PREPARATION AND EVALUATION OF COPPER GAMBIR COMPLEXES AS ANTIFOULING AGENT

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PREPARATION AND EVALUATION OF COPPER GAMBIR COMPLEXES AS ANTIFOULING AGENT

by

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LIST OF ABBREVIATIONS AND SYMBOLS

AAS	Atomic absorption spectrometry
AFS	Anti-Fouling Sytems
CE	Catechin equivalent
CuG	Copper gambir complexes
EDX	Energy Dispersive X-ray
EPS	Extracellular polymeric substances
FTIR	Fourier transform infrared spectroscopy
GAE	Gallic acid equivalent
IMO	International Maritime Organisation
LC_{50}	Lethality concentration fifties
ppm	parts per million
SEM	Scanning electron microscope
SPC	Self-polishing copolymer
TBT	Tributyltin
TBTF	Tributyltin fluoride
TBTO	Tributyltin oxide

TPTF	Triphenyltin fluoride
TFC	Total flavonoid content
TPC	Total phenolic content
TPLA	Triphenyl lead acetate
UV- Vis	Ultraviolet visible spectroscopy

PENYEDIAAN DAN PENILAIAN KOMPLEKS KUPRUM GAMBIR

SEBAGAI AGEN ANTIFOULAN

ABSTRAK

Tumbuhan *Uncaria gambir* telah dikaji dan mempunyai ciri-ciri sebagai agen antifoulan dalam cat *antifouling*. Empat pelarut berbeza yakni etil asetat, etil asetat: etanol (1:1), 50% etanol dan air telah digunakan untuk mengekstrak *Uncaria gambir* bagi mengetahui jumlah kandungan fenolik (TPC), jumlah kandungan flavonoid (TFC) dan kandungan tannin terkondensasi dalam ekstrak gambir. Pengekstrakan gambir dengan pelarut etil asetat: etanol (1:1) (v / v) didapati menghasilkan 32.3% hasil ekstrak dengan TPC, TFC dan kandungan tannin terkondensasi yang tertinggi. Ekstrak gambir didapati bertindak balas dengan kuprum(II) sulfat pentahidrat menghasilkan kompleks kuprum gambir (CuG) dan telah dikaji prestasinya sebagai pigmen antifoulan. Kesan dos kuprum, pH larutan, suhu tindak balas dan masa tindak balas dalam pengoptimuman keadaan sintesis kompleks kuprum gambir (CuG) dikaji. Spektrum FTIR dan analisis AAS menunjukkan bahawa larutan CuG pada pH 5.0 mengandungi ion kuprum terendah di dalam kompleks CuG dengan kehadiran kuprum hidroksida yang rendah. Kompleks CuG yang telah disintesis pada suhu 60 °C dan 30 minit masa tindak balas didapati mengandungi ion kuprum yang lebih tinggi di dalam kompleks CuG pada pH yang lebih tinggi. Analisis permukaan melalui SEM menunjukkan perubahan ketara dalam morfologi ekstrak gambir setelah ianya membentuk CuG. Permukaan gambir ekstrak yang licin telah berubah ke bentuk yang kasar dan berbutir dengan kehadiran

kuprum. Dalam ujian ketoksikan, didapati bahawa CuG yang dihasilkan pada pH 5.0 dan pH 6.0 dengan nilai LC_{50} 39.87 μg/mL dan 52.34 μg/mL masing-masing mempunyai kesan yang lebih baik pada kematian udang air masin daripada kuprum pirition yang merupakan biosid komersial di dalam cat antifoulan. Ujian lapangan di dalam air laut sebenar menunjukkan bahawa panel dan jaring ikan yang telah disalut dengan cat antifouling yang mengandungi kompleks CuG sebagai pigmen antifoulan dapat menghalang pertumbuhan organisma foulan pada permukaan selepas 3 bulan rendaman dalam air laut yang sebenar.

PREPARATION AND EVALUATION OF COPPER

GAMBIR COMPLEX AS ANTIFOULING AGENT

ABSTRACT

Uncaria gambir plant has been studied and characterized as antifoulant agent in antifouling paint. Four different solvents namely ethyl acetate, ethyl acetate: ethanol (1:1), 50 % ethanol and water were used to extract *Uncaria gambir* in order to find out the total phenolic content (TPC), total flavonoid content (TFC) and condensed tannin content of the gambir extract. Extraction of gambir by ethyl acetate: ethanol (1:1) (v/v) solvent produced 32.3 % yield and the highest TPC, TFC and condensed tannin content. Gambir extracts were reacted with copper(II) sulphate pentahydrate to produce copper gambir (CuG) complex and were evaluated as antifoulant pigment. The effect of copper dosage, pH solution, reaction temperature and reaction time in optimization of synthesis condition for copper gambir (CuG) complex were studied. The FTIR spectra and AAS analysis showed that CuG complex produced at pH 5.0 had shown the lowest copper content and low presence of copper(II) hydroxide. Synthesized CuG complexes at 60 °C and 30 min reaction time revealed that copper content in CuG complex increased as the pH solution of CuG increased. CuG complexes at pH 5.0 and pH 6.0 were chosen for comparison. Surface analysis through SEM portrayed significant changes in the morphology of gambir extract after reacted to produce CuG. The smooth surface of gambir extract was transformed into rough and granular shapes due to the presence of copper. In the toxicity test, it was shown that CuG prepared at pH 5.0 and pH 6.0 with

LC_{50} values of 39.87 µg/mL and 52.34 µg/mL, respectively have a better lethality effect on brine shrimp than copper pyrithione, a commercial biocide used in antifouling paint. It was revealed in field test in real seawater that the panels and fish nets that have been coated with antifouling paint containing CuG complex as antifouling pigment were able to prevent the growth of fouling organism on its surface after 3 months of immersion.

