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Reinventing nano drug delivery systems for hydrophilic active ingredients in *Ayurvedic* lipid based formulations containing poly herbal decoction

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ABSTRACT

Background: Anu Tailam, an Ayurvedic medicated oil where 'anu' meant for atom and 'tailam' meant for oil and virtually meant for 'oil of subtle or atomic size particles'. Since the major active ingredients in this formulation are incorporated from the polyherbal decoction, it is expected to contain predominantly water soluble ingredients.
Objectives: It is hypothesized that these polar active botanical ingredients are present in the formulation should be either suspended in the form of submicron particles or entrapped in the submicron vesicular structures since the formulation did not show any precipitation or phase separation instead showed a monophasic oily liquid with very little moisture.
Materials and Methods: In the present investigation, the micro architecture of the anu tailam is studied via column chromatography and high performance thin layer chromatography to prove the contents are polar hydrophilic compounds followed by optical microscopy, photon correlation Spectroscopy (PCS) and environmental scanning electron microscope (ESEM) to study the particle/vesicle size of the formulation.
Results: In this study, it was proved that the formulation contained only polar ingredients and can be extracted in polar solvents like methanol and ethanol. It was also found that the formulation taken for study contained nano particles of the active botanical ingredients embedded in a network of vesicular structures of the lipid base.
Conclusion: The selected Ayurvedic formulation 'anutailam' found to contain novel nano drug delivery system to deliver water soluble ingredients across barriers.

1. Introduction

Small hydrophobic molecules can partition across biological membranes down a concentration gradient whereas hydrophilic molecules generally require some sort of selective transport system to cross the lipid bilayer. The modern formulation techniques did not have many methods that address absorption problems of highly water soluble drug molecules except injectable preparations [1]. *Ayurveda*, an ancient system of medicine originated from India during *Vedic* culture around 3000 years ago [2], has developed a group of lipid based formulations for hydrophilic molecules that are delivered through oral, nasal and topical routes. *Anu Tailam*, an *Ayurvedic* lipid based formulation (medicated oil) where '*anu*' meant for atom and '*tailam*' meant for sesame oil and virtually meant for 'oil of subtle or atomic size particles'. It is prepared from the decoction of 23 herbs and a fine paste of these 23 herbs (Kalka) but 12 times lesser the number of herbs taken for decoction along with goat's milk and sesame oil [3]. This formulation is categorized under a group of formulations called *sneha kalpana* (*Ayurvedic* lipid based formulations) in which ghee or oil is boiled with prescribed *kasayas* (polyherbal decoctions) and *kalkas* (fine paste of botanicals) as stated in the *Ayurvedic* texts until all the water is evaporated depend upon the *paka* (cooking stage) [4]. References for *anu tailam* were found in oldest of Ayurvedic treatises like *Charaka Samhita, Sushruta Samhita, Ashtanga Hrudaya, Sahasrayoga*. This preparation is used as *nasya* (nasal delivery) since ancient times for the cure

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of *Twakraukshya* (dryness of the skin), *Palita* (Greying of Hairs), *urdhvajatrugata roga* (disorders body parts above clavicle), *Skantha Shushkata* (emaciation of shoulder), *Greeva shushkata* (Cervical aridity), *Vaksha shushkata* (Thoracic aridity) [5,6].

Since the major active ingredients in this preparation are coming from the poly herbal decoction, the formulation may expect to contain predominantly water-soluble ingredients. Moreover, the formulation did not show any precipitation or phase separation and showed a monophasic oily liquid with very little moisture. It is hypothesized that the active botanical ingredients (ABIs) of the formulation may present either suspended in the form of submicron particles or entrapped in the submicron vesicular structures. To substantiate the claim, the *anu tailam* was fractionated using column chromatography into polar and nonpolar fractions followed by high performance thin layer chromatography to prove that the contents are polar hydrophilic compounds. The microarchitecture of the formulation was investigated using optical microscopy, photon correlation Spectroscopy (PCS) and environmental scanning electron microscope.

