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Development and Characterisation of Natural Deep Eutectic Solvents for Solubility Enhancement of Mesalamine

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Abstract

Background: Mesalamine is an amino salicylate drug widely used in the treatment of inflammatory bowel disease. Its limited solubility may present challenges during formulation development. Natural Deep Eutectic Solvents (NADES) have emerged as simple and environmentally friendly solvent systems with potential for enhancing drug solubilization.

Objective: The present study aimed to develop and compare NADES formulations prepared from naturally derived components for improving the solubility of mesalamine.

Methods: NADES formulations were prepared using citric acid: glycerin:D-glucose (CA: Gly: Glu) and tartaric acid: glycerine: fructose (TA: Gly: Fru) at selected molar ratios. Mesalamine was subjected to preformulation studies, solubility evaluation, UV–Visible spectrophotometric analysis, λmax determination, calibration curve preparation, and assay. The UV spectrum was recorded between 200 and 600 nm, and the absorption maxima were observed at 252.8 and 329.6 nm. The calibration curve demonstrated good linearity ($R^2 = 0.995$). Mesalamine solubility in the prepared NADES formulations was evaluated spectrophotometrically.

Results: The CA: Gly: Glu (1:2:1) formulation showed the highest absorbance (0.569), followed by CA: Gly: Glu (1:4:1) (0.542). The TA: Gly: Fru (1:2:1) and (1:4:1) formulations showed absorbance values of 0.256 and 0.150, respectively, compared with 0.279 for mesalamine in water. Among the formulations tested, CA:Gly:Glu (1:2:1) demonstrated the greatest solubilization potential.

Conclusion: The findings suggest that NADES, particularly the CA: Gly: Glu (1:2:1) formulation, may serve as a promising and environmentally friendly solvent system for enhancing the solubilization of mesalamine. The 1:2:1 ratio and 0.150 for the 1:4:1 ratio. Overall, the citric acid-based NADES formulations showed better solubilization potential than the tartaric acid-based formulations under the conditions studied.

Keywords: Mesalamine, Natural Deep Eutectic Solvents, NADES, solubility enhancement, citric acid, tartaric acid, glycerine, UV–Visible spectrophotometry.

1.INTRODUCTION:

Mesalamine (5-aminosalicylic acid, 5-ASA) – This is a widely used anti-inflammatory medication used to treat inflammatory bowel disease [1] (IBD), including mild to moderate Crohn's disease and ulcerative colitis. It acts by inhibiting the synthesis of inflammatory molecules such as prostaglandins and leukotrienes [2], by decreasing oxidative stress, and by eliminating free radicals from the intestinal walls [3]. While mesalamine is a primary treatment for IBD, its effectiveness can be affected by its limited water solubility, which may impact how it dissolves and is formulated [4]. Solutions of poorly soluble drugs have gained paramount importance in pharmaceutical research [5]. Different approaches have been tried, including size reduction of particles, preparation of solid dispersions [6], use of cyclodextrin complexes, application of nanotechnology, and designing novel solvent systems to improve the solubility of drugs [7]. Natural Deep Eutectic Solvents (NADES) are one type of environmentally friendly solvent that shows great potential for pharmaceutical applications among these methods [8]. Natural Deep Eutectic Solvents are created by blending two or more naturally occurring materials (organic acids, sugars, amino acids, and polyols) in a particular ratio [9]. They form tight hydrogen bonds, and the mixture has a lower melting point than the components. Tartaric acid, citric acid, glycerol, glucose, fructose, sucrose and choline derivatives are common ingredients of NADES [10]. NADES are attractive alternatives to conventional organic solvents due to their non-toxic characteristics, biodegradability, non-volatility, ease of preparation and environmental sustainability [11]. One of the most significant applications studied by researchers in pharmaceutical research is the use of NADES to improve the solubility and dissolution of different active pharmaceutical ingredients [12]. NADES have special hydrogen-bonding network and adjustable physical and chemical properties, enabling them to interact with drug molecules, which enhances the apparent solubility [13]. Moreover, the composition of NADES can be tailored by varying the kinds and proportions of the hydrogen bond donors and hydrogen bond acceptors, optimised for particular drugs [14]. There are functional groups that are available to form hydrogen-bonding interactions in Mesalamine, making it an appropriate candidate to increase the solubility of Mesalamine with the use of NADES [15]. The better solubility may facilitate the formulation process and offer an important insight into the implications of investigating green solvents in drug delivery studies [16]. However, there is limited information on using NADES to improve mesalamine's solubility [17]. This study is aimed at developing and characterizing the Natural Deep Eutectic Solvents from natural sources such as citric acid, glycerol, glucose, tartaric acid and fructose in order to evaluate their ability to improve the solubility of mesalamine [18]. The physical and chemical properties of the prepared NADES will be analysed and the solubilizing ability will be tested by using UV–Visible spectrophotometry [19]. The findings of this study may lead to the creation of eco-friendly, sustainable, efficient solvent systems for pharmaceutical uses, and aid in the future formulation of dosage form of mesalamine [20].

