–155 °C at 550 rpm
for 5 h. While 0.0265 M Zinc (II) Acetate dihydrate in fractions
0.018, 0.0020, 0.0025, 0.004 M were added in reaction mixture (B)
at the same time interval at 140–145 °C at 550 rpm for 4 h. Weight
in milligram of metal ions incorporated in polymers matrix is given in
Table 1.

The progresses of the reactions were monitored similarly as
mentioned for COP synthesis. Acid value is given in Table S1. On
completion of the reactions, two clear visible layers were ob-
tained on both chamber A & B. Blue oily layer in chamber A (CPC)
and orange layer in chamber B (CPZ) were obtained; subsequently
they were separated and purified by the same above COP process.
The ﬁnal product of reactions is accomplished via condensation
polymerization with the release of small molecule i.e. acetic acid.
The major advantage of condensation polymerization is the lack
of broad molecular weight distribution during the polymerization,
which helps to get controlled molecular weight polymer [43]. Fur-

| Table 1 | Weight in milligram of metal ions incorporated in polymers matrix. |
|---------|---------------------------------------------------------------|
|         | Cu in CPC | Zn in CPZ |
| Weight (milligram) of Metal | 810.675 | 477.3975 |
| Moles | 0.045 M | 0.0265 M |
ther, the building blocks of animals, polypeptides DNA, RNA is also formed via condensation polymerization.

3. Characterizations

The Fourier transform infrared (FT-IR) spectra of the Castor Oil (COL), COP, CPC and CPZ were determined using a Perkin-Elmer Spectrum GX FT-IR spectrophotometer (Perkin-Elmer, Beaconsfield, UK, in the range of 4000–400 cm$^{-1}$) wavenumber. All data were taken at room temperature. UV–visible spectral analyses were performed by a Multiskan GO spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland) and all spectra were recorded from wavelength 200 to 800 nm. The nuclear magnetic resonance (NMR) spectra were performed in the solvent Chloroform d-6 (1 mL). All the NMR spectra were recorded at 30 °C on a 400 MHz Bruker AVANCE 400 spectrophotometer (Bruker BioSpin, Rheinstetten, Germany) for both $^1$H and $^{31}$P NMR.

4. Antibacterial methodology

Antibacterial activity was analyzed using agar well plate diffusion methods [44]. Bacterial cultures were procured from microbial culture collection at national center for cell science, Pune, India. Bacterial inoculates B. cereus (MCC2243) Gram +ve and E. coli (MCC2412) Gram –ve bacteria were prepared by growing a single colony in nutrient broth medium and adjusting the turbidity to 0.5 McFarland standards. A 200 μL portion of bacterial culture was spread onto 25 mL Mueller–Hinton agar medium plates, and different concentrations (10, 20, 30, 40 μg/ml$^{-1}$) of synthesized compound was taken from the stock solution of 1000 μg mL$^{-1}$ were put on added on the sterilized disk of 5 mm size. Samples in solution were used to investigate the antibacterial activity. Standard antibiotic (streptomycin) 10 μg/ml$^{-1}$ used as a control. These plates were incubated at 37 °C for 24 h. The results were expressed as the mean diameter of inhibition zone in mm ± standard deviation. All experiments were performed in three times.

5. Results and discussion

5.1. FT-IR spectra

The FT-IR spectra of COL, CPC and CPZ are shown in Fig. 1. For COL, the major peaks i.e.; the peak at 3380 cm$^{-1}$ corresponding to hydroxyl group, at 1736 cm$^{-1}$, C = O of ester of triglycerides and 1628 cm$^{-1}$ is due to C = C at 2925 & 2849 cm$^{-1}$ appeared due to the asymmetric and symmetric vibrations of C–H bond, while bending vibration of C–H gives peak at 1465 cm$^{-1}$. The intense peak at 722 cm$^{-1}$ is due CH$_2$ rock [45], and for CPC and CPZ, an intense additional peaks at 1171 cm$^{-1}$ with a shoulder 1070–1090 is due to the vibrations of $P = O$ and P-O-C respectively [46]. While the Metal oxygen bonds appeared for CPC and CPZ at 590, 484 cm$^{-1}$ respectively that clearly indicates the coordination of COP with Cu and Zn [47]. The decrease in the intensity of hydroxyl peak along with the shifting of peaks positions of C = O ester and C = C of triglyceride chain by 35–45 cm$^{-1}$ clearly suggest the incorporation of Cu and Zn in COL Framework.

