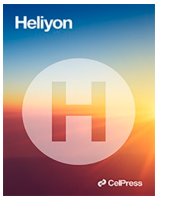
Heliyon 10 (2024) e32275

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Individual sol-gel microencapsulation of benzoyl peroxide and tretinoin enables controlled release onto the skin

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ARTICLE INFO

Keywords:
Microencapsulation
Silica
Encapsulation
Release profile
Acne
Rosacea

ABSTRACT

A combination of benzoyl peroxide (BPO) and tretinoin is recommended for treating acne; however, concurrent administration can be irritating, and coformulation is prevented by BPO-mediated oxidation of tretinoin. In rosacea, benzoyl peroxide has been shown to be efficacious; however, its use has been limited by poor tolerability. To overcome these limitations, the active ingredients can be encapsulated within silica microcapsules. The US Food and Drug Administration has approved 2 products using this technology, a combination of encapsulated benzoyl peroxide and encapsulated tretinoin product for acne vulgaris and encapsulated benzoyl peroxide to treat inflammatory lesions in rosacea. The active ingredients are released through small channels in the silica shell, gradually releasing the active ingredients to the skin. This study describes the stability and release profiles of encapsulated tretinoin and encapsulated benzoyl peroxide from the silica shell in physiologically relevant conditions and provides differentiation from traditional formulations.

1. Introduction

Tretinoin (all-trans retinoic acid) is a potent retinoid and keratinization inhibitor that decreases the cohesiveness of follicular epithelial cells [1]. This activity inhibits the formation of microcomedones and thereby prevents mature comedones and inflammatory lesions from forming [2]. Retinoids also promote the normal desquamation of the follicular epithelium [3]. In addition to its comedolytic effects, tretinoin has anti-inflammatory properties by binding to all 3 subtypes of retinoic acid receptors α, β, and γ) [4]. However, the use of tretinoin has been limited by poor tolerability, including dry skin, erythema, pruritus, and pain [4].

Benzoyl peroxide (BPO) is a powerful oxidizing agent with localized bacteriostatic, sebostatic, and keratolytic effects [3,5]. In acne treatment, the probable mechanism of BPO action is its antimicrobial activity against *Cutibacterium acnes* bacteria in the sebaceous follicles [6,7]. Unlike antibiotics, BPO does not cause bacterial resistance [6,8]. However, local irritation is a side effect that often compromises effective therapy by limiting patient adherence [5,9].

To overcome these limitations, 2 topical dermatological drug products, a combination of encapsulated benzoyl peroxide and encapsulated tretinoin cream (E-BPO/T) and encapsulated benzoyl peroxide cream (E-BPO cream, 5%), were developed by encapsulating the active ingredients within silica microcapsules. Encapsulation has 2 primary objectives: (1) overcoming active pharmaceutical ingredient (API) stability issues and (2) improving the tolerability of products on the skin.

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https://doi.org/10.1016/j.heliyon.2024.e32275
Received 18 December 2023; Received in revised form 5 March 2024; Accepted 30 May 2024
Available online 1 June 2024
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The first product, E-BPO/T, 3%/0.1%, is a fixed-dose combination topical cream for treating acne vulgaris in patients aged 9 years and older. BPO and tretinoin are APIs with different pharmacological actions, and both are known irritants [10,11]. Unencapsulated BPO and unencapsulated tretinoin can be found in prescription and over-the-counter medications or washes used to treat acne. Encapsulation overcomes 2 limitations of traditional formulations. The first is that combing the 2 agents may lead to intolerability and poor patient adherence [12]. The second is that tretinoin is highly sensitive to BPO-mediated oxidation and is chemically unstable in the presence of BPO, preventing coformulation [13]. It was postulated that sol-gel microencapsulation might resolve the incompatibility of BPO and tretinoin by encapsulating each component separately to form barriers between each API and between each API and the skin. E-BPO/T was designed to treat acne effectively. The American Academy of Dermatology recommends either BPO or a retinoid as a first-line treatment for mild acne [6]. Topical treatment with a combination of BPO and a retinoid or antibiotic is recommended for moderate acne [6]. For severe acne, the initial recommendation is to use an oral antibiotic in addition to a combination of BPO and a retinoid or a topical antibiotic [6]. However, the concurrent use of tretinoin and BPO is likely irritating, and the products must be applied at different times [13]. Combining the individual active ingredients into one product may improve adherence and treatment outcomes for patients with acne.

E-BPO cream, 5%, was developed for topical treatment of inflammatory lesions of rosacea in adults. With encapsulation, E-BPO tolerability may be improved by preventing contact with the initial bolus of BPO to the facial skin without compromising its effectiveness as a topical antibacterial, keratolytic, and comedolytic agent. The exact mechanism of BPO in treating rosacea is unknown.

The stability and tolerability of E-BPO/T and E-BPO are achieved by isolating the active ingredients in separate microcapsules formed by the sol-gel process. Sol-gel is a chemical process in which a "gel" is created by a network growth from an array of colloidal particles dispersed in a liquid ("sol"), increasing viscosity until a rigid mass is formed ("gel"). Precursors for the sol-gel process include metal oxide or semimetal oxide salts or alkoxides [14]. The sol-gel process is advantageous because it allows the formation of semimetal oxides at low temperatures, which is suitable for all organic materials, including unstable ones [15]. Sol-gel processes are used in numerous applications, including topical applications like organic sunscreens [16].

One of the benefits of microencapsulation is that it allows for a slower release of active ingredients to the skin compared with traditional formulations that release all the active ingredients at once. In this paper, we describe the release profiles of encapsulated tretinoin and encapsulated BPO to show that microencapsulation results in a gradual release of active ingredients from the silica shells in physiologically relevant conditions.

2. Materials and methods

2.1. Silica for sol-gel

The sol-gel process can be pharmaceutically applied using various metal oxides or semimetal oxides, such as silica, titania, or alumina. These oxides differ in their isoelectric points; alumina, titania, and silica have isoelectric points at a pH range of 8.5–9.6, 5.2–6.8, and $\leq$2.5, respectively [17]. Performing the sol-gel process at a pH above the isoelectric point, at which the metal (or semimetal) oxide is negatively charged, is desirable because electrostatic repulsion between charged species prevents agglomeration. For this reason, silica is a suitable candidate for the sol-gel process for pharmaceutical applications because it is negatively charged at a wide pH range (pH $>$2). Silica is compatible with various drug dosage forms due to its hydrophilic nature. Moreover, the US Food and Drug Administration (FDA) has designated amorphous silica as a generally recognized as safe ingredient, and it is listed in the inactive ingredient guide of the FDA as a safe excipient for use as a food additive and in drugs [18,19].

