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Methods for Quantitative Extraction of Niclosamide Based on Supported Liquid Membrane and High Performance Liquid Chromatographic Determination

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By

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METHODS FOR QUANTITATIVE EXTRACTION OF NICLOSAMIDE BASED ON SUPPORTED LIQUID MEMBRANE AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION

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For the Glory of Almighty God

in the name of my Lord and Savior Jesus Christ

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ABSTRACT

METHOD FOR QUANTITATIVE EXTRACTION OF NICLOSAMIDE BASED ON SUPPORTED LIQUID MEMBRANE AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION

By

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Advisor: Dr. Negussie Megersa

From literature review it is possible to hypothesize that a method for the preconcentration and isolation of niclosamide from indigenous plant materials using supported liquid membrane (SLM) can be developed. Neutral molecules of niclosamide can be extracted from a flowing aqueous solution to a stagnant basic acceptor solution across a liquid membrane. The efficiency of the extraction process depends on the pH values of donor and acceptor solutions, the flow rate and the carry-over (COE) effects when ordinary SLM configuration is applied. Therefore the optimization of these factors was also suggested. Comparison of simple transport and carrier-mediated mechanisms can be made; it is suggested that simple transport mechanism would be more effective. The use of hollow fiber supported liquid membrane (HFSLM) mode of extraction is also suggested for comparison purpose. The applicability of the method of extraction should be investigated by spiking analyte sample solutions to the real sample. After developing and optimizing the method, application to the extraction and determination of niclosamide from Hagenia abyssinica (*koso*), Glinus lotoides L. (*meterre*), Embelia schimperi vatue (*enqoqo*), and Cucurbita pepo L. (*dubba*) was suggested.

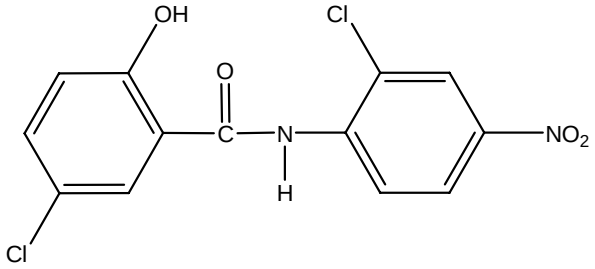
1. INTRODUCTION

1.1 Niclosamide

Pesticides are chemicals that inhibit or destroy undesirable life firms; which are considered a nuisance. They can be classified into several groups on the basis of the target they attack as follows: fungicides, insecticides, herbicides, nematocides, molluscides, bactericides, and rodentocides. They can also be grouped under three classes based on their chemical nature: inorganic, organic compounds not containing metal ions, and organic compounds containing metal ions [1]. The study of pesticides is required in order to determine the extent of their benefits and impacts to the environment as well as humans and optimize their applications. Niclosamide is a pesticide that acts as nematocide, mollucicide, and lampricide. The study of extraction of niclosamide is undertaken in this project work.

1.1.1 Properties of Niclosamide

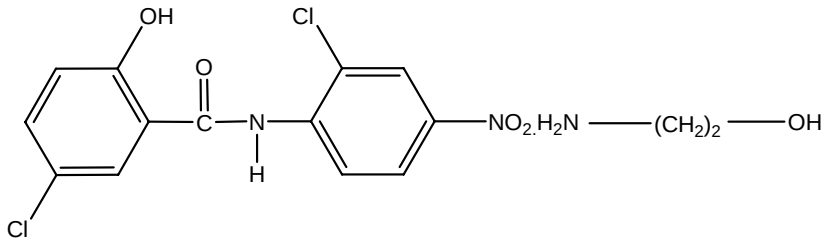
Niclosamide, 5-chloro-*N*- (2-chloro-4-nitrophenyl)-2-hydroxybenzamide /2', 5-dichloro-4'-nitrosalicylanilide/, is a well-known chemical substance that serves as molluscide and nematocide.



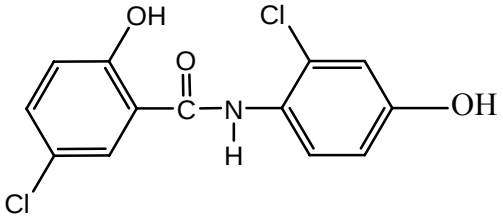
Niclosamide is a yellowish gray odorless crystalline solid, which melts between 224-229 °C. It is not very water soluble, 5-8 mg/L at 20 °C, sparingly soluble in ether, ethanol and chloroform, and soluble in acetone [2, 3]. The hydroxyl and the amide group play an important role in the acidity and/or basicity of the chemical substance. It is a weak acid [4] and has an ionizable proton with a pKa of approximately 6.25 as mentioned

elsewhere [5] or 8.5 as suggested by others [4]. The equilibrium concentration of ionized and un-ionized forms depends on the pH of the aqueous solution used to dissolve it [6]. Below pH 7 it is apparent that the NH group of niclosamide molecule is protonated to a great extent [5].

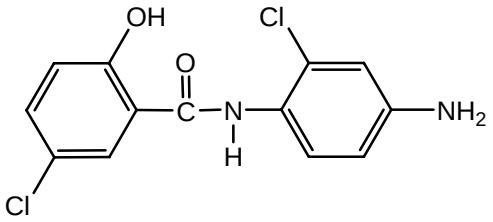
Pesticides when in contact with the environment undergo changes brought on by microbes, moisture, and sunlight. For example, moisture acts as a hydrolytic agent (for the generation of hydroxyl radical), whereas sunlight imparts photolytic energy upon the chemical to generate reactive ion species. Aminoniclsamide, hydroxyniclosamide and 5-chloro-salicylic acid are typical byproducts of niclosamide degradation [7, 8]. In tablets niclosamide undergoes a biodegradation in moist environments but it is stable in an aqueous solution for several months in the absence of light. The ethanolamine salt is stable to heat, hydrolyzed by concentrated acid or alkali, and stable in aquatic environments [2]. Niclosamide degrades under aqueous photolytic conditions. In neutral and basic solutions, niclosamide degradation is a result of photolysis only and is not affected by hydrolysis. Under aqueous photolytic conditions, its half-life is about 30 h in basic solutions and 11 h in neutral solutions. In acidic media it is even shorter. The first step in the photolysis of niclosamide is the cleavage of the amide bond to yield 5-chlorosalicylic acids and 2-chloro-4-nitroaniline [3, 8].



5-chloro-salicyl- (2-chloro-4-nitro) anilide 2-aminoethanol salt



Hydroxyniclosamide



Aminoniclosamide

Further degradates were formed by cleavage of both aromatic rings. Carbon dioxide was the major degradate. The other degradates were oxalic acid, maleic acid, glyoxylic acid, and glyoxal [3, 8].

1.1.2 Uses of Niclosamide

Niclosamide is a relatively selective, non-cumulative chlorinated aromatic amide pesticide [4]. It is used as an anti-parasitic drug that is effective against all the species of tapeworm infections. Its mode of action against tapeworm species is to uncouple oxidative phosphorylation and it blocks the glucose uptake and inhibits respiration in cestodes [4, 5]. However, the mechanism of action of niclosamide is not completely elucidated. The nitro group is fundamental for this activity. The reduced molecule (when the NO_2 group is changed to NH_2) has been shown to lose all mollusicidal and cestocidal properties, being also ineffective in decoupling respiration and electron transport linked phosphorylation [9]. The WHO Expert Committee, met in Geneva from March 7 to 11, 2005, on the selection and use of essential medicines, recommended that niclosamide be retained on the Model List as a reserve medicine. The review confirmed that no major safety concerns have been raised from the use of this item [10].

