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Formulation and Evaluation of Hydroxychloroquine Sulfate Nasal Spray for SARS Covid 19 Virus

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Keywords
SARS Covid 19, Hydroxychloroquine (HCQ) sulfate, Mechanism of Action of Hydroxychloroquine sulfate

Abstract
Rapid spread of SARS Covid 19 turned out to be a global emergency. Covid 19 RNA virus strain is from family Coronaviridae. Phylogenetic analysis of complete viral genome revealed that virus was most closely related to SARS like coronaviruses.Symptoms of viral infection include mild infection of upper repiratory tract, viral pneumonia, respiratory failure and death. Hydroxychloroquine (HCQ) has demonstrated positive results indicating a potential role against SARS Covid 19 virus. Mechanism of action includes ;interference in endocytic pathway, blockade of sialic acid receptors, restriction of pH mediated spike (S) protein cleavage at the Angiotensin converting enzyme (ACE 2) binding site and prevention of cytokine storm.Yet clinical trials have been carried out to evaluate its abilityand turning it into a needed drug. Existing literature hints the role of Hydroxychloroquine (HCQ) sulfate in Covid 19 for obtaining a therapeutic option for the pandemic.

1. Introduction

SARS Covid 19 emerged as one of major pandemic that spread across the world as on October 5, 2020, more than 35.1 million confirmed infections have been reported with approximately 1 million deaths (WHO: https://covid19.who.int/) [9]. The disease is caused by a novel coronavirus termed as SARS Covid 19 belonging to family Coronaviridae [9]. This virus was reported to be a member of the ß group of coronaviruses. The novel virus was named as 2019 novel coronavirus (2019-nCov) by the Chinese researchers but the official recognition was given by The International Committee on Taxonomy of Viruses (ICTV) named the virus as SARS-CoV-2 and the disease as COVID-19 [8]. Transmission rate of SARS-CoV-2 is higher than SRAS-CoV because of genetic recombination event at S protein in the Receptor Binding Region (RBD) region of SARS-CoV-2 may have enhanced its transmission ability [8].

NASAL SPRAY DRUG DELIVERY SYSTEM :

Nasal drug delivery has been recognized as a very promising route for delivery of therapeutic compounds including biopharmaceuticals. The nasal mucosa has also received attention as a viable means of systemic administration of analgesics, sedatives, hormones, cardiovascular drugs, and vaccines.Nasal route has been used for local delivery of drugs for treating nasal allergy, nasal congestion, or nasal infections.

1. Nasal Drug Delivery benefits over other drug delivery methods due to :
   - ✓ Simplicity of administration.
   - ✓ Avoidance of first pass metabolism.
   - ✓ Extended residence time by increasing viscosity of formulation.
   - ✓ Increased drug absorption with improved drug bioavailability by using penetration enhancers.
   - ✓ Rapid drug absorption and quicker onset of action.
2. To extend the time of dosage form with the mucosal layers of viscous formulation of Nasal spray was prepared so that it prolongs the

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duration of action for a period of time.

