

Assessment of Topical Formulations Skin Permeation Using Raman Spectroscopy

Peña-Velasco, Gabriela

Universidad Autónoma del Estado de Morelos,
Facultad de Farmacia, Cuernavaca, Morelos

Amador-González, Enrique

Universidad Nacional Autónoma de México,
Facultad de Química, México

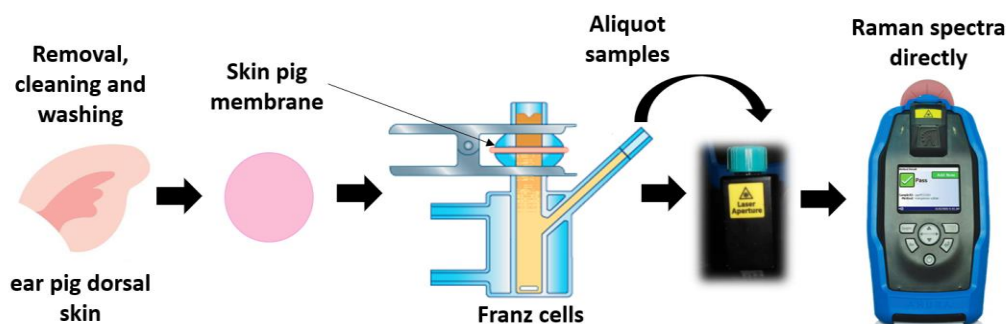
Melgoza-Contreras, Luz María

Universidad Autónoma Metropolitana,
Departamento de Ciencias Biológicas, México

Hernández-Baltazar, Efrén

Universidad Autónoma del Estado de Morelos,
Facultad de Farmacia, Cuernavaca, Morelos

GRAPHICAL ABSTRACT



ABSTRACT

The use of new noninvasive analytical techniques and procedures for the assessment of topical formulation skin permeation has been a challenge for pharmaceutical sciences. In recent years, Raman spectroscopy has been limited to the identification of components inside a sample or unknown substances. In this work, a handheld Raman spectrometer was used in the follow-up of active pharmaceutical ingredients (APIs) in topical formulations. Thus, in combination with Franz cells and Tape stripping procedures, permeation flux and retained drug amount in layers of skin were evaluated in hydrogels of lidocaine (LD) and meloxicam (MX) with myristate isopropyl (IPM). The proposed method allows lower analysis time, simple operation, collects direct measures of APIs without preview sample treatment, and avoids the use of solvents in support of eco-friendly.

The obtained results demonstrate the effective use of IPM as a permeation promotor agent increasing the permeation flux of 59.9 to 72.2 cm/min and 31.8 to 41.0 cm/min for LD and MX hydrogels, respectively. The developed analytical method by Raman spectroscopy obtained determination coefficients of $r^2 \leq 0.993$, an inter-day precision (repeatability) of $\%RSD \leq 5\%$, limits of detection (LODs) and quantification (LOQs) from 0.05 and 0.07 mg/mL, respectively. Finally, the advantages and limitations of this proposed quantification alternative were compared with other analytical methods, which could suggest their potential application and incorporation in standardized guidelines of skin permeation.

Keywords: Raman spectroscopy, hydrogels, skin permeation, lidocaine, meloxicam.

INTRODUCTION

In recent years, portable Raman spectrometers equipment has been further used in the pharmacy industry for identifying APIs, in real-time monitoring processes, and indirect quantification of powders [1–3]. In regard to bioanalysis, micro-spectroscopy and Confocal Raman micro-spectroscopy (CRM) are techniques that dominant to a great number of research [4–6]. Specifically for the permeation skin studies, until now, cells Franz diffusion with subsequent analysis for high-resolution liquids chromatographic or ultraviolet spectrometry, Tape Stripping coupled Confocal laser scanning microscopy (CLSM), Skin biopsy, and Suction blister, have been the mainly used techniques for the assessment permeation flux of API principles across the skin [7,8]. The majority with limitations such as ethical difficulties, high cost, variations in skin types (synthetic, animal or human), low reproducibility, laborious and high consumption time analysis, invasive processes [7,9]. Therefore, the importance of the use of new techniques that facilitate the assessment of permeation fluxes of APIs in topical formulations could make an important contribution to the field of pharmaceutic and cosmetology sciences. The purpose of the present research was the study the application of portable Raman spectroscopy combined with Franz cells and Tape stripping for assessment and monitoring permeation flux of two hydrogel formulations. Lidocaine (LD) and meloxicam hydrogels were used as models of topical pharmaceutical formulations, with IPM as an enhancer permeation agent.