2.Materials and Methods:

2.1. Materials:

Mesalamine (5-aminosalicylic acid) was provided as a gift sample by the pharmaceutical company. The citric acid, tartaric acid, glycerine (glycerol), D-glucose, and D-fructose were purchased from a reputed chemical supplier of analytical reagent (AR) grade. All the studies were conducted using distilled water. Chemicals and reagents were used as received, and all were analytical grade.

2.2. Methods:

2.2.1 Preformulation Study

2.2.1.1 Physical Appearance

Mesalamine was evaluated for its physical appearance by visual observation. Colour, smell, feel, and overall look of the drug were noted in normal laboratory conditions [21].

2.2.1.2 Solubility Study:

The solubility of mesalamine was determined in distilled water and the following prepared Natural Deep Eutectic Solvent (NADES) formulations [22]. In clean containers, 10 mL of each solvent was prepared to which an excess amount of mesalamine was added separately. Each mixture was stirred for 45 minutes at room temperature on a magnetic stirrer for dissolution. The samples were then stirred and were left to equilibrate for an extra 15 minutes. The solutions obtained were filtered through Whatman No. 1 filter paper to remove the undissolved drug. The filtrates were appropriately diluted with the respective solvent if necessary, and the absorbance was read at 329.6 nm, against the solvent blank, in a UV–Visible spectrophotometer. The amount of dissolved mesalamine was determined using the calibration curve. The solubility of mesalamine was compared between the different formulations of NADES and distilled water.

2.2.1.3 Determination of λmax of Mesalamine

An appropriate solvent was used to make a suitable stock solution of mesalamine. The prepared solution was scanned in the appropriate ultraviolet wavelength range (200 to 800 nm) on a UV–Visible spectrophotometer. The wavelength value at which the maximum absorbance was obtained was noted as λmax, and this value was used to estimate mesalamine quantitatively. Based on the present study, the mesalamine exhibited maximum absorbance at 329.6nm and hence 329.6nm was chosen to be used for further spectrophotometric analysis [23].

2.2.1.5. Preparation of Calibration Curve of Mesalamine

A stock solution of mesalamine was prepared by dissolving 10 mg of mesalamine in 10 mL of appropriate solvent to make a 1000 µg/mL solution. Appropriate aliquots of the stock solution were transferred to separate 10 mL volumetric flasks and made up to the mark with the same solvent to prepare standard solutions of different concentrations (10, 20, 30, 40 and 50 µg/mL). Each of the standard solutions was then measured on the absorbance spectrum at the wavelength of 329.6 nm by a UV–Visible spectrophotometer using the proper solvents as blanks. A calibration curve was plotted between absorbance and concentration of mesalamine, and the regression equation and correlation coefficient (R^2) were calculated [24].

2.2.1.6. Determination of Mesalamine Concentration

Absorbance of the unknown sample of mesalamine was obtained by UV–visible spectrophotometer at 252 nm with the blank of the solvent/NADES [25]. The calibration curve was used to obtain a regression equation for calculating the concentration of mesalamine. The percentage assay was determined using the following equation:

$$\text{Assay (\%)} = \frac{\text{Calculated amount of Mesalamine}}{\text{Theoretical amount of Mesalamine}} \times 100$$

2.2.2. Preparation of Natural Deep Eutectic Solvents (NADES)

The appropriate amount of mesalamine was added to the prepared NADES formulations and it was continuously stirred at the appropriate temperature to ensure the dissolution of the drug. The mixtures were left for 24 hours at room temperature. The samples were equilibrated and then filtered through Whatman No. 1 filter paper to separate undissolved drug. The filtrate was appropriately diluted with the appropriate solvent, and absorbance of the clear filtrates was recorded at 329.6 nm using a UV-Visible spectrophotometer with the appropriate solvent as the reference. The concentration of the mesalamine in the NADES was determined by the calibration curve, which had been prepared earlier. The solubility of mesalamine in the different NADES formulations was compared to distilled water [26].

