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IN-SILICO AND IN-VITRO ASSESSMENT STUDY OF ANTI- MICROBIAL, ANTI-INFLAMMATORY ACTIVITY OF NAPROXEN TOPICAL GEL

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ABSTRACT

Introduction: Naproxen is a nonsteroidal anti-inflammatory drug (NSAID) primarily used to reduce inflammation, pain, and fever by inhibiting the enzymes COX-1 and COX-2, which are involved in prostaglandin production. This action helps alleviate symptoms of inflammatory conditions like arthritis and musculoskeletal injuries. While naproxen is not directly antimicrobial, some studies suggest it might enhance the effectiveness of antibiotics by reducing inflammation or disrupting bacterial biofilms, though this is still an area of ongoing research. Its main therapeutic benefit remains its anti-inflammatory and analgesic properties. **Methods:** Basic polymer carbopol934 was used to prepare topical gel included with methanol extract of naproxen, anti-microbial activity was performed by checking ZOI in which marketed naproxen gel has taken as control group and anti-inflammatory activity by egg denaturation assay by taking Diclofenac as control group. **Results:** ZOI of formulation at 1000 µg/ml is 19mm and marketed formulation is 15 mm which shows the plain naproxen formulation shows more anti-microbial activity, anti-inflammatory activity was observed well in naproxen formulation (90%) rather than in diclofenac (1.4). **Conclusion:** This study was concluded that naproxen has high anti-inflammatory activity also mild anti-microbial activity.

KEYWORDS: Anti-Microbial, Anti-inflammatory, Topical gel, Naproxen.

INTRODUCTION

One popular nonsteroidal anti-inflammatory medicine (NSAID) that is well-known for lowering fever, discomfort, and inflammation is naproxen. It functions by preventing the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes from producing prostaglandins, which are substances that cause heat, discomfort, and inflammation.

Naproxen efficiently relieves the symptoms of a number of ailments, including headaches, menstrual cramps, tendonitis, bursitis, and arthritis, by blocking these enzymes. Despite not being an antibacterial directly, naproxen may indirectly affect microbial activity by lowering inflammation, which could increase the potency of some antibiotics, according to some research. It has also been investigated for its potential to break apart bacterial biofilms, which may increase an infection's resistance to therapy. However, the drug's primary role remains as an anti-inflammatory and analgesic agent, with limited evidence supporting its antimicrobial properties.[1]

Pre-formulation studies

Table No. 1: Pre-formulation studies of Naproxen.

Colour	White to off white powder
Odour	Practically odourless
Texture	Crystalline powder
Taste	Bitter taste
Solubility	Soluble in 25 parts of ethanol, 20 parts of methanol, 15 parts of chloroform, 40 parts of Ether and practically insoluble in water.
Melting point	147°C

Figure 1: Melting point of Naproxen drug.

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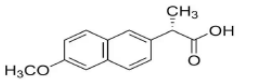
Name :	Naproxen
CAS Number :	22204-53-1
Synonym(s):	(S)-(+)-2-(6-Methoxy-2-naphthyl)propionic acid
Category of API's:	Nonsteroidal anti-inflammatory drug (NSAID)
Batch Number:	PADU0224

