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A Synthesis of Povidone-Iodine-Based Polymeric Nanoparticles for Intrauterine Applications

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Annotation: The present study investigates the synthesis and assessment of povidone-iodine-based polymeric nanoparticles (PVP-I-NPs) for potential application in the management of intrauterine uses. N-vinyl-2-pyrrolidone and methyl methacrylate used as a copolymer to create the nanoparticles. Iodine loading in ethanol was the next step in the production process, which involves free radical polymerization. They used UV-Vis spectroscopy, FTIR, SEM for characterization and Toxicity. The particles' diameters ranged from around 310 to 500 nm, according to the SEM examination. FTIR data demonstrated that iodine and the polymer successfully bound together by confirming the presence of distinctive functional groups. According to UV-Vis analysis, there were absorption peaks at 247 and 259.5 nm in line with iodine-polymer interactions-induced electronic transitions. Cytotoxicity experiments on CHO cell lines showed little adverse effects, confirming the nanoparticles' safety and suitability for biological uses. Accordingly, these results, confirmed that PVP-I-NPs is provided a useful substitute method for intrauterine antibiotic treatment. However, additional research is needed to

explore the vivo effectiveness and reproductive outcomes to treat complication inflammation of the female reproductive system in comparison with antibiotics and bacterial resistance.

Keywords: Synthesis, povidone, iodine, polymeric, nanoparticles.

Introduction

Polyvinyl pyrrolidone-iodine (PVP-I) is a water-soluble compound with bactericidal, fungicidal, and virucidal properties and use serves as a disinfectant and antiseptic in ophthalmology for skin preparation and wound treatment [1]. Researchers have developed hydrophobic povidone iodine nanoparticles (povidone-iodine NPs) to address water solubility and antibacterial stability issues in microbial pollution, the nanoparticles, synthesized using poly (N-vinyl-2-pyrrolidone-co-methyl methacrylate) nanoparticles, showed significant antibacterial activity against E. coli, S. aureus, and P. aeruginosa, offering potential applications in various industries [2]. Multidrug-resistant bacteria infections are increasing, prompting researchers to explore new antimicrobial agents like polymeric nanoparticles, utilizing advanced controlled polymerization methods to combat these growing infectious diseases [3]. The rise in antimicrobial resistance has led to the development of advanced strategies and methodologies, including nanotechnology, specifically nanocarriers, which enhance the delivery and effectiveness of antimicrobial agents, minimizing side effects and enhancing therapeutic impact [4]. Review explores the use of nanoparticles-hydrogel composites in local antimicrobial therapies, addressing the challenges of traditional antibiotics and antibiotic resistance, it highlights the potential of these composites in controlled drug release and prolonged hydrogel retention, highlighting future research directions [5]. Nanoparticles (NPs) are increasingly used as alternative treatment to traditional antibiotics for bacterial infections [6]. So, they could be used in antibacterial coatings, antibiotic delivery systems, and vaccines, however, their antibacterial mechanisms remain unclear [7,8]. This study explores the synthesis of the Polyvinyl pyrrolidone-iodine (PVP-I) in comparison with recent research which might be offering potential applications in biomedical fields in particular bacterial resistance and side effect of the different of types of the drugs [9]. Polyvinyl pyrrolidone-iodine (PVP-I) is a potent antiseptic and disinfectant used in various medical, surgical, and hygiene applications its stable complex with iodine allows controlled release of free iodine, minimizing irritation PVP-I's broad-spectrum antimicrobial activity targets bacteria, viruses, fungi, and spores, making it suitable for intrauterine use [6].

