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RESEARCH ARTICLE

KINETICS STUDY OF MYCOLACTONE ALKALINE HYDROLYSIS BY CONDUCTOMETRIC MEASUREMENT

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ABSTRACT

The present study reports a kinetics study of alkaline hydrolysis of a secreted Mycolactone by Mycobacterium ulcerans with the Phytomedicine called "MATHESIA" using the Conductometric measurement during the hydrolysis reaction. We also explored the influence of MATHESIA concentration on the rate constant of the hydrolysis reaction. Experiments were conducted in varying Mathesia concentrations from 173 to 17.30µg/ml. The results obtained demonstrate that the enhancement of reactant diffusion leads to an increased rate of Mycolactone hydrolysis. This effect was observed across a range of rate constants, specifically from 62.147 to 153.894$M^{-1}.s^{-1}$ when the concentration of MATHESIA decreases from 173 to 34.60 µg/ml, beyond this point (34.60 µg/ml), further dilution led to an inverse effect, resulting in a decrease of the rate constantfrom 139.48 to 56.123 $M^{-1}.s^{-1}$when the concentration of MATHESIA decreases from 28.83 to 17.30 µg/ml.This decrease could be attributed to the significant dilution of the solution, subsequently reducing the concentrations of reactants within the medium.

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INTRODUCTION

Buruli ulcer is a severe and devastating skin disease caused by Mycobacterium ulcerans infection. It is one of the most neglected diseases among mycobacterial infections and less common than tuberculosis and leprosy (Asiedu, 2000; Johnson, 2005; Hong, 2008; Demangel, 2009). The occurrence of Buruli is mainly increasing and spreading in tropical countries, and its incidence may exceed that of leprosy and tuberculosis in highly affected areas. This disease results in progressive necrotic lesions extending to 15% of a patient's skin surface if left untreated. Surgical intervention has been the only practical curative therapy for Buruli ulcers. Treatments based on combinations of rifampicin, streptomycin, or amikacin have been reported to prevent the growth of bacteria in the early stages of the disease.[4] Mycobacterium ulcerans, like most pathogenic bacteria, produce toxins that are essential to disease development. However, there has been no evidence to update to suggest toxin production by Mycobacterium tuberculosis and Mycobacterium leprae. The toxin structure produced by Mycobacterium ulcerans is composed of two polyketide macrolide molecules designated as Mycolactone A and B (George, 1999).

Several studies have shown that the toxin of Mycobacterium ulcerans may lead to detachment and death of cells (fibroblasts, epithelial, endothelial, and keratinocytes) (Laxmi Dhungel, 2021). In a recent study, we showed that the in vivo anti-mycobacterial activity of M. ulcerans is significantly improved by raising the pH of some antibiotics commonly used to treat tuberculosis by adding ethanolamine. The anti-mycobacterial activity improvement could be attributed to the hydrolysis of esters functions in the Mycolactone and stopping its action on tissues (Nkasa, 2021). Therefore, we propose to study the kinetic hydrolysis of Mycolactone in an alkaline medium by Conductometric analysis to determine the rate constants. This kinetic study could lay the first milestone in elucidating the Phytomedicine "MATHESIA" mode of action on M. ulcerans.

MATERIALS AND METHODS

Material: The Phytomedicine "MATHESIA," mainly used for Mycobacterium ulcerans infections in DR Congo, was obtained from the Industrial and Technological Group (GITCO) based in Kinshasa/DR Congo. The secondary metabolites screening done in the hydro-alcoholic solution of

this Phytomedicine shows the presence of tannins, polyphenols, saponins, and reducing sugars (Nkasa, 2020; Kabedi Bajani Marie Jose, 2018). It is known that PhytomedicineMathesia's solution, once prepared, is usually very viscous.

Methods

Mycolactone extraction: The Mycolactone used for the Conductometric analysis was extracted from a 16-week-old culture of Mycobacterium ulcerans as follows. Mycobacterium ulcerans was grown on BBL Lowenstein-Jensen medium with PACT supplemented with glycerol and egg mixture according to the procedure described by George *et al*. (1998) 16-week-old colonies extracted from the culture medium and inactivated at 90°C for 1 hour were centrifuged for 15 min and suspended in 20 mL of the chloroform-methanol mixture (2:1).

Mycolactone was then isolated using the standard Folch extraction Method (Folch Jordi, 1957; Nassim Hammoudi, 2019). The organic layer containing lipids was concentrated, and Mycolactone was purified by HPLC (Alliance 2690, Waters) using water-acetonitrile 1:4. Conductivity measurements of different solutions were obtained using a digital conductometer brand WTW 82362 Weilheim.

