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Influence of Process Conditions on Measles Virus Stability

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ABSTRACT

Recombinant measles viruses are currently tested in clinical trials as oncolytic agent to be applied in cancer therapy. Contrary to their use as vaccine where 10^3 infectious virus particles per dose are needed, for cancer therapy 10^9 virus particles should be provided per dose. This leads to other challenges for the production process when compared to vaccine production. This study presents measles virus stability with regard to conditions during production and storage of the virus. Relevant process parameters such as temperature (4-37°C), pH (pH 4-11), conductivity (1.5 to 137.5 mS cm^{-1}) and oxygen partial pressure were analyzed. The infectivity of measles virus particles decreased highly at 37 and 32°C, while at 22 and 4°C it remained stable for several hours or even days, respectively. The thermal inactivation reactions followed first order kinetics and the thermodynamic parameters enthalpy and entropy were estimated. Towards changes in pH measles virus particles were very sensitive, while no inactivation could be observed with varying conductivity. Measles virus incubation at an oxygen partial pressure of 100% did not lead to any loss of infectivity. The results show which parameters should be considered and controlled strongly in the production process to further raise measles virus yields for the high amount needed in cancer therapy approaches.

Keywords: Measles Virus, Virus Stability, Virus Inactivation, Oncolytic Agent

1. INTRODUCTION

As a member of the order *Mononegavirales* (family: *Paramyxoviridae*), Measles Virus (MV) is an RNA virus with a single stranded genome in negative-sense orientation. The genome is wrapped in a Ribonucleoprotein (RNP) complex and enveloped by a lipid layer containing the viral glycoproteins hemagglutinin and fusion protein (Navaratnarajah *et al*., 2009). The virus particles have a size of 120-270 nm. MV is the causative agent of the disease measles, which is highly infectious and characterized by the so called Koplik spots, high fever and a weakened immune system. Although the course of disease is often relatively mild, life-threatening complications such as Subacute Sclerosing Panencephalitis (SSPE) appear with relative low frequency, but regularly (Sabella, 2010). These complications can be prevented by vaccination with attenuated MV vaccine strains which have been

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used in several millions of doses and have revealed an excellent safety profile.

MV induces a Cytophatic Effect (CPE), i.e., cell-to-cell fusion and the formation of multi-nucleated giant cells, ultimately leading to induction of apoptosis and killing of infected cells. Current studies in cancer therapy try to take benefit of this inherent cytotoxicity, as derivatives of an MV vaccine strain are currently tested in clinical trials for cancer therapy (Msaouel *et al*., 2012). The ability of MV to kill cancer cells has been correlated to cell entry of attenuated MV via CD46; this receptor is frequently over-expressed on tumor cells (Anderson *et al*., 2004). Preclinical, unmodified and recombinant MV strains have revealed oncolytic potency in numerous models including xenograft tumor models of human lymphoma (Grote *et al*., 2001), ovarian cancer (Peng *et al*., 2002), or prostate cancer (Msaouel *et al*., 2009).

The infectivity of MV particles is the basic requirement for the success of oncolytic virotherapy. Moreover, the amount of virus particles for one therapeutic dose is up to 3 to 6 $\log_{10}$ units higher than those traditionally used for vaccination (Msaouel *et al*., 2012; Russel *et al*., 2010). For these reasons, stability studies of MV under production conditions (especially medium, oxygen saturation, ion strength, pH and temperature) are important to retain the infectivity during upstream and downstream processing. This will be essential for generation of the required doses of infectious virus particles.

Virus inactivation in general was reported to proceed similar to cell dying (Bauer and Henle, 1979; Johnson, 1974) or protein and enzyme inactivation (Weemaes *et al*., 1997; 1998). All reports about MV stability in general have been published decades ago. Temperature (Black, 1959; Boriskin *et al*., 1988; Musser *et al*., 1960; Rapp *et al*., 1965), pH (Black, 1959; Musser *et al*., 1960) and salt concentrations (Boriskin *et al*., 1988; Rapp *et al*., 1965) were the most important factors analyzed, then. Recently, some work has focused on stability studies for MV vaccines after production and purification (Burger *et al*., 2008; Ohtake *et al*., 2010). While these virus particles are stabilized with different agents or are even lyophilized, this stability studies are not representative for measles virus particles under production conditions. The key parameters for MV production for the use in cancer therapy were already discussed in a previous review (Weiss *et al*., 2012).

The current study aims to target MV stability of MV-batches for high-dose applications such as virotherapy. For process parameters during production and purification the presented data involve determination of temperature inactivation constants, analysis of the impact of pH, pO_2, or certain salts. The determined parameters may serve as a basis for optimizing MV production and purification in future applications such as oncolytic virotherapy requiring comparatively high doses of infectious virus.