2.2. Encapsulation

Encapsulation is when active molecules are stabilized through the structuring of systems capable of preserving their chemical, physical, and biological properties. Encapsulation may also enable their release or delivery under desired conditions [20]. Several possible methods are available to encapsulate an active ingredient, including interfacial polymerization and coacervation [21,22]. Interfacial polymerization is the formation of a polymer at the interface between solid/water or oil/water, for example. Coacervation is a technique that involves the formation of a homogeneous layer of polymeric wall material around the core material. Coacervation is achieved by altering the physicochemical properties of the wall material by changes in temperature, pH, or ionic strength.

Entrapment efficiency is the percentage of an active ingredient captured inside the microcapsules from the total active ingredient introduced into the product. Several parameters must be considered to achieve an encapsulated material with a high entrapment efficiency, such as solubility and stability at different temperatures and pH levels.

Encapsulation using the sol-gel technology is an interfacial polymerization process that can entrap organic molecules in sol-gel matrices while retaining much of their physical and chemical properties [15,23]. To encapsulate an active material via the sol-gel process, precursor units are polymerized at the solid/water or oil/water interface to produce a gel matrix surrounding the active material. Thus, a core/shell structure is formed, in which the organic molecules are in the core, and the microcapsule shell is comprised of silica. This process results in an aqueous dispersion of the active ingredient entrapped in silica shells. It is a stepwise process and is carefully controlled to enable the release of the active material at a desired rate.

2.3. Forming silica from an inorganic precursor (sodium silicate)

The formation of silica using the sol-gel process involves 2 consecutive reactions using an inorganic precursor, such as sodium

silicate salt (Fig. 1). These reactions are the acidification of sodium silicate to form silicic acid and the condensation of 2 hydroxyl-bearing species. Silicic acid ($Si(OH)_4$) polymerizes into particles that aggregate into chains and networks. The process begins with the polymerization of monomers to form sol particles, followed by the linking of particles into chains [24]. Many factors significantly affect these sol-gel reactions, including pH, solvent types and concentrations, salt concentration, and temperature.

2.4. Forming silica from an organic precursor (tetraethyl orthosilicate)

The formation of silica from an organic precursor, such as silicon alkoxides (eg, tetraethyl orthosilicate), via the sol-gel process involves 2 reactions (Fig. 2).

1. Hydrolysis of an alkoxy group
2. Condensation of 2 OH-bearing species or an –OH group and alkoxy group species

The hydrolysis reaction requires a catalyst, such as an acid or base. The condensation reaction proceeds to form siloxane bonds (Si–O–Si). The condensation reactions can occur in all 4 functional groups (-OH or –OR). Under most conditions, condensation commences before hydrolysis is completed because water and alkoxysilanes are immiscible.

2.5. Encapsulation method for BPO

BPO is found in high concentrations (2.5%–10%) in many commercial products. To achieve a high concentration of BPO in the final product (~5%), a microencapsulation system with a higher BPO concentration (~15%) was necessary. Therefore, BPO was encapsulated in its solid form in a water-dispersed system. The following factors were considered for the development process.

1. **Silica precursor–**Because the BPO is dispersed in water, an inorganic precursor, such as sodium silicate, was used.
2. **Surfactant–**Silica is negatively charged at neutral pH levels. Therefore, to electrostatically bind the silica to the surface of the BPO crystals, it was necessary to positively charge the surface with a cationic surfactant, such as cetrimonium chloride (CTAC).
3. **Polymer–**To improve microencapsulation, it was necessary to positively charge the shell surface continuously. The positively charged polymer polyquaternium-7 (PDAC) was selected due to its ability to interact with the negatively charged silica electrostatically, thus positively recharging the forming shell and enabling the addition of another silica gel layer.

The microencapsulation of BPO entailed the dispersion and de-agglomeration of BPO crystals in an aqueous solution of the cationic surfactant CTAC. Sodium silicate (Na_2SiO_3) solution was added as the silica precursor, followed by the addition of an acid cocktail (mixed solution of hydrochloric acid, lactic acid, and citric acid) to decrease the pH and form silicic acid (H_2SiO_3). "Sol" colloidal particles were formed, and silicic acid was polymerized until a rigid network, the silica shell (the "gel"), was formed (Fig. 1).

Because silicic acid "sol" particles are negatively charged at neutral pH, they electrostatically bind to the surface of the BPO-CTAC positively charged particles, where the condensation reaction occurs, forming a silica gel layer around the active ingredient. Next, PDAC was added to allow the addition of silica gel layers. In this manner, the encapsulation process continued for 5 to 100 cycles to obtain a continuous coating around the BPO crystals. Fig. 3 shows the chemical interactions between the molecules that form the shell structure around the benzoyl peroxide crystal.

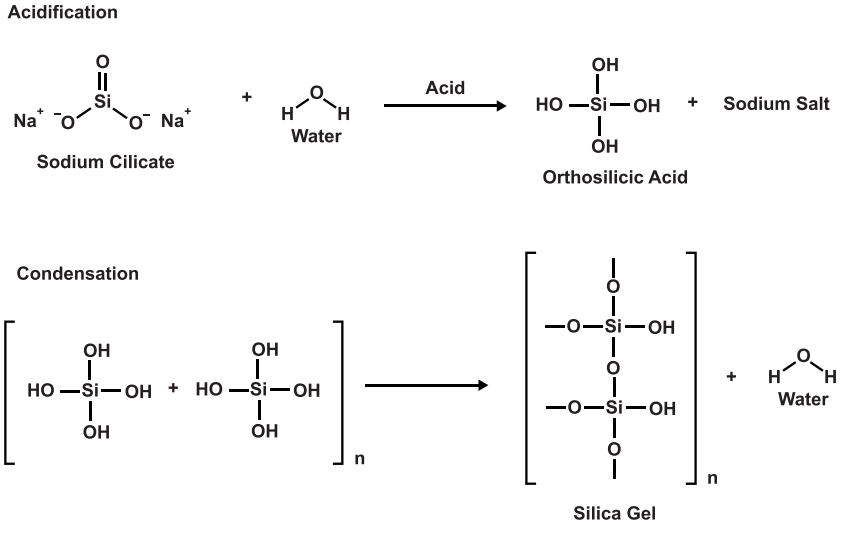

Fig. 1. Acidification and condensation of sodium silicate.

