Rediscovering Nano drug delivery systems in Ayurvedic lipid based formulations

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Sneha kalpanas (Medicated oleaginous preparations) are a group of Ayurvedic lipid based formulations in which ghee or oil is boiled with prescribed kasayas (polyherbal decoctions) and kalkas (a fine paste of botanicals) as stated in the Ayurvedic texts until all the water is evaporated. It is hypothesized that the polyherbal decoction contains only water soluble active ingredients that are dispersed in the form of submicron globules without the assistance of any additives and forms a monophasic oily liquid with absence of water. A model formulation Gugglutiktaka Ghrita was studied to understand the microarchitecture of this group of formulations. Photon correlation spectroscopy, Optical microscopy and Environmental scanning electron microscopy were used to examine the formulation for the presence of submicron sized particles and vesicular structures. The results showed that the formulation contained nanoparticles of the size 49.31±11.33 d.nm with PDI of 0.156 with 100 % peak intensity. The particle morphology showed that the active botanical ingredients may have been incorporated into the vesicular structures which in turn were dispersed in the oil phase. The ancient Ayurvedic system developed its own nanotechnology to deliver the water soluble ingredients across the biological barriers in a lipid base in the form of nanoparticles

Keywords: Nano delivery, Nano particles, Nano vesicles, Ayurvedic, Ghrita

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Ayurveda is one of the world’s oldest medical systems which originated in India more than 3,000 years ago and remains one of the country’s traditional healthcare systems1,2. Sneha kalpanas (Medicated oleaginous preparations) are Ayurvedic lipid based formulations in which ghee or oil is boiled with prescribed kasayas (polyherbal decoctions) and kalkas (fine paste of botanicals) according to the formula as stated in the Ayurvedic texts until all the water is evaporated. The cooking stage of these medicated oleaginous preparations decides the route of administration, and those prepared with mridu paaka (soft consistency of the residue) are used for nasal insufflations (Nasya). Madhyama paaka (semisoft consistency of the residue) is used for enema and oral administration. Khara paaka (rough consistency of the residue) is used for bathing and external applications. These preparations will generally have the colour, odor and taste of the ingredients used. Preparations for internal use are expected to maintain their potency for about sixteen months3,4. From the analysis of these types of formulations, it is found that the medicaments contained in these formulations are polar and mostly extracted with polar solvents like methanol. The results of the analysis also confirm the complete absence of non-polar medicaments in the final formulation. The medicaments in the lipid base seem to have been incorporated from the polyherbal decoction and the fine paste of botanicals and believed to be polar medicaments based on the fact that the major ingredients are transferred from the polyherbal decoction. It is also observed that there is no phase separation or precipitation in the final preparation and appears as a monophasic oily liquid5. It is interesting to note that these polar ingredients are incorporated into a lipid base without any known adjuncts to form a homogenous mixture with the oil phase. The above observation gives rise to a hypothesis that the polar ingredients may be entrapped in nano/micro sized vesicular structures dispersed in the oil phase. In order to investigate this hypothesis, it was decided to study the architecture of the formulation. The emphasis was given to particle size analysis and scanning electron microscopical
studies to explore the construction of the formulation falling under sneha kalpanas in Ayurvedic formulations. Guggulu Tiktaka Ghrita (GTG) has been used as a model formulation to study the characterization of this group of formulations via photon correlation spectroscopy, optical microscopy and scanning electron microscopy.

Methodology

All the reagents and solvents used were analytical grade and purchased from Sigma-Aldrich. The model ghrita “Guggulu tiktaka ghrita (GTG)” was purchased from AVN Arogya Ayurvedic Pharmacy, India. Pure cow’s ghee was obtained from LGC Sdn Bhd, Malaysia and used as a reference for the analysis with GTG.

Guggulu tiktaka ghrita

The quantity of cow ghee (Go-ghrita) was taken based on the quantity of the plant materials used. The ratio of the major ingredients used for the preparation of Guggulu tiktaka ghrita (GTG) is four parts ghee (sneha), one part of fine paste of botanicals (kalka) and sixteen parts polyherbal decoction (Kashaya). All the three ingredients, the decoction, fine paste of botanicals and cow ghee were mixed together and boiled under mild flame until the water is evaporated.

