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Formulation and evaluation of mucoadhesive Nigella Sativa and Olive oils for vaginal infections

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ABSTRACT

In this study, we prepare microspheres containing Nigella Sativa and Olive oil by spray drying method and these microspheres were added to carbopol gel. Spray dried microspheres added to Carbopol gel were evaluated for surface morphology and showed to be shriveled, In-vitro release studies showed 90.15 % and 98.34 % releases for NSSOM and NSSOMgel, release kinetics showed that the drug release followed First Order ($R^2 = 0.9826$) and Korsmeyer's Peppas ($R^2 = 0.9950$) respectably. During storage at 3-5⁰C or room temperature (15-25⁰C) for 12 months, surface morphology and content of Nigella Sativa and Olive oil had no notable changes. The conventional commercial preparations, such as creams, foams, gels, irrigations and tablets have limitations such as leakage, messiness and low residence time; often require multiple daily doses to ensure the desired therapeutic effect and which contribute to poor subject or patient compliance. Attempts have been made to develop microspheres mucoadhesive vaginal gel that can meet the clinical as well as the user's requirements.

Key words: Nigella Sativa, Olive oil, Microspheres, Vagina, Carbopol.

INTRODUCTION

The vagina, as a site for drug delivery, offers certain unique features that can be exploited in order to achieve desirable therapeutic effects.[1]. However, this route has not been broadly exploited because of the broad inter-individual variability, disturbing some physiological factors like the pH and the presence of limited vaginal secretions that further vary depending on age and menstrual cycles.[2]

The conventional commercial preparations, such as creams, foams, gels, irrigations and tablets have limitations such as leakage, messiness and low residence time, often require multiple daily doses to ensure the desired therapeutic effect and which contribute to poor subject or patient compliance[3]. By contrast, the scientific facts concerning the possibilities of drug delivery via the vagina is incomplete to date, there are only a limited number of vaginal formulations available, although various possibilities are at present being investigated.

Even after introduction of new antimicrobial agents for clinical use an alarming increase in bacterial resistance to existing agent's demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antimicrobials.

Nigella sativa is a herbaceous plant found in the Middle East, Europe and Western and Middle Asia. Its seeds have a great medicinal importance and have been reported to exhibit many pharmacological effects that include anti-parasitic, antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory activities. The seeds have also been used to treat bacterial, fungal and parasitic infections. Also since ancient times, Olive Oil is being used for the treatment of various kinds of ailments. Research has proved that Olive is antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant, effective in treating cardiovascular disorders.

Microspheres of both the oils in a polymeric matrix are an alternative that has been used by several researchers in order to protect unsaturated fatty acids against lipid oxidation, thus increasing their shelf life.

Attempts are being made to develop microspheres mucoadhesive vaginal gel that can meet the clinical as well as the user's requirements.

MATERIALS AND METHODS

Nigella Sativa and Olive oil were purchased from local market, Al-Ahsa, Saudi Arabia, Carbopol 934 NF (Goodrich, Cleveland, OH), Gum arabic and glycerol were procured from M/S Loba Chemie Pvt. Ltd., Mumbai, and all chemicals used in experiments were of analytical grade.

Preparation of emulsions

The wall material (Gum Arabic) was dissolved in distilled water under magnetic agitation, one day before emulsification. Coarse emulsions were prepared by blending two oils (*Nigella Sativa* and Olive oil) in the wall solution, using a rotor blender at 10000 rpm for 5 min.