2. MATERIALS AND METHODS

2.1. Cells and Virus

Infectious recombinant MV particles of the strain MV_{vac2} GFP (P) were rescued and propagated as described previously (Devaux *et al*., 2007). MV was propagated in Vero cells (# CCL-81, ATCC) under standard culture conditions. Titers were determined according to (Karber, 1931; Reed and Muench, 1938). Cells were adapted to grow in serum free VPSFM medium (Gibco, Invitrogen) supplemented with 4 mM glutamine (PAA, Germany) over 5 passages and were expanded in T flasks at 37°C and 5% CO_2, without any substituents of animal origin. Infections were performed at a cell density of 50.000 cells cm^{-2} with an MOI of 0.02. Measles virus suspensions were collected from the supernatant after centrifugation (5 min, 300 g (Megafuge 1.0R, Thermo Scientific)).

2.2. Temperature Stability Studies

MV suspension were incubated at 4, 22, 32, or 37°C in the dark for the indicated time. After exposure at 4°C, residual virus titers were always determined immediately after sampling. After exposure at 22°C, 32°C or 37°C samples were first frozen at-20°C in a radiator block to immediately stop particle decay before titrating residual titers.

2.3. pH Stability Studies

MV particle suspensions were supplemented with appropriate volumes of 1 M NaOH or 1 M HCl to adjust the pH to the desired range. The virus solutions at pH 5, 6, 7, 8, 9 and 10 were titrated according to the $TCID_{50}$ method immediately after adjustment of pH and after 3 h incubation (at 4°C, in the dark), respectively.

2.4. Salt Stability Studies

MV suspensions were incubated with different salt solution at different concentrations. $MgSO_4$ and NaCl solutions at 1.5 M, 1 M, 0.5 M, 0.25 M and 0.1 M were analyzed. For this purpose, appropriate volumes

of 3 M stock solution of each salt were given to different aliquots of same virus suspensions to adjust salt concentrations. $TCID_{50}$ titration of residual infectivity was done immediately after salt supplementation and after 3 h of incubation. Conductivity measurements of adjusted suspensions were performed using the SevenGoDuoPro device from Mettler Toledo.

2.5. pO_2 Stability Studies

For the analysis of pO_2 stability, MV suspensions were diluted in ice cold phosphate buffer saline at pH 7.2. Conditions were chosen as currently tested for measles virus production (manuscript in preparation), using impeller stirrer in 1 L Stirred Tank Reactor (STR).

3. RESULTS

3.1. Measles Virus Thermal Inactivation

To investigate the MV inactivation rates at different temperatures, measles virus suspensions were incubated at 37°C, 32°C (potential temperatures for production), 22°C (room temperature), or 4°C (storing temperature) and virus titer were determined after defined time spans.

Figure 1 shows an increase of MV inactivation rates with increasing temperature. At 37°C and 32°C MV half-life were one and two hours respectively. When starting with a titer of 6.3×10^5 $TCID_{50}$/mL, the MV titer was below the limit of detection (10^2 $TCID_{50}$/mL) after an incubation of 23 h at 37°C. At 22°C and 4°C, MV was more stable indicated by higher half-life times (18 h and 5 d, respectively).

In **Fig. 1** MV inactivation is displayed semilogarithmic. An exponential regression was fitted to the data sets. The exponent of the respective regression lines gives the inactivation rate constant k. According to Arrhenius (Equation 1) the estimated k-values (lnk) against temperature (T^{-1}) resulted in a linear correlation (**Fig. 2**), where the slope represents the term E_A R^{-1}. Taking Equation 2-4 the thermodynamic parameters enthalpy and entropy can be estimated (Johnson, 1974):

$$k = A \cdot e^{\frac{E_A}{R \cdot T}} \quad (1)$$

Where:

A = The pre-exponential factor (h^{-1})
E_A = The activation energy (kJ mol^{-1})
R = The universal gas constant (kJ mol^{-1} K^{-1})
T = The temperature (K)

$$k = \frac{k_B \cdot T}{h} \cdot e^{\frac{-\Delta G}{R \cdot T}} \quad (2)$$

Eyring Equation, where:

k_B = The Boltzmann constant (kJ K^{-1})
h = The Planck's constant (J s)
ΔG = Gibbs energy (kJ mol^{-1})

$$\Delta H = E_A - R.T \quad (3)$$

ΔH is the enthalpy of activation (kJ mol^{-1}):

$$\Delta S = (\Delta H - \Delta G) \cdot T^{-1} \quad (4)$$

ΔS is the entropy of activation (kJ mol^{-1} K^{-1}).

The estimated thermodynamic parameters for MV inactivation are presented in **Table 1**. The enthalpy for measlesvirus inactivation was 104 kJ mol^{-1} (25 kcal mol^{-1}) and the entropy had a value of 0.08 kJ mol^{-1} (0.02 kcal mol^{-1}).