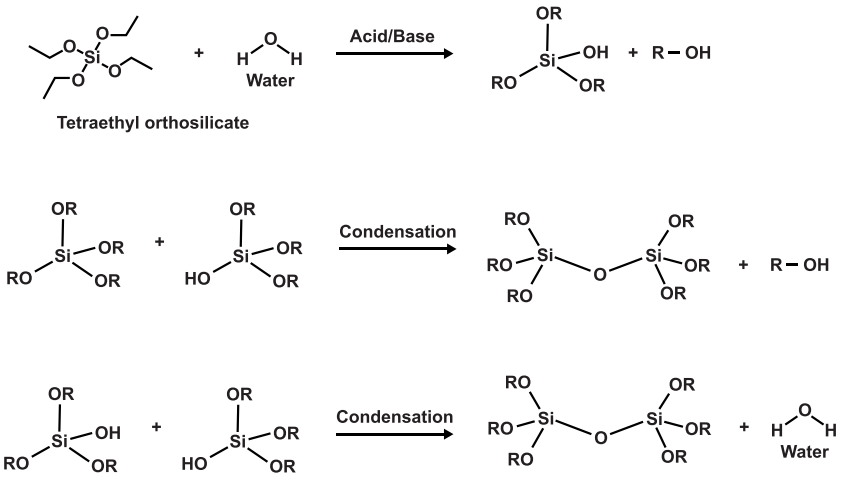

Fig. 2. Hydrolysis and condensation of alkoxides.

The detailed procedure of the BPO encapsulation has been previously described [25].

2.6. E-BPO cream, 5 %, manufacturing

The E-BPO cream was prepared by combining an aqueous phase containing a humectant (glycerin), a chelating agent (sodium edetate), and a preservative (phenoxyethanol) with an oil phase comprising an emollient (cyclomethicone), a thickening agent (cetyl alcohol), emulsifiers (mono- and diglycerides and macrogol stearate type I), and adding the E-BPO suspension to form the final cream.

2.7. Characterization methods for E-BPO microcapsules

A release method was developed to assess how process parameters affected the degree of encapsulation. This method measured the percentage of the API released into the extracting media at specific intervals. Two different release methods were developed to evaluate the BPO release from the suspension and from the final cream drug product. The method used to assess BPO release from the suspension was a fast in-process control method. The process used to evaluate BPO release from the final cream product was a performance testing method simulating "physiological" conditions.

2.8. BPO release method from E-BPO suspension

BPO was released into a mixture of acetonitrile:water (45:55) at ambient temperature. The amount of BPO released was determined

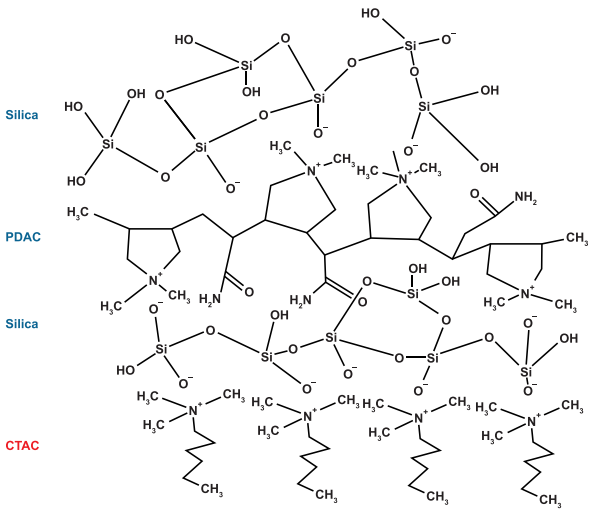

Fig. 3. A Schematic Representation of the Chemical Interactions Between CTAC, Silica, and PDAC in the Shell. CTAC, cetrimonium chloride; PDAC, polyquaternium-7.

by comparing it with an external standard using high-performance liquid chromatography (HPLC). The release profile was determined by sampling 4 times, representing the dynamics of the release (dissolution) at 10, 20, 40, and 60 min. At 10 min, the release profile of the nonencapsulated BPO crystals was demonstrated, while at 60 min, a more extensive overview and evaluation of process parameters could be analyzed.

2.9. BPO release method from cream formulation

BPO was released into an aqueous medium containing TWEEN 60 in a phosphate-buffered solution at physiological temperature. The amount of released BPO was determined by comparing it with an external standard using HPLC. E-BPO cream was designed to provide a release profile that ensured a moderate release of BPO from the microcapsules over time. Three times were selected for this purpose: 1 h immediately after application; 6 h, demonstrating approximately 50% release of the drug substance from the microcapsules; and 24 h, indicating complete release.

2.10. Encapsulation method for tretinoin

During the development process of encapsulating tretinoin using the oil-dispersed technology, which forms silica capsules around oil droplets in water, the following factors were considered.

1. **Oil**–Tretinoin crystals were dispersed in oil to prevent direct contact with water. Squalane oil was chosen due to the low solubility of tretinoin in squalane (one part of tretinoin is solubilized by 3846 parts of squalane), allowing tretinoin to be retained in solid form.
2. **Phase-changing material**–The oil phase was solidified by adding a phase-changing material to retain tretinoin in the core and reduce migration to the interface. White wax was used as the phase-changing material because squalane and white wax are miscible.
3. **Antioxidant**–Tretinoin is sensitive to oxidation, and adding an antioxidant within the same core would further prevent oxidation. Butylated hydroxytoluene (BHT) is oil soluble and can be dissolved in the capsule core and used as an antioxidant.
4. **Tretinoin loading–**Tretinoin loading is the tretinoin concentration in the oil phase. The effect of different loadings on tretinoin release was determined, and as seen in Fig. 4, the highest tretinoin loading (20%) had the slowest tretinoin release (lowest percentage of tretinoin released).
5. **Silica precursor**–Two silica precursors were selected: a water-soluble sodium silicate and an oil-soluble silicon alkoxide (TEOS). Silicon alkoxides have a structure of Si-$(OR)_4$. R can represent methyl, ethyl, propyl, and butyl groups. The longer the R group, the slower the hydrolysis of the sol-gel precursor. Hydrolysis of TMOS (R=methyl) is a very rapid reaction that does not allow time for gel formation and encapsulation. Hydrolysis of TEOS (R=ethyl) is slower, which enables slow gel formation and encapsulation in a