Material and method

Preparation of povidone-iodine-based polymeric nanoparticles (PVP-I-NPs)

During preparation of the pyrrolidone-co-methyl methacrylate nanoparticles P(MMA, PVP) NPs, and povidone-iodine- NPs followed method of the [2], also we optimized this method to get better product. So, weighed 1.2g of MMA and 0.8g of NVP and add 200ml of distilled water containing 100mg of KPS. Mix well using a magnetic stirrer. When the solution is placed on a magnetic stirrer at a temperature of 70 for 12 hours with continuous stirring using a magnet and under nitrogen gas, we have a white powder. Wash this powder with distilled water three times and then dry it using a rotary vacuum. We get the final product, which is in the form of a white powder. Then dry it under pressure until we get the final product, which represents P(NVP-MMA), (Figure 1).

Figure (1): represents P (NVP-MMA)

The second step is to prepare ethyl alcohol, which contains 1% iodine. We dissolve 1 gram of iodine in 100 ml of alcohol and place the solution on a magnetic stirrer. After the iodine is completely dissolved in the alcohol, 1 gram of P(NVP-MMA) is added and this solution is placed at a temperature of 60 for 12 hours. The resulting material is soaked in 100 ml of heptane for 24 hours. After that, the product is washed in heptane three times to remove iodine residues. Then it is dried at low pressure to obtain a solid product. In pale green color, this represents the prepared nanomaterial PVP-NPS, (Figure 2).

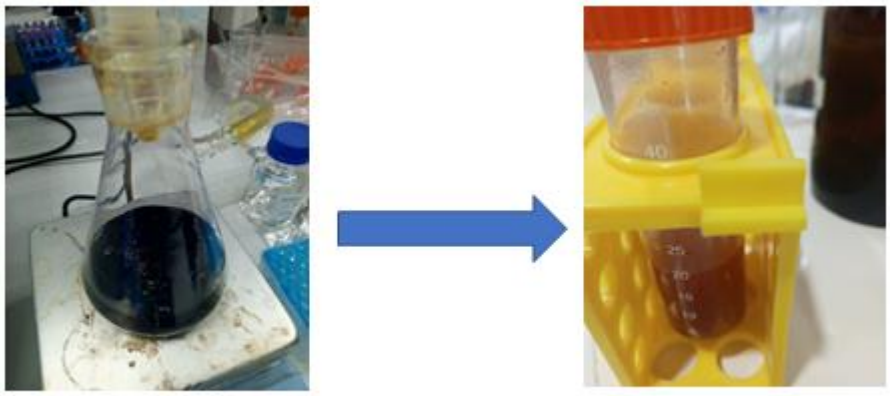

Figure (2): this represents the prepared nanomaterial PVP-NPs

Characterization of povidone-iodine-based polymeric nanoparticles (PVP-I-NPs)

Using UV-Vs spectrophotometer (Thermo® Scientific, America) to assessment of absorbance povidone iodine -NPS for confirm. The morphology of Povidone Iodine -NPS, was scanning electron microscope (SEM) (JSM-7001F, Japan). Moreover, Fourier transform infrared (FTIR) spectroscopy (Vertex 70, Germany) used to identify the functional groups capped on surface of the povidone iodine -NPS and test toxicity on Chinese hamster cell (CHO cell line -china).

MTT Assay Protocol for PVP-Iodine Nanoparticles Cytotoxicity Assessment

Materials Required

- **Cell Line:** Normal CHO (Chinese Hamster Ovary) cell line.
- **Test Compound:** PVP-iodine nanoparticles (stock solution and dilutions: 100 µg/mL, 75 µg/mL, 50 µg/mL, 25 µg/mL, and 0 µg/mL as control).
- **Cell Culture Medium:** Complete medium (e.g., DMEM or RPMI-1640 supplemented with 10% FBS and 1% penicillin-streptomycin).
- **Reagents:**

✓ Phosphate-buffered saline (PBS).

✓ MTT reagent (5 mg/mL in PBS) Elabscience® MTT Kit Manual (Cat. No. E-CK-A341).

- ✓ Dimethyl sulfoxide (DMSO) or Formazan Dissolution Buffer (from Elabscience kit).

