Carboxymethyl-starch:iodine Complexes as Virucidal Agents

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Abstract: Coronavirus disease (COVID-19), caused by the highly transmissible and pathogenic SARS-CoV-2 virus, led to the global pandemia. While various vaccines have been developed, drugs against SARS-CoV-2 have been primarily focused on repurposing existing antiviraldrugs. We report new a new formulation consisting of a carboxymethylstarch:iodine complex (CMS:I_2) as an alternative to the synthetic polymer polyvinylpyrrolidone-iodine virucidal agent. The CMS:I_2 showed a high mucoadhesion and an excellent sprayability, favoring the oropharyngeal administration. By hydrolysis with salivary amylase, CMS:I_2 liberates iodine exerting a strong virucidal activity against human coronavirus hCoV-OC43, an accepted model mimicking the SARS-CoV-2 virus.

The virucidal activity of CMS:I_2 was markedly augmented upon the addition of salivary α-amylase by on-site release of iodine. Importantly, none of the tested CMS:I_2 formulations impacted on cell viability. CMS:I_2 liberates iodine as virucidal activity. It is suggested that CMS:I_2 formulations could prevent the initial phases of SARS-CoV-2 infection by interfering with viral attachment and entry into target cells.

Keywords: Carboxymethylstarch:iodine complex; α-amylase, antiinfectivity, hCoV-OC43 coronavirus, COVID-19

(Title and abstract in Romanian)

Compexe Carboxymetil-amidon:Iod ca agenti virucizi.

Rezumat: Virusul SARS-CoV-2, extrem de transmisibil și patogen, a dus la pandemia globală COVID-19. Deși au fost dezvoltate diverse vaccinuri, tratamentele s-au concentrat în principal pe reutilizarea medicamentelor antivirale existente. Prezentam acum o nouă formula: un complex CarboxiMetilamidon/Starch:iod (**CMS:I_2**) ca alternativă la agentul virucid sintetic polivinilpirolidonă-iod, cunoscut ca Betadine®. CMS:I_2, bazat pe un material natural: Amidon, a demonstrat o mucoaderență ridicată, favorizând administrarea orofaringiană. Prin hidroliză cu amilază salivară, CMS:I_2

eliberează iod, exercitând o activitate virucidă puternică împotriva coronavirusului uman hCoV-OC43, un model similar cu virusul SARS-CoV-2.

Activitatea virucidă a CMS:I_2 a fost crescută *in vitro* la adăugarea de α-amilază salivară, cu eliberarea *in situ* de iod (I_2). Niciuna dintre formulările CMS:I_2 testate nu a avut impact asupra viabilității celulare. CMS:I_2 eliberează iod și își exercită activitatea virucidă. Se sugerează că formulările CMS:I_2 ar putea preveni fazele inițiale ale infecției cu SARS-CoV-2 prin interferența cu atașarea și pătrunderea virală în celulele țintă.
Cuvinte cheie: Carboxymethylstarch:iodine complex; α-amylase, antiinfective, hCoV-OC43 coronavirus, COVID-19, mucoadesiune, sprayability

Introduction

The COVID-19 pandemia stemming from Severe Acute Respiratory Syndrome coronavirus 2 (**SARS-CoV-2**) (Acter et al., 2020), has led to intensive research seeking to understand its epidemiology and pathogenic characteristics. SARS-CoV-2 and its variants have rapidly spread worldwide and there are no standard treatments (Lother et al., 2020). Non-pharmaceutical approaches (such as essential oils) have been proposed as virucidal agents (Elsebai et Albalawi, 2022; Wani et al., 2021) with different levels of success (Ayouni et al., 2021; Bohloli et al., 2023).

SARS-CoV-2 is highly transmissible, and infected individuals can be contagious days before and after the acute clinical phase of the disease (Mahyuddin et al., 2020). During infection, viral loads are elevated in the nasal cavity, nasopharynx, and oropharynx (Zou et al., 2020). The nasal cavity and the respiratory epithelium have the highest expression of ACE2 (angiotensin-converting enzyme) receptor, and this is the main receptor for the spike protein of SARS-CoV-2 (Sungnak et al., 2020). In addition to the nasal cavity, the oropharynx is an important way of contamination (Chavda et al., 2023; Hoffmann, 2023).

