SYNTHESIS, IN-SILICO PHYSICOCHEMICAL PROPERTIES AND ANTIMICROBIAL STUDIES OF PYRAZOLINE LOADED NANOPARTICLES

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ABSTRACT: Pyrazolines were prepared from the corresponding chalcones by using isonicotinylhydrazine as cyclizing agent. The synthesized compounds were characterized by spectral data and screened for antimicrobial activity. In-silico studies of physiochemical and drug-like properties revealed that these compounds have good bioavailability and drug-likeness properties. Compound PZ2 which exhibited good antimicrobial activity was incorporated into polymeric nanoparticles using PLGA polymer by solvent evaporation method. The prepared nanoparticles were characterized by particle size, entrapment efficiency (EE%), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and in-vitro release studies. Prepared nanoparticles were incorporated into different hydrogel systems, which were prepared by using Carbopol 934 and HPMC in different concentrations ratio 2:1, 1:1 & 1:2. Hydrogel formulations were evaluated for drug content, spreadability, viscosity and drug release studies. Hence, the studies confirmed that entrapment of pyrazolthe ine as antimicrobial drugs in nanoparticle systems has found to be an inventive alternative that improves therapeutic effectiveness and curtails undesirable side effects of the drugs. Further, incorporation of pyrazolone nanoparticles into hydrogel provides an additional benefit of advanced permeation features of antimicrobial constituents for transdermal application.

INTRODUCTION: In the development of a drug, drug delivery is one of the important criteria. Even though many potential compounds exhibit good pharmacological properties but finally fail to deliver the drug to the targeted site of action, thereby producing undesirable side effects, due to high drug doses. By developing alternative, targeting drug delivery systems such as nanoparticles, drugs can be transported directly to their site of action by crossing natural biological barriers as the blood-brain barrier or the skin.

This method provides a substantial advantage over traditional formulations since the less active ingredient is needed to have the same therapeutic effect, with improved safety profile ¹. Different types of nanoparticles including solid lipid nanoparticles, polymeric nanoparticles, dendrimers, and liposomes have been extensively examined as antimicrobial drug delivery platforms, of which numerous products have been introduced into pharmaceutical market².

The development of topical drug delivery is challenging because of the barrier function of skin, which inhibits the transport of drugs. The recent emergence of nanotechnology has opened up new opportunities to develop nanoparticles for topical and transdermal applications. If not all, some of these nanoparticles have been specifically developed for skin applications ³.
Nanoparticles have the advantages of biocompatibility, protecting drugs from the inactivation of external conditions, sustained release and the efficient permeation through the skin, have been demonstrated for their beneficial effect in the treatment of skin disorders 4,5.

Hydrogels are polymeric networks with a three-dimensional configuration capable of imbibing high amounts of water or biological fluids. Hydrogel nanoparticulate material would demonstrate the features and characteristics of hydrogel and nanoparticles separately possess, at the same time, i.e., hydrophilicity, flexibility, versatility, high water absorbivity, and biocompatibility of hydrogel particles and all the advantages of the nanoparticles, mainly long life span in circulation and the possibility of being actively or passively targeted to the desired purpose, e.g., tumor sites 6.

Pyrazolines constitute an important class of medicinally important molecules which can be attributed to their diverse pharmacological properties such as antimicrobial 7, antihyperglycemic 7, anti-inflammatory 8, antitumor 8, antitubercular 9, antioxidant 10.

Computational methods have key roles in drug discovery, for instance, virtual screening, in-silico prediction of physicochemical properties, Lipinski’s rule of five 11, drug-likeness, properties. This plays a vital role in the bioavailability and period of drug action, which enables the researchers to make better decisions faster in the ground of drug lead identification and optimization.

Various antimicrobial drugs have been prescribed to kill or inhibit the growth of microbes such as bacteria, fungi, and viruses. Even though the therapeutic efficacy of antimicrobial drugs has been well established, inefficient delivery could result in an inadequate therapeutic index and local and systemic side effects, including cutaneous irritation, peeling, scaling and gut flora reduction 2.