Kinetic measurements: TheConductometric method was used for calculating the hydrolytic rate constant for Mycolactone and NaOH and the Phytomedicine MATHESIA at different concentrations in the function of time. Second-order kinetics can be used for the hydrolysis of Mycolactone and is expressed by Equation 1.

$$k = \frac{1}{at} \times \frac{C}{a-C} \qquad (1)$$

Where "a" is the initial concentration and "C" is the concentration at time t

The terms "C" and "a" can be expressed in conductivity (χ). The conductivity of the reacting solution is proportional to the remaining concentrations of Na^+ and OH^-. Therefore equation 2 can be achieved by rearrangement of equation 1:

$$\chi_t = \frac{1}{ak}\left(\frac{\chi_0-\chi_t}{t}\right) + \chi_\infty \qquad (2)$$

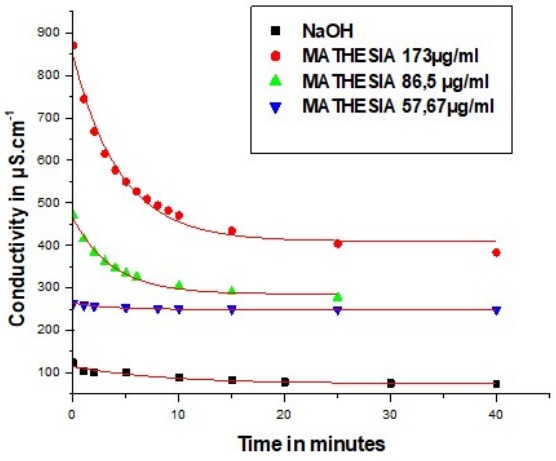
The value of the second-order rate constant can thus be calculated from the slope $\frac{1}{ak}$ of a linear plot of χ_t vs. $\frac{\chi_0-\chi_t}{t}$.

Experimental Procedure for kinetics study: The Mycolactone hydrolysis reaction was carried out in a glass vessel where the conductometer cell was immersed. The glass vessel and the cell were thoroughly washed with water to remove any interference agents, especially ionic species, which may affect the conductivity.

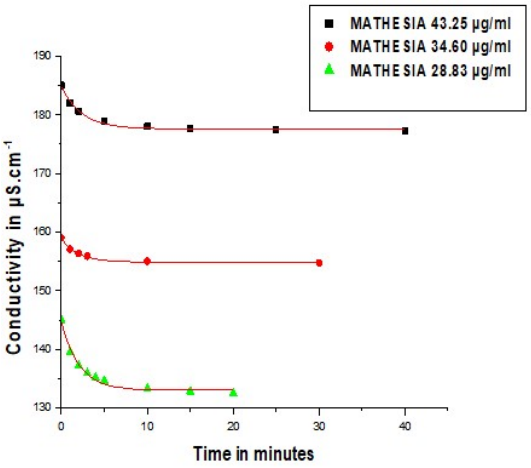
In a graduated cylinder, 10 ml of Mycolactone 0.005 M was added to 10ml of different concentrations of the Phytomedicine MATHESIA as well as in 10ml of NaOH 0.005 M. Conductivities were read at regular time intervals and were plotted versus time using equation 2 to obtain the rate constant.

RESULTS AND DISCUSSION

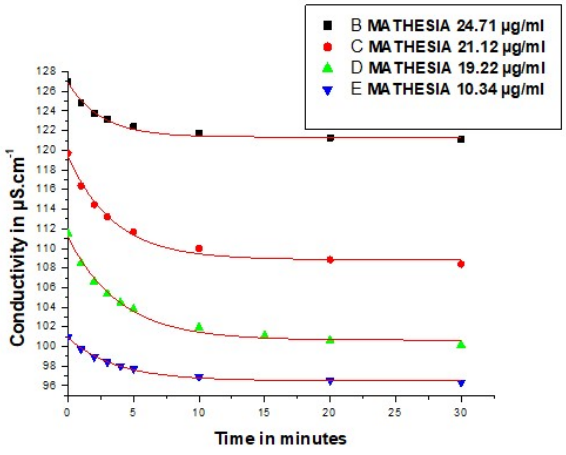
The following figures show the conductivity evolution as a function of the time during Mycolactone hydrolysis by NaOH and MATHESIA at different concentrations.



(a)



(b)



(c)

Figures 1a,b,c. Conductivity evolution of during hydrolysis of mycolactone by NaOH and MATHESIA at different concentrations

Table 1. Hydrolysis rate constant of Mycolactone as a function of MATHESIA concentration

Concentration µg/ml	Slope (1/ak)	k ($M^{-1}.s^{-1}$)	χ_0 ($\mu S.cm^{-1}$)	χ_∞ ($\mu S.cm^{-1}$)	R^2
173µg/mL	3,21816	62,147	870,0	341,432	0.99870
86.50µg/mL	3,03149	65,970	470,0	253,200	0.99940
57.67µg/mL	2,30398	86,806	265,0	247,606	0.99994
43.25µg/mL	1,70476	117,318	185,0	176,860	0.99995
34.60µg/mL	1,30383	153,894	159,0	154,500	0.99994
28.83µg/mL	1,43389	139,480	145,0	131,606	0.99995
24.71µg/mL	1,85002	108,107	127,0	120,657	0.99349
21.13µg/mL	2,68977	74,355	119,5	107,380	0.99954
19.22µg/mL	3,12797	63,939	111,5	98,990	0.99942
17.30µg/mL	3,56360	56,123	101,0	95,890	0.99630

The conductivity decreases with time until reaching a plateau corresponding to the end of the reaction for both the NaOH solution and the Phytomedicine Mathesia at different concentrations. Similar results were found by S. Khouri and A. Altwaip (Sa'ib, 2017).