EXPERIMENTAL SECTION

Materials

In all the experiments high purity deionized water was used. Lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl \cdot H_2O$) and Meloxicam ($C_{14}H_{13}N_3O_4S_2$) standards were high purity ($\geq 98\%$). For the preparation of hydrogels, sodium carboxymethyl cellulose, glycerin, and IPM were used as excipients. All the reagents used were purchased from Sigma Aldrich. For the assessment permeation flux of APIs, HAWP type 0.45 μ m Millipore nitrocellulose filter and vertical Franz type diffusion cells were used. LD and MX standards stock solutions were prepared at 100 and 2 mg/mL, respectively. From them and using the corresponding dilution of individual stock solutions were constructed the calibration curves for the quantification of target analytes and calculated statical parameters. Table 1 shows the mainly properties of model drugs studied.

Table 1 Properties of model drugs for hydrogel formulations.

	Lidocaine Hydrochloride	Meloxicam
Molecular weight (g/mol)	270.8	351.4
pka	7.7	4.08
LogP	2.44	3.54
Solubility (mg/mL at 25°C)	50	< 0.1
TPSA (Å²)	32.3	136

LogP = octanol-water partition coefficient, TPSA= Topological Polar Surface Area

Methods

Preparation of Hydrogels:

Two different formulations of LD and MX hydrogels were prepared and evaluated, respectively (with and without IPM added in them). Table 2 shows the composition of each formulation. Finally, the prepared hydrogels in an amber glass recipient at room temperature for 24 h before use were stored. The fabricated hydrogels were labeled as LD and MX for lidocaine and meloxicam hydrogel, respectively, the formulations with IPM added were identified as LD-IPM and MX-IPM, respectively.

Table 2 Composition of prepared lidocaine and meloxicam hydrogels formulations.

	LD Hydrogel	LD-IPM Hydrogel	MX Hydrogel	MX-IPM Hydrogel
Carboxymethylcellulose sodium (2%)	*	*	*	*
Glycerin (10%)	*	*	*	*
Meloxicam (0.3%)			*	*
Lidocaine hydrochloride (10%)	*	*		
IPM (1%)		*		*
Water q.s.	100 mL	100 mL	100 mL	100 mL

Procedure for the Obtention of Skin Membrane:

Used the test No. 428: Skin absorption: *in vitro* method from the *Guidelines for the Testing of Chemicals* [10], skin from human or animal sources can be used and it is essential that skin is properly prepared [11]. Pig skin has been reported with similar features to human beings, making it a suitable animal model for permeation tests [8]. Therefore, excised skin ear pig dorsal surface was used, purchased from the slaughterhouse in Cuernavaca, Morelos, México. Briefly, the pig skin membrane was prepared as previously reported [9], a first step of cleaning with distilled water, for removing traces of dirt, blood, and/or others. The membrane skin was extracted manually with a scalpel, separating the subcutaneous fatty layer from the skin (extracting the dermis without subcutaneous tissue) leaving the stratum corneum intact. The skin membrane obtained and conditioned with phosphate-buffered saline (PBS pH 7.4) was stored at -20 °C until use.

