INTRODUCTION

Innovations in cosmetic science have created scientifically advanced personal care products using polymers as thickeners, protective barriers, fillers, aesthetic enhancers, and delivery agents of other active ingredients.¹⁻³ One of the most widely used polymers for its humectant properties in the cosmetic industry is hyaluronic acid (HA). This natural, hydrophilic dipolsaccharide polymer was first
In 1934, hyaluronic acid (HA) was discovered in the vitreous humor of bovine eyes and later identified as a member of the glycosaminoglycan (GAG) family of polysaccharides. This large polymer of high molecular weight (10^3–10^6 kDa) has also been identified in the synovial fluid of the joints and is essential for cell development, tissue maintenance, and wound healing. HA is a vital molecule of the extracellular matrix, facilitating cell behavior by maintaining the extracellular space and providing an open, but hydrated, structure for the passage of nutrients. HA is technically a mild polyelectrolyte acid with cations but is also known as its salt form, sodium hyaluronate (or hyaluronic acid), which is the form most commonly found in the body. It is also designated as hyaluronan, which is a general term referring to all forms of HA.

Well-known for its hygroscopic properties and ability to retain large amounts of water (6 l per gram), HA is responsible for the hydration and lubrication of tissues, with 50% of the total body HA located in the skin. As the principal component of the skin’s extracellular matrix, it is found in high concentrations in the basal layer of the epidermis. In addition to increasing the skin’s water-binding capacity, HA also protects against free radicals, has anti-inflammatory properties, and promotes cell motility, intercellular communication, and skin healing. Young skin contains large amounts of HA, having a high turnover rate with approximately one-third of it degraded and reproduced every day. However, as we age, HA is not reproduced as quickly as it degrades, contributing to the atrophy, dehydration, and loss of elasticity characteristics of aged skin. In addition, ultraviolet exposure, pollutants, and stress lead to increased HA degradation and subsequent reduction in HA’s protection against free radicals, further contributing to premature aging.

For these reasons, HA has been considered the gold standard ingredient for improving skin hydration and combating age-related effects. Although HA-based skincare products have been shown to reduce fine lines and wrinkles and improve skin elasticity, turgor, and hydration, their effectiveness is inefficient, largely formulation-dependent, and short-lived between applications. Injectable HA is effective in improving age-related skin effects; however, injectables can be costly and inconvenient because they require a visit to a doctor’s office for administration. Although topical delivery is more convenient, cost-friendly, and readily available, results are inefficient and inconsistent, partially due to its poor skin penetration and surface deposition as well as its rapid degradation.

Cosmetic scientists have been exploring innovative ways to improve the topical delivery of water and HA to the skin. In order to increase water-binding capabilities and decrease degradation, injectable HA products were modified to alter their structural properties. Often these modifications involved cross-linking of HA by covalent bonding to improve the viscous elastic properties, which increased water binding. Subsequently, products for topical application also evolved to include cross-linking and creation of synthesized HA derivatives. Cross-linking HA into an interpenetrating polymer network (IPN) alters its water-retention capabilities by providing more individual and stable traps for water in a thermodynamically stable state. A synthesized HA derivative, sodium acetylhyaluronate (AcHA, also known as super hyaluronic acid), developed by Oka et al., has been shown to improve hydrophobic interactions by anchoring at the skin surface. This water-soluble, branched polymer structure raises the intrinsic water-holding capabilities of the stratum corneum and induces a strong skin-softening effect. However, AcHA is a water-soluble, branched polymer structure that is incapable of forming a three-dimensional scaffold.

The ability of an IPN to attract and retain moisture to the skin would create an improved delivery system for HA, generating and maintaining a well-hydrated stratum corneum—a key dermatological endpoint. Therefore, we set out to create an ionically cross-linked network with HA and other polymers in the presence of water in the hopes of achieving superior water-binding capacity in a topical delivery vehicle. Our goal was to add some structural hydrophobicity to improve surface deposition on the skin without synthesizing a new polymer. Here we report on the synthesis and in vitro characterization of a hydrophobically modified IPN developed using three polymers into a three-dimensional formation (3D3P) for entrapping HA and water. This 3D3P-IPN polymer is a new molecular network with a rigid polymer structure that functions as a super-humectant, attracting and binding water molecules more efficiently than the traditional cosmetic-grade forms of HA.

