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Preparation and characterization of novel carbopol based bigels for topical delivery of metronidazole for the treatment of bacterial vaginosis

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ABSTRACT

The current study reports the development of bigels using sorbitan monostearate-sesame oil organogel and carbopol 934 hydrogel. The microstructures and physicochemical properties were investigated by microscopy, viscosity measurement, mechanical analysis and differential scanning calorimetry analysis. Fluorescence microscopy confirmed the formation of oil-in-water type of emulsion gel. There was an increase in the strength of the bigels as the proportion of the organogel was increased in the bigels. The developed bigels showed shear-thinning flow behavior. The stress relaxation study suggested viscoelastic nature of the bigels. The developed bigels were biocompatible. Metronidazole, drug of choice for the treatment of bacterial vaginosis, loaded bigels showed diffusion-mediated drug release. The drug loaded gels showed good antimicrobial efficiency against *Escherichia coli*. In gist, the developed bigels may be used as delivery vehicles for the vaginal delivery of the drugs.

1. Introduction

Bacterial vaginosis (BV) is a common vaginal infection in which the vaginal pH is increased (>4.5) and is characterized by an increase in the vaginal discharge, with stinking smell [1]. The hydrogen peroxide producing lactobacilli, present in the vagina, provides natural disinfection. Lactic acid (produced by lactobacilli) helps maintaining the normal microflora balance and pH inside the vagina [2]. Apart from hydrogen peroxide and lactic acid, lactobacilli produce bacitracin (antimicrobial peptide) [3]. These natural antimicrobial agents suppress the growth of pathogenic bacteria, including *Gardnerella vaginalis, Mycoplasma hominis* and *Mobiluncus species* [4]. These pathogenic microbes are normally present in the vagina at low concentration levels. During BV, the level of lactobacilli is reduced. Hence, it is unable to perform its natural protective function resulting in the overgrowth of the suppressed anaerobic microbes [5].

BV may also be produced by *Escherichia coli*, a gram negative, anaerobic bacterium commonly found in the lower intestine, migrating into the vagina [3]. Normally, the vaginal mucosal lining provides protection against such bacterial movement. The protection may be compromised due to inadequate diet, decreased hormone levels, deprived health, frequent intercourse or abnormal microflora which in turn may result in the deterioration of the mucosal lining [6]. It is plausible that women with BV may develop urinary tract infections due to frequent sexual activities. *E. coli* is the major causative organism behind urinary tract infections [7]. Stamey and Timothy (1975) showed that increased vaginal pH was strongly correlated to the introital colonization with *E. coli* [8]. Similarly, Hooton et al. (1989) showed an increase in the vaginal fluid pH and altered vaginal microflora. They also suggested that the abnormal vaginal flora might be associated between urinary tract infections and sexual activity. Reduced levels of lactobacilli and overgrowth of pathogenic bacteria during BV have been reported to facilitate urinary tract infections by promoting colonization of urinary tract with *E. coli* [9]. Harmanli et al. (2000) also supported the above [10].

Metronidazole and clindamycin are the drugs of choice for the treatment of BV. The use of metronidazole is very common in developing and under-developed countries due to its low price as compared to clindamycin [11]. Oral and topical formulations of metronidazole have been described for the treatment of BV [12]. Mitchell et al. (2013) found a spectacular decrease in BV associated pathogenic microbes after treatment with metronidazole [13]. Aguin et al. (2013) investigated high-dose intravaginal metronidazole formulations for the treatment of recurrent BV [14]. Gels are more preferred for local therapy compared to oral medication. This helps avoiding the side effects related to the oral delivery.

Gels are semi-solid formulations containing both solid and liquid components. The solid component (gelator) is present as a network of aggregates, which immobilizes the liquid component. The gels are broadly classified into hydrogel and organogel based on the nature of the continuous phase. Hydrogels contain aqueous solvent, whereas,

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organogel contain apolar solvent as continuous phase [15]. Bigels are prepared by mixing hydrogel and organogel in defined proportions. The drug release rates from the organogels may be enhanced by many folds by converting the organogels into a bigel [16]. A bigel differs from an emulsion structurally. They offer significantly improved properties as a pharmaceutical or cosmetic formulation [16]. The improved physicochemical stability of the bigels is associated with the formation of extra-fine dispersions [17]. Recently, scientists are working towards the usage of bigels as formulations for the delivery of pharmaceutical and nutraceutical agents [18].

Carbopol 934 is a colloidal water-soluble, mucoadhesive, biodegradable and pH responsive acrylic acid polymer. It consists of chains of a polyacrylic acid crosslinked with 0.75% to 2% of crosslinking agent (e.g. polyallyl sucrose or polyallyl pentaerythritol) [19]. It is mainly used in the preparation of semi-solid and liquid formulations (gels, suspensions and emulsions) meant for topical application [20]. It is classed as a thickening agent and has been extensively used for modifying the release kinetics of the drugs [21]. Carbopol possesses bioadhesive property which is ascribable to its capability to form hydrogen bonds with the polysaccharide (mucin) present in the mucosal lining [22]. Puri et al. (2013) reported carbopol 934 based bioadhesive topical gel of 5-Fluorouracil with properties compared to the commercially available creams [23]. Rehman et al. (2013) developed Tramadol HCl matrix tablets using carbopol 934 as the rate-controlling agent [24]. Sorbitan monostearate (SMS) is a hydrophobic non-ionic surfactant which forms organogel when mixed with organic or apolar solvents (hexadecane, isopropyl myristate and some vegetable oils) [25]. SMS based organogels have been developed for pharmaceutical applications [25,26]. Sesame oil has been extensively used for therapeutic and cosmetic applications and is considered as a magic botanical potion [27]. The major components of sesame oil (sesamolin and sesamol) provide stability against oxidative deterioration [28].

