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Formulation and evaluation of topical gel containing combination of dapsone and acetazolamide

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***Abstract*---**The idea of innovation is to formulate a gel containing the combination of Dapsone and Acetazolamide for the treatment of Angioedema. Dapsone being a sulfone has antibacterial and antibiotic properties whereas Acetazolamide has a Diuretic property. So, a combination of these drugs helps to obtain a formulation with antibacterial, antibiotic and diuretic effect for the treatment of Angioedema. Various excipients such as a gelling agent, preservative, solubilizing agent etc. are used to form a stable and flawless gel formulation. The gel formulation prepared is used in the treatment of Angioedema that is a rapid oedema or swelling at specific areas such

as face, genitals, hands, feet's etc. The formulation has a comparatively greater effect for treatment of angioedema. Marketed formulations in treatment of angioedema have a specific action on the body such as antibiotic or diuretic whereas this new formulated gel has a combination of drugs that have a dual effect for the treatment of Angioedema. The swelling caused by the disease and the spread of infection is more effectively treated by this combination used in the gel formulation.

***Keywords*---**topical drug delivery system (TDDS), gel formulation, angioedema, HPMC, dapsone, acetazolamide, antibiotic, diuretic.

Introduction

Topical Drug Delivery System (TDDS) has now become a more progressive way for the treatment of diseases related to skin. This delivery system has a more target-oriented way of action. It includes formulations such as gels, creams, ointments, lotions etc. These formulations are directly administered at the site of skin injury, infection or diseases related to skin. Gels have a significant mechanism of action by optimal cutaneous and percutaneous drug delivery. They avoid the GIT (Gastro-Intestinal Tract) which helps in avoiding enzymatic activity and drug interactions with food and drinks thus avoiding the first pass effect. [1] Dapsone is a synthetic sulphone that has antibiotic and antibacterial property. It is a white crystalline powder that is odourless in nature. It is soluble in alcohol, methanol, acetone and Dil. Hcl. Dapsone acts against bacteria and protozoa in the same way as sulphonamides, that is by inhibiting the synthesis of dihydrofolic acid through competition with para-amino-benzoate for the active site of dihydropteroate synthetase. The anti-inflammatory action of the drug is unrelated to its antibacterial action and is still not fully understood. 5% of Dapsone gel is available in the market for the treatment of Acne Vulgaris. The antibacterial property of dapsone helps to cease the spread of infection. [2]

Acetazolamide is potent carbonic anhydrase (CA) inhibitor. It is a white crystalline powder which is odourless in nature. It is soluble in ethanol and slightly in water. The diuretic effect of acetazolamide is due to its action in the kidney on the reversible reaction involving hydration of carbon dioxide and dehydration of carbonic acid. The result is renal loss of bicarbonate (HCO3 ion), which carries out sodium, water, and potassium. The removal of water from the site of infection helps in treating swelling in case of angioedema. [3] The combination of Dapsone and Acetazolamide provide a dual effect in treatment of angioedema. The antibiotic and antibacterial effect of dapsone helps to cease the infection whereas the diuretic effect of acetazolamide helps in decreasing the swelling from the site of infection. Polymer or the gelling agent used is Hydroxy Propyl Methyl Cellulose (HPMC) that acts by stabilizing the gel molecules.

