INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Formulation and Evaluation of Mucoadhesive Anti-Infective Solution Containing Solubilised Tea Tree Oil for Vaginal Infections

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ABSTRACT

Vagina shows dynamic changes in its microbial population due to menstruation, douching, coitus, contraception and menopause. Vaginal health is majorly maintained by its acidic pH which is dictated by Lactic acid produced by Lactobacillus strains. In the present research work, we made an attempt to formulate a mucoadhesive anti-infective vaginal solution containing tea tree oil (*Melaleuca alternifolia*), dragosantol oil (*Roman Chamomile*) and menthol in solubilised form. Poloxamer 407 was used for oil stabilization and imparting the mucoadhesive property. Several formulations were formulated using Cold gel method. Lactic acid was used to impart the desirable pH. The formulations were evaluated for pH, specific gravity, viscosity, antimicrobial efficacy, inhibitory potential against Lactobacillus acidophilus, mucoadhesive property and active oil determination by gas chromatography. The formulation was found to show a desirable pH (2.95), specific gravity (1.06) and viscosity (1.8). The formulation was found to be effective against *Staphylococcus aureus*, *Escherichia coli, Aspergillus niger* and *Candida albicans*. The formulation did not show any inhibitory potential against the inherent vaginal microbe *Lactobacillus acidophilus*. Gas chromatography of mixture of standard tea tree oil and dragosantol oil showed a retention time of 22.2 minutes and 29 minutes whereas that of formulation showed a retention time of 22.4 minutes and 29.2 minutes respectively. The formulation was found to show desirable mucoadhesive property and can be used as an anti-infective mucoadhesive treatment in infectious conditions like vaginitis, candidiasis and bacterial vaginosis without adversely affecting the inherent vaginal microenvironment.

Keywords: Vagina, cold gel, mucoadhesive, tea tree oil, dragosantol oil.

INTRODUCTION

The age and estrogen level of a female determines the physiological pH of vagina. Lactic acid produced by the vaginal and cervical epithelial cells is responsible for maintaining an acidic pH of 3.8 to 4.5 in human vagina.[1] This vaginal acidity usually acts as a inhibitory factor for vaginal infections.[2] Vagina undergoes dynamic changes during perimenstruation, menses, douching, sexual activity, menopause and post-menopausal period.[3] Hence the health of vaginal and cervical epithelial cells and the lactic acid secretion by the same also show high fluctuations throughout the lifespan of human females. Furthermore vaginal infections like atrophic vaginitis, vaginosis, lactobacillosis etc severely affect the vaginal health by adversely affecting the vaginal microflora and consequently the vaginal pH.[4] First-line treatment against vaginal infections comprise of creams, ointments, etc containing drugs like Clotrimazole, Clindamycin and Metronidazole. The treatment is successful in eradicating the infectional symptoms but shows side-effects like vaginal discharge, symptomatic candidiasis and vulvo-vaginal irritation.[5]

Tea tree oil (*Melaleuca alternifolia*); an essential herbal oil, contains terpinen-4-ol as its main constituent.[6] Epidermal permeation studies have revealed that terpinen-4-ol easily permeates human epidermis depending on the applied formulation whereby solutions show highest permeation as compared to other topical preparations like emulsions, ointments or creams.[7] Hence we made an attempt to formulate an anti-infective mucoadhesive solution containing tea tree oil in solubilised state. The solution will not only aid in prevention and treatment of vaginal infections but also help to normalise the fluctuations in vaginal pH brought about by imbalance in the microflora.

The main focus of the current research work was to disperse tea tree oil in a cosolvent mixture of water and propylene glycol using Poloxamer 407 as a stabilizer and mucoadhesive agent. Dragosantol oil (*Roman Chamomile*) was added to have a synergistic anti-infective effect.Menthol was added

to impart a coolant action. Lactic acid was used to impart a pH stabilising action to the solution.

MATERIALS AND METHODS

Tea tree oil, Dragosantol oil and Chlorhexidine diacetate were obtained as gift samples from Bajaj Healthcare Ltd, Masjid Bunder; Mumbai, India. Lutrol F127 (Poloxamer 407) was obtained as a gift sample from BASF India (Turbhe) Mumbai, India. Menthol and sodium methyl paraben were purchased from Westcoast Laboratories, Mumbai, India. Propylene glycol and lactic acid were purchased from S. D. Fine Chemicals Ltd; Worli, Mumbai, India. Double -distilled water used was obtained from in-house distillation assembly.

