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Toxicological Study of Malic Acid-Propane 1, 2-Diol and Malic Acid-Propane 1, 2-Diol-Glycerol Copolyesters on Brine Shrimp Nauplii

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Malic acid-propane 1, 2-diol and malic acid-propane 1, 2-diol-glycerol copolyesters are synthetic polymers and it was reported that both of them are good enteric coating materials. The research work was conducted to investigate the toxicological study of both compound. The LC_{50} values on brine shrimp nauplii of malic acid-propane 1, 2-diol and malic acid-propane 1, 2-diol-glycerol copolyesters were found to be 5.00 $\mu g\ ml^{-1}$ and 5.62 $\mu g\ ml^{-1}$, respectively. It is slightly higher toxic than standard ampicillin trihydrate on brine shrimp nauplii.

Key words: Malic acid-propane 1,2-diol copolyester, malic acid-propane 1,2-diol-glycerol copolyester, brine shrimp lethality bioassay, toxicity

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Introduction

Recently there has been an increasing interest for the development of biodegradable polymer matrices for controlled and sustained release of drugs because such degradable carriers have the advantage of eliminating the necessity of their removal. Many of the existing biodegradable carriers are linear polymers (Heller, 1980), such as polylactic acid, polyglycolic acid and their copolymers (Pramanick and Roy, 1990) which are biodegradable and are being used for the specialized application, such as controlled release drug formulation (Hopfenberg, 1976; Rosenberg *et al*., 1983; Graham, 1978; Plimmer *et al*., 1977; Devi and Vasudevan, 1985) , insecticide and pesticide carriers as well as non toxic surgical implant materials. A cross-linked polymer with three dimensional network is expected to have a better control over the release of drugs from its molecular domain. A common prerequisite for all such polymer matrices is that they must biodegrade to nontoxic products to avoid adverse body reaction. Keeping the same view ahead we have attempted to synthesize polymers from malic acid-propane 1,2-diol and malic acid-propane 1, 2-diol-glycerol. It was reported (Bakr *et al*., 2000; Karim, 1995) that the compounds are good enteric coating materials.

To evaluate the toxicity level of the synthesized compound, studied the toxic effect on brine shrimp nauplii. Brine shrimp lethality bioassay is a recent development in the toxicity study for a compound (Meyer *et al*., 1982). Pharmacology is a simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Thus *in vivo* lethality in a simple zoological organism can be used as a convenient monitor for the screening and fractionation of synthetic compounds. The eggs of brine shrimp *Artemia selina* (Leach) are readily available at low cost and they remain viable for years in the dry state. Upon being placed in sea water, the eggs hatch within 48 hours to provide large number of larvae for experimental uses.

From the experiment, the LC_{50} values of experimental compounds and standard ampicillin trihydrate were determined. Ampicillin trihydrate, a widely used antibiotic, was taken as a marker compound.

Materials and Methods

Synthesis of copolyester: Malic acid and propane 1,2-diol in stoichiometric ratio together with p-toluene sulphonic acid as catalyst (approximately 0.4% of the total weight) were taken in a 100ml beaker. They were mixed well with a stirrer and the beaker was placed in a reaction vessel under vacuum to eliminate water as a by product. The reaction mixture was heated at 100-120°C for 4 hours until bubbling ceased indicating completion of the reaction. Heating was continued for one hour under the same condition (Pramanick and Roy, 1980). This polymer was solid at room temperature and was stored in a desiccator. In this way, another copolyester of the same reactant along with trace amount of glycerol was synthesized and stored. Synthesis and characterization of the copolyester has already been reported (Bakr *et al*., 2000; Karim, 1995).

Determination of toxic activity: The toxic effect of malic acid-propane 1, 2-diol and malic acid-propane 1, 2-diol-glycerol copolyesters and ampicillin trihydrate was determined by brine shrimp lethality bioassay (Meyer *et al*., 1982; Mclaughin and Anderson, 1988; Mclaughlin, 1990; Persoone, 1980).

Preparation of Sea water: 38 grams sea salt (NaCl, non-iodized) was weighed accurately, dissolved in distilled water to make 1000ml and then filtered off. The pH of seawater was maintained between 8 and 9 using $NaHCO_3$ (Meyer *et al*., 1982; Mclaughein and Anderson, 1988; Mclaughlin, 1990; Persoone, 1980).

Hatching of Brine shrimp eggs: Seawater was kept in a small two-chamber tank separated by a net which would allow movement of shrimp only. Shrimp eggs were added (1.5 gmL^{-1}) to one chamber of the tank. Constant temperature (35 ± 2)°C, sufficient light and oxygen supply were maintained to give the sufficient aeration. After 48 hrs matured shrimp as nauplii was collected and used for the experiment (Meyer *et al*., 1982, Mclaughin and Anderson, 1988, Mclaughlin 1990; Persoone 1980).