3.2. Measles Virus pH Stability

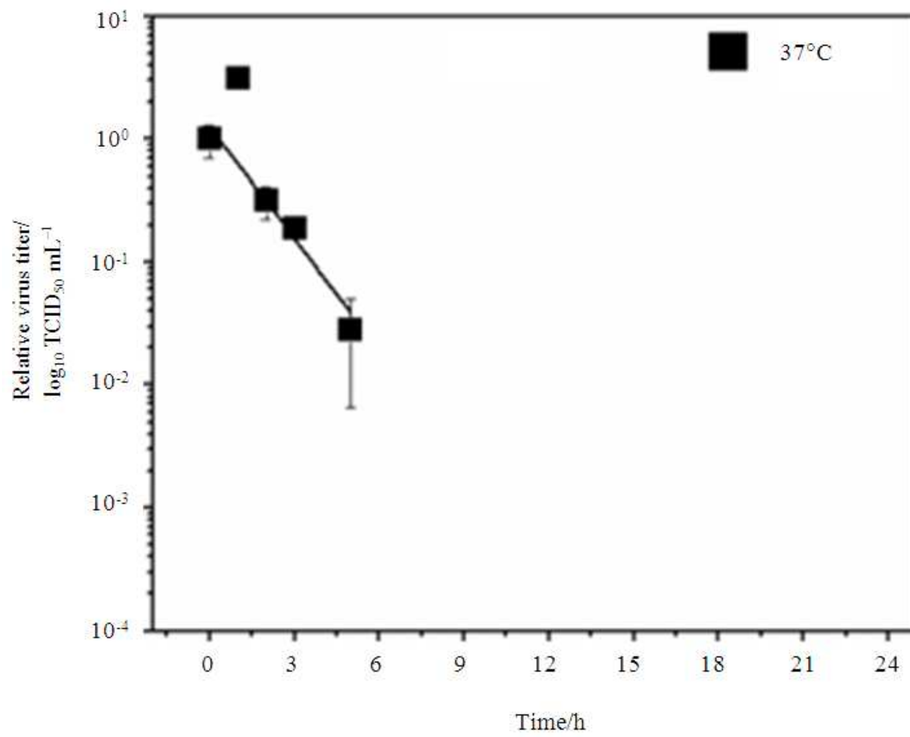
To estimate MV stability at various pH values, MV titers were determined at pH values between 4 and 11 (**Fig. 3**).

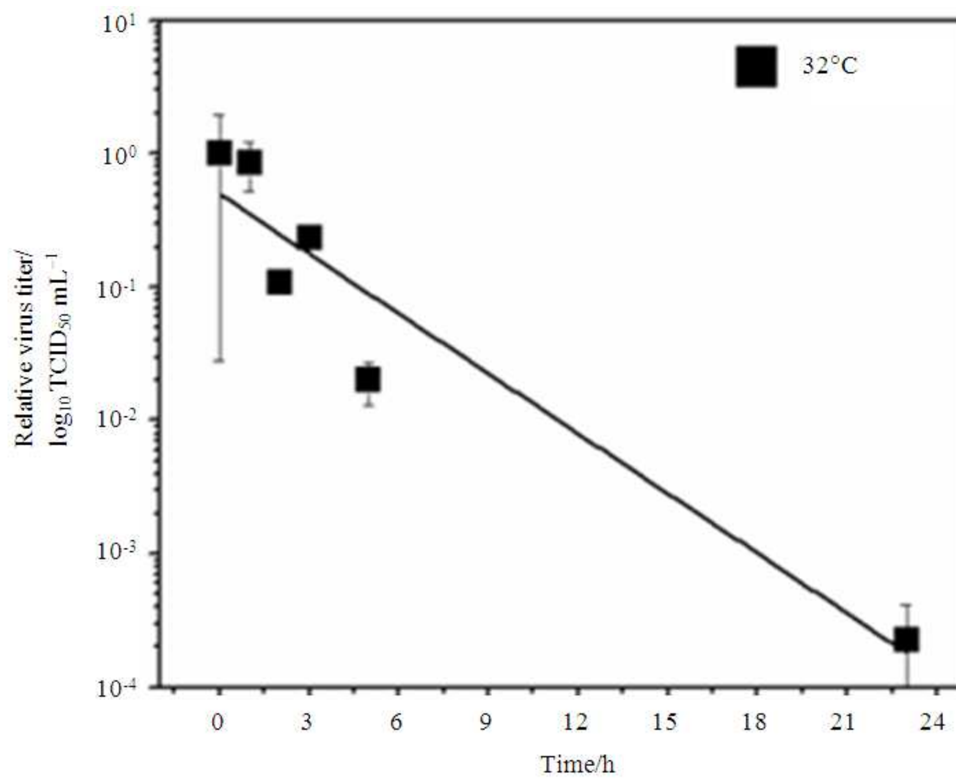
Directly after pH adjustment, the MV titer decreased below the limit of detection at pH 4 and pH 11. Therefore MV was referred to be completely inactivatedunder these conditions. In the acid pH range no infectious MV particles were found (pH 5) immediately after adjustment or a decrease of approx. 2 orders of magnitude appeared (pH 6). In the basic pH range, directly after pH adjustment to pH 8, 9, or 10, MV titers decreased by approx. one order of magnitude (1.1 to 1.6 log_{10} units). After 3h incubation time at acidic and basic pH MV titers were close to the detection limit in all approaches.

The highest titer was estimated at pH 7. Interestingly, only small changes in pH from 7.2 to 7.0 resulted in a titer decrease of one order of magnitude after 3h of incubation, highlighting the sensitivity of MV particles against pH changes.