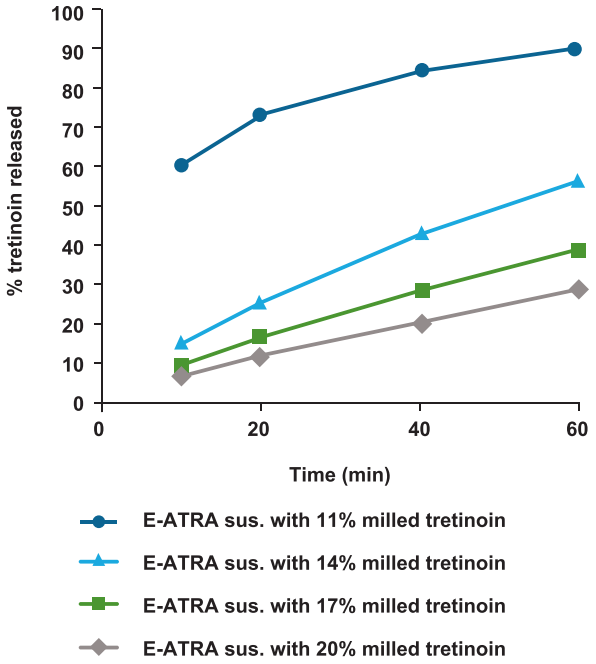

Fig. 4. Percentage of Tretinoin Release From E-ATRA Suspensions With Various Tretinoin Loadings. E-ATRA, encapsulated all-trans retinoic acid; sus., suspension.

reasonable time for a commercial process [24]. The oil-soluble TEOS builds the shell from the inside of the capsule. At the same time, water-soluble sodium silicate forms the shell from outside of the capsule, thus forming an efficient barrier between the tretinoin in the core and the water outside the shell.

2.11. Tretinoin microencapsulation and manufacturing

The manufacturing process of encapsulated all-trans retinoic acid (E-ATRA) suspension was performed as follows:

An oil phase was first prepared to contain tretinoin, the antioxidant BHT, and TEOS in squalane. The oil phase underwent milling, and then white wax was added and heated. The oil phase was then emulsified with an aqueous phase containing cetrimonium chloride, a cationic surfactant, resulting in positively charged oil droplets containing milled tretinoin crystals. A sodium silicate solution was added to the emulsion as a second silica precursor. The pH was reduced to the desired value by adding hydrochloric acid. A suitable pH value initiated the silica shell formation at the oil/water interface by 2 pH-dependent parallel processes: sodium silicate polymerization and TEOS hydrolysis and condensation. The first is a rapid process, and the second is slower. The optimal pH for acidic hydrolysis of TEOS is in the pH range of 3.0–5.0 [24,26].

A detailed microencapsulation procedure and materials have been previously described by Erlich and colleagues, 2020 [25].

2.12. E-BPO/T cream

E-ATRA was formulated with E-BPO suspension into a cream by combining an aqueous phase containing a humectant (glycerin), a chelating agent (sodium edetate), an emulsifier (macrogol stearate type I), and a preservative (imidurea) with an oil phase containing an emollient (cyclomethicone), a thickening agent (cetyl alcohol), a second emulsifier (mono- and diglycerides), and a gelling agent (carbomer homopolymer type C). Next, E-BPO and E-ATRA aqueous suspensions were sequentially added to form the final cream.

2.13. Characterization methods for E-ATRA microcapsules

The primary goal of tretinoin encapsulation was to minimize its degradation in the presence of BPO; therefore, several methods were developed to evaluate the quality of the encapsulation.

1. Tretinoin release from the microcapsules
2. Entrapment efficiency
3. Tretinoin assay and related compounds

Two different release methods were developed to measure the percentage of active pharmaceutical ingredients (API) released to the extracting media at specific time points to evaluate the effect of process parameters on the degree of microencapsulation.

2.14. Tretinoin release method from E-ATRA suspension

Tretinoin was released into a mixture of 1 g/L BHTin isopropanol (IPA) and water (59.3%:40.7% w/w) at 32 °C. The amount of tretinoin released was compared with an external standard using HPLC. The release profile was demonstrated by sampling at 4 time points representing the dynamics of the release (dissolution): 30, 60, 90, and 120 min.

2.15. Tretinoin release method from the cream formulation

E-BPO/T cream was designed to provide a release profile that ensured a moderate release of tretinoin from the microcapsules over time. This method determined the percentage release of tretinoin in the cream to the receptor medium (polysorbate 80, 2%, in phosphate buffer, pH 6.2 and 4 g/L BHT in 2-propanol, [66.7%:33.3% w/w]) at 32 °C. Three points were selected for this purpose: 0.5, 2, and 6 h. The 0.5-h point was chosen to assess the release, which starts immediately after application. The 2-h point was selected to demonstrate the pattern of the release profile. The 6-h point was chosen as the latest point in time because, during the development, it was observed that the released tretinoin degraded significantly after 6 h in the presence of BPO.

2.16. Entrapment efficiency

The E-ATRA suspension was dispersed in a mixture of BHT, 0.1%, in IPA:water (50:50 v/v) to determine the entrapment/microencapsulation efficiency in suspension. A sample was withdrawn and filtered, and the concentration of the free, nonencapsulated tretinoin dissolved in the medium was determined by comparison with an external standard by HPLC analysis. Mass balance against the suspension assay determined the encapsulated amount of tretinoin.

3. Results

3.1. E-BPO microcapsule studies and results (in suspension and E-BPO cream)

Scanning electron microscopy (SEM) characterization of the E-BPO suspension was performed. The E-BPO suspension was diluted with purified water until it became visually clear. One sample drop was placed on a silicon wafer and dried under a hood for several hours. The imaging was performed using an environmental electron microscope, Quanta FEG, FEI, and Apreo 2 SEM by Thermo Fisher Scientific.

Several E-BPO suspensions were characterized using SEM. Fig. 5 illustrates an image of E-BPO microcapsules in which most of the microcapsules exhibit a particle size of less than 10 μm. Fig. 5 also shows an image of a fractured microcapsule revealing the BPO crystal within the silica shell.

3.2. Effect of number of cycles and amount of sodium silicate and PDAC

Fig. 6 and Table 1 describe the BPO release results of E-BPO suspensions produced with the following process variations: the number of cycles, the amount of sodium silicate, and the amount of PDAC added at each cycle.

When the BPO was nonencapsulated (free-form), the BPO release was 90% after 1 min. In addition, the amount of time for BPO to release was increased with a higher amount of sodium silicate and PDAC added per encapsulation cycle and a higher number of cycles.

3.3. Effect of temperature

During the microencapsulation development of BPO, the temperature was observed to be the process parameter that most significantly affected BPO release. This effect was demonstrated by comparing the percentage of BPO release at 1 h for E-BPO suspensions manufactured at various temperatures during the encapsulation cycles. As shown in Table 2, when the temperature during encapsulation was higher, there was a lower percentage of released BPO after 1 h. For example, at 28 °C, the BPO release was 48%, whereas at 40 °C, the BPO release was 12%.

