

FORMULATING FOR EFFECT

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INTRODUCTION

The use of Tea Tree Oil in Australia spans many centuries. There is evidence to show that the leaves have been used by aboriginals for thousands of years for a variety of ailments. When Australia was discovered by the British, log book entries show the leaves were used as an infusion or tea in an attempt to control the scurvy from which the first fleet suffered and hence the name Tea Tree Oil. Unfortunately Melaleuca leaves contain no vitamin C but the name remained. In the 1920's Western scientists became aware of its antiseptic properties and it was issued to Australian Army Personnel during the second World War. With the discovery of antibiotics its use declined until recently. It has now been rediscovered as an effective natural antiseptic with a wide variety of uses in the pharmaceutical and personal care industry.

FEATURES OF TEA TREE OIL

Tea Tree Oil is a natural product, has a broad spectrum of activity and is environmentally safe with a long history of use. It has excellent antiseptic and wound cleansing properties and a low incidence of skin irritation. These features make it a versatile ingredient in pharmaceuticals, cosmetics, toiletries, pet care and household sanitation.

The oil consists of a complex mixture with over one hundred fractions identified, consisting of a mixture of monoterpenes, sesquiterpenes and terpene alcohols. The germicidal effect is mainly due to terpinene-4-ol although other compounds may act synergistically. Research indicates that the antimicrobial activity increases markedly as the terpinene-4-ol concentration increases up to 35%, then marginally to 40% concentration. No further increase in activity is observed at concentrations in excess of 40% terpinene-4-ol.

The Minimum Inhibitory Concentration (MIC) against commonly encountered gram positive and negative bacteria is typically in the range of 0.5 to 1.0%. TTO exerts its action by causing structural damage to the cell wall of the organism followed by denaturation of the cell contents. Unlike antibiotics, there is no evidence of genetically acquired immunity and the oil is effective in the presence of blood, pus, necrotic tissue and mucous discharge.

FORMULATION CONSIDERATIONS

SELECTION OF THE OIL

Perhaps the most important consideration in the development of products containing TTO is to select an oil of suitable quality. It has been noted that the activity of the oil depends in part on the terpinene-4-ol content. However high cineole levels have been associated with irritation of the mucous membranes. The chemical variants of *Melaleuca alternifolia* have been described as low, intermediate and high cineole forms. For optimal activity, only the low cineole level form should be utilised. On standing and after exposure to light and air, the terpenes convert to p cymene by an oxidative process. Antioxidants should thus be considered in formulated products. The pure oil is a clear, mobile liquid. If discolouration occurs then inferior distillation or contamination from the holding vessel should be suspected. The presence of weed or other impurities in the harvest will affect the colour. Odiferous compounds may also result from poor distillation techniques.

The factors affecting the quality of the oil include preharvest factors such as environment and agronomic factors, the age of the leaf and the time of extraction as well as post harvest factors such as the conditions of the extraction process, the storage conditions of the oil and most importantly, the formulation and storage of the formulated products. It is this latter consideration on which I now wish to focus.

SOLUBILISATION OF TEA TREE OIL

TTO can be solubilised in water utilising a variety of surfactants. In our laboratories we found it necessary to use four parts of polysorbate 20 to one part of TTO to produce a clear solution on subsequent dilution with water. This is in contrast to about one to one and a half parts of the surfactant polyoxyl 35 castor oil. The method of preparation is of vital importance. The method can be summarised as follows:

1. The TTO is first mixed with the surfactant and allowed to stand for several minutes.
2. Add small aliquots of water gradually with constant stirring. This results in a thick gel due to hydration.
3. Further gradual additions of water results in a lowering of the viscosity and a clear liquid.

Should the initial addition of water be done too quickly or the TTO not adequately mixed, then at best an opalescent solution results.

The addition of polyhydroxy alcohols such as glycerol or propylene glycol can effect solubilisation without having to go through the initial gelling phase. This can be achieved by triturating the glycerol with the mixture of the surfactant and TTO, prior to the addition of water. The addition of alcohol to the formulation greatly enhances solubilisation producing a clear solution. Typically a range of 5 to 10% alcohol is employed.

EFFECT OF SOLUBILISER ON ANTIMICROBIAL ACTIVITY

The effects of various surfactants and solubilising agents on the antimicrobial activity of a range of disinfectant and antiseptic products have been well documented in the literature. Our investigations aimed to determine the effect of varying the concentration of surfactants on the antimicrobial activity of TTO. The concentration of TTO remained constant at 0.5% and the surfactants were varied to achieve a ratio of Oil:Surfactant of between 1:1 to 1: 5 on a weight to weight basis. Simple aqueous solutions of TTO were prepared and subjected to a combined USP/BP Preservative Efficacy Test. The two surfactants investigated were Polyoxyl 35 Castor Oil and Polysorbate 20 and the results were as follows:

1. Polyoxyl 35 Castor Oil had no effect on TTO activity when used as the solubilising agent in the range of concentrations studied (Up to 2.5% surfactant with 0.5% oil). All formulations passed the BP test with the exception of *A. niger*. All passed the USP test.
2. Increasing the polysorbate 20:TTO ratio, decreased the activity of TTO as the ratio of surfactant to oil increased from between 1:1 and 1:5 (oil:surfactant). Higher levels of this surfactant result in failure of the BP/USP test for both some bacteria and yeast & mould.

These findings are in simple aqueous solution. In formulated products it can be anticipated that the situation will be even more complex.