By considering the above facts, the present study aimed to prepare and characterize hydrogel containing nanoparticles loaded with synthesized pyrazolines and to investigate their physicochemical properties and antimicrobial activity.

MATERIALS AND METHODS:
General Details: Melting points were determined by the capillary method and were uncorrected. The IR spectra are recorded by using Shimadzu Perkin Ekmer8201 PC IR SPECTROMETER using a thin film on potassium bromide pellets techniques and frequencies are expressed in cm⁻¹. The PMR spectra were recorded on BRUKER AVANCE II 400 NMR SPECTROMETER. All spectra were obtained in CDCl₃ and DMSO. Chemical shift values are reported as values in ppm relative to TMS (δ=0) as an internal standard. The FAB mass spectra were recorded on JEOL SX-102/DA-6000 Mass spectrometer using Argon/Xenon (6Kv, 10Ma) as the FAB gas.

Poly Lactic Glycolic Acid (PLGA 50:50) was procured from Sigma Aldrich, Bangalore and H.P.M.C K4M was obtained from Yarrow Chem products, Mumbai. Carbopol 934 was purchased from Hi-media laboratories Pvt. Ltd, Mumbai.

Synthesis and characterization of pyrazolines(3-(4-aryl)- 5- phenyl-4, 5-dihydro-1H-pyrazol-1-yl) (pyridin-4-yl)methanone: A mixture of chalcone (0.01 mol), isonicotinylhydrazide (0.01 mol) in ethanol (30 ml) in the presence of acetic acid (2 ml) was subjected to microwave irradiation for 7-8 min. With constant stirring, the reaction mixture was poured into cold water. The product thus obtained was recrystallized from ethanol. Ethyl acetate: Chloroform (8:2) is the solvent system for TLC 13.

**SCHEME 1: SYNTHESIS OF PYRAZOLINES (PZ1-PZ6)**

**Spectral Data:**

**PZ1**: (3-(4-bromophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl) (pyridin-4-yl)methanone: Brown Crystals; M.P (°C): 181-183; Yield (%):77; IR (KBr cm⁻¹): 3443 (C-H), 1652.1 (C=N), 1327.2 (C-N), 1594.7 (C=C), 889.9 (C-Br), 703.8 (C-Cl); ¹H NMR (δ ppm): 3.134 to 3.152 (dd, 1H, Ha), 3.673 to 3.682 (dd, 1H, Hb), 5.243 to 5.253 (dd, 1H, Hc), 7.76 to 7.99 (m, 12H, Ar-H);
Mass (m/z): (M⁺) 440, (M⁺ + 2) 442. Anal. Calcd for C, 57.23; H, 3.43, Found: C, 57.25; H, 3.44.

**PZ2: (3-(4-fluorophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl) (pyridin-4-yl) methanone:** Yellow Crystals; M.P (°C): 189-191; Yield (%): 79; IR (KBr cm⁻¹): 3443 (C-H), 1641.8 (C=N), 1332.2 (C-N), 1587.2 (C=C), 704.9 (C-Cl), 1232.9 (C-F); ¹H NMR (δ ppm): 3.102 to 3.111 (dd, 1H, Ha), 3.767 to 3.782 (dd, 1H, Hb), 5.231 to 5.239 (dd, 1H, Hc), 6.732 to 7.102 (m, 12H, Ar-H); Mass (m/z): (M⁺) 379, (M⁺ + 2) 381. Anal. Calcd for C, 66.41; H, 3.98, Found: C, 66.42; H, 3.97.

**PZ3: (3, 5-bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl) (pyridin-4-yl) methanone:** Yellow Crystals; M.P (°C): 175-177; Yield (%): 75; IR (KBr cm⁻¹): 3476 (C-H), 1647.9 (C=N), 1336.4 (C-N), 1556.5 (C=C), 704.9 (C-Cl); ¹H NMR (δ ppm): 3.167 to 3.181 (dqd, 1H, Hc), 3.453 to 3.463 (dd, 1H, Hb), 6.97 to 7.49 (m, 12H, Ar-H), 7.90 to 8.39 (m, 12H, Ar-H); Mass (m/z): (M⁺) 396. Anal. Calcd for C, 63.65; H, 3.82, Found: C, 63.67; H, 3.84.