Franz Diffusion Cell Permeation Studies:

Vertical Franz-type diffusion cells with an average area and volume of 3.31 cm² and 24.84 cm³ were used. Therefore, skin pig circular samples of around 3.31 cm² were placed between the donor and receiver chamber of a Franz cell, with the stratum corneum facing the donor chamber. The receptor compartment was filled with 25 ml of phosphate-buffered saline (pH

7.4), magnetically stirred at 400 rpm, and at 32°C was kept. Later, 500 mg of the test hydrogel formulation was applied to the skin surface in the donor cell and was sealed with parafilm to avoid evaporation. The LD and MX permeation flux were monitored for 2 and 6 h, respectively, taking samples (1 ml) at the desired time intervals and replacing the volume with the same amount of PBS. For comparative purposes, Franz diffusion cell study in synthetic membrane (HAWP type 0.45µm Millipore nitrocellulose filter) was realized using the same conditions and equipment previously described. The flux value (J_{ss}) was obtained from the slope of the linear regression analysis for each experiment. The apparent permeability coefficient (P_{app}) was calculated as previous reports [12,13] according to Fick law diffusion, taking initial drug concentration (C_0) using the equation (1):

$$P_{app} = J_{ss}/C_0$$

Furthermore, the profile concentration by skin deepness was studied by the Tape stripping technique. At the conclusion of the Franz diffusion cell permeation study, each skin sample was removed from the donor chamber and the residual drug delivery formulation was carefully removed. Thus, on the area that was in contact with the formulations, the technique of Tape stripping was performed as described previously in the literature [13–15]. Fifteen tapes were used for the remotion of the stratum corneum. Each tape strip was placed on a slide and directly measured (three points of the surface) by portable Raman spectroscopy (Figure 1) allowing analysis time ≤ 2 y 10 min for Franz cells and Tape stripping, respectively. All experiments for triplicate were carried out.

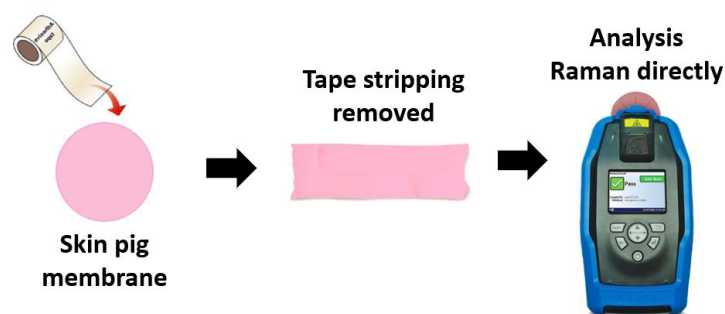


Fig. 1. Tape stripping *in vitro* procedure used in this work.

Spectroscopy Analysis Conditions:

The monitoring and quantification of target drugs LD and MX were carried out by Raman spectroscopy using TruScan Ahura Scientific handheld equipment with laser excitation wavelength light at 785 ± 0.5 nm in the Raman spectrum range 250 cm^{-1} to 2875 cm^{-1} , previous reports associated the use of this type of laser source with fluorescence less effect in biological samples [4].

As a mentioned preview, for the permeation study, the aliquot was placed in a vial. For Tape stripping, the strip was placed on a slide. In both cases, the samples without needing added solvents or calibration steps were directly measured for triplicate.

Statistical Analysis:

The analytical parameters obtained for the developed method were calculated and expressed with corresponding standard deviations. Finally, permeation data were analyzed by T-student test at a p -value of 0.05 of significance.

RESULTS AND DISCUSSION

In vitro Permeation Study of LD and MX Hydrogels

Pig skin samples and synthetic membrane (nitrocellulose filters) were used for these experiments. Figure 2 a) y b) shows results for study in synthetic membrane. The values P_{app} were highest for the prepared formulations with IPM added (Table 3), corroborating with previous reports [16–18] about their activity as an effective permeation promotor. The P_{app} calculated from the slope of the drug release curves were 59.9 and 72.2 cm/min for LD hydrogels and for MX, P_{app} = 0.48 and 0.61 cm/min, in both cases with and without IPM, respectively.

