Pharmaceutical Science and Technology
2024, Vol. 8, No. 2, pp. 18-23
https://doi.org/10.11648/j.pst.20240802.11

Research Article

Formulation and Ex Vivo Evaluation of Membrane Permeation Properties of a Mixed Acetaminophen Gel for Rectal Administration

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Abstract

The purpose of this work was to evaluate the permeation of a mixed native starch-based gel of Ipomoea batatas (Convolvulaceae), using acetaminophen as a tracer through a rat rectal membrane (ex vivo method). The formulated gel was composed of a 10 g solution of poloxamer 407 at 20% and 2.5 g of glycerolized potato starch. The gel obtained was a smooth, homogeneous mixed gel with no foam, air bubbles or lumps, there was no characteristic odor, and the gel was whitish in color. This gel was characterized at the physicochemical and rheological level by means of the viscosimeter KINEXUS, pH-meter EUTECH and the study of the permeation was carried out by means of the Ussing chamber of horizontal type (the dual chamber). The formulated mixed gel is thermogelling, rheofluidifying and viscoelastic with a gelling temperature of 23.83; the permeation study gave a relatively low permeation percentage of 0.16% but which can be improved. The different viscoelastic, rheofluidizing and thermogelling characteristics contained in this mixed gel as well as the pH did not influence the permeation of the active ingredient (AP) through the rat rectal mucosa.

Keywords

Rheology, *Ipomoea batatas*, Poloxamer 407, Sweet Potato, Permeation, Dual Chamber

1. Introduction

Tropical countries are rich in starch-rich plants. In the pharmaceutical field, starch is used in the formulation of various galenic forms (tablets, oral suspensions, cream, and toothpaste). [1]

This work follows the thesis of COULIBALY [2], which allowed the formulation of the mixed gel and the study of its stability. These results, although conclusive, remain insufficient. Indeed, this work did not address the issue of incorporation and permeation of active principle from the galenic form.

Thus the general objective of our work is to evaluate the permeation of acetaminophen (Paracetamol) through the rectal mucosa in the form of a mixed gel of poloxamer (PL) 407 at 20% and sweet potato starch.

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Received: 3 July 2024; **Accepted:** 30 July 2024; **Published:** 20 August 2024

2. Material and Methods

2.1. Material

1) Apparatus

Testut T 62 type Bingo scale with digital display and 0,001g accuracy for weighing potato tubers, Retsch® GM 300 mill (Germany) for grinding potato tubers, MEMMERT oven type UL40 n ℁800963 set at 45 ℃, (Germany) for drying the moistened starch, Retsch® sieve shaker set at 10 minutes and 70 revolutions, AS200 basic (Germany) for removing impurities from the potato starch powder and obtaining fine particles, OPTIKA ITALY optical microscope B-3383 POL, made in Italy, EUTECH pH-meter for pH at the physico-chemical control level, a magnetic stirrer (MULTISTIRRER®) series VELP scientifica6 (1000-10000 revolutions), UV-Visible Spectrophotometer (Drawell 8200, China) was used to quantify paracetamol in the receiving compartments after permeation study and finally the Horizontal Ussing Chamber or Dual Chambers (Biomechatronics, France).

2) Raw materials

Sweet potato starch powder, Biodistilled glycerol 99.5% lot: 161274109 made in France, Poloxamer 407 powder lot BCBS3351V (SIGMA life science) made in Germany, Paracetamol supplied by CIPHARM was used as a tracer molecule and Ringer lactate solution, lot 20.297.C02, per: 22.10.2023, which is available in pharmacies, was used as a survival solution.

3) Animal raw material

The rats used for the study were of the Wistar strain, weighing between 250g and 300g. They were raised and fed in the animal house of the pharmacology laboratory within the University's UFR of Pharmaceutical and Biological Sciences.

2.2. Methods

2.2.1. Preparation of Sweet Paste Starch Glycerol and Poloxamer 407 Gel at 20% and Paracetamol

In a vial containing 10g of poloxamer solution, add 2.5g of starch glycerol then put the vial in an ice bath under agitation, add progressively the acetaminophen powder and leave under the microvortex for 4h (complete dissolution of the acetaminophen) and finally, after complete dissolution, remove the vial and leave the preparation to stand at the laboratory temperature.

2.2.2. Dual Chamber Mounting Method for Permeation

1) Preparation of rectal tissue

The rats are fasted for at least 16 hours. They were then sacrificed after anaesthesia with ether. The rectum is removed and the faecal residues are removed by rinsing with osmosed water and then placed in physiological survival fluid (Ringer's solution).