**PZ4: (5-(4-chlorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl) (pyridin-4-yl) methanone:** Yellowish Brown Crystals; M.P (°C): 286-288; Yield (%): 72; IR (KBr cm⁻¹): 3451 (C-H), 1656.1 (C=N), 1327.2 (C-N), 1594.7 (C=C), 1333.4 (C-NO₂), 703.8 (C-Cl); ¹H NMR (δ ppm): 3.356 to 3.367 (dd, 1H, Ha), 3.563 to 3.675 (dd, 1H, Hb), 3.453 to 3.463 (dd, 1H, Hc), 6.97 to 7.49 (m, 12H, Ar-H); Mass (m/z): (M⁺) 406. Anal. Calcd for C, 62.00; H, 3.72, Found: C, 62.02; H, 3.70.

**PZ5: (3-(4-aminophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl) (pyridin-4-yl) methanone:** Yellow Crystals; M.P (°C): 203-205; Yield (%): 68; IR (KBr cm⁻¹): 3367 (C-H), 1647.5 (C=N), 1334.5 (C-N), 1574.5 (C=C), 704.9 (C-Cl), 1442.0 (C-NH₂); ¹H NMR (δ ppm): 3.167 to 3.181 (dd, 1H, Ha), 3.754 to 3.782 (dd, 1H, Hb), 5.262 to 5.271 (dd, 1H, Hc), 7.143 to 7.762 (m, 12H, Ar-H); Mass (m/z): (M⁺) 376. Anal. Calcd for C, 66.93; H, 4.55, Found: C, 66.93; H, 4.57.

**PZ6: (5-(4-chlorophenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl) (pyridin-4-yl) methanone:** Brown Crystals; M.P (°C): 234-236; Yield (%): 62; IR (KBr cm⁻¹): 3443 (C-H), 1656.0 (C=N), 1320.0 (C-N), 1572.3 (C=C), 3267.35 (C-OH), 703.8 (C-Cl); ¹H NMR (δ ppm): 3.304 to 3.315 (dd, 1H, Ha), 4.666 to 4.693 (dd, 1H, Hb), 5.443 to 5.483 (dd, 1H, Hc), 7.90 to 8.39 (m, 12H, Ar-H); Mass (m/z): (M⁺) 377. Anal. Calcd for C, 66.76; H, 4.27, Found: C, 66.78; H, 4.29.

**In-silico studies:**

**Physicochemical Properties:** Physicochemical properties and the presence of various pharmacophoric features, influence the behaviors of molecules in a living organism, including bioavailability. Thus, to achieve good oral drugs, we have investigated a series of pyrazoline derivatives for the prediction of their molecular properties, Lipinski’s ‘Rule of Five’ and drug-likeness properties by using Molinspiration server.

**Rule of Five’ Properties:** The rule states that most molecules with good membrane permeability have log P ≤ 5, MW ≤ 500, the number of hydrogen bond acceptors ≤ 10, and the number of hydrogen bond donors ≤ 5. A compound that fulfills at least three out of the four criteria is said to adhere to ‘Lipinski’s Rule of Five.’ A poor permeation or absorption is more likely when there are more than five H-bond donors and ten H-bond acceptors.

The compounds that were drawn using ChemDraw software were initially screened for Lipinski’s rule of 5 using Molinspiration server.

**Antimicrobial Activity:** The antimicrobial activity of synthesized compounds was determined by cup plate method. The *in-vitro* antimicrobial activity was carried out against 24 hr old cultures of three bacteria. The bacteria used were *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The zone of inhibition was determined for all the tested compounds. The antibacterial activity is proportional to the diameter (mm) of the zone of inhibition. The plates were incubated at 37 °C for 24 h.