2 MATERIALS AND METHODS

2.1 Synthesis of the 3D3P-IPN

Common formulation practice calls for dispersing and blending multiple polymers into skincare compositions for the purpose of improving hydration. The most recent definition as set forth by the International Union of Pure and Applied Chemistry (IUPAC) specifies an IPN as a polymer comprising two or more networks that are at least partially interlaced on a molecular scale but not covalently bonded to each other and that cannot be separated unless chemical bonds are broken. In basic terms, an IPN can be defined as a combination of two or more polymers in a network form where at least one is synthesized and/or cross-linked in the immediate presence of the other. Combining polymers in a way that allows the interlacing of the polymer networks on a molecular or near-molecular level makes it possible to achieve altered physical characteristics as compared to the same polymers simply blended together. Thus, synthesizing in juxtaposition a combination of two or more polymers in network form may offer unique skin-hydrating properties by the creation of a more rigid structure capable of improving water binding as well as modifications to the hydrophobicity of the hydrogel.

Our 3D3P-IPN was created by interlacing high-acyl and low-acyl gellan gums with a branched, hydrophobically modified cellulose and a linear medium-molecular-weight sodium hyaluronate. It was assembled by first unraveling dry-spooled gellan gums in water at a hydration temperature of 80°C under aggressive agitation conditions. When the gums reached full hydration, a solution of hydrophobically modified cellulose and a sodium hyaluronate in glycerin was introduced under slow agitation into the gellan gum solution.
After the three polymers become fully soluble in water at 80°C, they were interlaced by mechanical agitation. Once the polymer solution reached a high level of homogeneity, an activator solution of calcium ions was added in the form of pyroglutamic acid (PCA) salts to initiate cross-linking of the low-acyl gellan gum. Under agitation, the mixture was cooled to room temperature; this cooling allowed the formation of bonds for the final structuring of the high-acyl gellan gum. Additional common cosmetic ingredients were added including ceramides, preservatives, and fragrance. The end result was a three-dimensional, semisolid gel network that was further degraded down to a low-viscosity suspension of gelatinous particles by homogenization. Figure 1 represents a theoretical image of this three-dimensional gel network.

### Figure 1
Theoretical image of the 3D3P-IPN. The blue network fibers represent gellan gum structures reinforced with purple calcium cross-linked bridges (ionic bonds formed with the gellan gum carboxyl groups). The red and darker blue represent HA and hydrophobically modified cellulose polymers, orange represents cetyl modifications on the cellulose, and yellow represents the ceramides interlaced and entrapped inside the gellan structures.

### Table 1
Comparison of humectant ingredients in the 3D3P-IPN serums and a commercial reference product

| Parameter                        | Sample 1 3D3P-IPN laboratory preparation | Sample 2 3D3P-IPN scaled batch preparation | Sample 3 Commercial reference |
|----------------------------------|------------------------------------------|--------------------------------------------|-------------------------------|
| Appearance                       | Translucent gel particle suspension serum | Translucent gel particle suspension serum  | Homogenous opaque gel         |
| 3D3P-IPN content                 | 0.53%                                    | 0.53%                                      | none                          |
| Glycerin                         | 30%                                      | 30%                                        | Yes (% not determined)        |
| Polysaccharide polymers          | Sodium hyaluronate, cetyl hydroxyethylcellulose, and gellan gums | Sodium hyaluronate, cetyl hydroxyethylcellulose, and gellan gums | Sodium hyaluronate, hydroxyethylcellulose, and xanthan gum |
| Volatiles other than water       | None                                     | None                                       | Alcohol and cyclomethicone   |
| Electrolytes and osmolytes       | Magnesium PCA, calcium PCA, and sea salt | Magnesium PCA, calcium PCA, and sea salt   | Magnesium aspartate, zinc gluconate, copper gluconate, calcium gluconate, and betaine |
| Viscosity at near-zero shear rate| Thin serum ~0.5 Pa.s                     | N/A                                        | Cream-like gel ~5.5 Pa.s      |
|                                  | Low yield stress                         |                                            | Significant yield stress with shear-thinning rheology |

Note. 1 Pa.s = 1000 Centipoise.

