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DEVELOPMENT OF A NASAL SPRAY CONTAINING XYLOMETAZOLINE HYDROCHLORIDE WITH NITRIC OXIDE RELEASING SOLUTION FOR TREATMENT OF UPPER RESPIRATORY TRACT INFECTIONS

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ABSTRACT

The goal of this study is to create a nasal spray combining Nitric oxide gas and xylometazoline HCl that combines the nasal decongestant action of xylometazoline HCl with the antiviral activity of Nitric oxide to treat common upper respiratory tract infections such as the common cold, etc. Three distinct formulations comprising acidified nitrite solution, xylometazoline HCl, menthol, and camphor with three different pH values i.e. 2.6 ± 0.1, 3.4 ± 0.1 and 4.3 ± 0.1, were created to see if their properties interfered with each other. The stability of all three formulations was tested by monitoring NO gas release at 0 day, 15 days, 30 days, 45 days and 60 days intervals, respectively. The results showed that the formulations with pH 3.4 ± 0.1 were more stable and released NO gas even after 60 days. These findings provide the basis for starting further clinical studies on the pathophsiology for emerging infections.

KEYWORDS: *Xylometazoline HCl, Nitric Oxide, Upper Respiratory Tract Infections*

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INTRODUCTION

Upper respiratory tract infections (URTI) can be caused by a wide range of viruses and bacteria. These are responsible for a wide range of patient illnesses, including acute bronchitis, the common cold, influenza, and respiratory distress syndromes.

A URTI usually involves direct invasion of the upper airway mucosa by the organism. The organism is usually acquired by inhalation of infected droplets. Normal URTIs such as common cold is a very common disease infecting an adult 4-6 times/year and 6-8 times in children. Approximately, it causes 500 clinic visits per 1000 patients per year. Usually, the mucosa in the nasal cavity is the main site of infection, however infections may also initiate in the sinuses, the bronchi, the throat, or the ears. The common cold is generally caused by more than 200 viruses such as rhinovirus, corona virus, influenza, etc., which are continuously changing; where corona virus is responsible for severe symptoms while rhinovirus is responsible for mild symptoms which is responsible for 80% of all respiratory infections during peak seasons.

Common symptoms of URTIs include runny nose, cough, sore throat, nasal congestion, headache, low-grade fever, facial pressure, sneezing, discomfort, muscle aches and pain, etc.

As most of the symptoms go away on their own in one or two weeks, different products such as bronchodilators, anti-inflammatory drugs, decongestants, antihistamines, non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, etc. are used to relieve the symptoms. Furthermore, topical ointment such as Vicks® VapoRub™ can provide temporary relief from the symptoms. However, few studies have demonstrated that long term use of Vicks® VapoRub™ can lead to exogenous lipoid pneumonia and chemical leukoderma.

Xylometazoline hydrochloride (HCl) is a well-established nasal decongestant elimination half-life is 10-12 hours which is topically applied to relieve nasal congestion associated with acute or chronic rhinitis, common cold, sinusitis, hay fever or other allergies. It alleviates symptoms by restricting the big blood vessels that enlarge during the inflammation of any nasal infection or allergy.

These topical ointments, nasal decongestants, and analgesics offer only transient comfort from URTIs and do not aid in treatment by lowering virus loads in the upper respiratory tract.

Nitric oxide (NO), a free radical, available in the form of gas is key player in innate immunity, host defense mechanism, vasodilation, and neurotransmitter, in antimicrobial and antiviral activity with elimination half-life of few seconds. Studies revealed that NO has been shown to have non-specific antiviral effects in a range of viral infections, including HIV, influenza, wild type dengue virus, vaccinia virus (VACA), enterovirus, and coronavirus. NO or its derivatives reduces the replication of SARS-CoV at early stage and reduce the palmitoylation of the S protein, which is important for S-mediated cell–cell fusion, thereby reducing the attachment of S protein on the angiotensin-converting enzyme 2 (ACE 2) receptor. NO is mainly produced endogenously in endothelial cells of paranasal sinus and nasal mucosa from L-arginine and L-citrulline by a family of NO syntheses enzymes (NOS). NO regulates the ciliary beat frequency, when working perfectly, creating a sterile environment in the nasal cavity.