Formulation and Optimisation studies

Nine formulations were prepared and optimised till a clear solution of desirable consistency was obtained. [Table 1] Lutrol F127 (Poloxamer 407) was solubilised in distilled water by cold gel method till a clear solution was obtained.[4] Tea tree oil, dragosantol oil, menthol, chlorhexidine diacetate and lactic acid were solubilised in propylene glycol to obtain an active oil solubilised propylene glycol system. Sodium methyl paraben was solubilised in the poloxamer solution to obtain the final aqueous phase. The active oil containing propylene glycol system was added to the aqueous phase followed by mixing to give a clear oil-dispersed aqueous system.

Evaluation studies

i) pH

An adequate volume of the formulation was taken in a 50 ml beaker and the pH was recorded at 25° C using a standardised Siena Digital pH meter model no-UPH1. The readings were taken in triplicate.

ii) Specific gravity

Specific gravity of the formulation was measured by using specific gravity bottle of 10 ml capacity using purified distilled water as a standard. The later was calculated using the following formula:

Specific gravity of the formulation = Weight of 10 ml of formulation/ Weight of 10 ml of distilled water.

The readings were taken in triplicate.

iii) Viscosity

Viscosity of the formulation was measured using Ostwald's Capillary viscometer. The viscosity was measured at constant temperature of 25°C using distilled water as the standard. The readings were taken in triplicate.[8] The later was measured using the following formula:

$\eta_1 / \eta_2 = \rho_1 t_1 / \rho_2 t_2$

where:

η_1 = Viscosity of the formulation

η_2 = Viscosity of distilled water at 25°C

ρ_1 = Specific gravity of the formulation

ρ_2 = Specific gravity of distilled water at 25°C

t_1 = Time taken by formulation in seconds

t_2 = Time taken by distilled water in seconds

iv) Mucoadhesive strength determination

The mucoadhesive strength of the formulation was determined using a Brookfield CT3 Texture analyser. The probe used was TA/10 acrylic cone. Tension test was used with the trigger load of 0.0667 N and a test speed of 0.2 mm/s. A pre-test and post-test speed of 2 mm/sec was used as test specifications with a sample rate of 10 points per second. A 3 % sodium alginate dispersion in distilled water was used as a mucoadhesive reference against which the mucoadhesive efficacy of the formulation was determined.[4]

v) Antimicrobial efficacv studies

The antimicrobial analysis was conducted to determine the efficacy of the formulation against Gram-negative bacteria like *Escherichia coli*, Gram-positive bacteria like *Staphylococcus aureus* and fungi like *Candida albicans* and *Aspergillus niger*. The inhibitory potential of the formulation against Lactobacillus acidophilus (a Gram-positive bacteria responsible for buffering the vaginal pH in the physiological range) was determined. For each antimicrobial test, 1 loopful of the respective culture and 1 ml of the formulation sample was added to 10 ml of sterile peptone water aseptically and the growth of the particular micro-organism was observed visually.[9]

vi) Active oil content determination

The tea tree oil and dragosantol oil content of the optimised formulation were determined using Gas chromatography.The determination was done for the active terpinen-4-ol in tea tree oil and alpha-bisabolol in dragosantol oil. The instrument used was Chemito 8610 with flame ionisation detector. A VB-wax capillary column with length of 60 metres and a diameter of 0.53 mm was employed.Nitrogen was used as the carrier gas. The injector temperature was maintained at 240 degree celcius and the detector temperature was maintained at 250 degree celcius. 1 µl of the preparations (standard and sample) were injected and the oven temperature was maintained at 50 °C for 1 minute. The oven temperature was then raised at the rate of 5°C per minute to a temperature of 230°C. The oven was maintained at 230 °C for 8 minutes. The run time for a single run was standardised at 60 minutes.

Standard oil preparation for injection: 0.01 gm of tea tree and dragosantol oil was mixed and the oil mixture was diluted to 10 ml with hexane. This standard dilution was injected and a standard peak of tea tree oil and dragosantol oil was obtained respectively.

Sample formulation preparation for injection: 10 ml hexane was used to extract the active oils from 1 ml of the optimised formulation. This active extract was injected to obtain the active oil peaks in the formulation.[10, 11]

RESULTS AND DISCUSSION

i) Formulation and Optimisation studies

Formula IX was found to give the most optimised formulation. The selection of the optimised formulation was based on the system clarity. The system clarity and stability can be accounted to the efficient solubilisation of the two essential herbal oils by Poloxamer 407(Cold gel solubilisation) along with the synergistic solubilising action of Propylene glycol by cosolvency.

ii) pH

The pH of the formulation was found to be within the range of 2.9-3. The obtained pH value indicates that the formulation could be used for a therapeutic bacteriostatic action in the vagina. The pH of the formulation also ensures that it can be effective in restoring the alkaline vaginal pH (observed due to infection, coitus, menstruation etc) to its normal acidic value thus aiding in maintaining health of the vaginal epithelial cells. The desirable pH value can be attributed to the presence of Lactic acid in the formulation.