3.3. Measles Virus Stability at Different Salt Concentrations

To estimate whether MV inactivation at various pH values away from neutral pH was due to increased salt concentrations, MV infectivity was analyzed at different salt concentrations and conductivities.


37°C
Relative virus titer/
$\log_{10}$ TCID$_{50}$ mL^{-1}
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
0
3
6
9
12
15
18
21
24
Time/h


32°C
Relative virus titer/
$\log_{10}$ TCID$_{50}$ mL^{-1}
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
0
3
6
9
12
15
18
21
24
Time/h

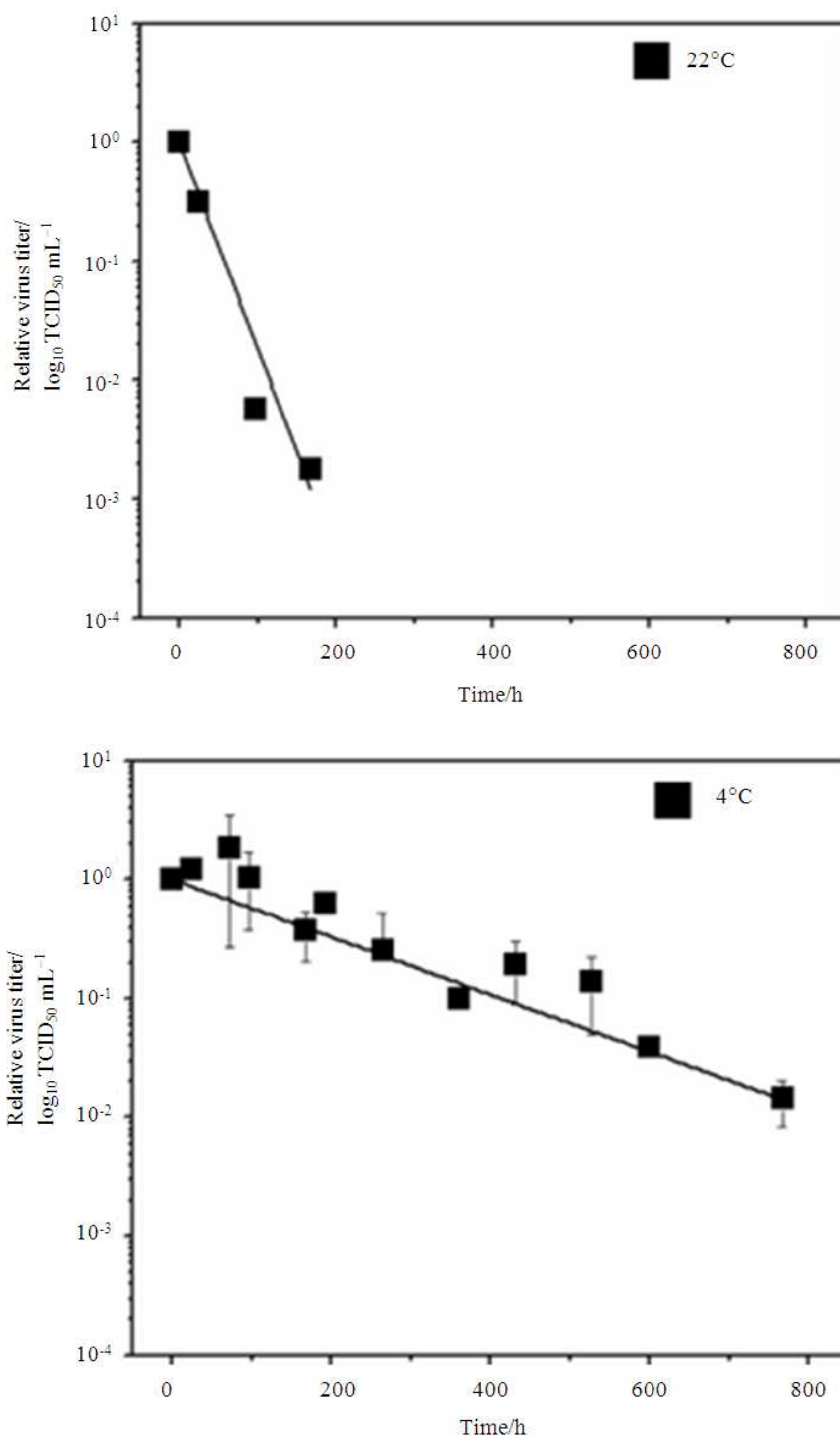


Fig. 1. Thermal inactivation of MV at 37°C, 32°C, 22°C and 4°C. Incubation studies were performed in triplicate, except for the temperature at 22°C. The virus titers are given as relative values divided by virus titer at time point zero of incubation. Lines represent exponential regression of respective data sets. Error bars indicate standard deviation.