Regarding permeation skin experiments, it was obtained the under the same procedure as for synthetic membranes. The obtained results (Figure 3 a) y b)) show effectively that an increase in permeation flux was kept in prepared formulations with IPM for both drugs, obtaining 41.0 y 0.473 cm/min P_{app} values for LD and MX hydrogels, respectively, in comparison with 31.8 y 0.44cm/min for LD and MX hydrogels without IPM. It is important to mention due to despite the diverse chemical features of LD and MX (mainly Log P, polarity, p_k), the IPM allowed the improvement in permeation through the skin due to having been related to a lower flux through it keeping drugs in the skin [19]. Moreover, is interesting observe the difference between MX and LD permeation through the skin, being less facilitated to the first them. Since, as can be seen in Table 2, the apparent permeability coefficient had an insignificance increase (0.44 to 0.47 cm/min); which could be explained due to its lipophilic character (Log P = 3.43), favoring a preference to be accumulated through the corneum stratus layers [20]. The effect of the use of IPM is shown in Table 3, the accumulated account of lidocaine and meloxicam were higher in all formulations with IPM added.

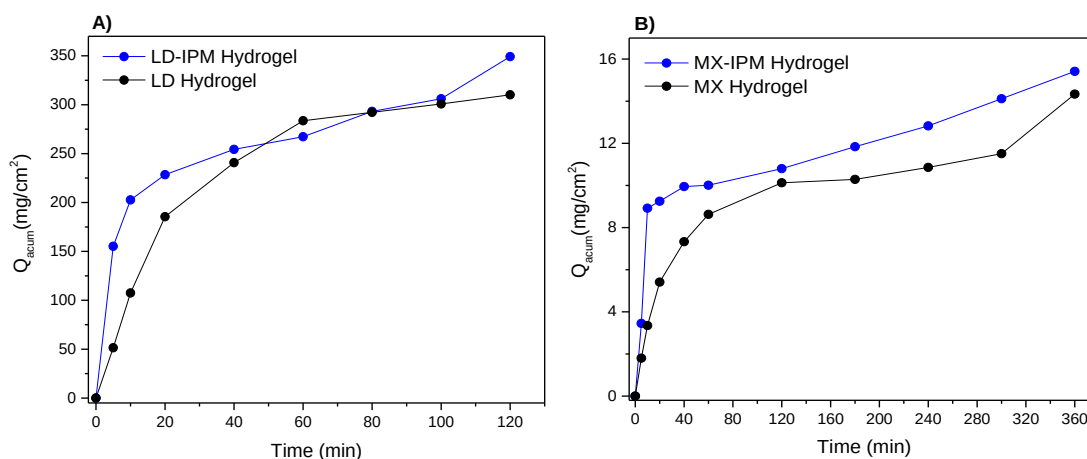
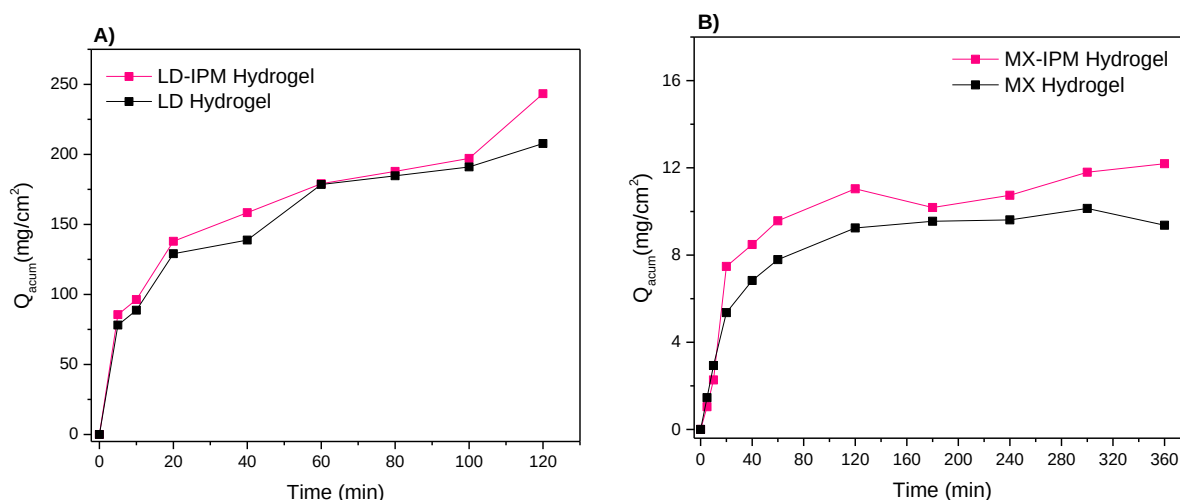