#### 2.2 Compositional and analytical characterization

Three samples were analyzed to assess their compositional and analytical characteristics. Sample 1 was a laboratory-formulated serum containing 0.53% 3D3P-IPN (including 0.12% sodium hyaluronate) and glycerin and an assortment of preservatives, ceramides, and osmolytic ingredients such as extracts and PCA salts. Sample 2 was the full-scale, manufactured, packaged, and batch-filled equivalent of Sample 1. Sample 3 was a commercially available skin-hydrating product containing similar ingredients to the 3D3P-IPN serum including glycerin and three polysaccharide polymers (sodium hyaluronate, hydroxyethylcellulose, and xanthan gum) in addition to a variety of preservatives, electrolytes, and osmolytic ingredients. A comparison of the ingredients from the three samples is detailed in Table 1. Sample 3 was selected as a benchmark because of its similar water content, commercial availability, claimed ability to hydrate the skin, and compositional ingredients similar to the 3D3P-IPN samples although in a more viscous form. The commercial sample is marketed as a masque to deliver maximum hydration to the skin when used as directed. Several techniques were used to determine each sample’s physical and analytical characteristics, which included microscopic analysis, rheology, Karl Fischer (KF) titration, differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA), and dynamic vapor sorption (DVS) techniques.
Microscopic analysis of the samples was conducted at Sunny BioDiscovery, Santa Paula, CA, USA. Sample preparation included combining 50 μL of each sample with 5 μL of 0.2% (weight:volume) aqueous solution of Toluidine Blue O (cat.# 198 161, Sigma-Aldrich, St. Louis, MO, USA). Each sample was thinly spread on a microscope slide and observed after 15 minutes of rest under an inverted Amscope IN300TC-FL microscope. Images were captured with a color Discovery 15 CMOS microscope video camera using ISCapture software (Fuzhou Tucsen Phototonics Company, Fujian, China).

Rheology of the samples was characterized using a TA Instruments AR-G2/AR2000ex rheometer fitted with a 40 mm two-degree cone by standard operational instrument techniques at the Rodan + Fields Innovation Lab, Berkeley, CA, USA.

The percentage of water concentration in each sample was determined by KF titration using a Mettler Toledo model C20 coulometric KF titrator and Aquastar CombiCoulomat fritless methanol solution (MilliporeSigma, Burlington, MA, USA) as the reagent. The KF titrator was qualified for use prior to analysis using Hydranal® Water Standard 10.0 (Honeywell Fluka, Mexico City, Mexico).

DSC analyses were carried out using a TA Instruments Q2000 instrument temperature calibrated using indium prior to analysis. Approximately 18 to 20 mg of Samples 1 and 2 and 7 to 8 mg of Sample 3 were placed in a crimped T-zero aluminum pan and cooled to −30°C prior to being heated to 350°C at a rate of 10°C per minute. The DSC cell was kept under a nitrogen purge of about 50 mL per minute during each analysis. Data analysis was performed using TA Universal Analysis 2000, version 4.7B (Mettler Toledo, Columbus, OH, USA).

TGA analysis was performed using a TA Instruments Q50 TGA cooled using a TA Instruments Refrigerated Cooling System 90 chiller. Approximately 40 to 70 mg of each sample was loaded onto a platinum sample pan and heated from ambient temperature to 350°C at a rate of 10°C permin. The instrument was controlled using Thermal Advantage Release 5.2.5 software and data analyzed using Universal Analysis 2000 for Windows version 4.7B.

DVS analysis was conducted using standard methodology. Briefly, a TA Instruments Q5000 DVS analyzer was used after calibration with standard weights and a sodium bromide standard for humidity. Approximately 30 to 50 mg of each sample was loaded into a metal-coated quartz pan and analyzed at 25°C with a maximum equilibration time of 1 h in 10% relative humidity (RH). The samples were then exposed to increasing RH over a period of 1200 min while being measured for weight gain with respect to humidity and time. The adsorption cycle was conducted stepping from 5% to 95% RH and the desorption cycle from 95% to 5% RH, both in 10%-step RH increments. Movement from each step to the next occurred (1) after satisfying the equilibrium criterion of 0.01% weight change in 30 minutes or (2) if the weight change was not met within 1 h. Percent weight change values were calculated using Microsoft Excel 2016. KF, DSC, TGA, and DVS analyses were conducted at Triclinic Labs Inc., Lafayette, Indiana, USA.

