بسم الله الرحمن الرحيم

LEACHING FROM PLASTIC CONTAINERS

BY

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(B. PHARM., UNIVERSITY OF KHARTOUM, 1998)

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Dedicated
To
My family

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الرشح من العبوات البلاستيكية

أطروحة مقدمة من: نهلة محمد أحمد عثمان بلية

(بكالوريوس الصيدلة- جامعة الخرطوم 1998م)

لنيل درجة الماجستير في الكيمياء التحليلية – قسم الكيمياء والعقاقير الطبية

كلية الصيدلة –جامعة الجزيرة

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المستخلص

تستخدم عدة أنواع من العبوات البلاستيكية المصنعة من مواد مختلفة في مجال الصناعة الدوائية مثال لذلك قوارير المحاليل الوريدية كما تستخدم في تعبئة المياه والمواد الغذائية والمشروبات. في الآونة الأخيرة كثر استعمال العبوات البلاستيكية في السودان. بجانب الأوعية الصيدلانية فإنها تستعمل لحفظ الاحتياجات المنزلية مثل الطعام ومياه الشرب مع وجود مواد سامة مختلفة قد تفرزها الأنواع البلاستيكية المختلفة .

في هذه الدراسة تم تحليل مياه منزوعة الايونات حفظت في أنواع مختلفة من الاوعية البلاستيكية وتحت ظروف تخزين مختلفة. كانت الملاحظة الرئيسة أن المياه في كل أنواع العبوات البلاستيكية (بولي ايثلين تريثليت في شكل قوارير أدوية ومياه، بولفينيل كلوريد في شكل قوارير محلول بروتوني ، بولي بروبيلين في شكل أوعية محاليل وريدية وبولي ايثلين في شكل أكياس) المستعملة تحت كل الظروف التخزينية (تجميد، درجة حرارة ثابتة، تبريد، درجة حرارة الغرفة وأشعة الشمس) أظهرت خاصية امتصاص للأشعة الفوق بنفسجية. أخذ هذا كدليل واضح على أن بعض المواد الممتصة للأشعة فوق البنفسجية قد رشحت. أتضح أن أشعة الشمس المباشرة تساعد على عملية الرشح هذه مما يؤكده انخفاض رقم الحموضة مع وجود مواد قابلة للأكسدة أو وجود ملح أمونيوم حسب نوعية البلاستيك المستعمل. لهذا فانه ينصح بوضع المواد المخزنة في أوعية بلاستيكية بالأخص البولفينيل بعيدا عن أشعة الشمس.

وجد أن المياه المخزنة في كل الأوعية البلاستيكية ومحفوظة في الثلاجة تحتوى ايونات رصاص بتركيز اكبر من العادي عدا القوارير الدوائية. وجد أن المياه المخزنة في أكياس البولي ايثلين تحتوي على 90 ميكروجرام/ لتر و190 ميكروجرام/ لتر عند التخزين في الثلاجة أو تحت أشعة الشمس على التوالي. من المعروف أن الرصاص من المواد السامة. عليه يجب اخذ ذلك في الاعتبار عند اختيار أوعية تخزين المياه والطعام.

أوضحت نتائج تحليل كل الأوعية البلاستيكية التي تم اختبارها أنه لايمكن تعقيمها عدا البولي بروبيلي(اوعية المحاليل الوريدية) وقد وضح ذلك من تغير شكلها الطبيعي بدرجة الحرارة. نتج عن محاولات التعقيم حي اد أو اختلاف بعض الصفات عن المواصفات لبعض الاختبارات مثل اختبار المواد المختزلة وذلك في المياه المعقمة في البولي ايثلين تريثليت (القوارير الدوائية).

أوضحت هذه الدراسة انه يجب التدقيق عند اختيار نوعية الأوعية البلاستيكية وكذا وسط التخزين إذا كانت هذه الأوعية سوف تستعمل لحفظ المواد الصيدلانية أو المياه أو المشروبات الأخرى . ويجب الامتناع عن استعمال البولي ايثلين كأوعية للطعام أو المياه.

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Abstract

Different types of plastics are used as containers for pharmaceuticals e.g. intravenous infusions and for food e.g. beverages. Plastic containers are now extensively used in Sudan. In addition to pharmaceuticals , they are used to keep household products, drinking water, food etc. Different types of toxic materials are known to leach out of different types of plastics. In the present study analysis of deionized water kept in different types of plastic containers at different storage conditions was conducted. The major observation was that water stored in all types of plastics used (PET drugs & water bottles, PVC, PP and PE) at all conditions (freezer, stability chamber, refrigerator, room temperature and sun light) was found to exhibit U V absorption. This is a clear indication that some U V absorbing materials leached out. Sun light seems to affect the process of leaching since storage under direct sun light resulted in water turning acidic or with oxidisable substances or with ammonium, depending on the type of plastic used. It is recommended that materials stored in plastic containers especially PVC should be stored away from sun light.

Higher than normal lead content was detected in water stored in all plastic containers (except drug bottles) kept in the refrigerator. Water stored in PE showed a higher concentration of lead (90µg/L) when stored in the refrigerator and even a higher concentration (190µg/L) when stored under direct sun light. Utmost care should be taken in choosing containers to store water and food, since lead is known to be toxic.

Result of analysis of the plastic containers revealed that all types tested (except PP infusion bottles) could not be sterilized. They showed physical changes upon sterilization. Sterilization by heat resulted in deviations from specifications in some tests e.g. reducing substances in PET drug bottles.

The present study clearly shows that care should be taken in selection of plastic type and storage condition, if plastic containers are to be used for pharmaceuticals or food. We should avoid using certain types of plastics, particularly PE as a container for food or water.

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List of Abbreviations

BBP	Butyl benzyl phthalate
BPA	Bisphenol A
CHEC	Children's health environmental coalition
DBP	Di-n-butyl phthalate
DEHP	Di- ethylhexyl phthalate
DEP	diethyl phthalate
DCHP	Dicyclohexyl phthalate
DHP	Di-hexyl phthalate
DINP	Di- isononyl phthalate
DNA	Deoxy ribonucleic acid
DPP	Di-n-pentyl phthalate
DprP	Di-propyl phthalate
EDs	Endocrine disruptors
EPA	Environmental protection agency
FDA	Food and Drug Administration
HDPE	High-density polyethylene
MEHP	monoethylhexyle phthalate
PCB	Polychlorinated biphenyl
PE	polyethylene
PET	Polyethylene terephthalate

PP	polypropylene
PVC	Polyvinyl chloride
RNA	Ribonucleic acid
TNPP	Tris (nonylphenyl) phosphite

1- Introduction

Plastic is a polymer which is a Greek word (poly= many, mer= part). Polymers are substances composed of molecules (monomer) containing one or more species of atoms linked to each other covalently (Holum,1968; Pharmaceutical codex, 1994). For example, polyethylene which is the material for packaging plastic, is derived from natural gas or a fraction of crude oil. (Goettlich, 2001).

1.1-Polymers are classified as :

1.1.1-Natural: such as DNA (deoxy ribonucleic acid), RNA(ribonucleic acid) , proteins, and cellulose. In 1800's scientists and inventors began to convert them into useful products.

1.1.2-Synthetic: Large scale production of polymers began in 1909 by Leo Bakeland.

If a polymer chain is cross-linked, the product will be more rigid and resistant to heat (thermo set) such as bowling balls, formica table tops, auto body parts and kitchen utensils. Thermoplastic polymer could be heated and remolded many times. Polyethylene, nylon, polyester, polyvinylchloride, and polystyrene, are thermoplastics used for clothing and plastic packaging material (bottles and bags). The two types differ in properties because thermoplastic polymer has long unconnected chains (randomly coiled) while thermo set polymers have many cross – links which occur by hydrogen bonding and lead to a very rigid structure.

1.1.2.1- Addition polymers: These are formed from alkenes. They are made from units (monomers) which are made of carbon and hydrogen atoms. Oxygen, halogen, and nitrogen atoms can be represented. Typical monomers are ethylenes (CH2 =CH2) eg: vinylchloride (CH2 =CHCL). These are linked together without loss of atoms. Monomers have C=C bonds which open up as the monomers join in chains. Ethylene molecules can join together under the proper condition of temperature, pressure and catalyst to form polyethylene (Figure1).