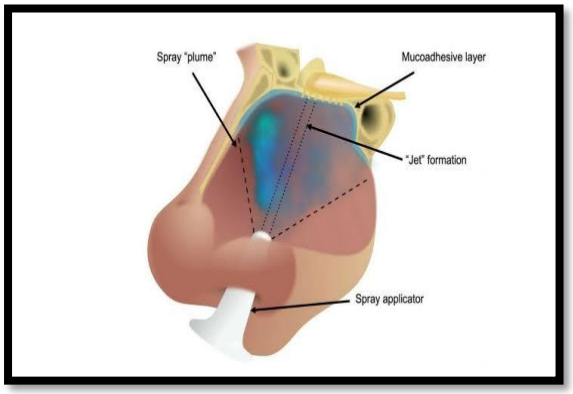


Fig 1: Nasal spray as a need for SARS Covid 19 virus

STRUCTURE OF SARS COVID 19

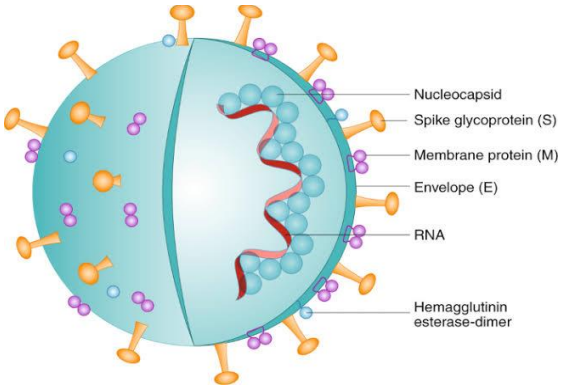


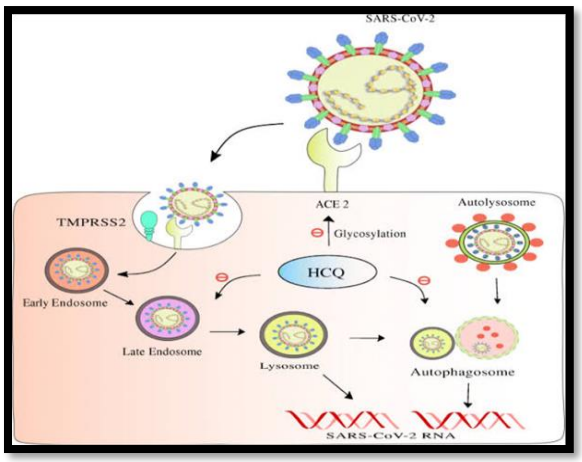
Fig 2: Structure of SARS covid 19

Structure : Coronavirus
Family : Coronaviridae
Order : Nidovirales
Information : Four main structural proteins contain spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. Most abundant structural protein is the membrane (M) glycoprotein; it spans the membrane bilayer three times, leaving a short NH2-terminal domain outside the virus and a long COOH terminus (cytoplasmic domain) inside the virion.
The spike protein (S) as a type I membrane glycoprotein constitutes the peplomers which is the main inducer of neutralizing bodies is S protein.

Symptoms of SARS Covid 19. [2]

1. Dysguesia
2. Hposmia
3. Fever
4. Altered mental status
5. Headache
6. Seizure, Encephalitis

MECHANISM OF ACTION OF HYDROXYCHLOROQUINE SULFATE (HCQ)



1. Inhibition of Glycosylation Process for ACE-2 Receptors and Blockade of Sialic Acid Receptors.

SARS-CoV-2 S protein binds to it, the ACE2 receptor undergoes glycosylation and gets activated. In this case, CQ/HCQ plays an essential role where it prevents the glycosylation of ACE2 receptors, thus preventing entry of SARS-CoV [4]

2. Inhibition Through Restriction of pH Mediated S Protein Cleavage at the ACE2 Binding Site.

S protein is composed of two subunit S1 & S2. The S1 subunit is responsible for mediating entry into the host cells through attachment at the ACE2 receptor. The cellular proteases like transmembrane serine protease II (TMPRSS2) facilitate the S priming, which leads to cleavage of S1/S2 and S2. Splice alterations occurring in the genome of TMPRSS2 due to single-nucleotide polymorphism could alter its expression levels, thus affecting rate of SARS-CoV-2 infection in patients[4].

3. Inhibition Through Prevention of Cytokine Storm

In Antigen Presenting Cells (APC) , HCQ inhibits the antigen processing by the histocompatability complex MHC Class II molecule to T cells in Fig 1.5

Due to this activated T cells decline causing reduction in production cytokines generated by T cells and B cells. [4]

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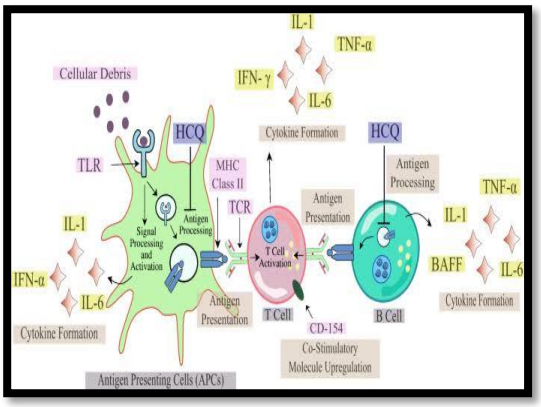

Fig 4: Cytokine storm

2. Methodology

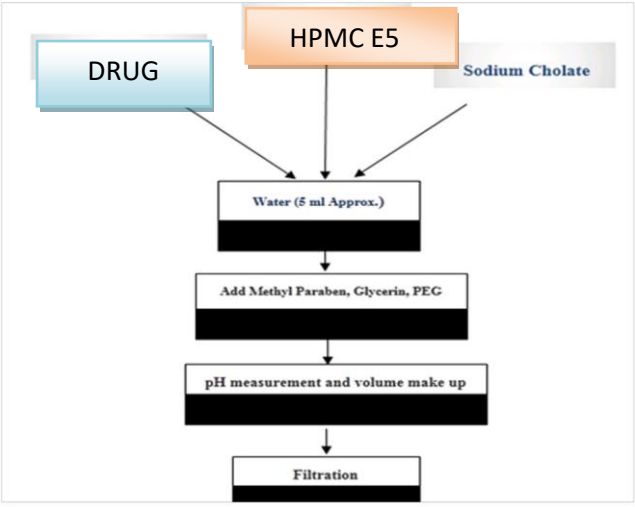

Fig 5 Nasal Spray Formulation (Flowchart)