➢ **Labware:**

- ✓ 96-well flat-bottom tissue culture plates.
- ✓ Sterile pipettes, tips, centrifuge tubes, and hemocytometer.

➢ **Equipment:**

- ✓ CO_2 incubator (37°C, 5% CO_2).
- ✓ Microplate reader (570 nm absorbance).
- ✓ Centrifuge.
- ✓ Inverted microscope.

Day 1: Cell Seeding

- ➢ CHO cells were harvested from a confluent culture flask using trypsin-EDTA.
- ➢ The trypsin was neutralized with complete medium, and the cells were centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded.
- ➢ The cell pellet was resuspended in fresh complete medium, and the cells were counted using a hemocytometer.
- ➢ The cell density was adjusted to 5×10^4 cells/mL (for cytotoxicity assays) or 2×10^4 cells/mL (for proliferation assays).
- ➢ 100 µL of the cell suspension was added to each well of a 96-well plate.
- ➢ Blank wells (medium only, no cells) and control wells (cells with no treatment) were included.
- ➢ The plate was incubated at 37°C, 5% CO_2 for 24 hours to allow cell adherence.

Day 2: Treatment with PVP-Iodine Nanoparticles

- ➢ The PVP-iodine nanoparticle stock solution was diluted to the desired concentrations (100, 75, 50, 25, and 0 µg/mL) in complete medium.
- ➢ The dilutions were vortexed or pipetted gently to ensure homogeneity.
- ➢ The medium was carefully aspirated from the 96-well plate.
- ➢ 100 µL of each nanoparticle dilution was added to triplicate wells.
- ➢ For the control group, 100 µL of complete medium (0 µg/mL nanoparticles) was added.
- ➢ The plate was incubated for 24-48 hours (depending on experimental design).

Day 3/4: MTT Assay

- ➢ MTT (5×) was thawed and diluted to 1× using MTT Diluent Buffer (e.g., 100 µL MTT + 400 µL buffer).
- ➢ The solution was protected from light.
- ➢ After treatment, 50 µL of 1× MTT solution was added to each well.
- ➢ The plate was incubated for 4 hours under the same conditions (37°C, 5% CO_2).
- ➢ For adherent cells: The medium was carefully aspirated, and 150 µL DMSO was added to each well. The plate was shaken gently on an orbital shaker for 10-15 minutes to dissolve the formazan crystals.

- For suspension cells: The plate was centrifuged at 1000 rpm for 5 minutes, and the supernatant was aspirated. 100 µL Formazan Dissolution Buffer (from the kit) was added, and the plate was incubated for 1-4 hours.
- The absorbance was measured at 570 nm using a microplate reader.
- Care was taken to ensure no bubbles were present in the wells before measurement.

Data Analysis

Cell Viability (%) = $[(OD_{sample} - OD_{blank})/(OD_{control} - OD_{blank})] \times 100\%$

Where:

- ✓ OD_{sample}: Treated wells
- ✓ $OD_{control}$: Untreated cells (0 µg/mL)
- ✓ OD_{blank}: Medium-only wells

The viability (%) was plotted against nanoparticle concentration to determine the IC_{50} (concentration inhibiting 50% of cells).

Key Notes

- ✓ MTT was stored in the dark at 2-8°C, and freeze-thaw cycles were avoided.
- ✓ Blanks (medium only) and controls (untreated cells) were always included.
- ✓ Preliminary tests were conducted to adjust cell density or MTT incubation time if necessary.
- ✓ PPE (gloves, lab coat) was worn, and work was performed in a biosafety cabinet.

Statistical analysis

Using SPSS program version 27 for analysis percentages with Chi-square test.