Materials and Methods

Materials

Dapsone and Acetazolamide were purchased from Aarti Distributors, Mumbai. HPMC K750 was obtained from Ashland Inc, Netherland. Other chemicals such as Tween 80, Methyl Paraben and Triethylamine were obtained in co-ordination from the Department of Pharmaceutical Quality Assurance. Dapsone is an antibacterial and antibiotic agent. Acetazolamide acts as a diuretic agent are main active pharmaceutical ingredient are used in the formulation. HPMC (Hydroxy Propyl Methyl Cellulose) is a polymer and is used as a gelling agent to form a stable gel formulation. The HPMC is mixed with water by continuous stirring to form a polymer complex which acts as a gel base for the formulation. Methyl Paraben used acts as a preservative and helps in maintaining the drug properties for a longer time. Tween 80 is used in as a surfactant; it helps in increasing the viscosity at acidic Ph. It also reduces the surface tension leading to increase in spreading and wetting properties of the gel. Tween 80 being in liquid form has an additive effect of increasing the volume of the formulation. Triethylamine used acts as an alkalizing agent or pH stabilizing agent. It is added in the formulation in quantity that is sufficient to make the gel neutral in nature to avoid any skin problems after application such as skin irritation, itching, redness etc. Purified water is added to the formulation with continuous stirring to avoid flocculation and formation of water bubbles. Water is added in sufficient quantity to make up the volume to desired quantity. These materials used were obtained in pure form and formulation was prepared. Each and every chemical was certified pure and free of any contamination or impurities. The purity of the drugs is important to formulate a stable gel, free from any contaminations or impurities.

Methods[7]

There are some of the processes like Hydrolysis and polycondensation (SOL-GEL), gelation, aging, drying, densification, and crystallization for the formulation of gel formulation. SOL-GEL: This process involves hydrolysis and polycondensation reactions. In this method a colloidal suspension is converted to a gel. It is compatible to polymer and polymerisation. DISPERSION: In this method the gelling agent or the polymer is dispersed in water with continuous stirring at 1200 rpm for 30 minutes. Then the API are directly added to the dispersion with a suitable preservative. We have used Dispersion method for successful formulation of the gel.

Dispersion method [8]

HPMC was dispersed in water with stirring at 1200 rpm for 30 minutes. Next step involves addition of API with continuous stirring. Dapsone and Acetazolamide are mixed with the dispersion of HPMC. Finally, the addition of excipients is done such as methyl paraben, Tween 80 and Triethylamine. Water is used as a vehicle to increase the consistency of the formulation and to make the volume to required quantity. During all the processes, continuous stirring of the formulation is required to avoid formation of water bubbles which may mess with the consistency of the formulation. The gel formulation then prepared is kept still for

30 mins to check whether it solidifies or not. This gel formulation prepared by dispersion method with firm and continuous stirring is free from flocculation or water bubbles. The polymer used is HPMC that acts as a gelling agent forms the dispersion with water and acts as a gelling base for the formulation. A stable gel formulation is prepared with the help of this dispersion method.

Formula

Table 1
Formulation Table for All Batches

Name of the ingredient	Batch I	Batch II	Batch III	Batch IV
Dapsone (gm)	0.375	0.375	0.375	0.375
Acetazolamide (gm)	0.25	0.25	0.25	0.25
Methyl paraben (gm)	0.01	0.01	0.01	0.01
HPMC K750 (GM)	0.025	0.050	0.075	0.125
Tween 80 (ml)	1.875	1.875	1.875	1.875
Triethylamine	Q. S	Q. S	Q. S	Q. S
Purified water	Q. S	Q. S	Q. S	Q. S

Analytical Tests Performed

Preformulation tests
UV-spectrophotometry: [9]

This technique is used to determine the absorbance of light in ultraviolet and visible ranges of electromagnetic spectra. A light in UV spectra is incident on the sample and the amount of light absorbed is determined. Method carried out in this test is as follows; Stock solution was prepared by weighing 10mg of drug and adding it in 0.1N Hcl. Dilutions were prepared of concentration of 2ppm, 4ppm, 6ppm, 8ppm, 10ppm and volume was makeup with 0.1N Hcl. Absorbance of each concentration was taken at 290 nm and calibration curve was plotted.

FT-IR Spectroscopy: [10]

This technique is used to determine the absorption of light in infrared spectra. It was used to determine different functional groups present in a drug or sample. FT-IR spectrum was recorded between 4000-400 cm^{-1}. This technique is mainly used in identification and confirmation of unknown materials.