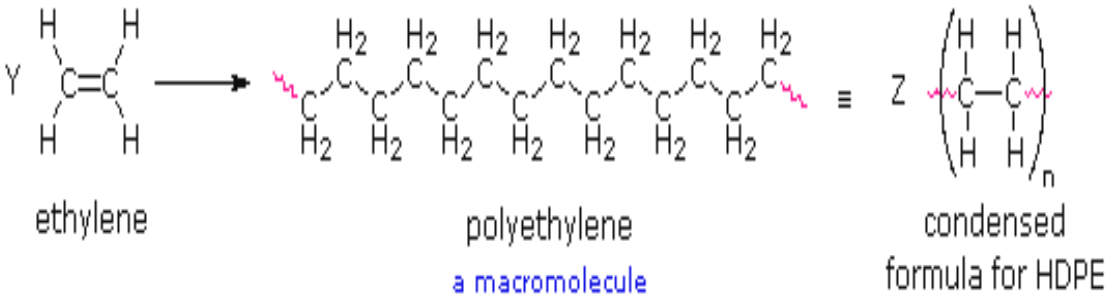


Figure1: Production of polyethylene (High density polyethylene)

This polymerization reaction is initiated in the presence of substances that have an unpaired electron (free radical). When free radicals react with a double bond , a chain reaction occurs (Holum,1968; Orana,1994)

$$CH_2=CH_2 + RO^{\cdot} \rightarrow RO{-}CH_2{-}CH_2^{\cdot} \xrightarrow{CH_2=CH_2} RO{-}CH_2{-}CH_2{-}CH_2{-}CH_2^{\cdot}$$

(Orana,1994)

Some polymerization occurs by means of carbonium ion which a catalyst that helps to generate as follows:

Catalyst + CH2 =CH2 → catalyst─CH2 ─CH2 + + CH2 =CH2 → catalyst─CH2 ─CH2 ─CH2 ─CH2 +

(Holum,1968)

1.1.2.2- Condensation polymers: These are formed from aromatic compounds, acid chlorides, carboxylic acids, and amines . They are formed by reaction of monomers. This reaction eliminates small molecules such as water or hydrochloric acid. There must be at least two functional groups on each reacting monomer.

1.2-Uses of polymers in medicine:

- Dacron Fabrics have been used in surgery to replace blood vessel segments.
- Nylon is used in specialized tubing and nylon sutures. (Orana, 1994).

Polyethylene is essentially a mixture of very- long-chain alkanes that makes it useful in synthesis of a variety of plastic house hold articles, laboratory apparatus, and hospital equipment. (Holum,1968).

In synthesis of plastics, ethylene is combined with solvents, co-monomers, additives, and other chemicals then polymerized to give the polymer which is extruded, palletized, or flocked to give what is called resin. The resin is sold, re-extruded and made into containers, films, and other products. Six resins types are used to make more than 92%of plastic packages (Goettlich, 2001).

1.3-Safe plastics:

- Polyethylene: Have low water absorption, excellent electrical resistance and high resistance to most solvents and chemicals. They are widely used in pharmaceutical industry and in hospitals as containers for liquid or dry products, sterile devices packaging and to molded parts for a variety of devices and equipment. e.g.: polyethylene terephthalate, high density polyethylene and low density polyethylene.
- Polypropylene: They could be used in every application for which polyethylene is used. Devices made of this type could be autoclaved for sterilization.

1.4-Plastics to be avoided:

Some carcinogenic effects occur due to additives added during manufacturing of these types. Such substances can leach and have been the main causes of adverse effect e.g: haemolysis of blood cells, thrombosis, hypersensitivity reactions, precancerous changes, and local tissue necrosis (Martindale, 2007).

- Polyvinyl: include vinyl chloride (the second most commonly used plastic in the world),vinyl acetate and vinylidene chloride. These are used to prepare materials ranging from soft flexible sheeting, to rigid hard tubing. They are characterized by high temperature resistance with softening points(70 -180 °C or higher), high solvent and chemical resistance, low water absorption and moisture permeability and non flammability. They are used in pharmacy and medicine fields as in the manufacture of blood bags and intravenous solution containers. The unplasticized form is used in fabrication of rigid parts for devices and administration equipments.
- Polystyrene: Used for fabrication of containers and syringes. They have low heat resistance.

- Polycarbonate: They are strong and have high temperature resistance. They have hardness properties similar to those of metals and can replace metals in numerous industrial applications(Remington's,2005).

1.5-Ways to reduce exposure to plastic toxins:

- Avoid processed foods and chemical additives.
- Eat only organic fruits and vegetables.
- Don't microwave food in plastic containers, use glass or ceramics.
- Filter home drinking water.
- If you eat meat and dairy, eat only organic meats and dairy, preferably grass-fed not grain-fed.
- Cosmetics and personal health care products can contain harmful chemicals so use only natural products and use less of these.
- Don't use artificial fragrances and perfumes.
- Avoid inhaling gasoline fumes - sit inside the car with the door closed.
- Eat seafood known to be low in polychlorinated biphenyl (PCB) and mercury contamination. Avoid most canned tuna - they contain unusually high levels of mercury. (Delicious organics,2005)

1.6-Reuse of water bottles:

The water and soft drink bottles are made of polyethylene terephthalate and are intended for single use. But some people reuse them. Frequent washing accelerate the breakdown of the plastic causing leaching of harmful chemicals into the water. Example of these chemicals is diethylhexyl adipate (plasticizer) which causes cancer in humans. People should be advised against reuse and that plastic bottles be recycled after a single use. People should also avoid softer opaque bottles for any liquid because they may leach chemicals even before washing. Freezing water bottles may cause plastic to leach into water (delicious organic,2005)

1.7-Closures for pharmaceutical bottles:

Closures for pharmaceutical bottles may take the form of threaded screw caps, which may be child resistant or tamper evident, using either preformed caps or caps formed during application for example: Roll-on pilfer-proof aluminum caps. The preformed caps may be made from tinplate or aluminum, but they should have an inner lining material (lacquer or liner). They may also be made from plastic: thermo set or thermoplastic. The use of thermo setting closure have been stopped and replaced by thermoplastic only (polypropylene and polyethylene).

A torque tester can be used to determine adequate closing tightness to prevent either over-or under tightening of the closure since over tightening makes up cap removal difficult and may induce stresses in the closure components. Under tightening will allow product to leak or be

contaminated by external substances. The composition of the closure system is complex and hence it may interact with component either by leaching or sorption.

1.8-Uses of plastic:

Plastic is widely used in health care industry due to its flexibility, and due to the two definite disadvantages of glass which are fragility and weight. Significant uses of plastic in pharmaceutical packaging have been made. For example plastics are being used in: syringes, bottles, vials and ampoules.

Other uses which would have never been technically feasible without use of plastic are in dwelling catheters, prosthetic devices, tracheotomy tubes, flexible blood collection containers, and semi rigid and flexible containers for intravenous, irrigation, and inhalation solutions.

An additional use is in secondary packaging (packaging that is not in direct contact with the product itself). For these uses in health care, plastic safety and adequacy raised many questions.

In case of plastic used in direct contact with a product the length of time that the medication and container are in contact may determine whether problems such as discoloration, leaching, and absorption or adsorption of a constituent of the product may arise. Both the product and the package containing it could change significantly from time of manufacture. Other factors that may affect the plastic packaging and/or product are:

product storage conditions, pH, temperature, type of polymer used, method of package preparation, light transmission and sterilization process. (Remington's,2005).

1.8.1-Plastic containers for pharmaceutical use:

Plastic containers and closures for pharmaceutical use are made of materials that may include some additives. They should not include any substance that can be extracted by contents: contents should not be absorbed into or adsorbed onto the container.

The most used polymers are: polyethylene (with or without additives), polypropylene, polyvinyl chloride, polyethylene terephthalate, polyethylene vinyl acetate, high density polyethylene, low density polyethylene and polystyrene.

The nature of the additives depends on the polymer composition and the method of manufacture of the container. Each source will have its own range of additives and processing conditions. (Pharmaceutical codex, 1994; British pharmacopoeia, 2007).

