

Influence of the pH in the transdermal delivery of hyaluronic acid

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Abstract. - In the formulation of a topical pharmaceutical product, several factors can influence the permeation profile, such as the physicochemical properties of the active ingredient, the pharmaceutical form, and other formulation parameters. A comprehensive understanding of the impact of these factors on product performance is essential for the successful development of a topical product. The objective of the present study was to investigate the influence of pH on the transdermal passage of hyaluronic acid (HA) across synthetic Strat-M® membranes. To achieve this, two formulations of hyaluronic acid were successfully developed: an aqueous solution and an aqueous gel. In both formulations, the pH was adjusted between 5 and 9, and the viscosities and contact angles of the HA aqueous solutions were determined. All formulations exhibited acceptable quality and remained stable for 90 days at room temperature and 40 °C. The results of this research indicated significant differences in the permeation of HA through the Strat-M® membrane due to the pronounced influence of pH. It was demonstrated that the aqueous solutions did not show variations in permeation, while the gel formulation exhibited an increase at pH 9, which represented double the permeation value through the membrane compared to the aqueous solution.

Keywords: Hyaluronic acid, pH, contact angle, transdermal passage, viscosity.

Influencia del pH en el pasaje transdérmico del ácido hialurónico

Resumen.- En la formulación de productos farmacéuticos de uso tópico, varios factores influyen en el perfil de permeación, incluyendo las propiedades fisicoquímicas del principio activo, la forma farmacéutica y diversos parámetros de formulación. Para lograr el desarrollo exitoso de una formulación es fundamental comprender cómo estos factores impactan su rendimiento. El objetivo de este estudio fue investigar la influencia del pH en la permeación transdérmica del ácido hialurónico (HA) a través de membranas sintéticas Strat-M®. Se desarrollaron dos formas farmacéuticas de ácido hialurónico: una solución acuosa y un gel acuoso, en las que se ajustó el pH entre 5 y 9. Se determinaron las viscosidades y los ángulos de contacto de las soluciones acuosas de HA. Todas las formulaciones presentaron una calidad aceptable y se mantuvieron estables durante 90 días a temperatura ambiente y 40 °C. Los resultados de la investigación evidenciaron diferencias significativas en la permeación de HA a través de la membrana Strat-M®, atribuibles al pH. Las soluciones acuosas no mostraron variaciones en la permeación, mientras que en el gel se observó un aumento en el pasaje a pH 9, alcanzando el doble del valor de permeación registrado en la solución acuosa.

Palabras clave: ácido hialurónico, pH, ángulo de contacto, pasaje transdérmico, viscosidad.

1. Introduction

Hyaluronic acid (HA) is a molecule consisting of units of D-glucuronic acid and N-acetylglucosamine linked in a dimeric structure, through β -(1 \rightarrow 3)-D-N-acetylglucosamine- β -(1 \rightarrow 4)- hyaluronic acid bonds (Figure 1). This heteropolysaccharide, which plays a decisive role in the physiology of many organs of the human body, is found in the skin where it exerts important physiological functions. Among them the maintenance of the viscoelasticity of the liquid

conjunctive tissues (synovial fluid in the joints or vitreous humor of the eye); the control of tissue hydration and water transport; the organization of proteoglycan in the extracellular matrix (ECM); tissue repair and various functions mediated by receptors in cell detachment, inflammation and tumoral development [1]. HA has specific physicochemical properties that make it a material widely used in biotechnological, biomedical, and cosmetic products [2] [3], due to its hydrating power that is given by the ability to form hydrogen bridges with water, thus helping to maintain of elasticity, turgor and moisture of the skin. [4]

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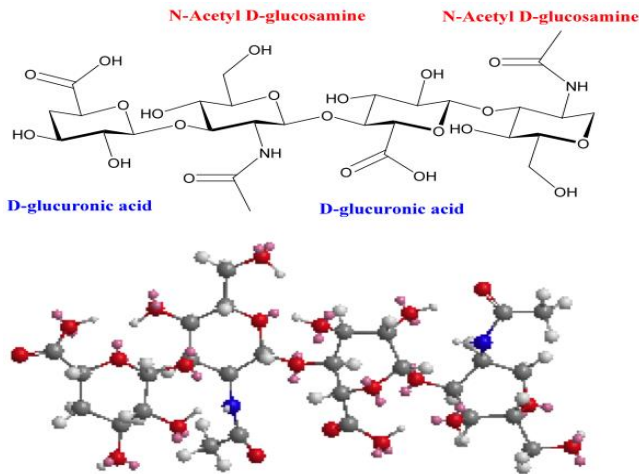


Figure 1. Hyaluronic acid general structure

The skin is the lining of the human body, being the largest organ in constant renewal. It consists of three layers, the most superficial called epidermis, followed by the dermis, and a deeper one known as hypodermis. The skin allows the passage of certain molecules from its surface, crossing the stratum corneum under the influence of a concentration gradient, and their subsequent diffusion through it to the epidermis and dermis and finally reaching the bloodstream. This process is known as absorption by passive diffusion, and is the mechanism most used by substances to cross biological membranes [5].