Further to enhance the antiviral effect of NO, hydroxypropyl methylcellulose (HPMC) has been added in the nasal spray which forms a gel-like matrix within the nasal cavity which inhibits the virus release from previously infected cells while NO prevents new virus to get attached to the cells and inhibits further replication.

To enhance the effect of nasal decongestant and provide a soothing effect in the nasal cavity, camphor & menthol can be used as they are active ingredients of Vicks® VapoRub™ which is used worldwide.

The purpose of mixing xylometazoline HCl and acidified nitrite solution into one product is to maximise each ingredient's benefits, especially xylometazoline HCl's decongestant effectiveness and NO's capacity to prevent viral infection. The intranasal topical medication improves breathing during ordinary URTIs and successfully eliminates the symptoms of nasal congestion. Additionally, NO has the ability to stop respiratory infections from multiplying and spreading. The aforementioned symptoms frequently involve viral infections, which hasten the progression of the underlying illness. As a result, patients benefit from a nasal spray that combines decongestant and antiviral characteristics as opposed to conventional xylometazoline HCl solutions.

An effect of utilized components has been summarized in table 1.

Table 1: Properties of Nitric oxide, Xylometazoline HCl, HPMC, Menthol and Camphor

Sr. No.	Component	Antiviral Properties	Antimicrobial Properties	Decongestant Properties	Cooling Properties
01	Nitric oxide	✓	✓		
02	Xylometazoline HCl			✓	
03	Menthol				✓
04	Camphor			✓	
05	HPMC	✓			

As a result, from a scientific and medical standpoint, a pharmaceutical product containing acidified nitrite solution, xylometazoline HCl, HPMC, menthol and camphor for the proposed indication and method of administration is favorable and reasonable.

MATERIALS AND METHODS

All process was carried out in a clean room environment to reduce the chances of contamination and glassware was sterilized using autoclave. All ingredients were weighed using calibrated Mettler Toledo Excellence Plus, Model No: XP205 weighing machine and pH was measured in Eutech pH 700 meter.

Acidified nitrite solution was prepared, in a ratio of 1.2% *w/v* $NaNO_2$ in mixture of 30% *w/v* $C_2H_8O_7$.

i. Preparation of 0.1% *w/v* Xylometazoline HCl (C16H25ClN2) solution: 0.1g of xylometazoline hydrochloride was precisely weighed and then added in 8 ml of 0.9% saline solution and thoroughly mixed using magnetic stirrer. After complete dissolution, additional saline was added to bring the solution to 10 ml. The pH of the mixture was determined and noted. In order to neutralise the hydrochloride activity of the xylometazoline, 0.01N sodium hydroxide was added frequently, and the pH was kept in range of 9.5- 9.8.

ii. Preparation of 0.1% *w/v* camphor (C10H16O) solution: 0.1g of camphor was precisely weighed and added in 0.7 ml of ethanol solution and thoroughly mixed using magnetic stirrer. After complete dissolution, additional ethanol was added to bring the solution to 1 ml.

iii. Preparation of 0.2% *w/v* Menthol (C10H20O) solution: 0.2g of Menthol was precisely weighed and mixed in 0.7 ml ethanol solution and mixed properly. More ethanol was added after complete dissolution, to make up the solution to 1 ml.

iv. Preparation of 0.2% *w/v* Hydroxypropyl methylcellulose (C56H108O30) solution: 0.2g of HPMC was accurately weighed and mixed in 7 ml 0.9% saline solution and mixed properly using magnetic stirrer. More saline was added after complete dissolution, to make up the solution to 10 ml.

v. Preparation of 0.01% *w/v* Benzalkonium chloride (C17H30ClN) solution: 0.01g of Benzalkonium chloride solution was precisely weighed and gently mixed into the 3 ml of 0.9% saline solution and mixed properly using magnetic stirrer. More saline was added after complete dissolution, to make up the solution to 10 ml.