CHAPTER 1

INTRODUCTION

Fouling is known as the undesirable accumulation of either living organisms known as biofouling or a non-living substance which are inorganic or organic on solid surfaces, which is immersed in an aquatic environment. Several types of fouling occurred included fouling of ship hulls, natural surfaces in the marine environment (marine fouling), fouling of heat transfer components through ingredients contained in the cooling water or gases, and development of plague or calculus on teeth (Yebra *et al.*, 2004).

Boats and ships spend most of their working life submerged in water; sea, river, lake, etc. Long period of time in water causes the hulls to become prone for micro-organisms in aquatic environment to accumulate known as biofouling. Biofouling is divided into two groups which are microfouling; biofilm formation and bacterial adhesion, and macrofouling; attachment of larger organisms such as barnacles, mussels, polychaete worms, bryozoans, and seaweed (Readman, 2006).

The fouling causes resistance on the hull which leading to increased fuel consumption and significant damage to the boat structure. Besides, accumulated biofoulers can form enormous masses that severely reduce ships' maneuverability and carrying capacity. It is necessary to prevent and control the fouling by apply some form of coating which can protect the hulls against infestation known as antifouling paints which applied to the hull at regular intervals.(Chambers *et al* ., 2006).

Antifouling paints contain biocides such as tributyltin (TBT) and cuprous oxide (Cu_2O) which held within the structure of the paint. TBT was introduced as a biocide in antifouling paints in the early 1960s. Initially, TBT was incorporated in conventional of 'free association' paints that relied on passive leaching from a soluble matrix to deliver surface toxicant to inhibit growth and had a lifetime of around two years. In 1970s, conventional paints were replaced with self-polishing copolymer (SPC). TBT copolymer in SPC paints provides both biocide and paint matrix, and this hydrolyses in seawater to release the TBT leaving an unstable surface layer that gradually erodes to expose a fresh layer of active paint (Lewis, 1998).

Environmental problems associated with the use of TBT in antifouling paint were detected in the early 1980s, where TBT were linked to shell abnormalities in oysters (*Crassostrea gigas*) and imposex in dogwhelks (*Nucella lapillus*) (Dafforn *et al*., 2011) which led to a worldwide ban of TBT by International Maritime Organisation (IMO). IMO introduced the "Anti-Fouling Sytems (AFS) Convention'' to ban the application of TBT on all vessels after 1 January 2003 and require its absence as an active coating on all vessels after 1 January 2008. AFS convention became internationally binding in September 2008 (IMO, 2001).

Copper-based antifouling has been used as immediate alternative as replacement for TBT-paint, due to the toxic effect of TBT-paint on non-target organism. The coating is designed to leach Cu_2O slowly into the marine environment, preventing any organism adhering to the paint by poisoning the settling organisms. However, the nature of a Cu_2O can have harmful effects, not only on the fouling organism but also on other marine life which unconnected with fouling activity, sharing the similarity to the problem caused by TBT-paint (Katranitsas *et al.*, 2003).

In aquaculture, application of biocidal coating based on copper oxide (Cu_2O) is still widely used. Net coatings are usually a basic version of coatings for vessels with small amounts active substances are released to prevent or kill the fouling organism. This type of coating has a short lifetime while the costs for treating nets including net replacement and reapply antifouling coating are expensive. Antifoulants are known sources of pollution from aquaculture and responsible are for elevated levels of copper close to fish-farms area. Another method in prevention of fouling problem in aquaculture is known as mechanical cleaning, which involves brushing, scraping or cleaning using water jets. However, these methods were found to be labour intensive and tedious (Willemsen, 2005).

In recent years, several studies have been conducted in order to evaluate the potential of natural products from plant as an antifouling agent. Antifouling compound from terrestrial plant such as phenols have the potential to be utilized as new environmentally friendly antifouling agent due to its properties including highly anesthetic, settlement inhibitory properties, growth inhibitors, attachment repellants, anti-mussel agents and anti-barnacle agents without showing biocidal properties (Omae, 2006b).

There are studies on tannin, a polyphenolic compound naturally found in plant such as quebracho, tea leaves, and oak. Quebracho tannin were reacted with metal elements such as copper and zinc to produce antifouling pigment in antifouling paint formulation, and have a performance as good as conventional antifouling paint with less damaging effect to the environment and on non-target organisms (Perez *et al.,* 2006; Bellotti *et al.,* 2010).

Gambir (*Uncaria gambir*) is known as a common medicine plant which consisted of several polyphenolic and flavonoid components. It contains catechin as the most abundant polyphenolic constituent which known to have many medicinal properties such as remedies for diarrhea and sore throat (Taniguchi *et al*., 2007a) and bactericidal activity (Hoshino *et al*., 2000). Polyphenol derivatives of (+)-catechin and (–)-epicatechin showed repellent activities against the blue mussel, *Mytilus edulis* (Omae, 2006b). Gambir containing catechin also has shown antibacterial properties on the Gram-positive bacteria such as *Streptococcus mutans* (plaque formation), *Staphylococcus aureus,* and *Bacillus subtilis (*R. Pambayun *et al*., 2007 & Lucida *et al*., 2007*)*.