Fig. 2 *In vitro* profiles hydrogels permeation of a) LD-10% and b) MX-0.3% using a synthetic membrane (nitrocellulose filters).

Table 3 Parameters obtained from *In vitro* permeation study MX and LD (mean $\pm$ SD, n = 3).

Synthetic Membrane			Pig skin Membrane		
Formulation	P_{app} (cm/min)	Q_{acum} (mg/cm ²)	Formulation	P_{app} (cm/min)	Q_{acum} (mg/cm ²)
LD Hydrogel	59.9 $\pm$ 0.13	310 $\pm$ 0.20	LD Hydrogel	31.8 $\pm$ 0.21	207 $\pm$ 0.71
LD-IPM Hydrogel	72.2 $\pm$ 0.15	349 $\pm$ 0.11	LD-IPM Hydrogel	41.0 $\pm$ 0.12	243 $\pm$ 0.32
MX Hydrogel	0.48 $\pm$ 0.09	14 $\pm$ 0.42	MX Hydrogel	0.44 $\pm$ 0.07	9.0 $\pm$ 0.37
MX-IPM Hydrogel	0.61 $\pm$ 0.05	15 $\pm$ 0.34	MX-IPM Hydrogel	0.47 $\pm$ 0.03	12.0 $\pm$ 0.19

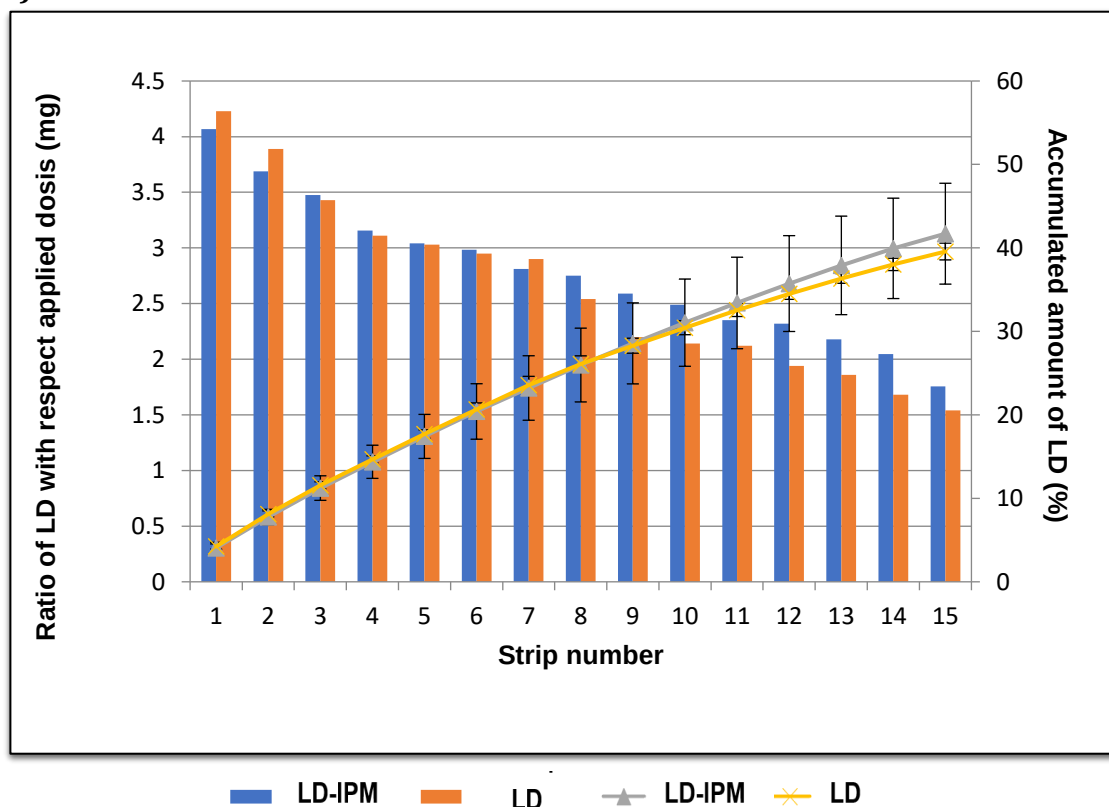
**Fig. 3 *In vitro* profiles hydrogels permeation of a) LD and b) MX using skin pig membrane.**

