bioRxiv preprint doi: https://doi.org/10.1101/2020.09.22.308098; this version posted September 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

Rapid and efficient inactivation of surface dried SARS-CoV-2 by UV-C irradiation.

Natalia Ruetalo[1], Ramona Businger[1], Michael Schindler[1*]

[1]Institute for Medical Virology and Epidemiology of Viral Diseases, University Hospital Tübingen, Tübingen, Germany

*Correspondence should be addressed to:

Michael Schindler, michael.schindler@med.uni-tuebingen.de

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.22.308098; this version posted September 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

Abstract

The SARS-CoV-2 pandemic urges for cheap, reliable and rapid technologies for disinfection and decontamination. We here evaluated the efficiency of UV-C irradiation to inactivate surface dried SARS-CoV-2. Drying for two hours did not have a major impact on the infectivity of SARS-CoV-2, indicating that exhaled virus in droplets or aerosols stays infectious on surfaces at least for a certain amount of time. Strikingly, short exposure of high titer surface dried virus (3*10^6 IU/ml) with UV-C light (0.66 mJ/cm²) resulted in a total reduction of SARS-CoV-2 infectivity. Together, our results demonstrate that SARS-CoV-2 is rapidly inactivated by relatively low doses of UV-C irradiation. Hence, UV-C treatment is an effective non-chemical possibility to decontaminate surfaces from high-titer infectious SARS-CoV-2.

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.22.308098; this version posted September 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

Introduction

SARS-CoV-2 has spread globally and there is an urgent need for rapid, highly efficient, environmentally friendly, and non-chemical disinfection procedures. Application of UV-C light is an established technology for decontamination of surfaces and aerosols (1-3). This procedure has proven effective to inactivate SARS-CoV-1 (4-6), several other enveloped and non-enveloped viruses as well as bacteria (7). Recently, it has also been shown that SARS-CoV-2 is sensitive to inactivation by UV-C irradiation (8-10). However, doses and exposure times necessary for total inactivation of SARS-CoV-2 were in a range precluding efficient application of UV-based methods to be employed for large-scale decontamination of surfaces and aerosols (10). We hence conducted a “real-life” application approach simulating the inactivation of dried surface residing infectious SARS-CoV-2 by a mobile handheld UV-C emitting device and an UV-C box designed to decontaminate medium-size objects. Our data shows that surface dried SARS-CoV-2 retains infectivity for at least two hours. Short exposure of high-titer surface dried SARS-CoV-2 to UV-C light lead to a total reduction of infectivity. Hence, UV-C irradiation is a rapid and cost-effective technology to decontaminate surfaces from high-titer SARS-CoV-2.

Material and Methods

Cell culture. Caco-2 (Human Colorectal adenocarcinoma) cells were cultured at 37 °C with 5% CO_2 in DMEM containing 10% FCS, with 2 mM l-glutamine, 100 µg/ml penicillin-streptomycin and 1% NEAA.

Viruses. The recombinant SCoV2 expressing mNeonGreen (icSARS-CoV-2-mNG) (11) was obtained from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) at the UTMB (University of Texas Medical Branch). To generate icSARS-CoV-2-mNG stocks, 200,000 Caco-2 cells were infected with 50 µl of virus stock in a 6-well plate, the supernatant was harvested 48 hpi, centrifuged, and stored at -80°C.

For MOI determination, a titration using serial dilutions of the virus stock was conducted. The number of infectious virus particles per ml was calculated as the (MOI × cell

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.22.308098; this version posted September 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

number)/(infection volume), where MOI = −ln(1 − infection rate).

UV-C light inactivation treatment. 35 uL of virus stock, corresponding to ~$4*10^6$ infectious units (IU) of icSARS-CoV-2-mNG were spotted (in triplicates) in 6-well plates and dried for two hours at RT. 6-well plates spotted with dried virus were treated with UV-C-light using the Soluva® pro UV Disinfection Chamber (Heraeus) for 60 seconds or the Soluva® pro UV Disinfection Handheld (Heraeus) for 2 seconds in a fix regime at 5 and 20 cm plate distance. In addition, a moving regime using a slow (3.75 cm/s) and fast (12 cm/s) speed at 20 cm distance was tested. As control, 6-well plates were spotted with the virus and dried, but not UV-treated. After UV-treatment, the spotted virus was reconstituted using 1 mL of infection media (culture media with 5% FCS). As control, 35 uL of the original virus stock were diluted to 1 ml with infection media and used as virus stock infection control.

