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RESEARCH ARTICLE

Formulation and evaluation of salicylic acid suspension using *Araucaria heterophylla* gum as a suspending agent

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Abstract: Suspensions serve as a means to improve the bioavailability of poorly soluble drugs, utilizing a variety of suspending agents derived from natural, synthetic, and semisynthetic sources. In this investigation, the potential of Araucaria heterophylla mucilage, a natural origin suspending agent, was explored to enhance the in-vitro permeability of salicylic acid, serving as a model poorly soluble drug. The suspensions were formulated using the trituration method with concentrations of 0.5%, 1%, and 2% of the suspending agent. Comparative analyses were conducted with acacia, a natural suspending agent, and HPMC 50 cps, a synthetic suspending agent, at equivalent concentrations. Physical and chemical assessments, encompassing particle size analysis, viscosity, physical stability studies, assay, and in-vitro drug permeability studies, were applied to all suspensions. The outcomes revealed that Araucaria heterophylla mucilage, when utilized as a suspending agent at a concentration of 2% (F6), exhibited a reduced particle size, favorable viscosity, heightened in-vitro drug permeability, and commendable physical stability compared to the other formulated suspensions. Consequently, F6 was deemed the final formulation, with Araucaria heterophylla mucilage recognized as the preferred suspending agent relative to acacia and HPMC, given its superior characteristics. Notably, F6 demonstrated a nearly 2.4-fold increase in in-vitro permeability compared to the suspension devoid of a suspending agent (F10)

Keywords: Suspension; Natural Suspending agent; Bioavailability; Araucaria heterophylla; Salicylic acid.

1. Introduction

Suspensions represent thermodynamically unstable, biphasic liquid pharmaceutical formulations devised by dispersing insoluble or indiffusible drugs within a specified solvent or liquid medium, facilitated by the incorporation of a suspending agent. The primary objective of formulating suspensions is to augment the solubility of poorly soluble drugs. Characterized by a diminutive particle size, suspensions afford a greater surface area, thereby facilitating increased drug dissolution and subsequently enhancing bioavailability [1]. The particle size of the suspended particles is contingent upon the method employed for suspension preparation, as well as the type and concentration of the suspending agent utilized. A diverse array of suspending agents can be sourced from natural, synthetic, and semi-synthetic origins. Notably, natural suspending agents are widely preferred in pharmaceutical suspensions due to their economic viability, extensive safety profile, compatibility, eco-friendly nature, and abundant availability [2].

In the present investigation, we opted for Araucaria heterophylla, a natural plant gum, as the natural suspending agent based on extensive scrutiny of scientific research literature. Araucaria heterophylla, commonly known as the Christmas tree, belongs to the Araucariaceae family. Indigenous to the southern hemisphere, it thrives in diverse environmental conditions, including India, where it is frequently cultivated for indoor greenery. The gum extracted from the bark, obtained through incision, has various pharmaceutical applications, notably contributing to the formulation of suspensions as a suspending agent. This is attributed to the gum's capacity to significantly enhance water viscosity upon dissolution, owing to its rich carbohydrate content [3]. Leveraging these characteristics, we formulated suspensions utilizing this natural gum and compared its physical and in-vitro release properties with those of suspensions prepared using another natural suspending agent, gum acacia, and a synthetic suspending agent, HPMC 50 CPS. In formulating the suspensions, we selected a model drug with poor water solubility to investigate the in-vitro drug release characteristics—an essential focus of our current research. We prepared a series of suspensions at varying concentrations, employing two natural suspending agents and a synthetic suspending agent at three distinct concentrations. These formulations were systematically compared for their physical attributes and in-vitro drug release characteristics, juxtaposed with a suspension devoid of a suspending agent.

In the present investigation, salicylic acid was selected as a model poorly soluble drug due to its slight solubility in water, leading to a notably low in-vitro drug dissolution rate. To address this limitation and enhance the in-vitro dissolution of salicylic

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acid, a suspension dosage form was formulated. The primary aim of this study was to formulate suspensions employing two distinct natural suspending agents, namely Araucaria heterophylla plant gum and Gum acacia, alongside a synthetic suspending agent, HPMC 50 CPS. The objective was to identify the optimal suspension with superior in-vitro dissolution properties among these formulations.