The prepared formulations were as follows:

NADES 1: Citric acid: Glycerine: Glucose (1:2:1)

NADES 2: Citric acid: Glycerine: Glucose (1:4:1)

NADES 3: Tartaric acid: Glycerine: Fructose (1:2:1)

NADES 4: Tartaric acid: Glycerine: Fructose (1:4:1)

2.2.3. Solubility Enhancement of Mesalamine in NADES

A measured amount of Mesalamine was added to the prepared NADES, and stirring was carried out continuously on a magnetic stirrer at 60-80°C until the solution formed was clear and homogeneous. The mixture was then allowed to cool to room temperature and kept for equilibration. The mixture was equilibrated and then filtered through Whatman No.1 filter paper to get rid of any undissolved drug. The filtrate obtained was highly transparent and was suitably diluted with the respective solvent and absorbance was taken at 329.6nm in a UV–Visible spectrophotometer with the corresponding NADES blank as blank. Using the calibration curve, the amount of mesalamine dissolved was determined. Formulation and Characterization of Natural Deep Eutectic Solvents for Enhancing Solubility of Mesalamine [27].

Data Analysis: -

To find out how well mesalamine dissolves in our specially made NADES mixtures, we used a calibration curve we had already prepared. We then compared this to how well mesalamine dissolves in pure water and some other solvents we picked. We did each test three times to make sure our results were accurate, and we showed our findings as an average, plus or minus a small amount of variation [28].

3. Results and Discussion:

3.1. Melting Point:

Sr.no.	Ingredients	Melting Point
1.	Citric acid	153°C
2.	Glycerine	18°C
3.	Glucose	146°C
4.	NADES	70–80°C
5.	Fructose	103°C
6.	Tartaric Acid	170°C

3.2. Solubility Study:

Sr.no.	Solvents	Solubility
1.	Water	-2.16
2.	PBS (6.8)	13.36

3.3. Determination of λ max of Mesalamine by UV–Visible Spectrophotometry:

The absorption of light by mesalamine was measured across a range of wavelengths, from 200 to 800 nanometres. It turned out that mesalamine absorbed light most strongly at a wavelength of 329.6 nanometres. So, this wavelength was chosen as the key wavelength for creating a calibration curve, determining the amount of mesalamine present, and doing further analysis using a UV-Visible spectrophotometer. This was all part of developing and characterizing natural deep eutectic solvents to improve the solubility of mesalamine.