COMPOSITION OF NASAL SPRAY FORMULATION

Table No 3 : Batches with Composition of Nasal spray formulation (10 ml) using Central Composite Design

Batch no.	HCQ (mg)	Conc of HPMC E5 (g)	Conc of Glycerine w/w	Sodium cholate (g)	Benzalkonium chloride	Distilled Water
B1	25	0.3	0.25	0.25	0.01	q.s
B2	25	0.37	0.25	0.25	0.01	q.s
B3	25	0.22	0.25	0.25	0.01	q.s
B4	25	0.35	0.2	0.25	0.01	q.s
B5	25	0.25	0.3	0.25	0.01	q.s
B6	25	0.3	0.32	0.25	0.01	q.s
B7	25	0.3	0.25	0.25	0.01	q.s
B8	25	0.3	0.25	0.25	0.01	q.s
B9	25	0.25	0.2	0.25	0.01	q.s
B10	25	0.3	0.25	0.25	0.01	q.s
B11	25	0.35	0.3	0.25	0.01	q.s
B12	25	0.3	0.17	0.25	0.01	q.s
B13	25	0.3	0.25	0.25	0.01	q.s

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3. Result:

A. PREFORMULATION STUDIES :

a. Identification of Drug:

Table No.4 Organoleptic Characteristic

Characteristics	Observation
Appearance	Characteristics
Color	White Colorless
Odor	Odorless

b. Melting Point Determination:

Table No.5 Melting Point

Sr. No.	Melting Point(°C)	Avg. Melting Point(°C)
1.	168	169
2.	169	
3.	170	

c. Solubility:

Table No. 6: Solubility of drugs

Sr.No	Solvent	Observed Solubility
1	Water	Soluble
2	Ethanol	Insoluble
3	Chloroform	Insoluble
4	Ether	Insoluble

B. FT-IR Spectroscopic Determination:

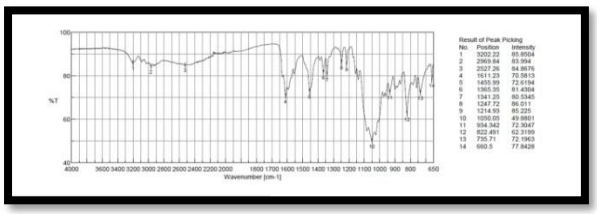


Fig 6: FTIR of Hydroxychloroquine sulfate

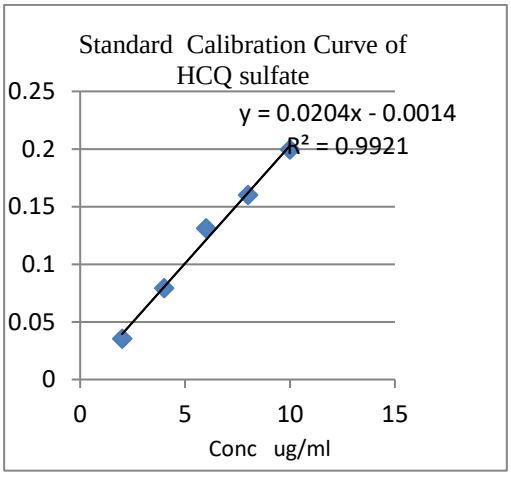
Hydroxychloroquine sulfate Table no. 7: IR Spectrum of Drug

Aromatic Ring	3202 cm^{-1}
C=C Aromatic Stretching	1455 cm^{-1}
C-Cl Stretching	660 cm^{-1}
C-OH stretching	1247 cm^{-1}
C-H Stretching	2969 $^{-1}$

Calibration curve of Hydroxychloroquine sulfate in Water:

Standard calibration curve of Hydroxychloroquine sulfate was drawn by plotting absorbance v/s concentration.Maximum wavelength of Hydroxychloroquine sulfate was found to be 265nm. The Equation of line was found to be y = 0.020x - 0.001 with correlation coefficient R^2 = 0.992

Table no.8 Calibration curve of Hydroxychloroquine sulfate at 265nm



C. Formulation Optimization Using Central Composite Design:

Central Composite Design was used to optimize HPMC E5 and Glycerine in a nasal spray to get optimum viscosity and % Drug Diffusion