DSC (Differential Scanning Calorimetry: [14]

A Differential Scanning Calorimetry, or DSC, is a thermal analysis technique that looks at how a material's heat capacity (Cp) is changed by temperature. A sample of known mass is heated or cooled and the changes in its heat capacity are tracked as changes in the heat flow. This technique helps in establishing a connection between the enthalpy and the physical properties of a substance.[15] The instrument used for DSC is a device thermal analysis instrument that

determines the temperature and heat flow associated with material transitions as a function of time and temperature.[16]

Postformulation tests

- pH determination:
  pH was recorded using digital pH meter. 5g gel was dispersed in 45ml distilled water at 27^{0}C and solution pH was measured.
- Conductivity:
  The conductivity of the sample depends on the dielectric constant of the solvent used. The conductivity may differ for each and every formulation depending upon the solvent used.

Particle size determination

It is a measurement of size distribution of individual particles of gel or any other formulation. It is a fundamental physical property that determine the physical nature and stability of the formulation.

Technique used
Laser diffraction spectrometry

It is the most widely used technique for determination of particle size distribution of any sample. This technique is widely used due to its rapid results and simplicity of use. It provides immediate experimental results compared to other techniques. Particle size distribution affects various physical parameters of a formulation are as follows; drug release rate for various sustained and delayed release formulations. Dissolution rate and bioavailability of various drugs. Dose uniformity and content of the formulation.

Figure 2

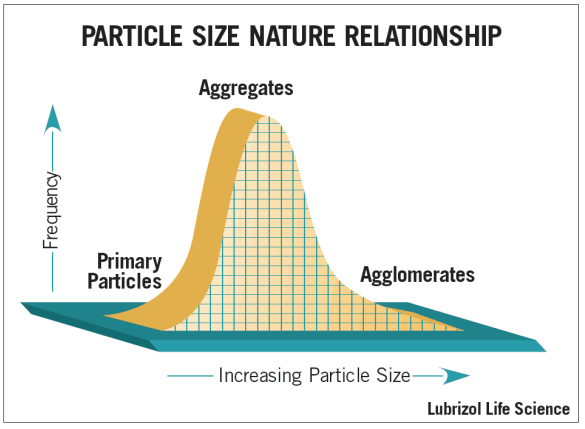


Figure: Relation between particle size and frequency of particles

Zeta potential: [17]

This process is used to determine the charge on the surface of particles present in the gel formulation. A greater positive and negative values of zeta potential indicate a good physical stability of the individual particles. This test helps in determining the physical stability of the formulation as well as the binding tendency of the particles of gel formulation. It is the key to electrostatic dispersion control. It is used to optimize the formulation and increase the stability of the formulation. It is used in determining long-term stability.

Table 2
Parameters of zeta potential

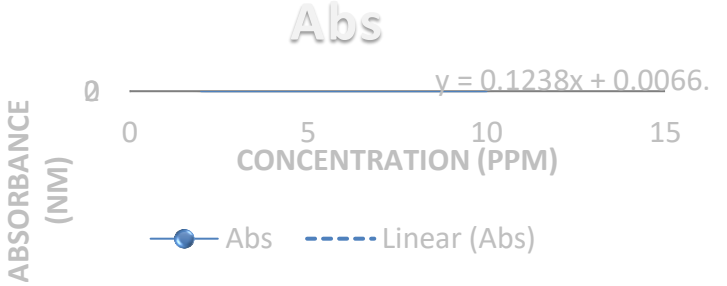
Manitude of Zeta potential (mV)	Stability behavior
0 to 5	Rapid coagulation or flocculation
10 to 30	Incipient instability
30 to 40	Moderate stability
40 to 60	Good stability
Greater than 61	Excellent stability

Result and Discussion

UV-spectrophotometry:[9]
Dapsone

Stock solution was prepared by weighing 10 mg of drug and adding it in 0.1N Hcl. Dilutions were prepared of concentration of 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10ppm and volume was makeup with 0.1N Hcl. Absorbance of each concentration was taken at 290 nm and calibration curve was plotted. Calibration curve was determined by plotting the values of Absorbance versus Concentration.