Preparation of different Formulations of Xylometazoline HCl based NO Releasing Nasal Spray:

Formulation 1: Combination of acidified nitrite solution with Xylometazoline HCl, HMPC and Benzalkonium chloride solution:

1.2% *w/v* sodium nitrite solution was added in screw cap glass bottle and the solution was stirred on magnetic stirrer at 500-600 rpm. 0.1% *w/v* xylometazoline HCl solution neutralized to pH 9.5 was added in the glass bottle containing 1.2% *w/v* sodium nitrite solution and stirred for 5 minutes.

0.2% *w/v* HPMC solution along with 0.01% *w/v* Benzalkonium chloride solution was added in the resultant solution and stirred for 5 minutes which resulted in a milky solution.

The pH of the resultant solution was set to 3.4 ± 0.1 by adding 30% citric acid solution drop wise. The resultant solution volume was made up to 100 ml using normal saline solution and pH was adjusted again when it was necessary.

Table 2: Utilized Composition for formulation 1

Ingredients	Amount
Sodium nitrite	1.2g
Xylometazoline HCl	0.1g
NaOH	q.s
HPMC	0.2g
Benzalkonium chloride	0.01g
Citric acid	q.s
Normal Saline	q.s
Total Volume	**100 mL**

Formulation 2: Combination of Acidified Nitrite Solution with Xylometazoline HCl, Menthol, HMPC and Benzalkonium chloride Solution:

Formulation 2 is prepared in the same manner as Formulation 1, exception that 0.2% *w/v* Menthol solution was added after 0.1% w/v xylometazoline hydrochloride solution was added. By adding 30% citric acid solution dropwise, the pH of the resulting solution was adjusted to 3.4 ± 0.1. The resultant solution volume was made up to 100 ml using normal saline solution and pH was adjusted again when it was necessary.

Table 3: Utilized Composition for Formulation 2

Ingredients	Amount
Sodium nitrite	1.2g
Xylometazoline HCl	0.1g
NaOH	q.s
Menthol	0.2g
Ethanol	1 ml
HPMC	0.2g
Benzalkonium chloride	0.01g
Citric acid	q.s
Normal Saline	q.s
Total Volume	**100 mL**

Formulation 3: Combination of Acidified Nitrite Solution with Xylometazoline HCl, Menthol, Camphor, HMPC and Benzalkonium Chloride Solution:

Formulation 3 is prepared in the same manner as Formulation 2, except that 0.1% w/v Camphor solution was added after 0.2% w/v Menthol solution was added. The pH of the resultant solution was set to 3.4 ± 0.1 by adding 30% citric acid solution dropwise. The resultant solution volume was made up to 100 ml using normal saline solution and pH was adjusted again if necessary.

Table 4: Utilized Composition for Formulation 3

Ingredients	Amount
Sodium nitrite	1.2g
Xylometazoline HCl	0.1g
NaOH	q.s
Menthol	0.2g
Camphor	0.1g
Ethanol	2 ml
HPMC	0.2g
Benzalkonium chloride	0.01g
Citric acid	q.s
Normal Saline	q.s
Total Volume	**100 mL**

To assess the stability of these formulations, further formulations of formulation 1, 2 and 3 were made in a similar manner with the pH set to 2.6 ± 0.1 and 4.3 ± 0.1.

Storage of Solutions

As shown in Figure 1, the solution was kept in a plastic bottle with a screw-on cap, a 100 microliter spray pump, and an actuator.

Figure 1: A Plastic Bottle Containing Delivery and Storage Solutions.

(i) Measurement of Nitric Oxide Gas Emission from SOLUTION

The NO release form solution was quantified using portable NO gas analyzer procured from Endee Engineers Pvt. Ltd, analyzer can detect up to a maximum of 500 parts per million (ppm) of NO.