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Table 9 : Responses of Formulation using Central Composite Design

Batch no.	X1:Concentration of HPMC E5	X2:Concentration of Glycerine w/w	Y1:Viscosity mPa	Y2:%Drug Diffusion	pH
B1	0.3	0.25	13.81	78.48	6.4
B2	0.37	0.25	21.02	73.47	6.6
B3	0.22	0.25	7.77	76.95	6.2
B4	0.35	0.2	16.85	74.01	6.3
B5	0.25	0.3	12.88	72.30	6.37
B6	0.3	0.32	15.65	76.74	6.28
B7	0.3	0.25	13.81	78.48	6.4
B8	0.3	0.25	13.81	78.48	6.4
B9	0.25	0.2	10.92	82.47	6.1
B10	0.3	0.25	13.81	78.48	6.4
B11	0.35	0.3	21.90	76.38	6.34
B12	0.3	0.17	12.53	79.34	6.29
B13	0.3	0.25	13.81	78.48	6.4

D. VALIDATION OF EXPERIMENTAL DESIGNS :

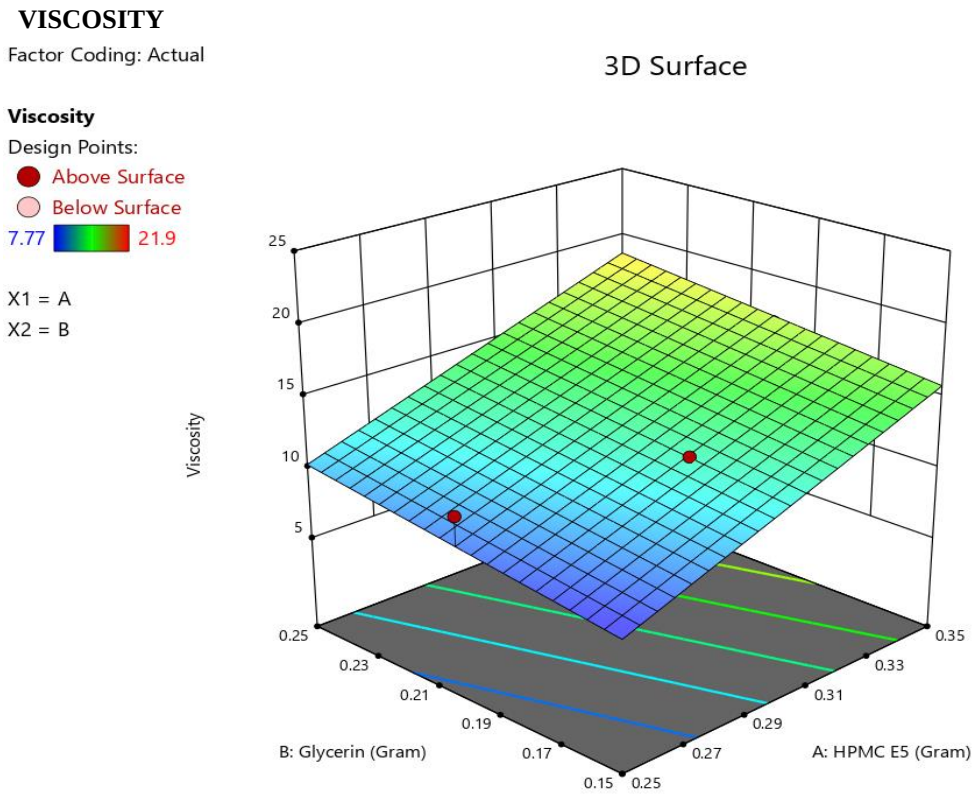

Figure No .7 : 3D Surface Graph of Viscosity

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Response	P-value	F-value	R^2	Predicted R^2 value	Remark
Viscosity	0.0001	61.12	0.9244	0.8477	Significant

% DRUG DIFFUSION :