Preparation of microspheres by spray drying method

Spray drying process was performed in a laboratory scale spray dryer Spray dryer B-191, Büchi, Flawil, Switzerland with a 2 mm diameter nozzle and main spray chamber of 500 mm X 215 mm. The emulsion was fed into the main chamber through a peristaltic pump, feed flow rate was 10 g/min, drying air flow rate was 70 m³/h and compressor air pressure was 0.06 MPa.[4, 5]

Preparation of Carbapol gel

As a vehicle for the incorporation of microspheres intended for vaginal purpose, Carbopol 974P NF as a more pronounced adhesive property. The gels (1% w/w) were prepared by the following procedure [6] Carbopol resin (1 g) was dispersed in distilled water (88 g) in which glycerol (10 g) was previously added. The mixture was stirred until thickening occurred and then neutralised by drop wise addition of 50% (w/w) triethanolamine, until a transparent gel appeared. Amount of triethanolamine was adjusted to get gel pH 4.2.

Incorporation of NSOO microspheres into 1% carbopol gel

Microspheres were incorporated into Carbopol gels following the procedure described by [6, 7]. Briefly, microspheres were mixed into vehicles by an electrical mixer (50 rpm, 10 min), with the concentration of microspheres in the gel being 10% (w/w, microspheres suspension/total). Prior to in vitro studies, samples were examined microscopically by Scanning Electron Microscopy to check the morphology of microspheres [4, 7]. Control gels (Carbopol gels mixed with 200 μ m calcein solutions in buffer instead of microspheres) were prepared under the same conditions. Concentration of calcein solution in the gel was 10% (w/w) [6, 7]

Surface morphology

The surface morphology of the microspheres was observed by scanning electron microscope (Joel, Japan). The microspheres were placed on graphite surface and coated with gold using an ion sputter (Joel) and were observed at 12 kV[8]

Invitro release studies

In vitro release patterns were studied using conventional dialysis technique. Nigella Sativa and Olive oil microspheres gel (NSOOMgel) were placed in a dialysis bag and dialyzed against 200 mL of phosphate saline buffer (PBS), pH 4.2 at $37 \pm 1^\circ\text{C}$. Sink conditions were maintained and throughout the course of study and were stirred continuously using a magnetic stirrer. Aliquots were withdrawn at specific time intervals and the same volume of dissolution medium was added to the flask to maintain a constant volume. [9] The withdrawn aliquots were estimated through fluorimetrically, and the data were used to calculate a cumulative drug release profile from the microspheres. The accumulated amount of drug released was calculated using a calibration curve.

Storage stability study

The NSOO microspheres gel were put into a bottle and stored for 12 months at $3-5^\circ\text{C}$, $15-25^\circ\text{C}$, and 37°C , respectively. The surface morphology of NSOO content were examined periodically [10, 11].

Rheological evaluation of gels containing microspheres

Flow properties of Carbopol gels with incorporated microspheres were determined on a LVDV-IIIUCP Ultra, Cone Spindle (CPE-40) (Brookfield, Germany). Measurements were performed at 20°C by using the cone/plate (0.05 mm) measuring system. Under the same conditions the flow properties of microspheres-free control gels were examined.[6]

RESULTS AND DISCUSSION

The conventional vaginal formulations, such as suppositories, gels, creams and foams cannot meet desirable distribution, bioadhesion, retention and release characteristics these can be improved by novel vaginal drug delivery systems. Several novel carrier systems were suggested to be appropriate for vaginal drug delivery, such as polycarbophilic gel [12], bioadhesive tablets [1, 13, 14], microspheres[15, 16] liposomes [6, 17, 18] and [19, 20]. To achieve the desirable therapeutic effect of Microspheres as drug carriers, they must be loaded with a sufficient amount of active compound. Therefore, microspheres with Nigella Sativa / Olive oil were prepared by spray drying methods and compared for surface morphology size and *invitro release studies* and Storage stability study.

The spray-drying method described here appeared to be a suitable and easy method to prepare NSOOM. Surface morphology reveals that the spray dried microsphere containing Nigella Sativa

and Olive oil were shriveled see figure 1, this may be due to the inlet temperature was above the solvent boiling point and also above the glass transition temperature of the polymer making it less rigid during drying which has also been reported by [21-23]

The in vitro release study helps us to understand the behavior of these systems in terms of drug release. The microspheres containing NSSO shows biphasic profile with initial burst effect followed by a prolonged period and NSSOMgel showed a triphasic release profile with initial slow release (latent) phase, burst phase, and faster release phase (Table -1), which was also proved by[24].