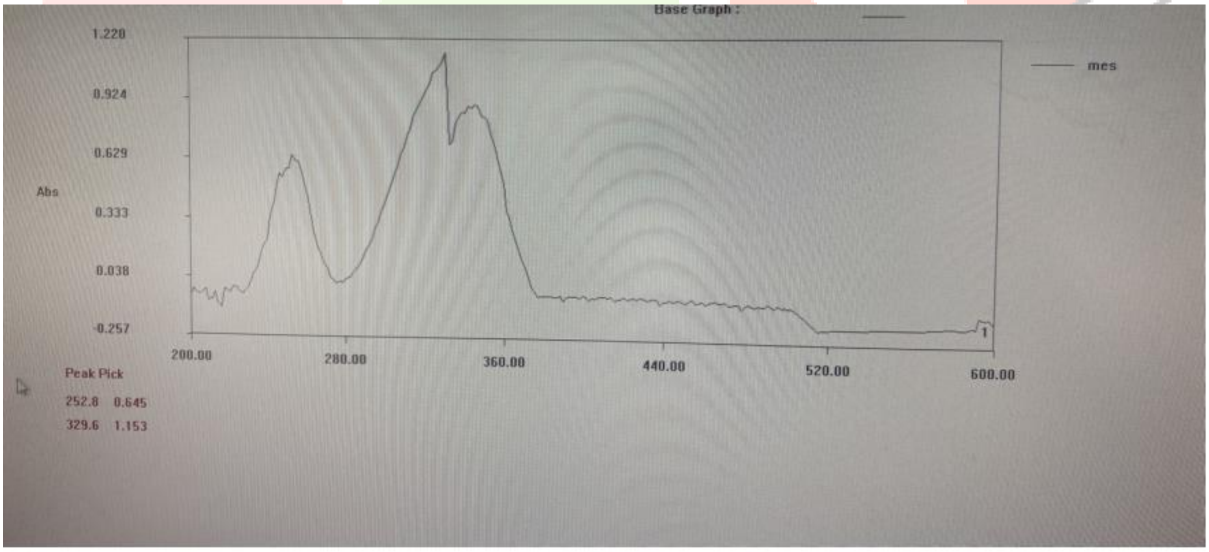
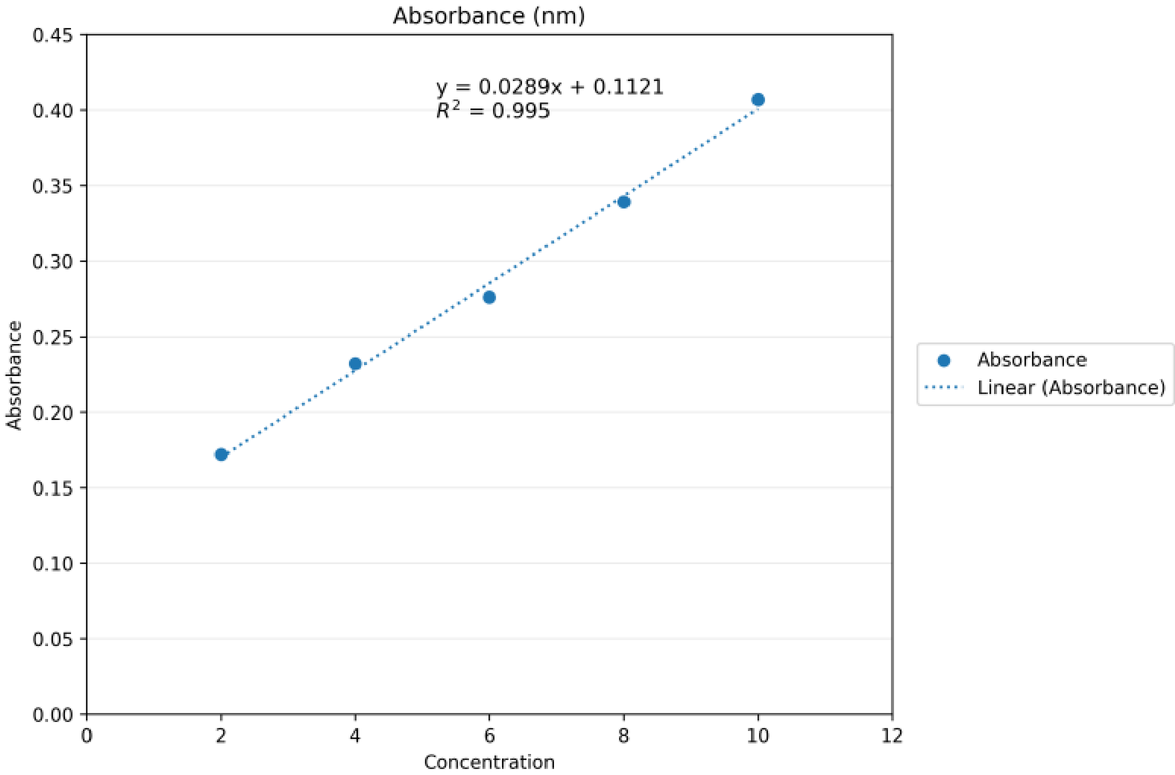


Figure. UV–Visible absorption spectrum of mesalamine. Peak picks: 252.8 nm (Abs = 0.645) and 329.6 nm (Abs = 1.153).

