Jungbunzlauer

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facts

Virucidal activity of L(+)-Lactic Acid against enveloped viruses

Abstract

Demand is increasing for efficient, yet more natural, sustainable and less hazardous active ingredients in surface and skin disinfectants. L(+)-lactic acid is one of few truly bio-based active substances – derived from renewable raw materials via fermentation and readily biodegradable. It is the ideal ingredient to improve the green profile of surface or hand disinfectants without compromising on efficacy.

This paper describes L(+)-lactic acid as an active ingredient with dual power against enveloped viruses and bacteria, with the main focus on its virucidal activity. An official EN standard test protocol was used to successfully demonstrate the virucidal activity of Jungbunzlauer's L(+)-lactic acid against enveloped viruses.

Introduction

Since the beginning of 2020 the world has seen a pandemic virus infection increasingly render normal life impossible. The respiratory illness typical of the COVID-19 pandemic is caused by the SARS-CoV-2 virus, more commonly referred to as corona virus.

Throughout human history viral pandemics have occurred at intervals, with the Spanish flu in 1918/19 being the deadliest in recent history. A different strain of the H1N1 influenza virus responsible for Spanish flu caused the Swine flu pandemic in 2009/2010, whereas the first worldwide corona virus outbreak was the SARS pandemic in 2002/2003.

Viral pandemics occur when new strains emerge against which many people have not yet developed the appropriate immune response in the form of antibodies. The immune system can be stimulated to build these life-saving antibodies through vaccination.

Besides vaccination and therapeutic drugs – which do not include antibiotics – the most important measure to combat a virus outbreak is to stop or slow down its spread. The route of viral spreading is often via droplets or aerosols. Consequently, social distancing, interrupting the hand-to-hand transmission route and hygiene are of extreme importance. Hygiene includes washing hands and using skin and surface disinfectants. Washing hands is an effective way to reduce the pathogenic load on the hands. Disinfectants, however, provide even better protection due to the presence of active substances.

A virus is a very small particle with its own genome but without its own metabolism. A virus therefore needs a host to replicate. Viral replication takes place inside the target host cells during infection. Once inside the host cell, the virus is invisible to antibodies or drugs.

Viruses come in two different forms: the enveloped and the non-enveloped virus. The non-enveloped virus consists only of the genome in the core of a very robust nucleocapsid. The enveloped virus has an additional relatively sensitive lipid bilayer. This envelope helps the virus to penetrate host cells, but also makes it more susceptible to destruction by soap, heat and disinfectants.

Antiviral efficacy inspired by nature

The first point of contact of the active substance, in this case L(+)-lactic acid, is with the outmost layer of the virus particle. The composition of this outmost layer significantly influences its susceptibility to being destroyed by disinfectants.[1] The outmost layer of an enveloped virus consists of lipids, proteins and glycoproteins.

The virucidal activity of lactic acid is thought to be based on multiple mechanisms. The polar molecule lactic acid interacts with the lipid bilayer membrane. For monocarboxylic acids the length of the aliphatic chain is key: the shorter the chain the more active the acid.[2] Organic acids have been described as acting not only on the membrane but also on the glycoprotein structure of the envelope. Glycoproteins are hydrophilic spikes that protrude from the surface of the outmost layer and are essential for interacting with the receptors of the targeted host cell. After penetrating the virus particle, lactic acid destroys or denatures the internal target sites.

Until recently the focus has always been on the antibacterial efficacy of disinfectant formulations. This is reflected in the fact that many disinfectants claim antibacterial but not antiviral efficacy. The current pandemic is leading to a shift in perspective. Now antimicrobial substances that are effective against both bacteria and viruses are of interest.

Lactic acid is one of these substances. It is an active substance with power to combat both viruses and bacteria. L(+)-lactic acid is produced by fermentation of natural and renewable resources and is readily biodegradable. As a liquid, it is easy to handle and to dose in surface and skin disinfectants, which are often liquid.