3.4. Comparison with nonencapsulated BPO commercial gel

BPO release from E-BPO vs nonencapsulated BPO in the final formulation was assessed, comparing E-BPO, 5%, with a nonencapsulated commercial BPO cream. The effect of BPO microencapsulation on its release was observed as the percentage of BPO release over time. The E-BPO had a lower percentage release than the nonencapsulated commercial BPO cream (Fig. 7).

3.5. Stability studies of nonencapsulated tretinoin and BPO

To demonstrate the instability of nonencapsulated tretinoin in a combined formulation of BPO and tretinoin, 2 formulations were produced and were subjected to stability. A formulation of BPO and tretinoin gel, 2.5%/0.1%, exhibited 9.4% tretinoin degradation following 1 month of storage at 25 °C and 60% relative humidity (RH) and 63.4% tretinoin degradation at 40 °C and 75% RH. The degradation of BPO under these conditions was 0.8% and 5.9%, respectively. A formulation of BPO and tretinoin cream, 2.5%/0.05%, had a tretinoin degradation of 42% following 5 days at accelerated conditions of 40 °C and 75% RH.

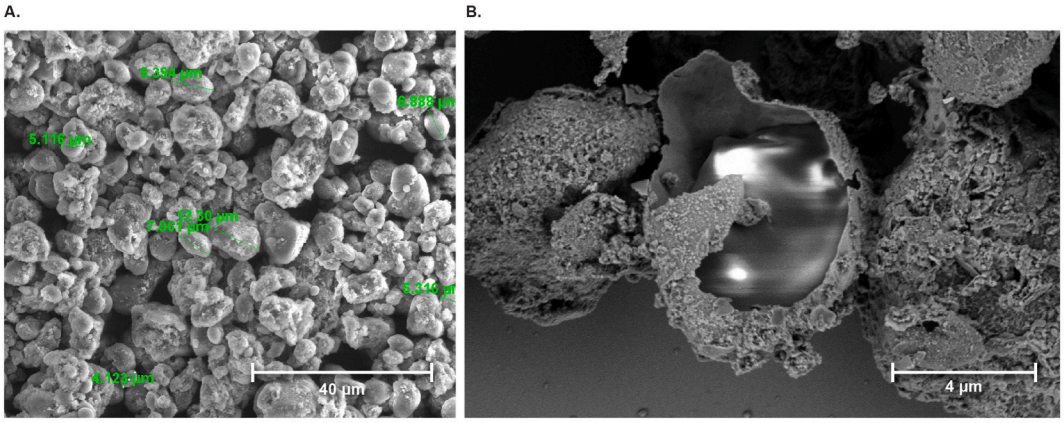

Fig. 5. SEM Images of E-BPO Microcapsules. E-BPO, encapsulated benzoyl peroxide; SEM, scanning electron microscopy.

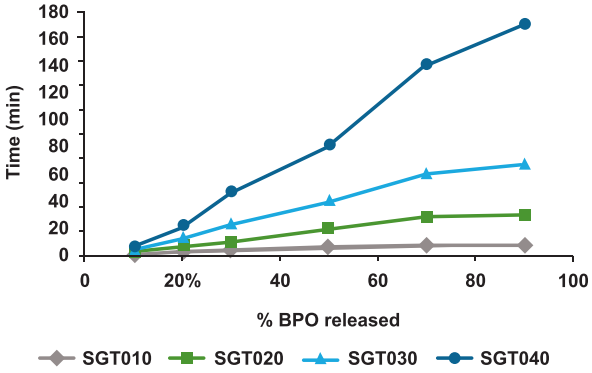

Fig. 6. Effects of the Number of Cycles and Amount of Sodium Silicate/PDAC on BPO Release. BPO, benzoyl peroxide; PDAC, polyquaternium-7.

Table 1
Effect of number of cycles and amount of sodium silicate/PDAC on BPO release.

Sample	SS & PDAC amount	Number of cycles	Time (min) at which 10%, 20%, … BPO is released					
			10%	20%	30%	50%	70%	90%
Free BPO	–	0			0.5			1
SGT010	1 g	20	1.2	3	4	7	7.5	7.7
SGT020	2.5 g	20	3	7	10.3	19.3	28	29.5
SGT030	2.5 g	30	4.6	12.3	23	40	60	68
SGT040	2.5 g	40	7	21	47	80	140	170

BPO, benzoyl peroxide; PDAC, polyquaternium-7; SS, sodium silicate.

Table 2
BPO release from E-BPO suspension.

Encapsulation temperature	BPO release after 1 h from E-BPO, 15 %, suspension
25 °C	68%
28 °C	48%
32 °C	26%
38 °C	17%
40 °C	12%

BPO, benzoyl peroxide; E-BPO, encapsulated BPO.

3.6. Encapsulation method for tretinoin

The primary goal of encapsulating tretinoin was to minimize tretinoin degradation in the presence of BPO, which is a strong oxidizer [7,9]. Because the oxidation reaction can only occur in the liquid phase, the selected encapsulation method required tretinoin in a solid form. During the development of tretinoin, different encapsulation technologies were evaluated. First, similar to BPO encapsulation, tretinoin crystals were dispersed in water with a cationic surfactant, and a silica shell was formed around them by repetitive cycles of a single water-soluble silica precursor, acidification, and a positively charged polymer. As shown in Fig. 8, the water-dispersed system demonstrated a fast release, and 87% of the tretinoin was released after 30 min. In contrast, the oil-dispersed technology showed a more gradual release profile. Furthermore, the water and oil suspensions were formulated with E-BPO into 2 different creams. The tretinoin stability results of the cream after 1 month with the water-dispersed system had a tretinoin degradation of 15.4% at 25 °C and 60% RH and a tretinoin degradation of 27.9% at 30 °C and 65% RH (Table 3). In comparison, the tretinoin stability of the oil-dispersed encapsulated tretinoin suspension demonstrated only 2.9% degradation following 1 month at 25 °C and 60% RH. Hence, the method for encapsulating tretinoin via the oil-dispersed technology was superior because tretinoin that is released too quickly will be degraded more rapidly by BPO.

3.7. E-ATRA microcapsules studies

Several E-ATRA suspensions were characterized using SEM and prepared as described for E-BPO suspensions. The imaging was performed using Zeiss GeminiSEM 500. Fig. 9 shows several tretinoin microcapsules with a spherical structure and a capsule diameter of about 20–25 μm. Fig. 10 presents an image of a fractured microcapsule revealing the inner tretinoin crystals within a coarse silica shell. The exact morphology by cryo-SEM has been observed previously [25].