Sr No.	Concentration (in ppm)	Absorbance (in nm)
1.	2	0.245
2.	4	0.512
3.	6	0.755
4.	8	0.992
5.	10	1.243

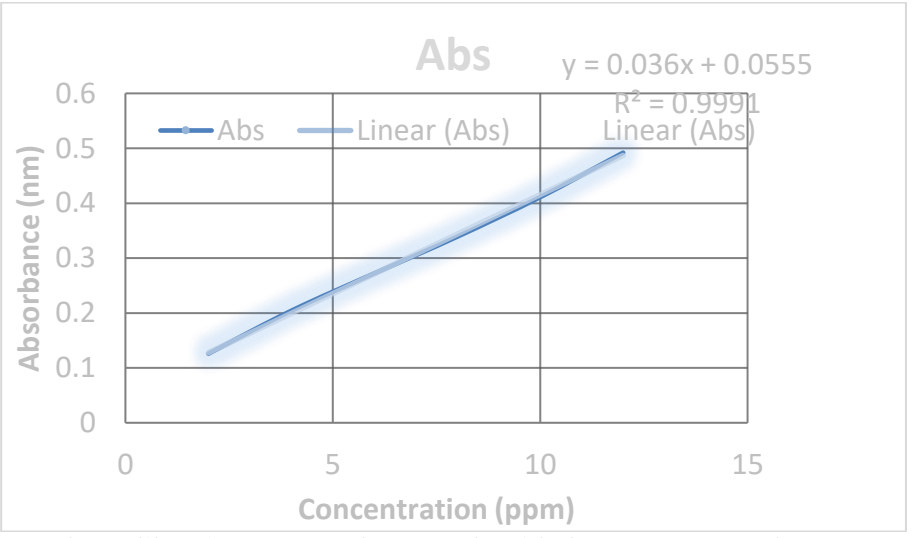


Graph: Calibration Curve of Dapsone by UV-Spectrophotometry

Acetazolamide

Acetazolamide (10 mg) was accurately weighed and dissolved in 100 ml of 0.00 1 N HCl to give a stock solution of concentration 100 g/ml. This was the primary stock solution of 100 g/ml. It was shaken to get the drug dissolved. UV spectrum was recorded in the wavelength range 278.94nm. Calibration curve was determined by plotting the values of Absorbance versus Concentration.

Sr No.	Concentration (in ppm)	Absorbance (in nm)
1.	2	0.126
2.	4	0.204
3.	6	0.272
4.	8	0.339
5.	10	0.411
6.	12	0.492

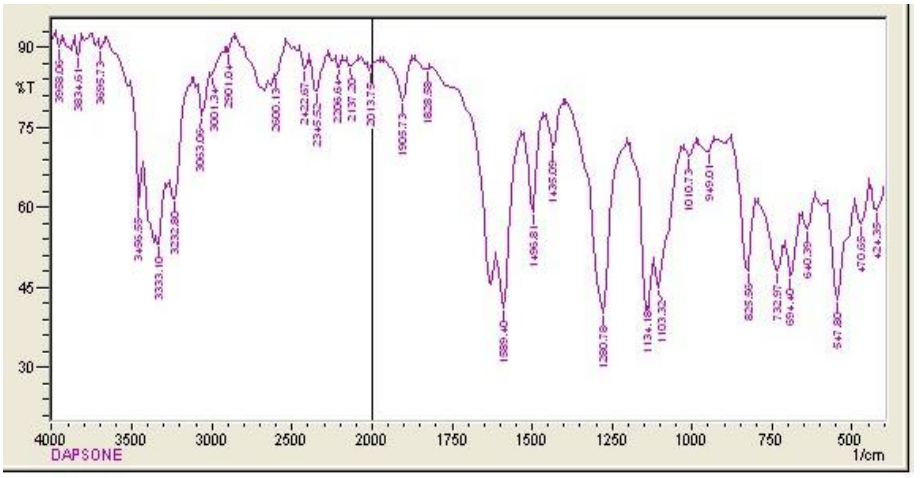


Graph: Calibration Curve of Acetazolamide by UV-Spectrophotometry

FT-IR Spectroscopy

The FT-IR spectroscopy was done and the following spectra were observed:

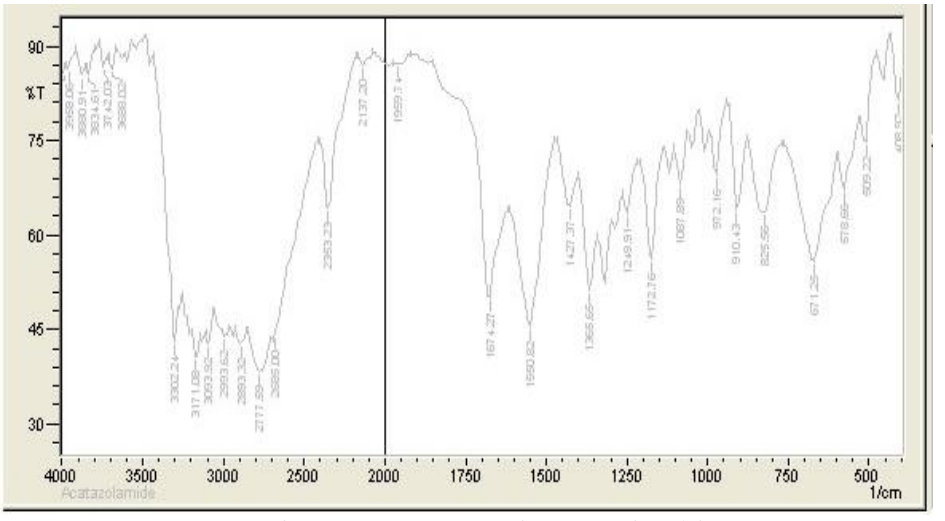
Dapsone



Graph: FT-IR spectra of Dapsone

Identification and confirmation of active pharmaceutical ingredients was carried out by observing the obtained FT-IR spectra. Dapsone showed characteristic peak values at 3063.06 (=C-H stretching); 3333.10 (N-H stretching); 1589.40 (C=C stretching); 1280.78 (C-N stretching) and 1134.18 (S=O stretching). These peaks values were determined by observed spectra of Dapsone as shown in above graph.

Acetazolamide



Graph: FT-IR spectra of Acetazolamide

Acetazolamide showed characteristic peak values at 3302.24 (C=O stretching); 2353.23 (C=N stretching); 1365.65 (S=O stretching) and 1674.27 (R-C(=O) NH_2 stretching). These peak values were determined by observed spectra of Acetazolamide as shown in the above graph.