Aerosol-associated viruses that can persist for hours on surfaces and there is a growing need to develop new easy to apply approaches to reduce transmission of the virus. Standard precautions, including making masks and gloves, may not be sufficient. Recent studies had the impact of iodine-based compounds for disinfection. as part of a transmission reduction plan. For instance, Guidelines from the American Dental Association recommended the use of mouthwash with povidone-iodine **PVP:I** (also known as Betadine®) before dental procedures, in order to prevent COVID-19 transmission (Tessema et al., 2020). PVP-I preparations are well known to be effective against several viruses, bacteria, fungi and protozoa due to the antimicrobial properties of iodine (molecular **I_2**). From more than a century, iodine has been used for identification of starch. It produces a blue complex with iodine, described as amylose-iodine (Saenger, 1984).

Based on an enhanced ability of carboxymethyl starch to include iodine, the formulation

of a CMS:I_2 complex was proposed as an alternative to the synthetic polyvinylpyrrolidone carrying iodine (PVP-iodine). Given the reported antiviral potential of PVP-iodine against SARS-CoV-2 (Anderson et al., 2020), we investigated the virucidal potential of the novel CMS:I_2 complex on the hCoV-OC43 virus, which belongs coronavirus family, including SARS-CoV-2 with respiratory transmission routes.

HRT-18 cells are a well-documented and established cell line for propagating HCoV-OC43 (Schirtzinger et al., 2022). These cells are highly sensitive to infection, allowing for consistent viral replication.

This report is preceded by several others (Tajer *et al*, 2025a,b; Ispas-Szabo *et al*., this issue) reporting various CMS (at different degrees of substitution).

Experimental

Materials - Commercial and "Home-made" alpha-amylase (α-amylase: crude saliva centrifuged and filtered) have been used for comparison.

Preparation of carboxymethyl starch (CMS) powder - To allow chemical modification, starch was first gelatinized by heating in an alkaline aqueous phase (Le Tien et Mateescu, 2017). Carboxymethylation was carried out by treating the gelatinized starch with sodium monochloroacetate (SMCA) in conditions previously reported (Tajer *et al*, 2025a) to obtain a substitution degree of 0.15 (determined by reverse titration, as per Stojanovic *et al.*, 2005).

Preparations of virucidal agents - Carboxymethyl starch with was loaded with iodine following the vapor loading procedure recently described (Tajer et al, 2025a). Briefly, 1 g powder of CMS was placed for « self-loading » in a beaker containing 2 g of solid molecular iodine (I_2) and the system was maintained in an inclosed desiccator, for one week. The obtained CMS:I_2 powder (with 90 mg iodine/g powder) was used for this investigation. For comparison, Betadine 1 % (povidone-iodine), a commercial product for sore throat gargling and Lugol reagent containing iodine), were used as controls.

Cell lines - For infectivity and virucidal effects, cultured HRT-18, epithelial cell derived from a colorectal adenocarcinoma, were used.

Human coronavirus hCoV-OC43 amplification. A modified version of hCoV-OC43 called hCoV-OC43RLuc (with a Renilla luciferase reporter gene inserted in the NS2 gene) allowed monitoring of infection through the measurement of luciferase activity.

Cell viability and in vitro infection assays – were carried out in conditions recently described by Tajer et al (2025b).

i) Viral replication: was evaluated by Luciferase activity (RLU);

ii) *Cell viability*: was evaluated with the 2,3-bis-(2-methoxy-4-nitro-5-sulfo-phenyl)

assay (XTT). It was run in order to examine whether CMS:I_2 or the other control virucides affected or not the viability of HRT-18 cells while exerting virucidal effects. Control samples included virus-free cells (Mock control) was set at 100 % viability. CMS:I_2, Betadine or Lugol and amylases (60 Units/mL, commercial or home-made human saliva) were added alone or together with hCoV-OC43RLuc after 30 min of treatment. Cells were also infected with non-treated viruses (Virus [NT]).