The acidifying step plays an important role in shell formation, and the primary process parameter found to affect tretinoin release

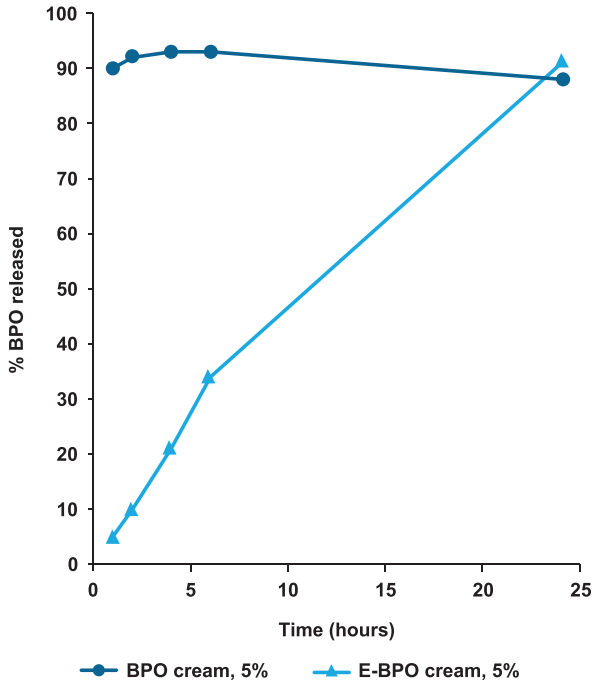

Fig. 7. Comparison of benzoyl peroxide release profiles of encapsulated benzoyl peroxide (E-BPO) cream, 5%, and unencapsulated benzoyl peroxide (BPO) cream, 5%.

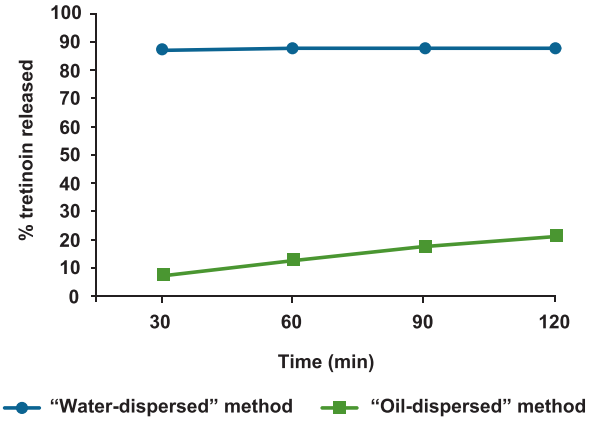

Fig. 8. Tretinoin released from encapsulated tretinoin suspensions prepared by 2 different technologies.

Table 3
Tretinoin stability results from E-BPO/T cream, 3%/0.1%, formulated with "water-dispersed" or "oil-dispersed" E-ATRA suspension.

E-ATRA suspension type	Tretinoin assay, % w/w		
	Time zero	1 month at 25 °C/60% RH	1 month at 30 °C/65% RH
"Water dispersed"	0.104	0.088 (15.4% degradation)	0.075 (27.9% degradation)
"Oil dispersed"	0.103	0.100 (2.9% degradation)	Not tested

E-ATRA, encapsulated all-trans retinoic acid; E-BPO, encapsulated benzoyl peroxide; RH, relative humidity.

from the microcapsule was pH. Therefore, the release of tretinoin from the microcapsules was assessed at different pH levels during encapsulation.

Table 4 shows a substantially higher percentage of tretinoin release at the 60-min point when the acidifying step occurred at pH 3.8 compared with pH 3.9. These results were consistent with the observation that a slower release was measured when the final pH was

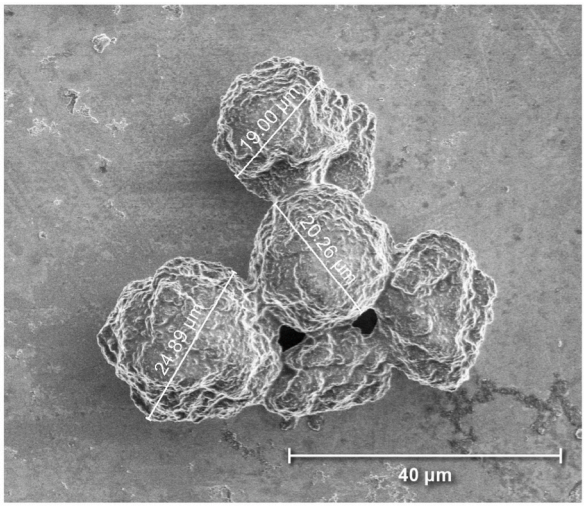

Fig. 9. Tretinoin Microcapsule image under scanning electron microscopy.

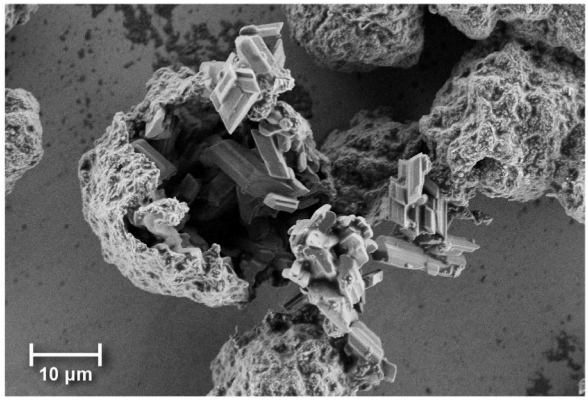

Fig. 10. A fractured tretinoin Microcapsule displaying the core shell structure, revealing the inner tretinoin crystals (A) within the silica shell (B).

Table 4
Percentage of Tretinoin Released From Suspensions Prepared at Different pH Levels.

pH	% tretinoin released after 60 min
4.2	16
4.1	15
4.0	19
3.9	26
3.8	35

raised to 4.2.

3.8. E-ATRA microcapsules studies and results (in E-BPO/T cream)

The comparison of tretinoin release from E-ATRA vs nonencapsulated tretinoin is shown in Fig. 11. The tretinoin release from the encapsulated tretinoin, 0.1%, in E-BPO/T exhibited a different release profile than the commercial nonencapsulated tretinoin cream. The release profile of tretinoin in the E-BPO/T cream demonstrated a relatively slow release, with approximately 10% released at 30 min and 50% released after 2 h. In contrast, the nonencapsulated product showed 100% release at 30 min under the same dissolution conditions.