Fig. 2 shows in vitro release for NSOO, NSOOM and NSOOMgel respectively. NSSOM in carbopol gel release in 5.2% first 0.5h and this slow release may be due to the drug inside the microspheres and also can be explained by a combination of the two mechanisms – diffusion through the gel and its hydrolytic degradation. However, NSSOM release from carbopol gel was completed in 12h. During the same period about 38% of the total NSSO in microspheres release in the first 0.5h, which reflected the significant amount of NSOO adsorbed on or near the surface of the microspheres. In clinical practice this would lead to 'burst effect', which enables the preparation to show fast effect to the patients. However NSSO release from microspheres was also completed 12 h. In comparison with NSSOM, NSSOMgel composite, the NSSO release is very fast, in approximately 54% release in 0.5h, numerical data shown in Table 2. This type of release may be clinically more therapeutic concentration also not good for the patient. The results indicated that the NSOOMgel had a well-controlled release efficacy.

The data obtained from in vitro release studies were fitted to various kinetic equations as shown in Table 1 (i.e., First order, Baker and Lonsdale, Hixon and Crowell, Korsmeyer's Peppas and Higuchi) to determine the mechanism of drug release and release rate using a macro written for graphing tool sigma plot demo version 9.01 (see Fig 3 and 4). The correlation coefficient value R^2 is taken into account to decide upon the relevance of the model/curve fit which will best describe the extent of fit. According to the R^2 values given by different data fits for NSOOM and NSOOMgel the First order model was to be an ideal fit having $R^2 = 0.9826$ and Korsmeyer's Peppas model having $R^2 = 0.9950$ respectively (Table-3). According to First Order and Korsmeyer's Peppas fit, the release of the drug is decided upon by the diffusion of the polymeric matrix and the drug release is governed by a variation of Fick's law of diffusion.

The release pattern showed classical Fickian diffusion the release was influenced by the initial swelling of the microspheres and releasing drug particles adsorbed in the surface aiding in a 'burst' release (Lu et al., 2003). Later on the release pattern is more controlled and sustained for longer period of time due to diffusion for NSOOM and the release pattern for NSOOMgel is diffusion for some time and later on behaves like sustained release.

During storage at 3-5°C or room temperature (15-25°C) for 12 months (Huo et al., 2005), surface morphology and content of NSOO had no notable changes (see Fig. 5). However, at 37°C and RH 75% the agglutinative phenomenon was observed.

Carbopol 974P NF would be taken as the resin of choice for preparing gels for vaginal application because of its good bioadhesive properties. Empty Carbopol hydrogels (blank) and NSOOMgel were tested for basic rheological properties. Both the Carbopol gels showed a similar behaviour. The yield point was detected at a shear stress of approximately 50 Pa. Interestingly, up to approximately 120 Pa the 980 NF type showed a constant slope (i.e. constant viscosity) of shear stress vs. shear rate, representing a Bingham-type fluid. The Carbopol 974P

NF gel was found to be more of a Casson type fluid with continuously decreasing viscosity, thus indicating successive loss of polymer entanglement upon increasing shear stress. However, both Carbopol types show a pronounced loss of viscosity for samples with incorporated microspheres and for samples containing buffer instead of microspheres. The changed viscosity is probably due to the presence of cationic ingredients (sodium ions) in the buffer which are not compatible with the anionic Carbopol resins (Dittgen et al., 1997).

The rheological measurements performed in this study were preliminary and for orientation purposes, however, further rheometrical testing would be worthwhile. The knowledge of the rheological properties of such pharmaceutical dosage forms is valuable for their characterization, especially if one is considering a possible scale-up.