Results

Povidone-iodine-Based Polymeric Nanoparticles by UV spectrometer

Our findings showed that analysis Povidone-iodine-Based Polymeric Nanoparticles by UV-Vis spectrometer. The measurement properties were settled: wavelength Range: 200.00 to 800.00 nm: indicates the wavelengths scanned by the instrument to assess the sample's absorbance of the Povidone-iodine-Based Polymeric Nanoparticles. Also, the instrument type was UV-1600 Series: as specifies the model of the spectrophotometer utilized for the measurements, the measuring mode absorbance recorded the sample's absorbance across various wavelengths. As, the Slit Width: 2.0 nm refers to the width of the instrument's entrance slit, which influences the resolution of the wavelength. The light Source change wavelength at 340.8 nm which the instrument transitioned between light sources.

Results displayed the sample exhibits four distinct absorbance peaks at the following wavelengths: Peak 1: 369.00 nm, Peak 2: 296.50 nm, Peak 3: 247.00 nm, and Peak 4: 233.00 nm respectively, (figure 3). So, the absorbance values for each peak documented, and the P/V ratio reflects the relative prominence of each peak. This test for Povidone-iodine-Based Polymeric Nanoparticles exhibited has high specificity, quality and purify, without impurifications.

Figure (3): This presented the analysis Povidone-iodine-Based Polymeric Nanoparticles by UV-Vis spectrometer

The scanning electron microscopy (SEM) of the povidine iodine base polymeric nanoparticle.

The scanning electron microscopy (SEM) image Polymeric nanoparticles composed of Povidone-iodine are characterized by a particle size ranging from 263.3 nm to 500.6 nm, confirming their classification within the nanoscale. Regarding their surface structure and physical properties, the images reveal that these particles possess a rough and irregular surface. This feature was significant as it may enhanced the effective surface area, which was crucial for applications in the medical and pharmaceutical fields. The irregularity of the surface could also improve interactions with biological fluids, thereby facilitated a more efficient released of iodine. In terms of their impact on biological activity, Povidone-iodine nanoparticles serve as potent antimicrobials, with their efficacy being influenced by both size and shape. The diminutive and nanoscale dimensions of these particles augment their absorption capacity and interaction with bacteria and fungi, thereby enhanced their effectiveness as antiseptics. picture (A)analysis to picture (B) For a material characterized by a porous structure and an irregular surface: The image illustrates the existence of substantial pores and voids, highlighting the material's porous characteristics. Regarded the potential applications of a highly porous material: Enhanced iodine released: the increased porosity expanded the effective surface area, facilitated a sustained released of iodine. Absorption capacity: the porous configuration may assist in the incorporation of supplementary substances, included antibiotics or active agents. Medical uses: this material could be advantageous in wound healing or serve as a long-lasting antimicrobial agent, (Figure 4).

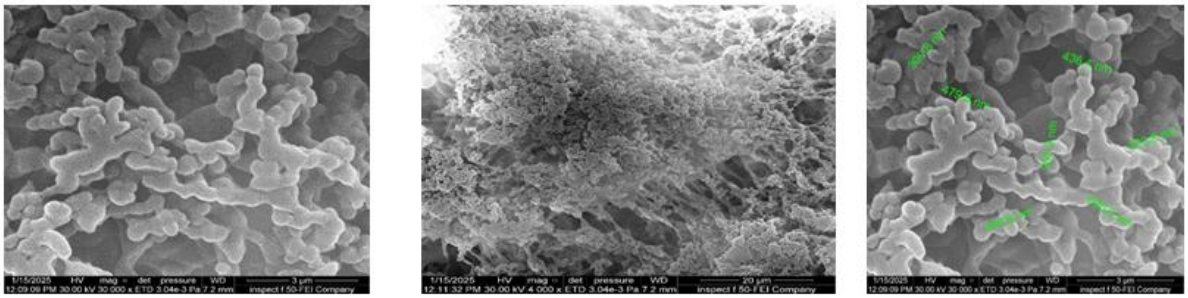

Figure (4): This presented the scanning electron microscopy (SEM) of the povidine iodine base polymeric nanoparticle.

Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectrum indicated the existence of multiple functional groups within the Povidone-iodine-based polymeric nanoparticles, encompassing aliphatic chains, aromatic rings, amide groups, and potentially ether or alcohol functionalities. These observations are consistent with the expected constituents of the nanoparticles, included polyvinylpyrrolidone (PVP) and other polymeric or stabilizing agents employed in the formulation.

- C–H stretching vibrations, which are frequently linked to aliphatic hydrocarbon structures, are indicated by a spectral peak that appears at 2996.86 cm^{-1}. These vibrations may be the result of hydrocarbon chains contained in the nanoparticles' polymer matrix.

The stretching vibration of C=C bonds, a characteristic commonly present in aromatic compounds, is represented by another peak, which was detected at 1619.21 cm^{-1}. The presence of aromatic rings in the main chain of the polymer or any aromatic additives employed during formulation might be the cause of this.

The C-H bending vibration in methylene groups (-CH2-) is associated with the peak at 1427.75 cm-1, which further supports the existence of aliphatic chains in the polymer structure. Indicating the presence of amide groups, most likely arising from the PVP component of the nanoparticles, the peak at 1310.64 cm-1 is representative of the C-N stretching vibration in secondary amides.

The C-O stretching vibration in ethers or alcohols is linked to the peak at 1044.00 cm-1, which may be related to ether or alcohol functionalities present in the polymer or its additions. The C-C stretching vibration in aromatic compounds is often associated with the peak at 950.08 cm-1, confirming the existence of aromatic moieties in the nanoparticles.

Lastly, the peak at 697.20 cm-1 further supports the existence of aromatic rings in the polymer structure as it is typical of the C-H bending vibration in aromatic compounds. It is also crucial to remember that iodine usually exhibits modest absorption bands in the mid-infrared spectrum, therefore its presence in the nanoparticles could not be clearly seen in the FTIR spectrum. It is also important to take into account the peaks' location and strength, (Figure 5).

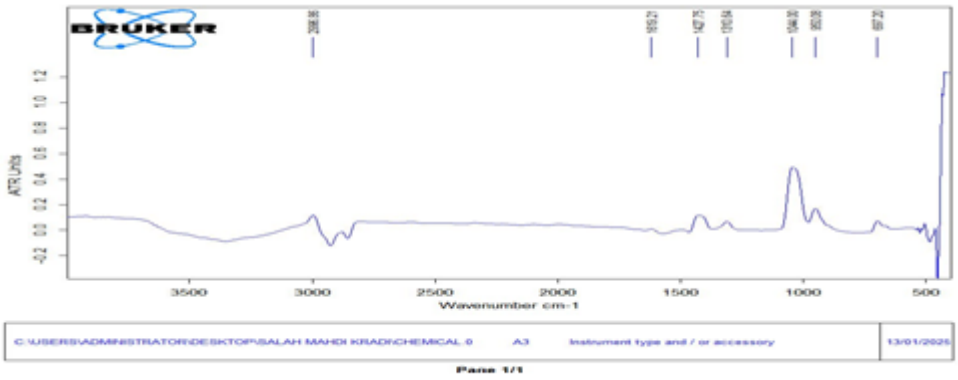

Figure (5): The present Fourier-transform infrared spectroscopy (FTIR)

MTT Test Results

The MTT test results demonstrate the cytotoxicity of PVP-INP at various concentrations (25, 50, 75, and 100 µg/ml) by measuring the inhibitory percentage and cell viability relative to the control group. The optical density (O.D) values for each concentration were recorded in triplicate (O.D1, O.D2, O.D3), and their mean was calculated to determine the inhibitory percentage.

