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Formulations and Development of Nanoemulsion based Gel Loaded and Piperine-Loaded with Phytoconstituents for the treatment of Urinary Tract Infection

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Abstract - The intravaginal delivery of a nanoemulsion gel comprising polyphenon 60 (P60) and cranberry (CRB) has been developed for the treatment of urinary tract infections. Utilizing an ultra-sonication technique, cranberry and polyphenon 60 were combined into a single nanoemulsion gel (NBG). Because of its low solubility in water, the alkaloid piperine has limited therapeutic effectiveness. Using oleic acid (oil), Cremophore EL (surfactant), and Tween 80 (co-surfactant), piperine nanoemulsions were generated in this work by the use of high-energy ultrasonication. Researchers used TEM, release, permeation, antibacterial, and cell viability tests to determine the best nanoemulsion (N2), which they predicated on the smallest possible droplet size and the highest possible encapsulation effectiveness. The droplet size of the finalized NE was 58±1 nm. In addition, radiolabeled P60+CRB NBG demonstrated a markedly increased absorption of radio labelled actives by the kidney (3.20±0.16) and urine bladder (3.64±0.29) in biodistribution tests conducted intravaginally. According to the results, piperine nanoemulsions might be a better nanodrug delivery technology than the ones now in use.

Keywords: Nanoemulsion, Gel Loaded, Piperine-Loaded and Urinary Tract Infection

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INTRODUCTION

The kidneys, bladder, and ureters are all affected by an infection known as a urinary tract infection (UTI). Infection develops when bacteria invade and thrive in the urinary tract. When bacteria and other microbes enter the urinary system via the urethra, they infect the bladder and, if ignored, may cause chronic kidney infection. Different types of urinary tract infections (UTIs) may be classified according to the location of the infection. The two most frequent forms of lower UTIs are cystitis, which affects the urinary bladder, and urethritis, which affects the urethra. The leading cause of UTIs in the upper urinary tract is pyelonephritis, which is an infection of the ureters and kidneys. Among all infections that may affect any organ, urinary tract infections (UTIs) rank second globally. There are about 150 million cases of UTIs reported annually, with many victims succumbing to infections brought on by germs that are resistant to antibiotics. The disease is more widespread there since so few people know about UTIs. Researchers Maheswary and Chitralekha (2018) found that over 30 million people in India have a urinary tract infection (UTI) each year.

Sepsis, which may develop from a urinary tract infection (UTI), is a major killer on a global scale. The anatomical variations between sexes make urinary tract infections (UTIs) more common in women. Urinary tract infections (UTIs) may strike at any moment, affecting 10% of American women. Recurring infections are often caused by the same germs since drugs do not completely eradicate the microbe. Urinary tract infections (UTIs) may be caused by either Gram-positive or Gram-negative bacteria; however, Gram-negative bacteria account for more than 95% of UTI cases. While Klebsiella pneumoniae and Pseudomonas spp. are other bacteria that may cause infections, the most common ones are Escherichia coli and Staphylococcus saprophyticus, accounting for 85% and 10% of cases, respectively. The specific adhesins produced by E. coli bacteria are responsible for infecting host cells. These adhesion proteins target the bladder's inner lining epithelial cells.

When a urinary tract infection (UTI) is in its early stages, the bacterium E. coli is already in the bladder. However, if the bacteria are not adequately treated and eliminated, it can progress to a more

complicated UTI, which in turn can cause kidney damage or even failure. Many antibiotics, including bactericidal, bacteriostatic, and urinary antiseptic varieties, are now considered to be the gold standard for treating UTIs. Many different forms of administration are available for these drugs, including oral tablets, liquid suspensions, capsules, and injectables. Urinary tract infections (UTIs) may be caused by either Gram-positive or Gram-negative bacteria; however, Gram-negative bacteria account for more than 95% of UTI cases. While Klebsiella pneumoniae and Pseudomonas spp. are other bacteria that may cause infections, the most common ones are Escherichia coli and Staphylococcus saprophyticus, accounting for 85% and 10% of cases, respectively. The specific adhesins produced by E. coli bacteria are responsible for infecting host cells.