Drug plastic interaction may involve permeation, leaching, sorption, chemical reaction, or alteration of physical characteristics of the polymer or the product. Permeation of gases or water vapor through polymer has a marked effect on the stability of the product. If a product that contains volatile ingredients is stored in plastic containers, loss of volatile components may occur. Leaching of additives into the dosage form causes product contamination when the dosage form is in solution. Factors that affect absorption or adsorption of drug components to the polymer layer include: chemical structure, pH, concentration of ingredients, area and time of contact and temperature.

1.9-Additives used in plastics:

Thermoplastics could be modified and their properties enhanced by addition of specific additives. They may act synergistically: any two additives may produce undesirable effects when combined , the reasons for which Food and Drug Administration (FDA) requires

combinations to be totally evaluated before marketing in product form. Many tests should be done to establish safety and stability. These test include: chemical, pharmacological and biological test.

1.9.1-Types of additives (Remington's, 2005) :

1.9.1.1 • Lubricants:

Are added during molding and extrusion operation. Example is zinc stearate in case of polyethylene.

1.9.1.2 • Stabilizers:

Are added to prevent degradation by heat and light and to improve its aging characters. But they have many problems: Some have tendency to migrate to the surface of the molded part during storage. Others have limited solubility in aqueous media and could be extracted into products. Examples include: organometalic compounds, fatty acid salts and inorganic oxides .

1.9.1.3 • Plasticizers:

Are used for softness and flexibility. They are used in vinyl, celluloses and propionates. They can easily migrate to the surface of the polymer and then get extracted by the product. Therefore they should be carefully selected. Examples include: butyl benzyl phthalate (BBP), di-n-butyl phthalate (DBP), di-n-pentyl phthalate (DPP) and di-hexyl phthalate (DHP).

1.9.1.4 • Antioxidants:

Are special types of stabilizers used in retarding oxidation. They can migrate under certain environmental conditions. Their combination with other additives may lead to undesirable chemical reactions. Example includes tris (nonylphenyl) phosphite (TNPP).

1.9.1.5 • Antistatic agents:

Are added to prevent the buildup of static charges on the plastic surface. . Examples include epoxy ethane amine type and glycerol stearate.

1.9.1.6• Slip agents:

Are primarily added to polyolefin (polyethylene and polypropylene) to reduce the coefficient of friction. At the end they result in antitack and antiblock characteristics. Examples include: oleyl palmitamide, stearyl stearamide and faty acide amide (eurcamide).

1.9.1.7 • Dyes and pigments:

Are added as colouring agents. They may also leach into product.

Due to the undesirable effects of combination and migration of additives, the final product and package should be evaluated for safety and stability under various time and storage conditions simulating those the product is expected to be subjected to. Evaluation should include compatibility of the primary plastic container with its secondary packaging.

Prolonged exposure to ultra violet light enhances the migration of certain additives and accelerates the aging characteristics of the plastic and decrease the shelf life of the product. Examples include: lead chromate and lead molybdate.(Remington's, 2005).

1.10- Thermosetting plastics:

They are made from condensation reactions between formaldehyde and substances such as melamine, phenol, and urea. They are used in pharmaceutical industry as closures for glass and/ or plastic containers, in applications where sterilization by steam is required. (Remington's, 2005).

The institute for agriculture and trade policy(Co-op America, 2004-2005) has issued a smart plastic guides that include the following recommendations for using any type of plastic:

• Avoid using plastic containers in the microwave.

• Beware for cling wraps, specially for microwave use.

• Avoid plastic bottled water, if possible.

• If you use plastic water bottles, take precautions. If you use a polycarbonate water bottle do not use for warm or hot liquids to reduce leaching of bisphenol A (BPA) .

• Discard old or scratched water bottles. Water bottles from polyethylene terephthalate (PET) or high-density polyethylene (HDPE) are recommended for single use only. For all types of plastic, you can reduce bacterial contamination by thoroughly washing daily. However, avoid using detergents that can break down the plastic and increase leaching of chemical additives.

The rapid development and immature systems of foetuses and children make them susceptible to damage from toxins. So pregnant women and parents should exercise extra caution with plastic. Children's Health Environmental Coalition (CHEC) advises choosing cloth and wooden toys and avoiding plastic toys, which are often made of Polyvinyl chloride (PVC) and can leach harmful chemicals when chewed.

CHEC also recommends avoiding polycarbonate bottles which are generally rigid, and choosing bottles made of tempered or polyethylene(PE) and polypropylene(PP). CHEC also recommends using nipples made of clear silicone rather than yellow rubber, because silicone hides less bacteria and is heat resistant. (Co-op America, 2004-2005).

1.11- Adverse effects of plastics:

Plastics used in medicine and pharmacy may cause various adverse effects either by direct contact with tissue or indirectly via solutions stored in plastic containers and disposable syringes. Adverse effects may also rise when workers are exposed to plastic by inhaling fumes during manufacturing or by handling the materials. The pure polymer plastic appear of low toxicity, but some carcinogenic effects occur due to prolonged implantation. Substances added during manufacturing to impart specific physical properties are toxic such as plasticizers(phthalates). Such substances can leach and have been the main causes of adverse effects e.g.: haemolysis of blood cells, thrombosis, hypersensitivity reactions, precancerous changes, local tissue necrosis(Martindale,2007) and developmental and reproductive damage (Co-op America, 2004-2005).Incineration of polyvinylchloride leads to emesis of dioxin, which are known to cause cancer, reproductive and developmental immune problems. According to Environmental Protection Agency (EPA) short-term styrene exposure can

cause nervous system effects such as loss of concentration, weakness, and nausea. Long-term exposure causes liver and nerve damage and cancer. Polycarbonates can leach bisphenol-A(BPA) an endocrine disruptor compound. Recent review studies regarding BPA's effects (environment health perspectives,August,2005) have found that more than 80% of published studies reported effects including alteration to brain chemistry, structure, behavior, the immune system and male and female reproductive systems(Co-op America, 2004-2005).

1.12- Leachable materials:

1.12.1-Plasticizers:

Phthalates are plasticizers that impart flexibility and durability to plastic products including: building materials, food packaging, clothing, toys, children's products, blood bags, intravenous fluid bags, infusion sets, and other medical devices(Huber *et al.*, 1996; Goettlich, 2001). Phthalates are not covalently bounded to plastic matrix and leach out of it when they come in contact with lipophilic substances. They are also released directly into environment during production and use and after disposal of phthalate containing products. (Staples *et al.*, 1997; Kumar,1999). Phthalates bioaccumolate in invertebrates, fish, and plants (Staples *et al.*, 1997). They are regulated as toxic substances under environmental laws that limit the discharge of chemicals to air, land and water (Ruth,2004). Phthalates are animal carcinogens and can cause fetal death, malformation, and reproductive toxicity. Toxicity profile and potency vary by specific phthalate. Di- ethylhexyl phthalate (DEHP) and di- isononyl phthalate (DINP) have received considerable attention, because (DEHP) is used in toys and medical devices, and (DINP) is a major plasticizer used in children's toys (Shea *et al.*, 2003) .

Other types of phthalates are : butyl benzyl phthalate (BBP), di-n-butyl phthalate (DBP), di-n-pentyl phthalate (DPP), di-hexyl phthalate (DHP), di-propyl phthalate (DprP), dicyclohexyl phthalate (DCHP) and diethyl phthalate (DEP). (Goettlich, 2001) . Phthalates are also known as endocrine disruptors (EDs). Which include: cosmetics, sunscreens, perfumes, soaps, detergents, solvents, dental sealants, pharmaceuticals such as birth control pills, clear plastic baby bottles, some water bottles, oil refining, burning coal, and cigarette smoke. Many EDs health effects in humans are not proven but have been shown in animal studies (Goettlich, 2001).

