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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Review

Solidified reverse micellar solutions (SRMS): A novel approach for controlling drug release from various lipids based drug delivery systems

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Solidified reverse micellar solutions (SRMS) are reverse micelles containing lecithin and a triglyceride, for example, SOFTISAN®142, which is hydrogenated coco glyceride. SRMS transform into a lamellar mesophase after melting on contact with water; this transformation enables controlled release of solubilized drugs. They offer potentials for sustained drug delivery of both hydrophilic and lipophilic drugs. SRMS have the advantage of providing more flexibility in controlling the drug release and protecting the encapsulated ingredients from chemical degradation. SRMS based systems influence the absorption of active ingredients through different mechanisms to modify the release of active ingredients, and improve drugs bioavailability. The types of SRMS-based drug delivery systems include solid lipid nanoparticles (SLN), solid lipid microparticles (SLM), tablets and suppositories amongst others. The work exhaustively reviews the advances in SRMS based carriers. Its formulation methods, characterisation and delivery systems were discussed in details.

Key words: Solidified reverse micellar solutions (SRMS), lipids, wide angle X-ray diffraction analysis (WAXD), small angle X-ray diffraction analysis (SAXD), lipid absorption.

INTRODUCTION

There is growing interest and investment in the use of lipid-based systems in drug discovery and product development to effectively overcome physical and biological barriers related to poor aqueous solubility and stability, membrane permeability, drug efflux and availability (Westesen and Siekmann, 1998). Solid lipids have the advantage of providing more flexibility in controlling the drug release and protecting the encapsulated ingredients from chemical degradation. Also, they allow for the incorporation of hydrophilic as well as hydrophobic drugs (Lippacher et al., 2001; Hu et al., 2020). Many waxes (for example, stearic acid, mono-, di- and tri-glycerides, glyceryl behenate, glyceryl monostearate, hydrogenated castor oil, among others) have been extensively investigated for sustaining the release of various drugs (Wadher et al., 2010).

Reverse micellar solutions (RMS) are lipidic solutions consisting of lecithin (30% w/w) dissolved in an oily

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Abbreviations: RMS, Reverse micellar solutions; **LRMS,** liquid reverse micelles; **SRMS,** solidified reverse micellar solutions; **LDDS,** lipid based drug delivery system; **P-gp,** P-glycoprotein; **WAXD,** wide angle X-ray diffraction analysis; **SAXD,** small angle X-ray diffraction analysis; **TEM,** transmission electron microscopy; **PCS,** photon correlation spectroscopy; **SLN,** solid lipid nanoparticles; **m.p.,** melting points; **SLM,** solid lipid microparticles; **NSAIDs,** non-steroidal anti-inflammatory drugs; **CMC,** critical reverse micelle concentration.

vehicle, for example, isopropylmyristate or middle-chain trigly-cerides and transform into a lamellar mesophase on contact with water (Papantoniou and Müller-Goymann, 1995; Friedrich et al., 2000). This application-induced transformation into a semisolid system of liquid crystals enables controlled release of solubilized drugs (Müller-Goymann and Hamann, 1993; Schneeweis and Müller-Goymann, 1997; Friedrich and Müller-Goymann, 2003). On contact with water, the liquid reverse micelles (LRMS) exhibits an application induced transformation into a semi-solid system of liquid crystalline microstructure. The structure of the liquid crystal has been identified by polarized light microscopy as a lamellar mesophase (Friedrich and Müller-Goymann, 2003). Solidified reverse micellar solutions (SRMS) are reverse micelles containing lecithin and a solid lipid such as the triglyceride (softisan® 154), and softisan® 154, which is a completely hydrogenated palm oil. SRMS offer potentials for sustained drug delivery of both hydrophilic and lipophilic drugs and also transform into a lamellar meso-phase after melting on contact with water. This trans-formation enables controlled release of solubilized drugs. Both LRMS and SRMS offer a high solubilization rate of different types of drugs (Friedrich and Müller-Goymann, 2003). SRMS based carriers have been investigated, and successfully employed to achieve controlled release of hydrophilic and lipophilic drugs (Schneeweis and Müller-Goymann, 2000; Friedrich and Müller-Goymann, 2003; Umeyor et al., 2012 a; Chime et al., 2012; Chime et al., 2013a,b). SRMS can also be formed using modified natural lipids containing lecithin and dika wax (Chinaeke et al., 2013), lecithin and Moringa oil, lecithin and goat fats (Uronnachi et al., 2013), lecithin and beeswax (Momoh et al., 2012) and lecithin and soy oil respectively at the ratios that could yield SRMS (Chinaeke et al., 2013).

SRMS were believed to be formed by combination of lecithin (30%) and oily vehicle or triglycerides (60 - 70%) (Schneeweis and Müller-Goymann, 2000). However, Friedrich and Müller-Goymann (2003), Umeyor et al. (2012 a), Momoh et al. (2012) Uronnachi et al. (2013) and Chime et al. (2013) demonstrated respectively that SRMS could be formed using 1:1, 2:1, 1:2, 3:1 and 2:3 ratios of lecithin and solid fats.

Most lipid based formulations today are available as normal release formulations, and this poses serious problems of patient noncompliance leading to poor disease management. This led to research into the field of controlled lipid based delivery system with huge success recorded by the discovery of SRMS. SRMS offer high solubilization capacities for different types of drugs in contrast with simple triglyceride systems (Friedrich and Muller-Goymann, 2003). However, the increase in water content causes a change in shape and size and finally a phase transformation from the reverse micellar solution into a lamellar liquid crystal. Solubilization of the drug in its free acid form results in almost spherical micelles, while solubilization of drug in its sodium salt form results in cylindrical micelles. The lamellar liquid crystals which form on contact with aqueous media can be used for sustained release, as the diffusion coefficient of the drug within the liquid crystals is smaller by factor 100 than that within an oily solution. The apparent diffusion coefficient of the drug depends on the thickness of the liquid crystalline interface which is also influenced when either the free acid or the salt is solubilized in the system (Muller-Goymann and Hamann, 1993).

Lipid-based formulations and SRMS based systems influence the absorption of active ingredients through different mechanisms to modify the release of active ingredients, improve drugs bioavailability, stimulate the lymphatic transport of active ingredients, and interact with enterocyte based transport (Fouad et al., 2011). SRMS-based formulations have been shown to enhance the bioavailability of drugs administered orally in addition to controlling the rate of drug release and have been used for once daily sustained release formulations (Umeyor et al., 2012a, Nnamani et al., 2010; Chime et al., 2013, Momoh et al., 2012, Uronnachi et al., 2013). The proven safety (GRAS) of lipid based carriers makes them attractive candidates for the formulation of pharmaceuticals (Attama and Nkemnele, 2005; Attama et al., 2009). Lipid formulations in general, provide increased drug solubilization for water - insoluble drugs. If the drug is dissolved in the lipid matrix (for example, SRMS), the drug absorption is observed to be better. Drug suspended in the lipid matrix has been shown to get absorbed better than the conventional solid dosage forms (Sarkar, 2002; Hou et al., 2003; Gao et al., 2004; You et al., 2005; Obitte et al., 2012; Brown et al., 2013). This could be due to the ease of wetting of the hydrophobic drug particles in the presence of lipid matrix. The presence of surfactant in the formulation may ease the wetting further. Also entrapment of drug in the micelles may be enhanced due to the presence of lipid matrix (Joshi and Shah, 2008). For poorly water soluble drug molecules, whose dissolution in water is likely the rate limiting step to overall oral absorption, the primary role of ingested lipids and their lipolytic products is to impact the drug dissolution step by forming different colloidal particles with bile components, which are able to maintain a larger quantity of hydrophobic drugs in solution via micellar solubilization (Porter, 2007). The primary mechanism of action which leads to improved bioavailability is usually avoidance or partial avoidance of slow dissolution process which limits the bioavailability of hydrophobic drugs from conventional solid dosage form (Pouton, 2000). Lipid-based excipients such as glycerides, fatty acids, ionic and non-ionic surfactants are known permeability enhancers (Aungst et al., 1996; Kuentz, 2012), which may be due to increased membrane fluidity. Permeability enhancement may also be achieved by the interaction of lipid based drug delivery system (LDDS) with efflux transporters. A well-known efflux transporter at the apical membrane of human intestine is the P-glycoprotein (P-gp). Excipients with

inhibiting effects on efflux pumps are found in the group of medium chain glycerides, polyethylene glycols, polysorbates and polyethoxylated castor oil or block copolymers of the type Pluronic. Surfactants have been shown to inhibit P-gp because of their amphiphilic structure (Bogman et al., 2003; Aungst et al., 1996; Pang et al., 2007; Kuentz, 2012).

MATERIALS USED FOR SRMS FORMULATION

Basically, SRMS are formed mainly using lecithin (for example, Phospholipon® 90G, a purified soybean lecithin with at least 90% (w/w) phosphatidylcholine, phospholipon® 90H, completely hydrogenated soybean lecithin with at least 90% (w/w) phosphatidylcholine) and a triglycride (softisan® 100, 133, 134, 138, 142, a hydroge-nated coco-glycerides and softisan® 154, a hydrogenated palm oil) at ratios 1:1, 1:2 and 2:1 (Friedrich and Müller-Goymann, 2003; Umeyor et al., 2012; Chime et al., 2013). Oils like soy oil, isopropyl myristate, coco nut oil and Moringa could be used in the right ratio in formulating SRMS (Schneeweis and Müller-Goyman, 2000; Chinaeke et al., 2013). Some waxes like bees wax, dika wax and fats from animals for example, goat fat may also be used in the formulation of SRMS (Momoh et al., 2012; Uronnachi et al., 2013).

FORMULATION OF SRMS

SRMS are normally formulated by fusion using a magnetic stirrer hot plate. A binary mixture (usually, 1:1, 1:2 and 2:1) of lecithin and triglycerides for example, softisan, respectively are normally used for SRMS preparation. The lipids are melted together and stirred with a Teflon coated magnet at a temperature of about 60 - 90°C, depending on the SRMS composition. The molten lipids are stirred until a transparent melt is obtained. Then, the homogeneous mixture will be stirred at room temperature until solidification (Friedrich and Müller-Goymann, 2003).

CHARACTERISATION OF SRMS

Differential scanning calorimetric analysis

Melting transitions and changes in heat capacity of the SRMS could be determined using a differential scanning calorimeter. About 1-5 mg of SRMS could be placed in the aluminum pan, hermetically sealed and the thermal behaviour determined in the range of 10-190°C at a heating rate of 5 or 10°C/min (Nnamani et al., 2010; Umeyor et al., 2012 a).

Depending on a variety of factors, lipids may exist in one crystalline form or it may be a mixture of several different crystal modifications. Lipids are polymorphic and transform systematically through a series of successive crystalline forms without change in chemical structure (O'Brien, 1998). The polymorphic transitions may be influenced by the addition of one or more substances (or other fats) to the SRMS (Attama et al., 2006). Consequently, certain properties necessary for improved performance as drug delivery bases may be influenced. It is important to know the thermal characteristics, crystal habit, texture, and appearance of a new lipid matrix when determining its suitability for use in certain food or pharmaceutical application. DSC is the most widely used thermo-analytic technique for studying lipids and their mixtures. It gives information about the temperatures and energy associated with their fusion and crystallization, phase behavior, polymorphic transformations, and data to estimate solid fat contents (Tan et al., 2000; Solís-Fuentes et al., 2005; Attama et al., 2006). Figure 1 shows the DSC thermograms of SRMS 100 (lecithin softisan 100 mixtures), (b) SRMS 142 (lecithin S142 mixtures, with 0, 30, 40, 50 and 60% (w/w) bottom to top) (Friedrich and Müller-Goymann, 2003). The crystallinity index (CI%) of the SRMS could be calculated using the Equation (1) (Freitas and Müller, 1999; Attama et al., 2006):

$$\mathrm{CI}(\%) = \frac{\mathrm{Enthalpy}_{\mathrm{SRMS}}\left(\frac{\mathrm{J}}{\mathrm{g}}\right)}{\mathrm{Enthalpy}_{\mathrm{PL}}\left(\frac{\mathrm{J}}{\mathrm{g}}\right)} 100\, f_{PL} \quad (1)$$

Where, $\mathrm{enthalpy}_{PL}$ is the fusion enthalpy of pure lipid for example, softisan, $\mathrm{enthalpy}_{SRMS}$ is the fusion enthalpy of the lipid matrix admixture (SRMS) and f_{PL} is a correction factor which takes into account the concentration of pure lipid. The SRMS with lower CI are preferable due to presence of spaces in the SRMS for drug localization therefore, leading to high payload of drug.