3.4. Preparation of Calibration Curve of Mesalamine:

A calibration curve for mesalamine was prepared by measuring the absorbance of standard solutions at 329.6 nm using a UV–Visible spectrophotometer. The absorbance increased with an increase in mesalamine concentration, indicating a linear relationship within the selected concentration range. The calibration curve was prepared by plotting absorbance against concentration, and the regression equation and coefficient of determination (R^2) were calculated. The developed calibration curve was subsequently used to determine the concentration of mesalamine in the samples analysed during the study.

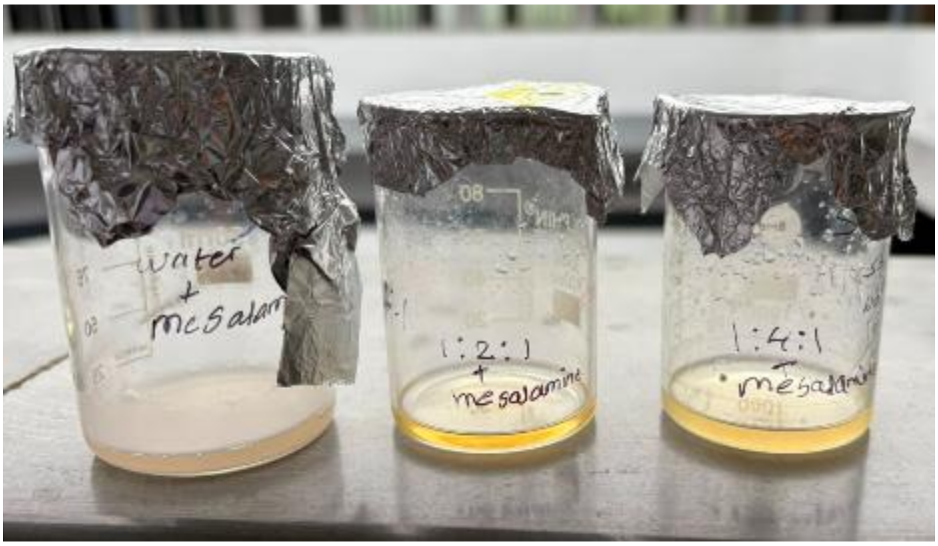


Assay of mesalamine:

3.5. UV–Visible Spectrophotometric Analysis

We measured how much light the filtered mesalamine samples absorbed at 329.6 nm using a special tool called a UV-Visible spectrophotometer. This tool had a 1 cm quartz cuvette. For each NADES mixture, we used a blank NADES solution without mesalamine as a reference. We calculated the amount of mesalamine in each sample using a calibration curve. Then, we compared the results to the aqueous sample to see how much the NADES mixture helped dissolve the mesalamine. This comparison allowed us to evaluate the solubility enhancement of mesalamine in the NADES formulations. By doing this, we could understand how well the NADES mixtures worked to increase the amount of mesalamine that could be dissolved.

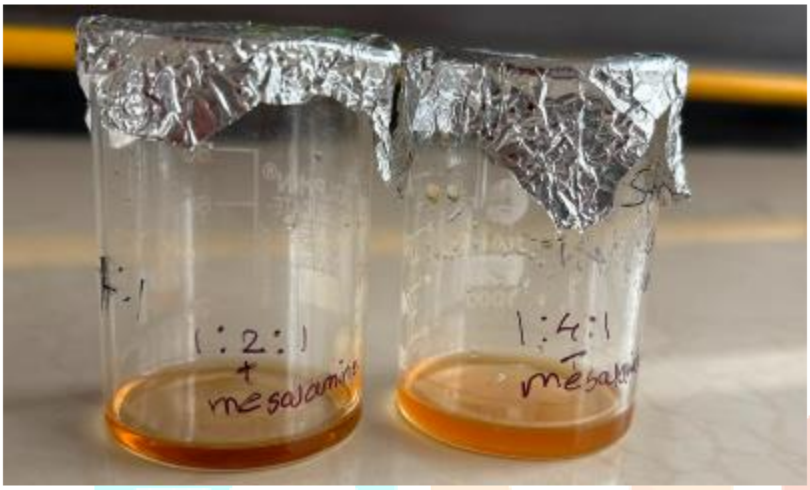
Preparation of Natural Deep Eutectic Solvent (NADES):