Formulated as the ethanolamine salt, it is also one of the most effective and widely used mollucicides for the control of snail vectors of schistosomiasis. Niclosamide is toxic to aquatic vertebrates and crustaceans but has very low toxicity to mammals. As can be observed from the degradation property explained above, it is non-persistent in the aquatic environment and has slight effect on aquatic plants and zooplankton [4, 5]. Because residues of niclosamide in aquatic organisms are rapidly eliminated once a stream treatment is complete, there is little concern that niclosamide will bioaccumulate in the food chain. It can also be used to control sea lamprey populations [6]. Despite its general use it has toxicity to certain non-target organisms including some plants [9]. Some negative implications to its use from environmental point of view have also been notified [11].

1.1.3 Synthesis and Potential Natural Sources of Niclosamide

According to the literature review, natural source of niclosamide was not mentioned. But it was first synthesized in 1959 from 5-chloro salicylic acid and 2-chloro-4-nitro aniline [12]. This is the common method of production of the pharmaceutical substance (such as Yomesan and Phenasal) [13]. When compared to its photolytic hydrolysis products the first degradates are the same as the two molecules used for its synthesis.

Some living plants in Ethiopia have been used as traditional medicines for treating tapeworm infestations. Four of them [14-17] are familiar in many places. It is suspected that niclosamide might be responsible for the anthelmintic effect of these plants. Extracting the chemical substance from such natural sources may also be safe and economically feasible. Thus further study on the methods of extraction of niclosamide is worth consideration.

1.2 Sample Preparation Techniques for Niclosamide

1.2.1 General Overview

The analytical procedure has several steps. Sampling, sample preparation, separation, quantitation, statistical evaluation, and decision. The sampling step includes deciding where to get samples that properly define the object or problem being characterized, and choosing a method to obtain samples in the right amounts [18]. There are two objectives in sample preparation step. (1) To achieve a good cleanup of the sample. (2) To enrich analytes in the sample [19]. During the separation step of the analytical process, the isolated complex mixture containing target analytes is divided into its constituents typically by means of a chromatographic or electrophoretic technique. Quantitation or quantification is the determination of the amounts of the identified compounds.

Sample preparation is the process of transforming a sample into a form that is suitable for analysis. This process might involve dissolving the sample, extracting the analyte from a complex matrix, preconcentrating a very dilute analyte to a level that can be measured, chemically converting analyte to easily detectable form (derivatization), and removing or masking the interfering species [20].

Simple separation procedures include centrifugation, crystallization, distillation, and filtration. Extraction, chromatography and electrophoresis are rather complicated analytical methods of separation. Chromatography and electrophoresis are categorized under instrumental methods of analytical separations. Here we consider extraction processes in particular. Extractions use two immiscible phases to separate a solute from one phase into the other. The distribution of a solute between two phases is an equilibrium condition described by partition theory. More typical laboratory extractions are of organic compounds out of an aqueous phase and into an organic phase. Distribution of a solute, S, between two immiscible solvents (such as aqueous and organic phases) is an equilibrium condition that is described by the following equation:

$$S_{aq} \rightleftharpoons S_{org}$$

The equilibrium constant for this equilibrium condition is:

$$K = [S_{org}] / [S_{aq}] \qquad (1.1)$$

where $[S_{org}]$ and $[S_{aq}]$ are the solute concentrations in the organic and aqueous phases, respectively, and the equilibrium constant, K, is called the partition or distribution coefficient. Partitioning of a solute between two phases is the basis for extractions and chromatographic separations

Liquid-liquid extraction (LLE) is the traditional technique for extraction of organic analytes from aqueous solutions. With LLE, large enrichment factors can be obtained despite certain drawbacks [21]. Niclosamide can be extracted from complex matrix by liquid-liquid extraction [22- 25].

Solid phase extraction (SPE) was used for cleanup niclosamide [25]. SPE is based on sorption of the analyte on to a sorbent or solid phase packed in a small column or flat membrane (disk). SPE involves bringing a liquid or gaseous sample in contact with a solid phase or sorbent where by the analyte is selectively adsorbed onto the surface of the solid phase [18, 26, 27]. Solid phase micro extraction (SPME) which needs a small volume of sample to achieve enrichment [28] was not used yet for niclosamide extraction according to the literature review.

1.2.2 Membrane-Based Sample Preparation Techniques

An area enjoying much attention by various research groups is developing membrane-based extraction techniques that are simple, cheap, and miniaturized [21]. A membrane can be defined as a semi-permeable active or passive barrier, which, under a certain driving force, permits preferential passage of one, or more selected species or components, *e.g.* molecules or particles. The separation process is accomplished by differences on how various components in the sample respond to a driving force such as concentration gradient, pressure gradient or electrical flux [29].

The membranes used for the extraction of liquid samples in the field of analytical chemistry are made of hydrophobic polymers of non-porous or micro porous character [29]. In porous membrane the solutions on both sides of the membrane are in physical contact through the pores of a membrane; and, in non-porous membrane techniques the membrane forms a separate phase (polymeric or liquid) between the donor and acceptor solutions. A clear difference between these two is that selectivity for porous membrane processes is mainly based on pore size and pore size distribution. In both of these cases, the chemistry of the membrane material can influence the selectivity and the flux of the process [21, 30].

In non-porous membranes separation is accomplished when the molecules to be separated are dissolved in the membrane. The transport of molecules is determined by the physical and chemical properties of the compounds in the membrane phase, which includes their solubility and diffusion coefficient. Hence the extent of interaction between the membrane and the compounds is a more important factor to be considered than the membrane pore size and molecular size of the analyte. Consequently, non-porous membranes can be regarded as relatively selective membrane.

The established idea of a membrane is a thin, semi-permeable and solid barrier. A liquid membrane (LM) is nothing like this. It is a liquid phase, usually organic, interposed between two miscible aqueous solutions [31]. A porous membrane impregnated with a liquid forms a special sort of non-porous membrane called “supported liquid membrane” (SLM). This uses the pores of the polymeric porous membrane as a support for an organic solvent, thus creating a three-phase system**,** with the organic solvent layer sandwiched between the donor and acceptor aqueous solutions. Where one

of the phases (usually the acceptor) was also an organic solvent, a two-phase system was adapted and the resulting technique was (more commonly) termed "microporous membrane liquid-liquid extraction" (MMLLE). The use of membranes presents the advantages of high selectivity; clean extract formation and a high degree of enrichment. Trace analysis is thus facilitated by additionally reducing solvent consumption. The polymeric membrane unit can be either flat or hollow fiber and most techniques involved some kind of on-line connection of the membrane extraction units to analytical instruments [30].

1.2.2.1 Supported Liquid Membrane (SLM)

Basic Principles

The main principles of SLM extraction are explained elsewhere [21]. An organic solvent is immobilized in the pores of an inert support material, separating the aqueous donor and the acceptor phases (Figure 1). The analytes are partitioned from the aqueous sample stream into the organic membrane and are then re-extracted into the aqueous acceptor phase. The driving force is the difference of the analyte concentration between the donor and acceptor phases. In order to maintain the concentration gradient across the two phases, the solutes must be able to exist in two forms: in a nonionic form on the donor side to be extracted into the membrane and in an ionic form on the acceptor side in order to be irreversibly trapped.

This is most simply achieved by pH adjustments in the two aqueous phases, and the method is, therefore, particularly well suited for ionizable compounds such as medium-to-weak acids and bases. SLM extraction can provide very selective enrichment. Selectivity can be fine-tuned by proper choice of the conditions in the three phases as seen in Figure1. This creates a selectivity window such that by the time the analytes are enriched in the acceptor phase, an indirect structural recognition is achieved and only analytes belonging to the same family are generally trapped at a time. Macromolecules are discriminated on the basis of their size while charged compounds are too polar to dissolve into the organic liquid. Neutral molecules merely distribute between the three phases without any enrichment.