Previous studies have shown that catechin is able to interact and form complex with various metal element such as copper and aluminium (McDonald *et al*., 1996; De-song *et al*., 2004; Pollock *et al*., 2009; Karamac, 2009). The formation of catechin-copper(II) complexes resulted in an increase in the bactericidal activity of catechin against Gram-negative bacteria such as *Escherichia coli* (Hoshino *et al.,* 2000; Smith *et al.,* 2003). These findings suggested that catechin from gambir extract could be employed as bioactive pigments for new antifouling technologies.

This study aims to evaluate the potential of *Uncaria gambir* extract and Copper gambir complexes as an antifoulants agent in antifouling paint. The effectiveness of these compounds may contribute to the reduction of marine pollution and generate an eco-friendly antifouling paint. The objectives of the present study are as follows:

1. To determine the most effective solvent to extract phenolic compounds from *Uncaria gambir.*

2. To synthesise and characterize copper gambir complexes (CuG) by means of FTIR spectroscopy, AAS analysis, SEM-EDX and its solubility in artificial seawater at room temperature.

3. To determine CuG toxicity against brine shrimp larvae as control marine organism.

4. To formulate and characterize of antifouling paint with the inclusion of CuG as an antifouling component.

5. To evaluate the potential of CuG as antifouling pigment in antifouling paint by immersion test in real sea water.

CHAPTER 2

LITERATURE REVIEW

2.1 Biofouling

Biofouling is the accumulation of microorganisms, plants, algae, or animals on submerged surfaces which lead to the impairment or degradation of surfaces or equipment as a result of the growth of living organisms. According to Flemming and Ridgway (2009), the term “biofouling” is referred to as the adhesion of microorganisms to surfaces and biofilm development while Gademann (2007) described biofouling as non-specific attachment of biological material such as proteins, carbohydrates, prokaryotic cells and higher organisms to surfaces upon their exposure to any biological fluid.

Marine biological fouling is defined as the undesirable accumulation of organisms on surfaces immersed in seawater (Perez *et al.*, 2007). Organisms such as bacteria, protozoans, algae, and crustaceans can accumulate in large numbers on surfaces like pipes, tanks, and ships' hulls, resulting in corrosion, clogging, contamination, or a decrease in the efficiency of moving parts. The numerous fouling organisms may be divided into microfouling and macrofouling according to their size. Macrofouling community consisting of either 'soft fouling' or 'hard fouling' may develop and overgrow the microfouling (biofilm).

Soft fouling comprises algae and invertebrates, such as soft corals, sponges, anemones, tunicates and hydroids; whilst hard fouling consist of invertebrates such as barnacles, mussels and tubeworms (Callow and Callow, 2002)

The sequence of fouling states is generally considered in five main stages (Delauney *et al.*, 2010):

1) the first event is the adsorption of organic and inorganic macromolecules immediately after immersion, forming the primary film ;

2) second, the transport of microbial cells to the surface, and the immobilization of bacteria on the surface ;

3) in the third stage, the bacterial attachment to the substratum is consolidated through extracellular polymer production, forming a microbial film on the surface ;

4) the fourth stage corresponds to the development of a more complex community with the presence of multicellular species, microalgae, debris and sediments on the surface ;

5) the final stage is the attachment of larger marine invertebrates such as barnacles, mussels and macro-algae.

2.1.1 Microfouling

Biofouling begins with adsorption of organic molecules onto newly submerged surfaces, followed by the accumulation of bacteria, diatoms, and other microorganisms bound together in a film of extracellular polymeric substances. Biofilms develop on surfaces within hours of immersion, increasing in density and structural complexity over time (Zardus *et al.,* 2008). Bacteria are among the first organisms to foul surfaces as they form biofilms which is complex, clusters and three dimensional in nature and serve as a focus for the attachment and growth of other organisms, such as invertebrates, sessile plants, and animals. Biofilms can enhance larval settlement of marine invertebrates and attachment of algal spore (Prabhakaran *et al.,* 2012).

According to Chambers *et al.* (2006), when a chemically inert substrate is immersed in seawater, an almost immediate accumulation of organic carbon residues adsorb onto the wetted surface. The forces that promote the adsorption and conditioning of the surface include electrostatic interactions and Van der Waal's forces. Pioneering microorganisms can now attach to the surface forming a biofilm. Contact and colonization between the microorganism and the surface is promoted by the movement of water through Brownian motion, sedimentation and convective transport, although organisms may also actively seek out substrates due to propulsion using flagella.

Bacteria and other colonising microorganisms secrete extracellular polymeric substances (EPS) to envelope and anchor them to the substrate thereby altering the local surface chemistry which can stimulate further growth such as the recruitment and settlement of macroorganisms.

The biofilm generated is a mass of microorganisms and their EPS which creates a gel matrix as shown in Figure 2.1 which provide enzymatic interaction, exchange of nutrients, protection against environmental stress and an increased resistance to biocides.