2. Material and methods

2.1. Materials

Salicylic acid, Guma acacia, HPMC 50 CPS, benzoic acid and ferric nitrate are procured from Molychem Pvt Ltd, Bangalore. All the chemicals are of analytical and pure grade.

2.2. Analytical methods

The analytical method was performed to estimate the drug content in dosage forms or drug solutions with the help of selected analytical equipment i.e., UV-Visible spectrophotometer [4]. The procedure was as follows:

2.2.1. Determination of λmax

Salicylic acid was dissolved in 10 ml of water within a 10 ml volumetric flask, utilizing sonication for 5 minutes to ensure homogeneity. The resultant stock solution achieved a concentration of 1000 µg/ml. Subsequently, aliquots of 0.25 ml, 0.5 ml, 0.75 ml, 1 ml, and 1 ml were drawn from the stock solution and transferred to individual volumetric flasks, each being adjusted to a final volume of 10 ml with water. This process resulted in solutions with concentrations of 25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml, and 125 µg/ml, respectively.

For further analysis, a 2 ml aliquot of the median concentrated solution (75 µg/ml) was transferred to a test tube. To this, 2 ml of a 0.5% ferric nitrate solution was added. The resulting mixture was then subjected to scanning between 450-670 nm using a UV-visible spectrometer at various wavelengths, with the aim of determining the λmax of salicylic acid

2.2.2. Construction of standard calibration curve

A solution of salicylic acid was prepared by dissolving 10 mg in 10 ml of water within a 10 ml volumetric flask, utilizing sonication for 5 minutes to ensure proper dissolution. The resulting stock solution had a concentration of 1000 µg/ml. Subsequently, aliquots of 0.25 ml, 0.5 ml, 0.75 ml, 1 ml, and 1 ml were withdrawn from the stock solution and transferred to individual volumetric flasks, each being adjusted to a final volume of 10 ml with water. This process yielded solutions with concentrations of 25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml, and 125 µg/ml, respectively.

To further analyze these concentrations, 2 ml aliquots were drawn from each solution and individually placed into 10 ml test tubes. Subsequently, 2 ml of a 0.5% ferric nitrate solution was added to each test tube. The resulting mixtures were allowed to stand for 5 minutes to develop color. Following this, all solutions were subjected to scanning at 540 nm to obtain the absorbances of each individual concentration solution.

2.3. Formulation of salicylic acid suspension

2.3.1. Preparation of Aruacaria heterophylla plant gum

The gum exudates were collected from the incisions made on the bark of *Araucaria* tree. The collected gum was dried and pulverized. The powder was dispersed in distilled water using a mechanical stirrer for 4h. The fibrous materials were removed from the dispersion by filtration through a muslin cloth. The extract was treated with aliquots of acetone to precipitate the gum. The precipitate was separated and dried in a vacuum desiccator at 50 °C for 48 h. The dried precipitate was pulverized using a laboratory blender, passed through sieve number #80 to get uniform particles and stored in air tight container [5].

2.3.2. Preparation of salicylic acid suspension

The design of all suspension formulae was as follows, F1, F2 & F3 formulations were designed by taking 0.5%, 1% & 2% of acacia gum as a natural suspending agent. F4, F5 & F6 formulations were designed by taking 0.5%, 1% & 2% of Araucaria heterophylla as suspending agent. F7, F8 & F9 formulations were designed by taking 0.5%, 1% & 2% of HPMC 50 CPS as a synthetic suspending agent. F10 was prepared without suspending agent & considered as deflocculated suspension. All the suspensions are prepared by considering the formulae showed in Table 1 by using following procedure. Salicylic acid and benzoic acid were finely triturated with the aid of mortar and pestle. Mucilage of the gums was prepared by hydration using some portion of the distilled water. The mucilage of suspending agent was added in same increments to the powdered drug and triturated until homogeneous slurry was obtained. This was transferred to a 100 ml beaker and the remaining vehicle was used to rinse the mortar to make up the required volume.