Preparation of Natural Deep Eutectic Solvent (NADES)

3.6. Solubility Enhancement of Mesalamine in NADES:

Sr.No.	Formulation (CA: Gly: Glu)	Absorbance	Solubility (mg/ml)
1	(1:2:1)	329.6	0.569
2	(1:4:1)	329.6	0.542
3	Water	329.6	0.279



Sr.No.	Formulation (TA: Gly: Fru)	Absorbance	Solubility (mg/ml)
1.	1:2:1	329.6	0.256
2.	1:4:1	329.6	0.150

3.7. Discussion

In order to improve mesalamine's solubility, this study looked into the usage of Natural Deep Eutectic Solvents (NADES) as an alternative solvent system. Citric acid, glycerine, and D-glucose (CA: Gly: Glu) and tartaric acid, glycerine, and fructose (TA: Gly: Fru) were used in varying molar ratios to create NADES formulations. The generated formulations' capacity to increase mesalamine's solubility was assessed in comparison. Mesalamine's physical and physicochemical properties were assessed during preformulation research. Two absorption peaks were visible in the UV-visible spectra at 252.8 and 329.6 nm, with the maximum absorption being seen at 329.6 nm. As a result, 329.6 nm was chosen for the spectrophotometric analysis that followed. With a correlation coefficient (R^2) of 0.995, the calibration curve showed high linearity over the chosen concentration range, suggesting a reasonable linear connection between absorbance and mesalamine concentration. The solubility analysis revealed that the NADES formulations under investigation had different solubilization potentials when compared to water. The absorbance of mesalamine in water was 0.279. The 1:4:1 formulation had an absorbance of 0.542, whereas the 1:2:1 formulation had the highest absorbance of 0.569 among the CA: Gly: Glu systems. By contrast, the absorbance values of the TA: Gly: Fru systems were lower, at 0.256 for the 1:2:1 formulation and 0.150 for the 1:4:1 formulation. According to the comparison results, under the examined experimental settings, the CA: Gly: Glu formulations showed a higher potential for mesalamine solubilization than the TA: Gly: Fru formulations. CA: Gly: Glu (1:2:1) was thot to be the most promising formulation out of all those examined because it had the highest observed absorbance. Interactions between mesalamine and the elements of the NADES system, especially hydrogen-bonding interactions, which might affect the drug's solvation within the eutectic medium, may be responsible for the increased solubilization. Overall, the results point to NADES as a viable and eco-friendly method for increasing mesalamine's solubility. To determine the chosen NADES formulation's stability, physicochemical characteristics, and appropriateness for pharmaceutical applications, more characterization and optimization are needed.

3.8. Conclusion

This research showed that Natural Deep Eutectic Solvents, or NADES, can be a good alternative to help make mesalamine more soluble. The team made different NADES mixes using citric acid, glycerine, and glucose, as well as tartaric acid, glycerine, and fructose. They then compared these mixes using a special tool called UV-Visible spectrophotometry, which measures how much light is absorbed by a substance at a specific wavelength, in this case 329.6 nm. By doing this, they were able to see how well each NADES mix worked to increase the solubility of mesalamine. When we looked at the different mixes, the one with CA, Gly, and Glu in a 1:2:1 ratio had the strongest effect, with an absorbance of 0.569. The next best was the 1:4:1 mix of CA, Gly, and Glu, which had an absorbance of 0.542. For comparison, when we just used mesalamine in water, the absorbance was much lower, at 0.279. The TA, Gly, and Fru mixes didn't do as well, with the 1:2:1 ratio giving an absorbance of 0.256 and the 1:4:1 ratio giving an even lower absorbance of 0.150. So, when we compare the two systems, we find that the CA: Gly: Glu NADES system is better at dissolving mesalamine than the TA: Gly: Fru system, at least under the conditions we tested. Looking at all the different formulations, we think that CA: Gly: Glu (1:2:1) is the most promising one, based on how it looked in the spectrophotometer. This study suggests that NADES could be a good, environmentally friendly way to improve how well mesalamine dissolves. To really make sure this system works for developing new medicines, we need to do more research on its physical and chemical properties, see how stable it is, and try to optimize the NADES formulation we've chosen.

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