Table 1: Results of malic acid-propane 1,2-diol and malic acid-propane 1,2-diol-glycerol copolyesters and ampicillin trihydrate on brine shrimp lethality bioassay

Conc. of sample (C) $\mu g\ ml^{-1}$	log C	Number of applied Shrimps in 1st, 2nd	Number of survivors in 1st 2nd and 3rd vival	% of mortality in 1st, 2nd and 3rd vival	Mean value of mortality	LC_{50} $\mu g\ ml^{-1}$
Malic acid-propane 1,2-diol copolyester						
5	0.7	10, 10, 10	6, 5, 4	40.0, 50.0, 60.0	50.0	
10	1.0	10, 10, 10	4, 5, 3	60.0, 50.0, 70.0	60.0	
20	1.3	12, 12, 10	3, 4, 3	75.0, 66.7, 70.0	70.5	5.00
40	1.6	10, 10, 11	3, 2, 3	70.0, 80.0, 72.7	74.2	
80	1.9	10, 10, 10	1, 2, 3	90.0, 80.0, 70.0	80.0	
Malic acid-propane 1,2-diol-glycerol copolyester						
5	0.7	10, 11, 10,	4, 5, 5	40.0, 54.5, 50.0	48.1	
10	1.0	10, 11, 10	4, 5, 3	60.0, 54.5, 70.0	61.5	
20	1.3	11, 10, 11	4, 3, 3	63.6, 70.0, 72.7	68.7	5.62
40	1.6	10, 10, 10	2, 2, 3	80.0, 80.0, 70.0	76.6	
80	1.9	10, 10, 11	0, 2, 2	100.0, 80.0, 81.8	87.2	
Ampicillin trihydrate						
5	0.7	10, 10, 10	5, 6, 5	50.0, 40.0, 50.0	46.7	
10	1.0	10, 10, 10	5, 5, 4	50.0, 50.0, 60.0	53.3	
20	1.3	10, 10, 10	4, 3, 3	60.0, 70.0, 70.0	66.7	6.31
40	1.6	10, 10, 10	2, 2, 3	80.0, 80.0, 70.0	76.7	
80	1.9	10, 10, 10	2, 1, 3	80.0, 90.0, 70.0	80.0	
Control	0.0	13, 10, 11	13, 10, 11	00.0, 00.0, 00.0	00.0	

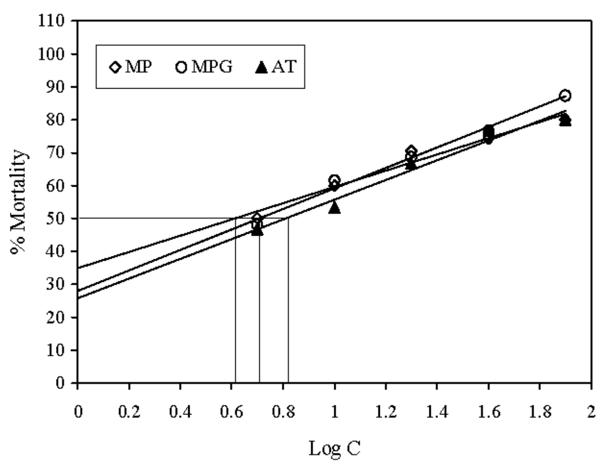
Preparation of test samples: At 3mg of each sample (Malic acid-propane 1,2-diol and Malic acid-propane 1,2-diol-glycerol copolymers and Ampicillin trihydrate) were weighed accurately and dissolved in 0.6ml dimethyl sulfoxide (DMSO) to get the concentration of 5μg μL^{-1}.

Application of the test sample and brine shrimp nauplii to the vials: The experiment was done into 5 groups. Each group contained 3 vials containing approximately 10 nauplii in 5ml sea water. The concentration of the sample in each vial of the group was made 5, 10, 20, 40 and 80μg ml^{-1}. The same assay procedure was carried out for the standard ampicillin trihydrate. For control group, 3 vials containing approximately 10 brine shrimp nauplii in 5ml sea water with 20 μL DMSO to each vial.

Counting of nauplii: After 24 hours the vials were observed and the number of survived nauplii in each vial were counted using magnifying glass and recorded. From the data the percentage mortality of brine shrimp nauplii was calculated at each concentration of the experimental samples, standard and control groups.

Results and Discussion

The results of toxicity of experimental samples and standard ampicillin trihydrate on brine shrimp are shown in Table 1. The



MP = Malic acid, Propane 1, 2-diol copolyester
MPG = Malic acid, Propane 1, 2-diol, Glycerol copolyester
AT = Ampicillin Trihydrate

Fig. 1: Brine shrimp lethality bioassay

experimental and standard samples showed that different mortality rates at different concentrations and it was found that the mortality rate of brine shrimp nauplii was increased with the increase of the concentration of samples. A graph of logarithm of concentration versus percentage of mortality was plotted and a best fitted curve was drawn which shown an almost linear correlation. The LC_{50} values were calculated (Goldstein *et al.*, 1974) by extrapolation of graph (Fig. 1).

The LC_{50} values of malic acid-propane 1, 2-diol and malic acid-propane 1,2-diol-glycerol copolyesters and ampicillin trihydrate were found to be 5.00, 5.62 and 6.31μg ml^{-1} respectively. So, it is clear that the experimental compounds are more toxic on brine shrimp nauplii than the standard compound (Meyer *et al.*, 1982). We also infer that malic acid-propane 1, 2-diol copolyester is a more toxic than malic acid-propane 1, 2-diol-glycerol copolyester. The presence of glycerol in the copolyester of malic acid and propane 1,2-diol reduces the toxicity. Therefore, extensive toxicological study is necessary before using such compounds as a carrier material.

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