Table 1. Formulation table for salicylic acid suspension

Formula	Salicylic acid (g)	Acacia (g)	*Araucaria heterophylla* gum (g)	HPMC 50 CPS (g)	Benzoic acid (g)	Distilled water (ml)
F1	1	0.5	-	-	0.1	100
F2	1	1	-	-	0.1	100
F3	1	2	-	-	0.1	100
F4	1	-	0.5	-	0.1	100
F5	1	-	1	-	0.1	100
F6	1	-	2	-	0.1	100
F7	1	-	-	-	0.1	100
F8	1	-	-	0.5	0.1	100
F9	1	-	-	1	0.1	100
F10	1	-	-	2	0.1	100

2.4. Evaluation of salicylic acid suspension

2.4.1. Particle size analysis

Particle is an important parameter for a suspension because the bioavailability of drug is indirectly proportional to the particle size. In this study mean particle size of all suspensions were calculated using microscopic method [6].

2.4.2. Viscosity

Viscosity is a very important parameter for a topical solution with respect to its flow properties, spread ability and intactness. The viscosity was determined by using capillary viscometer by comparing with water as a standard.

2.4.3. Assay

A volume of 1 ml from each suspension was introduced into separate 10 ml volumes of water and subjected to sonication for 5 minutes. From the resulting solutions, 2 ml was drawn into a 10 ml test tube. Subsequently, 2 ml of a 0.5% ferric nitrate solution was added to each test tube. The mixtures were thoroughly shaken and allowed to stand for 5 minutes to develop color. Following this, all the samples were subjected to scanning using a UV-Spectrophotometer at 540 nm to obtain the test absorbance. The percentage assay for all formulations was then calculated using the following formula:

$$\%\ Assay = \frac{Test\ Absorbance}{Standard\ Absorbance} x\ \frac{Standard\ dilution}{test\ dilution}\ x\ 100$$

2.4.4. Sedimentation volume (F)

Sedimentation volume is used to know the stability of the suspension and theprocedure for the sedimentation volume is as follows, 10 ml of suspension was taken in 10 ml measuring cylinder and sediment volume wasobserved every day for seven days and sedimentation volume was calculated by using below equation then the stability of suspension and best suspension among prepared suspensions were concluded by constructing a graph by taking time in days on X- axis and sedimentation volume, F on Y- axis [6]

Sedimentation volume, F = Ultimate volume of suspension/Initial volume of suspension

2.4.5. Degree of flocculation (β)

The degree of flocculation was studied to know the stability of prepared suspensions by comparing them with deflocculated suspension (F10) of salicylic acid and to select the best suspension among them by using following formula below [6]

$$\text{Degree of flocculation}(\beta) = \frac{Sedimentation\ volume\ of\ flocculated\ suspension}{Sedimentation\ volume\ of\ deflocculated\ suspension}$$

2.4.6. In vitro drug permeability studies

In-vitro drug permeability studies were carried out by following simulating diffusion Franz cell model. In this a 10 ml beaker was tied with a cellophane membrane such that three ml of suspension applied to the cellophane membrane that comes inside the beaker. This beaker was made to touch the surface of 25 ml water present in a 25 ml beaker. The water present in the beaker was maintained at 37±0.5^{0}C and agitated at 25 rpm by placing on a thermostatically controlled magnetic stirrer [7]. From 25 ml water solution two ml of sample was withdrawn for every hour till 8 hours. The 2 ml of withdrawn samples were placed in a 10 ml test tube and 2 ml 0.5% ferric nitrate solution was added and kept aside for 5 minute to develop colour. Then, the coloured mixtures were properly diluted and absorbances were taken for all samples by using UV- Spectro photo meter at 540 nm.