**UV Spectrophotometer Analysis of Synthesized Pyrazolines:** The synthesized pyrazoline was dissolved in a small amount of ethanol, followed by the addition of phosphate buffer of pH 6.8 and then diluted. λmax was obtained by scanning the solution at UV-Visible range. Then the absorbance of the different serial diluted samples was measured at the
\( \lambda_{\text{max}} \), and a standard calibration curve was plotted with concentration against absorbance.

**Preparation of Polymeric Nanoparticles:** The selected compound PZ2 which showed good antimicrobial properties was incorporated into polymeric nanoparticle by the solvent evaporation method. Table 1 using PLGA used as a polymer. The organic phase contains PLGA and pyrazolines (25 mg) in DMSO, and the aqueous phase was of 0.5% of PVA (surfactant) in water. The organic phase was then added dropwise at the rate of 1 ml/min into an aqueous phase while stirring. The nanoparticles suspension was kept under continuous stirring at 300 rpm for 3 h at 30 °C to allow the complete evaporation of DMSO, leaving behind the colloidal suspension of PLGA nanoparticles containing pyrazolines in the aqueous phase.

The nanosuspension was centrifuged at 12,000 rpm (Remi, Mumbai, India) for 30 min at 4 °C to get the final nanoparticulate containing encapsulated pyrazolines. The nanoparticulate was washed with deionized water twice to remove the unentrapped drug from the surface of nanoparticles.

**TABLE 1: COMPOSITION OF PYRAZOLINE LOADED POLYMER NANOPARTICLE**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug PZ2 (mg)</th>
<th>Polymer (mg)</th>
<th>Surfactant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP1</td>
<td>25</td>
<td>25</td>
<td>0.5</td>
</tr>
<tr>
<td>NP2</td>
<td>25</td>
<td>50</td>
<td>0.5</td>
</tr>
<tr>
<td>NP3</td>
<td>25</td>
<td>100</td>
<td>0.5</td>
</tr>
<tr>
<td>NP4</td>
<td>25</td>
<td>200</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Characterization of Polymer Nanoparticles**

**Formulation**: Particle Size, PDI and Zeta Potential: The average particle size, polydispersibility index (PDI) and zeta potential of NEs was determined using zeta sizer by dynamic light scattering (Nano ZS, Malvern Instruments, UK).

Six replicates were measured, and values were expressed as mean ± standard deviation (SD). The PDI value indicates the particle size distribution of nanoparticles in a given sample. The zeta potential of a particle is the overall charge that the particle acquires in a particular medium. Knowledge of the zeta potential of SLNs helps to assess the stability of the formulation during storage.

**Entrapment Efficiency of the Polymeric Nanoparticles:** Polymeric nanoparticles dispersion was centrifuged at 20000 rpm for 30 min using a cold centrifuge at 4 °C. The clear supernatant was decanted. Polymeric nanoparticles were washed with water and recentrifuged to remove an unentrapped drug. The two supernatants were combined, and after suitable dilution, the absorbance was recorded at 250 nm by keeping blank as phosphate buffer pH 6.8. The entrapment efficiency of the pyrazolines in the polymeric nanoparticles was calculated using the formula:

\[
\text{Entrapment Efficiency %} = \frac{\text{Total amount of drug added} - \text{Amount of drug in supernatant} \times 100}{\text{Total amount of drug added}}
\]

**Scanning Electron Microscopy (SEM):** Shape and surface morphology of the nanoparticles were studied using SEM (JEOL, JSM 50A, Tokyo, Japan). An appropriate amount of Colloidal dispersion of SLNs was mounted onto metal (aluminum) using double-sided adhesive tape and fractured with a razor blade. The samples were sputter-coated with gold/palladium for 120 sec at 14 mA under argon atmosphere for secondary electron emissive SEM and observed for morphology, at an acceleration voltage of 15 KV.

**Transmission Electron Microscopy (TEM):** Transmission electron microscopy (TEM) was used in describing the structure and morphology of the nanoparticles. The form and size of the nanoparticle were revealed by using a combination of bright-field imaging at increasing magnification and diffraction modes. A drop of the diluted nanoparticle was directly deposited on the holey film grid and observed after drying.