The control group, with an O.D mean of 0.828, served as the baseline for 100% viability and 0% inhibition. Key observations include:

- At the highest concentration (100 µg/ml), the inhibitory percentage was 5.19%, corresponding to 94.81% cell viability
- At 75 µg/ml, the inhibitory effect was 2.66% with 97.34% viability
- The 50 µg/ml and 25 µg/ml concentrations showed minimal inhibition (0.85% and 0.12% respectively)
- Standard deviations across triplicates were consistently low (0.004-0.044), indicating high data reliability

Table (1): Results of Inhibitory percentages

PVP-INP Concentration (µg/ml)	O.D1	O.D2	O.D3	O.D Mean	Inhibitory Percent (%)
100.0	0.789	0.782	0.784	0.785	5.19
75.0	0.834	0.759	0.825	0.806	2.65
50.0	0.797	0.789	0.877	0.821	0.84
25.0	0.843	0.857	0.781	0.827	0.12
Control	0.843	0.819	0.822	0.828	0.0

Table (2): Statistical Analysis of Inhibitory and Viability percentages

Concentration (µg/ml)	O.D Mean	Inhibitory %	Viability %	Standard Deviation (O.D)
Control	0.828	0.00	100.00	0.012
25	0.827	0.12	99.88	0.038
50	0.821	0.85	99.15	0.044
75	0.806	2.66	97.34	0.039
100	0.785	5.19	94.81	0.004

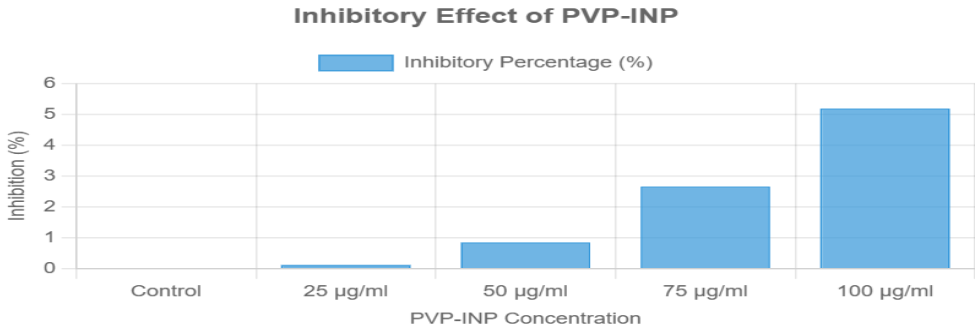

Figure (6): Cell viability across concentrations

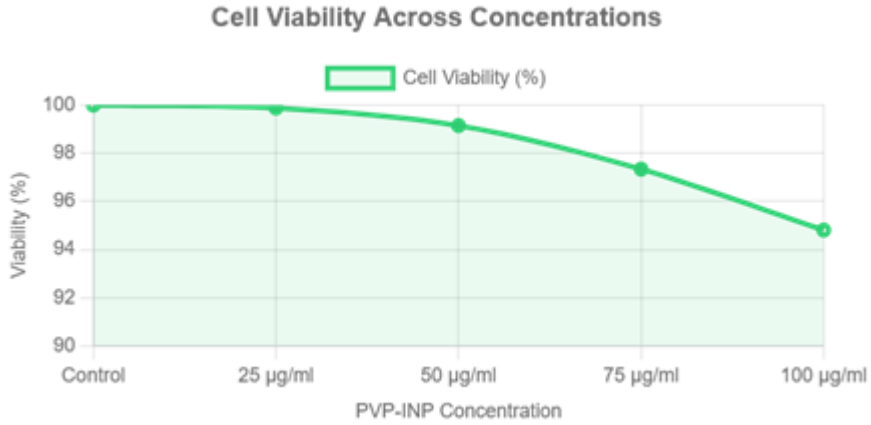

Figure (7): Inhibitory effect of PVP-INP

MTT assay indicates that PVP-INP exhibits very low cytotoxicity across all tested concentrations, with cell viability remaining close to control levels (94.81-99.88%). The marginal inhibitory effects suggest PVP-INP is non-toxic or minimally toxic under these experimental conditions, making it a potentially safe candidate for further applications. However, additional assays with higher concentrations may be valuable to fully characterize the safety profile and identify any potential cytotoxicity thresholds.