3. Results and discussion

3.1. Anayltical studies

The UV-visible spectrophotometric analysis revealed that the λmax for salicylic acid in water was determined to be 540 nm. Additionally, the R²-value for the standard calibration curve [4], as depicted in Figure 1, was found to be 0.9996. This observation indicates that the salicylic acid concentrations within the range of 75-150 µg/ml adhere to Beer-Lambert's law. Consequently, all formulations containing salicylic acid can be effectively analyzed using a UV-Visible Spectrophotometer within the colorimetric wavelength range.

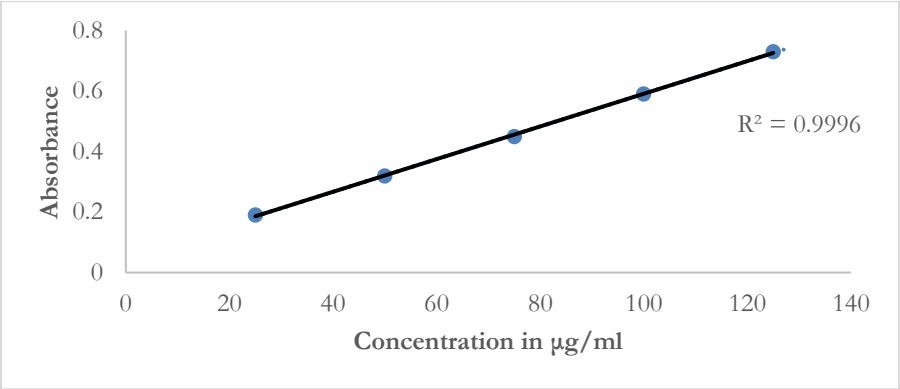

Figure 1. Calibration curve values for salicylic acid in water at 540 nm

3.2. Evaluation of suspensions

3.2.1. Particle size analysis

The mean particle size of salicylic acid was between 13.3 to 36.6 µm with the help of microscopic studies at our laboratory. The mean particle size was high for F10 and low for F5 & F6. And observed that, the low mean particle size for *Araucaria heterophylla* compared to acacia & HPMC 50 CPS. The ascending order for mean particle size as follows for the prepared salicylic acid suspensions. The results are shown in Table 2

Araucaria heterophylla gum < HPMC 50 CPS < Acacia

3.2.2. Viscosity

5 the relative viscosity for all the formulations from F1 to F10 was between 1 to 1.7 cps. The relative viscosity was observed highest for formulation F5 & F6 and lowest for formulation F10. Finally, it was observed that the relative viscosity was high for *Araucaria heterophylla* gum compared to acacia & HPMC 50 CPS. The descending order of relative viscosity for various suspending agents in this study was as follows. The results are shown in Table 2

Araucaria heterophylla gum> HPMC 50 cps > Acacia

3.2.3. % Assay

It was observed that the % assay values for all salicylic acid suspensions (F1-F10) were between 98.07 to 99.62%. All formulations were within the limits of assay with respected to the IP, the % assay values as per IP should be in between 95 to 105% for salicylic acid. Hence, all formulations have passed the test with respect to the IP. The results are shown in Table 2

Table 2. Results of evaluation parameters for the prepared suspensions

Formulation	Mean particle size (µm)	Viscosity(cps)	% assay
F1	32.6	1.02	98.28
F2	28.4	1.08	98.83
F3	20.8	1.17	98.24
F4	16.7	1.4	98.07
F5	13.3	1.7	98.48
F6	13.3	1.7	99.18
F7	21.2	1.17	99.75
F8	20	1.2	98.62
F9	16.3	1.4	98.84
F10	36.6	1	99.62

3.2.4. Sedimentation volume (F)

F9 formulation had highest sedimentation volume and F11 formulation had lowest sedimentation volume. As well it was observed F9, F7, F6 and F3 formulation have shown better sedimentation volume among all salicylic acid suspensions. The results are shown in Figure 2.