**Preparation of Hydrogel Containing Polymer Nanoparticles loaded with Pyrazoline:** The optimized pyrazoline loaded polymer nanoparticle (NP3) was incorporated in the hydrogel matrix. The hydrogel-forming polymers were dissolved in a small amount of double distilled water in various proportions separately, as shown in Table 2 and the remaining ingredients, *i.e.* glycerine and sodium benzoate was added. The prepared nanoparticle formulation was added to it, and they weighed up to 50 g. Then, probe sonicated at an amplitude of 25, 30 sec. The above formulation was allowed to stand for 24 h at room temperature. The pH of this
Characterization of Hydrogel Formulations:

Determinant of Drug Content: The amount of drug contained in the prepared hydrogel was determined by dissolving 100 mg of the prepared hydrogel in 10 ml in acetone and diluted by using phosphate buffer of pH 6.8. This mixture was analyzed by using UV spectrophotometer at 239 nm against phosphate buffer as blank.

pH Determination: Determination of pH is an important criteria for topical formulations, to ensure non-irritating nature and it was determined at ambient temperature with digital pH meter.

Determination of Spreadability: The spreadability of prepared hydrogel formulation was determined 48 h after preparation by measuring the spreading diameter of formulation between the two glass plates after 1 min. A weight of 500 mg of hydrogel was placed within a circle of diameter 1 cm premarked on a glass plate over which a second glass plate was placed. The increase in diameter as a consequence of weights added leading to the spreading of the gel was noted. The spreadability can be calculated by using the formula,

\[
\text{Spreadability (S)} = m \times \frac{1}{t}
\]

Where; \( S \) = Spreadability, \( m \) = Weight tide to upper slide, \( l \) = Length of the glass slide, \( t \) = Time is taken to separate the slide from each other.

Measurement of Viscosity: It was determined at different angular velocities at 32.0 ± 0.1 °C using spindle number 4 (Brookfield DV-II + Pro viscometer).

In-vitro Drug Release Study of Hydrogel:

Amount of release of the pyrazoline from the hydrogel formulations were evaluated for 12 h. Dialysis bag (12 Kda, Hi-Media) soaked in deionized water for 12 h was utilized for the study. In-vitro release of the drug across the dialysis bag was performed by using diffusion cell (containing 100 mg of the sample) and 80 ml of phosphate buffer pH 6.8 as the dissolution medium (n=6). The dissolution medium was maintained at 37 ± 0.5 °C, and the medium was stirred at 100 rpm with the help of small Teflon coated magnetic bead. Aliquots of the medium were withdrawn at a suitable time interval and were replaced with the same volume of fresh medium to maintain the sink condition. These samples were filtered through 0.45 µm membrane filter, and the collected samples were analyzed using UV-Vis Spectrophotometer at the \( \lambda_{\text{max}} \) of 370 nm.

RESULTS AND DISCUSSION:

Synthesis and Characterization of Pyrazolines:

Pyrazolines were synthesized from chalcones by cyclizing with hydrazine hydrate Fig. 1 and were characterized. The IR spectrum of the synthesized compounds revealed the presence of C –N stretching at 1315-1339 cm\(^{-1}\), and C=N (aromatic) stretching at 1641 -1659 cm\(^{-1}\). Further, in their \(^1\text{H} \) NMR spectrum, the appearance of a signal at \( \delta \) 3.1 (dd, 1H, HA), 3.7 (dd, 1H, HB), 5.2 (dd, 1H, Hx), confirms the presence of the pyrazoline ring (PZ2).

Physicochemical Properties: The compounds possessed desired physicochemical properties with no violations from the standard ranges and are reported in Table 3. The number of rotatable bonds must be less than 10. Drugs that penetrate CNS should have lower polar surface areas less than 140, than other kinds of molecules. The results showed that all the compounds have a promising oral bioavailability.

