Preparation, Characterization and In Vitro Release Test (IVRT) Study of Tretinoin-Loaded Microspheres in a Topical Gel Product

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PURPOSE

Over five decades, tretinoin has been used in the treatment of different indications, including acne vulgaris, however, topical tretinoin may cause skin irritation in some patients and is susceptible to photo degradation. Innovation led to the formulation of tretinoin in porous microparticles (microspheres) that offered controlled drug release, decreased irritation, and improved stability. Developing a generic product for tretinoin may involve utilizing the same microsphere product (i.e., Microsponge®) utilized in the reference product, or manufacturing the microspheres in-house. In both cases, the microspheres in the test product may exhibit different physicochemical characteristics than the reference product. These variations in physicochemical characteristics may result in different bioavailability and may impact the bioequivalence of the test product compared to the reference product (also see poster ID 895060).

OBJECTIVE

The objective of this study was to compare the physiochemical properties between the in-house prepared tretinoin microspheres with the microspheres that were separated from Retin-A Micro® (tretinoin) gel, 0.1%, and to evaluate the influence of physiochemical differences among microspheres on tretinoin release via in vitro release test (IVRT).

METHODS

Tretinoin-loaded microspheres were prepared by mixing tretinoin and Microsponge® 5640 in acetone followed by evaporating the solvent to achieve tretinoin concentrations at 0.5%, 1% and 2% w/w, respectively in the microspheres. The drug-loaded microspheres were characterized for particle size distribution and surface morphology by comparing them to the blank Microsponge® 5640, and the microspheres that were in (and separated from) Retin-A Micro® gel, 0.1% (Valeant Pharmaceuticals North America LLC., NJ) The particle size distribution was assessed using laser diffraction analysis. Surface morphology of microspheres was visualized by optical and scanning electron microscopy (SEM). Drug distribution in the internal space of the microparticles were evaluated by Raman mapping coupled with chemometric analysis. Drug release (IVRT) from the in-house drug-loaded microspheres 0.5%, 1% and 2% w/w, the separated microspheres, and Retin-A Micro® gel, 0.1% was studied using Vison® Microette™ Hanson vertical diffusion cells for 12 hours.

RESULTS

Morphology and surface topography of microspheres

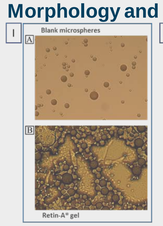

Fig I. Polarized light optical images at 200X magnification of blank microspheres (A), and microspheres separated from Retin-A Micro® gel (B).

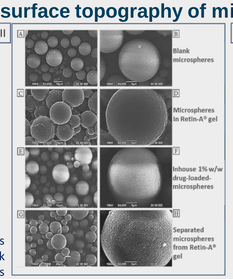

Fig II. SEM images at 800X magnification of blank microspheres (A and B), Retin-A Micro® gel (C and D), inhouse 1% w/w drug-loaded- microspheres (E and F), and separated microspheres from Retin-A Micro® gel (G and H).

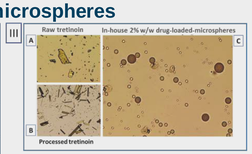

Fig III. Polarized light optical images at 200X magnification of raw tretinoin (A), processed tretinoin (B), and inhouse 2% w/w drug-loaded-microspheres (C).

- Both in-house drug-loaded microspheres and the microspheres from Retin-A Micro® gel were found to be of spherical shape, polydisperse, and free from aggregates as seen by optical and SEM.
- The surface of the drug-loaded microspheres was less smooth compared to the blank microspheres.
- No pores could be visualized on the surface of the microspheres using SEM at 5000X magnification
- Optical imaging of 2% w/w drug-loaded microspheres did not show any sign of tretinoin crystallization.

Particle size distribution of microspheres

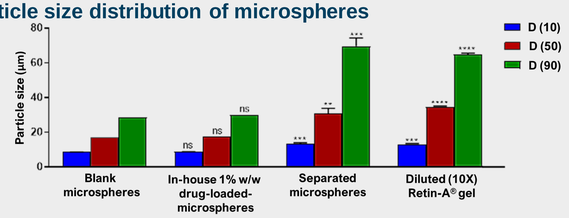

Fig IV. Volume-weighted mean particle size of blank microspheres, inhouse 1% w/w drug-loaded- microspheres, separated microspheres from Retin-A Micro® gel, and 10X diluted Retin-A Micro® gel (n=6). (ns = not significant; * p < .05, ** p < .01, *** p < .001, and **** p < .0001). The significant differences are relative to the D(10), D(50), or D(90) of the blank microspheres.

- The average particle size D (90) of the blank microspheres was 28.30 µm.
- After loading with the drug, the size of the microspheres increased by 1.6 µm.
- The microspheres in Retin-A® gel had a relatively larger size of 69.57 µm.