The toxicity of each phthalate ester depends on conversion of parent compound to toxic metabolite. The amount of the toxic compound varies with the route of exposure:e.g. ingestion, dermal absorption, or intravenous exposure. When (DEHP) is administered orally in Rodents, it is rapidly metabolized by pancreatic lipases in the lumen of the gut to the toxic metabolite monoethylhexyle phthalate (MEHP) which is rapidly absorbed. Dermal absorption is poor. Inhaled (DEHP) is absorbed as parent compound then metabolized to (MEHP), and both are distributed broadly through out tissues (national toxicology program,2003) . Environmental monitoring data are available for (DEHP). The major source is food contaminated during growth, production, processing, or packaging. The highest level is in fatty food. The second highest source is indoor air where (DEHP) adheres to aerosols particles. Because of its low solubility in water and low vapor pressure, little amount of (DEHP) is found in out door air or water . It is estimated that exposure to (DEHP) in general population (excluding occupational exposure, medical exposure, and non dietary ingestion in children) is in the range of 3-30 µg/kg body weight /day (Meek and Chan, 1994; Huber *et al.*, 1996; Doull *et al.*,1999) According to National Institute of

Health report, (DEHP) is reasonably anticipated to be a human carcinogen. (Delicious organics, 2005). Phthalates have been shown in animal studies to cross placenta and pass into breast milk (Parmar *et al.*, 1985; Dostal *et al.*, 1987; Sarivastava *et al.*, 1989) so it leads to prenatal and breast feeding exposure. It is estimated that the total intake of (DEHP) excluding non dietary ingestion, is higher in all children younger than 19 years (Meek and Chan, 1994). Non dietary ingestion of phthalates can occur when children suck or chew on phthalate-containing toys (Rastogi, 1998; Marin *et al.*, 1998; Steiner *et al.*, 1998) This source of exposure is difficult to quantify directly. In the United States and Canada, this uncertainty of predicting exposure level led to removal of all phthalates from infant bottle nipples, teether, and infant toys intended for mouthing (National toxicology program, 2003;Ruth, 2004). (DEHP) is the only phthalate currently used in medical devices (National toxicological program, 2003) Polyvinyl chloride medical devices contain 20-40% (DEHP). It has been known since the early 1970s that (DEHP), (MEHP) are infused with blood products (Jaeger and Rubin,1973; Marcel,1973; Contreras *et al.*, 1974;Vessman and Rietz,1974) and during hemodialysis (Gibson *et al.*, 1976; Lewis *et al.*, 1978; Grestoft *et al.*, 1979).

Much of concern about phthalates was raised in 1980's. Each phthalate has a different toxicity profile. The liver, kidneys, thyroid, and testes are common targets from oral exposure.

(DEHP) causes liver cancers (National toxicology program, 2003). It also causes skeletal, cardiovascular and eye abnormalities, neural tube defects, and intrauterine death.(price *et al.*,1986, 2003;Tyl *et al.*,1988; National toxicology program, 2003), DINP causes kidney and liver cancer in Rodents (National toxicology program, 2003).The most sensitive

system is the reproductive tract.. Pathologic changes in the testes and decreased sperm numbers are consistent effects. Changes in weight of testes, vacuolization of sertoli cells and atrophy of seminiferous tubules have been observed in Rodent pups exposed to (DEHP) in vitro via dietary exposure (Lamb *et al.*, 1987; Poon *et al.*, 1997). Pregnant and lactating women might deliver higher levels of (DEHP) and (MEHP) to their infants via placental transfer and breast milk than estimated for the general population, which is more dangerous to males with developing reproductive tracts (Corets *et al.*, 1987).

1.12.2-Anti oxidants:

Food and water containers may be a potential source of unwanted and possibly harmful chemicals. Antioxidants are often used to preserve the durability and asthetic quality of these plastic containers. A popular antioxidant is a material called tris (nonylphenyl) phosphite (TNPP) which contains three units of nonylphenol per molecule of this agent. When introduced several years ago, these materials were considered inert. Since the recent discovery of their potential to be endocrine disrupters, concern over human exposure to this chemical has increased. In a research study, nonylphenol (NP), octylphenol (OP) and their respective ethoxylates (polymers with 1 to 5 repeating ethoxy groups) were measured in spring water bottled in three different types of plastic (HDPE, PET, and PVC). NP was present in water from HDPE and PVC containers. OP was found in water from HDPE extracts in lower amounts, and none of the ethoxylates was detected in any of the samples. None of these chemicals was detected in tap water. These results suggest that environmental exposure may not be the only source of NP. NP is used as antioxidant and plasticizer in some plastic products. After the discovery of its endocrine-disrupting

potential, concern over human exposure to this chemical has increased. This may be because reaction with residual chlorine results in the formation of chlorinated by-products. Migration of NP from HDPE containers to milk surrogate was also evaluated; results indicated that the amounts of NP leaching into milk might be similar to that of bottled water. (Rosales, 2004).

1.12.3- Stabilizers:

Stabilizers(organometalic compounds, fatty acids and inorganic oxides) have the greatest degree of toxicity and if these are used in conjunction with a plasticizer in a plastic formulation, the toxicity potential is greatly increased due to an increased opportunity of migration from the plastic. (Wallace *et al.*, 2004).

1.12.4- Others:

A research by a Canadian scientist working in Germany involved 132 brands of bottled water from 28 containers made from polyethylene terephthalate (PET), concluded that the concentration of certain chemicals such as antimony , increased with the time the water stays in the plastic bottles .

Antimony is a white metallic element that in small doses can cause nausea, dizziness and depression .In large doses it can be fatal. Most of Canadian bottles samples had initial antimony levels of about 160parts per trillion ,but six month after sitting in plastic the level had doubled. However it was still bellow the drinking- water standard set by health Canada at 6000 part per trillion (The Canadian press,2006) .

Antimony trioxide is a catalyst often used in the production of (PET), so it can migrate out into the content (Wikipedia, 2006) . Migration of antimony from (PET) into food measured by inductively- coupled plasma mass spectrometry amounted to 4.0 µg/kg which was also less than the limit (Fordham. *et al.*, 1997).

Many chemical additives that give plastic its desirable performance properties have negative human health effects, which include direct toxicity as in cases of lead, cadmium, and mercury.

Examples of contaminants have been reported including styrene from polystyrene, plasticizers from (PVC), antioxidants from polyethylene, and acetaldehyde from (PET) (Goettlich,2001).

Acetaldehyde is one of the leaching chemicals. When it is produced, some of it remains dissolved in the walls of the container and then diffuses in the product stored inside causing an off taste in bottled water. Low concentration (10-20 parts per billion) can produce that taste (Wikipedia, 2006).

A total of 19 migrant from (PET) bottles wall has been identified by gas chromatography/ mass spectrometry analysis. Acetaldehyde was found in carbonated mineral water and lemonade in concentrations of 11-7.5 mg/L (Linssen. *et al.*, 1995).

Migration of ethylene glycol from PET stored at 32°C for 6 month into the food stimulant (3.0% acetic acid) was studied by gas chromatography. It was found to be produced and observed at the level of a bout 94 µg/bottle (Kashtock and breeder, 1980).

A leachability study designed to evaluate the nature and/or amount of any chemical migrating from the plastic material to the component should be implemented. The study should evaluate substances that migrate into the component for the length of claimed shelf-

life. The product should be evaluated at regular intervals, such as at one, three, or six months or at one or two years, until the length of the shelf-life has been met. Analytical techniques such as liquid chromatography/mass spectrometry (LC/MS) to evaluate nonvolatile organics, gas chromatography/mass spectrometry (GC/MS) to evaluate semi volatile organics, and inductively coupled plasma (ICP) spectroscopy to detect and quantitate inorganic elements could be used. Information on substances identified from extractable chemical evaluation can be used to help prepare standards specific for the plastic container being studied during leachability studies. Methods validation for detection of leachable chemicals in content must be based on industry practices and International Conference for Harmonization (ICH) Guidelines. (Albert, 2004).

1.13-Stability of pharmaceutical products **(Pharmaceutical codex,1994):**

Any pharmaceutical product should be stable. The product should be efficious, safe, and acceptable to the patient. Stability of a pharmaceutical product is related to its resistance to the various chemical, physical, and biological reactions that may change the original properties of the product during transport, storage, and use. Stability in quantitative terms is the shelf-life of a pharmaceutical product kept in its closed container under specific conditions and is commonly defined as the time from manufacture or preparation until the original potency or content of active ingredient has been reduced by 10%. More stringent limits may be needed if the degradation products are more toxic or irritant than the drug. Chemical reactions proceed more readily in liquid state than in solid state. Degradation of the active constituents in a medicinal product results usually in loss of potency. There are many

chemical reactions that result in degradation of drug substances and excipients. The most common ones are: oxidation, hydrolysis, dehydration, isomerisation, racemisation and polymerization.