Figure 1: Scanning electron microscopy of NSOO microspheres prepared by spray drying method. They had Shriveled surface

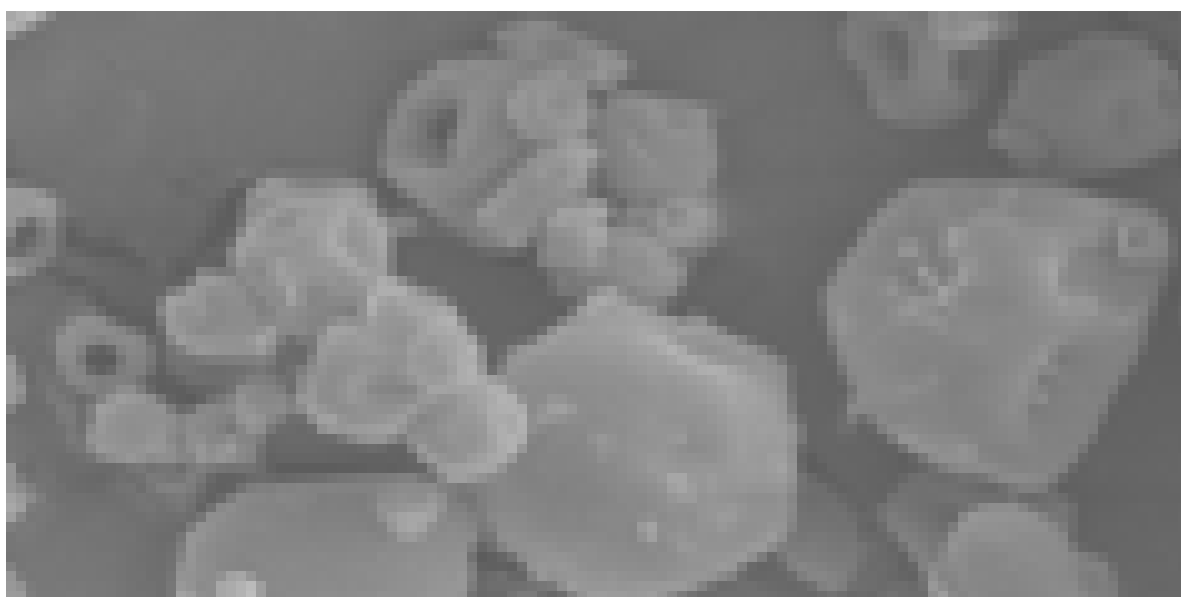


Figure2: Cumulative amount of drug release (□) NSOO (□) NSOOM (+) NSOOMgel

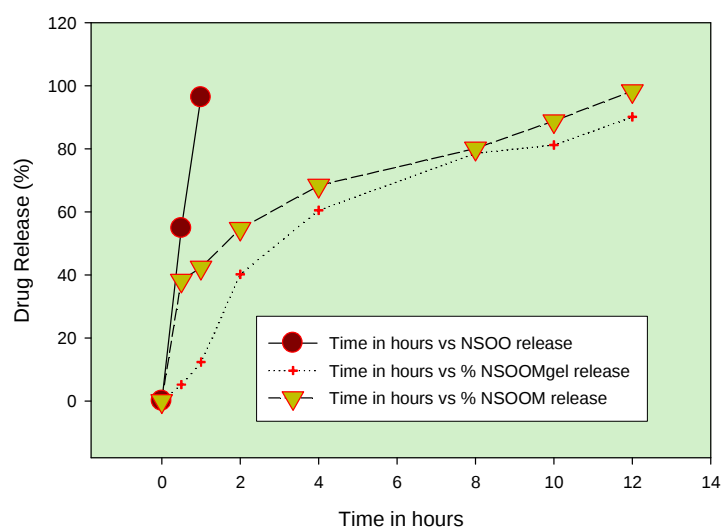


Figure3: In vitro release profile of NSOOM - Curve fit to different models

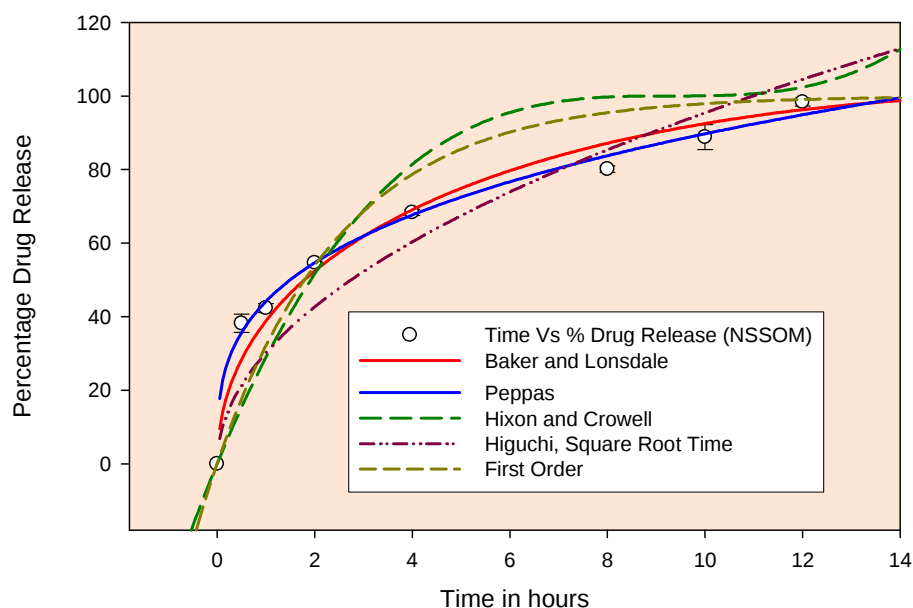


Figure 4: In vitro release profile of NSOOMgel - Curve fit to different models