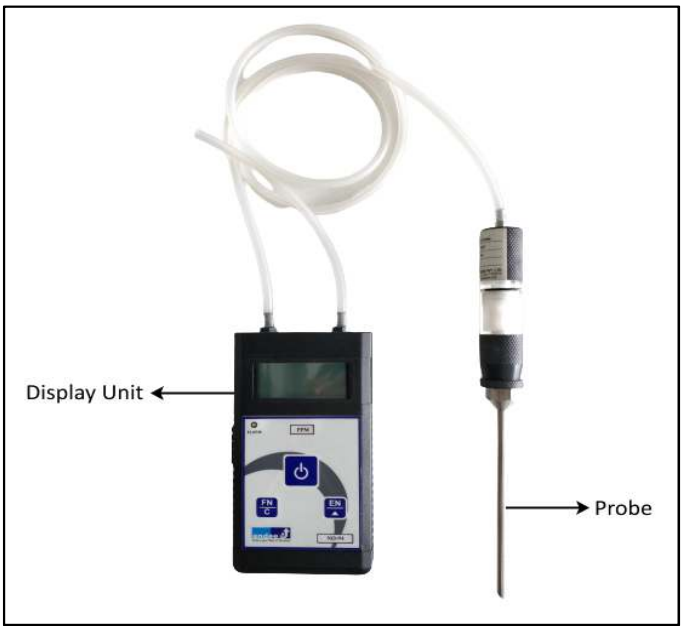

Figure 2: Portable NO Gas Analyzer.

As shown in Figure 2, portable NO gas analyzer comprised of a probe, a connector tube and an analyzer. The probe consists of long stainless-steel rod which is used for suction of NO gas which is transferred to the analyzer using the connector tube. The analyzer contains the sensor which detects the NO gas and displays the results in PPM level on the screen.

To measure the NO release from the prepared solution, single spray of 100 µL solutions was administered in a glass bottle and screw caped. The solution was allowed to settle down for 60 seconds and then uncapping the bottle, the probe of portable NO gas analyzer was inserted in the bottle and the NO gas was measured using analyzer.

(ii) Stability of different Formulations

All formulations were stored in plastic bottle as shown in Fig.1 and packed in outer box and kept in stability chamber at 30 ± 2 °C and relative humidity of 65 ± 5% as per ICH Q1(R2) guidelines.

One sample of each formulation was collected at intervals of 0 day, 15 days, 30 days, 45 days and 60 days in order to monitor the visual qualities, NO gas release, pH, and microbial growth.

RESULTS AND DISCUSSION

Measurement of Nitric oxide Gas Release from FORMULATION 1, 2 and 3 with pH 2.6 ± 0.1 at 0 day, 15 days, 30 days, 45 days and 60 days Interval Time

Formulation 1, 2 and 3 were prepared as mentioned above and pH was set to 2.6 ± 0.1. To measure the NO gas release from prepared solution, 100 µL spray of formulation 1, 2 and 3 were administered in a three different bottle respectively.

Using the portable NO gas analyzer, the NO release was measured during 0 day, 15 days, 30 days, 45 days and 60 days interval time which is mentioned in Figure. 3.

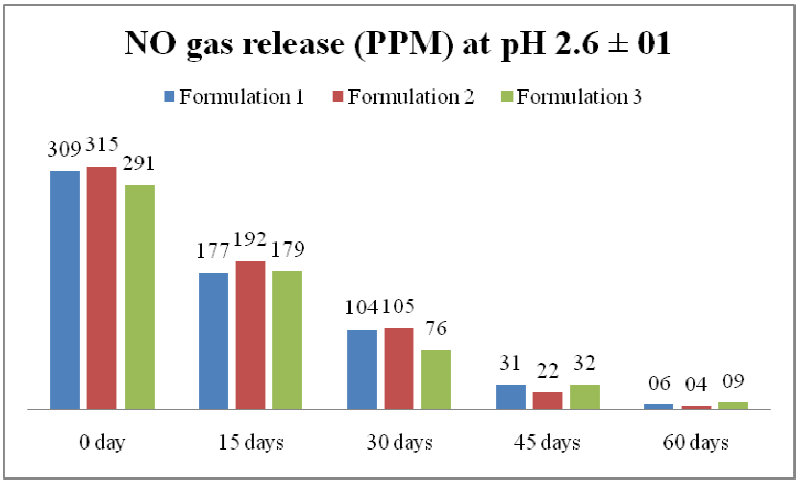

Figure 3: NO Gas Release at 0 day, 15 days, 30 days, 45 days and 60 days Intervals from Formulation 1, 2 and 3 with pH 2.6 ± 0.1.