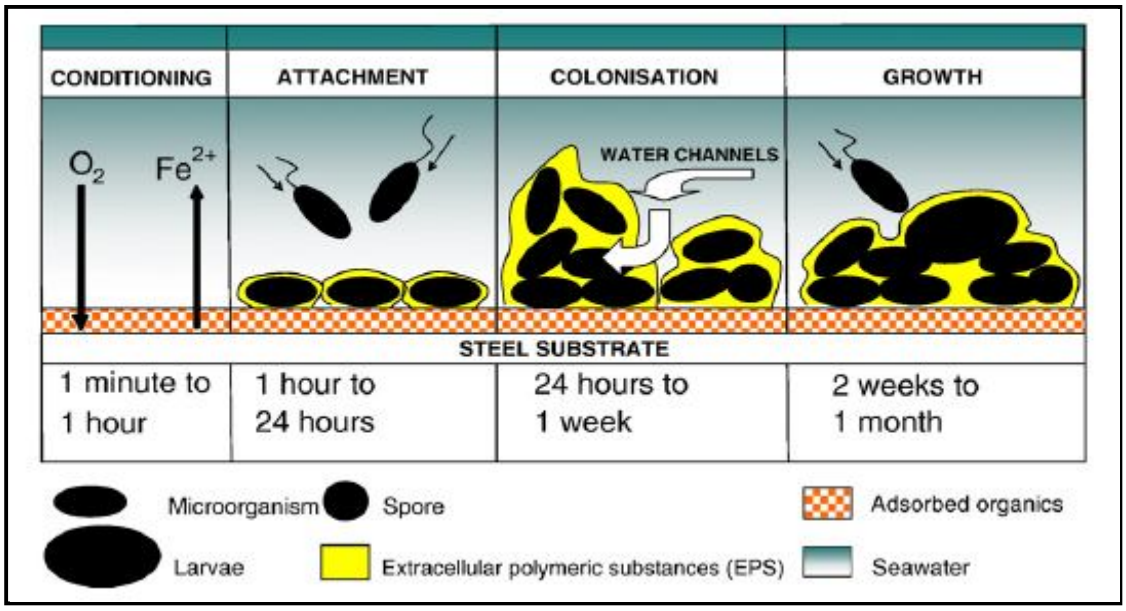


Figure 2.1 Schematic of biofilm formation (Chambers *et al.*, 2006)

2.1.2 Macrofouling

The fouling layer reaches the maximum development with the adhesion of marine animals such as Crustacea (Barnacles), Molluscs (Mussels and Clams), Anellids (Serpulids) and Coelenterates (Hydroids). The most important crustaceous fouling species is balanus, which has a planktonic larva that can produce a strong extracellular material at the surface called cement, and adheres strongly to many materials (Petrucci and Rosellini, 2005).

Mussels are considered the most characteristic macrofouling species. They are a bivalva species with two shells and form big colonies on a large number of materials and are the main species responsible for clogging of industrial pipes. Mussels use byssus threads composed mainly of collagen that has a hydrophilic polyphenolic adhesive protein which crosslinks in an oxidation–reduction reaction that occurs in the presence of an enzymatic catalyst. It is very difficult to destroy and detach mussel shells from pipe walls due to their strong adhesion (Petrucci and Rosellini, 2005; Callow and Callow, 2002). Barnacles adhere by using a hydrophobic protein which crosslinks using cysteine residues. At the critical larval developmental stage of the barnacle, known as cyprid, a temporary adhesive is used while exploring the surface for a place to settle and permanently adhere (Chambers *et al.*, 2006).

The green algae *Enteromorpha*, has a temporary and permanent attachment phase to its lifecycle. The motile zoospore stage can temporarily adhere in the same time actively searching for a suitable substratum. When the optimal substrate is detected, it

transforms into the immotile, settled cell phase which can permanently anchor itself and germinate producing a new plant. Spore germination often occurs within a few hours, giving rise to germlings. These are attached to the substratum by adhesive that is secreted by the rhizoids (Callow and Callow, 2002). The characteristic of macrofouling organism is depicted in Figure 2.2.

According to Yebra *et al.* (2004), the extent of biofouling depends on a several number of factors including water temperature, salinity, depth of waters and interactions between different microorganisms. Fouling is generally heavier in in regions with high water temperature, while reproduction of and growth of many species are completely suppressed at low-temperature period. For example, in tropical climates, where seasonal changes in conditions are relatively small, fouling may continue without interruption throughout the year, comparing to regions with variation in temperature due to seasonal changes. Most of common fouling organisms are unable to withstand low salinities which affect the growth rate, the maximum size attained, and causing several malformations. However, slime, algae, bryozoa and some species of macrofouling organism are commonly found in low-salinity waters.

Apart from that, it is also known that problem of fouling in deep waters is not as pressing as in coastal areas since bacteria and marine organism are much less plentiful in oceanic waters compared to coastal waters. This is related to amount of solar radiation which plays a very important role in the upper layers of the oceans and consequently for ship's fouling. Apart from influencing temperature and salinity, solar radiation also affects directly the rate if photosynthesis of the plants and thus control the nutrition of the animals.

Finally, the interactions between organisms also modify the process of fouling. Bacteria in host-associated biofilm cause significant mortality to their hosts by producing degradation of host tissue and increase the drag on their hosts. Bacteria and higher species may also compete for nutrients (Lehaitre *et al.*, 2005), block incident light, inhibit gaseous exchange and even secrete secondary metabolite that prevent the attachment.