Evaluation of UV-treatment. For infection experiments, 1×10^4 Caco-2 cells/well were seeded in 96-well plates the day before infection. Cells were incubated with the SARS-CoV-2 strain icSARS-CoV-2-mNG at a MOI=1.1 (stock) or the UV-treated and reconstituted virus in serial two-fold dilutions from 1:200 up to 1:51200. 48 hpi cells were fixed with 2% PFA and stained with Hoechst33342 (1 µg/mL final concentration) for 10 minutes at 37°C. The staining solution was removed and exchanged for PBS. For quantification of infection rates, images were taken with the Cytation3 (Biotek) and Hoechst+ and mNG+ cells were automatically counted by the Gen5 Software (Biotek).

Viral titers (number of infectious virus particles per ml) were calculated as the (MOI × cell number)/(infection volume), where MOI = −ln(1 − infection rate). Infection rates lower than 0.01 were used as a cutoff and set to 0 in order to avoid false positive calculations.

Software and statistical analysis. GraphPad Prism 8.0 was used for statistical analyses and to generate graphs. Figures were generated with CorelDrawX7. Other software used included Gen5 v.3.10.

Results

We set up an experimental approach to evaluate the effect of UV-C treatment on the stability

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.22.308098; this version posted September 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

of SARS-CoV-2. Simulating the situation that exhaled droplets or aerosols from infected individuals contaminate surfaces, we produced a high-titer SARS-CoV-2 infectious stock and dried 35µL of this stock corresponding to ~4*10^6 IU/ml in each well of a 6-well plate. The plates were then either non-treated or exposed to five UV-C regimens (Fig. 1a). These include inactivation for 60 s in a box designed to disinfect medium-size objects, 2 s exposure at 5 cm or 20 cm distance with a handheld UV-C disinfection device and finally an approach simulating decontamination of surfaces via the handheld UV-C device. For this, we performed slow and fast-moving at a distance of ~20 cm, with “slow” corresponding to a speed of ~3.75 cm/s (supplemental movie 1) and “fast” at ~12 cm/s (supplemental movie 2). UV-C irradiance (254 nm) in the box with an exposure time of 60 seconds corresponds to an irradiation dose of 800 mJ/cm²; for the handheld (HH) at 5 cm the UV-C dose at two second irradiation time is 80 mJ/cm² and at 20 cm is 16 mJ/cm². From the speed of the “slow” and “fast” moving regimens we calculate a UV-C dose of 2.13 mJ/cm² (slow) and 0.66 mJ/cm² (fast).

Subsequently, dried virus was reconstituted with 1 mL infection media and used to inoculate naïve Caco-2 cells at serial dilutions to calculate viral titers. Taking advantage of an infectious SARS-CoV-2 strain expressing the chromophore mNeonGreen (11), we quantified infected (mNG+) and total (Hoechst+) cells by single-cell counting with an imaging multiplate reader. Of note, even short UV-C treatment of the dried virus in the context of the moving “fast” regimen completely inactivated SARS-CoV-2, as no infected cells were detected based on fluorescence protein expression (Fig. 1b). Titration of two-fold series dilutions of the UV-treated and non-treated control samples, as well as the freshly thawed strain as reference, revealed that (i) drying for two hours does not have a major impact on the infectivity of SARS-CoV-2 and (ii) all five UV-C treatment regimens effectively inactivate SARS-CoV-2 (Fig. 1c). Calculation of viral titers based on the titration of the reconstituted virus stocks revealed a loss of titer due to drying from ~4*10^6 to ~3*10^6 IU/ml and effective 6-log titer reduction of SARS-CoV-2 by all employed UV-C treatment regimens (Fig. 1d). Altogether, our data demonstrate that UV-C regimens that expose high-titer SARS-CoV-2 to doses down to 0.66 mJ/cm² are sufficient to achieve complete inactivation of the virus.