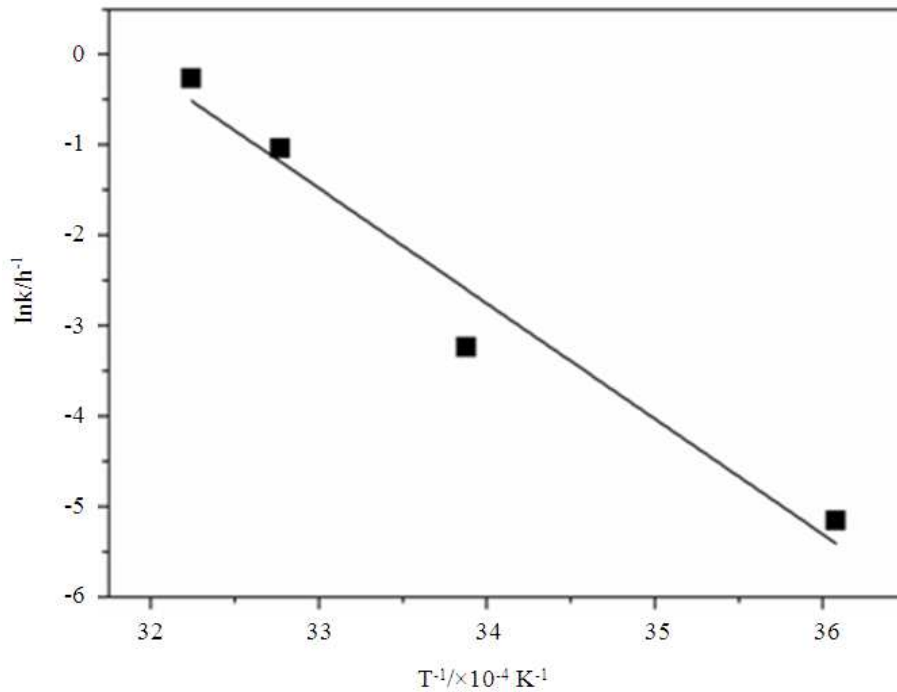


Fig. 2. Arrhenius plot for measles virus thermal inactivation. Values for inactivation constant k were estimated by exponential regression of data sets from **Fig. 1.**

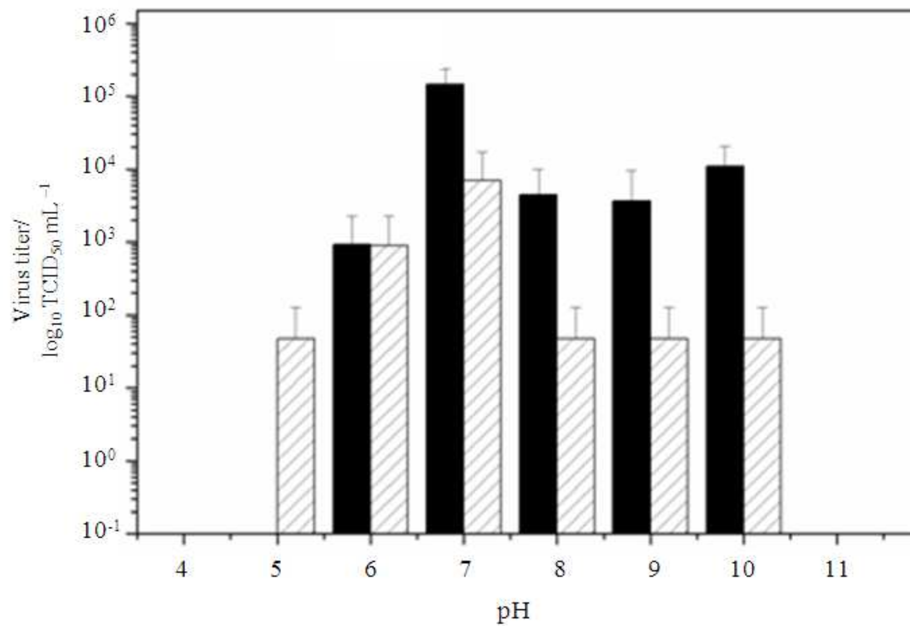


Fig. 3. Inactivation of MV at different pH. For pH adjustment of MV suspensions, respective volumes of 1 M NaOH and 1 M HCl were added. Virus titers were determined directly after pH adjustment (black bars) or after 3 h incubation in the dark at 4°C (hatched bars).