Tretinoin Recovery

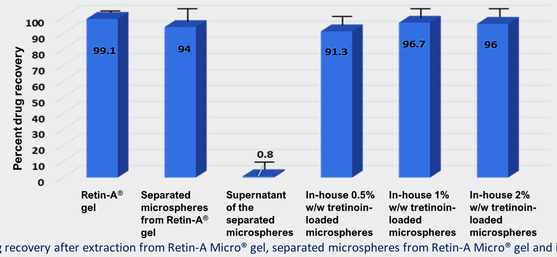

Fig V. Drug recovery after extraction from Retin-A Micro® gel, separated microspheres from Retin-A Micro® gel and inhouse 0.5%, 1%, and 2% w/w drug-loaded-microspheres. The numbers on the columns represent the average of three replicates for each study group. Standard deviations did not exceed 5% of stated values.

- 99% and 94% of tretinoin were recovered from Retin-A Micro® gel and separated microspheres from Retin-A Micro® gel
- Only 0.8% of tretinoin was found in the supernatant of the separated microspheres.
- Tretinoin was mostly present within the microspheres with minimal partitioning into the gel matrix.
- Over 91% of tretinoin was loaded into the microspheres for concentrations at 0.5%, 1%, and 2% w/w.

In vitro release test of tretinoin from the microspheres

Membrane selection

Table 1: Basic properties of the tested synthetic membranes

Membrane	Composition	Thickness (µM)	Pore size (µM)
Tuffryn	Hydrophilic polysulfone	145	0.45
Nylon	Hydrophilic nylon	170	0.45
Durapore	Hydrophilic polyvinylidene fluoride	125	0.45
Nuclepore	Track-etched polycarbonate	50	0.20

Diffusion rate of tretinoin through the tested membranes

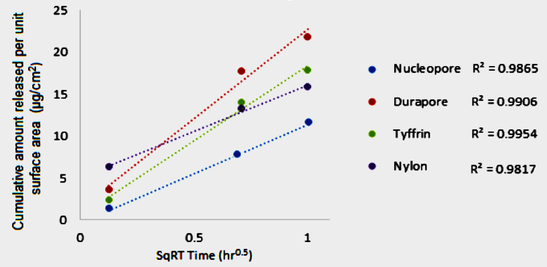

Fig VI. Diffusion rate of tretinoin from its 30 µg/mL tretinoin solution through Tuffryn, Nylon, Durapore, and Nucleopore. Data represent the average of three replicates for each study group.

- Tested membranes can be ranked according to the rate of tretinoin transport as Durapore (21.2 µg/cm²/h$^{0.5}$) > Tuffryn (18.1 µg/cm²/h$^{0.5}$) > Nucleopore (11.6 µg/cm²/h$^{0.5}$) > Nylon (11.0 µg/cm²/h$^{0.5}$).

Table 2: Percent recoveries of tretinoin after incubation of tretinoin solutions of 21µg/mL and 0.1µg/mL concentrations with various synthetic membranes.

Membrane	Theoretical concentration [µg.mL^{-1}]	Measured concentration [µg.mL^{-1}]	Recovery [%]
Whatman OE66 Cellulose acetate	0.130	0.131	101
	23.640	22.322	94
Millipore Fluoropore	0.130	0.133	102
	23.640	22.566	95
Millipore Durapore	0.130	0.134	103
	23.640	22.737	96
Whatman Nuclepore Track- Etch	0.130	0.139	**107**
	23.640	22.665	**96**

Table 3: Transport parameters of tretinoin across various synthetic membranes

Membrane	Transported amount after 3 h [µg·cm^{-2}]	
	Mean	RSD [%]
Whatman OE66 Cellulose acetate	29.61	2.97
Millipore Fluoropore	27.80	5.86
Millipore Durapore	29.71	7.18
Whatman Nuclepore Track- Etch	**30.59**	3.55

- Nucleopore membrane showed the highest percent of recovery and lowest affinity to bind with tretinoin when compared to the other tested membranes.
- Therefore, the IVRT experiments for Retin-A Micro® gel and microspheres were performed with Nucleopore membrane.

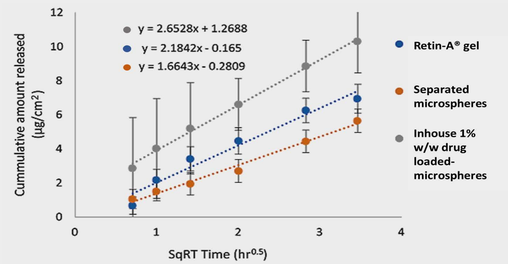

Fig VII. Release data of tretinoin from Retin-A Micro® gel, separated microspheres from Retin-A Micro® gel, and the inhouse 1% w/w drug-loaded-microspheres (n=4).

- Tretinoin release from in-house microspheres was faster than that from the separated microspheres ($p < 0.0001$).

Fig VIII. Release data of tretinoin from inhouse 0.5%, 1%, and 2% w/w drug-loaded-microspheres (n=4).

- Increasing tretinoin loading in the microspheres led to a significant ($p<0.05$) increase in the rates and amounts of tretinoin release.

CONCLUSION

- The IVRT method was found to be sensitive to the differences in tretinoin release rates.
- The drug release rates were influenced by the particle size and drug loading of the microspheres.
- Particle size, specific surface area, pore structure, and extent of drug loading of the microspheres may be critical physicochemical characteristics of tretinoin topical products containing microspheres.
- Tretinoin release from microspheres may be influenced by these critical physicochemical characteristics.

ACKNOWLEDGEMENT & DISCLAIMER

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