These adhesion proteins target the bladder's inner lining epithelial cells. When a urinary tract infection (UTI) is in its early stages, the bacterium E. coli is already in the bladder. However, if the bacteria are not adequately treated and eliminated, it can progress to a more complicated UTI, which in turn can cause kidney damage or even failure. Many antibiotics, including bactericidal, bacteriostatic, and urinary antiseptic varieties, are now considered to be the gold standard for treating UTIs. Many different forms of administration are available for these drugs, including oral tablets, liquid suspensions, capsules, and injectables. Nitrofurantoin blocks the bacterial creation of DNA, RNA, and proteins from a biological perspective. It triggers cell lysis and influences cell wall formation as well. Bactericidal beta lactam antibiotics like aminopenicillins and cephalosporins work by inhibiting the enzymes that bacteria use to lyse their cell walls. Bactericidal drugs, such as cephalosporins, cause cell wall collapse by inhibiting the synthesis of the peptidoglycan layer. When treating UTIs, physicians often prescribe the antibiotic Ciprofloxacin.

LITERATURE REVIEW

Hayley J Denison (2020) Considerations for safety, including withdrawal and side effect profiles, cost, and patient preference should be considered before prescribing or suggesting an antifungal for oral or intravaginal administration. Assuming there has been no record of adverse reactions or contraindications, women who are paying for their own treatment should have all the information they need to make a well-informed decision. If health services are paying for it, decision-makers should consider which oral antifungals are more cost-effective and which ones are more convenient.

Gilbert Lazarus (2021) Included in this meta-analysis were eight trials totaling 666 participants; five of these trials examined CAB-LA, while four examined RPV-LA. In terms of adverse events (AE), significant AE, and withdrawals due to AE, CAB-LA and RPV-LA were determined to have safety profiles similar to placebo. In pharmacokinetic investigations, CAB-LA and RPV-LA shown potential in limiting viral replication. Among the several dose regimens tested, male subjects showed the best response to intramuscular (IM) injections of 600 mg CAB-LA Q8W compared to 800 mg Q12W, while RPV-LA 1200 mg IM Q8W outperformed all others. Although preliminary results from RPV-LA studies seem promising, further research is required to confirm these conclusions. Positive safety and pharmacokinetic data have been seen with the two novel drugs, CAB-LA and RPV-LA. Various cancer-preventive medications are now in the Phase 3 testing phase.

Raphael J. Landovitz (2018) Cabotegravir (CAB) is an antiviral medication that is currently being developed for the treatment and prevention of HIV. There are two methods for administering CAB. One is an intramuscular (IM) injection of a long-acting injectable solution that keeps the medicine in the bloodstream for a longer period of time. For the HIV Prevention Trials Network 077 (HPTN 077), researchers looked studied the pharmacokinetics, safety, and tolerability of CAB LA in healthy men and women from eight nations in South America, Africa, the Middle East, and the US. At the dosages and intervals tested, CAB LA had no negative side effects. Product withdrawals were rare even though ISRs were widespread. All genders met the study's pharmacokinetic criteria when given 600 mg of CAB LA eight times weekly. On the basis of the results of the pharmacokinetic and safety investigations, trials are under ongoing to determine if CAB LA is useful in treating or preventing the spread of HIV.

Jennifer A. Robinson (2018) Vaginal film formulations have been developed to improve adherence and, perhaps, allow episodic administration, as a result of the poor acceptability and non-adherence of daily vaginal gels, despite the fact that oral preexposure prophylaxis with tenofovir (TFV) disoproxil fumarate/emtricitabine reduces HIV acquisition rates. In this two-arm, cross-over research, ten healthy women were given either 40 mg of fast-dissolving tenofovir film or 40 mg of semisolid tenofovir 1% gel. The pharmacokinetics and antiviral activity of the medicine were studied for seven days after administration. On the first day of therapy, film demonstrated higher concentrations in vaginal and plasma samples compared to gel in cervicovaginal and plasma samples. Previous reports of 5-hour steady-state TFV-DP concentrations in cervical tissue after oral administration of a single dose of Truvada® were lower than these. The tenofovir film may be a better option than the oral and gel versions of the drug. Clinical studies on people have not yet shown this treatment's efficacy.

Andrea R. Thurman (2021) Oral dosage forms of antibiotics, including tablets, capsules, and suspensions, are effective in treating UTIs. The hepatic portal vein carries ingested medications to the liver, where they undergo metabolism. As a result, there are several limitations associated with this type of drug administration. To make them less available in the circulation and at the site of action,

metabolising enzymes in the liver convert active medicines into inactive ones. Current research focuses on identifying potential new mechanisms to circumvent the liver's first pass metabolism and provide direct targeted medication delivery. The intravaginal route of administration is one of the least studied delivery methods, but it is also one of the closest to the urinary tract organs. With its precise precision, it can deliver steroidal contraceptives and medications to the vaginal cavity and urinary system, making it useful for treating UTIs and other similar conditions. A 7.5-centimeter-long muscular tube, the vaginal cavity sits between the rectum and the bladder. Layers one, two, and three of the vaginal wall are the epithelium, the muscular coat, and the tunica adventia. The vagina is made more pliable by the muscular coat's abundance of smooth elastic fibres.