In the current study, we developed bigels containing carbopol 934 hydrogel as the external phase and SMS-sesame oil organogel as the dispersed phase to combine the beneficial effects of both the carbopol hydrogels and organogels. Bigels are relatively novel formulations and are not much explored in the field of drug delivery. Recently, a comparative study of organogels, hydrogels and bigels was reported by Ibrahim et al. (2013) for the transdermal delivery of diltiazem hydrochloride [18]. Almeida et al. (2008) reported enhanced moisturizing effect of bigels when oleogels were mixed with hydrogel [29]. Novel self-assembled bigels prepared by arrested demixing in binary colloidal mixtures were reported by Varrato and Michele et al. (2012) [30]. Further, the authors explained the dynamics of aggregation of the bigels and related their observations with the change in structure and mechanical properties [16]. Being a relatively new category of delivery vehicles, bigels can be explored as potential drug delivery matrices. The novelty in the developed bigels lies in the improved stability compared to the hydrogels and organogels. We were also able to tailor the release of the drug in controlled manner which can be achieved by altering the proportion of the hydrogel and the organogel. The developed bigels were characterized by diverse techniques such as microscopy, viscosity, mechanical properties and differential scanning calorimetry. Metronidazole (model antimicrobial drug) was incorporated in the bigels. The release of metronidazole from the bigels was investigated, and the antimicrobial efficacy of the drug containing formulations was determined.

2. Materials and method

2.1. Materials

Carbopol 934 and SMS were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Food grade sesame oil (Tilsona®) was obtained from Recon Oil Industries Pvt. Ltd., Mumbai, India. Metronidazole was provided as a gift by Aarti drugs, Mumbai, India. Microbial culture of *Escherichia coli* (NCIM 2563) was received from National Collection of Industrial Microorganisms (NCIM), Pune, India. Nutrient agar was purchased from Himedia laboratories Pvt. Ltd., Mumbai, India. Purified water of Milli-Q quality was used throughout the study.

2.2. Methods

2.2.1. Preparation of bigels

The aqueous phase (carbopol hydrogel) and apolar phase (SMS-sesame oil organogel) were prepared separately. Carbopol hydrogel (1% w/w) was prepared by dissolving 1 g of carbopol in 99 g of water (60 °C, 500 RPM). SMS-sesame oil organogel (15% w/w) was prepared by dissolving 15 g of SMS in 85 g of sesame oil (60 °C, 500 RPM) and subsequently cooled to 25 °C. Bigels were prepared by dropwise addition of molten organogel in carbopol hydrogel (60 °C, 500 RPM) until a homogenous mixture was obtained (Table 1). Based on the composition, the mixture either formed a gel or remained as a phase separated system when cooled to room-temperature. The gel formation was confirmed by tube inversion test. The developed gels were evaluated for their organoleptic properties (odor, color, texture, and taste) and pH [31].

Metronidazole containing organogel was prepared in the same way. The drug loaded organogel was used for the preparation of the bigels. The amount of metronidazole in the bigels was kept constant at 1% w/w.

2.2.2. Stability studies

The accelerated stability of the bigels was checked by freeze-thaw method as per the reported literature [32]. In short, the bigels were alternatively kept at −20 °C (freezing) and 70 °C (thawing) for 15 min. The study was performed for 5 cycles, and the gels were checked visually for any signs of destabilization after each cycle. The gels were allowed to reach room-temperature upon completion of five cycles and finally evaluated for its organoleptic characteristics [33].

The long-term stability of the prepared bigels was estimated as per the ICH guidelines. The samples were incubated at 30 °C ± 2 °C/65% RH ± 5% RH for 6 months (intermediate stability). The bigels were observed for any changes in the visual appearances (e.g. color change, phase separation or syneresis) at regular intervals of time [3].

2.2.3. Microscopic studies

The microarchitecture and the nature of the bigel were determined using Fluorescence Stereo Microscope (M205 FA, Leica, Germany) [32]. The samples for the fluorescence microscopy was prepared by dissolving fluoral yellow (0.1% w/w) in sesame oil. The samples were analyzed using green filter. The droplet size analysis of the dispersed phase was done using ImageJ (v 1.43) software, National Institute of Health, USA [34].

2.2.4. Mechanical properties

The flow properties of the bigels were studied using a cone and plate viscometer (Bohlin visco 88, Malvern, UK) at room-temperature [35].

Table 1
Compositions of bigel formulations (for 20 g).

Formulations	Carbopol hydrogel (g)	Organogel (g)	Metronidazole (g)	Organogel (% w/w)
CG1	17.78	2.22	–	11.11
CG1M	17.78	2.02	0.2	–
CG2	16	4	–	20
CG2M	16	3.8	0.2	–
CG3	14.55	5.45	–	27.27
CG3M	14.55	5.25	0.2	–
CG4	13.33	6.67	–	33.33
CG4M	13.33	6.47	0.2	–