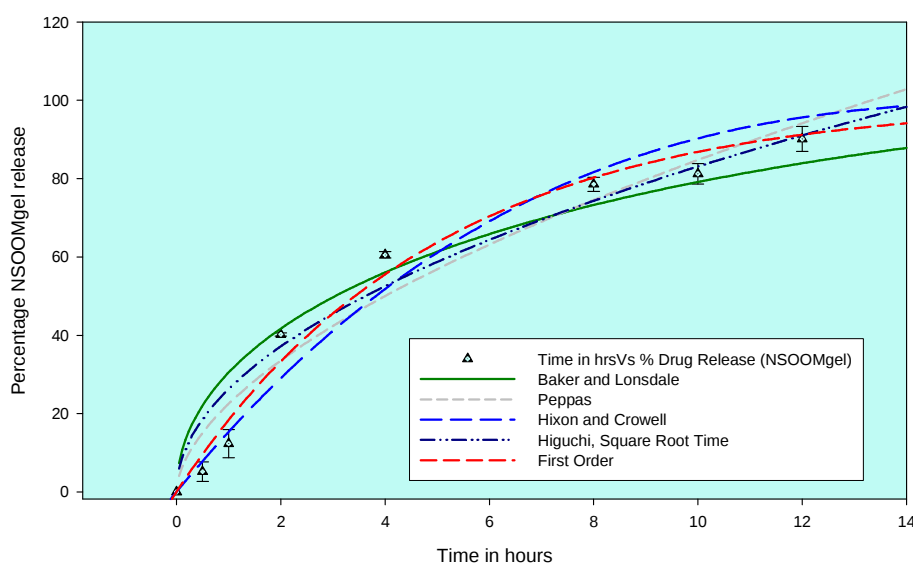


Table1: Applied release models [25]

Model	Equation
Baker-Lonsdale	$\frac{2}{3} \left[1 - \left(1 - \frac{F}{100} \right)^{2/3} \right] - \left(\frac{F}{100} \right) = kt$
First order	$F = 100(1 - e^{-k_1 t})$
Higuchi	$F = k_H \sqrt{t}$
Hixson-Crowell	$F = 100[1 - (1 - k_{HC} t)^3]$
Peppas	$F = k_p t^n$

F , amount of drug released in time t , k_{LB} , k_L , k_H , k_{HC} , k_p release rate constants, n release exponent.