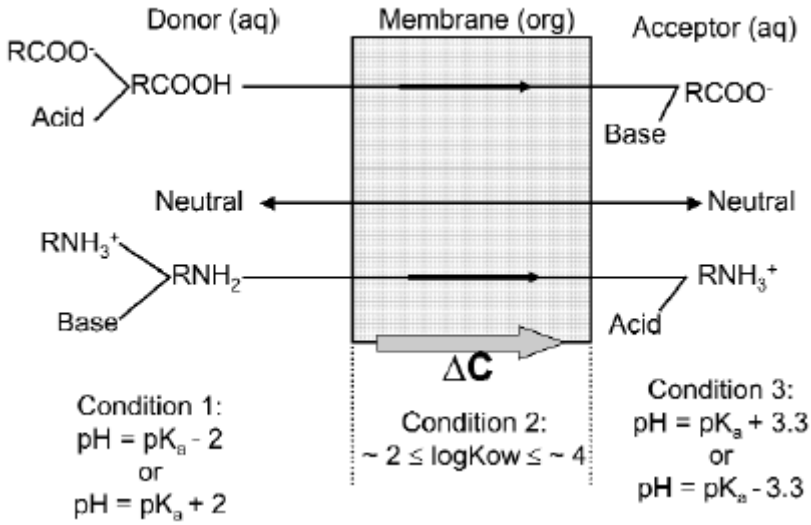


Figure 1: Principles of SLM extraction of ionizable organic compounds where the transport mechanism is simple permeation [21]

The theory of SLM extraction has been thoroughly discussed by several researchers [32-35]. The process of extraction in liquid membrane processes are frequently expressed by two important quantitative parameters, namely, extraction efficiency and enrichment factor. The extraction efficiency is defined as the fraction of analyte extracted from the donor phase into the acceptor phase.

Extraction efficiency is controlled by several parameters among which the most important ones are the partition coefficient of the analyte between the aqueous donor phase and the organic membrane liquid, pH in donor and acceptor, and donor flow rate. For acidic analytes the pH in the aqueous donor needs to be below the pKa values of the analytes rendering them uncharged phase; and the pH of the acceptor must be at least 3.3 pH units larger than the pKa values. For the basic compounds the pH of the flowing donor solution should be at least 2 pH units more than the highest pKa value; that of the acceptor side should be at least 3.3 pH units below the pKa of the analytes in question [19,21, 32, 33].

Transport Mechanisms in SLM Extraction

In liquid membrane extraction techniques, in general, the permeation of analytes through the membrane takes place in a stepwise manner. The following five distinct steps occur in

the process [36]: (i) analyte diffusion from the donor bulk to the membrane surface, (ii) analyte dissolution into the membrane, (iii) analyte diffusion through the membrane, (iv) desorption of analyte form the membrane, and (v) analyte diffusion from the membrane surface to the bulk of the acceptor.

Analytes, in SLM extraction procedure, are transported across the membrane through these steps according to two main classes of transport mechanisms, i.e., passive transport and carrier-mediated transport. These transport mechanisms are based on the need to maintain charge neutrality of analytes at the donor-membrane interface and facilitate mass transfer across the membrane.

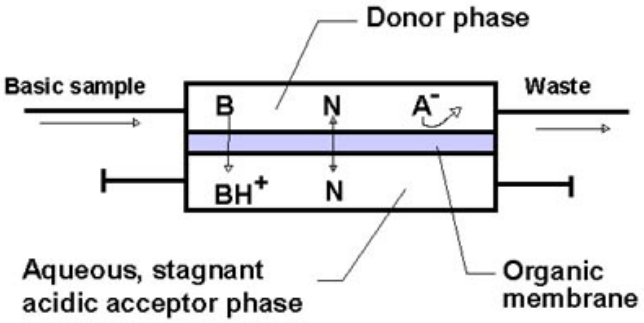


Figure 2: Schematic representation of "unfacilitated" transport mechanisms of solute through liquid membrane, extraction of a basic analyte B [35].

(a) Passive Transport Mechanism: In this transport mechanism, which is also called a simple permeation or diffusive transport, the mass transfer of analytes across the membrane is based on the difference in solubility of the matrix components in the membrane phase (Figure 2). Analyte molecules dissolve into and diffuse through the membrane due to their concentration gradient. They are then trapped in the acceptor phase, by reacting with appropriate chemical species. This way, the concentration gradient can be maintained during the entire extraction and enrichment of the diffusing species. This is sometimes called "unfacilitated transport" [31]. The main area of

application of unfacilitated transport mechanism is the extraction of small organic acids and bases that can be easily switched between neutral to charged forms by pH adjustments

(b) Carrier-Mediated Transport Mechanism: Often, selective transport based on relative differences in solubility in the membrane and trapping in the acceptor phase may be difficult to achieve. In another case, the solubility of the analyte may be too low to give efficient extraction even when the trapping in the acceptor can easily be realized. A good approach in such a case is to incorporate a mobile carrier into the membrane that selectively binds the analytes. The appropriate solubility of the permeate in the membrane phase is not required since the permeate interacts with the carrier molecule, C, dissolved in the membrane phase. This carrier should be totally insoluble in both the donor and the accepter phases and should react specifically and reversibly with the permeate. The idea of incorporating a carrier also allows SLM extraction to be applicable to a variety of compounds such as permanently charged chemical species like metal ions. It also gives different versions of carrier-mediated transport mechanisms such as simple carrier transport, coupled co-transport, and coupled counter-transport. These are elaborated elsewhere in some detail [21, 31, 37].

Simple Carrier Transport: The carrier in the membrane forms a complex with the analyte in the donor that diffuses to the acceptor, where the analyte is converted to a nonextractable form. The complex formation will increase both solubility in the membrane and speeds up the diffusion process. Thus concentration gradient across the membrane increases as compared to the simple permeation. Alkali metal transport by crown ethers through the hydrophobic membrane is a good example of this facilitated diffusion transport mechanism. The simple carrier transport may also take place with a chemical reaction in the acceptor. This type of transport was used in the extraction of

short chain aliphatic carboxylic acids from acidic donor solution to an alkaline acceptor solution with liquid membrane containing tri-*n*-octylphosphine oxide (TOPO) as a neutral carrier.

Counter-Transport Mechanism: Based on ion-exchange processes that occur at the membrane interfaces, the transport of the permeate (analyte), A, is coupled with the transport of the counter ion, D, in the opposite direction (Figure 3). At the donor phase boundary the analyte associates with the carrier, C, in the membrane solvent to form a complex that can be transported through the membrane. Upon releasing the analyte, the carrier combines with D from the acceptor solution, at the acceptor phase boundary and travel back for repeating the cycle. By providing a much higher concentration of counter ions against the concentration of the permeate, the permeate could be transported even in the direction opposite to its concentration gradient.

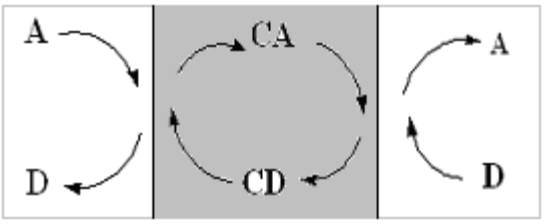


Figure 3: Schematic representation of counter-transport mechanisms of solute through liquid membranes (slightly modified from Ref. 31).

Permanently charged species, amino acids, for example, can be extracted in the form of neutral complexes using ion-pairing reagents in the sample phase or as carriers in the liquid membrane. At the membrane-acceptor interface, the analytes are exchanged for counter-ions present at high concentrations [38]. A transport based on carrier-mediated transport coupled with cationic carrier dissolved in the membrane phase can be applied to the enrichment of short peptides. The peptide is transferred over the organic, liquid membrane as its anionic form enhanced by complexation with the cationic carrier (Aliquat 336 - quaternary ammonium salt). NaCl was used as a source of chloride anion in the acceptor phase [39].