Wide angle X-ray diffraction analysis (WAXD)

WAXD analysis of lipid matrices gives information on the crystalline state of the matrices, as it reveals the dimensions of the short spacing of the unit cells. WAXD analysis gives further insights on the preferred orientation and crystallinity of the samples with emphasis on the orderliness of the crystal arrangement (preferred orientation) and the ratio of the crystalline properties to the non-crystalline properties (Attama et al., 2006). Distorted crystal arrangements of the individual lipids within the SRMS are desirable in order to create spaces for drug localization. This is a desirable quality in particulate drug delivery systems since it enhances the drug loading capacity of the lipids and also improves the overall drug encapsulation efficiency. Figure 2 shows the WAXD diffractograms of SRMS 142 consisting softisan 142 and 60% P90G (Friedrich and Müller-Goymann,

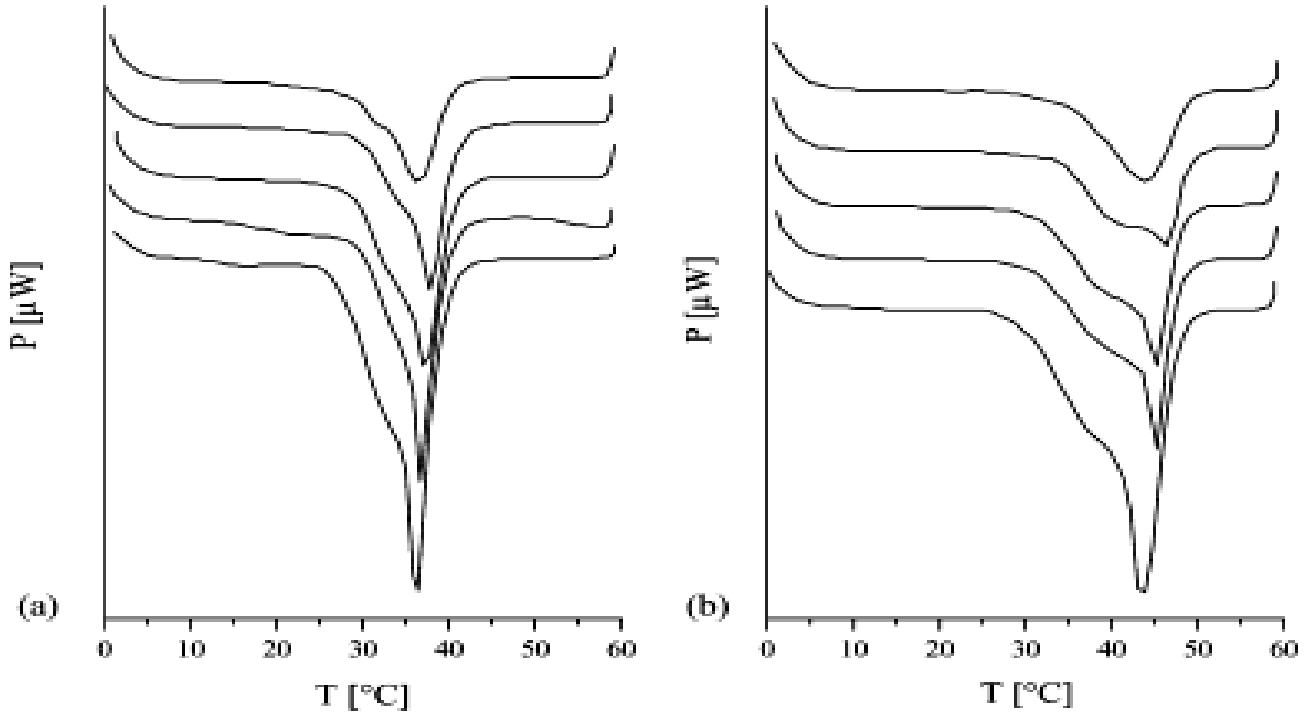


Figure 1. DCS thermograms of (a) SRMS 100 (lecithin S100 mixtures) and (b) SRMS 142 (lecithin S142 mixtures, with 0, 30, 40, 50 and 60% (w/w) bottom to top), S: softisan® (Friedrich and Müller-Goymann, 2003).

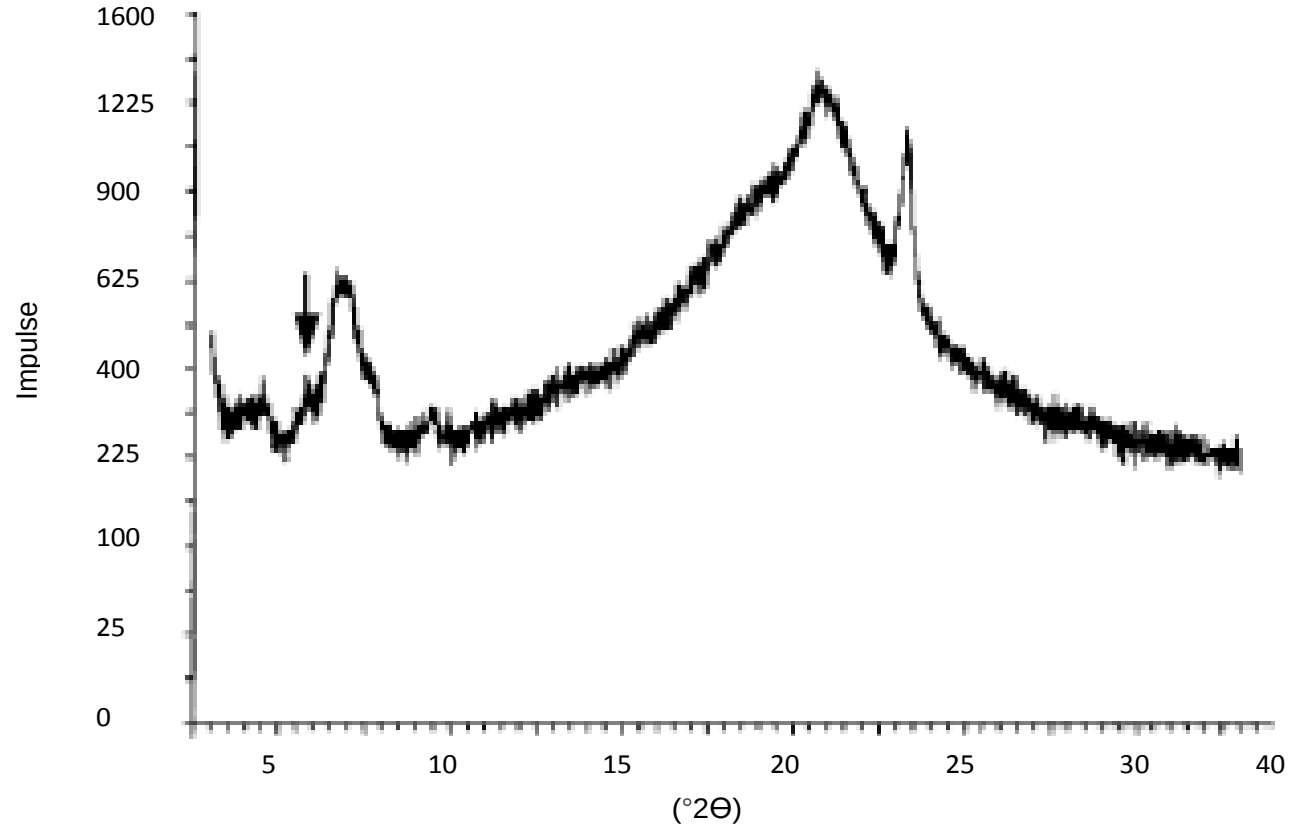


Figure 2. WAXD diffractograms of SRMS 142 consisting softisan 142 and 60% P90G (Friedrich and Müller-Goymann, 2003).

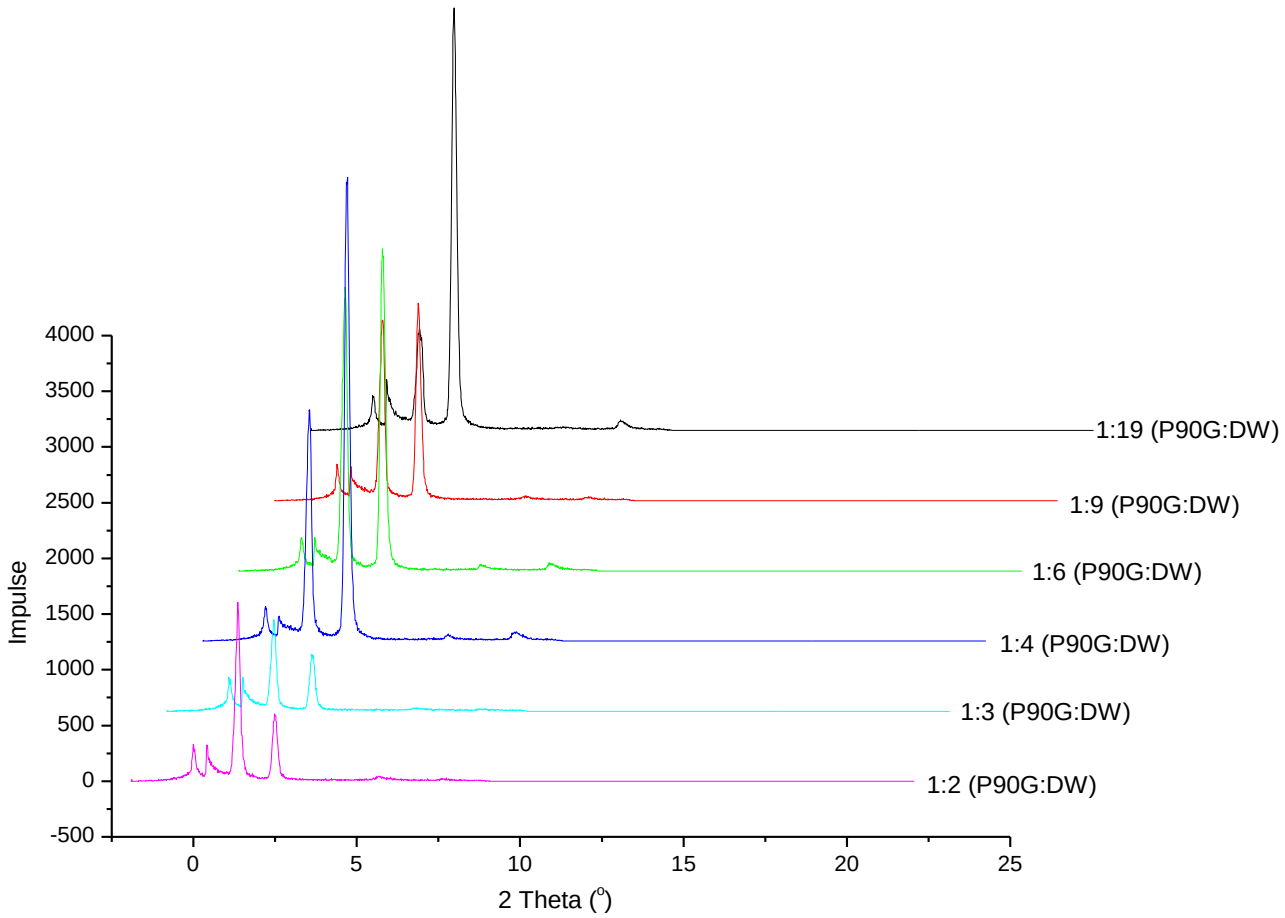


Figure 3. SAXD differactograms of different batches of lipid matrices formulated with different ratios of P90G and dika fats; DW - Dika wax (Chinaeke et al., 2013).

2003). The interlayer spacing in the SRMS may be calculated from the reflections using Bragg's equation (Equation 2):

$$n\lambda = 2d \sin\theta \quad (2)$$

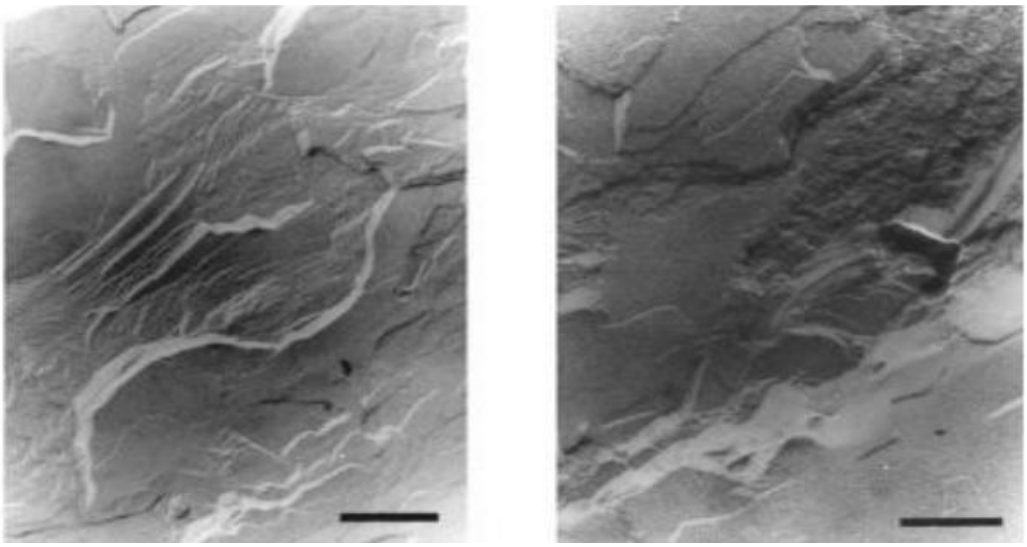
Where, λ is the wavelength of the incident X-ray beam, n is a positive integer which describes the order of the interference and θ is the scattering angle. The parameter *d*, otherwise called the interlayer spacing, is the separation between a particular set of planes of the crystal lattice structure (Attama et al., 2006).

Small angle X-ray diffraction analysis (SAXD)

SAXD is used to analyze the long range order of the crystalline structure of the lipid matrices. Interlayer spacing is the separation between a particular set of planes of the crystal lattice structure (Attama and Müller-Goymann, 2006). Many lipids are known to arrange themselves in layered structures with a repeat distance of few nanometers, thus giving rise to Bragg reflections in the small angle region. The repeat distances correspond to the thickness of the lipid layers (Attama et al., 2006). If the diffractograms produced by the SRMS are lamellar, it shows that the crystal arrangements of the individual lipids were disorganized. Lipid matrices with a certain degree of disorder are considered to be ideal for formulation of microparticulate lipid carriers due to their high active ingredient payload capacity (Attama et al., 2006; Attama and Muller-Goymann, 2007). Figure 3 shows the SAXD diffractograms of SRMS formulated with Phospholipon 90G and dika wax (Chinaeke et al., 2013).