EFFECT OF OIL CONCENTRATION ON ANTIMICROBIAL ACTIVITY

In this series the surfactant used was polyoxyl 35 castor oil at a constant concentration of 1.0% to solubilise the TTO. The oil concentration was varied between 0.1% and 1.0%. Again simple aqueous solutions were prepared and subjected to a combined BP/USP test. A summary of the results is as follows:

TTO Concentration	Result (Combined BP/USP Test)
0.1%	Fails some bacteria Fails <i>Candida</i> and <i>A.niger</i>
0.3%	Passes bacteria Passes <i>Candida</i> Fails <i>A.niger</i>
0.5%	As for 0.3%
1.0%	As for 0.3%

These results indicate that in colloidal systems and formulated products the nature of the surfactant employed is important with respect to the antimicrobial activity of the oil. It appears that polyoxyl 35 castor oil is superior to polysorbate 20 in that less surfactant is required to achieve satisfactory solubilisation of the oil and it has less effect on the antimicrobial activity of the solubilised oil. Simple aqueous solutions of TTO solubilised with polyoxyl 35 castor oil will

pass the USP Preservative Efficacy Test at a level of 0.3%. This level of oil passes the BP Test with the exception of *A.niger* as there was no 2 log reduction in count in 14 days. Higher concentrations of TTO up to 1.0% does not appear to improve the activity against *A.niger*.

EFFECT OF OTHER ADDITIVES ON ANTIMICROBIAL ACTIVITY

Other commonly used excipients were examined for their effect on the antimicrobial activity of TTO. Simple aqueous solutions were again used of 0.5% concentration, solubilised with polyoxyl 35 castor oil. The materials examined included EDTA, Oil of Thyme, propylene glycol and butylene glycol. All materials were examined in a range of concentrations, and it was found that none of the materials examined had any observable effect on the antimicrobial activity of the oil.

PARTITIONING EFFECT IN FORMULATED PRODUCTS

It is well accepted that the antimicrobial activity resides in the aqueous phase of a preserved two phase system and is therefore dependent on the equilibrium concentration of the preservative in this phase. It is expected that TTO will partition between the oil and water phases present in a two component system in accordance with the partition coefficient and the relative ratio of oil and water in the system. To estimate the likely concentration in the aqueous phase of a simple two component system, the partitioning behaviour of TTO between water and Crodamol GTCC (Caprylic/Capric triglycerides), a typical oily component of cream formulations was investigated. The effect of surfactant concentration was also noted.

Initial aqueous solutions were prepared using polyoxyl 35 castor oil. The ratio of surfactant to TTO varied between 1:1 and 1:3 on a weight basis and no surfactant as a control. The solubilised mixtures were mixed with an equal volume of Crodamol GTCC and the final concentration of TTO in the mixture was 0.5%. The mixtures were mixed on a vortex mixture and allowed to equilibrate over 3 days with occasional shaking. The ratio of TTO in the aqueous to organic phases was determined by GC assay of Terpinene-4-ol after centrifugation. The results were as follows:

TTO CONCENTRATION (Aq.Phase)	WITH SURFACTANT	WITHOUT SURFACTANT
INITIAL	0.5%	0.5%
FINAL	0.4%	0.01%

With no surfactant present nearly all the TTO had migrated into the oil phase. In the presence of the surfactant the concentration of the TTO in the aqueous phase remains at about 0.4%. The partitioning appeared to be independent on the concentration of the surfactant in the range studied. This has important implications for formulated products. It is thus essential that appropriate microbial evaluation be undertaken for all TTO products. It is recommended that specific organisms be used depending on the use of the product. eg. *P.acnes* and *C.albicans* for acne and antifungal products respectively.

PACKAGING

Perhaps as important as microbial evaluation is the need for stability testing on the final formula-

tion in the proposed pack for marketing. Glass whenever possible is the most appropriate for TTO products. In LDPE a loss of terpinene-4-ol occurs at room temperature after 3 months. At 45oC after 3 months, the terpinene-4-ol is barely detected. Even in low concentrations of TTO the oil migrates through LDPE walls and the solvent attacks the external features on the container such as the label print. HDPE has been reported to be satisfactory for products containing around 20 to 25% TTO. The maximum concentration acceptable will be a function of the nature of the product and the wall thickness of the plastic. Deformation has also been reported due to reactions between TTO and plasticising resins. If plastic caps are employed it is important to use impenetrable liners.

STABILITY

THE PURE OIL

Tea Tree oil is reasonably stable at Room Temperature when stored in brown glass or stainless steel. It is however sensitive to heat, light and air. On storage there is a drop in terpinene-4-ol, alpha terpinene and gamma terpinene and an increase in para cymene. High levels of this compound can indicate poor storage, old oil or bad extraction techniques. The use of antioxidants in long term storage should be considered.

FORMULATED PRODUCTS

The importance of adequate stability testing on the final product in the proposed marketing container cannot be overstated. Batches should be stored at 4oC, 30oC, 40oC and possibly cycling temperature conditions and monitored at regular intervals for appropriate physical characteristics. Chemically, it is suggested that terpinene-4-ol, cineole and oxidation products are monitored and preservative efficacy testing is performed at the beginning and end of the stability study.

PRODUCT FAILURE

Reasons for products failing stability testing are many and varied. Most product failures in the past have been associated with inadequate packaging components and poor stability testing prior to product launch. This has resulted in several unsatisfactory products from a physical viewpoint. Problems which have occurred include panelling of bottles, migration of TTO through pack walls resulting in deformation of external decorations including label print. Regulatory authorities have given little attention to chemical or microbial activity of formulated products as most of the products which have appeared are limited in their claims to simple antibacterial claims and not as a treatment for specific conditions such as candidiasis or acne. However as clinical testing on formulated products increases there will be increasing attention to the role of formulation in the effectiveness of TTO in specific conditions. The main points to consider in product failure from a performance point of view are as follows:

1. Inadequate packaging
2. Inactivation of TTO by solubilisers, surfactants and other excipients.
3. The grade of TTO used.
4. The method of manufacture including the order of addition of the components.