Many drugs and excipients may degrade when exposed to light (photochemical reactions).

Many factors may affect the stability: pH, nature of the solvent, general acids and bases, ionic strength and drug concentration. (Pharmaceutical codex,1994).

In case of pharmaceutical containers, the container should not interact physically or chemically with the formulation so as to alter the strength, quality, or purity of its contents.

The choice of the container and closure may affect the stability of its content. Some of the packaging elements are subject to physical and chemical changes which may be time-temperature dependant. In stability, the appearance of the container with special emphasis on inner walls, the migration of ingredients onto the plastic or into rubber closure, the migration of plasticizer or other components into the formulation, the possibility of two-way moisture penetration through the container walls, the integrity of the tac-seal, and the back-off torque of the cap must be studied.

The present work is concerned with plastic containers so as to evaluate their stability and their effect on stability of the contents (deionized and distilled water) will be discussed.

Plastic containers are most popularly used for storing pharmaceutical products. Selection of the type of the polymer to be used in synthesis of container may affect the suitability of plastics for pharmaceutical use. Many tests are used e.g. biological procedures for determining the suitability of plastic for packaging products intended for parentral use.

Materials from the plastic can leach into the formulation, and materials from formulation can be absorbed onto, into, or through the container wall.e.g:

1. Volatile oils, flavoring and perfume agents are permeable through plastic to varying degrees.
2. Components of emulsions and creams have been reported to migrate through the walls of some plastics.
3. Loss of moisture from formulation.
4. Gases like oxygen, carbon dioxide may migrate through the container wall from the air to affect the preparation.
5. Solid dosage forms, when stored in plastic container may be affected by moisture penetration.

Closures must also be studied in stability. Closures must form an effective seal for container, they must not:

- Physically or chemically react with the content.
- Absorb materials from the formulation or leach ingredients into the content. (Remington's,2005).

1.14- Analysis of water:

Water is chemically stable but it acts as a ligand, as an acid or base, and as oxidizing or reducing agent . Also it acts as a solvent for many substances due to its small size, strong permanent dipole, high di-electric constant, and availability of protons for hydrogen bonding (Remington's, 2005). Potable water is water that is fit to drink. It is mainly derived from surface sources such as lakes, rivers, and streams or from under- ground sources such as springs or wells. Its chemical composition depends upon the source from which it is drawn (pharmaceutical codex, 1994) .Naturally occurring water contains dissolved minerals

indigenous to the region. Such water is described as mineral water. Also it contains varying amounts of suspended matter: clay, sand, microorganisms, fragments of plants and animals. Also it contains iron, calcium, magnesium, sodium, and potassium ions and their counter ions, bicarbonate, sulfate and chloride. In addition, it contains dissolved atmospheric gases, ammonia and metabolic products.

Many processes should be done for natural water to be potable. These processes are:

1. Removal of insoluble matters by coagulation or filtration.
2. Destruction of pathogenic microorganism by aeration or chlorination.
3. Improvement of palatability by aeration and filtration using charcoal.
4. Partial removal of dissolved salts such as carbonates (Ca++ and Mg++) and hydroxide (Fe+++) by adding lime or ammonia.
5. Fluoridation by adding sodium fluorosilicate.

In emergencies water may be purified by boiling for 15-20 minutes (Remington's, 2005).Water supply monitoring programs indicate that more than one billion people have no access to safe water(WHO,1996). This situation has a negative effect on public health in developing countries. (WHO,1998).Sterile water may be sterilized at the time of production but loses this characteristic if it is improperly stored. The major impurities in water are calcium, iron, magnesium, manganese, silica, and sodium which are usually combined with carbonate, sulfate, or chloride.

Hard waters are those containing Ca++ and Mg++. Bicarbonates are the major impurities in the alkaline water. Most of these impurities are removed by ion-exchange (deionization, demineralization) processes (Remington's, 2005).

- Shelf-life of water, depends on the original quality of the water, the temperature at which it is stored and how much light it is exposed to. Treated water out of the tap needs nothing added and should have shelf-life for about 10 years. Untreated water from well should be stored with about 16 drops of chlorine bleach/ gallon. (Peter,2000).

Water should be clear, colorless, odorless and tasteless. Purified water kept in different types of plastic containers should comply with the following Pharmacopoeial tests:

Acidity or alkalinity, oxidisable substances, chlorides, sulphates, ammonium, calcium and magnesium, residue on evaporation, nitrates, and heavy metals.(B.P, 2007)

1.15- Rationale:

Establishing the safety of container- closure systems is of key importance to the medical, pharmaceutical, water, food etc. industries. It is not less important than the contents themselves.

The pure plastic appears to be of low toxicity, but many additives in plastic products appear to have negative health effect. Phthalates are regulated as toxic substances (Ruth, 2004).

However, harmful effects did occur in animals with prolonged exposure or in those to which high amounts of the chemicals were administered. These effects include reproductive problems, birth defects and damaged sperm and liver in mice. (Delicious organics, 2004).

Plastic materials are widely used as water and food containers. This practice is relatively recent in Sudan. Drinking water, frozen and refrigerated food, edible oil, milk and other food substances are usually kept in plastic containers of different types and from different sources. The possible health hazards of such practice are not to date studied.

1.16- Objectives:

- Determination of the quality of water kept in plastic containers made of different types of plastic.
- Determination of some materials e.g. phthalates (if any) leaching from plastic containers into water.
- Study of the changes (if any) in plastic containers containing water upon storage.

2- Materials and methods

2.1- Materials:

- Distilled, and deinoized water (Balsam pharmaceutical company, Sudan)

- Ammonium-free water (prepared according toB.P,2007)

- Nitrate-free water (prepared according toB.P,2007)

-Anti- pumping agent, lead nitrate, mercuric iodide, nitric acid (SPG= 1.42), phenolphthalein, potassium chloride, potassium nitrate, potassium permanganate, starch soluble (BDH chemicals Ltd Poole, England) .

-Barium hydroxide (Hopkin and Williums, England) .

-Ammonium acetate, ammonium chloride, barium chloride, bromothymol blue, diphenylamine, methyl red, Mordant black Π, nitrogen- free sluphuric acid 95-97% w/w, potassium iodide, silver nitrate, sodium bicarbonate, sodium carbonate, sodium chloride, sodium editate, sulphuric acid 96% w/w, thioacetamide (Merck, Germany) .

- Ammonia (Loba chemie, India).

- Hydrochloric acid 37% (Applichem, Germany).

- Sodium hydroxide (Panreac European union).

- Glycerol (Fauth &CO.KG- Manheim, Germany).

- Sodium thiosulphate (Scharlau, Spain) .

2.2- Apparatus:

- Water- bath, GFL, Germany.

- Stability chamber neutronic humidity control oven (Newtronic equipment PVT. LTD,India.)

- Autoclave (Tecnomara FVS, Germany.)

- Ultra Violet/ Visible spectrophotometer:

  -Single beam (Spectronic unicam, Helios, England.)

  - Double beam (Perkin-Elmer lambda, 12, Germany.)

- pH meter (Multical pH 538, Germany .)

- Analytical Balance (Sartorius cp 224s, Germany.)

- Atomic spectroscopy (Perkin Elemer, AAnalyst 700, USA)

2.3- Experimental methods:

Water was filled into different types of plastic containers, that are widely used. Water used was deionized and distilled.

The types of plastics used were:

- Polyethylene terephthalate (PET) in the form of drug bottles and water bottles
- Polyvinyl chloride(PVC) in the form of peritoneal dialysis bags.
- Polypropylene (PP) in the form of intravenous fluid bottles.
- Polyethylene (PE) in the form of bags.

2.3.1-Water tests(B.P, 2007):

According to the methods described in B.P, 2007, different tests were carried out for the water kept in the plastic containers . These tests included: acidity and alkalinity, presence of oxidisable substances, chlorides, sulphates, ammonium, calcium, magnesium, nitrates, heavy metals, residue on evaporation in addition to ultraviolet spectrum(200-400nm),. Tests were carried out on different days(zero, fifteen, thirty, sixty, and ninety) and at different storage conditions (freezer, stability chamber at 40°C, refrigerator, room temperature and direct sun light). The same tests were used for water kept in borosilicate glass. pH of water and lead content were measured at day zero and day ninety using pH meter and atomic absorption instrument respectively.