Transmission electron microscopy (TEM) and Photon correlation spectroscopy

TEM could reveal typical features of the triglyceride lattice such as planar layers in the SRMS. Photon correlation spectroscopy (PCS) measurements may be performed with a Zetasizer. Figure 4 shows the TEM



(a) (b)

Figure 4. TEM micrographs of: (a) softisan 100 (bar= 303 nm), (b): SRMS 100 consisting of softisan 100 and 50% of P90G (bar = 182 nm) (Friedrich and Müller-Goymann, 2003).

micrographs of (a): softisan 100 (bar= 303 nm), (b): SRMS 100 consisting of softisan 100 and 50% of P90G (Friedrich and Müller-Goymann, 2003).

Determination of drug solubility in the SRMS

For investigations on drug solubilization in the SRMS, the drug is added to the melt of the SRMS and then solubilized under stirring at a temperature used in formulating the SRMS and the solubility limit of the drugs in the SRMS melt determined both macroscopically and microscopically. Also, solubility may correspond to the highest drug concentration at which a transparent melt is be obtained (Friedrich and Müller-Goymann, 2003; Galal et al., 2004).

TYPES OF SRMS-BASED DRUG DELIVERY SYSTEMS

Solid lipid nanoparticles (SLN)

Friedrich and Müller-Goymann (2003) studied the effect of lipid matrix composition, homogenization speed, surfactant composition on the properties of nanosuspensions. They found out that for production of SRMS-based nanosuspensions, a polysorbate 80/SRMS ratio of 1:5 is sufficient for particle size reduction. Homogenization on cold with resulting product temperatures far below the melting points (m.p.) of the systems causes broad particle size distributions. They also found that to achieve small nanoparticles with a narrow particle size distribution, homogenization on hot is not required. Instead, the suspension temperature has to be just near the m.p. for more flexibility of the solid lipids or for a partial melting. This could be controlled by varying the homogenization pressure at room temperature. A pressure of 1000 bar results in a temperature near the m.p. of SRMS100 (formed with lecithin and softisan 100), that of 1500 bar in a temperature near the SRMS142 (formed with lecithin and Softisn 154) m.p. They also concluded that an increase in transition temperature after production caused an increase in particle size because of particle agglomeration or growth (Friedrich and Müller-Goymann, 2003).

Solid lipid microparticles (SLM)

SLM based on SRMS have been developed recently in order to control the release of drugs. Nnamani et al. (2010) formulated SRMS142-based solid lipid microparticles of glibenclamide and the findings showed that SRMS142 generated an imperfect matrix with numerous spaces that accommodated glibenclamide in a concentration-dependent manner up to 60.58%. The blood glucose–lowering effect of the SLMs was higher than that of a commercial sample. The results also showed that P90Gylated-softisan® 142 conjugate, otherwise referred to as SRMS142, have numerous advantages: wetting, solubilization, drug stabilization, emulsification, and modified release. Umeyor et al. (2012) also formulated SRMS-based SLM for intramuscular administration of gentamicin. SRMS formulated with Phospholipon® 90G and softisan® 154 were used to prepare gentamicin-loaded SLMs and results revealed high encapsulation efficiency of about 92%

and sustained release of drug for once daily administration were obtained. Momoh et al. (2012) also produced ibuprofen-loaded SLMs based on SRMS and reported sustained release properties in addition to good *in vivo* anti-inflammatory properties. Uronnachi et al. (2013) also worked on the pharmacokinetics and biodistribution of zidovudine loaded in a solidified reverse micellar delivery system and also reported good *in vivo* bioavailability of zidovudine in addition to controlled release properties. Chime et al. (2012 and 2013 a) worked on indomethacin-loaded SLMs-based on SRMS 154 and diclofenac potassium-loaded SLMs based on SRMS 154. The results showed high encapsulation efficiency of up to 90%, good loading capacity of SRMS, gastro protective potentials, enhanced *in vivo* bioavailability and good sustained release properties for once daily administration (Chime et al., 2012; 2013 a).

SRMS-based tablets

Solid lipid tablets based on SRMS have recently been produced by molding (Umeyor et al., 2012 b; Chime et al., 2013 b). In this method, softisan® 154 and lecithin were utilized. The drug was dissolved or dispersed in the lipid matrix and tablets were produced by molding using tablet mold. Gentamicin oral tablets were been produced by this method using lipid matrix based on solidified reverse micellar solutions consisting of phospholipid and triglycerides (Umeyor et al., 2012b) and the results show that SRMS-based tablets containing gentamicin were successfully prepared by fusion melt-solidification method which is simple, reproducible, scalable and cheap (Umeyor et al., 2012b) and tablets exhibited sustained release properties. SRMS-based tablets could be an alternative to the conventional parenteral dosage form of gentamicin. Some non-steroidal anti-inflammatory drugs (NSAIDs) based on SRMS have also been produced (Chime et al., 2013b). Diclofenac potassium and indomethacin solid lipid tablets have been produced and results showed that the tablets had sustained release properties for once daily administration in addition to ulcer inhibition potentials. Diclofenac potassium tablets based on SRMS showed good hardness and friability profiles, sustained release properties and possessed good anti-inflammatory and anti-nociceptive/analgesic effects. The formulations also exhibited good gastro-protective properties, as it inhibited the ulcerogenic potentials of diclofenac potassium by about 85% (Chime et al., 2013b). The *in vitro* release profile of diclofenac potassium tablet based on SRMS was comparable to the release profile of a market brand, coated diclofenac potassium. However, formulations showed higher sustained drug release. Advantages of lipid based tablets include: low cost of ingredients, low cost of technologies (equipment and labour requirement for the production of lipid dosage forms are minimal, unlike the conventional tablets) and improved oral bioavailability and reduced side effects of drugs (Chime et al., 2013b).

Suppositories

Controlled release of SRMS-based suppositories containing metoclopramide-HCl was formulated by Schneeweis and Müller-Goymann (2000). The SRMS consisted of 70% Witepsol W35 and 30% (w:w) lecithin. A 1% (w/w) metoclopramide-HCl (MCP) was solubilizied in the SRMS. After melting and on contact with water or any physicological aqueous media the SRMS exihibits an application induced transformation into a semisolid system of liquid crystalline microstructure. Due to a low coefficient of diffusion in this mesophase a controlled release of the drug may be possible. The release profiles of the *in vitro* experiments showed zero order kinetics and a sustained release of the SRMS-suppositories (SRMS-supp.) in comparison with commercial suppositories (Schneeweis and Müller-Goymann, 2000).

Advantages of SRMS based carriers

The advantages of SRMS include:

1) Generally, SRMS offers controlled release of drug, thereby enhancing patients' compliance and leading to better disease management and reduced toxicity.
2) The formulations can be tailored to meet a wide range of product development.
3) Feasibility of various administration routes.
4) Enhanced *in vivo* bioavailability.
5) Enhanced physical stability.
6) High carrier capacity.
7) Ease of formulation and scale up.
8) Protects loaded liable drugs against drug degradation.

Drug release from reverse micelles

When the reverse micellar delivery system comes into contact with an external fluid of the environment such as water or other biological fluid, a burst or gradual release of the ionic amphiphiles may occur. A concurrent release of the additional ionic amphiphiles and the agent of interest follow. The ionic amphiphiles released dissolve in the aqueous fluid media forming ionic monomers. Upon release of agent(s) of interest, depending on the prevailing pH of the fluid environment and the pKa of the chemical compound, ionised molecules are formed. These ions carry permanent charges opposite to that of the polar region of the ionic amphiphiles. The oppositely charged polar groups of the ionised agents of interest and amphiphiles attract each other. At some point when sufficient ionic monomers of the amphiphile are attracted

to the charged species in the aqueous fluid, aggregation and reverse micelle formation occurs. This point is believed to be the critical reverse micelle concentration (CMC) (Mac-Gregor and Markham, 2010). These reverse micelles, in the aqueous fluid environment, eventually form colloidal microemulsions. In the human gastro-intesyinal tract (GIT), such reverse micelles are in direct contact with the lipophilic membranes of the absorbing mucosal cells. Due to the inherent lipophilicity of the outer surface of the reverse-micelles, they partition rapidly into these membranes, thereby facilitating absorption. Once the reverse micelles partition into the lipophilic membrane, the concentration of the amphiphilic molecule component of the reverse micelles diminish below the CMC. The reverse micelles undergo disaggregation and release the polar agent within their core. The kinetics of transport and transmembrane release of these agents may be essentially zero order or near about zero order (Mac-Gregor and Markham, 2010).

CONCLUSION

SRMS could safely deliver and sustain the release of drugs and has advantages over polymeric delivery systems. It is easy to formulate and scale up and is produced using completely biodegradable lipids. The materials required for the production of SRMS are relatively cheap and available and could enhance patients' compliance by reducing side effects and preventing repeated dosing. SRMS could also be used in formulating liquid as well as semisolid and solid dosage forms.

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Review

Bioenergy production and food security in Africa

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Food and energy insecurities are the two greatest problems in Africa. Per capita energy consumption in Africa is less than 10% of that of United States of America while 18 out of 23 countries where starvation and malnutrition are most severe in the world are in Africa. Although various African governments have been making efforts to boast agricultural productivity, crop yields remain very low. Most governments do not even have accurate statistics on the number, location and types of crops produced by smallholder farmers that produce more than 80% of foods in Africa. This makes it very difficult to plan and implement any government support to the farmers. Sub-Saharan African countries have very high potential for production of different forms of bioenergy because the climatic conditions favour production of many energy crops. The big question has always been whether to produce bioenergy from food crops, especially in Africa with high acute food shortages. Large scale production of bioenergy may lead to competition with food crops for land, labour and other agricultural inputs. However, data from various sources indicate that Africa has abundant and underutilized arable land which can be effectively used for mass production of energy crops. Furthermore, shortage of labour cannot be a problem given the present very high rate of unemployment in most African countries. The benefits of bioenergy production in Africa outweigh the possible adverse effects on food security. Bioenergy production will create demand for, and stabilize the prices for crops, thereby increasing the earning of the farmers. This will in turn, facilitate industrialization in other sectors of economy through provision of affordable, renewable and clean energy. In order to minimize possible negative effects of bioenergy production on food security, land allocation for energy crop production can be regulated. Energy security cannot be separated from food security and the two should be seen as complimentary rather than as competitors.

Key words: Bioenergy production, food security, energy.

INTRODUCTION

In view of the non-renewable nature of fossil fuels and the various environmental problems associated with drilling, distribution and use of fossil fuels, a lot of attention has been focused on renewable energies. Among the various types of renewable energies, bio-energies have very high potentials because the major raw materials can be produced in most ecological zones at will, at all time and in desired quantities. They can be processed into gaseous, liquid and solid forms, thus making them suitable for various industrial, domestic and transportation applications. They are also environmentally friendly in that they are biodegradable and thus do not

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Abbreviations: IEA, International Energy Agency; **AGRA,** Alliance for a Green Revolution in Africa; **FAO,** Food and Agriculture Organisation; **GM,** genetically modified; **GDP,** gross domestic product.

constitute major soil or water pollutants while most of them are either carbon neutral or carbon negative. However, there has been a lot of debate on the effects of large scale bioenergy production on food security, especially in Africa where hunger and malnutrition have persisted for decades (Cotula et al., 2008; Jumbe et al., 2009). This paper highlights the current situation of energy and food production in Africa, discusses the interrelationship between energy security and food security, the potentials of bioenergy production in Africa, as well as the possible negative and positive effects of bioenergy production on food security in Africa. A vertical integrated bio-energy production system which can be used to achieve both energy and food securities in rural African communities and thus reduce poverty is proposed.

ENERGY SITUATION IN AFRICA

Africa is a very energy poor continent. Electrification rate in Sub-Sahara Africa is only 30.5% while only 14.2% of those living in rural areas are connected to power grid (WEO, 2011). Furthermore, the International Energy Agency (IEA) estimated that the per capita power consumption in Africa is only 7.7 kWh/capita which is far less than 14.421 kWh/capita in Latin America or 87.216 kWh/capita in the United States of America. Furthermore, only four countries in Africa (Algeria, Nigeria, Libya, and South Africa) have significant amount of fossil fuels (oil, gas or coal). Nigeria, Libya and Algeria oil reserves are estimated to be 37.1×10^9 bbl, 48.5×10^9 bbl and 12.12×10^9 bbl; and at the present rate of production, they are expected to last for only 41, 76 and 22 years, respectively (www.opec.org).

Africa has remained the poorest continent with estimated 265 million malnourished and 273 million people living on less than US$1 per day (APPPB, 2010). According to FAO (2012), 18 out of 23 countries where hunger is most severe in the world are in Africa. Energy availability and costs have an overwhelming influence on national development. No nation can develop without reliable and affordable energy sources. Energy availability determines the type of industries, and the cost of energy has significant effects on the cost of production and distribution of goods and thus the global competetiveness of companies. Energy availability affects not only industrial and agricultural development but also education and training (teaching and research). It is arguable that the lack of access to reliable and affordable energy source is a major problem limiting industrial development in Africa. Many teaching and research literature materials are now in the internet but cannot be accessed by many Africans because of lack of electricity. Many research projects cannot be implemented in many African communities because of lack of reliable and stable power supply. Energy supply also affects production and distribution of many social amenities such as water, and health care delivery. Thus, energy security is pivotal to African development.

The big question is therefore how Africa can achieve energy security at both national and local levels. Important aspects of energy security include availability, reliability and affordability. However, in quest to achieving energy security, the environmental and social implications of various energy sources must also be considered. It is therefore necessary to develop renewable energy alternatives as a means of diversifying their energy sources and export commodities, and reducing environmental pollution and degradation associated with drilling, distribution and use of fossil fuels.

FOOD SECURITY IN AFRICA

Food security simply means a condition where people have guaranteed access to safe and nutritionally balanced food in appropriate quantities over time by production, purchase or both. The four main components of food security include physical availability of food, food access (purchasing power/affordability and social/religious liberty to purchase and eat), food use (knowledge and liberty to eat appropriate quantity and quality of food) and stability of food supply over time. Food security can be at individual, family, group or national level. The ultimate goal of food security is to eliminate hunger and malnutrition.