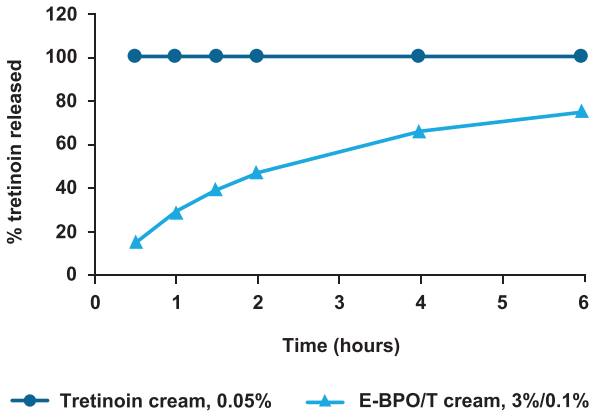

Fig. 11. Comparison of the Tretinoin Release Profiles of Tretinoin and BPO Cream, 0.1%/3%, and Tretinoin Cream, 0.05%. The Study Was Performed by Adjusting E-BPO/T to the Tretinoin Dose. BPO, benzoyl peroxide; E-PBO/T, encapsulated benzoyl peroxide and encapsulated tretinoin.

3.9. Entrapment efficiency and tretinoin stability

The entrapment efficiency of an E-ATRA suspension was modified by applying high-shear homogenization and sampling fractions at various times. The detailed procedure is provided in US patent US-2021361608-A1 [27]. The various fractions of suspensions were each formulated into E-BPO/T creams. The stability results of these creams following 2 weeks at 40 °C and 75% RH are presented in Table 5. The level of all-trans-5,6-epoxy retinoic acid, the primary oxidation degradant of tretinoin, and the total related compounds were monitored during the study. Results showed that lower entrapment efficiency of tretinoin was directly associated with increased chemical degradation.

3.10. Effect of tretinoin loading on tretinoin stability

The effect of tretinoin loading on the E-ATRA microcapsules and the chemical stability of tretinoin was examined. Suspensions with different tretinoin loadings were formulated into other creams with encapsulated BPO. As shown in Fig. 12, increased tretinoin loading reduced the percentage of tretinoin release in both the suspension and cream over time.

The tretinoin assay and related compounds in creams with different tretinoin loading are provided in Table 6. With higher tretinoin loading, the chemical stability of tretinoin in the cream was higher, as is evident from the change in assay and increase in related compounds after 2 weeks at 40 °C and 75% RH.

3.11. Shelf life

The stability results of tretinoin in E-BPO/T cream are provided in Fig. 13. The data points of different colors represent different cream batches, and the red line represents the average results for all the batches tested at each testing period. The results show that the tretinoin assay was stable within specification limits of 0.09%–0.11% during 24 months at 2 °C–8 °C. Furthermore, the levels of 5,6-epoxy retinoic acid over 6 months at accelerated conditions of 25 °C and 60% RH are provided in Fig. 14.

4. Discussion and conclusion

For the first time, sol-gel technology has been successfully introduced into 2 FDA-approved topical drugs, E-BPO/T for acne treatment and E-BPO for rosacea treatment. Sol-gel microencapsulation is a process that can be customized to match the active ingredient's specific chemical and physical characteristics and the desired duration of its effects. The microcapsules serve as a barrier between the active ingredient and the epidermis and result in the gradual release of BPO and tretinoin from the microcapsules onto the

Table 5
Tretinoin assay and related compounds in E-BPO/t creams containing E-ATRA suspensions with various EE levels.

EE of E-ATRA suspension	T=0			After 2 weeks at 40 °C/75% RH			
	Assay, %	5,6- epoxy retinoic acid, %	Total related compounds, %	Assay, %	5,6- epoxy retinoic acid, %	Total related compounds, %	Decrease in assay, %
98 %	0.0949	0.15	0.15	0.0932	0.76	1.32	1.8
94 %	0.0908	0.11	0.11	0.0878	1.01	1.86	3.3
86 %	0.0913	0.14	0.14	0.0865	1.19	2.17	5.3

E-ATRA, encapsulated all-trans retinoic acid; E-BPO, encapsulated benzoyl peroxide; EE, entrapment efficiency; RH, relative humidity; T, time.

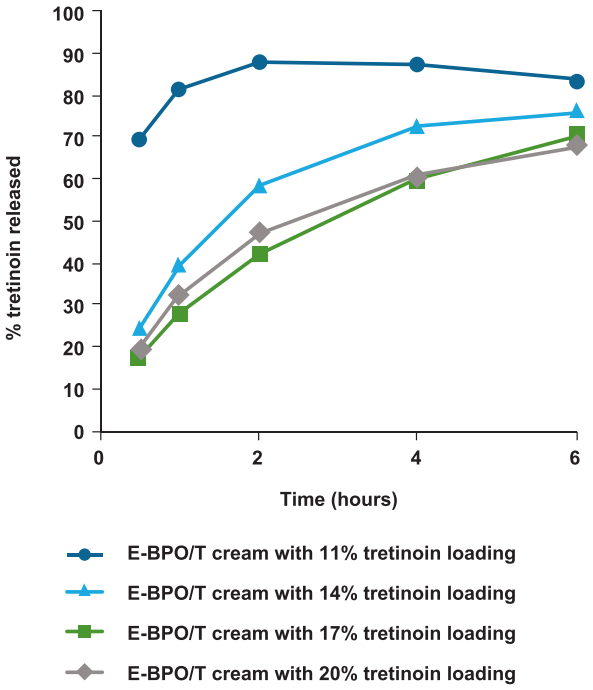

Fig. 12. Percentage of Tretinoin Released From E-BPO/T Cream With Various Tretinoin Loadings. E-ATRA, encapsulated all-trans retinoic acid; E-BPO, encapsulated benzoyl peroxide.

Table 6
Tretinoin assay and related compounds in creams at different tretinoin loadings.

Tretinoin loading, %	T=0		After 2 weeks at 40 °C/75% RH			
	Assay, %	5,6-epoxy retinoic acid, %	Assay, %	5,6-epoxy retinoic acid, %	Total related compounds, %	Decrease in assay, %
11 %	0.0995	<0.1	0.0883	1.5	4.3	11.3
14 %	0.1031	<0.1	0.0988	0.61	1.76	4.2
17 %	0.0982	<0.1	0.0948	0.5	1.49	3.5
20 %	0.0968	<0.1	0.0948	0.36	0.96	2.1

RH, relative humidity; T, time.