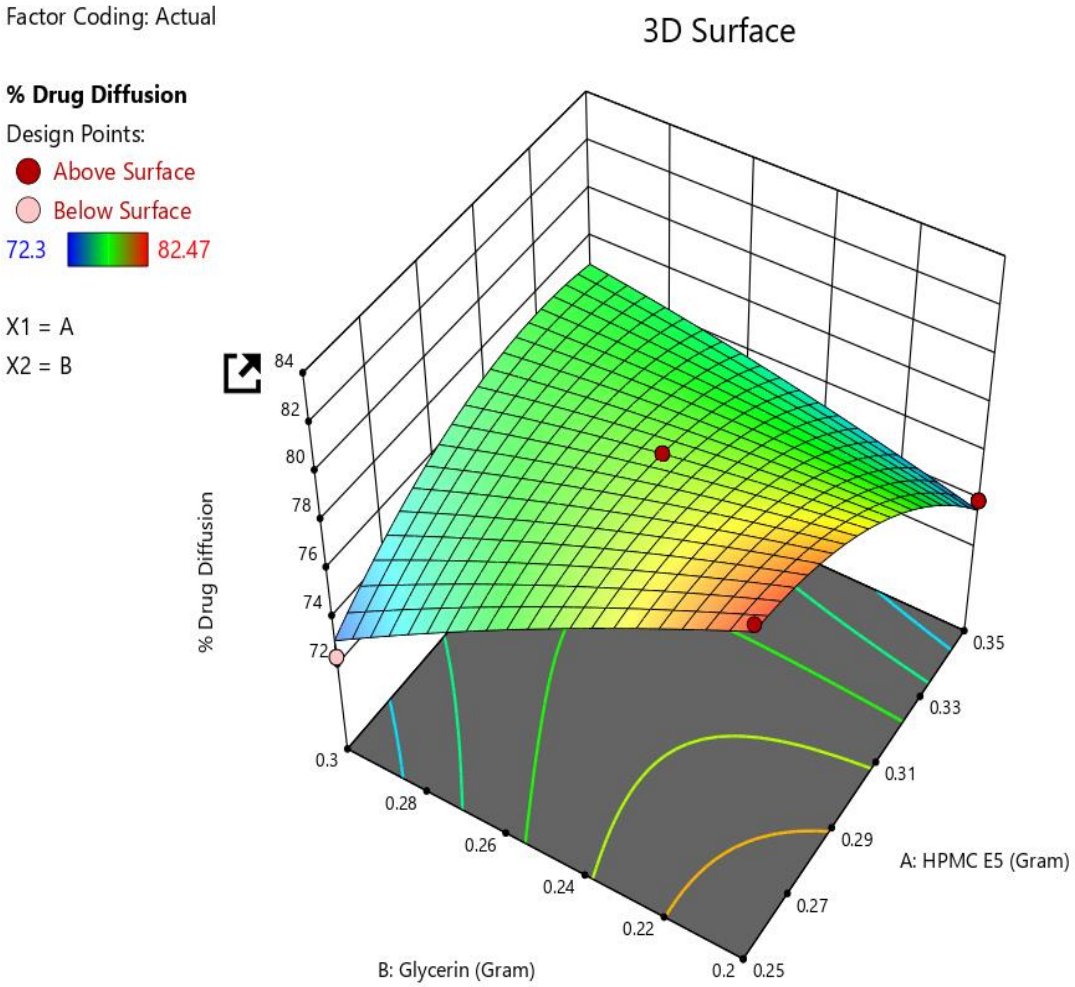

Figure No .8 : 3D Surface Graph of Drug Diffusion

Response	P-value	F-value	R^2	Predicted R^2 value	Remark
Drug Diffusion	0.0001	51.16	0.97	0.81	Significant

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4. Evaluation

1. Clarity :

The clarity of formulation was determined by visual inspection under black and white background. The prepared formulation was found to be clear (++).

2. pH :

The normal physiological pH range of nasal mucosa lies within the range of 4.5-6.5.The pH of the formulation was found to be 6.4±0.1 which lies within the normal physiological range.

Sr.No.	pH(n=3)	Avg .pH
1	5.9	
2	6.6	6.49±0.10
3	6.8	

3. Viscosity :

When the concentration of HPMC E5 and Glycerine the viscosity of nasal formulation increases linearly. The optimized batch from the Design Expert was found to be **B7**. The Viscosity of optimized batch B7 calculated by Ostwald's Viscometer was found to be **13.81 cp**.

4. Drug Diffusion :

In vitro drug diffusion of all batches was performed by using cellophane membrane. The drug diffusion of Hydroxychloroquine sulfate for optimized batch B7 was found to be **78.48 %**.

5. Conclusion

HPMC E5 and Glycerine were used as independent variables in central composite design for optimization of nasal spray for Hydroxychloroquine sulfate. B7 gave significant and good results with respect to viscosity and drug diffusion study. B7 shows the optimum viscosity of 13.81cp and drug diffusion through the cellophane membrane that is 78.48% respectively. In conclusion, it can be said that a stable nasal spray of Hydroxychloroquine sulfate was formulated which will bypass the first pass metabolism, improve the bioavailability by enhancing the drug diffusion, and give a prolonged release of the drug at the site by increasing the viscosity.

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