Virucidal formulations in demand

There is currently a high demand for fast-action hand disinfectants, as well as disinfectant formulations that work on surfaces. Most hand disinfectants are formulated as gels to ensure 30 to 60 seconds contact time with the substance. They often contain ethanol or isopropyl alcohol as their active substance. These alcohols show virucidal efficacy in concentrations from 60 to 95 percent but they raise concerns with regard to fire safety and transportation. Moreover, disinfectants with a high alcohol content may lead to dry skin if applied regularly. This makes the moisturising property of lactate, which is a component of the skin's natural moisturising factor, particularly relevant.[3] Jungbunzlauer's sodium L(+)-lactate and potassium L(+)-lactate have a high water-holding capacity and a proven positive effect on skin hydration even after removal of the natural moisturising factor.[4]

The serious ecological consequences arising from the accumulation of plastics in nature and the finiteness of fossil raw materials are causing many consumers to give preference to products containing natural ingredients. Yet most common hand disinfectant gels are still formulated with thickeners based entirely or partially on fossil sources, even though natural alternatives are available.

These alternative thickeners include xanthan gum, a naturally occurring polysaccharide produced through non-GMO and sustainable fermentation of corn-derived glucose syrup. Its most important functional property is the ability to control the viscosity and rheology of water-based products. Xanthan gum solutions are highly shear thinning, which means they exhibit high viscosity at rest, but an easy and homogenous flow under shear forces. Used as a thickener in hand gels xanthan gum can provide an agreeable skin feeling during rub in, combined with prolonged contact time on the skin.

An additional desirable feature for hand disinfection gels is crystal-clear transparency. This can be achieved by using a special clear solution grade of xanthan gum that provides all the stabilising and shear thinning properties of regular xanthan gum without imparting the typical turbidity. Jungbunzlauer xanthan gum can be used as natural-origin thickener in skin disinfectant gels with lower alcohol content, and can be combined with L(+)-lactic acid.

Jungbunzlauer lactic acid, sodium lactate, potassium lactate and xanthan gum are ECOCERT and COSMOS approved raw materials for use in natural cosmetics and detergents.

Whilst biocides have never been more important in contributing to a safer and cleaner world, customers are increasingly looking for more natural, sustainable, gentle and safer formulations.

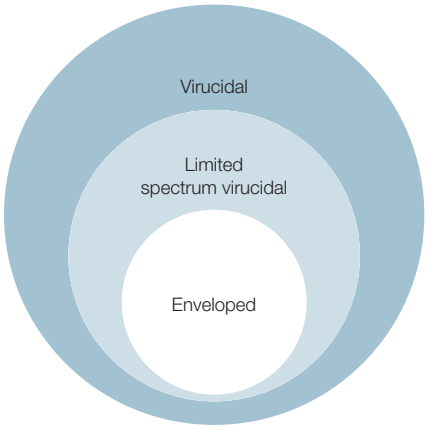
The need for green alternatives is also driven by regulatory and safety developments. A dedicated paragraph about regulations and safety is included in this article at a later stage.

The goal of this article is to share efficacy data on the antiviral activity of lactic acid. For more information on the dual power of lactic acid as a green alternative biocide please refer to the Facts leaflet entitled "Lactic Acid – Efficient disinfection inspired by nature" and other publications focusing on antibacterial efficacy data.[5,6]

Experimental part

The EN14476 Phase 2/Step 1 quantitative suspension test is designed to evaluate the virucidal activity of chemical disinfectants and antiseptics, including those intended for use in the medical area. The protocol describes a method to determine the virucidal efficacy of biocidal active substances. It is one of the test protocols recommended in the ECHA Guidance on Regulation (EU) No 528/2012, the Biocidal Products Regulation (BPR), especially for product type (PT) 1 and PT 2.[7]

Figure 1: Claims for virucidal activity of products. “Virucidal” includes both the smaller claims “limited spectrum virucidal” and “active against enveloped viruses”.