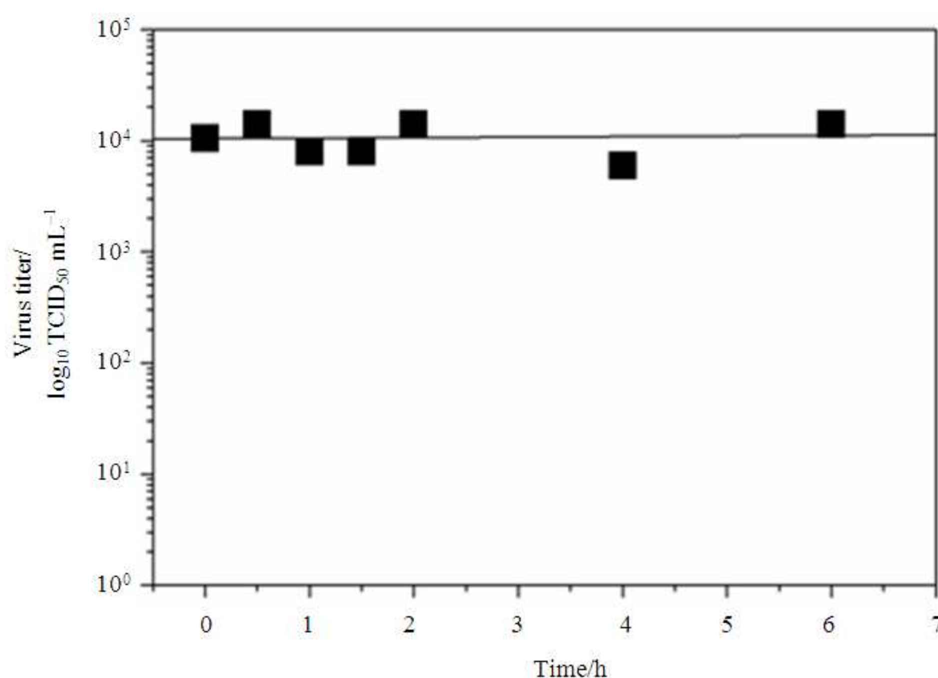


Fig. 4. Oxygen stability of MV particles. Measles virus suspension was stirred in an STR at 70 rpm, pH 7.2, 0.1% (w/v) Pluronic F68 and 100% pO_2; samples were taken at the indicated time points and subsequently titrated. The horizontal line at $1x10^4$ $TCID_{50}$/mL is displayed for easier comparability of different values.

Table 1. Estimated thermodynamic coefficients for measles virus thermal inactivation

ΔH/kJ mol^{-1}	ΔS/kJ mol^{-1} K^{-1}	ΔG/kJ mol^{-1}
104	0.08	78
ΔH/kcal mol^{-1}	ΔS/kcal mol^{-1} K^{-1}	ΔG/kcal mol^{-1}
25	0.02	19

Table 2. Measles virus stability at different conductivities in diluted water and in NaCl and $MgSO_4$ salt solutions

		Log_{10} $TCID_{50}$ mL^{-1}	
Sample name	Conductivity/mS cm^{-1}	Directly	After 3 h incubation
Reference	013.28	6.10±0.17	6.47±0.23
1.5M NaCl	137.50	6.19±0.42	6.60±0.44
1M NaCl	094.30	6.21±0.15	6.31±0.55
0.5M NaCl	054.70	6.07±0.06	6.46±0.53
0.25M NaCl	037.80	5.92±0.30	6.39±0.69
0.1M NaCl	023.99	5.83±0.11	6.66±0.43
1.5M $MgSO_4$	059.80	5.96±0.47	6.05±0.27
1M $MgSO_4$	052.90	6.13±0.43	6.62±0.35
0.5M $MgSO_4$	040.10	6.18±0.35	6.26±0.28
0.25M $MgSO_4$	032.10	6.57±0.23	6.18±0.54
0.1M $MgSO_4$	030.42	6.98±0.43	6.06±0.11
Dilution factor (aquadest):			
2	006.71	6.37±0.26	6.12±0.51
5	002.78	6.39±0.19	6.01±0.19
10	001.49	6.15±0.13	5.77±.0.25

In **Table 2**, virus titers determined at different conductivities and salt concentrations are displayed. Neither measles virus suspensions with NaCl, or $MgSO_4$ at 0.1 to 1.5 M, respectively, nor up to 10-fold dilutions with distilled water resulted in significant virus titer decrease. Therefore, measles virus was observed to be very stable at conductivities between 1.5 to 137.5 mS cm^{-1}. Thus, a decrease of virus titer under different pH conditions can be addressed to different proton concentrations, not salt ions or conductivities.

3.4. Measles Virus Oxygen Stability

As MV is produced by an aerobically host cell, oxygen supply and sensitivity might play an important role for MV production. For this reason an exemplary analysis was done to estimate MV stability under production conditions. Measles virus suspension was stirred in STR at pH 7.2, 70 rpm, 8°C and 100% pO_2 **Fig. 4**.

Within 6 h, the virus titer did not vary to a significant level, so no inactivation has been observed due to high oxygen partial pressure. Under the above described conditions, oxygen concentration according to MV particles inactivation is not a critical factor for MV production.

4. DISCUSSION

For the application of measles virus in cancer therapy up to 1,000,000 more infectious virus particles are needed compared to vaccination (Russel *et al*., 2010). For this reason, first, an effective oncolytic measles virus production process should be established or the measles virus has to be highly concentrated with preserved infectivity to get these high titers. Second, since a successful therapy will be dependent on the infectivity of the virus particles, measles virus stability is an important issue and has to be integrated into MV production considerations. In this study stability studies of infectious MV suspensions in serum free medium are presented aiming to analyze the impact of conditions like pH or temperature that play an important role during production of MV in upstream and downstream processing. Finally, critical parameters were identified to further raise MV yields for the relative high amount needed e.g., for cancer therapy approaches (Russel *et al*., 2010).