"**Co-transport" mechanism:** The driving force for the transport of the permeate in this mechanism (Figure 4) is provided by the difference in concentration of the co-ion, D, between the donor and acceptor solutions. If the concentration of the co-ion is much higher than the concentration of the permeate, A, the permeate could be transferred in the direction opposite to its concentration gradient ("uphill" transport). For example, hydrogen dichromate, which exists in ionic form as $2H^+$ and $Cr_2O_7^{2-}$, is extracted from the acidic donor phase with tryoctyl amine, R_3N, by forming the complex $(R_3NH)_2Cr_2O_7$. The complex traverses the membrane to the basic acceptor and gives up the protons and the dichromate anion.

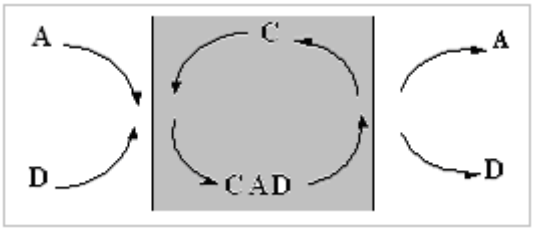


Figure 4: Schematic representation of co-transport mechanisms of solute through liquid membranes (slightly modified from Ref. 31).

Factors that Influence the Extraction Process

These include trapping in the acceptor phase, solvation power of the membrane and donor flow rate besides other factors such as membrane thickness, geometry of the donor, and acceptor channels of the SLM module [21]. Transformation of the analytes dissolved in the membrane into a non-extractable form in the acceptor is termed as 'trapping'. The trapping in the acceptor phase is seen as critical and is desirable that analytes are virtually ionized; otherwise extraction efficiency is not constant and decreases with time.

The solvation power of the membrane should give large analyte partition coefficients (K_D), but studies have shown that too high K_D values result in slow release into the acceptor phase. At lower donor flow rates, the contact time of the analytes with the membrane is longer so highest extraction efficiencies are obtained. At high donor flow rates, the extraction efficiency decreases as the contact time of the analytes with the membrane reduces. However, for moderately polar analytes (with log k_{ow} > 2), high

donor flow rates results into an increase in high enrichment factors since the amount extracted per unit time increases. This reduces the extraction time [21, 35, 40].

Membrane Solvents and Supports in SLM Extraction

One of the major factors that determine the selectivity and efficiency of the SLM extraction is the type and composition of the organic liquid immobilized in the porous membrane support, as the transport rate of analytes is related to their partition coefficients between the aqueous and organic phases. The points that have to be considered when choosing the membrane liquid have been described elsewhere [41] and are summarized below.

1. The organic solvent should have low solubility in water. Since a thin film of the solvent comes in contact with a large volume of aqueous solution, even a slight solubility will result in losses of the solvent and risk for leakage between the two separated solutions, thus shortening the lifetime of the membrane.

2. Solvents of low viscosity are preferred because it results in a large diffusion coefficient of analyte molecules in the membrane.

3. The analytes should have high enough partition coefficients in the membrane solvent of choice to give a high flux. At the same time, other substances present in the sample should have low partition coefficients to make the analyte enrichment as selective as possible

Di-n-hexyl ether [42] and undecanone [43], which are moderately polar solvents, and the non-polar solvent n-undecane [40] are typical solvents that have been employed in SLM extractions as membrane liquids.

The type of the porous support material in which the membrane liquid is immobilized is also equally important in SLM extraction processes. As it is reported in the literature [36], the ideal support should be very thin (< 10 μm), should have high porosity (> 50%), a mean pore size of less than 0.1 μm and a narrow pore size distribution. Furthermore, it

should be stable and easy to handle. Membranes with PTFE materials are found to be suitable and used in most applications of SLM extraction.

Advantages and Limitations of SLM Extraction

SLM extraction offers a number of advantages over other sample preparation methods in trace analysis [37, 44]. The principal advantage of SLM extraction is the use of minimum organic solvents. This makes the technique environmentally friendly and cheaper as the cost of buying and disposing large quantities of organic solvents is reduced. SLM extraction also leads to high degree of selectivity and cleanup from complicated matrices. The selectivity is based on differences in solubility of the analytes in the membrane liquid and their diffusion into the acceptor phase in addition to the difference in the size of the molecules. The more flexible nature of liquid membrane extraction creates a selectivity window for the enrichment of analytes belonging to the same family by changing the organic solvent, changing or modifying the extraction process using carriers or ion pairing agents in various modes, adjustment of the pH in the donor and acceptor phases and changing the support material.

The major problem often cited in this technique is instability of the membrane. This is caused by solvent or carrier loss during extraction as a result of dissolution of the membrane liquid into the flowing aqueous sample and differences in osmotic pressure between the phases. This problem results in decline of analyte flux or even in leakage of one of the aqueous phases into the other. For analytical purposes, especially with di-*n*-hexylether or *n*-undecane as membrane liquids, SLMs are stable from a week to a month. Convenient regeneration of the membrane using the same hydrophobic porous support has also been demonstrated either in situ by Thordarson et al. or by Dzygiel et al. after demounting the support [21].

Incomplete transfer of analytes through the membrane to the acceptor is another major problem commonly encountered in this extraction technology [40]. Adsorption of

solute molecules on the connecting tubes, flow system or on the surface of the membrane support is the first reason for the incomplete transfer and is termed as carry-over effect COE). Slow diffusion of solute molecules through the liquid membrane can be cited as another reason for the incomplete transfer. The effect due to this portion of the left over analytes caused by slow diffusion is termed as membrane memory effect (MME). A liquid giving a high partition coefficient might also give high memory effects. Both of these reasons of incomplete analyte transfer result in inclusion of notable fraction of solute molecules to the acceptor phase during subsequent extractions unless the flow system is thoroughly washed and allowed to stand for some time between each extraction.

The extraction time required to secure maximum accumulation of analytes from large sample volumes is relatively longer, and this can also be another limitation to liquid membrane extraction [37]. The extraction time can be minimized by processing samples at higher flow rates provided that it will not result in the reduction of the membrane lifetime.

1.2.2.2 Extraction Using Hollow Fiber Supported Liquid Membrane (HFSLM)

We already said that the polymeric membrane unit could be either flat or hollow fiber. The set up of the flat membrane unit is presented in section 2.3. The porous, hydrophobic, hollow fibers are impregnated with an organic phase to create both SLM and MMLLE systems. This new extraction methodology that uses hollow fiber supported liquid membrane (HFSLM) is also termed as hollow fiber liquid phase micro extraction (HF-LPME). HF-LPME proved to be an attractive alternative to other micro extraction concepts because, apart from being simple, inexpensive, fast and virtually solvent free, it resulted in increased sensitivity. Because of the disposable nature of the hollow fiber, it also eliminated the possibility of carry-over between analyses [30, 45].

There are two sampling modes that can be used with HF-LPME: two phase; and, three phase [30]. In the two-phase HF-LPME sampling mode, analyte ***i*** is extracted from

an aqueous sample (donor phase) through a water-immiscible solvent immobilised in the pores of the hollow fiber into the same organic solvent (acceptor phase) present inside the hollow fiber (Figure 5, *i*). The extraction process of the two-phase HF-LPME for analyte ***i*** may be illustrated as follows:

$$i_d \leftrightarrow i_{org}$$

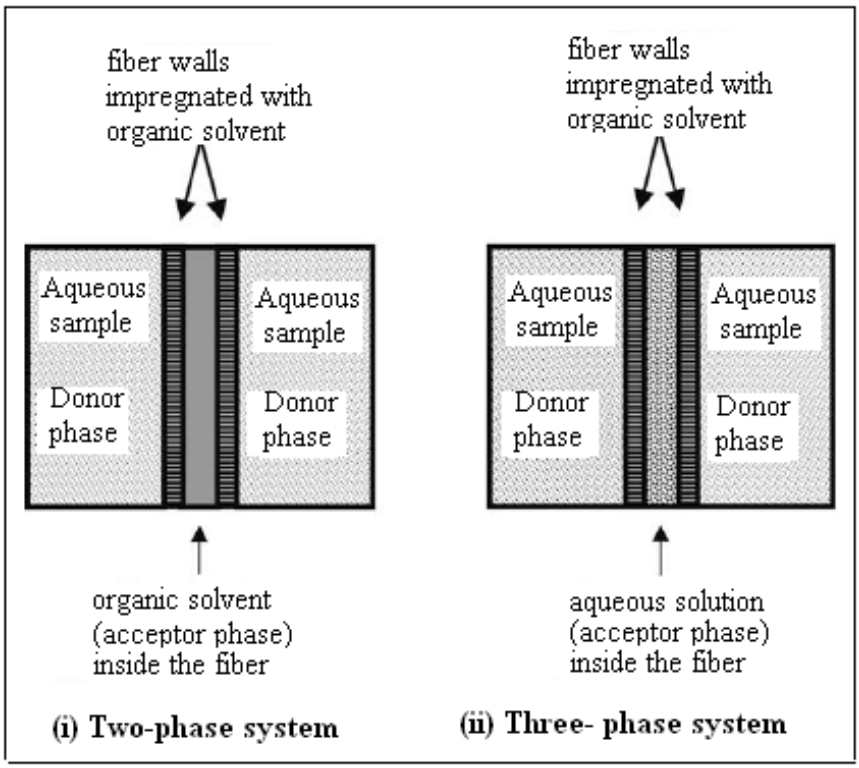


Figure 5: Cross section of the hollow fiber inside the aqueous sample during (i) Two-phase and (ii) Three-phase LPME (slightly modified from Ref. 30).

and is characterised by the distribution ratio Korg/d, which is defined as the ratio of the concentrations of analyte ***i*** in the organic and donor phase at equilibrium conditions.

Successful two-phase HF-LPME requires large distribution ratios for target analytes. Such Korg/d values correspond to moderately or highly hydrophobic compounds containing acidic or basic groups, or neutral compounds of similar hydrophobicity. In two-phase HF-LPME the final extract is an organic phase, compatible with analytical techniques, such as GC or HPLC.

In the three-phase LPME sampling mode, analyte ***i*** is extracted from an aqueous solution (donor phase) through the organic solvent immobilised in the pores of the hollow fiber (organic phase) into another aqueous phase (acceptor phase) present inside the lumen of the hollow fiber (Figure 5, *ii*). The organic phase in this case serves as a barrier between the acceptor and the donor aqueous solutions, preventing mixing of these two phases. The three-phase sampling mode is usually combined with a HPLC or a capillary electrophoresis (CE) system, as the acceptor phase is aqueous.

Overall, the three-phase HF-LPME extraction process for analyte ***i*** may be illustrated as follows:

$$i_d \leftrightarrow i_{org} \leftrightarrow i_a$$

The three-phase HF-LPME process is characterised by Korg/d and Ka/org, which are the distribution ratios at equilibrium between the organic phase and the donor phase, and the acceptor solution and the organic phase, respectively. The overall distribution ratio Ka/d between the acceptor and the donor phase can be written as:

$$K_{a/d} = K_{org/d}.\ K_{a/org} \qquad (1.2)$$

Adjustment of the composition of the donor and acceptor phases is critical for successful three-phase HF-LPME. Large Ka/d (>>1) can be achieved when the analytes in the acceptor phase are converted by reactions, such as protonation or complexation, to species that will have very small affinity for the organic phase. In this way, back extraction of analytes from the acceptor to the donor solution is prevented. Hence, the three phase system will also extract acidic or basic compounds having a low Korg/d, as long as the Ka/org value is high, thus preserving the requirement for large overall distribution ratio. This is very important, because the applicability range of HF-LPME may be extended to ionisable analytes having a low Korg/d by simply changing from two-phase to three-phase HF-LPME sampling mode.

There are several configurations of arranging the fiber during extraction with HF-LPME sampling modes [30]. In one configuration one end of the hollow fiber is used for injection/collection of the acceptor solution and the other one is left suspended in the

donor-sample solution. This is possible by using the tip of the needle of a micro syringe as a support for the fiber (Figure 6). The micro syringe in this case serves to support the hollow fiber, introducing/collecting the acceptor solution, and as a sample-introduction device for subsequent analysis. The free end of the hollow fiber can be flame sealed. Prior to immersion in the donor solution, the fiber should be dipped in the organic solvent to impregnate it. Under stirring, the analytes are extracted from the donor phase through the organic solvent in the pores of the hollow fiber, and finally into the acceptor solution inside the fiber [30, 46].

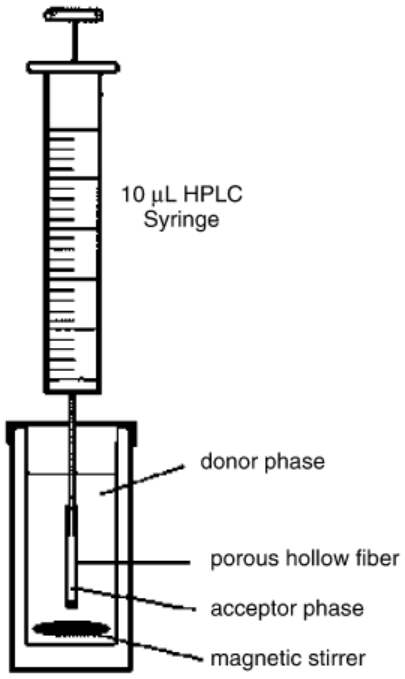


Figure 6: Schematic representation of a configuration where a micro syringe is used for supporting the hollow fiber, introducing/collecting the acceptor solution, and serves as a sample introduction device for subsequent analysis [30].

The hollow fibers employed in HF-LPME should be hydrophobic as well as compatible with the organic solvent being used. Almost all published reports use polypropylene capillary membranes. The most common type of such fibers has an inner diameter of the order of 600 µm, compatible with the µl volumes of the acceptor solution required for micro extraction. Its wall thickness (200 µm) provides excellent mechanical stability and simplifies the preparation of the extraction units. Finally, the nominal and maximum pore sizes (0.2 µm and 0.64 µm, respectively) of these capillary membranes

ensure efficient micro filtration, allowing penetration of only small molecules (target analytes) through the pores of the hollow fiber [30].

Agitation of the sample is routinely applied to accelerate the extraction kinetics. Increasing the agitation rate of the donor solution enhances extraction, as the diffusion of analytes through the interfacial layer of the hollow fiber is facilitated, and improves the repeatability of the extraction method. In HF-LPME, the acceptor solution is confined within the fiber, and it can tolerate very high agitation speeds. In both the two-phase and three-phase HF-LPME modes, sample agitation, by using either vibration or magnetic stirring, dramatically increased extraction. Vibration of the sample had the advantage that it eliminated the possibility of sample contamination through the use of Teflon-coated magnetic stirrers. Where magnetic stirring was used, application of the maximum stirring speed was found to promote the formation of air bubbles that tended to adhere to the surface of the hollow fiber, thus accelerating solvent evaporation and introducing imprecision in the measurements [30].

1.3 Analytical Determination Methods of Niclosamide

1.3.1 Chromatographic Analysis of Niclosamide

High Performance Liquid Chromatography (HPLC) has been repeatedly used for the determination of niclosamide as reported in the literature [6, 7, 8, 25, 47, 48]. HPLC methods are extremely sensitive and can analyze substances, which are polar and difficult to vaporize for gas chromatographic analysis. They are simple and suitable for routine analysis and have low detection limits. The analysis can be performed at ambient temperature [1, 4]. The structure of niclosamide confirms that UV-vis detection is possible.

Quantitative chromatography is based upon a comparison of either the height or the area of the analyte peak with that of one or more standards. If conditions are properly controlled, both of these parameters vary linearly with concentration. A graph showing detector responses as a function of analyte concentration is called a calibration curve or a standard curve. The most straightforward method for quantitative chromatographic analysis involves the preparation of a series of standard solutions that approximate the

composition of the unknown. Chromatograms for the standards are the obtained and peak heights or areas are plotted as a function of concentration. A plot of the data should yield a straight line passing through the origin; analyses are based upon this plot. Frequent standardization is required for highest accuracy [49].