1. PHYSICAL AND CHEMICAL PROPERTIES

Molecular Structure	
Molecular Formula	$C_{14}H_{14}O_3$
Molecular Weight	230.26

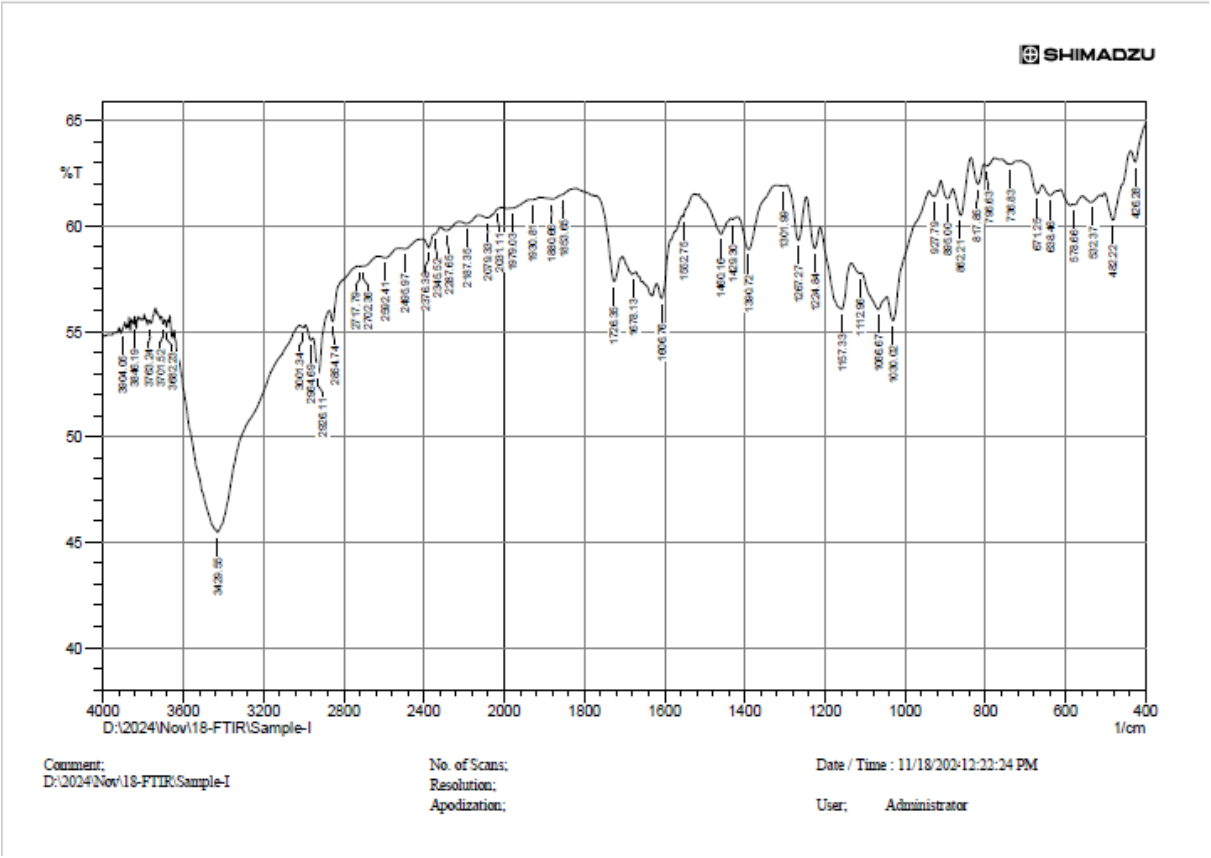
Parameter	Specification	Result
Appearance	White to Off-White Powder	White Powder
Solubility	Slightly soluble in ether; Soluble in methanol, chloroform; Freely soluble in alcohol	Complies

2. ANALYTICAL DATA

Purity (GC)	98.0 - 102.0%	99.99%
Specific rotation [a]20/D	+ 83.0° - + 89.5°	Complies
Melting Point	152–158 °C	Complies
Loss On Drying	max. 0.5 %	Complies
NMR	Confirm to structure	Complies
Storage Temperature	Store at room temperature below 30°C	

Caution - Not Fully Tested • Research Use Only • Not For Human or Veterinary Use

Figure 2: COA of Naproxen Drug.



Graph 1: FTIR of Naproxen Drug.