RESEARCH METHODOLOGY

Sigma Aldrich supplied the polyphenon 60 or GTCs and the reagent grade Tween 20. Naturex of DBS in New Jersey, USA, graciously donated cranberry powder. A product of CDH (P) Ltd, India, was oleic acid and glycerol. Milli-Q water from Millipore, USA, was used. Vinnocell (Cheshire, England) was the source of the oleic acid.BASF of Ludwigshafen, Germany, supplied the chromophore EL.Gattefosse supplied transcutol and ligament.ABITEC supplied CAPMUL.BDH was sourced for the PEG400 and eucalyptus oil.Sigma Aldrich was consulted for the procurement of ethyloleate, tween80, poloxamer188, and poloxamer 127.

Screening of Components.

The nanoemulsion was prepared by choosing each component—oil, surfactant, and co-surfactant—according to their greatest solubility. We conducted the solubility test using a variety of oils (oleic acid, eucalyptus oil, ethyl oleate, capmul, and labrafil), surfactants (poloxamer 188, poloxamer 127, and Cremophore EL), and co-surfactants (Tween 80, transcutol, PEG 400). There was an excess of piperine in each vial along with oil, surfactant, and a co-surfactant. Before being shaken in a water bath for 72 hours, the samples were vortexed to ensure homogeneity. After centrifuging the samples at 5,000 rpm for 1 hour, the supernatant was collected. An ultraviolet (UV) spectrophotometer was used to detect the quantity of piperine in each component after diluting the supernatant with methanol.

Formulation of Nanoemulsion

We tested many oils, surfactants, and co-surfactants to see how polyphenon 60 and cranberries would dissolve. Cranberries were dissolved in oleic acid to create the oil phase of the nanoemulsion, which was then combined with tween 20 to create the surfactant and glycerol to form the co-surfactant. Aqueous phase was prepared by dissolving P60 in milli-Q water. Then, pre-emulsion was formed by adding drops of the aqueous phase to the oil phase while stirring continuously. The pre-emulsion was first homogenised by spinning it at 10,000 rpm for 20 minutes in an ice bath using a Tissue Master 125 homogenizer from Omni International, Georgia. Then, it was subjected to high energy ultra-sonication using a Bench Top Ultrasonicator from Hielcher, Ultrasound Technology, Germany, with an amplitude of 70% for 286 seconds, cycling between 0.3 seconds of ON and 0.7 seconds of OFF. Using a Malvern Zetasizer (Malvern, Worcestershire, UK), the produced nanoemulsion was examined for particle size and zeta potential. The nanoemulsion was first diluted with HPLC water at a ratio of 1:50 v/v before measuring particle size and zeta potential.

The oil, surfactant, and cosurfactant for nanoemulsions were selected based on the greatest solubility of piperine, oleic acid, and Cremophore EL, respectively. The Smix phase, which is a combination of surfactant and co-surfactant, was chosen based on the emulsification efficiency. The nanoemulsion was made with a little tweak to the high-energy ultrasonication method.31 After combining the chosen oil and Smix, piperine was added to create the coarse emulsion. While the oil and water phases were still mixed, the aqueous phase was added while the mixture was vortexed continuously for one minute.32 The finished coarse emulsion phase was then subjected to further ultrasonication in a water bath for 30 seconds each cycle with a sonication amplitude of 40%.33 In order to determine the best composition, the produced nanoemulsions were placed in a glass vial and tested. One last step was to characterise the stable nanoemulsions using a variety of characteristics.

DATA ANALYSIS

Phantom 60+ CrB Nanoemulsion Preparation A combination of 41 mg/ml of medication (P60=11 mg/ml; CRB=30 mg/ml), 5% weight of oil, and 16.4% weight of emulsifier was used to create optimised NE (Table 1). We found that a 30% amplitude and 300 s of sonication duration worked well. A PDI of 0.2 and a zeta potential of -16 mV were revealed by the particle size analysis, which revealed particles with a size of 58 nm.