Rule of Five Properties: Based on the experimental values, it was inferred that all the compounds successfully satisfied all the parameters of Lipinski’s rule of five. Compounds with log P values less than 5 mean that they can readily get pass ester/phosphate groups in skin membranes. The results obtained are given in Table 1. The calculated values of log P for the derivatives ranged from 3.06 to 4.7. The lipophilic aptitude of a
compound increases with the increasing log P. The C log P values of all the compounds show a variation which can be attributed to the presence and position of different substituents on the phenyl ring. The series under investigation has not only most of the compounds possessing less number of hydrogen bond donors (<5) but also does possess a considerable number of acceptors (<10).

**TABLE 3: PHYSICOCHEMICAL PROPERTIES OF PYRAZOLINE DERIVATIVES**

<table>
<thead>
<tr>
<th>Compound Code</th>
<th>tPSA</th>
<th>nrobs</th>
<th>Mol. Wt</th>
<th>Log P</th>
<th>nONs</th>
<th>nOHNHs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PZ1</td>
<td>45.56</td>
<td>3</td>
<td>440</td>
<td>4.7</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>PZ2</td>
<td>45.56</td>
<td>3</td>
<td>379</td>
<td>4.15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PZ3</td>
<td>45.56</td>
<td>3</td>
<td>396</td>
<td>4.66</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>PZ4</td>
<td>91.39</td>
<td>3</td>
<td>406</td>
<td>3.94</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>PZ5</td>
<td>71.59</td>
<td>3</td>
<td>376</td>
<td>3.06</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>PZ6</td>
<td>65.79</td>
<td>3</td>
<td>377</td>
<td>3.50</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

**Antimicrobial Activity of Pyrazolines:** Agar cup plate method was used for determination of antimicrobial activity and the results were summarized in Table 4. From the results, it was evident that the activity was due to the presence of the halogen group.

**TABLE 4: ZONES OF INHIBITION (mm) OF PYRAZOLINES AGAINST BACTERIAL STRAINS**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gram-negative bacteria</th>
<th>Gram-negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacillus subtilis</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>PZ1</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>PZ2</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td>PZ3</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>PZ4</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>PZ5</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td>PZ6</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>28</td>
<td>30</td>
</tr>
</tbody>
</table>

**UV Spectrophotometer Analysis of Synthesized Pyrazolines:** The pyrazoline solution was scanned between the wavelengths of 200-400 nm using a UV spectrophotometer and the maximum absorbance was observed at 370 nm.

The standard calibration plot of the pyrazoline was prepared in phosphate buffer pH 6.8 and showed good linearity and with acceptable regression values ($R^2 = 0.999$) at the concentration range of 2-10 µg/ml Fig. 1.

**Preparation and Characterization of Polymeric Nanoparticles:** Polymeric nanoparticles were prepared by the solvent evaporation method with different ratios of pyrazoline and polymer PLGA. All formulated polymeric nanoparticles were subjected for different characterizations such as % entrapment efficiency, particle size and size distribution and results were stated in.

**Particle Size, PDI, Zeta Potential and Entrapment Efficiency (EE):** All the formulations had shown particles in nanosize range 102-539 nm. Particle size was found to increase with the increase in concentration of polymer due to an increased frequency of collisions, resulting in fusion of semifomed particles and producing finally an overall increase in the size of the nanoparticles, as shown in Table 5, which is in agreement with the studies reported by Jeffery et al., 24 & Benoit et al., 25 The different nanoparticle formulation had entrapment efficiency in the range of 34.12-72.23%. The results revealed that increasing the concentration (weight) of the polymer also improved the pyrazoline entrapment efficiency significantly till 100 mg of polymer (NP3).
Moreover, the high concentration of polymer in the emulsion droplets led to an enhancement of the efficiency of drug entrapment, because the high viscosity of the organic phase tends to restrict migration of the inner organic pyrazoline phase to the external water phase. Finally, at high polymer concentration (200 mg), the viscosity was so high that the efficiency of emulsion stirring was reduced and allowed the production of large particles (539.6 nm in mean diameter) with reduced entrapment efficiency (63.12%). Polydispersibility index (PDI) of formulations was found to be in a range of 0.185-0.539. The low PDI values indicated that nanoparticles size was uniform within each formulation. Surface charge measured in terms of zeta potential plays a major role in nanoparticles stability as the standard criteria. The zeta potential of the synthesized nanoparticles was found to be -2.08 mV to -4.58 mV. Zeta potential greater than +25mV and less than -25mV correlates to the higher stability of the nanoparticles. At larger zeta potentials, colloidal nanodispersions are expected to be stable as the charged droplets within them repel one another more strongly, thus overcoming the natural tendency to aggregate which was concluded from the results of zeta potential.