The cutaneous structure is an excellent way of applying topical products, with huge advantages, such as its ease of use. Other benefits are that the first step of hepatic metabolism is avoided thus reducing the risk of systemic effects, it turns out to be a painless administration and it avoids the pain inherent to intramuscular or subcutaneous administration, resulting more comfortable for the patient [6].

The skin absorption process will depend on the barrier function that resides in the most superficial layer of the epidermis, called the corneal layer. This is a simple structure in which the flattened cells, called corneocytes, are composed mainly of keratin, surrounded by an extracellular medium of lipids organized in the form of multiple lamellar bilayers that modulate the insensible loss of water and condition the entry of substances.

In topical application of products, it is pursued that the active principle arrives at the site of action so that it exerts a systemic or local effect. This will depend, mainly, on the physicochemical characteristics of the molecule, such as the lipid/water partition coefficient, the diffusion coefficient, its lipophilicity and chemical stability, as well as the pKa and pH, which influences the degree of ionization [7].

The pH of the stratum corneum is acid, with a range between 4 and 6, so it is called the acid mantle. The stratum corneum was first described by Schade & Marchionini in 1928 as a thin film composed of fatty acids, amino acids and other organic acids deposited on the surface of the skin. Several studies have shown that its acidity is given by exogenous mechanisms that include the generation of free fatty acids by bacterial lipases and sebum derivatives, as well as by compounds derived from the eccrine glands [8].

Historically, it was thought that acidic pH was only a defense mechanism of the skin against microorganisms. However, recently, its active participation in the maintenance of the barrier function has been demonstrated, since it is involved in the synthesis of lipids and in the formation of the lamellar structures of the stratum corneum. On the other hand, it has been shown that an increase of pH in the skin causes a disruption of the barrier, associated to the increase of the activity of the serine protease enzymes, as well as the reduction of the enzymes involved in the formation of ceramides, thus affecting the integrity, cohesion and desquamation from the stratum corneum [9].

In the present work, the effect of pH variations in the transdermal passage of the HA was studied through the Franz diffusion cells, using Strat-M® synthetic membranes. In addition, the effect of pH on the contact angle of the aqueous solutions of the HA and on the viscosity of the two pharmaceutical forms was determined. To this end, two systems were used: an aqueous solution and an aqueous gel of hyaluronic acid, at a concentration of 7000 ppm (mg/L). All the systems were formulated in the Laboratory of Formulation, Interface, Rheology and Processes (FIRP).

2. Methodology

2.1. Franz cell diffusion

The Franz cell (Figure 2) is a device used in the *in vitro* kinetic permeation assays. It consists of an upper compartment (donor), where the system under study is placed (vehicle with the active) and a lower compartment (receptor) with a liquid, in this case saline buffer at pH 7.4; very similar to that of the extracellular fluid of mammals. The Strat-M® membrane is placed between the two compartments.

The Strat-M® membrane is constituted by two layers of polyether sulfone and polyolefin with mixtures of synthetic lipids that provide some resistance to the passage of substance which tries to recreate the composition of human skin [10]. The contact area in the cell is 0.7854 cm².

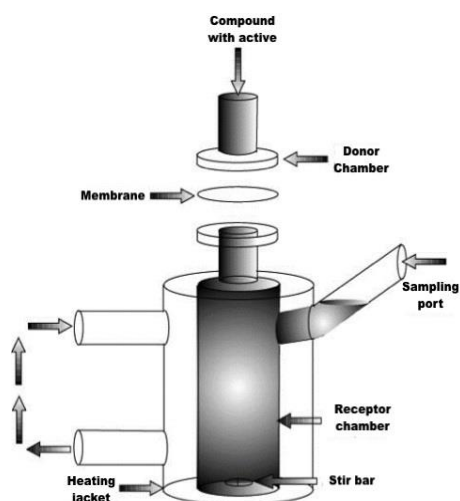


Figure 2. Franz diffusion cell

The lower compartment of the cell has a volume of 10 mL and has a lateral sampling arm, where aliquots are taken to analyze the accumulated concentration of the assets that have passed through the membrane. The cell has an external jacket that can be connected to a thermostatic bath with recirculation, in order to ensure a constant temperature during the release tests. The cells were maintained at (37 ± 0.5) °C in all the experiments, this temperature is generally accepted as the average body temperature [11].