iii) Specific gravity

The specific gravity of the formulation was found to be between 1.04-1.08. The specific gravity of the formulation can be accounted to be affected by varied factors like concentration of Poloxamer 407, propylene glycol and the extent to which the oils have been solubilised in the formulation.

iv) Viscosity

The viscosity of the formulation was found to be within the range of 1.6 cps to 2 cps. The viscosity of topical vaginal formulation is an important evaluation parameter as it dictates the spreadability and retention power of the same. The obtained viscosity value for the formulation indicates its higher spreading and topical residence time on the vaginal skin.

v) Mucoadhesive strength determination

The mucoadhesive nature of the formulation can be observed from Fig 1 (a-b). The formulation showed a gradual rise in the mucoadhesion (as indicated by distance travelled by the probe in the solution) after which it showed a plateau region. This indicates that mucoadhesion of the formulation reaches a constant value once a topical film of the formulation is formed.

vi) Antimicrobial efficacy studies

The formulation was found to be efficacious in inhibiting gram-positive bacteria like *Staphylococcus aureus*, gram-negative bacteria like *Escherichia coli* and fungi like *Candida albicans* and *Aspergillus niger*. The formulation was found to show no inhibitory potential against *Lactobacillus acidophilus* (inherently present in the vaginal microenvironment). Thus the antimicrobial studies indicate that the formulation could be used as an anti-infective in infections like vaginitis, candidiasis, bacterial vaginosis, vulvovaginal candidiasis and bacterial vaginosis without adversely affecting the inherent vaginal microenvironment.

vii) Active oil content determination

Gas chromatograms of the oil mixture of tea tree oil(terpinen-4-ol) and dragosantol oil (alpha-bisabolol) considered as standards showed the retention times of 22.2 minutes and 29 minutes respectively whereas those of optimised formulation showed peaks of tea tree oil(terpinen-4-ol) and dragosantol oil(alpha-bisabolol) at the retention times of 22.4 minutes and 29.2 minutes.[Fig: 2(a-b)] Thus the chromatograms of the optimised formulation confirmed the presence of the active oils.

Table 1: Formulas I to IX used for Optimisation Studies

Ingredient/Formula	I	II	III	IV	V	VI	VII	VIII	IX
Tea tee oil	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Dragosantol oil	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Menthol	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Chlorhexidine diacetate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propylene glycol	30	20	20	20	20	20	20	20	10
Lutrol F127	12	12	10	8	6	5	4	3	2
Sodium methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Lactic acid	1	1	1	1	1	1	1	1	1
Distilled Water(q.s.)	100	100	100	100	100	100	100	100	100