Tape Stripping Test

Tape stripping (TS) is a simple, low-cost, viable, and minimally invasive technique for the quantification of pharmaceuticals compounds through the skin, considering layers depth where they could be found by chemometrics analysis [7,14]. In this work, this technique was applied separately to the permeation in Franz cells study [21]. Figure 4 a) y b) shows the obtained results for the Tape stripping test from the MX and LD hydrogels topically applied in skin pig membranes. The $\geq 50\%$ amount of accumulated pharmaceutical compounds was retained in layers of skin for all formulations. As expected, the IPM added hydrogels formulations show lower drug retention in the corneum stratus layers, corroborating the obtained results in the Franz cells permeation study. The most interesting of this graphic is specifically, in the case of MX, a pronounced difference in the retention of the drug in the skin is observed in the formulation with isopropyl myristate (IPM) compared to the formulation without it (Q_{acum} with IPM = 1.6393 mg vs Q_{acum} without IPM = 2.3756mg), what could be seen as an opposite effect to the results obtained in Franz cells permeation study, however, could due to the short exposure time of the hydrogels formulations on skin membranes (2h for TS vs 6h Franz cells, respectively) moreover of lipophilic character of this formulation [22]. Moreover, as mentioned preview, the properties of model drugs are key factor, Table 1 shows

clearly the hydrophilic character of Lidocaine ($K_{ow} = 2.44$ and higher solubility in water) which that facilitate their dispersion in the vehicle, moreover lower values of molecular weight (270 vs 351 g/mol for LD and MX, respectively) and topological polar surface area of $32.3 \text{ \AA}^2$ in comparison to $136 \text{ \AA}^2$ of meloxicam, could explain the improve their pass across the layers skin. Therefore, meloxicam to be an API lipophilic strong API (solubility in water ≤ 0.1) and higher molecular weight have a highest trend to remain between layers skin, according to obtained results of TS and Franz cells experiments. In addition, the use of hydrophilic formulation could be relational with an improvement Tape stripping protocol due to not affecting the adhesion impairment [22].

A)