Food availability in Africa

Starvation has remained endemic in Africa in spite of all the previous “green revolutions” under different names aimed at increasing agricultural productivity in Africa. It is clear that such efforts have not yielded the expected results. One of the major reasons for the shortage of foods in Africa is the very low crop yields. Data from various sources confirm that average yields of various crops in Africa are less than half of the world average and in some crops, less than 20% of the yields in some developed countries (Chaurin et al., 2012; Langyintuo, 2013). In all the agro-ecological zones in Africa, there is still a huge gap between the potential and actual yields of crops (Pingali et al., 2010). Between 1961 and 2009, average cereal yields increased only from about 800 kg/h to about 1,500 kg/h (88% increase) in Africa. However, during the same period, the yields increased from 1250 kg/h to 3500 kg/h (180% increase) in Asia, and from 1,400 kg/h to 4000 kg/h (186% increase) in South America (Jama et al., 2013). By 1961, for example, yields of grains in the United States was already 2 ton/h but more than 50 years later, average yields of grains in Africa is only about 1.5 ton/h, in spite of all the apparent efforts being made by the Governments and different organizations to boast crop production in Africa. Even the

slight increase in agriculture production has been mainly due to expansion of cultivated land rather than increase in yields. Yet in some areas, expansion of cultivated land is restricted by land ownership and land tenure systems. The current climate changes will even reduce agricultural productivity in Africa where most farms are rain-fed.

Jane Karuku, the president of Alliance for a Green Revolution in Africa (AGRA) in the Food and Agriculture Organisation (FAO) reported on the state of Agriculture in Africa (AGRA, 2013) enumerating what smallholder farmers in Africa need to boast Agricultural productivity. These include supportive policies, access to better seeds, fertilizers, markets, finance, extension support, effective national research systems and better rural infrastructure. Almost all these have been addressed in one way or the other by successive Governments in Africa without desired results.

Soil fertility in Africa has been continuously declining due to poor management and yet fertilizers are either too expensive or not available to farmers. Currently, for most African countries, it is more cost effective to import fertilizers than to produce locally, until demand for fertilizer increases to support large-scale production (World Bank, 2006). Thus, more than 90% of fertilizers used in Africa are imported according to World Bank report (2012). Consequently, the rate of fertilizer application in Africa is less than 20% of what obtains in the United States of America or even most Asian countries (Jama et al., 2013). Some African countries have re-introduced fertilizer subsidies but this is not effective in most countries because of corruption, and long bureaucracies in distribution so that the farmers do not get them on time.

Most farmers in Africa still use farmer-saved seeds (seeds from the previous harvest) in spite of the very low yields from such seeds (Mabaya et al., 2013). There are many National and International Research Institutes engaged in development of improved seeds and planting materials in Africa but their capacity is still very limited. Besides, according to Parliamentary Office of Science and Technology (2004), Africa has the world's lowest capacity in personnel involved in agricultural research with only 70 researchers per million inhabitants. Seeds are still relatively expensive for the farmers and most of them still do not know the importance of using improved seeds and the fact that the yields of these seeds decrease with their filial generations. Extension service is none existence in many areas to educate and guide the smallholder farmers to maximize their productivities. There is still a very big controversy in the use of genetically modified (GM) crops. As at 2012, GM crops are grown in 20 developing countries and 8 industrialized countries (Clive, 2012), but only four African countries (Burkina Faso, Egypt, Sudan and South Africa) have started cultivation of GM crops. The total area of GM crops in Africa is 2,801,000 h but South Africa alone has 2,300,000 h of GM crops (82.1%). Most of the farms are rain-fed while most of the farmers lack the essential basic knowhow in agriculture.

Unlike many countries in Asia, Europe and Americas where farmers of some crops are guaranteed prices, farmers in Africa are left at the mercies of the rapid and unpredictable fluctuations in the prices of farm produce. There are both seasonal and annual fluctuations in the prices of produce in some rural areas. Post and on-farm losses and seasonal glut reduce farmers' incentives. In some cases, very high percentages of some crops are not even harvested because of lack of market and storage facilities. According to World Bank report (2010), 11 to 19% of maize is lost in Africa. The percentage losses are even higher for other crops such as cassava, fruits and vegetables. There are only very few or no functional silos and other storage facilities for storage and gradual release of the produce to the market, which would prevent market fluctuations. Furthermore, low cost and subsidized food imports weaken African agricultural markets as many countries subsidize and encourage food production for national security and the surplus are exported to Africa at very low prices, thus unduly completing with domestic production. It is also very important to note that poor distribution network and poor storage facilities are major factors limiting physical food availability in many places in Africa. Thus, while foods are rotting away in some places, the same food items are totally lacking in other places or are too expensive because of the high transportation costs.

Another major problem facing agriculture in Africa is the poor access to credit facilities. Although agriculture represents as much as 40% of gross domestic product (GDP) in some African countries, only 0.25% of bank lending goes to smallholder farmers (AGRA, 2013). Commercial banks are unwilling to give loans to farmers because of the high risks due to uncertainties in weather conditions as well as the non- guaranteed market and prices. There are some Agriculture banks in some countries mandated to give low-interest loans to farmers but these are not enough to meet the huge demand for credit. Besides, these loans are hardly given to the smallholder farmers that make up more than 80% of farmers in Africa because they are not able to meet up with the demands (collateral, among others) while most farmers are not even aware of the loans, and do not know what to do to access the loans.

The complexity of land tenure system in Africa is also a major problem limiting agricultural productivity. Land tenure system varies among African countries and even among the ethnic groups and communities within each country. Both customary (traditional) systems as well as state (statutory) systems operate depending on the place. These systems make it difficult to establish large farms, and in some cases, lands are leased only for short periods of time, thereby making long term investments difficult.

In the face of all these problems, Government expenditure on Agriculture is still very low. The Maputo Declaration

by the African heads of state in 2003 to spend at least 10% of their national budgets on Agriculture is not being implemented (Doward and Chirwa, 2009). In 2007, AU/NEPAD survey showed that 50% of African countries spend less than 5% of their national budget on Agriculture. Furthermore, in many African countries, less than 1% of their Agricultural GDP was spent on Agricultural R and D between 2000 and 2008 (Oluoch-Kosura and Sikei, 2013). We tend to believe that we know the problems facing agriculture in Africa, we express our willingness to tackle them yet we are not succeeding. Does this imply that the technologies and knowhow on agricultural production are so complex that Africans cannot learn and adapt? There is definitely a need for radical change in our mind set and approach to boast agricultural productivity in Africa.

HOW TO INCREASE AGRICULTURAL PRODUCTIVITY

Increasing agricultural productivity is important not just for food security but because agricultural sector accounts for a large share of gross domestic product in most African countries, and poverty is concentrated in rural areas where majority are employed in agricultural sector. Furthermore, boasting agricultural productivity is fundamental to development of bio-industries that use agricultural produce as the raw materials. The average agriculture growth rate in Sub-Saharan Africa (excluding South Africa) is estimated to be 4.0% but varies widely among the countries. It is very low in some countries such as Kenya (1.6%) and high in others such as Liberia (9%) and Mozambique (8.7%) (Keita, 2013).

Increasing agricultural productivity in Africa requires a lot in terms of infrastructural development (energy, roads, water and irrigation systems), research and development (development of improved varieties, and efficient agronomic practices) and policy changes aimed at attracting investment in the sector. Farmers must have access to finance, agricultural inputs (fertilizers, good planting materials, and agrochemicals), stable market with minimal transaction costs, and efficient extension service. Most reports on ways of increasing agricultural productivity recommend that Governments must investment on infrastructures (roads, water, power) as well as make favorable policies and interventions such as interest-free or low-interest loans, and subsidies on inputs such as fertilizers and seeds. However, these types of government interventions are very expensive to sustain over a long period of time. Government investment on infrastructure is fundamental to socio-economic development and must be pursued vigorously. However, agricultural subsidies must be seen as a take-off measure to boast agricultural productivity. In most cases, the government interventions are not effective because of poor implementation, corruption, lack of awareness on the part of the intended beneficiaries, and lack of accurate statistics.

The best way to sustain high agricultural productivity is to make farming lucrative even without government interventions. Farming is not a humanitarian venture and must be seen as an integral part of business system. As a business, profit is the most important incentive for investment in agriculture. The efforts of Governments to promote agriculture to achieve national food security must go hand in hand with pure agriculture business by private investors and all the policies and interventions by Governments must not negatively affect private investors in Agriculture. In other words, commercial farmers must be encouraged and protected by government policies. Investment is product demand driven and sustained only by profits. Demand is different from need. The need must be backed with purchasing power. Thus, it can be argued that although most Africans are hungry, there is low demand for food, thereby making foods too cheap to attract investment. In other words, individuals do not have the purchasing power and there is very low level of agro-processing and value addition, resulting in low demand for agriculture produce. The low demand can in turn be blamed for low agricultural productivity. Thus, the small-holder farmers must be encouraged to change from subsistence farming to commercial farming. Because of low profitability in agriculture, many young ones are not interested in farming and even many of those presently engaged in farming are taking it as the last option. The unemployment rate is high, yet most of the unemployed who even have access to land would rather remain idle than farm. Farming must be seen as a lucrative business. To make farming lucrative, the demand must be increased by increasing the purchasing power of individuals, promoting food processing and value addition and establishing ready and stable market for the products which will aid in stabilizing the prices. Primary products are bulky, cheap, perishable and most vulnerable to price fluctuations. Agricultural productivity is highly correlated with proximity to market. Dorosh et al. (2010) reported that total crop production relative to potential production is 45% for areas close to urban cities (markets) but as low as 5% in remote areas in many countries. Foods are produced in rural areas with low demand for the products and the costs of transportation to urban areas coupled with the high transaction costs due to long marketing chains significantly reduce the farmers' profits. Furthermore, reduction of risks and vulnerability is very necessary to increase agricultural productivity. Guaranteed market and prices is needed to absorb surpluses, sustain producer incentives and thus encourage investment. Although putting in place market-supporting institutions and policies can facilitate efficient marketing of products, guaranteed demand is the best option.

HOW TO ACHIEVE FOOD SECURITY

Increasing food production does not mean achieving food security. Based on the definition of food security, a poor

person can never be food secured since even if foods are available for sale, he cannot purchase. In other words, hunger (food insecurity at individual level) results from poverty rather than from absolute lack of food. Thus, the problem of poverty must be solved to achieve food security at individual levels. Even at national levels, foods are not available because the countries do not have money to import foods. Agriculture makes up high percentage of most African countries' GDP and yet people are starving and malnourished. Kroma (2013) reported that between 2003 and 2005, agriculture contributed 3.1, 23.1, and 45.8% of GDP for South Africa, Mozambique and Tanzania, respectively. In another report, although share of agriculture in GDP in Sub-Saharan Africa (excluding South Africa) was estimated to be 22.7%, it is very high in some countries such as Sierra Leone (57.6%), and Liberia (53.1%) (Keita, 2013).

Most of the foods are produced in rural areas, yet most of the poor and hungry people are living in rural areas. Agriculture contributes about 53% of total employment in developing countries (Meijerink and Rosa, 2007). In the case of Africa, World Bank report shows that about 80% of rural population in Sub-Saharan African is employed in Agriculture (World Bank, 2012). The figure however varies among the countries. For example, in 2011, agri-culture provided employment for 48.131% of people living in rural areas in Ghana but as high as 84.421% in Uganda (Keita, 2013). Some of them are poor because they produce and cannot sell (there is artificial surplus), or can only sell at very low prices because those who need the food cannot afford to buy at high prices. It can therefore be argued that most of the people living in African rural communities are poor because agriculture is not profitable. Improving the agricultural productivity and profitability is therefore very important as a means of poverty alleviation, increasing their purchasing power and thus their food security levels. Any attempt to increase food production must ensure that the smallholder farmers are protected, and that the measures increase employ-ment opportunities and earnings of the rural communities. Currently, the costs of energy, transport-tation, seeds, fertilizers and other agricultural inputs, and thus the cost of food production are higher in Africa than in other regions of the world. Government subsidies and intervenetions to bring down these costs are expensive, ineffective and non-sustainable. Economy of scale will bring down prices of seeds and fertilizers if their demands increase as a result of increased production. Thus, government can concentrate in provision of energy, construction of roads to reduce transportation costs and provision of other basic infrastructures.

Sustaining high agricultural productivity cannot be achieved if there is no market for the products. The market can only be guaranteed if people have the purchasing power or if there is industrial or export demand for the products. Bioenergy production is one way of creating ready and stable markets for the crops, and creating more jobs for the people which again will increase their purchasing power and thus their food security levels.