2.3.1.1-Acidity or alkalinity:

To 10 ml of the water under test , freshly boiled and cooled in borosilicate glass flasks, 0.05 ml of methyl red solution was added. (should not give a red colour .)

To 5 ml in test tubes, 0.05 ml of bromothymol blue solution was added. (should not give a blue colour.)

2.3.1.2- Oxidisable substances:

To 10 ml of the water under test in 25ml volumetric flasks ,1 ml of dilute sulphuric acid and 0.1 ml of 0.002 M potassium permanganate were added, then boiled for 5 min. (should give a faintly pink colour.)

2.3.1.3- Chlorides:

To 5 ml of the water under test in test tubes, 0.5 ml of dilute nitric acid and 0.1 ml of 1.7% w/v silver nitrate solution were added. The solution should stay for 15 min without any turbidity.

2.3.1.4- Sulphates:

To 5 ml of the water under test in test tubes, 0.05 ml of dilute hydrochloric acid and 0.05 ml of 6.1% w/v barium chloride solution were added. The solution should stay for 1 h without any turbidity.

2.3.1.5- Ammonium:

To 10 ml of the water under test in test tubes ,0.5 ml of alkaline potassium tetraiodomercurate solution was added. After 5 min, the solution was compared with standard prepared at the same time by adding 0.5ml of alkaline potassium tetraiodomercurate solution to a mixture of 2 ml of ammonium standard solution (1 ppm NH_4) and 8 ml of ammonium-free water (0.2 ppm). The solution was examined down the vertical axis of the tube .The solution should not be more intensely coloured than that of the standard.

2.3.1.6- Calcium and magnesium:

To 10 ml of the water under test , 0.2 ml of ammonium chloride buffer solution pH 10.0, 5mg of Mordant black 11 triturate and 0.5 ml of 0.001M sodium edetate were added. A pure blue colour should be produced.

2.3.1.7- Residue on evaporation:

10 ml of the water under test were evaporated on a water-bath and dried in an oven at 100°C to 105°C. The residue weight should not be more than 1 mg (0.001 per cent).

2.3.1.8-Nitrates:

5 ml of the water under test was transferred to a test-tube and immersed in iced water, 0.4 ml of a 10% w/v solution of potassium chloride, 0.1 ml of 0.1%w/v diphenylamine solution were added, and 5 ml of nitrogen-free sulphuric acid was added dropwise with shaking, . The tube was then transferred to a water-bath at 50°C, and kept for 15 min. The standard was prepared at the same time in the same manner using a mixture of 4.5 ml of nitrate-free water and 0.5 ml of nitrate standard solution (2 ppm NO_3). Any blue colour produced in the solution should not be more intense than that in the standard.

2.3.1.9- Heavy metals:

20ml of the tested water were heated in a glass evaporating dish on a water-bath until the volume is reduced to 2 ml. To 1.2 ml of the concentrated solution ,0.2 ml of acetate buffer pH 3.5 was added, then 0.12 ml of thioacetamide reagent was added. The solution was allowed to stand for 2 minutes. Any brown colour produced was not more intense

than that obtained by treating, in the same manner, a mixture of 1ml of lead standard solution (1 ppm Pb), and 0.2 ml of the water being examined. The standard solution should exhibit a slightly brown colour when compared to a solution prepared by treating in the same manner a mixture of 1 ml of water and 0.2 ml of the solution being examined.

2.3.1.10-UV spectrum:

UV absorbance of the sample under the test in the range 200-400nm, using deionized and distilled water in a borosilicate glass at the same condition as a blank, was carried out.

2.3.1.11- pH of water stored in plastic containers:

pH was determined for water (deionized and distilled) kept in different types of containers in different conditions at day zero and day ninety for all types of containers.

2.3.1.12- Lead content in water stored in plastic containers (measured by atomic absorption):

Presence of lead was determined using atomic absorption instrument. From standard solution containing lead at a concentration of 1000 mg/L, 10 ml were transferred to a volumetric flask and diluted to 100ml with water as stock solution. From this stock solution a series was prepared by diluting the volumes: zero, 2.5, 5, 7.5 and 10 ml in 50 ml volumetric flasks completing the volume with water. To each standard flask 1.5ml of diluted nitric acid (1:1) was added to inhibit ionisation. To each sample of the solution under test 3 ml of the diluted nitric acid was added. The instrument was adjusted at 217nm for lead lamb.

The five standard solutions were read before and after each 10 samples group and the calibration curve of each group was made.

2.3.2- Plastic containers analysis:

Solution S:

This solution should be used within 4 h of preparation. The container was filled to its nominal capacity with water (deionized and distilled) and closed with the usual means of closure, then heated in an autoclave, at a temperature of 121 ± 2°C for 30 minutes. It should be clear and colourless .

The blank was prepared by heating water (deionized and distilled) in a borosilicate-glass flask at the same temperature and for the same time used for the preparation of solution S.

Containers were tested, after being filled with deionized and distilled water and autoclaved; tests were done at day zero (control) and for samples of the containers with deionized distilled water kept at different storage conditions for ninety days which were empted, and refilled with fresh deionized dissteld water and autoclaved at 121 ± 2°C for 30 minutes. This was done to study the effect of storage at different conditions on the plastic container test. The tests carried out included, acidity and alkalinity, reducing substances, transparency, and UV absorbance (230 - 360nm).

2.3.2.1-Acidity or alkalinity:

To a volume of solution S corresponding to 4 per cent of the nominal capacity of the container in a conical flask, 0.1 ml of phenolphthalein solution was added. The solution should be colourless. Then 0.4 ml of 0.01M sodium hydroxide was added. The solution

should become pink. After that 0.8 ml of 0.01M hydrochloric acid, and 0.1 ml of methyl red solution were added. The solution should have an orange-red or red colour.

2.3.2.2-Absorbance:

The absorbance of solution S from 230- 360 nm was measured, using the blank as the compensation liquid. At these wavelengths, the absorbance should not be greater than 0.20.

2.3.2.3-Reducing substances:

To 20.0 ml of solution S in a conical flask, 1 ml of dilute sulphuric acid and 20.0 ml of 0.002M potassium permanganate were added. The mixture was boiled for 3 minutes and cooled immediately. 1 g of potassium iodide, was added and the solution was then titrated immediately with 0.01 M sodium thiosulphate, using 0.25 ml of starch solution, as indicator. The titration was also carried out using 20.0 ml of the blank . The difference between the titration volumes of sample and blank should not be greater than 1.5 ml.

2.3.2.4-Transparency:

A container previously used for the preparation of Solution S should be transparent and the solution itself should be clear and colourless.

3-Results

3.1 Water monograph analysis:

The quality of water contained in different types of plastic containers was examined. The plastic types used in this study were PET drug bottles, PET water bottles, PVC peritoneal dialysis bag, PP intravenous infusion bottles and PE sacks. Water was kept in the containers at different conditions: freezer, stability chamber, refrigerator, room temperature and direct sun light. Analysis of water was carried out at different time intervals: zero time, fifteen days, thirty days, sixty days and ninety days. Complete water monograph(B.P, 2007) was carried out. In addition the UV absorption (200-400nm) was measured for each sample.

3.1.1- Polyethele terephthalate(PET):

i- PET Drug bottles:

The nominal capacity of plastic drug bottles was 100 ml. 25 bottles were used for each condition. The results are shown in Table 1 as can be seen from the results all samples of water kept in PET (drug bottles) at different conditions: freezer, stability chamber, refrigerator, room temperature, and direct sun light passed the tests in the monograph of water analysis. However, containers in direct sun light became acidic at day ninety. And the UV spectra showed small peaks at different wavelengths ranged between 243-260nm.