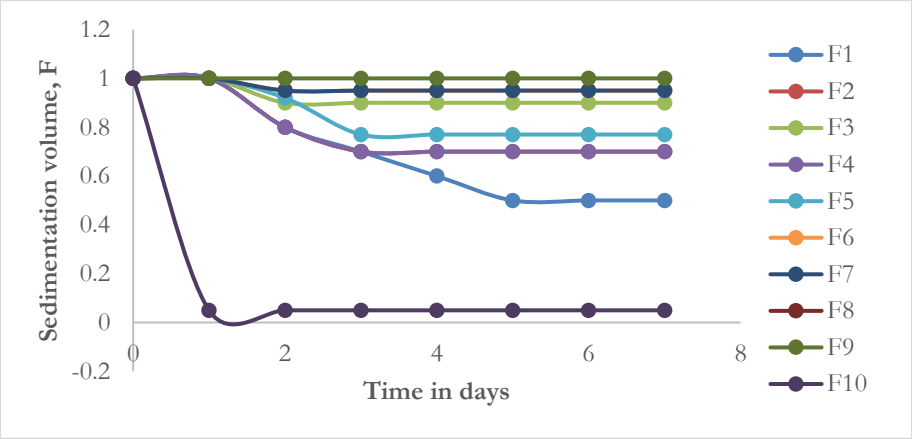

Figure 2. Sedimentation volume (F) for all the formulations

3.2.5. Degree of flocculation (β)

It was observed that all salicylic acid suspensions from F1 to F10 were showing highest β values as two. That means all suspensions were physically stable and were equally best with respect to the physical stability.

Table 3. Degree of flocculation for all the formulations

S.No	Flocculation (days)	β values								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
0	**0**	1	1	1	1	1	1	1	1	1
1	**1**	2	2	2	2	2	2	2	2	2
2	**2**	1.6	1.6	1.8	1.6	1.84	1.9	1.9	2	2
3	**3**	1.4	1.4	1.8	1.4	1.54	1.9	1.9	2	2
4	**4**	1.2	1.4	1.8	1.4	1.54	1.9	1.9	2	2
5	**5**	1	1.4	1.8	1.4	1.54	1.9	1.9	1.9	2
6	**6**	1	1.4	1.8	1.4	1.54	1.9	1.9	1.9	2
7	**7**	1	1.4	1.8	1.4	1.54	1.9	1.9	1.9	2

3.2.6. In vitro drug permeability studies

The assessment of cumulative % drug permeability at the 8th hour indicated that among all salicylic acid suspensions, F10 exhibited the lowest, while F8 demonstrated the highest cumulative % drug permeability. Within the formulations prepared using acacia as the suspending agent (F1 to F3), F3 displayed the highest cumulative % drug permeability at the 8th hour. Among formulations utilizing *Araucaria heterophylla* as the suspending agent (F4 to F6), F6 exhibited the highest cumulative % drug permeability at the 8th hour. Similarly, among formulations prepared with HPMC 50 cps as the suspending agent (F7 to F9), F9 displayed the highest cumulative % drug permeability at the 8th hour.

Upon further analysis, it was observed that F3 (with acacia as the suspending agent), F6 (with *Araucaria heterophylla* as the suspending agent), and F7 (with HPMC 50 cps as the suspending agent) were identified as formulations with the highest cumulative % drug permeability at the 8th hour. Additionally, F6 exhibited the highest cumulative % in-vitro drug permeability at the 8th hour among the formulations F3, F6, and F9. Overall, based on the cumulative % in-vitro drug permeability data for all salicylic acid suspensions, F6 emerged as the formulation with the best and highest cumulative % in-vitro drug permeability at the end of the 8th hour among all formulations. The results are shown in Figure 3.