BIOENERGY PRODUCTION IN AFRICA

Bioenergy is any form of energy from biomass materials. They include the solid primary energy (fire wood, charcoal and combustible wastes), gasses (biogas and bio-hydrogen), and liquid fuels (bioethanol and biodiesel). Although fire wood, and charcoal are currently the major source of energy in most African communities (IEA, 2009; Cotula et al., 2008) they are very inefficient and rely on cutting and drying of trees and thus environmentally unfriendly. They are linked to various forms of respiratory problems due to constant inhalation of carbon dioxide, carbon monoxide and other combustion gasses. There are many advantages of developing refined bioenergy industries in Africa. It will help to diversify energy sources and achieve energy security in rural areas. In some remote rural communities, it is cheaper and more feasible to produce bioenergy than to connect to the national power grid. Bio-hydrogen is the cleanest of all the bio-energies since its combustion does not result in any pollution. However, bio-hydrogen production is still very inefficient and expensive and a lot of R and D are still required before commercial bio-hydrogen production (Ogbonna and Tanaka, 2000). Biogas is produced by anaerobic digestion of organic wastes. The technology is very simple and digesters are cheap and easy to maintain. Most of the biogas projects in Africa are designed as waste valorization (conversion of wastes into valuable products) projects aimed at simultaneous treatment of the wastes and production of biogas as well as organic manure. Although there are many small scale biogas production facilities in Africa, most were established as either government or Non-govermental Organisations (NGO) projects rather than as pure commercial ventures. Thus, most of them do not have well-trained personnel to manage them, and are usually abandoned if there is no follow up. Because of the difficulty and costs of collecting wastes from distance places, biogas projects are usually very small and community based. The raw materials are almost free but for the cost of collection and transportation to production plants. They can be house hold, community, animal farm or biomass processing industrial based. Since wastes are the raw material, biogas production does not have any negative effect on food security and therefore highly desirable. Bioethanol and biodiesel have very high potentials in Africa. Bioethanol can be produced from any carbohydrate - containing biomass materials such as sugar crops, starchy materials, and lignocellulosic materials (Ogbonna et al., 2001; Ogbonna, 2004; Ogbonna, 2013). Although most of the current commercial bioethanol industries use food crops such as sugar cane, sugar beet, corn, and cassava, emphasis are shifting to non-food crops and bio-products

from crops such as cobs, molasses, bagasse, and fibers. However, the technologies for production of bioethanol from lingo-cellulosic materials are still inefficient and expensive. Currently, South Africa produces 65% of all the bio-ethanol in Africa and this is followed by only 5% produced by Egypt and Nigeria (DFID, 2007). Biodiesels can be produced by esterification of oils of animal, plant or microbial origin. Most of the biodiesels are currently produced from plant oils (jatropha, sunflower, rapeseed, and palm oil) but a lot of projects are on-going for development of commercial processes for biodiesel production by microalgae. Although there are some estimates and reports on some bioenergy production projects in Africa, there are limited reliable data on bio-energy production and utilization in Africa. There are many proposed projects but a lot of them end up at the planning stage or are never completed. Some of such projects were listed by Amigun et al. (2008).

POTENTIALS OF BIOENERGY IN AFRICA

The potential of bioenergy production in Sub-Saharan Africa is very high because almost all the ecological zones can support different types of energy crops. One of the major questions that must be answered why thinking of large scale production of bio-energy is the availability of land. Production of energy crops definitely means competition for land between energy crop and food crop production. Some have argued that large scale production of energy crop will significantly limit the land available for food crop production. On the other hand, there are a lot of reports, indicating that availability of land is not a problem for large scale bioenergy crop production in Africa. Fischer et al. (2002) estimated that there are 807 million of cultivatable lands in Africa and only 197 - 227 million were under cultivation by 1996. It has also been reported that about 70% of cultivatable land in Africa is not cultivated (Alexandratos and Bruinisma, 2012). They estimated that about 183 million hectares of land are under cultivation in Sub-Saharan Africa and about 452 million hectares of suitable land are not being cultivated. Gnansounou et al. (2007) also noted that in some countries in Africa, only 6% of cultivatable land is under cultivation, and UEMO (2008) concluded that availability of land is not a problem in most countries in Africa. According to FAO, area of land under annual or perennial crops increased by more than 50% in many African countries between 1990 and 2011. However, Alexandratos and Bruinisma (2012) predict only an increase of 50 million h in land under cultivation by 2050. To ensure that land availability does not affect food crop production, specified areas of land can be allocated to energy crop production, the exact area depending on the region. For example, ethanol yield from cassava flour is about 0.34 g/g of flour, which is equal to 0.11 g/g-fresh tuber (Ogbonna and Okoli, 2013). Assuming a density of 0.789 g/ml, ethanol yield is then 0.1394 mL/g-tuber. Thus, one ton of cassava will yield 139.4 L of anhydrous ethanol. With an average cassava tuber yield of 12 ton/hectare, one hectare of land will yield 1,672.8 L of anhydrous ethanol. Similarly, with average corn yield of about 1.979 ton/h in Africa, one hectare of land will yield 904.8L of corn ethanol (Ogbonna and Okolo, 2009) but using the world average corn yield of 5.158 ton/h (FAOSTAT, 2013), one hectare of land will yield 2,358.2 L of corn ethanol. Thus, using only ten percentage of uncultivated land in Africa (45.2 million hectares), Africa can produce 1.06×10^{11} L of ethanol per annum, which is higher than the current total fuel ethanol production in the world. In the same way, production of biodiesel using jatropha, for example, is 1,892 L/h (Chisiti, 2007) and 8.55×10^{10} L of biodiesel can be produced from ten percentage of uncultivated land in Africa per year.

It can therefore be argued that with proper planning, significant amount of bioenergy can be produced in Africa without significant effect on land available for food crop production. Furthermore, increasing crop yields will drastically reduce the area of land needed to produce the fixed amount of bio-energies. Average crop yields in Africa are very low because crop production is still characterized by low input/rain-fed crop system. It has been estimated that the yields of cereals in such a system is only about 13.2% of the potential yield in high input/rain-fed systems, and 8.3% of the potential yields in high input irrigated systems (Dorosh et al., 2010). With improved varieties, fertilizer applications and good agronomic practices, we can achieve more than double increase in our crop yields and this has been demonstrated in Millennium villages (Sanchez, 2010), thereby drastically reducing the required area of lands to produce a given volume of bioenergy. Furthermore, labour is generally cheap and available, considering the very high rate of unemployment in Africa.

However, even with availability of land and labour, it is still very important to consider the economic feasibility of large scale production of bio-energy in Africa. Amigun et al. (2008) noted that the economy of bioenergy depends on the cost of raw materials, the biofuel production costs, the cost of corresponding fossil fuel, and the strategic benefit of substituting imported fuel with locally produced biofuels. The cost of raw material has an overwhelming effect on the final cost of production (Ogbonna and Okoli, 2013). There can be more than 50% seasonal variation in the cost of raw materials (crops) used for bioenergy production. The conversion cost remains very low and depends on the technology and scale of production. Because of the problem of equipment maintenance and power supply, process automation should be reduced to minimum in rural communities in Africa. This also has an advantage of providing employment opportunities though the overall efficiency may be lower. The costs of corresponding fossil fuels vary greatly. In some countries such as Nigeria, there is Government subsidy on fossil fuels but in other countries, fossil fuels are very expensive,

making bioenergy industries more profitable.

Most bio-energies are insensitive to economies of scale because scale dependent variables such as labour costs represent only very small percentage of the production costs. On the other hand, raw materials which represent more than70% of the final production cost tend to be more expensive as the scale increases because of the increased costs of transporting raw materials from distance farms. Sourcing raw materials from far away farms will increase the cost of transportation, thus, nullifying the advantage of economy of scale. Thus there is often diseconomy of scale in bioenergy production. It is also difficult to find funds for large scale projects because of the huge capital investment costs required. Credit facileties are not easily available, and the interest rates are often too high for farming business. Thus, there is an optimum scale for bio-energy production and the optimum scale depends on the size of the feeding farms and their yields. In most places, small to medium scale bioenergy production facilities may prove more useful, even though the conversion costs may be higher.

Aside from economic viability, energy security issues, environmental and social issues must be considered while making decisions on establishment of bioenergy industries. Many developed countries support bioenergy industries either in form of tax exceptions or subsidies. For example, in France, tax exceptions for biofuels are 0.35 EUR/l for biodiesel and 0.50 EUR/l for bioethanol while subsidy on bio-ethanol is US $0.51/gal in the United States of America (ESMAP, 2005). Unfortunately, some of these government supports are difficult to implement in most African countries because of lack of reliable statistics and implementation capacity/frame work. Nevertheless, supporting such ventures can be an indirect way of genuine support for unemployed youths.

EFFECT OF BIOENERGY PRODUCTION ON FOOD SECURITY

It is very important to understand and appreciate the interrelationship between food security and energy security. Energy security and food security are highly interwoven and inseparable. Energy is needed for production and distribution of food and it is extremely difficult to achieve food security without energy security. As discussed, Africa is energy poor and improving energy production in the continent is fundamental to achieving food security. In Nigeria, for example, any slight increase in the pump prices of fuel has always resulted in sharp increases in the prices of foods and other commodities.

Large scale bio-energy production can have both positive and negative effects on food security. It is feared that if food crops are used for large scale bio-energy production, the increase in demand for the food crops will push the prices up and some school of thought have been insisting that the recent increase in the price of corn in the world market is due to large scale corn ethanol production in the United States of America. However, others have argued that the increase in the grain prices is due to changes in weather conditions rather than bio-energy production. Large scale bio-energy production can also lead to competition for land, labour and other agricultural inputs and may even deprive smallholder farmers their land due to either excessive increase in the prices of lands or government displacing small scale farmers and allocating the land to large scale farmers. These apparent negative effects of large scale bioenergy production on food security cannot be overlooked but can be significantly reduced by proper planning and policies to minimize competition between food and energy production. Land use can be regulated whereby specified area of land is allocated to bioenergy production and the land allocation reviewed from time to time, depending on demands. Small scale farmers should never be deprived of their land but encouraged to engage in a more lucrative farming. They should be encouraged to form cooperative societies for proper coordination and easy access to funds, extension services and various government interventions. Bioenergy companies can organize the smallholder farmers, contract them to produce for them and support them technically and financially.

On the other hand, there are some possible positive effects of bioenergy production on food security. Bioethanol and bio-diesel industries will lead to development of the agricultural sector by creating a stable demand, attracting investment, and thus stimulate R and D that will eventually lead to increases in yields and income for the farmers. Currently, production of food crops fluctuates with weather, especially in most African countries where most farms are rain-fed. Bioenergy production is a very good means of absorbing excess products on good harvest years, thereby stabilizing prices. Thus, it will create stable market and prices for the crops, resulting in higher income for smallholder farmers who are currently the majority in many countries and very poor due to unstable and low prices for their products. Crop production is the major source of household incomes in rural areas. Thus increasing the profitability of farming will drastically reduce poverty which is fundamental to achieving food security. Large scale bioenergy production will also create jobs both in the agricultural sector and the bioenergy production facilities. Madakadze et al. (2013) noted that increasing the level of farm productivity is a prerequisite for economic development. There is a lot of wasted capacity due to high rate of unemployment. Many graduates of Agriculture are without jobs and yet lack of human capital is discussed as one of the major problems facing agriculture in Africa. Large scale bio-energy production will create jobs to absorb all these unemployed graduates. Bioenergy production will lead to total agricultural transformation and mobilize the available knowledge and skills that now lie wasted in many African countries. Bioenergy production can also lead to increase in energy security which in turn will encourage investment

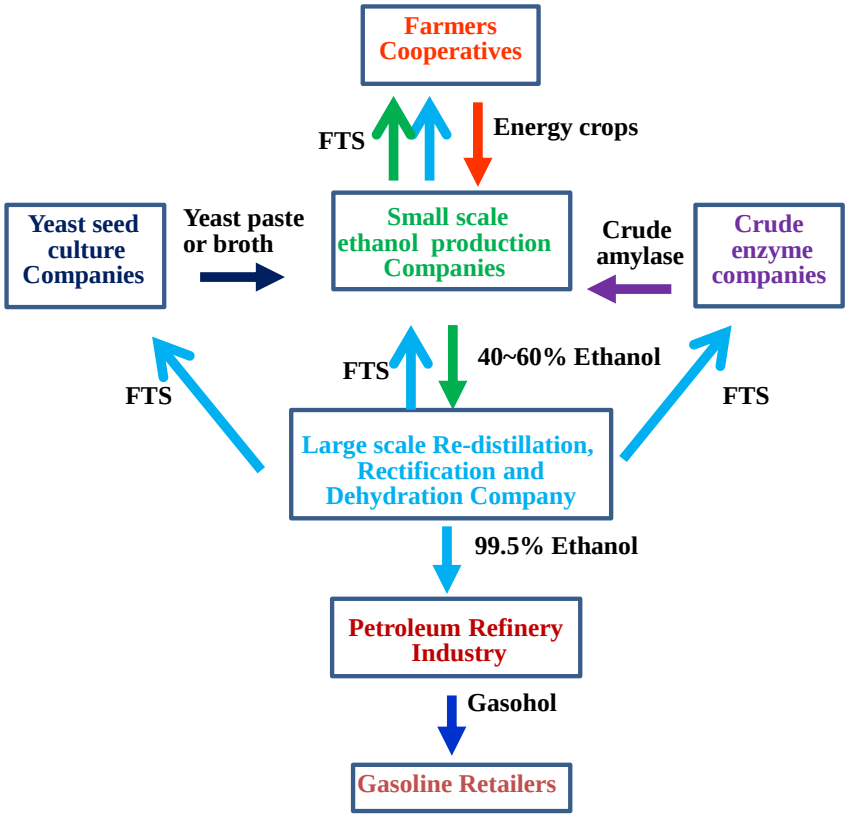

Figure 1. A vertical integrated system for community-based bio-ethanol production. FTS, Financial and technical support.

in other sectors of the economy and thus create more jobs. Bioenergy can stimulate demands for agricultural inputs such as seeds, fertilizers, and other agrochemicals so that suppliers can operate on large scales for reduced unit costs. On the whole, the net effect of bioenergy production on food security would therefore depend on country, locality within the same country and economic groups. However, the above discussions point to the fact that the possible positive effects far outweigh the negative effects.