The setup of the EN14476 test takes the different virucidal classes into consideration and has model viruses for each class (figure 1). EN 14476 focuses on the antiviral activity evaluation of hand hygiene products, such as hygienic hand wash preparations or hand rubs, surface disinfectants, instrument and laundry disinfectants. Depending on the intended use of the biocidal product in question, additional EN test methods may be applicable. These additional test methods, however, will not be discussed here.[8]

Only disinfectants that pass for all virus classes may carry the claim “virucidal”. As depicted in figure 1, two weaker claims are available which distinguish viruses according to their lipophilic character and the presence of an envelope as described in the introduction. Examples of viruses with a more lipophilic character are the murine norovirus and the adenovirus. A successful pass in tests against the murine norovirus and the adenovirus permits the claim “limited spectrum virucidal”.

This article focuses on enveloped viruses with a less lipophilic character. A model to test efficacy against this type of virus is the modified vaccinia virus ankara (MVA, ATCC VR-1508). Passing this test permits a claim of “virucidal activity against enveloped viruses”.[8] In each case the setup offers a choice of two different conditions. While clean conditions use 3 g/L of bovine serum albumin as the interfering substance, dirty conditions are represented by 3 g/L of bovine serum albumin plus 3 ml/L of erythrocytes.

To pass the EN14476 test a disinfectant or disinfectant solution has to have the ability to reduce the virus titre by at least 4 log steps within the given time period at 20°C. This is equal to a 99.99% inactivation of the virus.[8] All EN14476 tests were carried out at the laboratory Dr. Brill + Partner GmbH, Institute for hygiene and microbiology, Bremen, Germany, which is accredited according to DIN EN ISO/IEC 17025.

Results and discussion

A scheduled approach to determine usage levels of lactic acid in disinfectants with any kind of virucidal activity was set up. Since enveloped viruses are usually the easiest to inactivate, tests were started against MVA in accordance with EN14476. The more challenging (dirty) conditions including sheep erythrocytes as interfering substance were selected.

Table 1: Results of screening step 1 against vaccinia virus (MVA, dirty conditions, 20°C).

Formulation	Contact time 60 s	Contact time 5 min
2.90% Lactic Acid	Pass	Pass
0.90% Lactic Acid	Pass	Pass

The results for the different solutions are given in table 1. Simple solutions of lactic acid diluted with water were used in this step. Even low concentrations of lactic acid seem to be able to inactivate the MVA. However, these preliminary results are not sufficient to substantiate a claim.

As a second step, lower concentrations of lactic acid were used in accordance with the full EN14476 test method. Again dirty conditions were used. A positive result could be obtained for low active concentrations of lactic acid – below 1% active substance. The formulations given in table 2 therefore avoid the need for classification, labelling and packaging (CLP) labelling while still offering an additional route to combat enveloped viruses such as SARS-CoV-2.

Table 2: Results of full EN14476 against vaccinia virus (MVA, dirty conditions, 20°C).

Formulation	Contact time 60 s	Contact time 5 min
0.90% Lactic Acid	Pass	Pass
0.45% Lactic Acid	Fail	Pass

As shown by the results given in the tables above, the activity of lactic acid against enveloped viruses is clearly proven. Since lactic acid is also registered as a biocide under the EU Biocidal Products Regulation (BPR) this enables numerous formulations with a variety of other ingredients for disinfectant applications.

Given the ongoing pandemic, there is an urgent need for disinfectants that are potent against the enveloped virus SARS-CoV-2. Formulation A, containing 1.5% lactic acid and 44% ethanol plus 0.56% xanthan gum as thickener, was tested according to the full EN14476 method against MVA (table 3). The pH of the formulation was set to 3.5 with potassium lactate. The lactate was used to add a moisturising benefit to the formula. Lactates are a component of the skin's natural moisturising factor.[3] This is highly relevant because solutions with high alcohol concentration can dry the skin.[9] Besides possible irritation, poor skin conditions can increase the risk of infections later on.[10] Topical application of potassium lactate is able to restore the natural moisturising factor after it has been removed by water.[4]

Table 3: Formulation A for the full EN14476 tests. The pH of the formulation was set to 3.5 with potassium lactate.