Statistics - Statistical tests were performed using one-way ANOVA multiple comparisons with GraphPad Prism software.

The results on cultured HRT-18 cells confirmed that neither CMS:I_2 nor Betadine nor Lugol did not affect cell viability/metabolism (data presented in Tajer et al., 2025b)
On infected HRT-18 cells CMS:I_2 had a slightly lower virucidal effect than Betadine and Lugol (Fig 1), At low concentrations CMS:I_2 was not anti-infective, but at a high concentration it exerted a strong virucidal activity, as Betadine and Lugol (Fig 1).

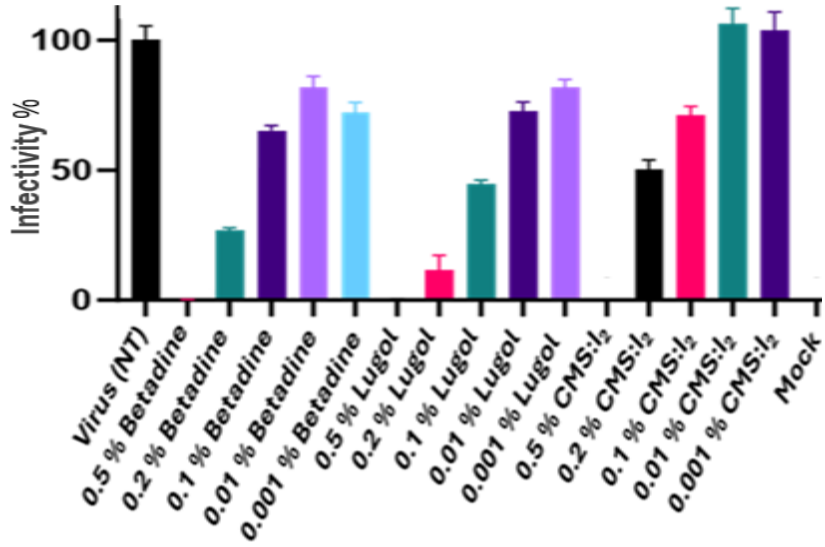

Fig.1- Comparative virucidal effects of CMS:I_2, Betadine or Lugol at different concentrations on hCoV-OC43RLuc virus.

When HRT-18 cells were exposed to hCoV-OC43RLuc treated with CMS:I_2 at various concentrations in the presence of home-made saliva preparations (containing salivary alpha-amylase), the virucidal effect of CMS:I_2 was much stronger (Fig. 2). With the CMS:I_2 at concentrations of 1 % and 5 %, in the presence of saliva, hCoV-OC43 replication was drastically (almost totally) reduced to 0.03 % and 0.006 % respectively, compared to

untreated infected control cells set at 100 % (Tajer et al, 2025b). This powerful virucidal capacity of CMS:I_2 in presence of salivary amylase appears due to the enzymatic hydrolysis of starch chains: consequently, iodine is released *in situ*, opening the possibility to use CMS:I_2 as a sprayable solution for decontamination of the oropharyngeal area. Moreover, this virucidal activity of CMS:I_2 in presence of saliva was clearly higher than those found with Betadine or Lugol at the same concentrations (Tajer *et al*., 2025b).