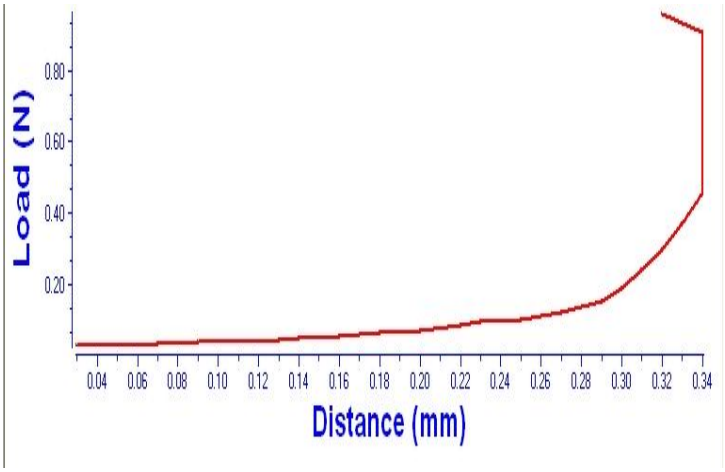

Fig. 1a: Mucoadhesive strength of 3% Sodium alginate

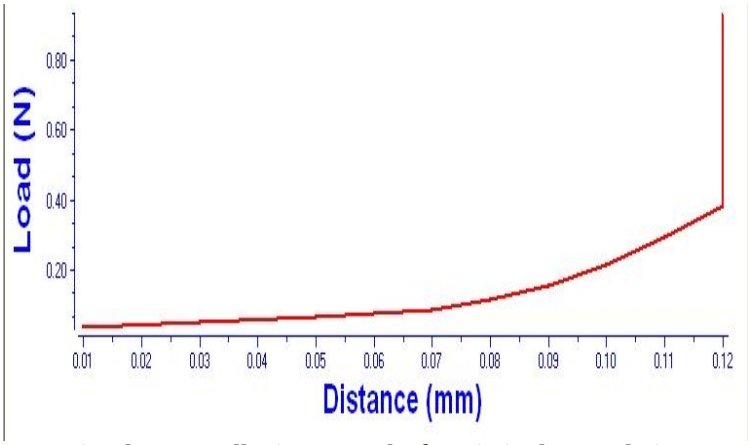

Fig. 1b: Mucoadhesive strength of Optimised Formulation

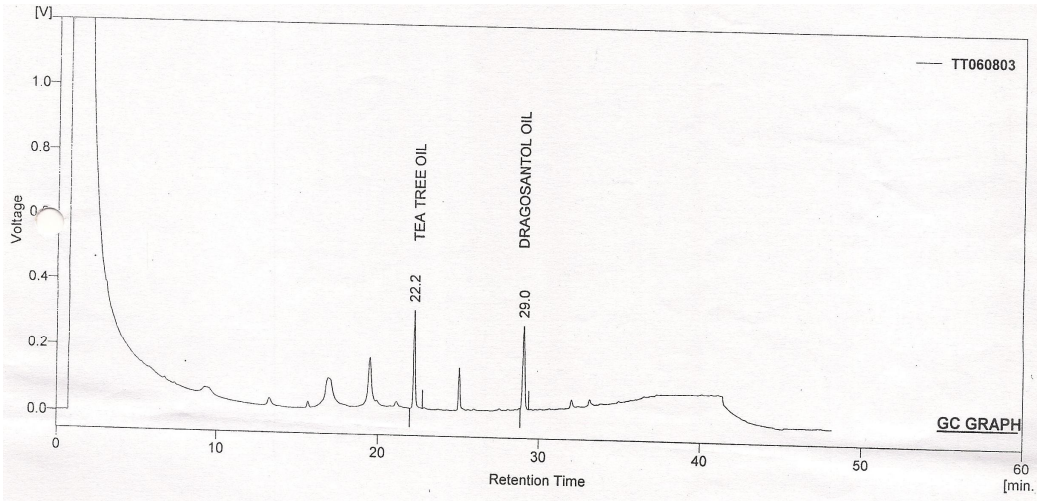

Fig. 2a: Gas Chromatogram of Oil mixture of standard tea tree oil and dragosantol oil

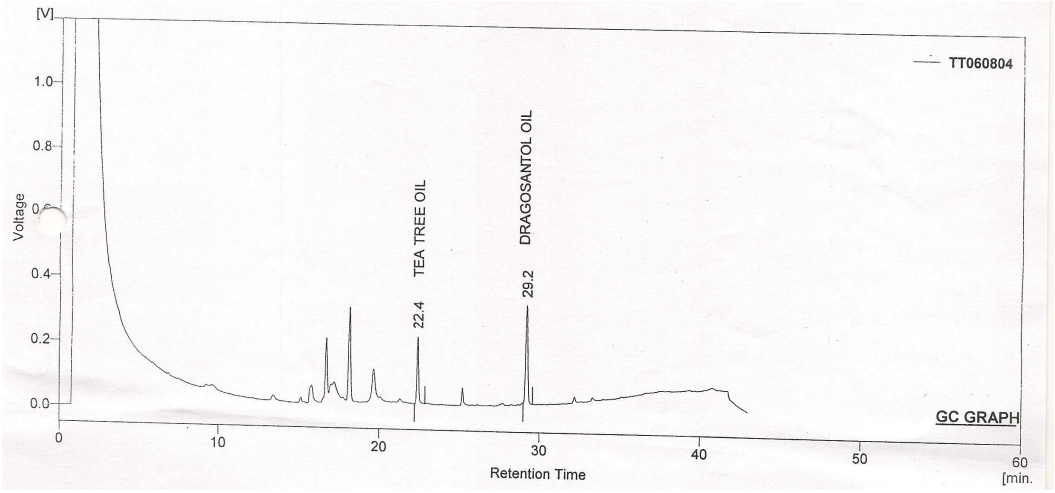

Fig. 2b: Gas Chromatogram of Optimised Formulation

CONCLUSION

The present research work indicated that a stable aqueous mucoadhesive system containing anti-infective essential oils like tea tree oil and dragosantol oil in a solubilised form and a mucoadhesive like Poloxamer 407 can be formulated using a synergistic approach of solubilisation by Cold gel method and cosolvency. The resultant anti-infective solution can be used as an efficient bacteriostatic as well as aid to balance the fluctuated vaginal pH in conditions like vaginosis, vaginitis, candidiasis etc without adversely affecting the inherent microenvironment of the vagina.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Pharmaceutical Sciences and Technology at the Institute of Chemical Technology, Matunga, and Mumbai for allowing them to use the departmental facilities for conducting the research work. Additionally, the authors are grateful to Bajaj Healthcare Ltd, Masjid Bunder, Mumbai and BASF India, Turbhe, Mumbai for providing with gift samples of tea tree oil, dragosantol oil and Poloxamer 407 respectively.

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