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.22.308098; this version posted September 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

Discussion

Disinfection of surfaces and aerosols by UV-C irradiation is an established, safe and non-chemical procedure used for the environmental control of pathogens (1-3, 12). UV-C treatment has proven effective against several viruses including SARS-CoV-1 (4-6) and other coronaviruses i.e. Canine coronaviruses (13). Hence, as recently demonstrated by others (8-10) and now confirmed by our study it was expected that SARS-CoV-2 is permissive for inactivation by UV-C treatment. One critical question is the suitability of this technology in a "real-life" setting in which the exposure time of surfaces or aerosols should be kept as short as possible to allow for a realistic application, for example in rooms that need to be used frequently as operating rooms or lecture halls. Furthermore, in such a setting, we assume that the virus is exhaled from an infected person by droplets and aerosols, dries on surfaces and hence represents a threat to non-infected individuals. We simulated such a situation and first evaluated if surface dried SARS-CoV-2 is infectious. Drying for two hours, in agreement with previous work (14), did not result in a significant reduction of viral infectivity indicating smear-infections could indeed play a role in the transmission of SARS-CoV-2 (Fig. 1). On the other hand, our virus-preparations are dried in cell culture pH-buffered medium containing FCS, which might stabilize viral particles. Hence, even though this is not the scope of the current study, it will be interesting to evaluate if longer drying or virus-preparations in PBS affect the environmental stability of SARS-CoV-2. Irrespective of the latter, UV-C-exposure of dried high-titer SARS-CoV-2 preparations containing ~3*10^6 IU/ml resulted in a complete reduction of viral infectivity (Fig. 1). In this context, it is noteworthy that we achieved a 6-log virus-titer reduction in a setting simulating surface disinfection with a moving handheld device. With the "fast"-moving protocol (see supplemental video 1) we were exposing surfaces at a distance of 20 cm with a speed of 12.5 cm/s resulting in a calculated UV-C dose of 0.66 mJ/cm² at 254 nm. This is substantially less than the previously reported 1048 mJ/cm² necessary to achieve a 6-log reduction in virus titers when exposing aqueous SARS-CoV-2 to UV-C (10). In another study, using a 222 nm UV-LED source, 3 mJ/cm² lead to a 4-log reduction of infectious SARS-CoV-2 (9) and 20 s deep-ultraviolet treatment at 280 nm

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.22.308098; this version posted September 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

corresponding to a dose of 75 mJ/cm² reduced 3-logs (8).

Comparing these values to other pathogens, SARS-CoV-2 seems particularly sensitive towards UV-C light. To achieve a 3-log titer reduction, 75-130 mJ/cm² are necessary for adenovirus, 11-28 mJ/cm² for poliovirus, and bacteria as for instance Bacillus subtilis require 18-61 mJ/cm² (7). This is in-line with susceptibility of SARS-CoV towards UV-C in aerosols at 2.6 mJ/cm², whereas adenovirus or MS2-bacteriophages were resistant to such a treatment (1).

Altogether, we establish the effectiveness of UV-C treatment against SARS-CoV-2 in a setting designed to simulate realistic conditions of decontamination. The easy, rapid, chemical-free, and high efficacy of UV-C treatment to inactivate SARS-CoV-2 demonstrates the applicability of this technology in a broad range of possible settings.

Author contributions

NR and MS designed the experiments; NR performed the experiments with support from RB; NR, RB and MS analyzed the data; NR and MS drafted the figures and wrote the manuscript; MS developed the manuscript to its final form; MS planned and supervised the study; all authors read, edited, and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest

Funding

This work was supported by grants to MS from the Baden-Württemberg foundation (BW-Stiftung), the Deutsche Forschungsgemeinschaft, the MWK Baden-Württemberg as well as by basic funding provided to MS by the University Hospital Tübingen. Heraeus provided the Soluva UV-disinfection chamber and the UV-handheld disinfection device and contributed funding for consumables. The funders had no role in study design, data analysis or decision to publish the data.