**TABLE 5: EFFECT OF PYRAZOLINE AND PLGA RATIO ON PARTICLE SIZE, PDI, ZETA POTENTIAL AND % ENTRAPMENT EFFICIENCY**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Particle Size (nm)</th>
<th>PDI</th>
<th>Zeta Potential (mV)</th>
<th>Entrapment Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP1</td>
<td>102.9 ± 2.18</td>
<td>0.185</td>
<td>-2.08</td>
<td>34.12 ± 1.26</td>
</tr>
<tr>
<td>NP2</td>
<td>153.8 ± 3.21</td>
<td>0.212</td>
<td>-3.24</td>
<td>46.28 ± 1.72</td>
</tr>
<tr>
<td>NP3</td>
<td>214.2 ± 2.06</td>
<td>0.203</td>
<td>-3.58</td>
<td>70.94 ± 1.08</td>
</tr>
<tr>
<td>NP4</td>
<td>539.6 ± 2.51</td>
<td>0.532</td>
<td>-4.39</td>
<td>63.12 ± 3.06</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n= 3).

Based on results of particle size and entrapment efficiency, polymeric nanoparticle formulation NP3 containing pyrazoline with 100 mg of PLGA polymer was selected as optimized formulation as it has nanosize range particles (214.2 nm) with low PDI (0.203) and high entrapment efficiency (70.94%).

**Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM):** The SEM studies reveal that polymeric nanoparticles were spherical with average particle size around 225 nm as shown in **Fig. 2a**. The transmission electron microscope revealed a positive image in which nanoparticles appeared dark with bright surroundings as shown in **Fig. 2b**. TEM images would give a better understanding of the real geometric size of the particles.

**FIG. 2A: SEM IMAGE AND 2B: TEM IMAGE OF OPTIMIZED FORMULATION OF PYRAZOLINE LOADED PN (NP3)**

**Formulation and Characterization of Hydrogel Containing Polymer Nanoparticle Loaded with Pyrazoline:** Different polymeric nanoparticle (NP3) incorporating hydrogel formulations HGN1, HGN2, HGN3 were prepared by varying ratios of HPMC 15 LV: Carbopol 934 hydrogel-forming matrix 1:2, 1:1 and 2:1 respectively. The prepared hydrogels were characterized by its appearance; pH, viscosity, spreadability and drug content **Table 4**. The drug content of the nanoparticulate hydrogel formulation was in the range of 95.6 ± 0.38% to 98.4 ± 0.23%. The results showed that the drug was
uniformly distributed throughout the formulation and drug loss was minimized while formulating nanoparticulate hydrogel. The pH values of different nanoparticulate hydrogel formulations were found to be in a range of 6.4-6.7 (nearly neutral), permitting the use of the formulation on the skin. The spreadability of the all formulations exhibited slips and drag phenomenon with higher diameters. By results of spreadability and % entrapment studies, the HGN3 formulation was found to be optimum which was further supported by a drug release study.

**TABLE 6: CHARACTERIZATION OF NANOPARTICLE INCORPORATED HYDROGELS**

<table>
<thead>
<tr>
<th>Form code</th>
<th>Appearance</th>
<th>Drug content</th>
<th>pH</th>
<th>Spreadability</th>
<th>Viscosity (m.PaS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGN1</td>
<td>Glossy, pale-yellowish colour</td>
<td>97.8 ± 0.23</td>
<td>6.4 ± 0.2</td>
<td>5.1 ± 0.32</td>
<td>4312 ± 0.21</td>
</tr>
<tr>
<td>HGN2</td>
<td>Glossy, pale-yellowish colour</td>
<td>98.4 ± 0.21</td>
<td>6.7 ± 0.3</td>
<td>5.6 ± 0.22</td>
<td>4131 ± 0.18</td>
</tr>
<tr>
<td>HGN3</td>
<td>Glossy, pale-yellowish colour</td>
<td>95.6 ± 0.38</td>
<td>6.5 ± 0.1</td>
<td>5.9 ± 0.42</td>
<td>4021 ± 0.16</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n= 3).