The formulation 1 with pH set to 2.6 ± 0.1 released 309 ± 10 ppm at 0 day which reduced to 177 ± 10 ppm after 15 days. After 30 days the formulation was able to release 104 ± 10 ppm which further reduced to 31 ± 10 ppm after 45 days and 06 ± 10 ppm after 60 days.

The formulation 2 with pH set to 2.6 ± 0.1 released 315 ± 10 ppm at 0 day which reduced to 192 ± 10 ppm after 15 days. After 30 days the formulation was able to release 105 ± 10 ppm which further reduced to 22 ± 10 ppm after 45 days and 04 ± 10 ppm after 60 days.

The formulation 3 with pH set to 2.6 ± 0.1 released 291 ± 10 ppm at 0 day which reduced to 179 ± 10 ppm after 15 days. After 30 days the formulation was able to release 76 ± 10 ppm which further reduced to 32 ± 10 ppm after 45 days and 09 ± 10 ppm after 60 days.

Measurement of Nitric Oxide Gas Release from Formulation 1, 2 and 3 with pH 3.4 ± 0.1 at 0 day, 15 days, 30 days, 45 days and 60 days Interval Time

Formulation 1, 2 and 3 were prepared as mentioned above and pH was set to 3.4 ± 0.1. To measure the NO gas release from prepared solution, 100 µL spray of formulation 1, 2 and 3 were administered in a three different bottle respectively. Using the portable NO gas analyzer, the NO release was measured during 0 day, 15 days, 30 days, 45 days and 60 days interval time which is mentioned in Figure 4

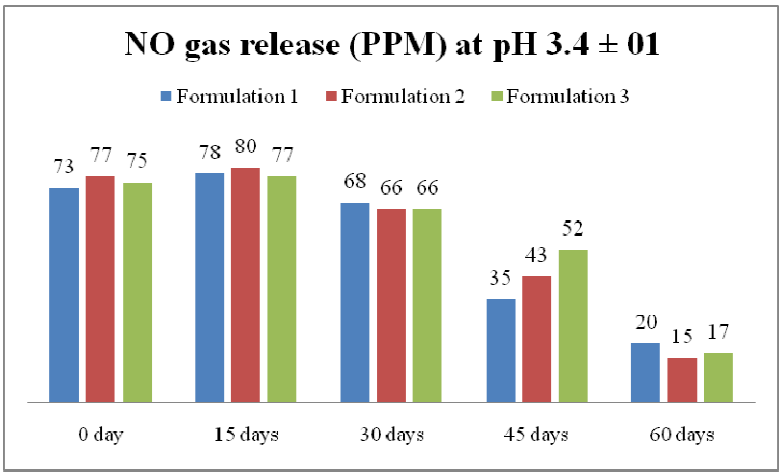

Figure 4: NO Gas Release at 0 day, 15 days, 30 days, 45 days and 60 days Interval time from Formulation 1, 2 and 3 with pH 3.4 ± 0.1.

The formulation 1 with pH set to 3.4 ± 0.1 released 73 ± 10 ppm at 0 day which increased to 78 ± 10 ppm after 15 days. After 30 days the formulation was able to release 68 ± 10 ppm which further reduced to 35 ± 10 ppm after 45 days and 20 ± 10 ppm after 60 days.

The formulation 2 with pH set to 3.4 ± 0.1 released 77 ± 10 ppm at 0 day which increased to 80 ± 10 ppm after 15 days. After 30 days the formulation was able to release 66 ± 10 ppm which further reduced to 43 ± 10 ppm after 45 days and 15 ± 10 ppm after 60 days.

The formulation 3 with pH set to 3.4 ± 0.1 released 75 ± 10 ppm at 0 day which increased to 77 ± 10 ppm after 15 days. After 30 days the formulation was able to release 66 ± 10 ppm which further reduced to 52 ± 10 ppm after 45 days and 17 ± 10 ppm after 60 days.