Table 1: Results of analysis of water kept in PET drug bottles at different storage conditions :

Storage condition	Time (days)	Water monograph			UV absorption		
		comply	Doesn't comply	comment	+ve	-ve	λmax
Freezer	00	√				√	
	15	√			√		258
	30	√			√		259
	60	√			√		256
	90	√			√		257
Stability chamber	00	√				√	
	15	√			√		259
	30	√			√		258
	60	√			√		258
	90	√			√		243
Refrigerator	00	√				√	
	15	√			√		252
	30	√				√	
	60	√			√		256
	90	√			√		258
Room temperature	00	√				√	
	15	√			√		254
	30	√			√		257
	60	√			√		257
	90	√				√	
Sun light	00	√				√	
	15	√			√		260
	30	√			√		258
	60	√				√	
	90		√	acidic	√		259

ii- PET water bottles:

The nominal capacity of each bottle was 500 ml, so 5 bottles were needed for each condition. As can be seen from the results all samples of water kept in PET (water bottles) at different conditions passed the tests in the monograph of water analysis. However containers in direct sun light became acidic at day ninety, and the UV spectra showed small peaks at different wavelengths (Table 2) ranged between 252-260nm.

Table 2: Results of analysis of water kept in PET water bottles at different storage conditions :

Storage condition	Time (days)	Water monograph			UV absorption		
		comply	Doesn't comply	comment	+ve	-ve	λmax
Freezer	00	√				√	
	15	√			√		256
	30	√			√		260
	60	√			√		256
	90	√			√		257
Stability chamber	00	√				√	
	15	√			√		253
	30	√			√		256
	60	√			√		252
	90	√			√		257
Refrigerator	00	√				√	
	15	√			√		258
	30	√			√		257
	60	√			√		256
	90	√			√		258
Room temperature	00	√				√	
	15	√			√		252
	30	√				√	
	60	√			√		259
	90	√				√	
Sun light	00	√				√	
	15	√			√		258
	30	√			√		257
	60	√			√		259
	90		√	acidic		√	

3.1.2-Polyvinyl chloride (PVC) peritoneal dialysis bags:

The nominal capsacity was 2000 ml, so 5 bottles were needed for each condition. From the results obtained all samples of water kept in PVC at different conditions were found to pass the monograph tests for water analysis. However, containers in direct sun light became acidic with oxidisable substances at day ninety, and the UV spectra showed small peaks at different wavelengths ranged between (323-328nm) (Table 3).

Table 3: Results of analysis of water kept in PVC peritoneal dialysis bags at different storage conditions:

Storage condition	Time (days)	Water monograph			UV absorption		
		comply	Doesn't comply	comment	+ve	-ve	λmax
Freezer	00	√				√	
	15	√			√		328
	30	√			√		328
	60	√			√		323
	90	√				√	
Stability chamber	00	√				√	
	15	√			√		328
	30	√			√		328
	60	√			√		323
	90	√				√	
Refrigerator	00	√				√	
	15	√			√		328
	30	√			√		328
	60	√			√		326
	90	√				√	
Room temperature	00	√				√	
	15	√			√		326
	30	√			√		328
	60	√			√		328
	90	√				√	
Sun light	00	√				√	
	15	√			√		328
	30	√			√		328
	60		√	Acidic& oxidisable substances	√		326
	90		√	Acidic& oxidisable substances		√	

3.1.3-poly propylene (PP) intravenous infusion bottles:

The nominal capacity of these bottles was 500 ml. 5 bottles were taken for each condition. As can be seen from the results all samples of water in PP at different conditions were within the test limits stated in the monograph of water analysis. However, containers in direct sun light became acidic with ammonium salt at day ninety, and the UV spectra showed small peaks at different wavelengths ranged between (320-328nm) (Table 4).

Table 4: Results of analysis of water kept in PP intravenous infusion bottles at different storage conditions :

Storage condition	Time (days)	Water monograph			UV absorption		
		comply	Doesn't comply	comment	+ve	-ve	λmax
Freezer	00	√				√	
	15	√			√		326
	30	√			√		328
	60	√			√		328
	90	√				√	
Stability chamber	00	√				√	
	15	√			√		328
	30	√			√		328
	60	√			√		327
	90	√			√		320
Refrigerator	00	√				√	
	15	√			√		326
	30	√			√		328
	60	√			√		328
	90	√				√	
Room temperature	00	√				√	
	15	√			√		328
	30	√			√		327
	60	√			√		327
	90	√				√	
Sun light	00	√				√	
	15	√			√		328
	30	√			√		328
	60	√			√		322
	90		√	Acidic& ammonium	√		326

3.1.4-poly ethylene (PE) sacks :

5 sacks were chosen for each condition. It was found that all samples of water kept in PE sacks at different conditions passed the monograph tests of water analysis. However, the UV spectra showed small peaks at different wavelengths ranged between (250-257nm) (Table 5).

Table 5: Results of analysis of water kept in PE sacks at different storage conditions :

Storage condition	Time (days)	Water monograph			UV absorption		
		comply	Doesn't comply	comment	+ve	-ve	λmax
Freezer	00	√			√		255
	15	√			√		255
	30	√				√	
	60	√				√	
	90	√			√		254
Stability chamber	00	√			√		255
	15	√			√		253
	30	√				√	
	60	√				√	
	90	√				√	
Refrigerator	00	√			√		255
	15	√			√		257
	30	√				√	
	60	√				√	
	90	√				√	
Room temperature	00	√			√		255
	15	√			√		250
	30	√				√	
	60	√				√	
	90	√				√	
Sun light	00	√			√		255
	15	√				√	
	30	√				√	
	60	√				√	
	90	√				√	

3.2- Other analysis of water:

3.2.1-pH of water stored in plastic containers:

pH was determined for deionized- distilled water stored in different types of plastic containers at different conditions(freezer, stability chamber, refrigerator, room temperature, and sun light pH determination were made at day zero and ninety. The results are shown in (table 6). The pH at day zero was 5.45± 0.04.

Table 6: pH of water stored in different types of plastic containers for ninety days :

Conditions	Plastic container type				
	PET drug bottle	**PET water bottles**	**PVC**	**PP**	**PE**
Freezer	5.33±0.04*	5.41±0.09	5.43±0.02	5.32±0.13	5.73±0.10
Stability chamber	5.24±0.03*	5.23±0.02	5.42±0.12	5.61±0.04	5.83±0.30
Refrigerator	5.41±0.03	5.56±0.09	5.76±0.40	5.54±0.30	5.14±0.07
Room temperature	5.36±0.08*	5.44±0.06	5.34±0.02	5.45±0.03	5.58±0.50
Sun light	5.05±0.02*	5.20±0.30*	4.06±0.05*	4.23±0.13*	5.79±0.80

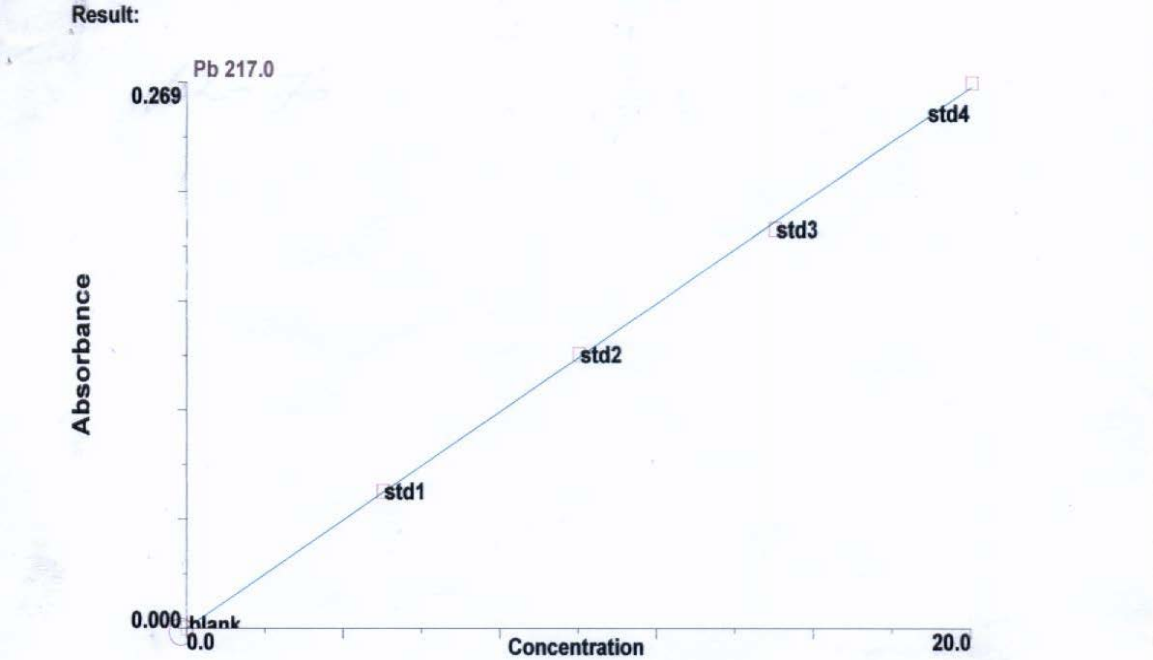
* ≡ Differences were significant (sig < 0.05)(see Appendix A)