General characterization

The formulation was investigated for organoleptic characteristics like color, odor, taste and texture in comparison with the ghee to ascertain the presence of active ingredients qualitatively. The determination of viscosity was performed by using Brookfield Viscometer (DV2T) and the refractive index was determined by At ago Refractometer (RX-5000 α). The moisture content determination was achieved using an AND Moisture Balance (AND MS-70).

Dynamic light scattering

Measurement of particle size was carried out using dynamic light scattering (Photon correlation spectroscopy). The formulation was investigated for their particle or vesicle size and their distribution by dynamic light scattering (DLS, Photon correlation spectroscopy) using Malvern Zetasizer version 7.10. The sample was diluted fresh with liquid paraffin (1 %) and used for the analysis. The polydispersity evaluates the size distribution of the particles/vesicles and the degree of the homogeneity.

Optical light microscopy

Optical microscope was employed to investigate the presence of microstructures as well as to ensure the absence of large structures. The sample was prepared by diluting the GTG with liquid paraffin (1 % solution). The microscopic analysis was performed using Nikon microscope Eclipse 55i attached to Nikon camera (DS-Fi2). The images were captured in conventional bright field mode and 40, 100, 200, 400 and 1000 fold magnifications were employed.

Environmental scanning electron microscopy

This method was chosen to study the internal structure considering the fact that the sample to be analyzed was an oil sample. Quanta Environmental SEM (FEI Quanta 450 FEG) was used to analyze the sample.

Results and discussion

Sneha kalpanas are Ayurvedic lipid based formulations that require three ingredients for the effective preparation. The ingredients are sneha (ghee or oil), drava (liquid, which may be decoctions, expressed juice, milk, etc.) and kalka, the fine paste of the botanicals, usually in very small amounts that are used to prepare the decoction. The ratio of the major ingredients, unless specified otherwise, is four parts sneha, one part kalka and sixteen parts drava (liquid).

During the preparation, the fine paste of botanicals and decoction are mixed together with oil or ghee and boiled on mild fire and stirred continuously to ensure the uniform heating until the aqueous portion is evaporated. This process ensures the absorption of the active botanical ingredients (ABIs) of the herbs into the oily base. The completion of the preparation is tested with the help of a ladle to determine the paaka (cooking stage). The paaka is categorized into mridu (soft) if the residual paste is soft and waxy when rolled between fingers, madhyama (moderate) if the paste is semisoft and fires with a crackling noise when put into fire and khara (hard) if it burns without crackling noise. The ideal condition of the medicated oil is attained when uniform froth comes out and subsides in case of medicated ghee.

General characterization of the formulation, GTG in comparison with ghee (butter oil) which was used as a base for the formulation via color, odor and taste indicated that the formulation has acquired the properties of herbs. The change in viscosity, refractive
index of the formulation in comparison to the ghee indicated that the ABIs were incorporated from the polyherbal extract during the processing (Table 1). The moisture content analysis indicated that the formulation has only tiny amounts of moisture that rule out the possibility of biphasic system.

It is believed that the ABIs contained in the formulation are polar since they are incorporated from the aqueous extract. They are made miscible with the lipid base by an Ayurvedic process without any excipients. If nonpolar ABIs are present in the formulation, they would have been dissolved in the oily phase, but all the ABIs present is proven to be polar and rules out the possibilities of self-emulsifying systems. When this formulation was analyzed qualitatively by solid phase separation of ABIs and subsequent HPTLC fingerprinting, it was observed that all the ABIs of this formulation were found in the polar fraction of the lipid base extracted in methanol. The non-polar fraction which was soluble in highly non-polar organic solvents like petroleum ether did not show the presence of any ABIs (Fig. 1). The macroscopic analysis of the formulation neither showed any phase separation nor the presence of any suspended particles of ABIs instead showed a clear monophasic oily liquid\textsuperscript{10,11}.

The above finding supported the hypothesis that the ABIs are polar and may be distributed with the lipid base in the form of confined encapsulating structures of micro to nano size particles. To substantiate the claims, the microarchitecture of the formulation was studied through dynamic light scattering for particle size analysis, optical microscopy and scanning electron microscopy for topological analysis of the formulation.