Figure 5: The stability studies of NSOOMgel

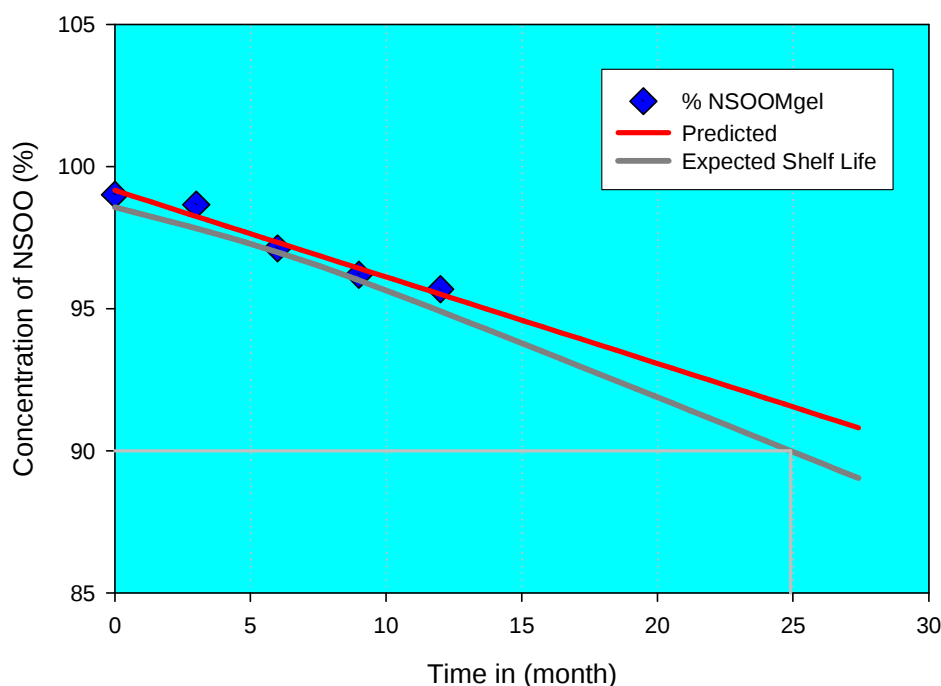


Table 2: Drug release profile of Nigella Sativa and Olive oil as a function of time

Time in hours	% NSSO release	% NSOOM release	% NSOOM gel release
0.5	54.75	5.2	38.21
1	96.34	12.36	42.35
2		40.23	54.65
4		60.54	68.32
8		78.57	80.12
10		81.23	88.84
12		90.15	98.34

NSSO - Nigella Sativa and Olive oil

NSSOM - Nigella Sativa and Olive oil microspheres

NSSOMgel - Nigella Sativa and Olive oil microspheres in gel

Table 3: In vitro curve fits for various release systems for gelatin microspheres

Sl No.	Equation	NSOOMgel R ²	NSOOM R ²
1.	First Order	0.9247	0.9746
2.	Higuchi, (Square Root Time)	0.9574	0.9950
3.	Hixon and Crowell	0.9639	0.8028
4.	Peppas	0.9502	0.8957
5.	Baker and Lonsdale	0.9826	0.8666

CONSLUSION

The conventional commercial preparations, such as creams, foams, gels, irrigations and tablets have limitations such as leakage, messiness and low residence time; often require multiple daily doses to ensure the desired therapeutic effect and which contribute to poor subject or patient compliance. Attempts have been made to develop microspheres mucoadhesive vaginal gel that can meet the clinical as well as the user's requirements.

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