To prepare niclosamide standard solution, the powder can be dissolved in methanol containing 10% formic acid [47]. A three or four point standard curve is used to quantify the niclosamide concentration and interval standards were included approximately every tenth injection for quality control. Niclosamide concentrations of samples are calculated based on comparison of peak area responses with those of standards [6, 25]

The retention time depends on several conditions as mentioned in the literature. Using C_{18} cartridge and 1mL/min flow rate; the retention time is 17.5 min with mobile phase of phosphate buffer and methanol (75; 25, v/v) at a pH of 3.6 and UV detection at 254 nm [47], about 28 min with a gradient mixture of trifloroacetic acid and acetonitrile mobile phase and UV detection at 265 nm [48] or 220 nm [7], and 25.9 min with a gradient mixture of 58 mM sodium acetate buffer (pH 3.8) and acetonitrile mobile phase and at UV detection of λ_{max} 360 nm [6, 25]. The last one was niclosamide analysis in rainbow trout and catfish with detection limits 0.0107 μg/g and 0.0063 μg/g respectively.

Thin layer chromatography was used frequently as method of analysis for confirmation and identification work after HPLC result [3, 7, 8, 48].

1.3.2 Other Methods of Analysis of Niclosamide

A number of qualitative and quantitative techniques for the determination of niclosamide have been reported. These include spectrophotometeric techniques [50-53], polarography [54, 55] and voltammetry [4, 5]. Of these, techniques, the spectrophotometric methods that involve complex formation or derivatization are non-selective and subject to high interference. Sastry, C.S.P. and co-workers reduced niclosamide with Zn and HCl followed by reaction with metol and $K_2Cr_2O_7$ at pH 3.0 to give a colored product having maximum absorbance at 720 nm [50]. Other workers determined niclosamide in tablets by dissolution of the tablets in NaOH solution followed by measurement of the absorbance at 375 nm [52]. The work by Abdel Fattah, S.A. was based on the reduction of the nitro group in niclosamide to the amino group by treatment with zinc powder and

HCl in ethanol solution. The product reacted with p-benzoquinone and produced a pink compound, which absorbed at 506 nm [53].

The polarographic method has very narrow linear range. Solomon, T. and Zewude, H. qualitatively and quantitatively determined niclosamide at 10^{-4}-10^{-7} M in 0.1 M NH_4OAC / ethanol based on D.C. and differential pulse polarography [54]. According to Mishra, A.K. and Gode, K.D. niclosamide was extracted from tablets using methanol and the solution with an appropriate buffer was polarographed at the dropping mercury electrode vs. SCE at 25^0. Here niclosamide could be detected up to a level of 5-10 μg/mL [55].

Cyclic and square-wave voltammetry are sensitive and selective but they are commonly used in pharmaceutical analytical problems. Alemu, H. et al. applied voltammetric method for the determination of niclosamide in tablets. Following optimization of voltammetric parameters, pH and reproducibility, a linear calibration curve over the range 1 x 10^{-6} - 1 x 10^{-4} M niclosamide was achieved. The detection limit was found to be 8 x 10^{-7} M [5]. In a similar experiment, a calibration curve over the range 5 x 10^{-8} - 1 x 10^{-6} M was achieved with a detection limit of 2.05 x 10^{-8} M [4].

1. 4 Scope of the Present Study

Many people in Ethiopia are traditionally accustomed to eating raw meat. Peoples from southern Shewa, Gurage, and Walaita zones are typical examples. Hence they are frequently exposed to tapeworm infestations. For medical treatment, they use traditional medicines extracted from some living plants. The main plants used are Hagenia abyssinica (*koso*), Rosaceae, [14], Glinus lotoides L. (*meterre*), Molluginaceae, [15], Embelia schimperi vatue (*enqoqo*), Myrsinaceae, [16], and Cucurbita pepo L. (*dubba*), Curcurbitaceae, [17]. The various forms of the medicine, currently available on market are expensive. On the other hand, a success in extraction of the medicinal substance from the plants mentioned above would be of great advantage. In addition the natural products seem to be safer as far as the quantity to be medically prescribed is well known. The raw materials are also cheap and plenty in species

According to the literature review, the supported liquid membrane was not yet utilized for extraction of niclosamide. Most conventional extraction processes take

advantage of only one difference in physical and chemical properties of the components of the sample. Supported Liquid Membrane (SLM) extraction technique has high degree of selectivity since it takes advantage over two or more properties at the same time. Therefore, it is valuable to apply this method for the preconcentration of niclosamide from plant materials. HPLC methods are simple and suitable for routine analysis and have low detection limits. The structure of niclosamide confirms that UV-vis detection is possible.

The main objective of this study, therefore, is to develop a method of extracting niclosamide from indigenous plant materials based on Supported Liquid Membrane (SLM) technique and determination of the analyte using High Performance Liquid Chromatography (HPLC) technique equipped with UV- detection.

2. EXPERIMENTAL: SUGGESTED SAMPLING, EXPERIMENTAL SET-UP AND PROCEDURES

2.1 Sampling and Sample Pretreatment

Touring the representative regions, namely southern Shewa, Gurage, and Walaita zones and gathering information regarding the local applications of the traditional medicines is a lengthy process for appropriate sampling. The season of flowering and seed ripening of some of the plants matters for direct sampling from the locality. The other alternative is to buy the plant materials from markets that represent different localities. As first hand information an inquiry about their usage was made with some of the people in the surrounding.

An early flowering part of "Kosso" plant is used. The people in some locality use two living plant additives one, Pygeum africanum (*mi'essa*), Rosaceae [56], to make it laxative, and the other, Grewia ferruginea (*dhoqonuu*), Tiliaceae [57], to make it more slippery in the alimentary canal. It is ground when dry and mixed with water. No filtration is made; the heterogeneous mixture is directly given to the patient. The fresh mixture is used as medicine; otherwise it turns to be poisonous upon staying in water. "Kosso" is the most bitter and effective against tapewarm and other worms in the stomach or intestine. Our plan was to collect "Kosso" excluding the additives.

Either the fresh or dry seed part of "enkoko" is used. It is mixed with water after grinding. No filtration is done; it doesn't become harmful upon staying a while. The dry seed of "metere" is ground and directly eaten with soft food; it is not mixed with water. The seed part of "dubba" is slightly roasted and directly eaten alone since it is not sour or bitter.

We bought samples of the plant materials from Waliso, Walqitte and Dilala. The collected plant materials should be finely ground using mortar and pestle. The powder should be soaked in water and filtration will be carried out to remove any solid component. Thus a clear aqueous solution should be made ready for SLM extraction. The "kosso" sample should be extracted immediately after pretreatment.

2.2. Preparation of Standard, Reagents, and Working Solutions

2.5 mg of the standard niclosamide was dissolved in 25 mL of CH_3OH containing 10% HCOOH. This makes a stock solution of 100 mg/L that is kept in a refrigerator. Working solutions of the analyte mixture were prepared by diluting the required volumes of the stock solution with distilled water. The $NaH_2PO_4.2H_2O/Na_2HPO_4$ and glacial CH_3COOH/CH_3COONa buffer systems that are used as donors in SLM were prepared in distilled water and were stored in refrigerator. $NH_4H_2PO_4$ or NaH_2PO_4 that were used, as mobile phase buffers in chromatographic analysis were freshly prepared, filtered and degassed.