2) Rectal tissue assembly

The removed rectum is cut into small pieces of approximately 1.5cm^2. The resulting cylinder is then opened longitudinally along the mesenteric border. The fragment obtained is very gently spread over the exchange surface of one of the two chambers (recipient side) so that once the chambers are joined; the mucosal and serosal sides of the tissue studied delimit the 1cm2 exchange point between the chambers. The other chamber (donor side) is superimposed on the first and the whole is screwed together.

3) Permeation studies

The recipient chamber is filled with 5 ml of lactated Ringer's solution, then 2.5 g of the gel is introduced into the donor chamber containing 4 mg of paracetamol. The whole device is tempered at 37 °±05 C. Oxygenation of the medium is ensured by a permanent bubbling of carbogen. Samples of 1ml on the recipient side are taken at regular intervals (0, 30, 60, 90 and 120 min). Each sample is replaced with the same amount of lactated Ringer's solution in the recipient compartment and the samples are quantified using a UV-visible spectrophotometer.

4) Calculation of permeation parameters

The total cumulative quantity (Qt) of the active ingredient (paracetamol) that has diffused from the donor compartment to the recipient compartment is given by relationship (1) [3, 4]

$$Q_t = Vp(\sum_{n=0}^{n} Cn - 1) + V_r C_n \quad (1)$$

Where Vr is the volume of the receiving compartment, Vp is the volume taken from the receiving side at different times, C_{n-1} and C_n are the respective concentrations determined at the receiving side.

The percentages of the cumulative quantities at different times are calculated from the following relationship:

$$\%Q_t = (Q_t/C_0)*100 \quad (2)$$

Where C_0 is the initial amount of paracetamol in the donor compartment.

2.2.3. Characterisation of the Mixed Thermogel

1) Macroscopic characterization

48 hours after the preparation of the gel, the parameters evaluated are:

a. The appearance of the gel: homogeneity, presence or absence of lumps as well as the detection of air bubbles; carried out by spreading the gel on a plane,
b. The smell (by smell)
c. and finally the color (observation with the naked eye).

2) Physico-chemical characterization

pH determination

The pH was determined using a neo-Tech SA electronic pH meter, after calibration the glass electrode was immersed in a beaker containing a 10% solution of the gel; the measurement was carried out three times.

3) Rheological characterization

Determination of gelling temperature and viscoelastic parameters

The instrument used was the MALVERN INSTRUMENTS KINEXUS rheometer linked to data processing software, Space Kinexus. The tests were carried out in oscillation mode, over a temperature range of 4 ℃ to 50 ℃ at a frequency of 1 Hz. The geometries used were CP4/40 SR3673 SS cone and PL61 ST S2448 SS plate. The parameters to be evaluated were elastic modulus (G'), viscous modulus (G") and complex modulus (G*) as a function of temperature in order to assess the elastic and viscous characteristics of the gel at different temperatures. The gelation temperature was the temperature at which the value of the elastic modulus became much higher than that of the viscous modulus.

Assessment of the fluid typology (type of flow)

It was determined with the same rheometer used for the determination of the gelation temperature. The temperature was maintained at 25 ℃, the flow type analysis consisted of following the evolution of the viscosity over a shear rate range from 0.1 to 100 s^{-1}.

Centrifugal stability

The gel was centrifuged at 1000rpm, 3000rpm and 5000rpm. The appearance of the gel was observed with the naked eye. Three tests were carried out.

Heat stability

This test was carried out in a water bath thermostated at 50 ℃ for 30 minutes. The gel was characterized by eye. Three tests were carried out.

3. Results

The gel has been manufactured with a 20% solution of poloxamer 407 and starch glycerol. The gel contains 10 g of this poloxamer 407 solution and 2.5 g of starch glycerol.

1) MACROSCOPIC CONTROL

Appearance of the gel: The resulting mixed gel is smooth and homogeneous, with no foam, air bubbles or lumps,

Odour: no characteristic odour Color: the gel is whitish in color.

Figure 1. *Mixed gel of PL407 at 20% /starch glycerol.*

2) PHYSICAL AND CHEMICAL CONTROLS

Chemical control was carried out by measuring the hygiene potential. At a temperature of 25 ℃ the measured pH is 7.2 and at 37 ℃ the hydrogen potential value is 7.5. The hydrogen potential at 25 ℃ and 37 ℃ are approximately equal.

3) HEAT AND COLD STABILITY

The resulting gel is stable at 15 ℃, 25 ℃, 37 ℃ and 45 ℃ with no change in color or smell and no phase separation.