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.22.308098; this version posted September 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

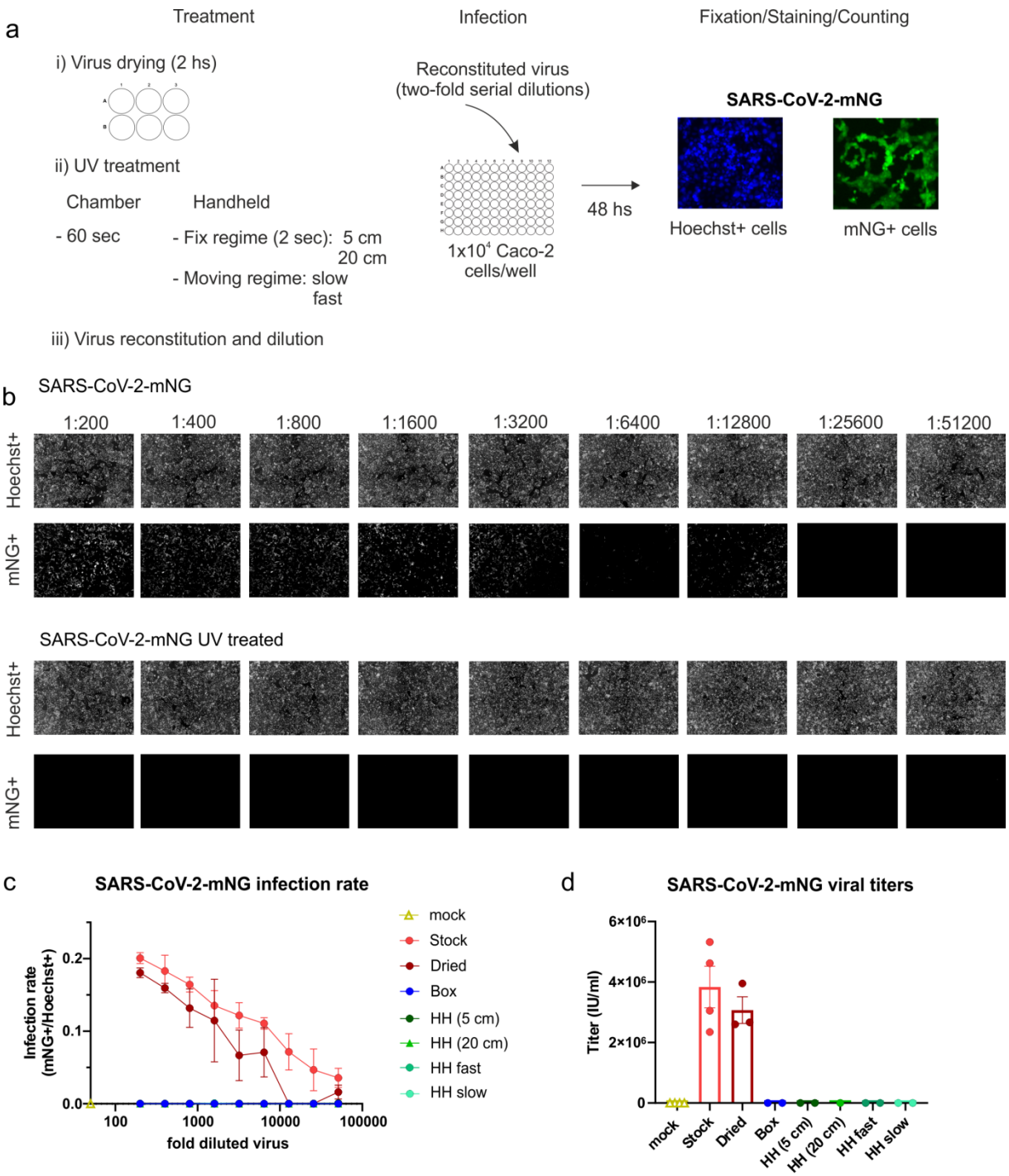
References

1. Walker CM, Ko G. Effect of ultraviolet germicidal irradiation on viral aerosols. Environ Sci Technol. 2007;41(15):5460-5.
2. Qureshi Z, Yassin MH. Role of ultraviolet (UV) disinfection in infection control and environmental cleaning. Infect Disord Drug Targets. 2013;13(3):191-5.
3. Simmons S, Dale C, Holt J, Velasquez K, Stibich M. Role of Ultraviolet Disinfection in the Prevention of Surgical Site Infections. Adv Exp Med Biol. 2017;996:255-66.
4. Duan SM, Zhao XS, Wen RF, Huang JJ, Pi GH, Zhang SX, et al. Stability of SARS coronavirus in human specimens and environment and its sensitivity to heating and UV irradiation. Biomed Environ Sci. 2003;16(3):246-55.
5. Tsunetsugu-Yokota Y. Large-scale preparation of UV-inactivated SARS coronavirus virions for vaccine antigen. Methods Mol Biol. 2008;454:119-26.
6. Darnell ME, Subbarao K, Feinstone SM, Taylor DR. Inactivation of the coronavirus that induces severe acute respiratory syndrome, SARS-CoV. J Virol Methods. 2004;121(1):85-91.
7. Malayeri AM, Madjid; Cairns, Bill; Bolton, James. Fluence (UV Dose) Required to Achieve Incremental Log Inactivation of Bacteria, Protozoa, Viruses and Algae. IUVA News 2016;18. 4-6.
8. Inagaki H, Saito A, Sugiyama H, Okabayashi T, Fujimoto S. Rapid inactivation of SARS-CoV-2 with deep-UV LED irradiation. Emerg Microbes Infect. 2020;9(1):1744-7.
9. Kitagawa H, Nomura T, Nazmul T, Omori K, Shigemoto N, Sakaguchi T, et al. Effectiveness of 222-nm ultraviolet light on disinfecting SARS-CoV-2 surface contamination. Am J Infect Control. 2020.
10. Heilingloh CS, Aufderhorst UW, Schipper L, Dittmer U, Witzke O, Yang D, et al. Susceptibility of SARS-CoV-2 to UV irradiation. Am J Infect Control. 2020.
11. Xie X, Muruato A, Lokugamage KG, Narayanan K, Zhang X, Zou J, et al. An Infectious cDNA Clone of SARS-CoV-2. Cell Host Microbe. 2020;27(5):841-8 e3.
12. Weber DJ, Kanamori H, Rutala WA. 'No touch' technologies for environmental decontamination: focus on ultraviolet devices and hydrogen peroxide systems. Curr Opin Infect Dis. 2016;29(4):424-31.
13. Pratelli A. Canine coronavirus inactivation with physical and chemical agents. Vet J. 2008;177(1):71-9.
14. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. N Engl J Med. 2020;382(16):1564-7.

Figure and Legend

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.22.308098; this version posted September 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

242



243

244 **Figure 1. Inactivation of SARS-CoV-2 by UV-C light treatment.** (a) Experimental layout of
245 the different UV-treatments and the infection assay employed using the green-fluorescent
246 virus SARS-CoV-2.mNG. (b) Primary data showing the results of the infection assay using
247 the non-treated stock virus as a positive control and the UV-treated virus (HH, fast-moving
248 regime). In the upper row, the total amount of cells for each well of the two-fold serial dilution

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.22.308098; this version posted September 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

of virus is shown as Hoechst+. In the lower, infected cells are visualized indicated as mNG+ cells. (c) Infection rate curves for UV-irradiated SARS-CoV-2-mNG using different UV-treatments. The graph shows the infection rate at each two-fold serial dilution, calculated as the number of infected cells (mNG+) over the total number of cells (Hoechst+) for the non-treated viral stock (n=4), dried viral stock (n=3), and dried and UV-irradiated virus using five different UV-treatments (n=2). Data are presented as mean +/- SEM of the number of biological replicates indicated above. (d) SARS-CoV-2-mNG viral titers after UV-treatment. The graph shows the viral titers calculated in IU/mL for the mock-infected, non-treated, and dried stock as well as the dried and UV-irradiated virus under the different treatments. The number of biological replicates is directly plotted and indicated in 1c. Data are presented as mean +/- SEM.

Supplemental Movie 1. UV-irradiation using the Handheld device, slow-moving regime. SARS-CoV-2-mNG was spotted in a 6-well plate, dried for two hs and UV-irradiated as shown in the video. Speed is calculated at approx. 3.75 cm/s.

Supplemental Movie 2. UV-irradiation using the Handheld device, fast-moving regime. SARS-CoV-2-mNG was spotted in a 6-well plate, dried for two hs and UV-irradiated as shown in the video. Speed is calculated at approx. 12.5 cm/s.