Solubility parameters

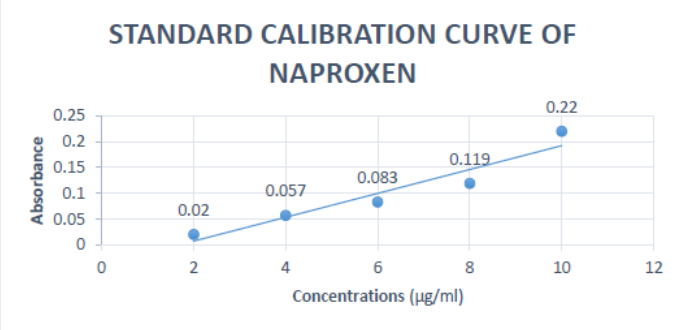
Making a 6.8 pH phosphate buffer involves dissolving 28.8g of disodium hydrogen phosphate and 11.45g of potassium di-hydrogen phosphate in enough distilled water to reach 1000ml. The pH is then measured. To get a concentration of 1000 µg/ml of naproxen, weigh 50 mg of naproxen and add it to 50 ml of 6.8 pH buffer. Making the secondary stock solution: To get a concentration of 100 µg/ml solution, pipette off 1 ml of the original stock solution and dilute it with buffer up to 10 ml. working standard preparation: Pipette 0.2, 0.4, 0.6, 0.8, and 1.0 of the secondary stock solution above into separate test tubes. Then, dilute each test tube with buffer solution until it reaches a concentration of 2,4,6,8, and 10 µg/ml. The absorbance of above dilutions were measured by UV Spectrophotometer at a wavelength of 277 nm, the standard graph was plotted by taking concentration on x-axis and absorbance on y-axis.[2]

Figure 3: UV Spectrophotometer.

Table 2: Absorbance of Naproxen drug dilutions.

S. No	Concentration	Absorbance
1.	2 µg/ml	0.02
2.	4 µg/ml	0.057
3.	6 µg/ml	0.083
4.	8 µg/ml	0.119
5.	10 µg/ml	0.220

Drug: Naproxen λmax=277nm



Graph 2: Standard Calibration Curve of Naproxen.

***Insilco* studies:**

Insilco studies provide valuable resources for scientific research and medication development, allowing computer-based modeling and simulation of biological systems and processes. Although they have many advantages, they should be used in conjunction with experimental procedures for a comprehensive research.[3] Molecular docking is most important technique to study protein and ligand binding techniques. In this process we used cb dock 2 free docking website and discovery studio to analyse the amino acids and bonds that are binded.[4]

Here the selected protein molecule is obtained in pdfqt format in website Protein Data Bank (RCBS-PDB) and ligands which are selected are extracted from web page pubchem in sdf format, these structures and now further used in CB dock 2 webpage. The structures extracted from the above step are now put in CB dock 2 web page as mentioned and due to auto docking technique it binds the ligand and protein and a combination structure is formed, highest negative vina score possess highest binding capacity, Now the structure complex is downloaded and put in discovery studio, this helps in removing water molecules and find the binding site and type of bond present.

Molecular docking on Naproxen with carbopol943

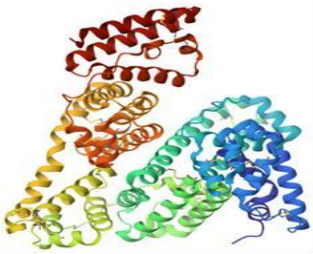


Figure 4: Structure of naproxen.

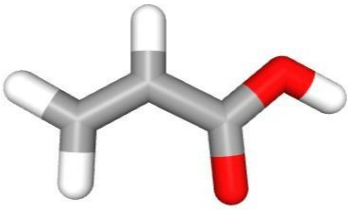


Figure 5: structure of carbopol 934.

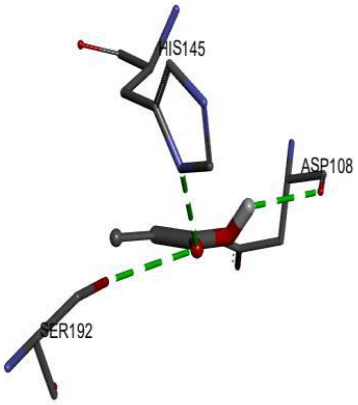


Figure 6: Structure of naproxen with carbopol 934.