3 | RESULTS

3.1 | Compositional characterizations

On visual, nonmagnified examination using a glass plate for spreading each sample, the 3D3P-IPN samples appeared as a thin, translucent serum with a visible suspension of gelatinous particles resembling another known cross-linked hyaluronic acid, Hylasome® (Luromed LLC, Orangeburg, NY, USA). Sample 3, the commercial

![FIGURE 2](image_url) Microphotographs of 3D3P-IPN (A, C, D) and a commercial moisturizer with similar ingredients (B). Note the globular, 3D network structure of 3D3P-IPN (A, C), which is absent in the noncross-linked moisturizer (B). The globular structures of 3D3P-IPN are aligned with thin scaffolding structures (see arrows) visible in a different focal plane (D), absent in the commercially available moisturizer. Magnification: X200

![FIGURE 2](image_url)
benchmark, was opaque, smooth, and viscous with no signs of suspended particulates or gelatinous globules. Under X200 magnification and stained with Toluidine Blue O to improve visualization of HA distribution, both 3D3P-IPN samples revealed a gelatinous, globular, three-dimensional arrangement of open fluid pockets that were interconnected with thin scaffolding structures (Figure 2). Sample 3, the commercial benchmark, appeared as a homogenous sheet with no observable suspended particulates, gelatinous globules, open pockets, or scaffolding-like structures such as those were observed in the 3D3P-IPN samples (Figure 2).

The rheological flow characteristics are compared in Figure 3 and Table 1. The 3D3P-IPN sample demonstrated almost no yield stress under shear, only minor shear-thinning properties, and a very low viscosity. Sample 3 exhibited substantially greater yield stress, with a viscosity that was 11 times greater compared with 3D3P-IPN sample. Sample 3 exhibited substantial shear-thinning properties evidenced by a typical pseudoplastic curve plot line (viscosity with respect to shear; Figure 3). The KF analysis indicated that all three samples contained similar concentrations of water, between 58% and 61% (Table 2). The concentration of water is important to establish in order to further explore the comparisons of bound-water behavior between the samples by gravimetric testing.

### 3.2 thermoanalytical and gravimetric characterizations

The DSC thermograms for all three samples are summarized in Table 3 with graphical representation shown in Supplemental Figure S1. Comparison of the DSC results shows the two 3D3P-IPN samples are similar; however, Sample 3 exhibited very different results. The 3D3P-IPN samples show one large endotherm at ~80°C and a smaller, yet important, endotherm at ~220°C consuming about 190 J/g. However, Sample 3 had a large endotherm at ~64°C followed by a series of three much smaller endotherms (adding up to a consumption of about 35 J/g) at increasingly higher temperatures. All the endothermic events are broad, suggesting that they are related to a loss of volatile components from the samples. As water is the primary volatile, it can be assumed that the first and second endotherm of Samples 1 and 2 represent water. The first large endotherm of Sample 3 can be attributed to a mixture of water, alcohol, and cyclomethicone. The boiling point of glycerin occurs at 290°C and is not likely represented in this analysis. Establishing the freezing behavior of water by DSC was unsuccessful in all samples, likely due to the limitations of the instrument and the complexity of composition of polymers in combination with electrolytes and humectants.

Comparison of the TGA results for the three samples shows two distinct weight-loss events for the laboratory and manufactured 3D3P-IPN samples; however, the commercial reference sample shows three distinct weight-loss events (Table 4 and Supplemental Figure S2). These weight-loss events occurred over different temperature ranges between the 3D3P-IPN samples and the commercial reference sample. In the 3D3P-IPN samples, the first weight-loss event is suspected to be from a loss of free and/or semibound water. The second weight-loss event occurred from the loss of more tightly bound water, glycols, and preservatives. In the commercial reference sample, the first weight-loss event is suspected to be from a loss of cyclomethicone, alcohol, and unbound water. The second event is

### Table 2 Karl Fischer titration results of the 3D3P-IPN serums and a commercial reference product

| Sample               | Sample weight (g) | Water content (%) | Mean water content (%) | Standard deviation (%) |
|----------------------|-------------------|-------------------|------------------------|------------------------|
| Sample 1 3D3P-IPN laboratory preparation | 0.0618 0.0669 0.0687 | 58.68 57.43 57.98 | 58.03 57.98 | 0.63 0.63 |
| Sample 2 3D3P-IPN full-scale batch preparation | 0.0442 0.0630 0.0526 | 61.05 56.03 60.80 | 59.31 2.80 |
| Sample 3 Commercial reference | 0.0789 0.0590 0.0835 | 60.27 60.25 61.03 | 60.52 0.44 |

FIGURE 3 Rheology characteristics of the 3D3P-IPN serums and a commercial reference product
hypothesized to be from semibound water and the third event from bound water, glycols, and preservatives.