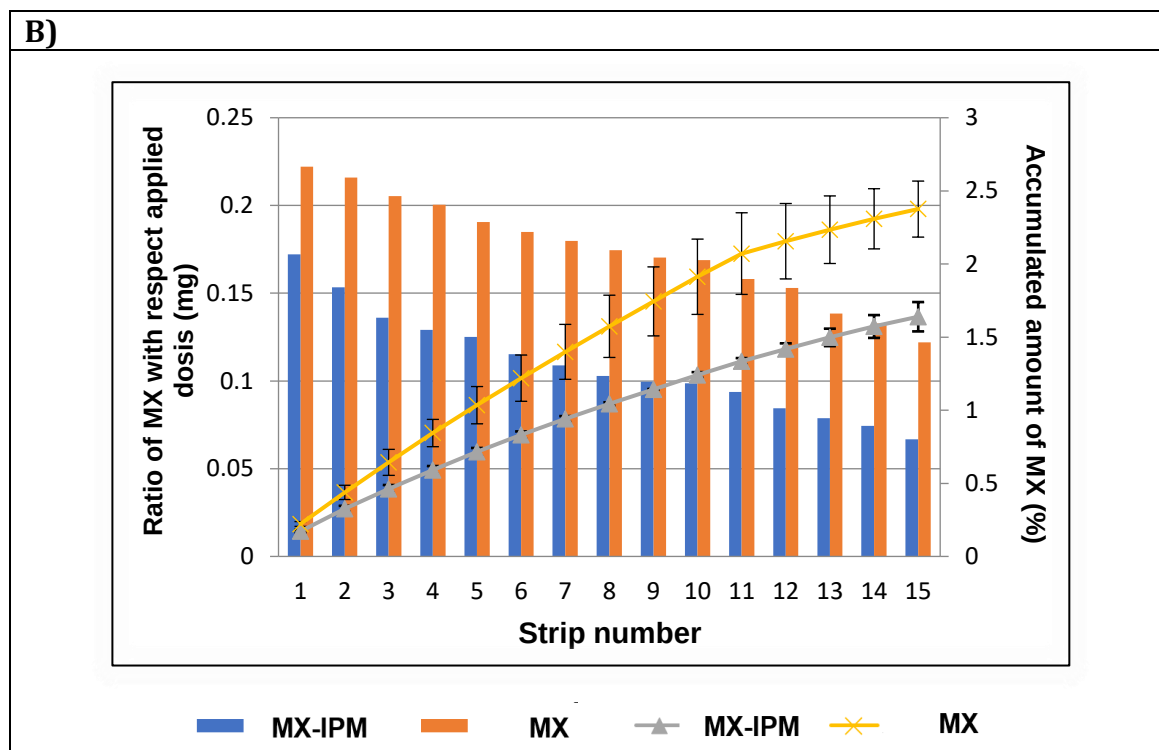


Fig. 4 Tape stripping test results of hydrogels of a) Lidocaine and b) Meloxicam. (The values are the amount of drug retained in SC and related to drug concentration detected in each applied tape and analyzed by Raman spectroscopy).

For the LD hydrogels Tape stripping test, no greater difference was observed in the cumulative amount obtained for both formulations Q_{acum} with IPM = 41.71mg vs Q_{acum} without IPM = 39.57 mg, previous reports associated a greater potentiating effect of IPM in LD formulations in combination with other permeation promoters agents [23]. However, less amount of LD retained in the first superficial layers of the skin is observed in the formulation without the permeation promoter agent, indicating a lower depth of penetration of it, corroborating IPM function [24]. The tape stripping study shows that 50% of drug models remained between corneum stratum (SC) layers, being MX the API highest retained for their lipophilic properties (Table 1).

Analytical Parameters for Drugs Quantification by Raman Spectroscopy

According to USP 43 - <1225>, the validation procedure is key for insurance that the developed quantification method the requirements for its intended analytical applications [25]. The obtained analytical parameters are present in Table 4. Linearity, precision, recovery, limits of detection (LOD), and quantification (LOQ) were calculated for Franz cells permeation experiments.