5.2. Identification metal containing-COL; CPC and CPZ by UV–Vis spectral analysis

UV spectrum of COL is shown in supplementary Fig. S1, the peaks at 295 and 315 nm is due to $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$ transitions respectively, while Fig. 2 shows interaction of COL with metal ion Cu (II) and Zn (II). For CPC, the intraligand charge transfer peaks appeared due to $n \rightarrow \pi^*$ (324 nm), and $\pi \rightarrow \pi^*$ (390 nm) and the broad peak in between 680 and 720 nm associated with a shoulder at 490 nm is due to Jahn Teller distortion. The distortion lowered the symmetry, the Jahn Teller effect is more pronounced in six-coordinate complexes [47,48], moreover the distorted geometry of Cu (II) was strongly supported by magnetic susceptibility measurement Table S2. While in case of CPZ, Fig. 3 shows peaks at 322 nm is due to $\pi \rightarrow \pi^*$ transition and the peak between 380 and 410 nm is due ligand to metal charge transfer (LMCT) of Zn (II) [47]. The n → π* is vanished in CPC and CPZ. It is needless to prove; this is due to the interaction of Cu (II) and Zn (II) with COP. Nonetheless the existence of intense π → π* intra-ligand transitions band in CPC and CPZ is strongly supported by FT-IR spectra.

5.3. Physico-chemical study of COL, CPC and CPZ

Primarily the progress of the reactions for oil-based polymer is monitored my TLC and by determining physico-chemical characteristics. The critical characterization was acid and saponification value determination. The physico-chemical properties are given in Table S 1. The data for specific gravity, refractive index and inherent viscosity were increasing in the order COL < CPC < CPZ, while hydroxyl, saponification and iodine value were found in opposite trend, this could be attributed to increases of chain length and hence unsaturated concentration also increases due to polymerization and metal incorporation in COP chain [15,49,50]. The maximum acid value is for CPC (3.98 mg/KOH) followed by CPZ (3.84 mg/KOH). These data endorsed the interaction of Cu & Zn metal acetates in the backbone of the COP main chain as shown in Fig. 2. Moreover these results strongly makes an agreement with the literature [47]. The greater risk due to insolubility of new material in aqueous medium is unquestionably a real threat in developing new pharmaceuticals and industrial products. COL has long been used medically as a laxative and as an excipient. The insolubility of COL in aqueous medium remains a major concern to make it a drug even after having a positive drug linkage value Fig S2.

The critical criterion of any potential drug is that it must get absorbed and interact with the antigen or radical species. Since insolubility will influence both pharmacokinetic and pharmacodynamic properties of any potential drug in repressive way and it can dramatically reduce productivity in drug discovery, development and its delivery. Although COL is miscible with absolute ethanol, methanol, ether, chloroform, acetic acid but the percentage of miscibility has increased due to metal ion coordination with modified
Fig. 2. UV-Visible spectra of CPC & CPZ.

Fig. 3. Reaction scheme for synthesis of CPC/CPZ.
5.4. Determination of the magnetic susceptibility of CPC and CPZ

The magnetic susceptibility is given Table S2 for Cu (II) and Zn (II) in CPC and CPZ respectively. The magnetic moment of Cu (II) in CPC complex is 1.99 BM, this subnormal value is due to the distorted octahedral geometry of CPC which is a result of lowering its symmetry [51]. Zn (II) is in $d^{10}$ configuration CPZ polymer; diamagnetic. The coordinating environment in CPC & CPZ polymer are hexa coordinate and tetra coordinate respectively. The proposed geometry of CPZ is tetrahedral. Moreover in crowded environment; macromolecules or large monomer; Zn (II) is more likely to from COL; COP. Both CPC and CPZ have shown partial solubility in aqueous medium.
tetrahedral than octahedral [52]. The proposed generic structure of CPC and CPZ is shown in Fig. 3 along with its reaction scheme.