Measurement of Nitric Oxide Gas Release from Formulation 1, 2 and 3 with pH 4.3 ± 0.1 at 0 day, 15 days, 30 days, 45 days and 60 days Interval Time

Formulation 1, 2 and 3 were prepared as mentioned above and pH was set to 4.3 ± 0.1. To measure the NO gas release from prepared solution, 100 µL spray of formulation 1, 2 and 3 were administered in a three different bottle respectively. Using the portable NO gas analyzer, the NO release was measured during 0 day, 15 days, 30 days, 45 days and 60 days interval time which is mentioned in Figure 5.

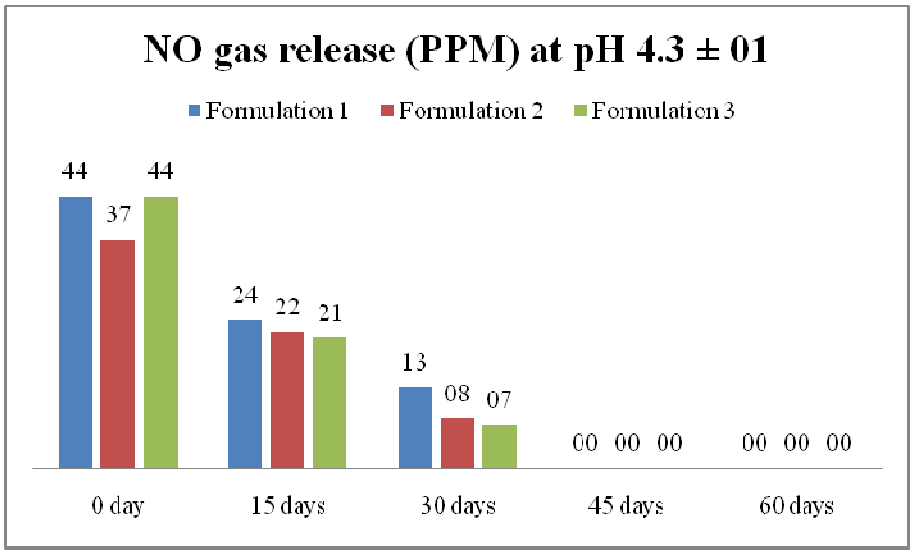

Figure 5: NO Gas Release at 0 day, 15 days, 30 days, 45 days and 60 days Interval time from Formulation 1, 2 and 3 with pH 4.3 ± 0.1.

The formulation 1 with pH set to 4.3 ± 0.1 released 44 ± 10 ppm at 0 day which reduced to 24 ± 10 ppm after 15 days. After 30 days the formulation was able to release 13 ± 10 ppm which further reduced to 00 ppm after 45 days.

The formulation 2 with pH set to 4.3 ± 0.1 released 37 ± 10 ppm at 0 day which reduced to 22 ± 10 ppm after 15 days. After 30 days the formulation was able to release 08 ± 10 ppm which further reduced to 0 ppm after 45 days.

The formulation 3 with pH set to 4.3 ± 0.1 released 44 ± 10 ppm at 0 day which reduced to 21 ± 10 ppm after 15 days. After 30 days the formulation was able to release 07 ± 10 ppm which further reduced to 00 ppm after 45 days.

Stability Data

Sample of each formulation were kept at 30 ± 2 °C and relative humidity of 65 ± 5% as per ICH Q1 (R2) guidelines. At an interval of 0 day, 15 days, 30 days, 45 days and 60 days, one sample of each formulation was taken to observe the visual observation, NO gas release, pH and microbial growth. Table 5 presents the stability data of each formulation with pH 2.3 ± 0.1 at defined intervals, whereas table 6 describes the stability data for each formulation with pH 3.4 ± 0.1 at defined intervals and table 7 describes the stability data of each formulation with pH 4.3 ± 0.1 at defined intervals.