2.3 Experimental Set-up Based on SLM

We applied the flat polymeric membrane unit for SLM extraction in this particular experiment. The preparation of liquid membrane and the experimental set-up are similar to those described elsewhere [40]. The arrangement of the membrane unit is shown in Figure 7. The membrane holder consists of two circular polytetrafluoroethylene (PTFE) blocks (diameter 120 mm, thickness 8 mm). In each block a groove, arranged as Archimedes' spiral (depth 0.25 mm, width 1.5 mm and length 250 cm, with a total volume of about 0.95 mL), is machined and liquid connections are provided. Both sides

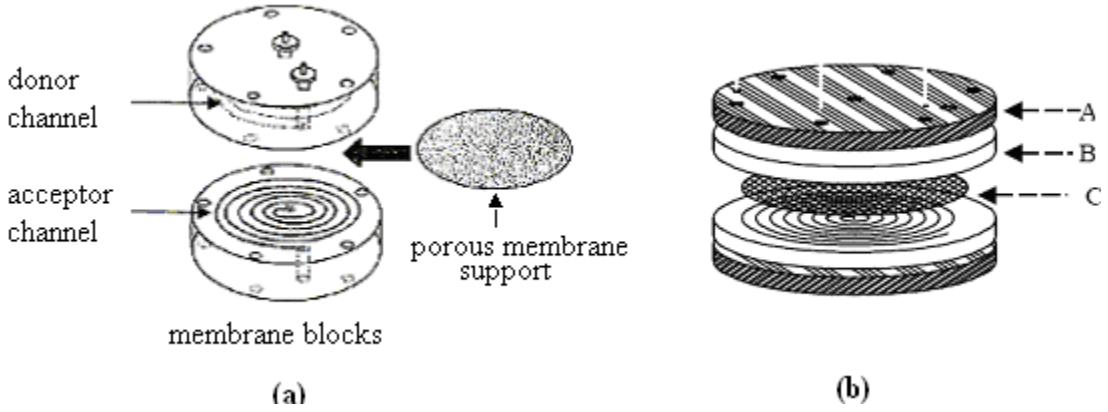


Figure 7: The membrane unit; a) the set-up of the parts to make the donor and acceptor compartments. b) A= aluminium blocks, B= channeled PTFE blocks,
C= PTFE membrane (modified from Ref. 35, 37, and 40).

of the holder are backed with aluminum blocks of 6 mm thickness, in which threads for the clamping screws are machined to make the assembly stable. In addition, the donor channels of PTFE block are equipped with an O-ring, outside the grooves, for preventing the leakage of solutions.

The liquid membrane support is Millipore with an average pore size of 0.2 μm, a total thickness of 175 μm of which about 115 μm is polyethylene backing and a porosity of 70%. The liquid membrane is prepared by immersing the porous polymeric membrane support in the membrane liquid for 30 minutes. The soaked membrane is placed between the two PTFE blocks with its rough side facing the donor channel. The whole assembly is clamped together tightly and evenly with six screws. Thus, the two channels are separated by the liquid membrane forming the donor and the acceptor compartments.

Two peristaltic pumps are used to deliver the donor and acceptor solutions independently to the membrane unit at controlled flow rates. The tubes used for pumping solutions are acid-resistant. The configuration of the flow system is shown in Figure 8.

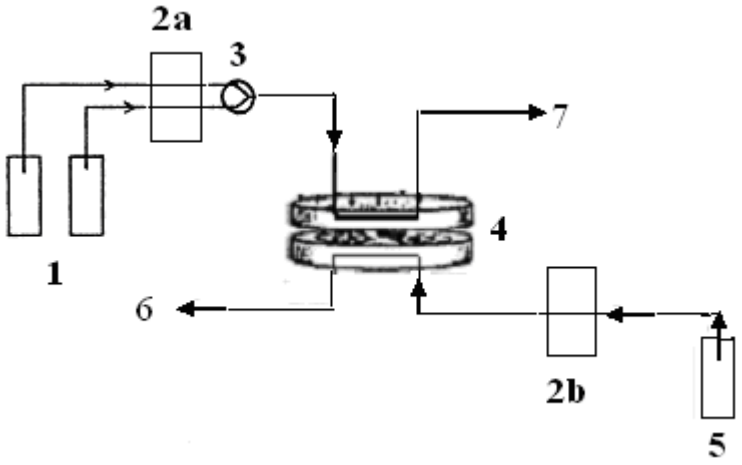


Figure 8: Simplified arrangement of the flow system for liquid membrane extraction. (1) Containers for sample solution and donor buffer; (2a) and (2b) peristaltic pumps for the donor and acceptor phases, respectively; (3) PTFE T- connection (4) membrane extraction unit; (5) container for the acceptor solution; (6) container for extract collection and; (7) waste (modified from Ref. 40).

2.4 Procedure of Extracting Standard Samples

Two possibilities were suggested for extracting standard solutions of niclosamide. They are simple transport mechanism and carrier mediated transport mechanism.

2.4.1 Using Simple Transport Mechanism

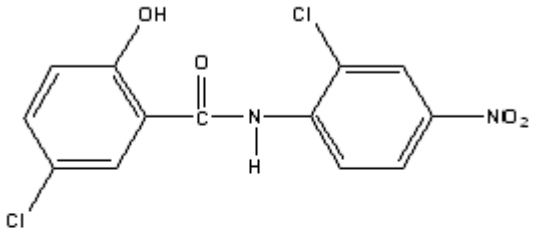
Niclosamide can be extracted as a neutral molecule. Thus we applied simple transport mechanism without carrier in this experiment. Since it is weakly acidic in water, taking the pKa 8.5, NaH_2 $PO_4.2H_2O$ and Na_2HPO_4 buffer system was used to provide pH 6.5 for donor solution. 0.001 M NaOH with its pH adjusted to 11 was used for acceptor solution. If extraction in this way is not found to be successful, we can consider the pKa 6.25. In this case donor solution of about pH 5 can be provided by glacial acetic acid and sodium acetate; the acceptor solution of pH 10 can then be prepared using sodium hydroxide.

In the extraction procedure, first the membrane support, PTFE, was soaked in 1 mL undecane for 30 minutes. After installation, the donor and acceptor channels were flushed one after another with distilled water to remove excess of the organic solvent from the surface of the membrane. This was done till clear water was seen in the pumping tubes. Next, the acceptor solution was pumped at a rate of 0.2 mL/min into the acceptor channel for 10 minutes. The buffer and analyte solutions were separately pumped at a flow rate of 0.5 mL/min each; T- connection was used for mixing them before sending them to the donor channel. This was done at a total donor flow rate of 1 mL/min for 20 minutes. Next, the donor buffer was pumped alone for 10 minutes.

After additional 10 minutes were given to provide sufficient diffusion time for the analyte through the membrane, 2 mL solution containing the extract was collected by pumping the acceptor solution. Three replicate samples were collected in vials. All the extracts were found in the basic pH range when tested with universal indicator. Thus they were adjusted to neutral pH by drops of acid using Pasteur pipette. Finally they were stored in a refrigerator till HPLC analysis is done. The analysis could not be done due to shortage of time.

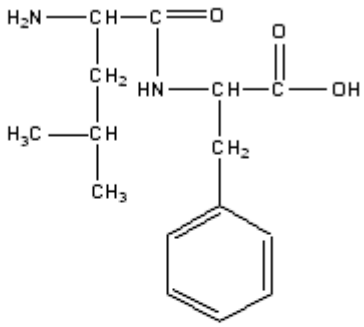
2.4.2 Using Carrier Mediated Transport Mechanism

Niclosamide can also be extracted in a charged form using a transport catalyst. In basic medium the NH group is not affected but the OH group is deprotonated. In this case, a positive carrier is required. In acidic medium NH group is protonated and a negative carrier is required. Counter ions are required in the acceptor and NaCl can provide these. The suggested extraction of niclosamide in a charged form can be compared with that of leucinephenylaniline (Leu-Phe), the work already done by Drapala A., et al [39]. Leu-Phe and niclosamide have quite similar functional groups as shown below. The pKa of niclosamide is calculated using the software "ACD/Chemsketch 133 Richmond St.West, Toronto, Canada".