The parameter temperature is a very important factor, as temperatures around 30°C are minimally required for the host cell maintenance metabolism and even for virus production. Contrary MV (Edmonston strain) has been reported to be very sensitive towards temperatures above 30°C, (Black, 1959; Kohn and Yassky, 1962; Musser *et al*., 1960; Rapp *et al*., 1965). A half-life of two hours was determined at 37°C for the Edmonston strain, according to about 100 % loss within 4 h (Black, 1959), while a decrease in virus titer of 90 % within 16 h was reported by (Kohn and Yassky, 1962) and within 13 h by (Musser *et al*., 1960). The AIK-C MV strain at 28°C incubation was completely inactivated within 3 days (a loss of over 7 orders of magnitudes) and at 4°C the virus titer decreased about 2 $\log_{10}$ units within 10 days and remained after that stable for another 2 days (Trabelsi *et al*., 2012). The Edmonston strain lost its infectivity at 23°C with a rate of approx. one order of magnitudes in 24 h (Black, 1959). In accordance with the data presented here, inactivation at 37°C or 32°C occurs with a half-life of just 1 or 2 h, respectively, where an inactivation at room temperature or at 4°C was significantly slower (**Fig. 1**).

The medium of the MV suspension seems to be an important factor for MV stability at different temperatures. The present studies were done in serum free VPSFM, as this medium is suitable for MV production under serum free conditions (own unpublished data). As already mentioned above, the half-life determined in the current set-up were quite comparable to those reported in Eagle's medium supplemented with 10% calf serum (Black, 1959), where a half-life of two hours was determined at 37°C. In medium 199 without serum, a drop of infectivity of 2.4 $\log_{10}$ units has been demonstrated after 7 h incubation at 25°C. At 37°C no virus inactivation could be observed (3.2 +/-0.2 $\log_{10}TCID_{50}$ mL^{-1}) but after 24 h a drop of two $\log_{10}$ units appeared (Musser *et al*., 1960). Nevertheless, this is the only report about a higher stability at a higher temperature for measles virus. This is in contrast to the data presented in this study.

Interestingly, a higher inactivation rate was reported at 6°C, when MV particles were prepared in serum free medium. At this temperature, MV titers decreased to 10% of original titers in serum free preparations within 4 weeks, while in serum containing medium the virus titer was more or less stable (5 +/-0.3 $\log_{10}$ $TCID_{50}$ mL^{-1}) (Musser *et al*., 1960). In water-suspensions, MV preparations lost 90% of infectivity within 24 h at 4°C and 50% within 1 h at 25°C (Rapp *et al*., 1965). This shows that measles virus particles in suspension need certain supplements to retain their infectivity. While serum was reported to be very effective, other supplements like sugar, amino acids and divalent ions were already reported to preserve measles virus infectivity (Trabelsi *et al*., 2012).

The thermal inactivation reactions follow first order kinetics for all analyzed temperatures (**Fig. 1**), which is also reported for tobacco mosaic virus (Ginoza, 1958), Sindbis virus (Barnes *et al*., 1969) and baculovirus (Gotoh *et al*., 2008). The corresponding Arrhenius plot (**Fig. 2**) indicates only one process parameter that contributes to MV inactivation due to temperature sensitivity in our experimental setting. A two component mode mechanism of inactivation in which inactivation proceeds in the first place in a faster manner and then a slower degradation is observed that follows first order kinetics had been reported for lyophilized measles vaccine (Allison *et al*., 1981; Colinet *et al*., 1982). For unprocessed MV at 45°C and 50°C this so called two component mode has also been shown for the Schwarz and Mantooth strain (Albrecht and Schumacher, 1972) and at 4°C for the AIK-C strain (Trabelsi *et al*., 2012). For the Edmonston MV strain a two component inactivation was suggested only by the data at45°C or 50°C (Rapp *et al*., 1965).

In a previous study (Woese, 1960), an enthalpy of 20 kcal mol^{-1} and an entropy of -19 cal mol^{-1} $°C^{-1}$ had been determined for Tobacco Mosaic Virus (TMV) RNA which was designated to be associated with RNA inactivation. In general RNA inactivation was accompanied by a small value for enthalpy and a negative value for entropy. In the same study, positive entropy values between 3 to 950 cal mol^{-1} $°C^{-1}$ of thermal inactivation were reported for proteins, bacterial and plant viruses and other animal viruses (Woese, 1960). The thermodynamic parameters for MV inactivation as determined in this study are summarized in **Table 1**. The enthalpy and entropy for thermal inactivation of MV were 25 kcal mol^{-1} and 20 cal mol^{-1} K^{-1}, respectively. According to Woese (1960), the values in this study could suggest that measles virus inactivation is accompanied by both RNA and protein inactivation. For the enthalpy of measles virus heat inactivation values of 18 to 70 kcal mol^{-1} (23°C to 56°C) were reported in the literature (Woese, 1960). As the enthalpy is greater than zero the reaction is so called endothermic, which means that for measles virus inactivation energy must be absorbed into the system. According to the second law of thermodynamic, an entropy larger than zero indicates an irreversible reaction, meaning that in this study MV thermal inactivation is estimated to be a non-reversible process. Measles virus thermal inactivation as a reversible process, according to entropy of zero, has never been reported and such back formation without any permanent alteration of the system or its surrounding is not expected for a biological system.