Table 1. Conditions and quantities of drugs and excipients selected for formulation of Nanoemulsion and characterization

Nanoemulsion		
Drug candidate	**P60+CRB**	**Composition**
Oil	Oleic Acid	10%
Surfactant	Tween 20	20%
Co-surfactant	Glycerol	3.52%
Aqueous Phase	Mili Q water	66.48%
Label claim	41 mg/ml	P60 = 11 mg/ml CRB = 30 mg/ml

Formulation Parameters	
Homogenization Speed	10,000 rpm
Homogenization Time	30 min
Time of Ultrasonication	300 sec
% Amplitude	30%
Charaterization Parameters	
Droplet size	58±1 nm
PDI	0.2±0.015
Zeta potential	-16±0.2 mV

Where, P60: Polyphenon 60; CRB: Cranberry; PDI: Particle distribution index

Development and characterization of Nanoemulsion based gel

Nanoemulsion based gels (NBG) were prepared using chitosan in three distinct doses. The primary criteria for selecting chitosan gel (1% concentration) soaked in lactic acid (1% concentration) were its transparency and a pH value that is near to the physiological range (3.5-4.5 for vaginal pH). All of the finished chitosan gels were included in Table 2 along with their pH values and final compositions.

Table 2. Composition of different gels with their pH values and homogeneity

Ingredients (for 10g of gel)	Formulation codes		
	CH 1%	CH 1.5%	CH 2%
Chitosan (g)	0.10	0.15	0.20
Lactic acid (ml)	1.15	0.15	0.20
Nanoemulsion (ml)	8.85	8.85	8.85
pH	3.2±0.2	3.7±0.2	4.9±0.1
Homogeneity	✓	✓	✓

Where, CH is the different concentration of Chitosan containing formulations

Rheological studies of selected nanoemulsion based gel

(Figure 1) shows the apparent viscosity characteristics of all the gels that were chosen. As is typical of polymeric systems, none of the three gels exhibited Newtonian, but rather pseudo-plastic, behaviour. At 0.01 s-1, the results showed viscosity values ranging from around 141 Pa.s to 1060 Pa.s, and at 100 s-1, they decreased to about 0.5-2 Pa.s. Figure 2 shows that it varies throughout the three gels from 0.1 to 10 Hz, the frequency range that was studied. The fact that CH 1.5% gel has a higher elastic component and lower tan δ values suggests that it may be better able to remain in place once applied.

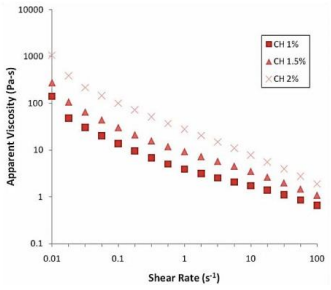

Figure 1. Viscosity profiles as a function of shear rate for tested gels prepared with different concentrations of chitosan CH 1%, CH 1.5%, CH 2%.

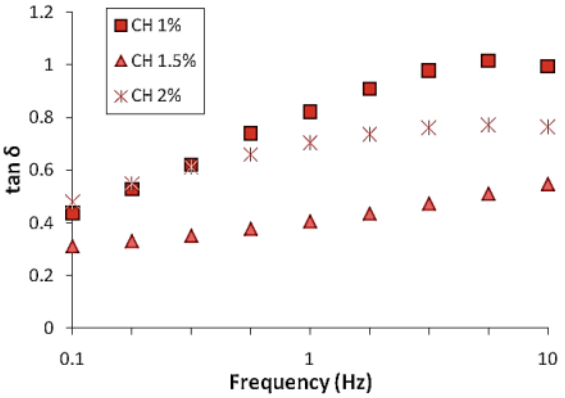

Figure 2. tan δ as a function of frequency for tested gels prepared with different concentrations of chitosan CH 1%, CH 1.5%, CH 2%.

Screening of Components.

An essential factor in influencing a drug's solubilizing ability is the selection of the oil, surfactant, and co-surfactant. The drug loading is increased when the solubilizing capability is raised. Figure 3 shows the solubility data for several oils, surfactants, and co-surfactants. The oils (Oleic acid, Eucalyptus oil, Ethyl oleate, Capmul, and Labrafil) and surfactants (Poloxamer 188, Poloxamer 127, and Tween 80) are also shown. It was discovered that oleic acid was the most soluble of the oils. Figure 3. Solubility data of piperine in different nanoemulsions components. Data shown as mean ± SD (n = 3).

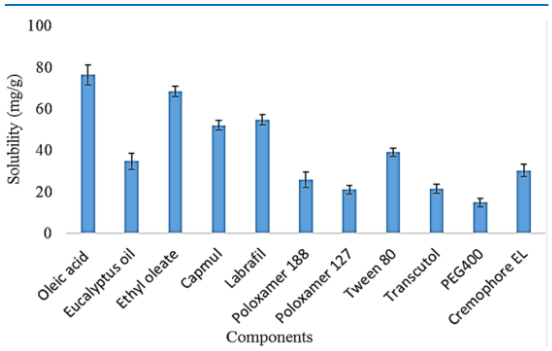

Figure 3. Solubility data of piperine in different nanoemulsions components. Data shown as mean ± SD (n = 3).