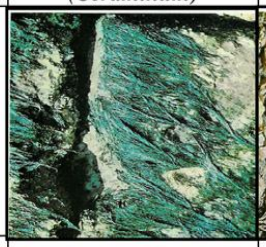
Groups	Algae (plants)	Invertebrates (animals)							
Subgroups	(a) green, (b) brown and (c) red	Hard shell organisms				Grass type organisms	Small bush organisms	Spineless organisms	
Designation	*(a) Enteromorpha, Ulva and Cladophora), (b) (Ectocarpus and Fucus, and (c) (Ceraminum)*	*Balanus*	*Barnacles*	*Molluscs*	*Fouling bryozoans*	*Hydroids or bryozoans*	*Hydroids or bryozoans*	*Ascidians*	*Sponges and sea anemones*
Example of typical aspect									
Designation	*Green algae*	*Balanus*	*Calcareous polychaetes*	*Molluscs*	*Fouling bryozoans*		*Bryozoans*	*Ascidians*	
Example of typical aspect									
Short description	Only plants that become attached to immersed surface: a) close to surface; b) at mid depth; and c) at depth	Attached trunco-conical or cylindrical crustaceans	Barnacles are Balanus that are fixed to surfaces via a stem	Bivalves containing a spineless animal in their interior	Calcareous incrustations that multiply from a central individual	Organisms that cover surfaces with an open grass or fur	Like bushes of several centimetres and with branches	Constituted by a spineless bag with two tubular openings or starry plates	Spineless and spongy aspect (sponges) and sea anemones

Figure 2.2 Characteristic of macrofouling organism (Almeida *et al.*, 2007)

2.2 Problem created by biofouling

Biofouling occurs everywhere but is most economically significant to the shipping industries, since high levels of fouling on a ship's hull results in an increase in roughness and significantly increases drag, reducing the overall hydrodynamic performance of the vessel and increases the fuel consumption. Increased fuel consumption, hull cleaning, paint removal and repainting, dry docking time and associated environmental compliance measures all contribute to the increased costs within the shipping industry (Callow and Callow, 2002; Chambers *et al.*, 2006; Dafforn *et al.*, 2011; Schultz *et al.*, 2011).

By comparison, macrofouling is generally of greater concern for offshore structures, ships and oceanographic instruments than microfouling due to its greater contribution to weight or hydrodynamic loading (Lehaitre *et al.*, 2005). In aquaculture, bio fouling problem areas include fouling on infrastructure (immersed structures such as cages, netting and pontoons) and stock species (farmed shellfish such as mussels, scallops and oysters). Biofouling greatly reduces the efficiency of materials and physically damages equipment causing abrasion, brittleness and increased load, thus flow can be significantly reduced directly reducing foods supply. The selling of biofouled shellfish is also affected on aesthetic grounds because the fouling is not compatible with product processing or packaging methods. Significant losses of cultures are also attributable to deoxygenation and degradation products when biofouling communities die or simply swamp the cultures/cages preventing oxygen and waste product exchange (Willemsen, 2005).

2.3 Antifouling protection

Antifouling is the process of preventing the accumulations of organisms from forming or attaching to a surface. Antifouling strategies are depending on the type of the object or surface that needs to be protected. Generally, moving objects and platforms rely upon antifouling coatings while condenser tubings and cooling water intake systems are protected via the addition of a biocide into the water column. The variety methods of antifouling in prevention of fouling phenomena are as following (Nandakumar and Yano, 2003):

1) antifouling coatings by means of toxic coating and non-toxic coating

2) treatments with biocide by means of oxidizing and non-oxidizing types

3) electrochemical methods by conductive paint electrode and low power pulsed laser irradiation

4) manual cleaning of the surfaces through physical and mechanical methods

5) temperature control

6) water flow according to the principle that shear stress of water exceeds the shear strength of the organisms

Among all the methods of antifouling listed, antifouling paints or coatings have been and remain the primary strategy and common methods for combating biofouling in the marine industry (Magin *et al.*, 2010; Willemsen, 2005).

2.3.1 Antifouling paint

Anti-fouling paint or bottom paint is a specialized coating applied to the hull of a ship or boat in order to avoid the growth of organisms that attach to the hull and can affect a vessel's performance and durability. Antifouling paints are generally consists of three parts; the polymeric binder, the water-soluble resin and toxic species (Nandakumar and Yano, 2003). The binder forms the matrix of the paint, which holds the paint components together and facilitates firm attachment to the surface. Antifouling paint or coatings are necessary in order to avoid the colonisation of surfaces by biofoulers and consequently the high costs relative to transport delays, hull repairs, cleaning of desalination units and biocorrosion (Maréchal and Hellio, 2009).

The earliest antifouling application recorded in history was in 1500-300 BC where the early Phoenicians developed the form of lead and coppers sheets to prevent biofouling on their wooden boats (Dafforn *et al.,* 2011). According to Readman (2006) and Callow and Callow (2002), the ancient civilizations of the Romans and the Greeks coated their vessels with lead sheathing secured by cooper nails while Columbus' ships are thought to have been coated with pitch and tallow. In the late 1600's, lead sheathing was replaced with the development of antifouling paints containing tar, grease, sulphur pitch and brimstone by the British Navy. One hundred years later, copper sheathing was used to prevent fouling through dissolution of the toxic metal ions.