Similar to Murine Leukemia Virus (MLV) production, the high degradation rate at 37°C (Nehring *et al*., 2006) presents a new challenge for the production process. To achieve the high amounts of infectious virus particels virus filtration for concentration is a possible solution, like it has been reported for MLV, (Nehring *et al*., 2004; 2009), densonucleosis virus (Czermak *et al*., 2008; Grzenia *et al*., 2006; Specht *et al*., 2004), recombinant baculovirus (Grein *et al*., 2012; Michalsky *et al*., 2009; 2010) and minute virus (Hensgen *et al*., 2010). MV purification in a scale of up to 60 L is already reported by Langfield *et al*. (2011), but especially the half-life of 2 and 1 h of MV particles at 32 and 37°C, respectively, suggests inline filtration with continues harvest and immediate cooling like it has already been reported for MLV particles (Nehring *et al*., 2009).

Referring to pH stability, the data in this report (**Fig. 3**) reveal that MV is very sensitive towards changes away from neutral (pH 7.2). This shows that process conditions have to be adjusted during upstream and downstream processing. Inactivation of MV due to pH sensitivity has already been presented, before. Similar to the results presented here, a total virus inactivation at pH >10 or < 5 has been reported (Musser *et al*., 1960). Stable virus titers were identified between pH 7 and 8, whereas between pH 5 and 7 or 8 and 9 inactivation was between one to two log_{10} units (Musser *et al*., 1960). Black reported a maximum virus titer of 7.9×10^3 pfu at pH 7.6, a stable titer from pH 6 to 10.5 (maximum decrease of 0.6 log_{10} units), but a comparably high inactivation at pH 4.4, 3.8 and 2.4 of 2.8 log_{10} units, representing a loss of 80% (Black, 1959). Such sensitivity towards pH changes was also reported for recombinant baculovirus. In this case particles aggregation due to pH changes has been suggested to be responsible for the observed virus inactivation (Grein *et al*., 2012). The data show that for an optimized production process pH regulation in narrow limits during upstream and downstream processing is essential to preserve measles virus infectivity.

As depicted in **Table 2**, MV titers did not significantly decrease with either a rising or falling conductivity. This stability of MV under different salt concentrations indicates that the sensitivity of MV to pH differing from neutral was not due to enhanced ion concentrations, but linked to proton concentration. The ions Ca^{2+}, Zn^{2+} and Mg^{2+} can be associated to different proteins and especially $MgSO_4$ was reported to improve MV stability or even production yield (Boriskin *et al*.,

1988; Rapp *et al*., 1965; Trabelsi *et al*., 2012). While a negative impact of $MgSO_4$ on measles virus could not be observed in the present study, it possibly improves the virus yield during production.

To date, no analysis concerning the sensitivity of MV towards oxygen has been published. However, this parameter is essential in bioprocess evaluation and scale up investigations as it is necessary for the growth of the mammalian host cells. Especially in scale up investigations dealing with high cell concentrations aeration and the oxygen partial pressure is an important factor. The negative effect of oxygen is given by the formation of oxygen radicals in redox reactions by several enzymes. Oxygen radicals are highly reactive and contingently destructive to biomolecules or its functional groups. Therefore, high oxygen concentrations potentially resulting in generation of larger amounts of oxygen radicals may be counterproductive for virus stability due to biochemical redox reactions e.g., with subsequent inactivation of viral surface proteins critical for infectivity (Lim *et al*., 1999). For these reasons it was analyzed, whether oxygen is a critical parameter for MV stability. For an exemplary test, small scale laboratory conditions were chosen, where measles virus production is currently evaluated (manuscript in preparation). Under these conditions MV titers were stable at an oxygen partial pressure of 100%, indicating resistance of MV particles against inactivation under high oxygen concentrations. The result presented shows that in scale up investigations a high oxygen demand of Vero cell cultures after infection is no critical factor after infection, whereby this enables working at high cell concentrations.

In summary, this study shows which parameters should be considered for further production evaluation. Identified critical parameters could be controlled strongly in the production process to further raise measles virus yields for the high amount needed in cancer therapy approaches. High thermal inactivation should be considered when thinking about time of harvest or continuous cooling during downstream processing. A half-life of 1 and 2h at 37 and 32°C, respectively, shows that harvest of measles virus particles should be performed several fold or even continuously. After that immediate cooling of the virus suspension is essential to retain the infectivity. pH sensitivity is also an important factor when thinking about automatic pH regulation during measles virus production. Interestingly, measles virus showed no sensitivity towards an oxygen partial pressure of 100% or varying conductivities between 1.4 and 138 mS cm^{-1}. Measles virus stability towards different salt concentrations could be an advantage in purification approaches using ion exchange membrane chromatography. This purification has already been reported for recombinant baculovirus (Grein *et al*., 2012) and densonucleosis virus (Specht *et al*., 2004).

5. CONCLUSION

The results presented show how process parameters could affect final infectious MV yields at production and purification.

Aiming for high amounts of infectious MV particles for the applications in cancer therapy, future studies should go in more detailed investigations for MV stability and should also integrate them into the evaluation of MV production.

6. ACKNOWLEDGEMENT

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