Ingredient	INCI	Supplier	Amount [%]
Water	Aqua		qs
Ethanol	Ethanol	Diverse	44
L(+)-Lactic Acid 88% Heat Stable, Biocidal Grade	Lactic Acid	**Jungbunzlauer**	1.70
Xanthan Gum FNCSP	Xanthan Gum	**Jungbunzlauer**	0.56
Potassium L(+)-Lactate 60%, Personal Care Grade	Potassium Lactate	**Jungbunzlauer**	qs

With the results given in table 4 it is shown that the active ingredients lactic acid and ethanol work well in combination with a skin moisturiser, potassium lactate, and a natural-origin thickener, xanthan gum, against enveloped viruses.

Table 4: Results of different concentrations of formulation A in a full EN14476 against vaccinia virus (MVA, dirty conditions, 20°C).

Test setup	Contact time 30 s
10% of formulation A	Fail
80% of formulation A	Pass

Regulations and safety

In the European Union, the biocidal market is regulated by Regulation (EU) No 528/2012, the Biocidal Products Regulation (BPR). This regulation ensures that only approved active substances and authorised biocidal products are placed on the market. This applies to biocides of all kinds including disinfectants, which fall within the first of five main groups. Product type (PT) 1 (human hygiene) and PT 2 (general disinfection) are both relevant in this context. Since 2015, suppliers of active substances have to be listed in Article 95. Jungbunzlauer meets this criterion.

L(+)-lactic acid is an approved active substance for PT 1 (human hygiene), PT 2 (general disinfection), PT 3 (veterinary hygiene) and PT 4 (disinfectants in the food and feed area). The approval of L(+)-lactic acid for PT 6 (preservation) is pending.[11] The BPR includes only L-(+)-lactic acid and ethanol as active substances of natural origin, whereby ethanol is still in the approval phase.

Since 2015 not only substances, but also mixtures and final consumer products require adequate CLP labelling in accordance with Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) to meet safety concerns.[12] This regulation also applies to biocides. For formulations containing L(+)-lactic acid with a pH >2, hazardous labelling is required for concentrations greater than 1%; from 1%–3% the “harmful/ irritant” pictogram is required and from ≥3% the “corrosive” pictogram is mandatory. However, these are only default values, which can be overruled by in-vitro testing.

Conclusion

With the corona pandemic, awareness of the importance of surface and skin disinfection has increased and hygiene will probably remain more important than it was before the crisis even once this pandemic is overcome.

The virucidal activity of Jungbunzlauer L(+)-lactic acid against enveloped viruses was successfully demonstrated using the EN14476 test, an official EN standard test protocol. First proof of principle results demonstrate its efficacy in aqueous solutions with a concentration of 0.90% and a contact time of one minute or a concentration of 0.45% and a contact time of five minutes. Neither concentration requires hazardous labelling, though the low pH value of these formulations means they are suitable for surface disinfection only.

With regards to skin disinfection, the efficacy of a greener skin disinfection gel containing the two active ingredients lactic acid and ethanol (pH 3.5, contact time 30 seconds) was demonstrated. It was further shown that incorporating the biodegradable thickener xanthan gum into formulations of this type simplifies application without impairing the disinfectant action. Additionally, the presence of lactate has a proven positive effect on skin hydration due to its high water-holding capacity.

It has been demonstrated that Jungbunzlauer L(+)-lactic acid has virucidal activity against all enveloped viruses, including corona viruses. As a fermentation-derived product, lactic acid meets market and consumer demands for green, gentle yet effective solutions. Jungbunzlauer lactic acid, sodium lactate, potassium lactate and xanthan gum are ECOCERT and COSMOS approved raw materials for use in natural personal care products and detergents.
Jungbunzlauer lactic acid is also approved under the BPR for the relevant PTs 1 and 2. It is a valuable green biocide with power against both enveloped viruses and bacteria.

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About Jungbunzlauer

Jungbunzlauer is one of the world's leading producers of biodegradable ingredients of natural origin. We enable our customers to manufacture healthier, safer, tastier and more sustainable products. Thanks to continuous investment, state-of-the-art manufacturing processes and comprehensive quality management, we are able to provide outstanding product quality.

Our mission "From nature to ingredients®" commits us to protecting people and their environment.

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