The DVS analysis showed that both 3D3P-IPN samples absorbed appreciably more moisture, 23.8% and 29.9%, respectively, than the commercial reference (13.9%) between 35% and 95% RH (Table 5 and Supplemental Figure S3). All samples showed weight loss up to 35% RH and weight uptake between 35% and 95% RH. Weight loss up to 35% RH was about 10% higher in the commercial benchmark as compared to the 3D3P-IPN samples. Weight-loss during the entire desorption segment of the analysis was similar for the 3D3P-IPN samples (28.7% and 33.1%, respectively), which was more than the commercial reference of 17.0% (Table 5). It was observed that the 3D3P-IPN samples were capable of losing less water under dry atmospheric conditions below 35% RH and absorbing about 2-fold the amount of water in increasing humidity conditions between 35% and 95% RH as compared to the benchmark sample. (Supplemental Figure S3).

## DISCUSSION

The ability of topical moisturizers and emollients to generate and maintain a well-hydrated stratum corneum is largely dependent upon their ability to bind and retain water at the skin’s surface. We believe that improvements can be made to traditional (those containing unaltered HA) and advanced (those containing altered HA such as AcHA) topical humectants that contain sodium hyaluronate, as water may not bind as well as previously assumed. The water content from topical humectants can quickly evaporate from the warm skin surface, depending on the influence of RH and temperature, leaving a dehydrated product film along with HA and other nonvolatile ingredients. Others have pursued improving topical humectant performance by modifying HA through covalently bonded cross-linking structures and covalently bonded hydrophobic modifications (e.g., AcHA). Although these altered forms of HA do offer superior hydration benefits compared to traditional linear HA, they do not combine both properties of improved water binding by cross-linking with improved affinity to the skin’s surface through hydrophobic modifications. However, we theorized

### TABLE 3  Differential scanning calorimetry results of the 3D3P-IPN serums and a commercial reference product

| Sample                      | Endotherm | Enthalpy (J/g) |
|-----------------------------|-----------|---------------|
| Sample 1 3D3P-IPN laboratory preparation | -83°C | 1282          |
| Sample 2 3D3P-IPN batch preparation      | -82°C | 1347          |
| Sample 3 Commercial reference             | -64°C | 1207          |

### TABLE 4  Thermal gravimetric analysis results of the 3D3P-IPN serums and a commercial reference product

| Sample                      | Weight loss (%) | Temperature range | Reason for weight loss                        |
|-----------------------------|-----------------|-------------------|-----------------------------------------------|
| Sample 1 3D3P-IPN laboratory preparation | 61.9 | Ambient–167°C | Free water and semibound water |
|                             | 36.3 | 167°C–330°C  | Bound water, and glycols, preservatives       |
| Sample 2 3D3P-IPN batch preparation | 64.0 | Ambient–167°C | Free water and semibound water |
|                             | 34.2 | 167°C–330°C  | Bound water, and glycols, preservatives       |
| Sample 3 Commercial reference | 35.7 | Ambient–105°C | Cyclomethicone, alcohol, free water          |
|                             | 45.1 | 105°C–200°C   | Semibound water                               |
|                             | 11.0 | 200°C–330°C   | Bound water, glycols, and preservatives       |

### TABLE 5  Dynamic vapor sorption results of the 3D3P-IPN serums and a commercial reference product

| Sample                      | Weight loss up to 35% RH | Weight uptake 35% to 95% RH | Weight loss 95% to 5% RH |
|-----------------------------|--------------------------|-----------------------------|--------------------------|
| Sample 1 3D3P-IPN laboratory preparation | 52.1% | 56.9% | 63.5% |
| Sample 2 3D3P-IPN batch preparation | 56.9% | 29.9% | 13.9% |
| Sample 3 Commercial reference | 23.8% | 29.9% | 17.0% |
greater water binding and improved affinity for skin deposition could be achieved through the development of an IPN. In addition, we wanted this development of a superior IPN-based humectant to be simplified and not involve reactive chemistries with potentially hazardous compounds (eg, formaldehyde, glutaraldehyde, and divinyl sulfone) that are not suited for standard, finished cosmetic product manufacturing. (US Patent Publication US8080641 B).