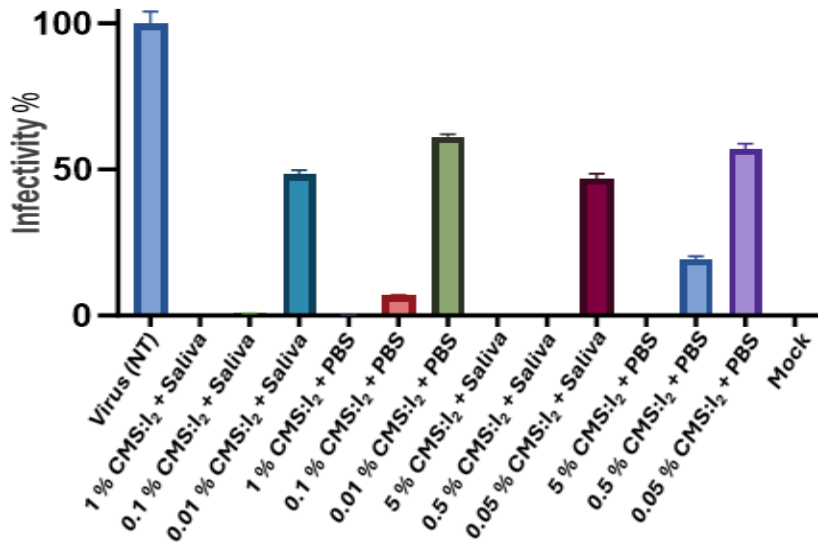

Fig. 2 - Virucidal effect of CMS:I_2 at various concentrations in the presence of human saliva on hCoV-OC43RLuc virus replication.

Worth to mention is that, despite the strong virucidal activity, the viability of host cells was not affected at all by the CMS:I_2 with alpha-amylase (Fig. 3).

We have previously reported unexpected properties of CMS:I_2 complexes, particularly related to iodine inclusion (Tajer *et al*, 2025a; Ispas-Szabo *et al*., this issue). Among the peculiar aspects was the spontaneous self-inclusion of iodine into the CMS to form the CMS:I_2 complexes.

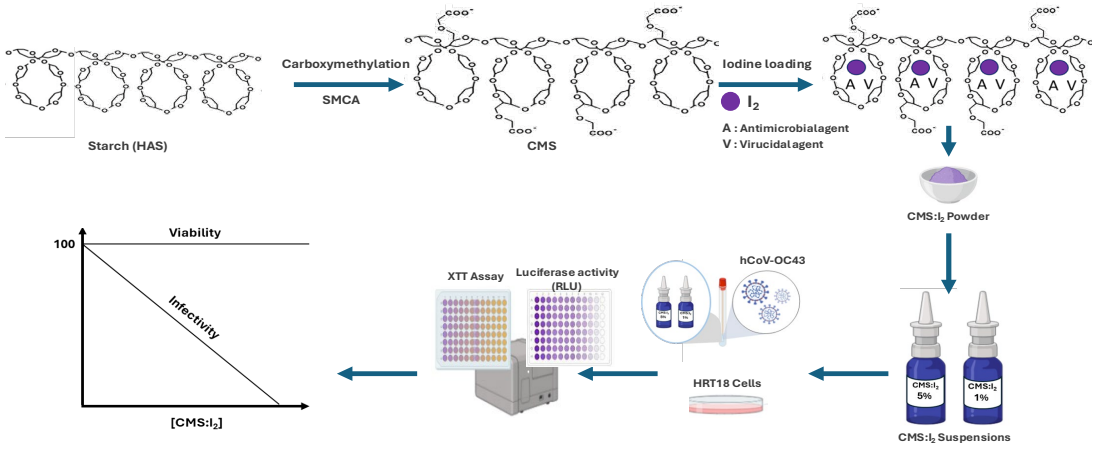

Fig. 3 - Schematical presentation of the virucidal CMS:I_2 action modulated by on site hydrolysis with salivary amylase.

The self-loading phenomenon was found only with CMS (DS 0.15) and not with other tested starch derivatives (such as cross-linked starch). A possible explanation is related to the expanded V-helix structures for the CMS derivatives. The CMS:I_2, when loaded with iodine, presents an enhanced mucoadhesion and a drastic decrease of viscosity when compared with the unloaded CMS derivative. The unexpectedly lowered viscosity is an important property, allowing thus an excellent spray ability using spray bottles for oropharyngeal administration.

In conclusion, it appears that CMS:I_2-based formulations could prevent the initial phases of SARS-CoV-2 infection, particularly the viral attachment and replication, reducing the risk of the downstream infection with respiratory viruses. This approach represents a way to reduce transmission of these viruses for individuals that recently were or may be in contact with infected people.

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