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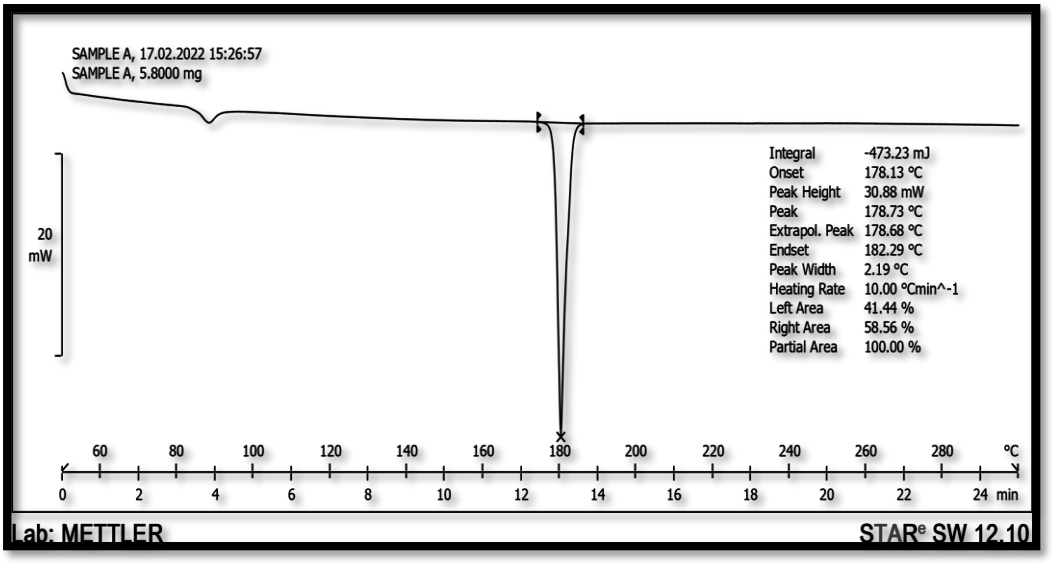
DSC (Differential Scanning Calorimetry):

SAMPLE A: Dapsone: The melting point of Dapsone is 175-178 ^{0}C.

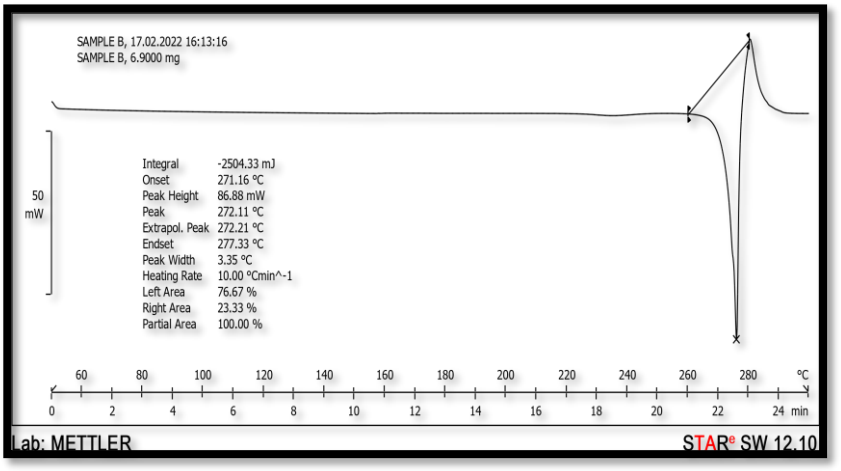


Graph**:** Differential Scanning Calorimetry of Dapsone

A curve is observed in the graph. The onset temperature is 178.13^0 C and the peak transition temperature is 178.73^0 C that is the melting point of Dapsone.so the thermal identification of Dapsone is done with the help of DSC.

Sample B: Acetazolamide:

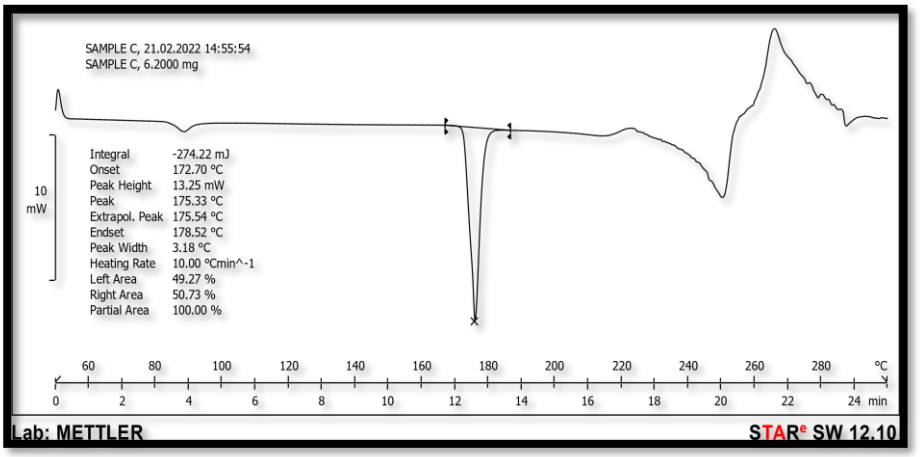
The melting point of Acetazolamide is 258-271^0 C.