VERTICAL INTEGRATED SYSTEMS FOR BIOENERGY PRODUCTION

As outlined before, most of the foods consumed in Africa are produced by smallholder farmers. For example, Salami et al. (2010) reported that about 75% of total agricultural output in Kenya, Tanzania, Ethiopia and Uganda are produced by smallholder farmers with average farm size of about 2.5 h. Any program aimed at increasing agricultural production in Africa must therefore be targeted towards protecting and empowering small-holder farmers. Thus, in order to avoid the adverse effects of bio-energy production on food security, and thus realize the various advantages of bio-energy production in Africa, the smallholder farmers must be fully integrated into the bioenergy production, rather than displacing them. Community based bioenergy systems are most appropriate for most rural communities in Africa. Examples of such systems are shown in Figures 1 and 2 for bio-ethanol and bio-diesel, respectively. In the case of bioethanol, large scale Bioethanol Company organizes and provides financial and technical supports to many smallholder farmers, small scale ethanol producers as well as small scale crude enzyme and yeast companies. Small scale ethanol production facilities are established within clusters of smallholder farms to minimize transportation costs for energy crops. The small scale producers buy the energy crops, ferment and distill into about 40~60% ethanol, using simple pot still distillation equipment. These types of pot still can be locally fabricated for reduced costs. These small scale ethanol companies also provide supports to the smallholder farmers. Since pure yeast cells and purified enzymes are often very expensive in most African countries, yeast and crude enzyme production Industries are also established to supply yeast and crude enzymes to the small scale producers. A large re-distillation, rectification and dehydration company buys the ethanol from the small scale producers at fixed prices, re-distill, dehydrate and then sell to petroleum companies for blending.

In the case of bio-diesel production, a big bioenergy

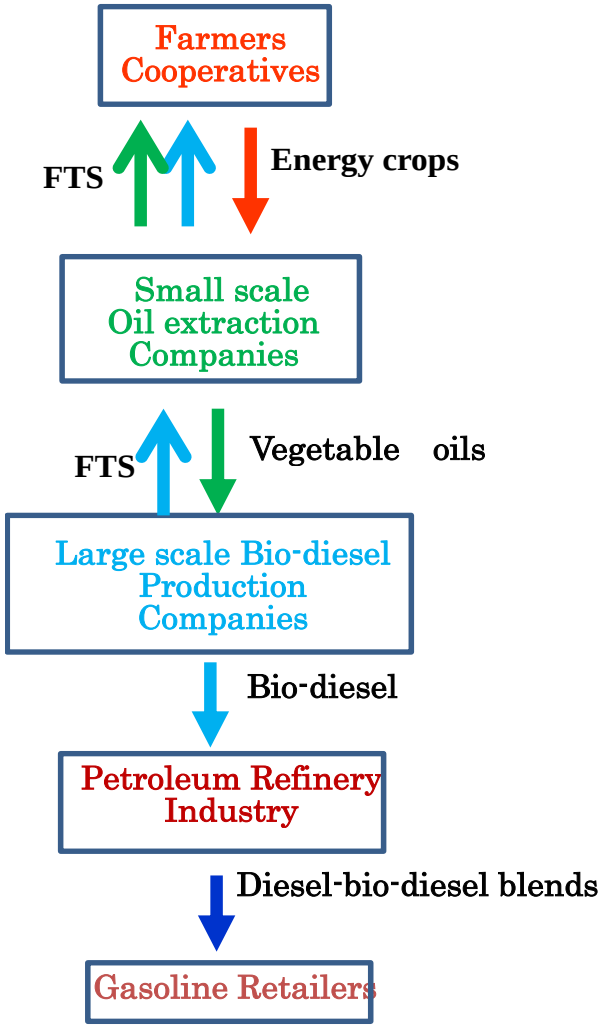

Figure 2. A vertical integrated system for community-based bio-diesel production. FTS, Financial and technical support.

industry also supports the smallholder farmers for increased productivity. Small scale oil extraction companies are established within farm clusters and sell their oil to large scale esterification companies for biodiesel production and distribution. The large scale biodiesel company also provides financial and technical supports to the farmers and small scale oil extractors. The vertical integrated bio-energy production systems have the following advantages:

1) The smallholder farmers are empowered, thus increasing their productivity and earnings.
2) Many jobs are created by the farmers, small scale ethanol producers, oil extractors, and the large bio-energy industries.
3) Transportation costs for the energy crops are significantly reduced.
4 The farmers are protected from fluctuations in prices, and have ready market that guarantees prices for their products.

CONCLUSION

One fundamental problem with policies and strategies for increased agricultural productivity in Africa is lack of reliable statistics on such vital things as the number of farmers, their farm sizes, their location as well as their major crops. Even the total national productions of some staple crops are very rough estimates. Keita (2013) noted that many countries cannot even provide reliable information on land area under cultivation, amounts of important crops produced, yields, amounts consumed, amounts processed, producer prices, and market prices. These types of information are very vital for planning but unfortunately, many countries do not have the capacity to collect and analyze information on farming activities.

Crop yields in Africa is still on average, less than 20% of their potential yields, while less than half of cultivatable lands in Africa are not cultivated. Thus crop production in African can easily be increased far more than their food requirement. With proper planning, large scale bio-energy production can be done without negative effects on food security. This will create jobs, reduce poverty and thus lead to the overall socio-economic development of the continent. However, this requires good policies, initial support and proper implementation.

Aside from bio-energy production from crops, micro-algae biotechnology can also be used for large scale production of bio-diesel without adverse effect on food security. Microalgae have higher photosynthetic efficiencies, thus higher productivities than higher crops, they are very diverse and thus can adapt to many ecological zones, they can be cultivated in non-agri-cultural areas, have high oil contents, and their cultivation can be coupled with waste water treatment, and thermal plants for carbon dioxide reduction. Relatively small area of land is required and Chisti (2007) estimated the productivities of microalgae oils to be 139,900 L/h/year and 58,700 L/h/year for microalgae with oil contents of 70 and 30% in photo bioreactors, while with open ponds, the productivities are 99,400 and 42,600 L/h/year for micro-algae with oil contents of 70 and 30%, respectively. These values are more than one order of magnitude higher than productivities with higher plants. The potentials of algae bio-diesel production have been extensively reviewed (Chisti, 2007; Huntley and Redalje, 2007; Brennan and Owende, 2010), and the climatic conditions in most parts of Africa are favourable for large scale cultivation of microalgae. However, the cost of production of microalgae oil is still very high due to the high costs of constructing and operating closed photo-bioreactors with good light supply on the one hand, and the problems of contamination, low cell growth rates and low biomass concentrations in open air photo bioreactors on the other hand (Ogbonna, 2003). Furthermore, the cost of harvesting from low standing biomass concentrations is still very high and thus requires process improvement. There is also a need for genetic improvement of microalgae strains for increased

growth rate, increased oil contents, and improved quality of the oils.

It is also important to note that the proposed vertical integrated system can be made more sustainable and profitable by integrating co-production of high value compounds such as potable alcohols, vitamins, organic acids, amino acids and various pharmaceuticals from the energy crops. Furthermore, efficient utilization and bio-conversion of wastes will definitely increase the profitability of the system. This includes conversion of the tuber peels and lignocellulosic wastes to organic manure, production of animal feeds from other solid wastes such as distillery wastes and solid wastes after oil extraction, recovery of glycerol during biodiesel production as well as recovery and purification of carbon dioxide for beverage industries.

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Review

Immunodiagnosis of pesticides: A review

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The word 'pesticide' is known worldwide for repelling or killing all kinds of pests in both agricultural and domestic settings but the harmful effects they cause on the immediate environment and living beings exposed to them has raised serious concerns and makes it more necessary to detect the minutest levels of contamination. Convenient, cost-effective and rapid pesticide detection systems are urgently needed, for which immunoassays are the best option. The present review deals with the immunodiagnosis of pesticides; various steps involved in development of an immunoassay focussing primarily on hapten preparation and conjugation strategies, generation of antibodies and assay optimization techniques in enzyme linked immunosorbent assay (ELISA) format. Their advantages over conventional methods and limitations have been discussed, followed by a bird's eye view of the multianalyte detection, immunosensors and other options-to-be-explored for better and more sensitive detection of pesticides.

Key words: Immunodiagnosis, pesticides, immunoassay.

INTRODUCTION

Pesticides are widely used for killing, preventing, destroying, repelling or mitigating insect pests, weeds, rodents, fungi, and other organisms that compete for food supply or threaten public health, cause nuisance and affect national economies, thereby giving a plenty of benefits to man and environment. More than 20000 pesticide products with nearly 900 active ingredients are registered by United States Environment Protection Agency (USEPA) for use as insecticides, miticides, herbi-cides, rodenticides, nematicides, fungicides, fumi-gants, wood preservatives and plant growth regulators (WHO, 2004). Their use helps in improving mankind by preventing food loss, making it more available to man, with longer storage life and lower costs. Though pesti-cides provide a relief to the mankind from pests, they have certain significant economic, environmental and public health impacts also. Widespread use of pesticides has raised sincere concerns over food and environmental contamination caused by them. A leading survey done by USEPA (Grube et al., 2011) revealed an approximate 5.6 billion pounds of annual pesticide usage worldwide. WHO (2009) estimated that globally, every year, 3 million people suffer health effects from exposure to pesticides and a minimum of 300,000 people die, of which, 99% belonging to low- and middle- income countries.

Several groups of pesticides are used commercially namely: Organophosphates, organochlorines, carbamates and pyrethroids. Organochlorines possess a chlorinated hydrocarbonmoeity;dichorodiphenyltrichloroethane(DDT), dieldrin, endosulfan being the common ones. Organo-

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Abbreviations: DDT, Dichorodiphenyltrichloroethane; **OP's,** organophosphate pesticides; **LC,** liquid-chromatography; **HPLC,** high performance liquid chromatography; **GPC,** gel permeation chromatography; **GC-MS,** gas chromatography-mass spectrometry; **LODs,** limits of detection; **TCP,** 3,5,6-trichloro-2-pyridinol; **ELISA,** enzyme linked immunosorbent assay; **TLC,** thin layer chromatography; **NMR,** nuclear magnetic resonance; **BSA,** bovine serum albumin; **OVA,** ovalbumin; **CAMM,** computer-assisted molecular modelling; **scFv,** single-chain variable fragment.

phosphate pesticides (OP's) are characterized by the presence of a phosphate-ester moiety in their chemical structure and account for approximately half of the pesticides used worldwide (ENVIS-NIOH, 2007). Their ability to degrade easily makes them an attractive alternative to the organochlorine pesticides, such as DDT, aldrin and dieldrin (Needleman, 2005) but OP's have greater acute toxicity, posing risks to people who may be exposed to large amounts (Costa, 2006). Commonly used organophosphates include parathion, malathion, chlorpyrifos. Pyrethroids are synthetic derivatives of pyrethrin and include cypermethrin, deltamethrin, among others. Another category includes carbamate pesticides, the derivatives of carbamic acid with carbaryl, bendiocarb, methomyl and propoxer dominating the category. Organophosphates and carbamates primarily act as neurotoxincants; they inhibit the enzyme acetylcholine-esterase, resulting in oversignalling, which leads to muscle paralysis and even death.

The dose of a pesticide is important in predicting the potential toxicity. Further, factors such as age, health and possibly gender may significantly lower the threshold for toxic effects. Even a single exposure to pesticide poses risks to man and other mammals, birds, fish and aquatic invertebrate species. Prolonged and multiple exposures to toxic concentrations raises the risks to wildlife as has been reviewed extensively by Parsons et al. (2005). Because of the widespread use of pesticides in agricultural and residential areas and the considerable toxic ill effects these cause on the persons exposed to it even at low levels, their detection even at minutest levels becomes a major concern. Hence the present review has been made with an aim to compile the various techniques used for detection of pesticides and concisely evaluate the recently developed state-of-the-art immunodiagnostic techniques over the conventional ones.

CLASSICAL TESTS USED TO DETECT PESTICIDES

Conventionally, gas-chromatography (GC), liquidchromatography (LC), high performance liquid chromatography (HPLC) and gel permeation chromatography (GPC) have been used with great ease and success for detection of pesticides. Zhu et al. (2006) applied gas chromatography-mass spectrometry (GC-MS) with negative chemical ionization (NCI) for the determination of five pyrethroid pesticide residues (fenpropathrin, cyfluthrin, fenvalerate, deltamethrin, cypermethrin) in traditional Chinese medicinal plants and obtained limits of detection (LODs) from 0.5 to 5 ppb with recoveries ranging from 70 to 120%. The method was considered rapid, sensitive and suitable for the analysis of large numbers of samples simultaneously. A deadspace solid-phase microextraction method developed by Chai et al. (2008) for the determination of various pesticides in vegetables and fruits by gas chromatography with an electron capture detector resulted in more than 80% recovery for all the investigated samples. In another study, ultrasonic-assisted dispersive liquid-liquid microextraction (UA-DLLME) followed with GC- flame ionization detection (FID) was applied for the analysis of cypermethrin and permethrin residues in pear juice (Du et al., 2010). The enrichment factors for cypermethrin and permethrin residues were 344 and 351, respectively and LODs ranged from 3.1 to 2.2 µg/kg, recoveries were 92.1 to 107.1%. The concentration of acephate, methamidophos and omethoate was determined in animal and fishery products and their processed foods and honey (Jia et al., 2010) using extraction of target analytes with ethyl acetate and pre-purification on a primary secondary amine (PSA) mini-column followed by detection using LC-MS in the electrospray ionizaton mode with recoveries of the method ranging between 71.4 and 98.4% ± 12.5%. Organochlorine pesticides residues have been determined in commercial fruit species by Cieslik et al. (2011) where target compounds were extracted and cleaned by QuEChERS (quick, easy, cheap, effective, rugged and safe) method followed by GC-MS analysis. The recovery rates of the method ranged from 70 to 120 ±17% in the majority of cases; limit of quantification varied between 0.001-0.013 mg/kg. An accelerated solvent extraction system (HPLC with GPC) was used for separation, detection, and quantification of the pesticides from the sediment-sample extracts followed by their detection by GC/MS (Hladik and McWayne, 2012). Recoveries in test sediment samples fortified at 10 µg/kg dry weight ranged from 75 to 102%; relative standard deviations ranged from 3 to 13%, LODs ranged from 0.6 to 3.4 µg/kg dry weight.