It is necessary to maintain a total contact between the different formulations of the HA to be studied,

the membrane and the solution contained in the receptor compartment (Buffer), so that the transfer area through which the permeation occurs is constant. That is why 6 cells were assembled in series with agitation with an Ika-Werke magnetic plate of 10 agitation points. All the tests were carried out in triplicate and the average of them is reported.

2.2. Hyaluronic acid

The HA used was a solution of 7000 ppm (mg/L) of Dekluron M 1% (1% solution of sodium hyaluronate) from Jam Dekker International. IMCD Benelux B.R. Holand.

In the same way, solutions of a gel at 7000 ppm (mg/L) of HA were used. The aqueous gel contains as a viscosifying agent a crosslinked polymer of acrylic acid carbomer, at a concentration of 0.30%. The final pH of each of the pharmaceutical forms was adjusted to the values of interest in the investigation: 5, 6, 7 and 9 respectively, with triethanolamine (Merck®).

The composition of the two pharmaceutical forms are presented in Table 1.

A precision Shimadzu UV Mini 1240 Uv-vis Spectrophotometer was used to determine the concentration of hyaluronic. The measurements were made at a wavelength of 256 nm.

Table 1. Composition of the pharmaceutical forms

Ingredients	Concentration (wt%)	
	Aqueous solution	Aqueous gel
Water	q.s. 100	
Hyaluronic acid	0.70	0.70
Viscosity agent	---	0.30
pH adjuster (5, 6, 7, 9)	q. s	q. s

2.3. Salty buffer

For the preparation of the saline buffer, 1,5125 g of Na₂HPO₄ and 2,0875 g of KH₂PO₄ (100% purity, Riedel-de Haën) are weighed, then added in a 250 ml volumetric flask, 80% of the required

volume of water is added distilled, mixing continuously.

2.4. Viscosity measurements

The viscosity measurements were carried out with a controlled voltage AG-G2 rheometer (TA Instruments) using conical plate geometries (cone diameter, 40 mm, cone angle 1). The measurement temperature was 37 °C.

2.5. Determination of the contact angle

The contact angle measurements of the HA solutions on the Strat-M® membranes were made with the Ramé Hart 200 goniometer. For the measurement, a drop of the solution is dropped on the surface of the Strat-M® membrane making a digital capture of the image. The contact angles were determined at room temperature.

3. Analysis and discussion of results

The results of the viscosity measurements are shown in the following table.

Table 2. Physicochemical properties and transdermal passage of HS formulations at different pH values

Systems	pH	Viscosity cP 1000 S ⁻¹	Transdermal passage ppm (mg/L)	Contact angle
Aqueous solution	5	3,01	307	---
	6	3,35	249	---
	7	3,11	297	---
	9	3,05	332	---
Aqueous gel	5	857	482	55
	6	1065	400	50
	7	1135	477	52
	9	159,7	632	57

The results of the transdermal passage tests are reported in Figure 3, for the aqueous solution, and Figure 4 for the aqueous gel. In Figure 3 it can be seen that the transdermal passage of HA is practically independent of pH and time. Only at pH 9 it slightly increases, after 50 minutes of contact. In aqueous solution there is no pH effect, the acid passage does not depend on the degradation or the

superficial load of the HA molecule, it is independent of the pH [12].

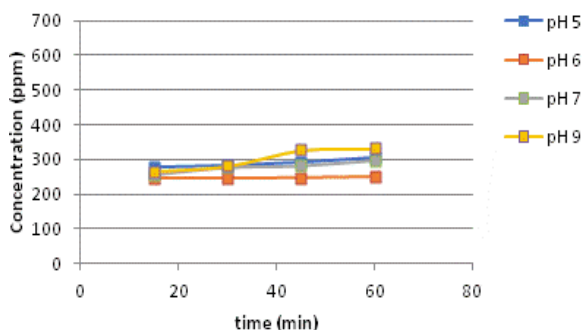


Figure 3. Results of the transdermal passage on the Strat-M® membrane for the aqueous solution of AH at 7000 ppm (mg / L)