5.5. Stability of CPC and CPZ

Stability of metal ion coordinated complexes depends on both the nature of metal ion and nature of ligands. Theoretical interpretation for the stability of CPC over CPZ complex is due to the smaller size of Cu (II) ion compared to the size of Zn (II) ion [53]. The distortion of CPC due δ configuration of Cu (II) makes it more likely to have better stability compared to the CPZ. Since the ligands are same in both polymeric complexes so the trend of stability is expected to be same. Moreover, distorted octahedral nature of CPC stabilizes the complex compared to CPZ which is tetrahedral nature.

5.6. Establishment of CPC and CPZ structure by spectral analysis (31P & 1H NMR)

Fig. 4 and 5 represent the 31P & 1H NMR spectra of CPC. In the 31P spectra of CPC, Fig. 4, the peaks appeared at δ = -3 ppm and δ = 1.99 ppm is attributed to the presence of two distinctive phosphorus nuclei. Phosphorous atom attached with the electron withdrawing group; copper ion attached site is noted at δ = -3 ppm while the second phosphorous atom is noted at δ = 1.99 ppm. While the same peaks were appeared for 31P spectra of CPZ, Fig. 4, at δ = -3 ppm and δ = 2.55 ppm respectively in the similar trend. The 1H spectra of CPC, Fig. 5 and CPZ, Fig. 5, the peaks appeared at δ = 1 and 11 ppmis due to the sp2 proton attached to the fatty acid chain. The signals at δ = 4.1 appeared for both CPC and CPZ may be ascribed to the sp2 proton of fatty acid chain. While the peak at δ = 5.3 ascribed to proton directly attached to oxygen atom and the protons attached to the carbonyl group [54].

The obtained chemical shift (δ) for CPC & CPZ are different and broaden compare to virgin COL, Fig. S3. The assigned and broadening of peaks of CPC & CPZ compare to COL is strongly in agreement with the coordination of Cu (II) and Zn (II) in modified-COL; COP [59]. The chemical shift (δ) of the protons of CPC & CPZ at different assigned peaks are precisely shown in Fig. 5, and S5 respectively, these data strongly supports the coordination of metal ions i.e. Cu (II) & Zn (II) to the main phosphorous polyol chain, Fig. 3. Nevertheless these data are well in agreement with the FT-IR and UV spectra.

6. Antibacterial study of COL, COP, CPC and CPZ

The antibacterial properties of synthesized COP, CPC and CPZ were evaluated against both B. cereus (MCC2243) (Gram +ve) and E. coli (MCC2412) (Gram -ve) bacteria in solution form. The different concentration taken for analysis are given in Table 2. The virgin castor oil compound (COL) showed no zone of inhibition or any strain of bacteria, which revealed that COL alone is inef-

![Fig. 6. Antibacterial nature of COP, CPC and CPZ against Bacillus cereus (MCC2243) and Escherichia coli (MCC2412).](image)
effective for antimicrobial activity. Further, the antimicrobial activity tested for COP, CPC, and CPZ have shown bactericidal nature. All of them exhibited the high degree of susceptibility against both the gram positive and gram-negative bacteria (B. Cerus and E. Coli). It has been observed that with the increased concentration of CPC material (10–40 μg/mL) an increase in the antibacterial activity is observed and the best performance was observed against E. Coli than the B. Cerus. The better performance of the CPC over COP material against both B. Cerus and E. Coli may be due to the greater interaction between metal ion and microbial agents. While in case of CPZ shown in Fig. 6, with the increased concentration of CPZ material (10–40 μg/mL), similar results were observed, on comparison with CPC, CPZ has shown outstanding performance against both gram-positive and gram-negative bacteria Fig. 7(a) & (b).