In designing this super-humectant, we pursued the creation of an IPN that would integrate the attributes of three different polysaccharides into one structure held together by gellan gum’s cross-linking ability. We demonstrated that a three-dimensional, three-polymer IPN can be created with gellan gum, cetyl-modified cellulose, and sodium hyaluronate stabilized with calcium salts. This 3D3P-IPN can be utilized as a topical delivery vehicle for humectants, osmolytes, natural moisturizing factors, ceramides, and other agents that can increase and sustain moisture levels and help counter the signs of dry skin by supporting keratinocyte differentiation and degradation of desmosomes responsible for dry, flaky skin.

In comparison against a standard commercial hydrator, we found the 3D3P-IPN exhibits significant differences in visual appearance, appearing as a suspension of gelatinous gel particles that can be observed with and without magnification. The Toluidine Blue O-stained HA did not appear to be distributed in a continuous structure in the commercial sample. In contrast, the commercial HA masque prepared with sodium HA has a smooth, soluble, and continuous appearance and does not visually resemble a particle suspension.

Our rheological comparison also suggested that 3D3P-IPN does not retain the expected viscosity, shear-thinning, and yield stress profiles of a typical HA hydrogel. Traditional hydrogels and gels expand and swell in water, generally exhibiting a greater viscosity, significant yield stress, and shear-thinning properties. The rheological profile of 3D3P-IPN is inconsistent with a typical hydrogel continuous-phase preparation. Microscopic observations explain the rheological profile, showing that 3D3P-IPN does not exist as a continuous hydrogel but as a blend of interlaced polymers in a broken semisolid suspension of globular gel particles weakly attached by scaffolding fibers. The globular gel particles may be good traps for bound water.

The DSC and TGA analyses revealed very different behaviors of water activity between the 3D3P-IPN samples and the commercial benchmark. While all three samples exhibited similar amounts of water loss (up to ~200°C) the two 3D3P-IPN samples appeared to bind water more tightly than the benchmark sample as evidenced by the maximum endpoints of the endotherms occurring at significantly higher temperatures. The DVS results further confirm this speculation, as the 3D3P-IPN samples lost approximately 10% less water up to 35% RH than the commercial benchmark. The 3D3P-IPN samples also absorbed more water as the humidity level increased than the commercial benchmark, demonstrating superior humectant properties.

KF analysis indicated that all three samples had similar concentrations of water; however, TGA results were very impressive, demonstrating that a viscous, humectant-rich hydrating masque did not have much bound water. Our thin 3D3P-IPN serum revealed substantially more bound water that transitioned in two major events, both occurring at higher temperatures. Therefore, a thin serum can theoretically deliver more hydration to a skin’s surface than a viscous masque.

While our work does not compare the water activity of 3D3P-IPN to newer, covalently modified HA products, we borrowed some of the characteristics of these advanced HAs in preparation of the 3D3P-IPN. However, our results demonstrated that our 3D3P-IPN serum produced superior humectant properties in both absorbing water and binding water more tightly in comparison to the commercial HA-containing benchmark. This achievement in improving the water-binding properties of HA was executed without complex synthetic modification of HA, but by utilizing standard cosmetic compounding processes and ionic bonding. This makes 3D3P-IPN an attractive option for improving the performance of HA-based hydrators without the need for more complex and expensive processing that involves synthetics and hazardous substances.

Due to the DSC instrument limitations and the complexities of the sample formulations, we were not able to successfully study the glass transition and water dynamics under cooling conditions. Our temperature range for DSC analyses was 30°C to 350°C, which was sufficient for evaporation endotherms of water. An additional limitation to our research was the inability to compare exacting cosmetic formulations between our 3D3P-IPN formulation with the benchmark product because of its complex system of many ingredients. This limited us to provide only directional insight on how specific ingredients may alter the thermodynamic behavior of water. While significant distinctions were observed in the DSC endotherms and TGA weight loss, we applied the same assumptions to all compositions undergoing thermodynamic transition. Although water is the major component, glycol, preservatives, or any other volatile may have impacted these transitions and therefore subsequent assumptions. In addition, our characterization methods did not explore the hydrophobic properties of an IPN derived from the addition of the hydrophobically modified cellulose. These modifications would be more relevant for exploring the product interactions and substantivity at the skin surface during application and likely less relevant in studying bound water.