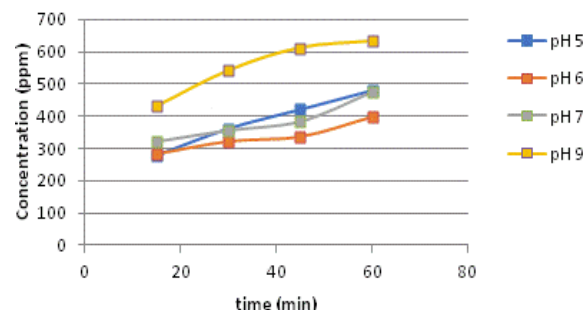


Figure 4. Results of the transdermal passage on the Strat-M® membrane for the aqueous AH gel at 7000 ppm (mg / L)

For the aqueous solutions of HA, no significant variation in viscosities was observed in the samples, suggesting that there is no relevant viscosity effect in the transport of the active ingredient across the membrane; the rheological behavior is not greatly influenced by pH. This is attributed to cooperative interactions between chains due to the reduction of the polymers net charge and may be related to the protonation of acetamide groups. These results correspond to those obtained by [13], in his research on the effects of pH on the behavior of diluted and semi-diluted aqueous solutions of hyaluronic acid.

In the transdermal passage, the alkaline pH of the formulation (pH 9) affects the ionization of the functional groups present in the hyaluronic acid molecule. The structure has carboxyl groups (-COOH) that can ionize, and a greater proportion of these groups will be in their ionized form (-COO⁻), which increases the negative charge of the

molecule. This increase in negative charge may enhance the solubility of hyaluronic acid in water, and also generate electrostatic repulsion between hyaluronic acid molecules, affecting their physical properties and their ability to form three-dimensional networks. This is important for their function in the viscosity and elasticity of gels, as well as in their interaction with other macromolecules in the tissue. [14] [15]. Likewise, the contact angles between the aqueous solution of the HA and the membrane (Figure 5 and Table 3) do not vary significantly with the pH value.

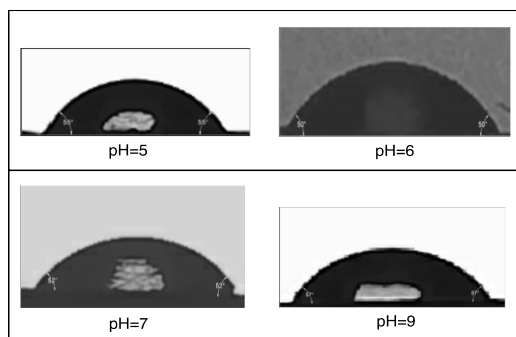


Figure 5. Contact angle of the HA aqueous solution on the Strat-M® membrane at different pH values

4. Conclusions

For the aqueous solution the passage of the HA is independent of time and pH while for the gel its viscosity is affected by pH changes, probably due to the effect of the latter on the viscosifying agent. The absorption of the HA through the Strat-M® synthetic membranes showed a dependency with the pH and the viscosity of the formulation for the gel formulation. At pH 9, the gel has the lowest viscosity and the largest passage. The consistency of viscosity in aqueous HA solutions highlights a very important aspect of its molecular interactions under various pH conditions, suggesting that despite the increased negative charge, the cooperative nature of the interactions within the polymer network allows for effective functionality. The potential impact of these properties on the solubility and mechanical characteristics of HA should be carefully considered when developing new formulations for therapeutic applications.

This study confirms that pH is a determining factor in the transdermal permeation of hyaluronic acid gel formulations at pH 9 show significant permeability compared to aqueous solutions, suggesting a way to optimize topical formulations containing HA. Additional more studies are recommended to explore the impact of pH in different formulations for pharmaceutical and dermocosmetic applications.