The potential influence of zinc for antibacterial tendencies is well known, while copper has become a potential material due to its ion forming tendencies and its active interaction with its neighbor. Both CPZ and CPC have shown greater zone of inhibition compared to COP due to the metal ion coordination with COP [56]. The proposed mechanism of antibacterial activity of CPZ and CPC is depicted in Fig. 8. The enhancement in the antibacterial activity of CPZ and CPC may be attributed to (i) polymer coating provide the stability to the metal ion and controls the release of metal ions that led to the increase in antibacterial activity (ii) electrostatic interaction between the metal ion and microorganisms that induces electron transport and oxidative phosphorylation at the cell membrane. (iii) Further, this electron transport led to the release of antimicrobial ions i.e. reactive oxygen species (ROS) such as OH-, O2<sup>-</sup> etc., which interacted with the cell wall of bacteria that help in the rupturing of bacterial cell and inducing antibacterial property. The release ions may occur because the metal ions are loosely held in the polymer matrix through phosphorylated coordination sites so as to pursue thermodynamically more favorable interactions with the cells, leading to the increase in antibacterial activity [57,58].

These results have been compared with the other reported similar work (Table 3) and found that the composite showed comparatively better antibacterial performance than those of reported and cited studies.

### 7. Conclusion

Catalytic phosphorylation of castor oil was successfully done and subsequently metal ions; Cu (II) & Zn (II) were incorporated in modified-COL (COP) at elevated temperature. The structures were established with FT-IR, UV-Visible, 1H NMR and 31P-NMR spectra. The castor oil polyol (COP), copper and zinc ions incorporated polymer i.e. CPC and CPZ showed potency of antibacterial activity against both B. cerus (MCC2243, Gram +ve) and E. coli (MCC2412, Gram –ve). Among CPC and CPZ polymer, the CPZ has displayed

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**Table 3**

Comparative performance of the present system with the similar reported work.

| S.No. | Composite system      | Bacteria | Zone of inhibition (mm) | Ref. |
|-------|-----------------------|----------|-------------------------|------|
| 1.    | CPZ                   | E. coli  | 22                      | Present study |
| 2.    | Polyurethane/TEOS     | E. coli  | 20                      | [59]  |
| 3.    | LMPOL/0.06CuO         | E. coli  | 21                      | [60]  |
| 4.    | Carboxymethylcellulose/CuO | E. coli | 14                      | [61]  |
| 5.    | Poly(ethylene glycoldiacrylate)/Cu | E. coli | 20                      | [62]  |

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**Fig. 7.** Zone of inhibition (in mm) induced by COP, CPC & CPZ in (a) Bacillus cereus and (b) Escherichia coli. (P < 0.05).

**Fig. 8.** Probable mechanism of antibacterial activity of CPC and CPZ polymer.
excellent antibacterial activity, explicitly against *E. coli* (MC24212, Gram –ve) bacteria. This excellent antibacterial activity of CPZ may be attributed to the ability to produce antibacterial ions, which interact electrostatically with the pathogens wall and causes cell rupture. These results strongly suggest that the metal ions incorporated polymer i.e. CPC and CPZ may find their application as environment friendly antibacterial agent. Moreover, on comparison with other similar system, CPZ is found efficient in killing bacteria.

Credit author statement
Professor Athar Adil Hashmi is corresponding author of the manuscript and will manage all future queries. He has supervised, reviewed and edited the manuscript. Md Iqbal Ahmed Talukdar has fabricated and characterized polymers (CPC & CPZ). He has also interpreted the data and written the manuscript. Irshad Ahmad and Tasneem Fatma have contributed equally to investigate antibacterial activities. Manzoor Ahmad Malik, Ovais Ahmad Dar, Md. Khursheed Akram have contributed equally in formal analysis. Sajid Iqbal has contributed in designing antibacterial mechanism.

Notes
The authors declare no conflict of interest with any person or any organization.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials
Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2020.129091.

Appendix
COL-Castor oil, COP-Castor oil polyol, CPC-Copper (II) incorporated polymer, CPZ-Zinc (II) incorporated polymer, Ecoli-Escherichia. coli and B.cereus-Bacillus cereus.

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