However, determination of metabolites cannot be taken as standard for estimating the exposure to a pesticide, since residues of metabolites frequently occur on fruits and vegetables, often at concentrations 10-20 times greater than the parent pesticide. Besides, structurally related pesticides can also be converted to same metabolites. A bacterium strain KR100 isolated from a Korean rice paddy soil by Kim and Ahn (2009) is able to degrade chlorpyrifos as well as chlorpyrifos-methyl to 3,5,6-trichloro-2-pyridinol (TCP). Moreover, conventional methods owe their high cost for set-up skilled labour and extensive sample preparation steps, so it becomes necessary to replaced them with more analytical and simpler techniques for which immunoassays are a good option. In an attempt to compare the efficacy of immunoassays with classical tests, Curwin et al. (2010) collected urine samples from 51 participants among farm families in Iowa to analyze the status of exposure to chlorpyrifos using enzyme linked immunosorbent assay (ELISA) and HPLC in tandem mass spectrometry. The immunoassay methods consistently had significantly higher geometric mean estimates for the metabolites. The LOD for TCP as a metabolite of chlorpyrifos ranged from 13.9-14.13 µg/L by HPLC and 2.91-2.99 µg/L from

immunoassay. The study confirmed that HPLC-MS/MS methods tend to be less sensitive, costly and time consuming as compared to immunoassays and also require sample pre-treatment. The same has been proved by Rubio et al. (2003) who compared ELISA and HPLC for the detection and quantification of glyphosate-spiked Nanopure, tap, and river waters. The ELISA had a detection limit 0.6 ng/mL whereas the HPLC method had a detection limit of 50 ng/mL.

RECENTLY DEVELOPED IMMUNOASSAYS AND THEIR ADVANTAGES OVER CLASSICAL TESTS

The development of immunochemical techniques revolutionized the practice of pesticide analysis in veterinary medicine, agriculture, clinical chemistry and other areas including environmental and food analysis and have begun to gain acceptance as rapid, simple, sensitive, specific and cost-effective tools. These can detect nano-gram scale *in situ*. Immunoassays rely on detection of analyte concentration in the sample by measurement of level of antigen-antibody binding. Highly sensitive detection of analytes can be made by enzyme immunoassays such as ELISA. Development of an immunoassay typically follows some basic strategic principles in which antigen-specific antibodies are generated and then used to capture the antigen present in a biological sample or vice versa. Availability of antibodies with the desired affinity and specificity is a prerequisite for the development of immunoassays and the foremost step in antibody production is the preparation of an immunogen with molecular weight appropriate enough to target the animal's immune response. The major steps in the development of an immunoassay include: Preparation of hapten and immunogen and its characterization; conjugation of hapten to macromolecular carrier and its purification; immunization of host animals for generation of antibodies and optimization of the assay.

Preparation of hapten and immunogen

Most pesticides are unable to elicit an appropriate immune response because of their low molecular weight. Hence, production of antibodies against pesticide-hapten can be made possible by covalent linking of the hapten to a carrier protein, through a coupling spacer, to synthesize the 'immunogen'. Hapten immunochemistry occupies a major role in the area of development of antibodies. The major points of influence in the preparation of an immunogen include site of coupling to the carrier, the length of coupling spacers, the selection of optimized carriers, the coupling procedure as well as the number of haptens bound to one carrier molecule (Dankwardt, 2001). It is optimal to use C_3-C_6 spacers; too long spacers may bend back over the carrier and prevent proper exposure of the hapten. A wide variety of immunogens and protein/enzyme conjugates have been prepared and used in immunoassays for various pesticides. Basic synthetic ways of preparation of the hapten derivatives (hapten design) have been explored (Figure 2) by Franek and Hruska (2005) and Tong et al. (2007). Out of various ways of preparing a conjugate, it is important to select the best, which can elicit maximum immune response.

Structural modification of pesticide

Generally the haptens are desired to contain (a) a reactive group (b) an aromatic ring (c) branch atom or (d) heteroatom ring. The hapten needs a reactive functional group such as -COOH, -NH_2 or -OH to directly couple with the carrier proteins. Else, it has to be modified first to introduce at least one of these reactive groups in its structure to couple with the protein. For example, as described by Manclus et al. (1994, 1996) there are two approaches to prepare chlorpyrifos-hapten depending on the site of attachment of spacer arm: modification of the aromatic ring (Hapten 1) viz. spacer coupling through the pyridyl ring by substitution of chlorine at the 6th position (ortho- to the nitrogen) or the thiophosphate group (Hapten 2) through replacement of the o-ethyl group with a suitable spacer arm keeping the pyridyl ring moiety intact (Figure 1).

Characterization of the hapten

The hapten can be characterized by thin layer chromatography (TLC) or nuclear magnetic resonance (NMR) spectral analysis. TLC is a low-cost, maintenance-free, fast, and reliable method and also uses limited volumes of solvents; NMR offers better solutions in terms of confirmation.

Coupling of hapten to macromolecular carriers

Various approaches are used to couple the hapten to the carrier depending upon the chemical structure of haptens (Figure 2) namely: (1) Carboxyl-containing haptens can be coupled with the carrier using N-hydroxysuccinimide active ester/carbon-diimine or Woodward reagent protocol; (2) Amino-containing haptens can be linked to carriers by employing glutaraldehyde, diisocyanate, halo-nitrobenzene, hiophosgenation, diimine ester, or diazotization protocol; (3) Hydroxyl-containing haptens can be directly connected to the carrier through succinic anhydride or azobenzoic acid protocol; (4) Carbonyl-containing haptens (ketone or aldehyde) are usually connectd to carriers using amino-ox-acetic acid protocol; (5) Homogeneous or heterogeneous difunction reagents

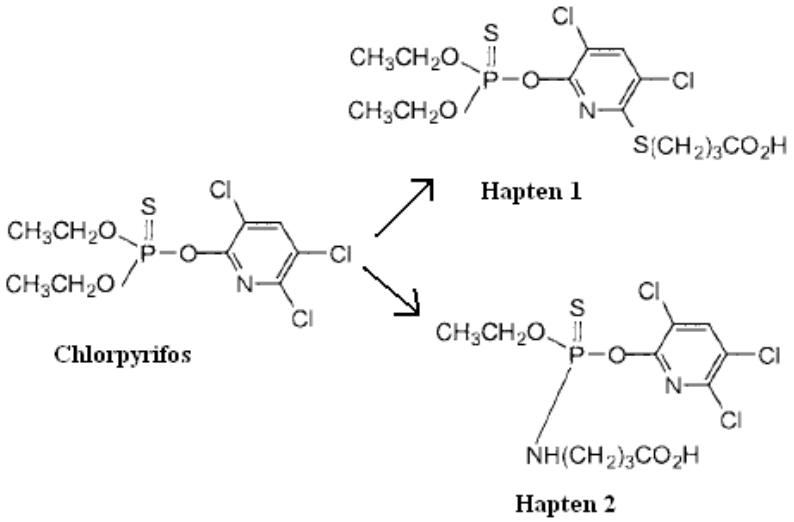

Figure 1. Synthesis of chlorpyrifos hapten (introduction of carboxylic group).

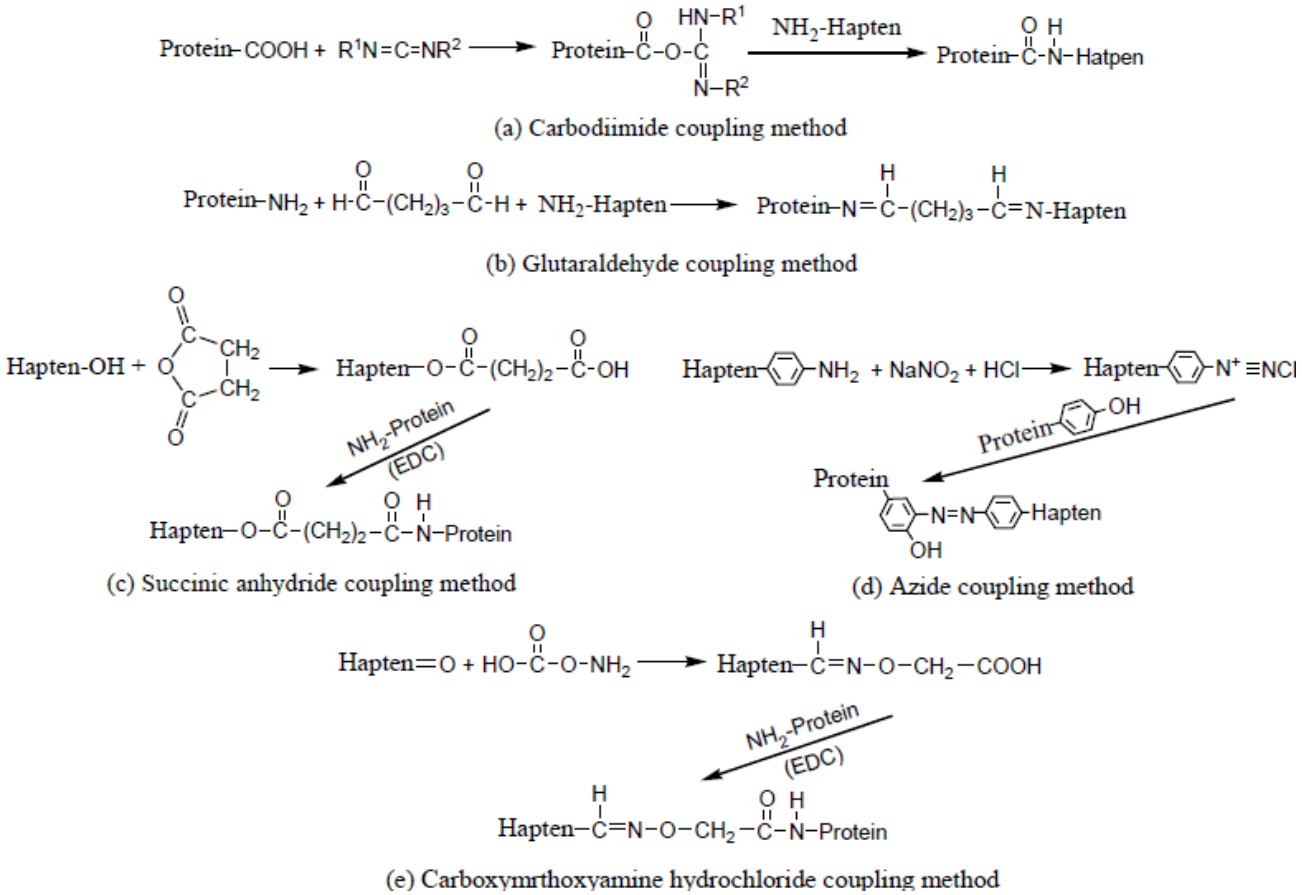

Figure 2. General principles of coupling of hapten to carrier protein (Tong et al. 2007).

can be used to synthesize the immunogen for mercapto-containing haptens (Tong et al., 2007).

Various proteins have been used by a number of analysts with ease and effectiveness, as carrier molecules with a variety of pesticides viz. bovine serum albumin (BSA) (Cho et al., 2002), keyhole limpet hemocyanin (KLH)

(Lee et al., 2003) and ovalbumin (OVA) (Zhang et al., 2008, Qian et al., 2009). A single polypeptide chain of BSA has 59 lysine groups containing side chain amino groups, out of which 30-35 are available for coupling to carboxyl group of hapten, which makes it an excellent carrier; OVA has 20 lysines and KLH has a very high number of lysines (300-600 are usually available for binding). It is always best to choose the carrier protein containing the optimum number of lysine residues for binding high number of binding sites in tertiary structure of the protein by masking the essential free amino groups. Moreover, the easy availability of BSA and its ability to solubilize in organic solvents under various pH range and ionic strength makes BSA a popular carrier protein. Also non-proteinaceous carriers viz. liposomes and dextran or synthetic molecules designed with appropriate functional groups such as poly-L-lysine and polyethylene glycol can be used as carriers in pesticide immunoassays.

However, the above trial and error-based procedures of hapten designing may give rise to antibodies lacking some features necessary to develop a useful immunoassay, besides being time-consuming and arduous. Immunochemists have recently come up with the computer-assisted molecular modelling (CAMM) as a useful tool for hapten design. CAMM offers assistance in predicting the spatial and electronic effects of molecular structure of hapten on its biological activity that are difficult or otherwise impossible to obtain, and can then be successfully applied in improving the sensitivity of immunoassays. Molecular modelling studies have been used to identify the best out of various potentially immunizing haptens for development of sensitive immunoassays against trichlorophenol (Galve et al., 2002), permethrin (Ahn et al., 2004), parathion (Liu et al., 2007) and semicarbazide (Vass et al., 2008). Applications of CAMM in hapten designing for immunoassay development along with limitations and prospects have been reviewed in detail by Xu et al. (2009). Xu et al. (2010) applied CAMM to model the hapten design to develop a broad-specific competitive indirect ELISA for fourteen O,O-diethyl organophosphorus pesticides. CAMM is foreseen as a practical and potential tool in rapid and more economical development of immunoassays.

Purification of conjugate

The conjugate to be used as immunogen needs to be obtained in the pure form to avoid production of nonspecific antibodies and unwanted cross-reactivities of the polyclonal antisera. The conjugate is separated from the uncoupled haptens by dialysis or gel filtration. Dialysis results in a well purified antigen and is a simple process and has been successfully used for separation of hapten-protein conjugates from uncoupled haptens for Acephate (Lee et al., 2003) and Fenthion (Zhang et al., 2008); bromophos hapten-protein conjugates were separated from the uncoupled haptens by gel filtration (Sephadex G-25), the same has been applied for the separation of chlorpyrifos-protein conjugates (Manclus et al., 1996). Reports are also available describing the separation of cyanophos-protein conjugates by gel filtration followed by dialysis (Park et al., 2002b).