5. References

- [1] C. Buckley, EJ. Murphy, TR. Montgomery, I. Major, "Hyaluronic Acid: A Review of the Drug Delivery Capabilities of This Naturally Occurring Polysaccharide," *Polymers*, vol. 14, pp. 1-23, 2022. DOI: [10.3390/polym14173442](https://doi.org/10.3390/polym14173442)
- [2] P. Ratajczak, O. Maciejak, D. Kopciuch, A. Paczkowska, T. Zaprutko, K. Kus, "Directions of hyaluronic acid applications in cosmetology", *J Cosmet Dermatol.*, vol. 22, pp. 862-871, 2023. DOI: [10.1111/jocd.15485](https://doi.org/10.1111/jocd.15485)
- [3] T. Pavici, G. Gauglitz, P. Lersch, K. Schwach-Abdellaoui, B. Malle, HC. Korting, M. Farwick, "Efficacy of cream-based novel formulations of hyaluronic acid of different molecular weights in anti-wrinkle treatment". *J Drugs Dermatol.*, vol. 10, pp. 990-1000, 2011. URL: https://www.researchgate.net/publication/51769103_Efficacy_of_CreamBased_Novel_Formulations_of_Hyaluronic_Acid_of_Different_Molecular_Weights_in_Anti-Wrinkle_Treatment
- [4] M. Essendoubi, C. Gobinet, R. Reynaud, J. Angiboust, M. Manfait, O. Piot, "Human skin penetration of hyaluronic acid of different molecular weights as probed by Raman spectroscopy", *Skin Res Technol.*, vol. 22, pp. 55-62. 2016. DOI: [10.1111/srt.12228](https://doi.org/10.1111/srt.12228)
- [5] V. Phatale, K. Klaudi Vaiphei, S. Jha, D. Patil, A. Alexander, "Overcoming skin barriers through advanced transdermal drug delivery approaches," *J Control Release*, vol. pp. 361-380. 2022. DOI: [10.1016/j.jconrel.2022.09.025](https://doi.org/10.1016/j.jconrel.2022.09.025)
- [6] B. Alhasso, U. Ghori, C. Barbara, "Systematic Review on the Effectiveness of Essential and

- Carrier Oils as Skin Penetration Enhancers in Pharmaceutical Formulations,” *Scientia Pharmaceutica*, vol. 90, pp. 1-25. 2022. DOI: 10.3390/scipharm90010014
- [7] M. Lukic, I. Pantelic, S. Savic, “Towards Optimal pH of the Skin and Topical Formulations: From the Current State of the Art to Tailored Products,” *Cosmetics*, vol. 8, pp. 1-18. 2021. DOI: 10.3390/cosmetics8030069
- [8] C. Suber, P. Humbert, C. Abels, M. Howard, “The Acid Mantle: A Myth or an Essential Part of Skin Health?” *Curr Probl Dermatol.*, vol. 54, pp. 1-10, 2018. DOI: [10.1159/000489512](https://doi.org/10.1159/000489512)
- [9] M. Farage, W. Hood, B. Enzo, M. Howard, “Intrinsic and Extrinsic Factors Affecting Skin Surface pH,” *Curr Probl Dermatol.*, vol. 54, pp. 33-47, 2018. DOI: 10.1159/000489516
- [10] P. Bolla, C. Bradley, J. Abhishek, S. Hanumanth, R. Jwala, “Evaluation of Formulation Parameters on Permeation of Ibuprofen from Topical Formulations Using Strat-M® Membrane,” *Pharmaceutics*, vol. 12, pp. 1-19, 2020. DOI: [10.3390/pharmaceutics12020151](https://doi.org/10.3390/pharmaceutics12020151)
- [11] G. Saiko, “Skin Temperature: The Impact of Perfusion, Epidermis Thickness, and Skin Wetness,” *Applied Sciences*, vol. 12, pp. 1-14. 2022. DOI: [10.3390/app12147106](https://doi.org/10.3390/app12147106)
- [12] V.D. Eliane, G. Giulia, K. Gijssge, B. Huibb, “Hyaluronan biopolymers release water upon pH-induced gelation,” *Phys. Chem. Chem. Phys.* vol. 22, pp. 1-5, 2020. DOI: [10.1039/D0CP00215A](https://doi.org/10.1039/D0CP00215A)
- [13] A. Maleki, A. Kjøniksen, B. Nystrom, “Effect of pH on the Behavior of Hyaluronic Acid in Dilute and Semidilute Aqueous Solutions,” *Macromol. Symp.*, vol. 274, pp. 131-140, 2008. DOI: [10.1002/masy.200851418](https://doi.org/10.1002/masy.200851418)
- [14] K. Hong-Seop, Ch. Ji-Youn, K. Yoon-Young, P. Moon-Soo, “Viscosity and Wettability of Hyaluronic Acid according to Antimicrobial Supplementation, Ionic Strength, and pH,” *Journal of Oral Medicine and Pain*, vol. 39, pp. 90-95, 2014. DOI: [10.14476/jomp.2014.39.3.90](https://doi.org/10.14476/jomp.2014.39.3.90)
- [15] B. Kim, S. Woo, Y. Park, E. Hwang, M. Moon, “Ionic strength effect on molecular structure of hyaluronic acid investigated by flow field-flow fractionation and multiangle light scattering,” *Anal Bioanal Chem.*, vol. 407, pp. 1327-34, 2015. DOI: [10.1007/s00216-014-8379-2](https://doi.org/10.1007/s00216-014-8379-2)