4) CENTRIFUGAL STABILITY

The mixed gel is stable to centrifugation with no pelleting and phase separation at 1000 rpm, 3000 rpm and 5000 rpm.

5) EVALUATION OF PERMEATION

Figure 2 shows the evolution of the cumulative concentrations of acetaminophen after the permeation study of the gel formulation where a fast phase is observed between t_0 and t_{30} min and then a slow absorption phase until t_{120} min.

On the other hand, Picture 3 shows that, during the permeation study time, the amount of paracetamol released is about 0.16%, which is relatively low. Since the cumulative concentrations are non-zero, acetaminophen undergoes membrane passage through the rat rectal mucosa.

a. Cumulative amount of paracetamol gel formulation

The measurement of the concentration of the solution collected after passage at different times (t_{30} to t_{120}) made it possible to produce the graph of the evolution of the quantity of paracetamol below.

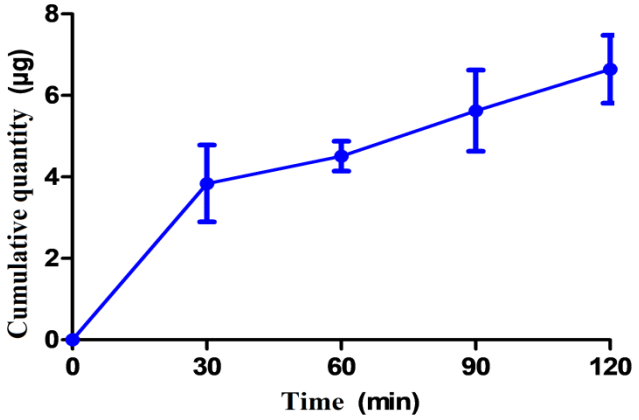

Figure 2. *Evolution of the cumulative amount of paracetamol gel formulation after permeation study through the incised rat rectum for 120 min in a horizontal Ussing chamber (Dual chambers). Values are means ± standard deviation; n=6.*

b. Percentage of paracetamol distributed

From the cumulative amount of acetaminophen after membrane passage from t30 to t120, the percentage of acetaminophen passing through the rectal mucosa was plotted. Figure 3 below shows the evolution of the percentage of acetaminophen in the medium.

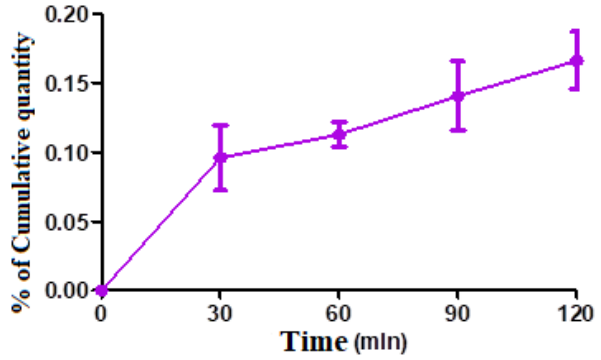

Figure 3. *Percentage of the amount of paracetamol in the gel formulation obtained after 120 min of permeation study through the incised rat rectum in horizontal Ussing chamber (Dual chambers). Values are means ± standard deviation; n=6.*

6) RHEOLOGY

The viscosity at 20 ℃ is 0.236 Pa s. At 24 ℃, the viscosity increases to 8169 Pa s. At 37 ℃, the viscosity increases to 6774 Pa s. The viscosity therefore increases with temperature.

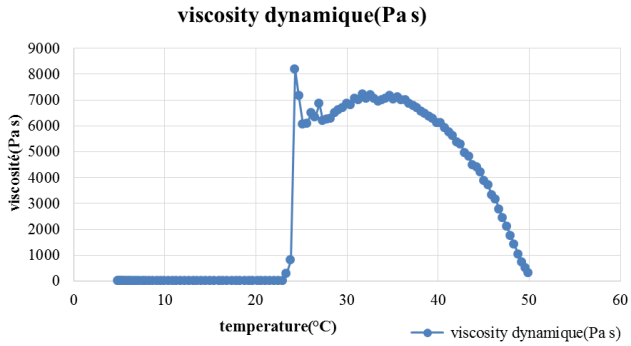

Figure 4. *Evolution of dynamic viscosity as a function of temperature (n= 3).*

FREEZING TEMPERATURE AND VISCOELASTICITY

The determination of the viscoelastic character of the formulated gel by an oscillatory scan under a range of temperatures allowed us to produce the graph in Picture 5 below.