Discussion

Our findings found that the synthesis of povidone-iodine polymeric nanoparticles (PVP-I NPs) was optimized using different monomer feed ratios, which significantly influenced their physicochemical properties. So, SEM analysis shown that PVP-I NPs synthesized with a 6:4 (MMA-NVP) feed ratio, and displayed a smaller size range (309.8–500.6 nm) in comparison with recent study [2] produced with an 8:2 ratios (531–983 nm). Accordingly, this size variation might be attributed to differences in cross-linking density, and polymerization kinetics, because a higher MMA content upsurges rigidity, and restricting particle growth, even though NVP boosts flexibility which are allowing larger nanoparticle formation [10,11].

Significantly, FTIR spectra examine detected and confirmed that there is successful incorporation of iodine into the polymeric matrix, with characteristic peaks at 2996.86 cm^{-1} (C-H stretch), 1619.21 cm^{-1} (C=C aromatic), and 1310.64 cm^{-1} (C-N amide). As a result, these outcomes agreed with previous studies, validating the structural integrity of the synthesized nanoparticles [12].

Moreover, UV-Vis assessment showed absorption maxima for the PVP-I NPs at 247 nm (due to PVP-I complex formation), 259.5 nm (related to n-π transitions of iodine-pyrrolidone interactions), and 369 nm (plasmon resonance of iodine nanoparticle formation). These characteristic peaks ascertain the successful synthesis of PVP-I NPs, confirming their stability and ability for controlled release of iodine [13,14]. In addition, the plasmon resonance band at 369 nm clearly indicates the presence of well-dispersed, nanosized iodine particles within the PVP matrix, which is essential in maintaining the long-term antimicrobial effect [15].also, the consistency of these absorption maxima was maintained along several repeated scans, which indirectly indicates negligible-antiparticle-aggregation and hence an indefinite colloidal stability of PVP-I NPs; this makes the PVP-I NPs useful for relevant biomedical and antimicrobial applications, facile synthesis of antimicrobial aloe vera-“smart” triiodide-PVP biomaterials [16]. Remarkably, toxicity assays against CHO cells showed that PVP-I NPs are safe even at higher concentrations; thus, they can be used within the uterus [17]. A controlled release of iodine avoids oxidative stress and damage to epithelial cells, as would an iodine solution [18]. Additionally, there are many studies conformed that PVP-I NPs act as long-lasting antiseptics that kill pathogens such as E. coli and T. pyogenes, which are typical agents of endometritis [19].

Conclusion

This suggest that they may reduce the use of antibiotics, hence mitigating resistance problems in livestock. For PVP-I NPs to be used in clinical veterinary practice, additional research needs to be undertaken, including assessment of PVP-I NPs in bovine endometritis models for evaluation of uterine healing, microbial clearance, and reproductive performance; development of mucoadhesive or thermosensitive gels to improve retention in the uterus and further extend antimicrobial effects; assessment on uterine immunity (e.g., modulation of cytokines) and comparison with standard antibiotics for efficacy; and finally, trials in dairy herds to validate safety, efficacy, and economic perspectives. Our findings, the size distribution of PVP-I NPs (309.8-500.6 nm) was prepared by the 6:4 polymerization MMA-NVP confirming iodine incorporation and release using (FTIR, UV-Vis). Also, the NPs showed low cytotoxicity with high antimicrobial efficacy against pathogens. However, to realize their potential for reducing antibiotic usage, further in vivo studies need to be undertaken for demonstrating therapeutic efficacy in veterinary use.

Ethical Approval

Ethical authorization was given following the Ethical Scientific Research committee at the College of Veterinary Medicine, University of Al-Qadisiyah (Approval number P.G/2314 at 26/5/2025).

Conflict of Interest: The authors state that there is no competing interest.

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