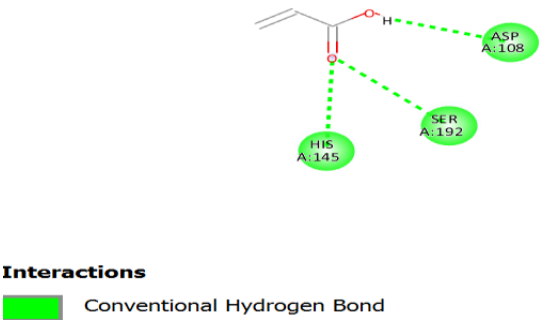


Figure 7: 2d structure of naproxen with carbopol.

CurPocket ID	Vina score	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)
C5	-3.9	3414	20, 26, 101	28, 24, 31
C1	-3.8	8974	0, 26, 107	35, 35, 27
C2	-3.8	8155	67, 21, 84	35, 35, 29
C3	-3.8	5911	50, 25, 97	35, 31, 31
C4	-3.6	4379	40, 14, 119	31, 35, 25

Figure 8: Binding score of naproxen with carbopol940.

RESULT

Molecular docking between naproxen and carbopol 940 with a binding score of -3.9 kcal/mol suggests a moderate interaction, where the two molecules bind with a relatively weak affinity. Naproxen, an NSAID, and carbopol 940, a polymer used in pharmaceutical formulations, may interact in a way that is not particularly strong, but still potentially relevant in some contexts. A binding score of -3.9 indicates a moderate level of affinity, and further analysis would be needed to explore the specific binding interactions and whether this interaction has practical significance for drug formulation or other applications.

MATERIALS AND METHODS

Table 3: Ingredients used in Naproxen topical gel.

Ingredients	Category
Naproxen (GC)	Anti-inflammatory and Anti-microbial
Carbopol934	Polymer
Glycerine	Plasticizer
Tri-ethanolamine	pH adjuster
Methyl Paraben	Preservative
Distilled water	Solvent

Method of preparation of Gel

The first step in making a topical gel is to dissolve a gelling agent (such carbomer, hydroxyethyl cellulose, or xanthan gum) in water. Usually, 0.5–2% of the gelling agent is used. The gel is then allowed to hydrate for 30–60 minutes, stirring gently to achieve adequate dispersion. To activate the gel's thickening capabilities, neutralize the mixture using a pH adjuster, such as triethanolamine, once the gelling agent has been hydrated. This will bring the pH down to about 7 for the best consistency. After that, swirl the gel base well to ensure that the active ingredient or ingredients are evenly dispersed. Preservatives should be added at this point if they are necessary (to stop microbial development).[5] If the gel is too thick, you can add additional solvent; if it is too thin, you can add more gelling agent. To guarantee a

uniform blend, stir the liquid for a further ten to fifteen minutes. Then, check the pH to make sure it is within the appropriate range. In order to avoid contamination and guarantee that the gel is stable for use, finally move the completed gel into the proper containers, such as tubes, jars, or airless pumps, and close them tightly.[6]

Formulation:

Table 4: Composition of Gel.

Ingredients	F1
Carbopol934	1gm
Distilled water	25ml
Naproxen	2g
Methanol	5ml
Methyl Paraben	0.1g
Glycerine	2ml
Tri-ethanolamine	1ml

Preparation of base gel

Weigh 1gm of Carbopol934 and add into three different beakers. Add 25ml distilled water into beakers. Continue stirring the Carbopol934 and water mixture up to 30-40 minutes using glass rod until uniform gel base is formed.[7] pH of base gel is observed as 5.2.

Figure 9: pH of Base gel.

Preparation of Naproxen topical gel

Weigh 2g of naproxen in different beakers. Add 5ml of methanol in each beaker and let the naproxen dissolve. In the base gel naproxen with methanol as solvent has been incorporated in the respective beaker (F1) and 2ml of glycerine and 0.1gm of methyl paraben is added, appropriate stirring is done to formulate uniform mixture in magnetic stirrer at 30rpm for 15- 20 min for good texture and consistency.

Figure 10: Naproxen gel F1 (2g).

Figure 11: Marketed naproxen gel.