In the mid 1800s, variety of paints was developed based on the idea of dispersing a toxicant in a polymeric vehicle (Yebra *et al.,* 2004; Dafforn *et al.,* 2011). One of the main reasons behind the development antifouling paint with toxicants was due to the

introduction of iron ships which copper sheathing caused corrosion of the iron (Readman, 2006). Paints were prepared by adding toxicants such as copper oxide, arsenic, and mercury oxide to various kind of binder including linseed oil, shellac, varnish, tar and resin, with the used of solvents included turpentine oil, naphtha and benzene (Yebra *et al.,* 2004).

During the 1950 to 1960 period, antifouling paints that were produced can be classified into insoluble matrix type and soluble matrix type according to their chemical characteristic of the binder and defined by their water solubility (Almeida *et al.,* 2007; Yebra *et al.,* 2004; Chambers *et al.,* 2006). For insoluble matrix paint, high molecular mass binders such as acrylic, vinyls or chlorinated rubber were used in paint formulation. These binders are insoluble in sea water and does not polish or erode after immersion in water. Due to their good mechanical strength characteristics, they can contained high toxicant amounts, whose particles can be in direct contact with each other and gradually released into sea water. The species dissolved by the sea water penetrating into the film have to diffuse through the interconnecting pores formed after dissolution of toxicant particles (Yebra *et al.,* 2004). These types of paint are generally resistant to atmospheric exposure in non-aqueous environment such as oxidation and photodegradation with 12-18 months of lifetime.

As for soluble matrix paint, rosins based binder were used to incorporate toxic pigments such as copper, iron, zinc oxides and previously arsenic and mercury. In contrast with insoluble matrix paint, this type of paint is soluble in sea water, with poor mechanical strength and only allows the inclusion of low concentrations of toxicant and the

application of relatively fine films. As a result of constant erosion in service, soluble matrix paint does not assure protection for more than 12-15 months.

Their main advantage is that they can be applied on smooth bituminous-based primers (Almeida *et al.,* 2007). The working schemes of soluble and insoluble matrix paints were shown in Figure 2.3 (Chambers *et al.,* 2006).

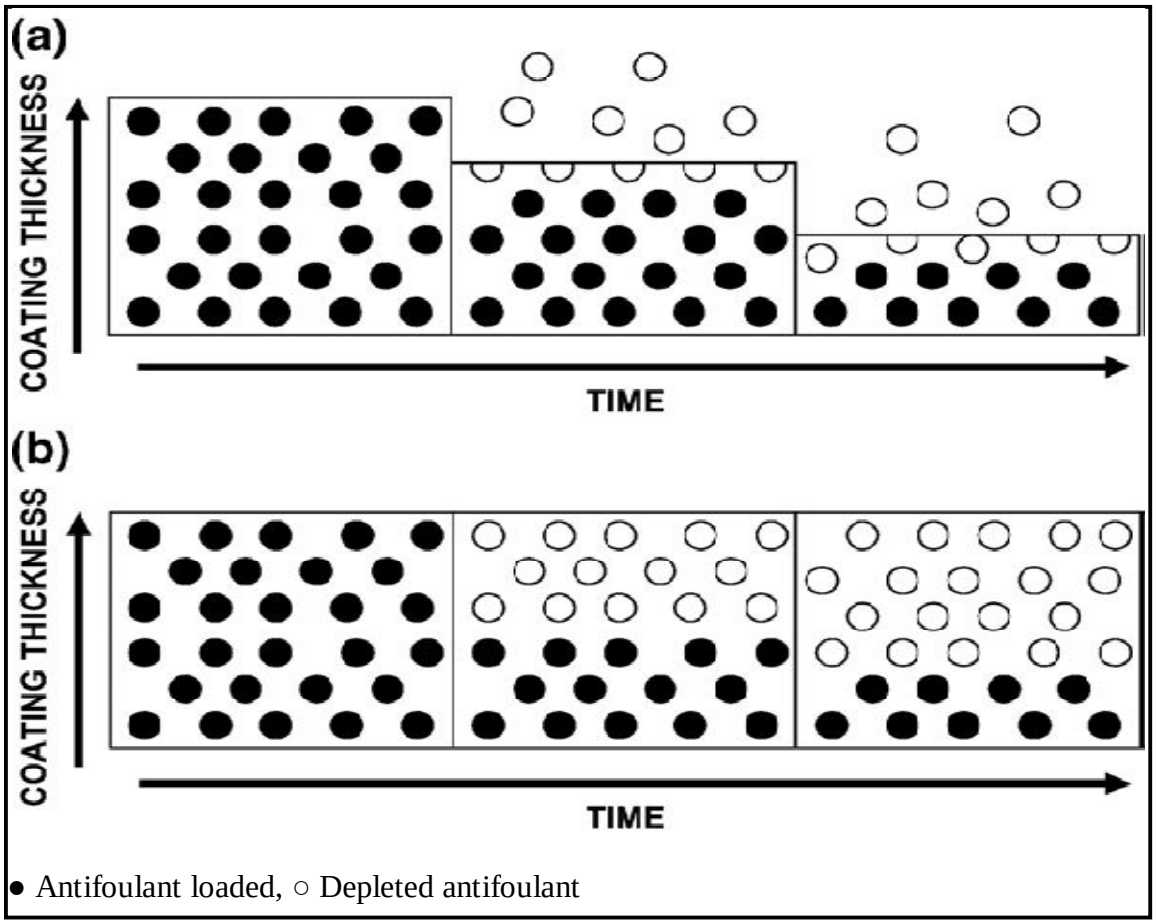


Figure 2.3 Schematic of (a) soluble matrix paint and (b) insoluble matrix paint (Chambers *et al.,* 2006)