The results from DLS clearly indicated that the formulation contained nano size particles. The average particle or vesicle size of the formulation was found to be 49.31±11.33 d.nm (mean ± SD; n = 3) with PDI of 0.156 with 100 % peak intensity indicating a very narrow distribution of particle size, whereas the average particle or vesicle size of the ghee was found to be 434.4 d.nm with PDI of 1.0 indicating broader size distribution showing 1594 d.nm with 59.6 % peak intensity, 4358 d.nm with 17.1 % and 68.07 d.nm with 16.4 % peak intensities (Fig. 2).

In support of the above finding, the optical microscopic pictures clearly indicated the distribution of uniform sized vesicles fluorescing yellow under the day light illumination in comparison to the ghee (butter oil) used as a lipid base (Fig. 3).

The ESEM images of the formulation showed the presence of submicron sized particles embedded in

\begin{table}[h]
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\begin{tabular}{|c|c|c|}
\hline
 & Ghee & GTG \\
\hline
Color & Slight Yellow & Brownish yellow \\
Odor & Fragrant & Characteristic, Fragrant \\
Taste & Characteristic & Characteristic, bitter \\
Nature & Smooth, oily & Smooth, oily \\
Viscosity (c. p.) & 25.80* & 45.90* \\
Refractive index & 1.3078* & 1.4635* \\
Moisture content (%) & 1.405* & 1.320* \\
\hline
\end{tabular}
\caption{General Characterization of GTG}
\footnotesize{*Results obtained on average of 3 values}
\end{table}

Fig. 1 — HPTLC behavior of polar and non-polar fractions of GTG Visualization of the TLC plates under UV light under 254 and 366 nm with track 4 and 5 correspond to the polar methanolic fractions showing the spots of separated components in comparison to the track 2 which correspond to the GTG. The track 3 correspond to the non-polar fraction did not show the presence of ABIs and showing the similar behavior as that of the ghee\textsuperscript{11} (Track 1) (Fig. 1). (Reproduced from Selvakumar D, Vijaya S, Indian Journal of Traditional Knowledge, 14(3) (2015) 365-369)
Fig. 2 — Particle size distribution of the formulation by photon correlation spectroscopy. The image showing the particle size distribution of the GTG diluted in liquid paraffin under dynamic light scattering.

Fig. 3 — Optical microscopy of the ghee (A) and GTG (B). Images A and B showing the distribution of vesicles in the oil fluorescing under illumination of light (10X100 magnification) of Ghee (butter oil) and GTG respectively.

the solid background (solid ghee) (A) and the diluted formulation (1 %) with liquid paraffin (B). The images showed the presence of spherical particles which further substantiate the finding that the polar ABIs were entrapped in the submicron vesicular structures (Fig. 4).

The Ayurvedic system described above was different and novel from the modern lipid based drug delivery systems (LBDDS), owing to their ability to formulate hydrophilic active ingredients in a lipid base without any specialized excipients. However, LBDDS includes lipid formulations such as lipid solution, lipid suspension and self-emulsifying systems for drugs having a poor aqueous solubility and can be formulated using the lipid-based systems composed of simple oil solutions to complex mixtures of oils, surfactants and co-surfactants12-15. Lipid based systems for hydrophilic macromolecule explained by Macrosol technology for the solubilisation of hydrophilic macromolecule in oily formulations in which the amphiphilic molecules such as oleic acid were used to entrap hydrophilic macromolecule by providing a protective sheath of amphiphile and then dispersed in the oil phase16. Solid-in-oil nano suspension (SONS) was described for hydrophilic substances like L-ascorbic acid in squalene using a combination of lipophilic and hydrophilic surfactants17,18. The above methods described the use of either amphiphiles or a combination of lipophilic and hydrophilic surfactants to bring out the final formulation in the oil phase. However, has yet to be proven successful to reach commercialization, but the Ayurvedic lipid based formulations were found to be superior in formulating hydrophilic active ingredients in oil, has been used successfully since ancient times.

**Conclusion**

In conclusion, there is evidence to say that the free fatty acids (amphiphiles) present in the ghee or plant edible oils were oriented in such a way that the polar ABIs were entrapped in confined structures during the preparation of Ayurvedic lipid based
formulations. Since the confined structures are in nano sizes, they easily penetrate the biological barriers and remain in the circulatory system to deliver the polar active substances over a prolonged period of time. Ayurveda an incomparable medical system that has many unearthed technologies of ancient wisdom, therefore, the scientific community and Ayurvedic practitioners should look into the artifacts of Ayurvedic formulations besides the therapeutic effects of this system.

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