The developed method was linear with determination coefficients (r^2) higher than 0.998, the range lineal was 75 - 91 and 1 - 2 mg/mL for LD and MX, respectively. Precision (inter-day) evaluated as reproducibility, lower than 5%, and recovery percentages in the range of 98 - 102% considering a method successful according to established in "Harmonized guidelines for single laboratory validation of methods of analysis" [26]. To distinguish between these two

applications of Raman spectroscopy, the method for the quantification of drugs in Tape stripping experiments by measuring drugs concentration contained in hydrogel formulations directly was developed. Table 4 shows indicate also analytical parameters calculated for Tape stripping test. Coefficients (r^2) higher than 0.993, the range lineal was 2 - 10 and 0.05 - 0.03 mg/mL for LD and MX, respectively. Precision (inter-day) evaluated as reproducibility, lower than 5%, and recovery percentages in the range of 95 - 102%. It is important to note that under current good validation practice regulations, users of the analytical methods described in the USP-NF are not required to verify the accuracy and reliability of these methods, but merely to verify their suitability under actual conditions of use [25]. Therefore, both quantification methods were satisfactorily developed and applicated to the monitoring of LD and MX through the skin, demonstrating the potential use of this spectroscopy technique as a viable, eco-friendly, and fast tool for the analysis of dissolved drugs or pharmaceuticals formulations.

Table 4 Analytical parameters for the quantification of MX and LD using portable Raman spectroscopy (n = 3).

Type experiment	Franz cells permeation study		Tape stripping	
	LD	MX	LD	MX
Regression equation	$12.622x - 28.714$	$795.43x + 8.7857$	$2464.3x - 48.714$	$62012x + 683.9$
r^2	0.9992	0.9998	0.9994	0.9936
Linear range (mg/mL)	75 - 91	1-2	2 - 10	0.05 - 0.3
LOD (mg/mL)	1.26	0.05	0.16	0.05
LOQ (mg/mL)	9.99	0.17	0.49	0.07
Precision (% RSD) *	1.15	1.53	4.9	4.4
Recovery (%)	99.22	100.46	99.94	100.53

*Calculated at the lowest level of linear range.

Comparison with Conventional Quantification Methods

Interestingly, Raman spectroscopy was observed to allow monitoring of drug permeation in the skin (according to USP 42) and provide results in accordance with those obtained by quantification traditional methods. Table 5 shows that the use of this technique yields similar results that studies carried out with techniques such as ultraviolet spectroscopy (UV), and high-resolution liquid chromatography (HPLC).

Table 5 Comparison of the analytical techniques for quantification of drugs in the permeation of topically formulations.

Formulation Type	Drug	Instrumental Technique	Sample Preparation (Solvents added)	Test	Analysis Time (min)	Ref
Cream, ointment.	Fludrocortisone and Diclofenac sodium	HPLC	Yes: Mobile phase Extraction of drug from tape	Tape stripping	39	[19]
Hydrogels	Diclofenac	HPLC	Yes: Mobile phase Extraction of drug from tape	Tape stripping	67	[2]
Hydrogels and organogels	Meloxicam	UV-Vis Spectroscopy	Blanks used (placebo gel)	Franz cells	NR	[10]
Hydrogels	Meloxicam and Lidocaine	Portable Spectroscopy Raman	None	Tape stripping Franz cells	10 and 2	This work

NR= Not reported.

CONCLUSIONS

The present research confirmed the successful use of a portable Raman spectrometer for monitoring the skin permeation of two hydrogel formulations. The use of advanced techniques could represent an alternative for the development and analysis of new topical formulations. Thus, the developed protocol designed in this work allowed the monitoring and quantification of lidocaine and meloxicam, two active pharmaceutical ingredients with different polarity in addition to IPM as an enhancer permeation inside of topical formulation. These experiments confirmed that lidocaine showed a higher and facilitated permeation across the skin could suggest their systemic action. Instead, meloxicam was higher retained (around 83%) between the SC layers being a better candidate drug for local action. Also, the use of IPM increased the permeation flux of both drugs, and their incorporation in hydrogels formulation was not affected the topical formulation analysis by Raman spectroscopy. Finally, one of the more significant findings to emerge from this study is an easy, fast, low-cost, and non-invasive method with huge potential for monitoring skin permeation drugs by use of portable Raman spectroscopy. Considerably more work will need to be done to determine its capability or effectiveness in detecting APIs in topical formulations and incorporating them into guidelines by the regulatory agencies.

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