The discovery of self-polishing copolymer (SPC) antifouling paints was made in the 1970s. It was also deemed as the discovery of polymers with very high performances as

the paints containing antifoulant and was used for a long time. These polymers allowed a constant release rate and maintained a low frictional resistance to seawater by keeping a polished smooth surface (Omae, 2006a). Tributyltin (TBT) self-polishing paints are based on an acrylic copolymer (normally methyl methacrylate) with TBT groups bonded to the main polymer backbone by an ester linkage (Yebra *et al.*, 2004) in which the polymer is soluble in seawater. According to Almeida *et al.* (2007), apart from the toxicants that react inside the copolymer, these paints include toxicant pigments, such as copper oxide, and thus present highly efficient antifouling properties in any service conditions at sea. After immersion, the soluble pigment particles come into contact with the sea water and start to dissolve.

Unlike insoluble matrix paints, in this type of paint, where the TBT methacrylate and methyl methacrylate copolymer is hydrophobic, the water is prevented from penetrating the film. Thus, the sea water barely manages to fill the pores created by the dissolution of the soluble pigment particles. In time, sea water dissolves more pigment particles, causing the releasable area to grow and making the copolymer film brittle and easily erodible by sea water, leaving a new fresh layer of active paint uncovered for subsequent release (self-polishing effect) (Dafforn *et al.*, 2011). Figure 2.4 shows the diagrammatic representation of self-polishing copolymer (SPC) paint working scheme. Among the advantages of this type of paint is the fact that it is not necessary to remove their porous residues, in contrast to soluble or insoluble matrix paints, or to apply a sealer or tie coat during recoating operations (Almeida *et al.*, 2007). SPC antifouling paints have a longer lifetime in comparison with soluble and insoluble matrix paints.

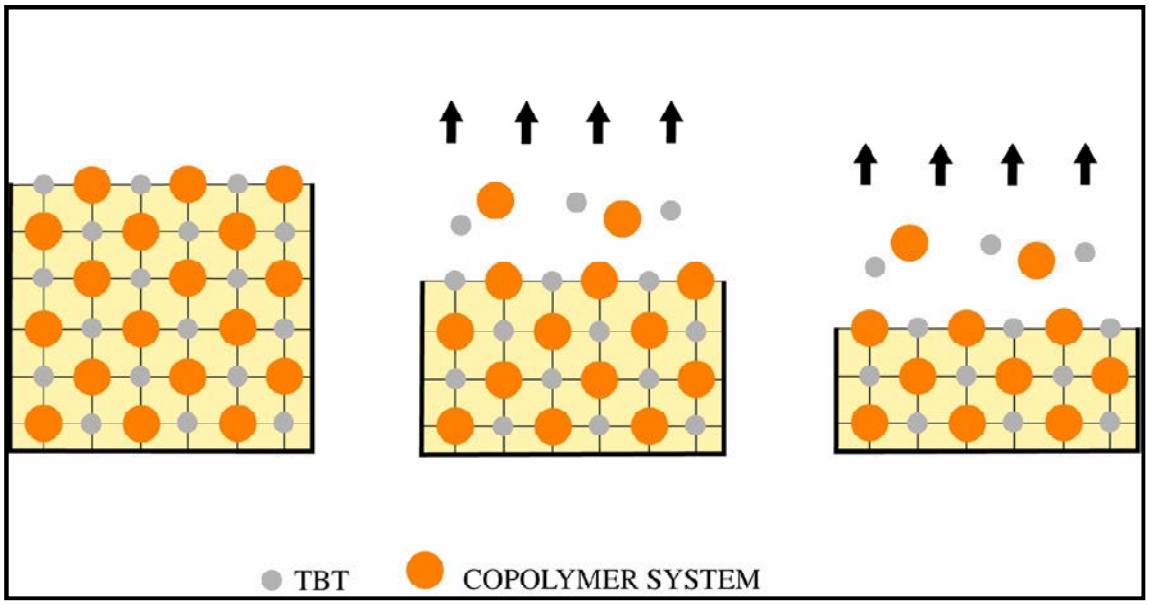


Figure 2.4 Diagrammatic representations of self-polishing copolymers (SPC) paint (Omae, 2003b)

2.3.2 The biocides: Tributyltin

Tributyltin compounds (TBT) are a group of compounds containing the $(C_4H_9)_3Sn$ moiety, such as tributyltin hydride or tributyltin oxide. Tributyltin oxide (TBTO) is an organotin compound that is mainly used as biocide and marine anti-biofouling agent. TBT derivatives that were recently used as a biocide in antifouling paint include tributyl tin fluoride (TBTF), triphenyl tin fluoride (TPTF), and triphenyl lead acetate (TPLA) (Nandakumar and Yano, 2003).

The discovery of TBT compounds was made in 1950s by the research group of Van der Kerk in Netherlands (Omae, 2006a; Yebra *et al.*, 2004). Tributyltin was first used in freshwater systems to eradicate molluscs harbouring the parasitic worm *Schistosoma*

and from the early 1960s TBT was introduced as a biocide in marine antifouling paints (Dafforn *et al.,* 2011)

TBT was initially incorporated in conventional or 'free association' paints, which relied on passive leaching from a soluble matrix or contact leaching to deliver surface toxicant to inhibit growth. In the beginning, organotin compounds were used as co-toxicants in high performance copper paints, but gradually came to be used as in all-organotin systems (Yebra *et al.,* 2004). Conventional paints had a limited lifetime of around two years and were superseded in the 1970s by self-polishing copolymer (SPC) paints with lifetime of more than 5 years (Dafforn *et al.,* 2011).