EVALUATION STUDIES

- **pH**:

pH is tested using pH meter. As the prepared gel is thick and highly viscous we are diluting the gel, by mixing 1gm of gel into 10ml of distilled water and ensure the gel is thoroughly mixed to get a uniform sample, and check the pH, adjust pH using Triethanolamine.[8]

- **Spreadability**

The parallel plate method for measuring the spreadability of a topical gel involves placing a small amount of the gel between two parallel plates. A known weight is applied to one plate, causing the gel to spread. The spreadability is determined by measuring the area the gel covers after spreading, which reflects how easily the gel can be applied and distributed on the skin.[9]

- **Anti-microbial test**

The bacterial inoculum was distributed across the media's surface using the spread plate technique, which involved punching borers (6 mm in diameter) in the agar to introduce the microorganisms into nutrient agar plates. After allowing 500µg/ml and 1000µg/ml of Naproxen topical gel, and their mixture of each concentration to diffuse out into the agar medium in separate Petri dishes, the zones of inhibition were observed to be uniformly circular, displaying a confluent lawn of growth, after a 24- hour incubation period at 37°C. The zone of inhibition (ZOI) diameter was then measured in millimetres using the Vernier scale.[10] Through zone of inhibition we are performing anti-microbial test compared with marketed naproxen gel with two concentrations of 1000µg/ml and 500µg/ml.

Figure 12: Nutrient Agar medium

Figure 13: laminar air flow bench.

Figure 14: BOD incubator.

- **Anti- inflammatory activity:** Egg Albumin Denaturation Assay

Prepare 1000µg/ml and 500µg/ml dilutions of Naproxen + Herbal extract gel With 0.2% DMSO, add 0.2ml egg albumin and 2.8ml of phosphate buffer [pH 6.8], in 2 test tubes and prepare 2 test tubes of standard Diclofenac sodium dilutions of 1000µg/ml and 500µg/ml, prepare one blank test tube with egg albumin, phosphate buffer, 0.2% DMSO solution. Incubate for 10 min at 37°C and heat at 70°C then cool and filter the denatured egg albumin and make up the volume to 5ml with Phosphate buffer, check the absorbance.[11]

RESULTS

Table 5: Results of Evaluation Tests.

S.No	Evaluation test	F1 (2g)	Marketed formulation
1.	pH	5.73	-
2.	Spreadability	4.5cm	-
3.	Anti-microbial test	1000µg/ml = 16mm 500 µg/ml =12mm	1000µg/ml =14mm 500 µg/ml =11mm

Figure 15: pH of Naproxen topical gel.

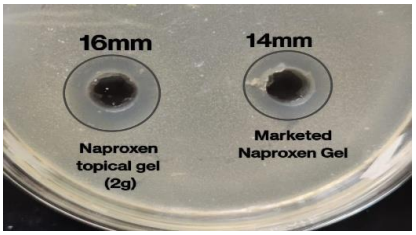


Figure 16: ZOI at 1000µg/ml.

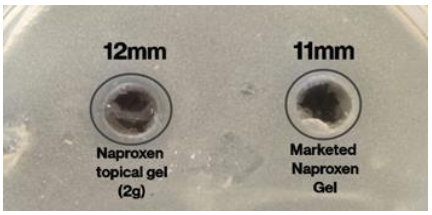


Figure 17: ZOI at 500µg/ml.

4. Anti-inflammatory test:

Table 6: Results of Anti-inflammatory activity.

Concentration	F1	Standard	Blank
1000µg/ml	1.83	1.4	0.05
500µg/ml	1.42	1.3	

$\%inh\ ibition\ = [AS/AC - 1]$ x 100

Concentration	F1	Standard	Blank
1000µg/ml	30.7%	1.4	0.05
500µg/ml	9.23%	1.3	

CONCLUSION

Here we conclude that based upon the evaluation parameters F1 formulation showed excellent Anti-microbial as well as Anti-inflammatory Activity as it showed increase in %inhibition of Albumin denaturation. F1 formulation has shown better activity when compared to that of Marketed Naproxen gel. The efficacy of F1 formulation was found to be comparable to that of Standard Diclofenac sodium solutions. So in upcoming future we can develop more potent topical as well as transdermal gel to reduce the contraindications and adverse effect caused due to oral drug delivery.

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