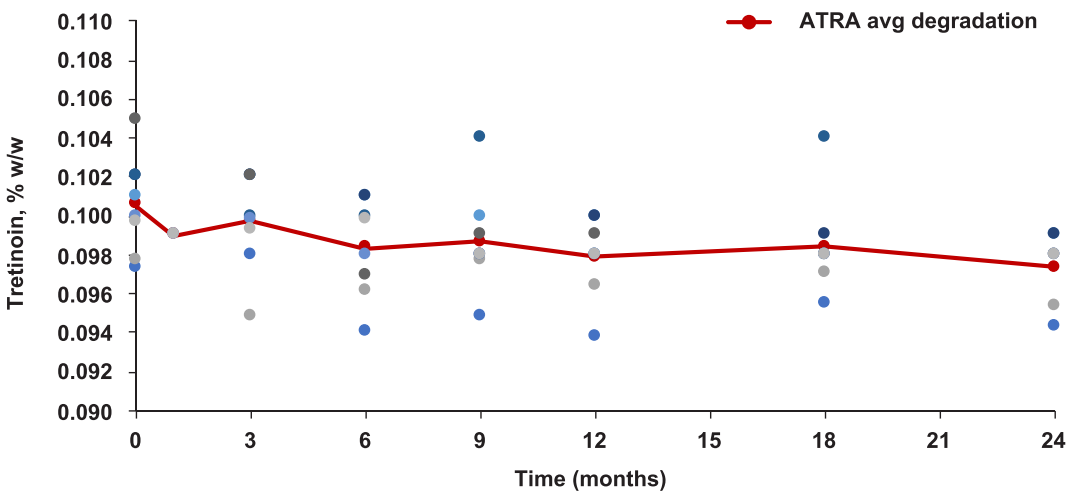

Fig. 13. Tretinoin Stability Results Over 24 Months at 2 °C–8 °C Storage Conditions. ATRA, all-trans retinoic acid; w/w, weight by total weight.

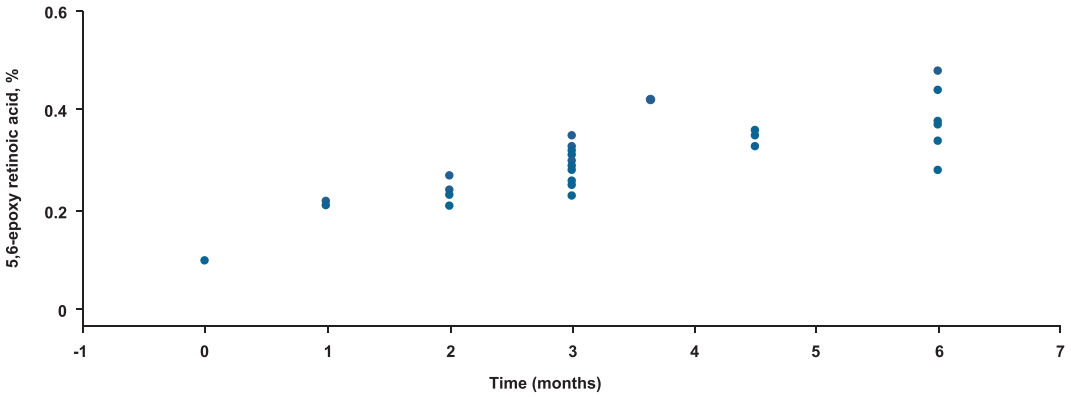

Fig. 14. Scattered diagram for levels of 5,6-epoxy retinoic acid after 6 Months of storage at the accelerated conditions of 25 °C/60% relative humidity.

skin, as shown in physiologically relevant conditions. This slow release likely contributes to the tolerability observed in the clinical trials. In E-BPO/T, the microcapsule barrier also stabilizes tretinoin in the presence of BPO because the 2 drugs are encapsulated separately.

The safety, efficacy, and tolerability of E-BPO and E-BPO/T were each evaluated in 2 randomized, double-blind, vehicle-controlled Phase 3 studies. E-BPO, 5%, was assessed in 733 adults with moderate to severe rosacea over 12 weeks [28]. Both primary endpoints were met (improvement in Investigator Global Assessment [IGA] score and improvement from baseline in inflammatory lesions) with a statistically significant improvement compared with vehicle. The most common adverse events reported were at the application site, including pain, pruritus, and erythema. Prior studies of BPO in rosacea found that while BPO was efficacious, it was not tolerated well by patients [5,9]. Because no head-to-head comparison of the safety and efficacy of E-BPO with unencapsulated BPO has been performed, these data cannot be compared.

E-BPO/T, 3%/0.1%, was evaluated in 858 participants 9 years and older with moderate to severe acne vulgaris for 12 weeks [29]. E-BPO/T showed significant improvement over vehicle in all 3 coprimary endpoints (IGA score and change from baseline in noninflammatory and inflammatory lesions). E-BPO/T cream was well tolerated in both studies.

In summary, these results demonstrate the promising potential of sol-gel technology to overcome API stability issues and improve skin tolerability to these products, thanks in part to the slow release from the silica shell. E-BPO/T cream, 3%/0.1%, and E-BPO cream, 5%, have provided remarkable progress in treating moderate to severe acne and rosacea by improving treatment adherence and patient outcomes. This technology has the potential to be utilized in other disease areas where treatment has been difficult due to tolerability or stability.

Role of the funding source

Study funding was provided by Sol-Gel Technologies, Ltd. All authors are employees of Sol-Gel Technologies, Ltd. Galderma Laboratories, Ltd. solely provided editorial support.

Data statement

The data is company proprietary knowledge and, therefore, will not be provided upon request.

Funding sources

Study funding was provided by Sol-Gel Technologies, Ltd.

Data availability

This data has not been deposited into a publicly available repository. The data used in this paper is confidential.

CRediT authorship contribution statement

Hila Hakak: Writing – original draft, Methodology, Investigation, Conceptualization. **Karine Neimann:** Supervision, Methodology, Investigation, Conceptualization, Writing – review & editing. **Ofer Toledano:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Maya Erlich:** Writing – original draft, Visualization, Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Ofer Toledano reports a relationship with Sol-Gel Technologies Ltd that includes: employment and equity or stocks. Maya Erlich reports a relationship with Sol-Gel Technologies Ltd that includes: employment and equity or stocks. Hila Hakak reports a relationship with Sol-Gel Technologies Ltd that includes: employment and equity or stocks. Karine Neimann reports a relationship with Sol-Gel Technologies Ltd that includes: employment and equity or stocks. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Alyssa Theodore, PhD, and Eileen Gamagami, PhD, of Simpson Healthcare, LLC, for their assistance in manuscript preparation. Their assistance was provided by Galderma Laboratories, Ltd.

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