Graph: Differential Scanning Calorimetry of Acetazolamide

A curve is observed in the graph. The onset temperature is 271.16^0 C and the peak transition temperature is 272^0 C that is the melting point of Acetazolamide.so the thermal identification of Acetazolamide is done with the help of DSC.

Sample C: Dapsone + Acetazolamide:



Graph: Differential Scanning Calorimetry of Dapsone and Acetazolamide in combination

A curve is observed in the graph. The onset temperature is 172^0 C. It shows the melting point of Dapsone. So, the combination shows a compatibility. So, the thermal compatibility of this combination is done with the help of DSC.

Post Formulation Analysis
PH Determination

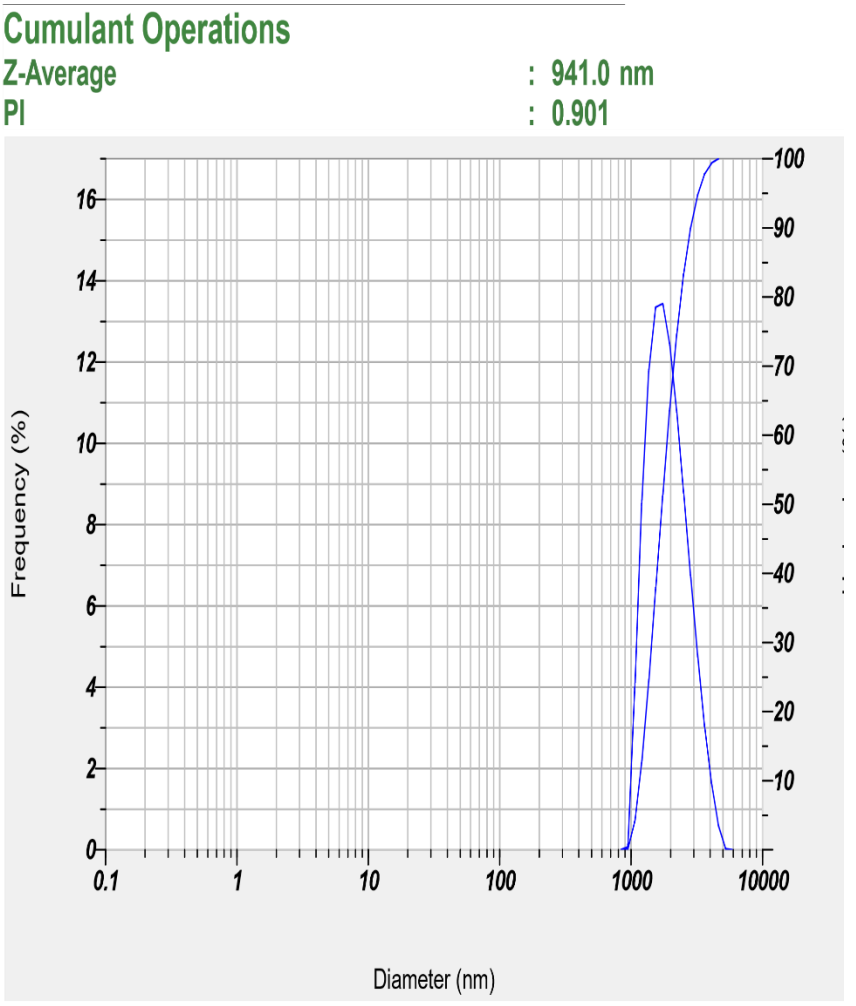
Batch no.	pH value
I	6.9
II	5.8
III	6.2
IV	6.4