PRODUCTION OF ANTIBODIES

Various warm blooded animals viz. mice, rabbit, goat or chicken can be immunized with the hapten-protein conjugate for pesticide. Rabbit is most widely used as they are easy to handle, respond quickly, produce adequate amount of antiserum and have a sufficiently long life span. Both polyclonal and monoclonal antibodies have been used for development of pesticide immunoassays so far. Though mAbs produced from hybridoma culture (Manclus et al., 1994, 1996) in laboratories offer the advantages of a steady supply and unvarying characteristics, many pesticide immunoassays still employ polyclonal ones (Cho et al., 2002; Brun et al., 2005) owing to the great effort and expense involved in mAb production. There is no standard protocol for immunization; generally, the animal is injected with small volumes of the inoculum at multiple sites and the immunization is repeated with booster doses at regular intervals (week or so). The animal is bled after each booster dose and antisera is collected.

A third possibility of obtaining antibodies is using recombinant antibody engineering techniques- that represent the next generation immunoreagents. Here, *in vitro* production of recombinant antibodies (rAbs)- or their fragments (for example, scFv or Fab) (Figure 3) is made by creating libraries of antibody gene segments followed by phage display, ribosomal display or yeast display, from which antibodies of desired specificities and affinities tailored by site-directed mutations can be selected. Single-chain variable fragment (scFv) antibodies have been produced by phage display against a number of pesticides including parathion (Chambers et al., 1999) methamidophos (Li et al., 2006) and fenitrothion (Luo and Xia, 2012).

DEVELOPMENT OF ASSAY

The antibodies so obtained can be used to develop immunoassays in different choices of formats. These may be broadly categorized as "competitive" and "non-competitive", depending upon their utilization of limited concentration of available antibody while non-competitive assay rely on the measurement of antibody binding sites getting occupied after they are allowed to react with the analyte; competitive assay is based on the competition between solid-phase (bound) and soluble antigen for

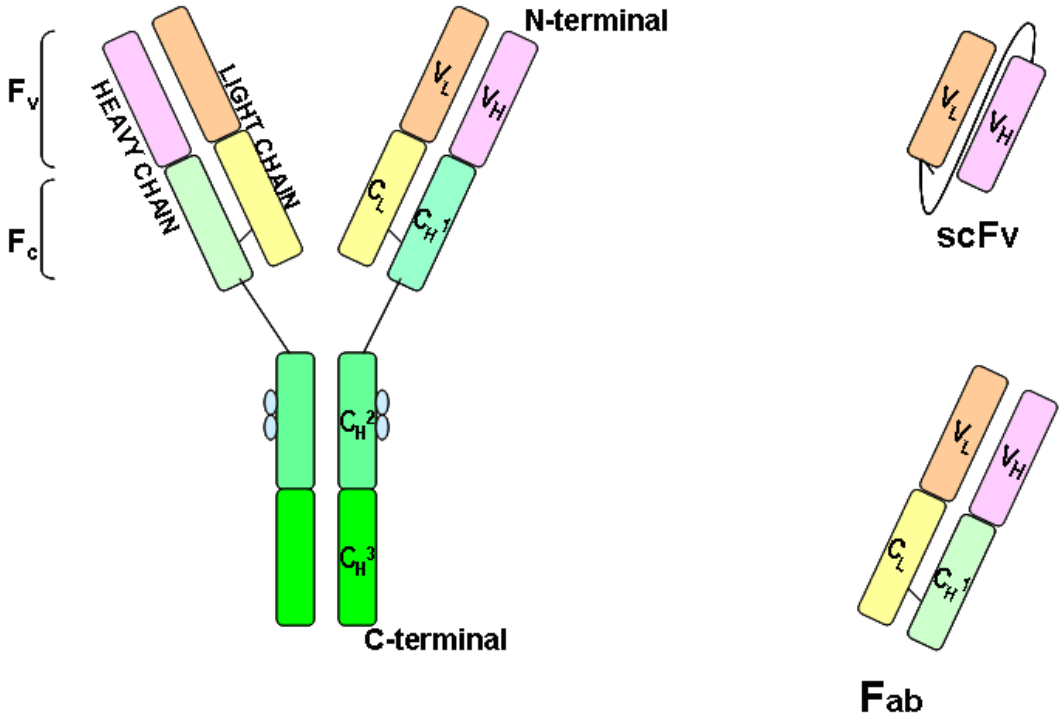

Figure 3. Structure of a typical antibody and antibody fragment types. C_H, Constant heavy weight; C_L, constant light weight; V_H, variable heavy chain; V_L, variable light chain; Fab, antigen binding fragment; scFv, single chain variable fragment.

limited antibodies and give an indication of the antibody binding sites left unoccupied and is more preferred for pesticide detection. With increase in concentration of pesticide added for competition, lesser amount of antibodies is available for binding with the bound antigen and hence there is a decrease in absorbance. A highly sensitive immunoassay will have a low detection limit (DL) and that calls for high affinity between the antibody and the analyte. Dankwardt (2001) presents a complete overview of the immunoassays developed for detection of various pesticides. Table 1 summarises the work done after 2001 by various immunochemists in development of highly sensitive ELISAs against various pesticides along with their limit of detection (LOD) and I_{50} values.

Enzyme assays have been used for the rapid screening of insecticides, but these assays may lack distinction between similar pesticides. Many workers have found cross-reactivity of ELISA developed for one pesticide towards certain other pesticides of the same category, probably due to structural similarity or same functional groups that act as antibody competitors or even against the carrier protein used for hapten-conjugation. So it is always essential to check the immunoassay for potential cross reactivities before applying. This is usually done by comparing the standard curves of the analyte under investigation with similar haptens, using analyte concentrations at 50% of the inhibition curve as the reference. Cho et al. (2002) found the ELISA for chlorpyrifos showed 66.6% cross reactivity with chlorpyrifos-methyl, 15.6% with bromophos-ethyl, 4.58% with bromophos-methyl and 3.05% with dichlofenthion. Park et al. (2002c) found the ELISA developed for bromophos to show cross-reactivity with chlorpyrifos and fenitrothion because of their similar aromatic structure. Brun et al. (2005) found the ELISA developed against chlorpyrifos to be cross-reactive against chlorpyrifos-methyl, bromophos-methyl and fenchlorphos. The commercial magnetic particle-based ELISA kit used by Sullivan et al. (2007) for the detection of chlorpyrifos showed cross-reactivity with chlorpyrifos-methyl (37%), while reactivity with other pesticides ranged from 1.6 to 10.7%.

CONCLUSIONS AND FUTURE PROSPECTS

ELISA can be potentially adopted as a routine test for the detection of pesticides for rapid and cost-effective mass screening of a large number of samples simultaneously.
So far immunoassays dominate the field of pesticide diagnosis, being the highest sensitive tests. Dipstick immunoassays based on competitive immunoassay format have great potential to become cost-effective and sensitive tool for on-site monitoring of pesticides. Its main advantage is its ease and effectiveness to use for field analysis and high sensitivity. Quantification of the pesticides

Table 1. List of ELISAs developed for detection of various pesticides (2002 onwards).

Pesticide	Antibody used	LOD	I_{50}	Reference
Chlorpyrifos	pAb	0.1 ng/mL	20 ng/mL	Cho et al. (2002)
Bromophos-ethyl	pAb	1.0 ng/mL	6.5 ng/mL	Kim et al. (2002)
Deltamethrin	pAb	1.1 ± 0.5 µg/L	17.5 ± 3.6 µg/L	Lee et al. (2002)
2-(2,4,5 trichloro phenoxy) propionic acid	pAb	0.05 µg/L	0.80 µg/L	Morais et al. (2002)
Isofenphos	pAb	70 ng/mL	580 ng/mL	Park et al. (2002a)
Bromophos	pAb	7 ng/mL	40 ng/mL	Park et al. (2002c)
Fenitrothion	pAb	0.3 ng/mL	6 ng/mL	Watanabe et al. (2002)
Fenthion	pAb	0.1 µg/L	1.2 µg/L	Cho et al. (2003)
Acephate	pAb	2 ng/mL	25 ng/mL	Lee et al. (2003)
Fenthion	pAb	0.03 ng/ml	0.05 ng/ml	Brun et al. (2004)
Isoproturon	pAb mAb	0.1 µg/L 0.003 µg/L	1.06±0.34 µg/L 0.07±0.04 µg/L	Kramer et al. (2004)
Cypermethrin	pAb	1.3 ± 0.5 µg/L	13.5 ± 4.3 µg/L	Lee et al. (2004)
Chlorpyrifos	pAb	0.3 ng/mL	271 ng/mL	Brun et al. (2005)
Cyhalothrin	pAb	4.7 µg/L	37.2 µg/L	Gao et al. (2006)
Pirimiphos-methyl	mAb	0.07 ng/mL	4.2 ng/mL	Yang et al. (2006)
Imidacloprid	pAb	30 ng/mL	995.4 ng/mL	Li et al. (2007)
Triazophos	mAb	0.02 µg/L	0.21 µg/L	Liang et al. (2007)
Pentachloronitrobenzene	pAb	7 ng/mL	37 ng/mL	Xu et al. (2007)
Triazophos	mAb	0.36 to 7.89 ng/mL	1.69 ng/mL	Jin et al. (2009)
Carbofuran	mAb	1.89 to 45.95 ng/mL	9.32 ng/mL	
2,4-dinitroaniline	mAb	0.05±0.03 µg/L	0.24±0.06 µg/L	Kramer et al. (2008)
2,6-dinitroaniline	mAb	0.11±0.08 µg/L	0.61±0.08 µg/L	
O-ethyl o-4-nitrophenyl phenyl phosphonothioate	pAb	0.9 ng/mL	8.4 ng/mL	Shim et al. (2008)
fenazaquin	pAb	1.8 ng/mL	42.13 ng/mL	Kyung et al. (2009)
Parathion	pAb	0.15 ng/mL	0.95 ng/mL	Liu et al. (2009)
Deltamethrin	mAb	1.2±1.3 ng/mL	17.0±3.3 ng/mL	Kong et al. (2010)
EPN	mAb	0.09 ng/mL	0.6 ng/mL	Shim et al. (2010)
Atrazine	pAb	0.1 ng/mL	17.5 µg/mL	El-Gendy et al. (2011)
Chlorpyrifos	mAb	0.1 ng/mL	3.3 ng/mL	Liu et al. (2011a)
Pretilachlor	pAb	6.9 ng/L	0.0359 mg/L	Liu et al. (2011b)
Imidaclothiz	mAb	0.0178 ± 0.0018 mg/L	0.0875 ± 0.0034 mg/L	Fang et al. (2011)
Triazophos	mAb	0.063 ng/mL	0.87 ng/mL	Jin et al. (2012)
Imidacloprid	pAb	0.03-0.16 ng/mL	1.2-3.0 ng/mL	Wang et al. (2012)

is carried out by measuring the dot colour by spectronic read-out or naked eye (Gabaldon et al., 2003). However the errors inherent in immunoassays may give false positives and lack absolute specificity as was originally presumed, besides exhibiting cross reactivity with similar epitopes, thereby misleading the results. But an approximation of the results can definitely be inferred from immunoassays, which can further be verified.

Another limitation of immunoassays is their restriction to detect single analyte per assay. Hence, new approaches for development of multianalyte immunoassays are being undertaken, for the advantages of simultaneous detection of multiple analytes in a single assay, high sample throughput, reduced sample consumption and lower cost per assay they offer. Important progress is expected in the field of ‘ambient analyte immunoassays’ based on Microspot detection systems. These systems rely on "ratiometric" analysis, involving measurement of analyte concentration from the ratio of signals emitted by two labelled antibodies- "Sensor" antibody, deposited as a microspot on a solid support which is exposed to the analyte-containing sample; and "Developing” antibody directed against either occupied or unoccupied binding sites of the sensor antibody. An array of sensor antibodies of different selectivity can be employed in the form of a chip and the fluorescent signal ratio emitted

from each discrete antibody couplet in the array can be used for multianalyte determination.

Gold- or selenium-nanoparticle based aggregation immunoassay technique is also being recently undertaken for multianalyte sampling of the pesticides. An enzyme immunoassay is performed on the antibody-nanoshell conjugates and aggregates of analytes of various morphologies present in the complex sample medium are formed on their surface thereby allowing simultaneous read-out of multiple analytes. A gold nanoparticle (GNP) based dipstick competitive immunoassay has been developed for several pesticides viz. an LOD of 1ng/mL has been developed for atrazine (Kaur et al., 2007), 27 ng/mL for DDT (Lisa et al., 2009), 3 ng/mL for 2,4-dichlorophenoxyacetic acid (Boro et al., 2011); though Salmain et al. (2008) found no significant difference in the IC_{50} and LOD values obtained from gold particle based immunosensor and the competitive inhibition ELISA using the same antibody and the antigen.

In order to increase their range, speed and sensitivity, recently ELISA techniques have been combined with biosensors, to form immunosensors, in which analyte concentration is directly determined by measuring the alteration in physical properties (electrical or optical) induced by the formation of an immune complex between the analyte and the antibody immobilized on the transducer carrier surface acting as a sensing device. Till date, immunosensors based on optical, piezoelectric (PZ), electrochemical and micromechanical designs have come up as the most sophisticated immunoassay format to detect trace amounts of pesticides (Suri et al., 2009). Opto-electronic based biosensors such as surface plasmon resonance (SPR) sensors, interferometer devices or grating couplers offer the benefits of real-time measurement of biomolecular interactions, portability, versatility and regenerability in multi-analyte detection. An SPR-based biosensor has been recently developed for multianalyte detection of sulfonamides (Bienenmann-Ploum et al., 2005) and for simultaneous detection of DDT, chlorpyrifos and carbaryl (Mauriz et al., 2007). Overall, it would not be wrong to say that despite the advances made in developing sensitive diagnostic tools for detection of pesticides, the quest for an even better one still goes on; the requirement for novel signal detection equipments still persists; the need for better and more sophisticated assays for pesticide monitoring in environmental and food analysis is call of the hour.

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