Niclosamide

pKa-OH = 7.45 ± 0.43
pKa-NH = 10.36 ± 0.46



Leucinephenylaniline

pKa-COOH = 3.14
pKa-NH2 = 8.41

So incorporation of the transport catalyst**,** cationic carrier (Aliquat 336- quaternary ammonium salt), in the membrane support during membrane preparation is recommended. The membrane support is immersed in a mixture of undecane and Aliquat 336 for 30 minutes. The acceptor is suggested to be aqueous solution of NaCl. The rest of the extraction procedure is similar to that of the simple transport mechanism. We didn't apply this alternative method of extraction since our result from the previous one was not determined.

2.5 Procedure of Chromatographic Analysis and Detection

Reverse phase HPLC using C_{18} column is preferable. A mixture of 75% (v/v) of about 0.04M $NH_4H_2PO_4$ /adjusted to pH 3.6 by H_3PO_4/ and 25% (v/v) of HPLC grade methanol are preferred for isocratic mobile phase. A gradient mixture of the mobile phase may also be applied. The suggested flow rate is 1mL/min. The UV detection is at 254 nm. NaH_2PO_4 could also be used instead of $NH_4H_2PO_4$. A series of three aqueous samples are prepared by diluting stock solution of the standard at concentration levels between 1 and 10 mg/L in distilled water. These are injected to the chromatograph to produce a calibration graph. Niclosamide concentrations in the extracts can be calculated on the basis of peak height responses with those of standards. We can also use a gradient mixture of sodium acetate buffer and acetonitrile mobile phase at UV detection of wavelength 360 nm.

2.6 Study of Experimental Parameters

The pH: We can study the pH effect of the donor flow system by preparing buffers in the pH range of 3.0 –7.0. The effect of the acceptor solution can also be studied by varying NaOH pH within the range of 9.0 – 12.

Flow rate: The influence of the donor flow rate on the extraction efficiency can be investigated within a range of 0.5 – 4mL/min. The donor flow rate suggested in the above procedure is just to start with. The acceptor flow rate is kept the same in all experimental procedures.

Concentration of the Transport Catalyst: The effect of the amount of the mobile carrier, mixed with the membrane liquid, on the efficiency can be investigated by extracting samples of the analyte using liquid membranes containing Aliquat 336 at different concentrations, i.e., 0 to 50 % v/v.

Carry-Over Effect: The sample mixture containing the analytes of interest is first extracted as described in sections 2.4.1 and/or 2.4.2. This is followed by extraction of the blank reagent water under identical conditions.

2.7 Extraction using Hollow Fiber Supported Liquid Membrane (HFSLM)

HFSLM extraction could be carried out and its extraction efficiency can be compared with the ordinary SLM extraction conducted as presented in the above procedure.

2.8 Application to Real Samples

Enrichment of the real sample extracts is carried under the same condition of the developed method of extracting the standard sample. The standard sample could be spiked to the plant material pretreated and SLM extraction will be carried out for justification of the developed method. Then detection of niclosamide in the enriched sample is attempted. Further experiments for confirmation and quantification will be carried out if the analyte is correctly identified in the real sample. The detection limit of the analyte for niclosamide in the real sample will also be determined.

3. DISCUSSION BASED ON LITERATURE REVIEW

3.1 Determination of Experimental Variables

The **extraction efficiency** of the technique can be calculated from experimentally measured quantities using equation 3.1 [32, 37]

$$E = C_A V_A / C_I V_I \qquad (3.1)$$

where C_I and C_A are the concentrations of the analyte molecules in the aqueous sample and enriched in the acceptor phase, and V_I and V_A are the volumes of the donor and acceptor solutions, respectively.

The **enrichment factor** of the SLM extraction process refers to the extent to which the extracted molecules are accumulated in the acceptor compartment. This can also be calculated from experimentally measured quantities.

It is calculated by equation 3.2 [32, 37]

$$E_e = C_A / C_I \qquad (3.2)$$

The **carry-over effect** (COE) can be evaluated from the peak heights using the following equation [37]

$$COE = Pb/ (Pb + Ps) \qquad (3.3)$$

where Pb and Ps are peak heights of the blank extraction and sample mixture, respectively.

3.2 Possible Effects of the Experimental Parameters on Extraction Efficiency, E

Effect of pH*:* The pH of the donor solution as suggested in the simple transport mechanism is within the range of 5-7. At pH 6.5, it is expected that the majority of niclosamide molecules are electrically neutral i.e. uncharged. Thus the pH of the acceptor

solution should be in the basic range for effective trapping by deprotonation. It is expected that E increases when the pH of the solution increases. For carrier mediated transport mechanism using Aliquat 336, E is expected to increase with increasing pH since greater number of analyte anions are formed in a more basic solution.

Donor flow Rate: Generally we mentioned that at lower donor flow rates, the contact time of the analytes with the membrane is longer so highest extraction efficiencies are obtained. At high donor flow rates, the reverse is true. However, high donor flow rates are expected to result in an increase in high enrichment factors since the amount extracted per unit time increases. This reduces the extraction time.

Carry-Over Effect: The greater the adsorption of analyte molecules on the flow system the greater will be the carry-over effects in subsequent extractions. This decreases the extraction efficiency since the rate of accumulation of the analytes in the acceptor phase decreases.

Concentration of Transport Carrier: For carrier-mediated liquid membrane extraction systems, the carrier concentration is a vital factor that affects E. A steady increase in extraction efficiency with Aliquat-336 concentration up to a level of about 50% (v/v) is expected. Further increase may affect the efficiency of the membrane solvent.

Concentration of the Counter-ion in the Acceptor solution: The driving force responsible for the mass transfer process is the difference in the concentration of the so-called counter-ion (in this case Cl^-) in the opposite direction. For high mass transfer the presence of a chloride anion gradient from the acceptor to the donor phase is essential. It can be suggested that higher chloride anion concentration results in a higher extraction efficiency but this is valid only for salt content in the acceptor phase below a certain molar concentration. At higher NaCl concentration the extraction efficiency is expected to reach a maximum as a result of reaching the limit of solubility of niclosamide in the acceptor phase causing backward transport.

3.3 Effect of the Nature of Real Sample

The standard sample contains few solvent chemicals besides niclosamide. But the real sample is a complex matrix containing many organic compounds in addition to niclosamide, if it ever exists in the plant material under investigation. Therefore the SLM extraction and the analysis of niclosamide in the plant extract are not quite simple as compared to the standard sample. Many peaks are expected in the chromatogram of the real sample and the retention time of niclosamide might be quite altered. The detection limit of the analyte in the real sample is also expected to be enhanced. Therefore careful observation and repeated experimentations are recommended.

3.4 Comparison of Experimental Results

Niclosamide is a weak acid as observed from its pKa values. Therefore it is likely that the majority of the analyte are transported in the organic liquid membrane as electrically neutral molecules within the suggested pH range of the donor buffers.

Thus the carrier mediated transport mechanism seems to be less effective in preconcentrating the analyte when we compare the two transport systems. Comparison of results from ordinary SLM and HFSLM extractions is expected to show that HFSLM extraction has higher extraction efficiency since it is free from MME and COE.

3.5 General Recommendations

A research result from this analytical technique can be used for drug development from the resource that is already available in the country. Again the method to be developed can be used for medical investigation and analysis of environmental water samples. Knowledge of the parameters affecting the extractability of niclosamide can also be utilized for further scientific research in extraction technology.

4. CONCLUSION

If this project is successful, it will demonstrate that SLM extraction can be used to preconcentrate trace amounts of niclosamide that might be available in a given living plant material or in a given environmental source of interest. The work also is important as one way of searching for the active ingredient in the traditional medicines mentioned. The comparative studies suggested in this review may also help us to select the best method of SLM extraction. Results of the quantitative determination of niclosamide extracts in a given amount of sample are also important to evaluate the dosage used in the traditional custom.

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DECLERATION

I, the undersigned person, declare that this project is my work and that all sources of materials used for the thesis have been duly acknowledged.

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This project work has been submitted for examination with my approval as university advisor.

Dr. Negussie Megersa _____