In SPC paints the TBT copolymer provides both the biocide and paint matrix. Self-polishing copolymer indicates the 'polishing' effect as the polymer dissolves away during normal vessel operation, TBT were released and killed settling fouling organisms and, at the same time, the surface becomes smoother. Being very lipid soluble, it is rapidly taken up by cells, where it inhibits energy transfer processes in respiration and photosynthesis (Callow and Callow, 2002).

During the late 1950s and 1960s periods, a new formulation using TBT proved to be excellent in fouling prevention. The efficiency of TBT, especially in "self-polishing" formulations, was remarkable and extremely successful, and the application of TBT-based paints rapidly expanded. Apart from fact that it did not cause galvanic corrosion on aluminium hulls, TBT was colourless, and periods between dry-docking were extended (Readman, 2006). However, TBT was shown to effect non-target organisms including a number of shellfish.

In 1974, the occurrence of abnormal shell growth in *Crassostrea gigas* (the Pacific oyster) along the east coast of England was first reported. However, it was not until the mid 1980s that researchers in France and the UK began to suggest that the use of TBT in the antifouling paints was adversely impacting a number of marine species other than the fouling organisms. The most sensitive invertebrate species, the dog whelk, *Nucella lapillus*, exhibits imposex (imposition of male sexual characters on the female) at TBT concentrations below 1 ng l^{-1}, and its disappearance from rocky shores in areas of high boating activity has also been attributed by the presence of TBT from antifouling paints (Omae, 2006a; Callow and Callow, 2002).

Legislation to ban the use of the organotins for small boats was first introduced in France in 1982 and followed by the UK in 1987. Similar legislations have been introduced worldwide since then, and led to a significant fall in the concentrations of organotins in seawater. The "International Convention on the Control of Harmful Antifouling Systems on Ships" adopted on 5 October 2001 by the International Maritime Organization (IMO) stated that: by January 1st, 2003, a ban on the application of TBT-based antifouling paints should be introduced, where all ships should not apply or re-apply organotin-containing antifouling paints; and January 1st 2008 is the last date for having a TBT-based antifouling paint on a vessel and all ships shall not bear such compound on their hulls. The Maritime Environmental Protection Committee (MEPC) also proposed that the IMO promotes the use of environmentally safe antifouling technologies to replace TBT (Omae, 2006a; Fent, 2006).

2.3.3 Alternative approaches for tributyltin (TBT) replacement

For many years tributyltin (TBT) was the preferred biocide used in antifouling paints. However, in the 1980s it has been proved that its continued use causing severe damage to shellfish communities and dog whelk populations. Therefore, in 1987, European countries had banned the use of TBT in antifouling paints on boats. The subsequent total global banned in 2008 on TBT-based coating by International Maritime Organization (IMO) led the antifouling paint industry into a very uncertain situation.

Copper compound such as cuprous oxide (Cu_2O), copper thiocyanate (CuSCN) and metallic copper were utilized as the principal biocide in the absence of the antifouling potency of TBT (Nandakumar and Yano, 2003 and Callow and Callow, 2002). Copper is an essential element required for the growth of all plants and animals. It occurs commonly in the environment and exhibits antifouling activity against organisms such as barnacles, tube worms and the majority of algal fouling species. However, copper-based antifouling paint also produces effects on marine environment and non-target organism (Katranitsas *et al.*, 2003). According to Voulvoulis *et al.* (1999), high concentrations of copper in water can caused a range of deleterious sublethal effects to algae, invertebrate phyla and other aquatic biota. Over the period of 1979-1991, oysters monitoring in Arcachon bay (on the Atlantic coast of France) demonstrated an increase in the copper content of oysters (*Crassostrea gigas*) in the vicinity of marinas and mooring areas. This phenomenon was caused by the growing use of copper-based paints (Voulvoulis *et al.*, 1999).

Although copper was regarded as the predominant antifouling biocide since the ban on TBT, the effectiveness was relatively short-lived, therefore dry dockings of vessels for cleaning and paint reapplication were required frequently (Dafforn *et al.,* 2011). In addition, copper biocides ecotoxicity, durability and cost factors might be considered as interim solutions rather than real alternatives for TBT (Raveendran and Mol, 2009).

There was also the development of non-toxic silicone-based coatings, known as fouling-release coatings as an alternative to biocide-containing paints. This type of coatings do not prevent settlement of fouling organisms, but rather to minimize the adhesion strength of attached organism such that when the vessel is in motion through the water, any accumulated biofouling will slough off as drags increases (Figure 2.5). The removal of biofouling on vessel can also be done through special cleaning procedures. Macroalgae and some hard foulers such as barnacles detach relatively easily from such surfaces, but diatom slimes, oysters and tubeworms are attached tenaciously and are not easily removed, even at high speed. Silicone elastomers are also expensive and prone to tearing, so are only employed at the present time for specific applications, such as on high speed or high activity vessels where release of biofouling is effective and in locations where toxic paints are prohibited (Callow and Callow, 2002; Dafforn *et al.,* 2011)