Through the synthesis of a 3D3P-IPN using simplified methods without hazardous compounds, we were able to increase the water-binding capacity of a low-viscosity HA-containing serum. The process was proven to be scalable in manufacturing. This 3D3P-IPN serum has the potential to deliver more hydration to the skin’s surface as compared to traditional HA-based formulations.

CONFLICTS OF INTEREST
Authors KR, KF, and TJF are employees of Rodan + Fields, San Francisco, CA. Author GPM was an employee of Rodan + Fields during the research presented within and the development of this manuscript.
REFERENCES

1. Patil A, Ferritto M, eds. Polymers for personal care products and cosmetics: overview. In: Polymers for Personal Care and Cosmetics. 1st ed. Washington, DC: American Chemical Society; 2013: 3-11. http://dx.doi.org/10.1039/9781782623984

2. Goddard ED, Gruber JV, eds. Principles of Polymer Science and Technology in Cosmetics and Personal Care. New York, NY: Marcel Dekker, Inc.; 1999: 68-688.

3. Lochhead RY. The role of polymers in cosmetics: recent trends. In: Cosmetic nanotechnology. American Chemical Society Symposium Series. Washington, DC: American Chemical Society; 2007:3-56. http://pubs.acs.org. Accessed July 25, 2018.

4. Meyer K, Palmer J. The polysaccharide of the vitreous humor. J Biol Chem. 1934;107:629-634.

5. Toole BP. Hyaluronan is not just a goo! J Clin Invest. 2000;106:335-336.

6. Salwowska NM, Bebenek KA, Żądło DA, Wcisło-Dziadecka DL. Physiochemical properties and application of hyaluronic acid: a systematic review. J Cosmet Dermatol. 2016;15:520-526.

7. Narurkar VA, Fabi SG, Bucay VW, et al. Rejuvenating hydrator: restoring epidermal hyaluronic acid homeostasis with instant benefits. J Drugs Dermatol. 2016;15:524-s37.

8. Dicker KT, Gurski LA, Pradhan-Bhatt S, Witt RL, Farach-Carson MC, Jia X. Hyaluronan: a simple polysaccharide with diverse biological functions. Acta Biomater. 2014;10:1558-1570.

9. Papakonstantinou E, Roth M, Karakulakis G. Hyaluronic acid: a key molecule in skin aging. Dermatoendocrinol. 2012;4:253-258.

10. Wiest L, Kerscher M. Native hyaluronic acid in dermatology – results of an expert meeting. JDDG. 2018;6:176-180.

11. Balazs EA, Laurent TC, Jeanloz RW. Nomenclature of hyaluronic acid. Biochem J. 1986;235.

12. Stem R. Hyaluronan catabolism: a new metabolic pathway. Eur J Cell Biol 2004;83:317-325.

13. Tammi R, Ripellino JA, Margolis RU, Maibach HI, Tammi M. Hyaluronate accumulation in human epidermis treated with retinoic acid in skin organ culture. J Invest Dermatol. 1989;92: 326-332.

14. Averbeck M, Gebhardt CA, Voigt S, et al. Differential regulation of hyaluronan metabolism in the epidermal and dermal compartments of human skin by UVB irradiation. J Invest Dermatol. 2007;127:687-697.

15. Trookman NS, Rizer RL, Ford R, Ho E, Gotz V. Immediate and long-term clinical benefits of a topical treatment for facial lines and wrinkles. J Clin Aesthet Dermatol. 2009;2:38-43.

16. McCall-Perez F, Stephens TJ, Herndon JC. Hyaluronic acid: efficacy and tolerability of a facial serum for fine lines, wrinkles, and photodamaged skin. J Clin Aesthet Dermatol. July, 2011;4:51-54.

17. Pavicic T, Gauglitz GG, Lersch P, et al. Efficacy of cream-based novel formulations of hyaluronic acid of different molecular weights in anti-wrinkle treatment. J Drugs Dermatol. 2011;10:990-1000.

18. Weindl G, Schaller M, Schäfer-Korting M, Korting HC. Hyaluronic acid in the treatment and prevention of skin diseases: molecular biological, pharmaceutical and clinical aspects. Skin Pharmacol Physiol. 2004;17:207-213.