2. Materials and methods

All the solvents used were analytical grade and purchased from Sigma–Aldrich. *Anu tailam* used in this research was procured from AVN Ayurveda Formulations Private Limited, Madurai, India. The crude sesame oil was procured from V.V.V.Anandham & Sons (India). The determination of viscosity was performed by using Brookfield viscometer (DV2T) and the refractive index was determined by Atago Refractometer (RX-5000 α). The moisture content was determined to confirm little or least presence of water in the formulation and was achieved through AND Moisture Balance (AND MS-70). The silica gel (60–120) for chromatography was purchased from Merck, India. The glass column fabricated with sintered glass filter was supplied by Borosil, India. Pre-coated silica gel GF_{254} aluminum plates for normal phase TLC were purchased from Merck, India to carry out HPTLC analysis on CAMAG HPTLC system using winCATS 1.4.7, Switzerland.

2.1. Column chromatographic fractionation of anu tailam

The separation of Active Botanical Ingredients (ABIs) from the *anu tailam* was performed using column chromatography in which 30 cm long and 10 mm internal diameter glass column was used. The column was rinsed with acetone, dried and packed with the silica gel for column chromatography (60–120 mesh) in petroleum ether. Chromatographic separation of the *anu tailam* into fractions was performed by the reported method [7]. The non-polar fraction and polar fraction thus obtained were subjected to HPTLC analysis.

2.2. High performance thin layer chromatography (HPTLC)

Two micro liter (2 μL) samples was applied as 8 mm band in four tracks on pre-coated silica gel 60 GF_{254} aluminum plates (10 × 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WINCATS software. The detection was performed using Densitometry TLC Scanner 3 at 254 nm and 366 nm in UV cabinet. The plates were developed in the TLC chambers pre-saturated with the mobile phase. Mobile Phase: Toluene: Ethyl acetate: Methanol (7:2:1).

2.3. Dynamic light scattering (photon correlation spectroscopy)

The formulation was investigated for their particle/vesicle size and their distribution by dynamic light scattering (DLS, Photon correlation spectroscopy) using Malvern Zetasizer version 7.10. The sample was used freshly for the analysis. The polydispersity evaluates the size distribution of the particles/vesicles and the degree of the homogeneity [8].

Table 1
General characterization of *anu tailam*.

Properties	Sesame oil	Anu tailam
Color	Golden yellow	Reddish brown
Nature and texture	Smooth and oily	Smooth and oily
Odor	Characteristic and fragrant	Characteristic and fragrant
Taste	Characteristic	Characteristic
Clarity	Clear	Clear
Viscosity (cP)	3.18	2.58
Refractive Index	1.4671[a]	1.4675[a]
Moisture Content (%)	N/A	0.1667[a]

[a] Results were obtained on an average of three readings.

2.4. Optical light microscopy

Optical light microscopy was used to investigate the presence of microstructures and also to ensure the absence of large structures [9]. The sample was used as such without any dilution. The microscopic analysis was performed using Nikon microscope Eclipse 55i attached with Nikon camera (DS-Fi2). The images were captured in conventional bright field mode and 40, 100, 200, 400 and 1000 fold magnifications.

2.5. Environmental scanning electron microscopy

E-SEM analysis was preferred to study the internal structure considering the point that the samples to be analyzed were oily samples. Quanta Environmental SEM (FEI Quanta 450 FEG) was used to analyze the sample under different magnifications.

3. Results

The general characterization *anu tailam* through the listed methods showed that the active ingredients in the polyherbal decoction were transferred into the lipid carrier (sesame oil).