By linearizing according to equation 2, the results recorded are shown in the following Figure 2 and Table 1:

Figure 2 shows the linear fit of the equation $X_t = \frac{1}{ak}\left(\frac{X_\infty - X_t}{t}\right) + X_0$ for different dilutions of MATHESIA.

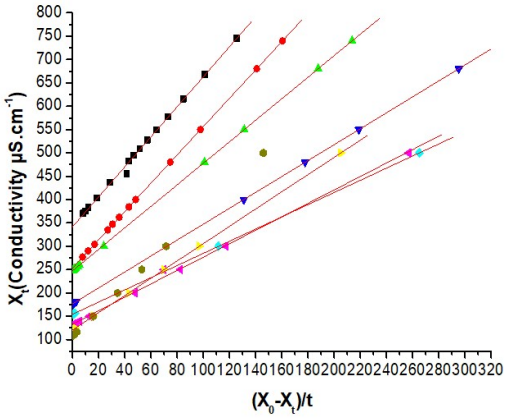


Figure 2. Linear fit of equation (2) for rate constant determination

From the slope of each line, it is possible to deduce the value of the constant rate k expressed in $M^{-1}s^{-1}$, knowing that the initial concentration a = 0.005 mol.L^{-1}. From this table 1, it is clear that the rate constant increases when the concentration of MATHESIA decreases (corresponding to an increase of the dilution factor) until it reaches a maximum; then it decreases, as can be seen in Figure 2 below:

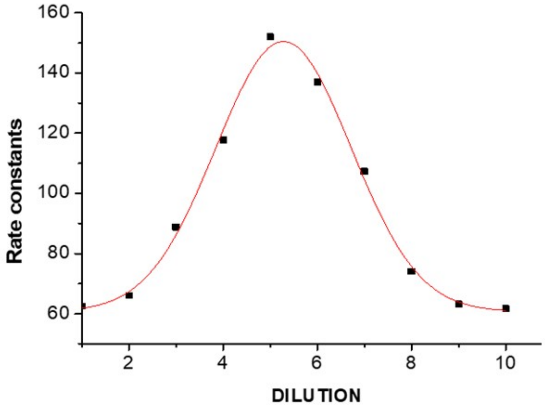


Figure 3. Evolution of Hydrolysis rate constant of Mycolactone as a function of the dilution factor

It has been observed that Phytomedicine Mathesia, when prepared at high concentrations, typically exhibits viscosity. Consequently, its practical utilization often involves diluted concentrations. As a result, the evolution of the hydrolysis rate constant appears to initially depend on the medium's viscosity, which decreases through dilution, subsequently promoting the diffusion of reactants and leading to an increased hydrolysis reaction rate. As the medium's viscosity approaches that of water, the reaction rate becomes more reliant on lower reactant concentrations. The rate law of the reaction hinges on kinetic parameters, including the medium's properties and the reactants' concentrations. The increase in the rate constant to a maximum value can be attributed to the diffusion of reactants, with the reaction being diffusion-controlled within a liquid medium (Frank, 1949; Manuel Dibak, 2019; Tyrrell, 1981). Conversely, the decrease in the rate constant is linked to a high solution's dilution, reducing reactant concentrations.

CONCLUSION

In this study, we investigated the kinetics of the hydrolysis reaction of mycolactone, a compound secreted by Mycobacterium ulcerans, using conductometric measurements. Our findings reveal a noteworthy trend: the hydrolysis rate constant of mycolactone increases with decreasing concentrations of MATHESIA until it reaches a peak. Remarkably, similar observations were documented in our prior in vitro investigation of MATHESIA's anti-mycobacterial activity (Nkasa, 2021). The outcomes presented in this study suggest that the mechanism of action of the phytomedicine MATHESIA extends beyond its antibacterial effects. It also exhibits the ability to hydrolyze the mycolactone secreted by M. ulcerans, thereby impeding its impact on muscle tissue. These findings shed light on a multifaceted mode of action for MATHESIA, potentially offering new insights into its therapeutic applications.

Conflict of interest statement: The authors declare no conflicts of interest.

Animal welfare and ethics statement: No animals were used in the case of this experiment.

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