Table 5: Stability Data of Formulation 1, 2 and 3 set at 2.3 ± 0.1

Formulation	Interval	Visual Appearance	NO Gas Release (ppm)	pH	Microbial Growth
pH 2.3 ± 0.1					
1	0 day	Milky	309	2.34	No
	15 days	Milky	177	2.21	No
	30 days	Milky	104	2.13	No
	45 days	Milky	31	2.03	No
	60 days	Milky	6	2.10	No

Table 5 Contd.,

2	0 day	Milky	315	2.37	No
	15 days	Milky	192	2.31	No
	30 days	Milky	105	2.27	No
	45 days	Milky	22	2.43	No
	60 days	Milky	4	2.34	No
3	0 day	Milky	291	2.35	No
	15 days	Milky	179	2.13	No
	30 days	Milky	76	2.20	No
	45 days	Milky	32	2.19	No
	60 days	Milky	9	2.13	No

Table 6: Stability data of formulation 1, 2 and 3 set at 3.4 ± 0.1

Formulation	Interval	Visual Appearance	NO Gas Release (ppm)	pH	Microbial Growth
pH 3.4 ± 0.1					
1	0 day	Milky	73	3.43	No
	15 days	Milky	78	3.45	No
	30 days	Milky	68	3.44	No
	45 days	Milky	35	3.48	No
	60 days	Milky	20	3.51	No
2	0 day	Milky	77	3.46	No
	15 days	Milky	80	3.43	No
	30 days	Milky	66	3.47	No
	45 days	Milky	43	3.49	No
	60 days	Milky	15	3.53	No
3	0 day	Milky	75	3.45	No
	15 days	Milky	77	3.41	No
	30 days	Milky	66	3.38	No
	45 days	Milky	52	3.45	No
	60 days	Milky	17	3.49	No

Table 7: Stability data of Formulation 1, 2 and 3 set at 4.3 ± 0.1

Formulation	Interval	Visual Appearance	NO Gas Release (ppm)	pH	Microbial Growth
pH 4.3 ± 0.1					
1	0 day	Milky	44	4.35	No
	15 days	Milky	24	4.38	No
	30 days	Milky	13	4.41	No
	45 days	Milky	0	4.43	No
	60 days	Milky	0	4.58	No

Table 7 Contd.,

2	0 day	Milky	37	4.34	No
	15 days	Milky	22	4.39	No
	30 days	Milky	8	4.44	No
	45 days	Milky	0	4.49	No
	60 days	Milky	0	4.52	No
3	0 day	Milky	44	4.37	No
	15 days	Milky	21	4.43	No
	30 days	Milky	7	4.48	No
	45 days	Milky	0	4.53	No
	60 days	Milky	0	4.61	No

As per above mentioned data, the formulations set with pH 3.4 ± 0.1 is more stable and release consistent gas compared to formulations set with pH 2.3 ± 0.1 and pH 4.3 ± 0.1

CONCLUSION

In order to combine the benefits of xylometazoline HCl's decongestant properties and NO's capacity to inhibit viral infection, an acidified nitrite solution that releases NO gas was combined with menthol, camphor, and HPMC. Three different formulations with pH 2.6 ± 0.1, 3.4 ± 0.1 and 4.3 ± 0.1 were prepared to check stability by measuring the pH and NO gas release at different intervals.

We found that the pH of the solution plays an important role in the release of NO gas. The formulations with pH 2.6 ± 0.1 released NO gas for a short duration of time in a high concentration (>180 ppm) that may cause toxicity in the human body. The formulations with pH 3.4 ± 0.1 released consistent NO gas for up to 60 days with a concentration of up to 80 ppm. The formulations with pH 4.3 ± 0.1 were only able to release NO gas for up to 15 days in a concentration of less than 45 ppm.

We also found that mixing xylometazoline HCl with or without menthol and camphor does not interfere with the NO releasing capacity of the acidified nitrite solution. This is because the NO elimination half-life is significantly shorter than that of xylometazoline HCl. As a result, the impact of NO upon administration will be considerably faster. Therefore, the properties of NO and xylometazoline HCl may not interfere.

Further to confirm the clinical effectiveness of these formulations, more clinical research examining the impact of xylometazoline HCl, menthol, and camphor in conjunction with NO is required.

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