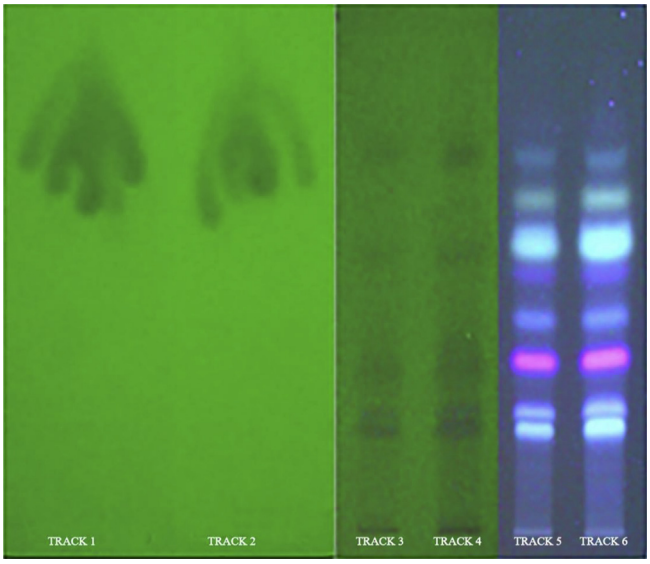

Fig. 1. HPTLC of sesame oil (Track 1), nonpolar fraction separated from *anu tailam* (Track 2) and polar fractions of *anu tailam* (in duplicates) visualized at 254 nm (Track 3 and 4), 366 nm (Track 5 and 6).

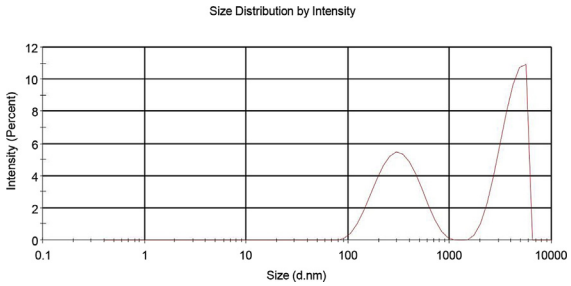

Fig. 2. Photon correlation spectroscopy of *anu tailam* showing the particle size distribution.

The moisture content of *anu tailam* indicated that the presence of minute amount of water. The results were presented in Table 1.

Column chromatographic separation of *anu tailam* into polar and nonpolar fractions followed by high performance thin layer chromatography showed that all the ABIs were present in the polar fraction whereas the nonpolar fraction did not show the presence of any ABIs (Fig. 1).

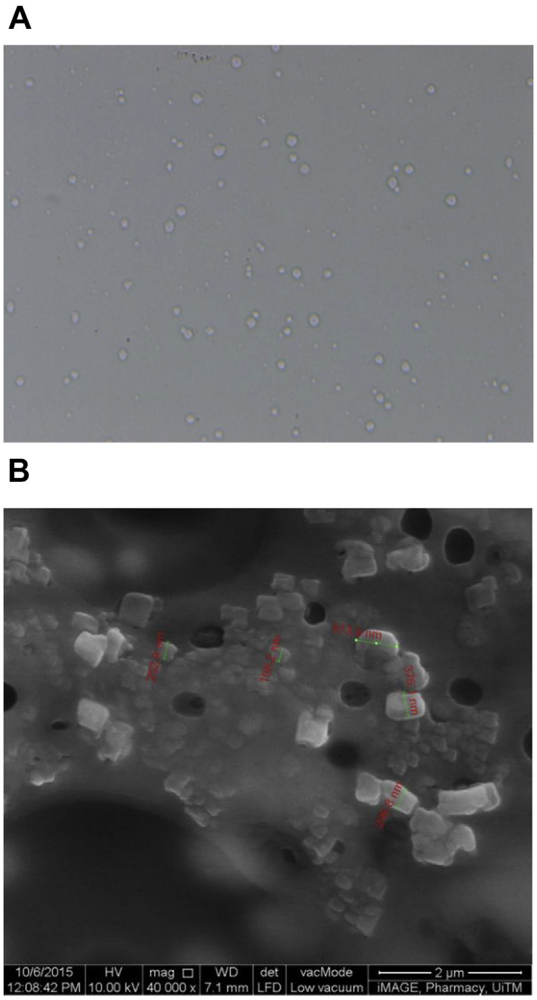

Fig. 3. (a) Optical microscopic picture of *anu tailam* at the magnification of 100 × 10 showing absence of large particles. (b) Environmental scanning electron microscope pictures showing nanoparticles of anu tailam (ranging from ~147 nm to ~573 nm; on the average of 335 nm).