19. Oka T, Uemura M, Ueno N, Yanaki T. Skin-softening effect of acetylated hyaluronate on stratum corneum. J Cosmet Sci. 1999;50:171-184.

20. Oka T, Ueno N, Yanaki T. Differential scanning calorimetry studies on the mechanism of skin-softening effect of sodium acetylhyaluronate. Polymer (Guildf). 2000;41:6055-6059.

21. Chen M, Gupta V, Anselmo AC, Muraski JA, Mitragotri S. Topical delivery of hyaluronic acid into skin using SPACE-peptide carriers. J Control Release. 2014;173:67-74.

22. Takigami S, Takigami M, Phillips GO. Effect of preparation method on the hydration characteristics of hylan and comparison with another highly cross-linked polysaccharide, gum Arabic. Carbohydr Polym. 1995;26:11-18.

23. Wohlrab J, Wohlrab D, Neubert RHH. Comparison of noncross-linked and cross-linked hyaluronic acid with regard to efficacy of the proliferative activity of cutaneous fibroblasts and keratinocytes in vitro. J Cosmet Dermatol. 2013;12:36-40. https://doi.org/10.1111/jocd.12024

24. Takigami S, Takigami M, Phillips GO. Hydration characteristics of the cross-linked hyaluronan derivative hylan. Carbohydr Polym. 1993;22:153-160.

25. Jenkins AD, Kratochvil P, Stepto RFT, Suter UW. Glossary of basic terms in polymer science (IUPAC Recommendations 1996). Int Union Pure Appl Chem. 1996;68:2287-2311. http://www.de-gruyter.com/view/j/jpac.1996.68.issue-12/pac199668122287/pac199668122287.xml. Accessed March 6, 2017.

26. Sperling LH. Interverpenetrating Polymer Networks and Related Materials. Boston, MA: Springer US; 1981. http://link.springer.com/10.1007/978-1-4684-3830-7. Accessed March 6, 2017.

27. American Chemical Society. Meeting (202nd: 1991: New York, NY, Klemperner D, Sperling LH, Leslie H, Utracki LA). Division of Polymeric Materials. Science and Engineering. Chemical Congress of North America (4th: 1991: New York, NY. Interverpenetrating polymer networks [Internet]. American Chemical Society: 1994:638. https://global.oup.com/academic/product/interverpenetrating-polymer-networks-9780841225822?cc=us&lang=en&. Accessed March 6, 2017.

28. Matricardi P, Cencetti C, Ria R, Alhaique F, Covelli T. Preparation and characterization of novel gellan gum hydrogels suitable for modified drug release. Molecules. 2009;14:3376-3391. http://www.mdpi.com/1420-3049/14/9/3376/. Accessed February 16, 2017.

29. Osmalek T, Froelich A, Tasarek S. Application of gellan gum in pharmacy and medicine. Int J Pharm 2014;466:328-340. http://linkinghub.elsevier.com/retrieve/pii/S0378517314001902. Accessed July 28, 2017.

30. Johnstone AH. CRC Handbook of Chemistry and Physics-69th Edition. Weast RC, ed. Boca Raton, FL: CRC Press Inc.; 1988.

31. Plazek DJ. Magnetic bearing torsional creep apparatus. Polym Phys. 1968;6:621-638.

32. Costello B. The AR-G2 magnetic bearing rheometer. 2005. http://www.tainstruments.com/pdf/literature/RL085_AR_G2_performance.pdf. Accessed November 6, 2017.

33. Kilpatrick-Livecor L, Polefka TA. Use of the dynamic vapor sorption meter to measure skin hydration properties, in vitro. Skin Res Technol. 2016;12:36-42. https://doi.org/10.1111/j.0909-725X.2006.00132.x.

34. Panagopoulou A, Molina JV, Kyritsis A, et al. Glass transition and water dynamics in hyaluronic acid hydrogels. Food Biophys. 2013;8:192-202. https://doi.org/10.1007/s11483-013-9295-2.

35. Saturnino C, Sinicropi MS, Parisi OI, et al. Acetylated hyaluronic acid: enhanced bioavailability and biological studies. Biomed Res Int. 2014;2014:921549. https://doi.org/10.1155/2014/921549.

36. Gibbs DA, Merrill EW, Smith KA, Balazs EA. Rheology of hyaluronic acid. Biopolymer. 1968;6:777-791.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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