In this order: ethyl oleate, labrafil, capmul, and eucalyptus oil. Utilising oleic acid (77.11 ± 4.7 mg/g), the highest level of solubility was demonstrated. Based on the solubility finding, we also chose the surfactant and co-surfactant. Tween 80 and Cremophore EL were the solvents with the highest solubilities, measuring 38.54 ± 4.5 mg/g and 29.8 ± 2.9 mg/g, respectively. Given piperine's excellent solubility, the following formulation components were chosen: oleic acid, tween 80, and cremophore EL.

Formulation of Nanoemulsion.

By adjusting the components, the piperine nanoemulsions were created using the ultrasonication procedure (Table 3). From the various nanoemulsions, six formulations (N1−N6) have been chosen for further characterization. The Smix ratio substantially affected the mean droplet size of the produced nanoemulsions ($p < 0.05$). Nanoemulsion droplet size is proportional to Smix ratio. Nanoemulsions with a larger droplet size (121 ± 3.8 nm) are produced by Smix at a 1:1 ratio compared to Smix (2:1; 105 ± 4.11 nm). With each successive increase in the Smix ratio, the droplet size rose (1:2, 1:3, and 3:2).

Table 3. Formulation Composition of Piperine Nanoemulsion

code	oil (%)	Smix	water (%)	piperine (mg)
N1	20	1:1	65	25
N2	20	2:1	58	25
N3	20	3:1	48	25
N4	20	1:2	53	25
N5	20	1:3	57	25
N6	20	3:2	61	25

Droplet Characterization.

According to Table 4, all of the created nanoemulsions had an average diameter in the nanorange. A nanoemulsion with a size ranging from 105 ± 4.1 nm (N2) to 250 ± 7.4 nm (F7) was discovered. In the produced nanoemulsions, there was a notable change in the average diameter ($p < 0.05$). Changes in nanoemulsion composition account for the observed size disparity. Also, the nanoemulsions (N2) had the smallest droplets because they had the ideal Smix ratio and a lower concentration of co-surfactant compared to surfactant (Figure 4). By acting as a barrier between the smaller droplets, Smix contributed to their reduction in size. There was also an evaluation of the formulations for PDI, which yielded a low value (<0.7). A lower PDI score indicates that the formulations are more homogeneous.34 Table 4 displays the ζ potential for the piperine nanoemulsion. The formulations exhibited negative ζ potentials ranging from -19 to -39 as a result of the cosurfactant's anionic group. The ζ potential value of the chosen piperine nanoemulsion (N2) was −32 mV. Stability is enhanced by a η potential with a higher negative or positive value (±30 mV).\

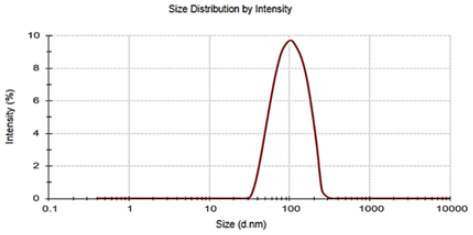

Figure 4. Droplet size of the optimized piperine nanoemulsion (N2).

Table 4. Evaluation Parameters of Piperine Nanoemulsion

code	mean diameter (nm)	polydispersibility index	ζ potential (mV)	pH	transmittance (%)	encapsulation efficiency (%)
N1	121 ± 3.8	0.34	−24	7.1	99.1	79.94 ± 3.3
N2	105 ± 4.1	0.21	−32	7.0	98.7	89.32 ± 1.9
N3	153 ± 3.8	0.19	−27	6.9	98.3	76.85 ± 2.2
N4	178 ± 5.7	0.27	−19	7.0	99.4	86.03 ± 1.4
N5	250 ±7.4	0.36	−22	7.6	99.1	87.88 ± 4.1
N6	236 ± 6.3	0.24	−28	7.5	98.6	79.1 ± 1.2

CONCLUSIONS

The current study set out to do just that—create NBG encapsulated with P60 and CRB—in order to heighten its antibacterial activity. With an MDS of 58 nm, PDI of 0.2, and zeta potential of -16 mV, an optimised oil-in-water NE of P60+CRB was created. A chitosan-based gel (1.5%) formulation was created and studied for P60+CRB NBG with the aim of increasing the formulation's residence duration at the site of action. Solubility and physicochemical properties were enhanced in the preparation of the piperine-loaded nanoemulsion by means of ultrasonication. The nanoemulsions that were created showed characteristics such as nanodroplet size, superior stability, optimal ζ potential, and transmittance (%).

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