Photon correlation spectroscopic results showed that 47.1% particles were in the average particle size of 335.5 d.nm and the rest 52.9% were in the average particle size of 4118 d.nm. The Z-average particle was found to be 640.4 d.nm with PDI value of 0.943 (Fig. 2). The optical microscopy results have shown the presence of discrete vesicular structures of the formulation signifying the distribution of particles and the absence of large size particles under the magnification of 100 × 10 (Fig. 3a). The environmental scanning electron microscope (E-SEM) was chosen because of the oily nature of the sample. The results have shown the presence of nano-size particles embedded in a network of vesicular structures of lipid base (Fig. 3b).

4. Discussion

The general characterization of the formulation did not show any phase separation or precipitation and presented as a mono phasic oily liquid. Also, it retained the characteristics of the lipid carrier despite the incorporation of the hydrophilic active botanical ingredients. Through column chromatic separation, it was assumed that the ABIs contained in the formulation were highly water-soluble since they are incorporated from the aqueous polyherbal extract (decoction). The HPTLC analysis confirmed the presence of ABIs only in the polar fraction. The ABIs were incorporated into the lipid base by *Ayurvedic* process without any excipients like surfactants and emulsifiers. The results of photon correlation spectroscopy clearly indicated the existence of nano size particles of the formulation. To further substantiate, the formulation was studied for its micro architecture using optical microscopy and environmental scanning electron microscope. If nonpolar ABIs were present in the formulation, they would have been dissolved in the oily phase but all the ABIs in the formulation were proven to be polar and completely extracted in polar solvents. These findings exclude the possibilities of being micro/nano self-emulsifying systems. The DLS and microscopical analysis were confirmed the existence of nanoparticles of ABIs in the formulation. They may be presented either entrapped in the nano vesicular structures or suspended in the form of submicron particles distributed in the sesame oil.

5. Conclusion

The present investigation of *Anu tailam* confirmed that the *Ayurvedic* lipid based formulations contained nano drug delivery systems. This discovery also confirms that the *Ayurvedic* system used advanced scientific techniques to prepare formulations designed to deliver ABIs across specific barriers such as blood brain barrier (BBB). The advent of this technology would revive interest in numerous drug candidates that had failed because of delivery problems. Reverse pharmacology and detailed pharmacokinetics should be investigated for the formulations falling under *sneha kalpana* to find out the novelty of these systems.

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Conflicts of interest

None.

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References

[1] Caryn LB, Leila M, Pushpa V, Suresh B, Helena RA, Michael JS, et al. Design of compounds that increase the absorption of Polar molecules. Proc Natl Acad Sci USA 1997;94:12218–23.
[2] Narayanaswamy V. Origin and development of Ayurveda: (A brief history). Ancient Sci Life 1981;1(1):1–7.
[3] Hari Sadashiva SP, editor. Astaanga hridaya of vagbhata, Sutrasthana; Chapter 20, Verse 37–39. 1st ed. Varanasi: Chawkhambha Surbharti Prakashan; 2011. p. 278–94.
[4] Savarikar SS, Ravishankar B. Bhaishajya Kalpanaa – the Ayurvedic pharmaceutics – an overview. Afr J Tradit Complement Altern Med 2010;7(3):174–84.
[5] Patil YR, Sawant RS. Study of preventive effect of pratimarsha nasya with special reference to anu tailam (an Ayurvedic preparation). Int Res J Pharm 2012;3(5):295–300.
[6] Garde GK. Sartha Vagbhata Marathi commentary. In: Ashtanga Hrudaya. 8th ed. Pune, India: Raghuvansi Publications; 1996.
[7] Selvakumar D, Vijaya S, Ng YE. Reverse engineering of Ayurvedic lipid based formulation, ghrita by combined column chromatography, normal and reverse phase HPTLC analysis. BMC Complement Altern Med 2015;15:62.
[8] Akbari B, Tavandashti MP, Zandrahimi M. Particle size characterization of nanoparticles- a practical approach. Iranian J Mat Sci Eng 2011;8(2):48–56.
[9] Kanyas S, Aydın D, Kizilel R, Demirel AL, Kizilel S. Nanoparticle and gelation stabilized functional composites of an ionic salt in a hydrophobic polymer matrix. PLoS One 2014;9(2):e88125. https://doi.org/10.1371/journal.pone.0088125.