Conductivity

Sr no.	200 ms	20 ms	2 ms	200 µs	20 µs
Batch I	000	00.2	0.19	190	1
Batch II	000	00.2	0.22	1	1
Batch III	000	00.2	0.22	1	1
Batch IV	000	00.1	0.18	177	1

Particle size determination

The test was done on 4 batches of gel and particle size data was obtained. Among them 4th batch had the best particle size. The graph of the 4th batch is as shown below:



Graph: particle size determination of Batch IV

Zeta Potential (17)

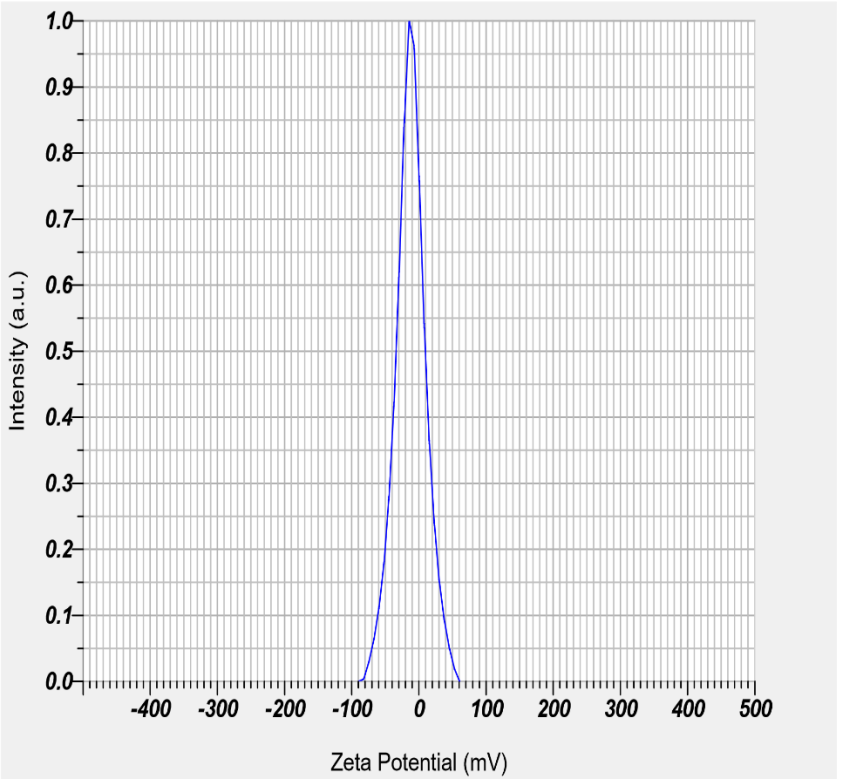
The test was done on 4 batches of gel and particle size data was obtained. Among them 4th batch had the best particle size. The graph of the 4th batch is as shown below:

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-12.1 mV	-0.000094 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : -12.1 mV

Electrophoretic Mobility Mean : -0.000094 cm^2/Vs



Graph: Zeta potential of Batch IV

Conclusion

The prepared formulation has a greater effect and stability compared to other present marketed formulations. Various marketed formulations present in the form of tablets, oral suspensions and i formulations have a synergic effect on treatment of angioedema. But the gel formulation prepared has a greater action because (1) The administration is done at the site of infection or cell injury so a greater drug absorption is caused which leads to more efficient action on the diseases. (2) The topical drug delivery avoids the First Pass Metabolism thus avoids the enzymatic degradation and acidic degradation of drug. (3) The

administered drugs are readily absorbed in the body and cause a dual action of antimicrobial as well as diuretic action which totally treat angioedema to minimize all its symptoms. (4) The systemic side effects caused are relatively low compared to other marketed formulations. Drug degradation leading to lower action of drug is minimized by use of preservative. So, a stable and long use gel formulation is obtained. Thus, a stable gel formulation is prepared that is effective in the treatment of angioedema.

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