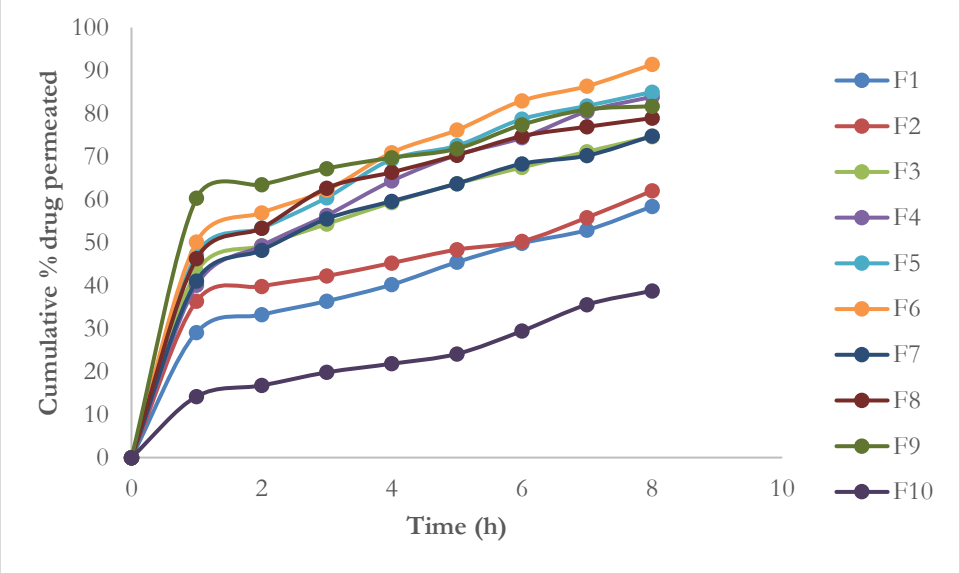

Figure 3. *In-vitro* cumulative % drug permeability data for salicylic acid suspensions

Based on the obtained results, it can be deduced that the selected analytical method was well-suited for evaluating salicylic acid in various formulations. This assertion is supported by the high R^2 value for the concentrations versus absorbances in the calibration curve, ranging between 0.997-0.997 and specifically noted as 0.9996. The method also demonstrated conformity to Beer-Lambert's law within the concentration range of 75 to 150 µg/ml, exhibiting linearity [8]. All salicylic acid suspensions were prepared using the trituration method, with formulations incorporating *Araucaria heterophylla* as the suspending agent being compared against those using a natural suspending agent (acacia) and a synthetic suspending agent (HPMC 50 CPS). This comprehensive comparative analysis encompassed various characteristics. The suspensions were prepared with suspending agent concentrations of 0.5%, 1%, and 2%.

The mean particle size for all salicylic acid suspensions ranged from 13.3 to 36.6 µm, with F5 and F6 exhibiting the smallest mean particle size at 13.3 µm. The relative viscosity across all formulations (F1 to F10) ranged from 1 to 1.7 cps, with the highest values observed for formulations F5 and F6. The physical stability of the suspensions was assessed through degree of flocculation studies, with all formulations deemed physically stable [9, 10]. F10 demonstrated excellent physical stability, as indicated by sedimentation volume studies. All formulations adhered to IP specifications concerning assay, falling within the 95 to 105% range. Notably, among all formulations, F6 exhibited the highest in-vitro cumulative % drug permeability.

4. Conclusion

In conclusion, the selection of F6 as the final formulation is supported by its favorable attributes, including excellent physical stability, a reduced particle size, heightened viscosity, and a notably high in-vitro cumulative % drug permeability. The choice aligns with the established correlation between particle size and bioavailability, where smaller particle sizes contribute to enhanced bioavailability. Additionally, a positive correlation exists between particle size and viscosity, with smaller particle sizes leading to increased viscosity, ultimately enhancing the suspension's adherence to the skin. Taking into account these interrelated factors, F6

emerges as the optimal formulation, showcasing desirable characteristics such as a diminished particle size, favorable viscosity, and heightened in-vitro drug permeability. F6 was formulated with *Araucaria heterophylla* as the suspending agent at a 2% concentration, leveraging its mucilage properties. Importantly, this formulation demonstrated a 2.4-fold increase in in-vitro drug permeability compared to the suspension prepared without a suspending agent (F10).

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