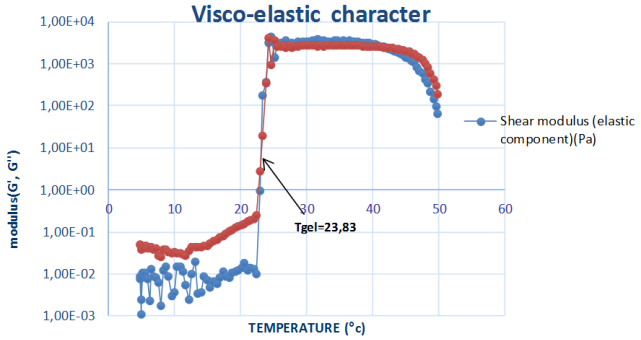

Figure 5. *Evolution of viscous and elastic modulus as a function of temperature.*

Below the gelation temperature, the elastic modulus is lower than the viscous modulus (G'<G") and above the gelation temperature, the elastic modulus is higher than the viscous modulus (G'> G").

The mixed gel of starch glycerol and Poloxamer 407 (PL 407) with 20% acetaminophen is a viscoelastic gel.

Table 1. *Evolution of G' and G" as a function of temperature (n=3).*

	15 ℃ Tgel (23.83)	37 ℃
G"(Pa)	341,5	2670
G'(Pa)	364,1	3230

G': elastic modulus G": viscous modulus

The evolution of the elastic modulus as a function of temperature shows that the mixed gel of 20% PL 407 and GA containing acetaminophen is a thermogelling and viscoelastic gel with an average gelling temperature (Tgel) of 23.83 ℃.

Evaluation of the fluid type

The study of the dynamic viscosity of the gels under the influence of shear stress allowed us to obtain the flow curves shown in Figure 6.

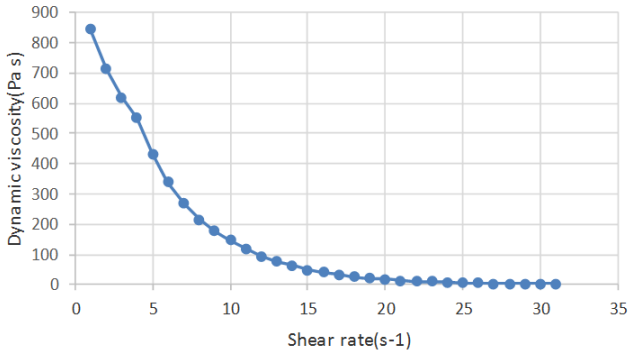

Figure 6. *Evolution of the dynamic viscosity as a function of the shear rate of the mixed gel.*

The plot of the evolution of the viscosity as a function of the shear rate shows a decrease in viscosity as a function of the shear rate from 0.1 to 30 s^{-1}. A mixed glycerol starch and poloxamer 407 gel containing acetaminophen can therefore be described as a rheofluidizing gel.

Finally, the mixed gel of starch glycerol and poloxamer 407 at 20% has rheofluidifying, thermogelling and viscoelastic behavior.

4. Discussion

The objective of this work was to evaluate the permeation of acetaminophen and to determine its percentage through the rectal mucosa in the form of a mixed gel of 20% P407 and sweet potato starch. A study was carried out to characterise the rheological behaviour, pH, stability to heat, cold and centrifugation, as well as macroscopic and microscopic observation. To assess the influence of these parameters on membrane permeation, the Ussing technique was used and permeation kinetics were determined after assay with a UV-visible spectrophotometer.

1) CARACTERIZATION OF MIXED GEL

To prepare the mixed gel, a 20% solution of poloxamer 407 was used. According to the work of COULIBALY Si é[2], the 20% poloxamer content in the solution would allow good dispersion of the starch glycerol. The formulation of the starch glycerol was carried out according to the European Pharmacopoeia formula.

The mixed gel obtained was homogeneous, smooth and pasty with no air bubbles and no lumps. The gel is whitish with no characteristic odour.

2) PHYSICOCHEMICAL PARAMETERS

a. DETERMINATION OF THE pH

The formulated mixed gel gives pH values that vary between 7.5 and 7.68 at 37 ℃. These results are within the pH range of the child's rectum, which is 7.2 to 12.1 [5]**.** Based on these pH results, the formulated gel presents no risk of irritation and is therefore safe for use in the rectum.

b. RHEOLOGICAL CHARACTERIZATION

The parameters used to characterize the rheology of the formulated gel were the gelation temperature (Tgel), the viscoelasticity and the rheofluidic character (which is shown by the evolution of the dynamic viscosity as a function of the shear rate).