**In-vitro Release Studies from Nanoparticulate Incorporated Hydrogel Formulation:** This study showed that the nanoparticle-loaded pyrazoline was released in a sustained manner from the hydrogel, due to the presence of the sustained release polymer Carbopol and HPMC Fig. 3.

The drug release of formulation HGN1 was found to be 44.67% due to the presence of a large concentration of Carbopol 934, on the other hand, formulation HGN2 and HGN3 shown increased release of 83.42% and 89.01% respectively, due to increase in HPMC concentration in the formulation which is more hydrophilic. By drug release study, formulation HGN3 was found to be optimized.

For the investigation of drug release kinetics, release data of all formulations were fitted to various kinetic models. The best linearity of all formulation HGN1, HGN2 & HSN3 were found in the first order, indicating that the release rate of the drug from the hydrogel system is dependent on the concentration of the drug present in the system.

The mechanism of drug release from hydrogels was studied by fitting the data into the Higuchi model and KorsmeyerPeppa’s exponential model. All hydrogels formulation showed good linearity with the Higuchi model as shown in Table 7. Here drug was diffused from matrix and release depends on the swelling rate of the polymer matrix.

**TABLE 7: KINETIC PARAMETER OF IN-VITRO DRUG RELEASE STUDIES OF HYDROGEL**

<table>
<thead>
<tr>
<th>Kinetic model</th>
<th>Pure Drug Solution</th>
<th>HGN1</th>
<th>HGN2</th>
<th>HGN3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero Order</td>
<td>R²</td>
<td>0.7462</td>
<td>0.9773</td>
<td>0.9498</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>4.2155</td>
<td>1.9456</td>
<td>3.342</td>
</tr>
<tr>
<td>First Order</td>
<td>R²</td>
<td>0.9306</td>
<td>0.9928</td>
<td>0.9983</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>0.0865</td>
<td>0.0111</td>
<td>0.0291</td>
</tr>
<tr>
<td>Higuchi</td>
<td>R²</td>
<td>0.8894</td>
<td>0.9809</td>
<td>0.9915</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>26.15</td>
<td>11.079</td>
<td>19.308</td>
</tr>
<tr>
<td>KorsmeyerPeppas model</td>
<td>R²</td>
<td>0.923</td>
<td>0.9272</td>
<td>0.9882</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>0.9589</td>
<td>1.8794</td>
<td>0.8861</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>0.903</td>
<td>0.5835</td>
<td>0.7625</td>
</tr>
</tbody>
</table>

Antimicrobial activity study by cup plate method of optimized formulations was compared with the synthesized pyrazoline, and results showed that they had a similar zone of inhibitions.
CONCLUSION: The present study reports the synthesis of novel pyrazolines from chalcones. The molecular properties were predicted, which showed that these active compounds could be used as templates for the development of new drugs. Compound PZ2 which exhibited good antimicrobial activity was loaded in polymer nanoparticles made with different ratios of pyrazoline and polymer PLGA. Among all NP3 showed particle size in nano range with high entrapment efficiency. NP3 was incorporated in hydrogel made by using different ratios of carbopol and HPMC (2:1, 1:1 2:1).

PLGA nanoparticles loaded with pyrazoline incorporated into the hydrogels showed sustained release of active constituents which will be a promising carrier for transdermal delivery of pyrazoline. The slow release of the pyrazoline revealed that drug remains localized for a longer period, thus enabling drug targeting to the skin and feasibility of using a dermatological formulation to improve skin wound healing, inflammation and prevent microbial infection.

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