The gelation temperature of the mixed gel with 20% poloxamer 407 was determined and indicates an average value of 23.83 ℃. This value is close to the Tgel of pure poloxamer. This high Tgel value is certainly due to the presence of acetaminophen in the mixed gel. The Tgel, despite its elevation, remains below the rectal temperature of 37 ℃.

The evolution of viscosity with temperature shows an increase in viscosity with temperature (Figure 4).

The formulated mixed gel is viscoelastic, rheofluidic and thermogelic. The Viscoelasticity was assessed by comparing the values of viscous modulus and elastic modulus. When temperatures are below the gelation temperature, the viscous modulus dominates, but when temperatures are above the gelation temperature, the elastic modulus dominates. Our results are consistent with those obtained by Kouam é[6]. The dominance of the viscous modulus at temperatures below Tgel would favour the spreading of the gel at the rectal mucosa. Also the dominance of the elastic modulus at temperatures above Tgel would favour the contact of the mixed gel to the rectal mucosa.

The study of the evolution of the dynamic viscosity as a function of the shear rate allowed us to highlight the rheofluidizing character of the acetaminophen mixed gel. This characteristic of the gel would favour a good flow of the gel in the rectal ampoule. Indeed, for increasing shear stresses at the administration site, the viscosity of the gel would be reduced and would therefore favour its spreading on the surface of the rectal mucosa.

The mixed gel is stable to heat centrifugation and is unstable at temperatures below 15 ℃, below which PL 407 becomes more fluid, causing precipitation of the starch glycerol that has been dispersed in the gel during preparation.

3) KINETICS OF MEMBRANE PERMEATION

After 2 hours of permeation study, the cumulative permeation percentage of acetaminophen was found to be 0.16%, which is relatively low. Since the cumulative concentrations are non-zero, acetaminophen passes through the rat rectal mucosa.

This low percentage indicates that the permeation of acetaminophen was influenced by factors such as (i) the concentration of PL 407 (ii) pH.

Indeed, drug diffusion from a poloxamer gel occurs through extra micellar aqueous channels [7]. The PL 407 solution used in the gel formulation imposes its mechanical characteristics on the whole gel. To this end, at laboratory temperature, the P407 solution sets in mass (pasty consistency). This behaviour takes place when the gel is placed in the donor chamber and has a direct influence on the diffusion of the active ingredient (AP) contained in the gel. Indeed, P407 is used in several formulations as a release modulator for the AP due to its rheological properties [8]. The authors have shown that an increase in PL 407 content resulted in a decrease in AP release [9, 10]**.** An increase in PL 407 concentration results in a reduction in the diameter of these aqueous channels, preventing mass diffusion of the acetaminophen contained in the gel. The use of P407 at 20% could explain this low percentage of membrane permeation.

On the other hand, among these works**,** [11] have shown that the diffusion of AP contained in a PL 407 gel varies according to the pH [12] and tends to decrease towards weakly acidic to neutral pH. The slightly basic pH of our gel at 37 ℃ could also explain this low permeation.

5. Conclusion

The objective of our work was to formulate a mixed acetaminophen gel, to determine some rheological characteristics and to evaluate its permeation through a rat rectal mucosa. The formulated gel consisted of 10 ml of 20% poloxamer 407 and 2.5 ml of starch glycerol. This formulation was performed cold in an ice bath. The characterization of the mixed gel from an organoleptic, physicochemical and rheological point of view revealed the stability of the mixed gel. The gel was indeed stable to heat and centrifugation. The rheological analysis allowed us to know that the mixed gel had a rhe-

ofluidifying, viscoelastic and thermogelling character.

The permeation tests carried out using the horizontal Ussing chamber allowed us to determine the percentage of permeation, which is relatively low. However, it is important to note that this percentage is not zero. This low permeation percentage of acetaminophen could be due to the poloxamer 407 used in the formulation which imposes its viscous behaviour on the whole gel or the apparatus.

The results of this work are encouraging for the development of a new sustained release acetaminophen delivery system for the management of fever and pain in children.

Abbreviations

AP	Actif Ingredient
PL	Poloxamer
Qt	Total Cumulative Quantity
Tgel	Gelation Temperature
PL 407	Poloxamer 407

Author Contributions

Dally Laba Ismael: Conceptualization, Validation, Writing – original draft, Writing – review & editing

Aka-Any Grah Sandrine: Resources, Validation

Baka Kouassi Wilfried Junior: Conceptualization, Methodology, Resources

Anin Apo Laurette Ingrid: Methodology, Writing – review & editing

Lia Gnahore Jose Arthur: Visualization

Conflicts of Interest

The authors declare no conflict of interest.

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