3.2.2- Lead content in water stored in plastic containers (measured by atomic absorption):

Presence of lead in water stored in different types of plastic containers for ninety days was determined by atomic absorption spectrometry. A series of solutions was prepared, by diluting: zero, 2.5, 5, 7.5 and10 ml of standard lead solution to 50 ml with water. To each standard flask 1.5 ml diluted nitric acid (1:1) was added as preservative. To each sample of the solution under test 3 ml diluted nitric acid was added. The instrument was adjusted at 217nm for lead lamb. The five standard solutions were read before and after each 10 tested sample groups and the calibration curve of each group was made(figure 2).

- Minimum detectable level of the instrument was 60.0 µg/L
- Concentration of lead (Pb) at day Zero was 29.0 µg/L
- The standard limit of lead according to EPA standards is 15.0µg/L.

The results are shown in table 7.



Calibration Curve Slope: 0.01337
Calibration Curve Intercept: 0.00014
Calibration Curve Correlation Coefficient: 0.999781
Calibration Curve Type: Linear, Calculated Intercept
Current Sample Concentration: -0.155 mg/L

Std #	Standard ID	Entered Conc.	Calculated Conc.	Action
Blank	blank	0	-0.010	Include
1	std1	5.0	5.034	Include
2	std2	10.0	10.108	Include
3	std3	15.0	14.724	Include
4	std4	20.0	20.144	Include

Figure2: calibration curve of the lead standard solutions.

Table 7: Lead concentration in water stored in different types of plastic containers at different Conditions for ninety days:

Condition	Standard µg/L	PET drug bottles µg/L	PET water bottles µg/L	PVC µg/L	PP µg/L	PE µg/L
Freezer	31.0	3.0±20.0	3.0±10.0	0.00	40.0*±230.0	5.6±20.0
Stability chamber	21.0	60.0*±20.0	20.0*±20.0	30.0*±20.0	20.0*±2.0	40.0*±90.0
Refrigerator	10.0	10.0±10.0	50.0*±80.0	20.0*±20.0	20.0*±10.0	90.0*±60.0
Room temperature	64.0*	20.0*±10.0	3.0±0.00	10.0±20.0	20.0*±40.0	30.0*±40.0
Sun light	42.0*	20.0*±10.0	10.0±40.0	10.0±40.0	20.0*±10.0	190.0*±30.0

* ≡ Differences out of control(see Appendix B)

3.3-Plastic container analysis:

Different types of plastic containers (PET drug and water bottles, PVC and PP) were filled with deionized and distilled water and autoclaved at 121±2˚C for 30 minutes, the tests were done at day zero (control) and for samples of the containers with deionized distilled water kept at different storage conditions for ninety days which were empted, and refilled with fresh deionized dissteld water and autoclaved at 121 ± 2°C for 30 minutes to give (solution S). This was done to study the effect of storage at different conditions on the plastic container test. The tests carried out include, acidity and alkalinity, reducing substances, transparency, and UV absorbance (230 - 360nm).

- water kept in a borosilicate glass flask was used as a blank, for reducing substances the needed volume of titrant (0.01M sodium thiosulphate) to titrate the blank = 18.5ml±0.07 . Results obtained were illustrated in Tables 8-11.

Table 8: Results of analysis of water kept in PET (drug bottles) containers at day zero and after being kept at different condition for ninety days. And after being put in an autoclave for 30 minutes:

Condition	Reducing substances ml	Alkalinity or acidity	Transparency	UV absorbance of solution S			Physical appearance
				λmax	ABS	Comment	
Control (day zero)	17.7±0.08	√	√	243	.08	comply	Shrunken
Freezer	19.3±0.27	√	√	242	.095	comply	changed
Stability chamber	17.6±0.8	√	√	244	.092	comply	changed
Refrigerator	17.8±1.6	√	√	243	.081	comply	changed
Room temperature	18.4±1.3	√	√	243	.087	comply	changed
Sun light	19.1±1.4	√	√	243	.079	comply	Shrunken

√ ≡ within specified limit

Differences were not significant (sig > 0.05)(see Appendix C)

Table 9: Results of analysis of water kept in PET (water bottles) containers at day zero and after being kept at different condition for ninety days. And after being put in an autoclave for 30 minutes:

Condition	Reducing substances ml	Alkalinity or acidity	Transparency	UV absorbance of solution S			Physical appearance
				λmax	ABS	Comment	
Control (day zero)	18.7±0.3	√	√	242	.045	comply	Shrunken
Freezer	19.2±0.3	√	√	-	-	Not comply	Shrunken
Stability chamber	19.3±0.5	√	√	243	.060	comply	Shrunken
Refrigerator	18.7±0.4	√	√	242	.045	comply	Shrunken
Room temperature	18.9±0.3	√	√	243	.060	comply	Shrunken
Sun light	17.8±0.5	√	√	243	.061	comply	Shrunken

√ ≡ within specified limit

* ≡ Differences were significant (sig < 0.05)(see Appendix C)

Table10: Results of analysis of water kept in PVC containers at day zero and after being kept at different condition for ninety days. And after being put in an autoclave for 30 minutes:

Condition	Reducing substances ml	Alkalinity or acidity	Transparency	UV absorbance of solution S			Physical appearance
				λmax	ABS	Comment	
Control (day zero)	17.7±1.7	√	√	328	.014	comply	3 shrunk and opened
Freezer	19.0±0.5	√	√	325	.010	comply	2 shrunk and opened
Stability chamber	19.0±0.3	√	√	324	.009	comply	√
Refrigerator	18.7±0.2	√	√	324	.017	comply	2 shrunk and opened
Room temperature	18.0±1.6	√	√	324	.047	comply	2 shrunk and opened
Sun light	17.0±2.2	√	√	325	.002	comply	2 shrunk and opened

√ ≡ within specified limit

Differences were not significant (sig > 0.05)(see Appendix C)

Table 11: Results of analysis of water kept in PP containers at day zero and after being kept at different condition for ninety days. And after being put in an autoclave for 30 minutes:

Condition	Reducing substances ml	Alkalinity or acidity	Transparency	UV absorbance of solution S			Physical appearance
				λmax	ABS	Comment	
Control (day zero)	18.4±0.7	√	√	332	.003	comply	√
Freezer	18.9±0.4	√	√	332	.002	comply	√
Stability chamber	17.3±2.2	√	√	324	.005	comply	√
Refrigerator	18.7±0.4	√	√	316	.010	comply	√
Room temperature	19.0±0.5	√	√	318	.004	comply	√
Sun light	18.2±0.8	√	√	319	.004	comply	√

√ ≡ within specified limit

Differences were not significant (sig > 0.05)(see Appendix C)

4- Discussion

The length of time a plastic container and a product e.g.medication are in direct contact may determine problems such as leaching. Many other factors may affect plastic packaging and products namly storage condition, pH, temperature, type of polymer used and sterilization process (Remington′s 2005).

Substances added to plastics during manufacturing for specific physical properties are toxic. Such substances can leach causing many adverse effects e.g.: haemolysis of blood cells, thrombosis, hypersensitivity reactions, precancerous changes, local tissue necrosis and developmental and reproductive damage (Martindale, 2007; co-op America, 2004-2005).

Recent review studies regarding bisphenol-A's (BPA) effects (environment health perspectives,August,2005) have found that more than 80% of published studies reported effects including alteration to brain chemistry, structure, behavior, the immune system and male and female reproductive systems(Co-op America, 2004-2005). Examples of such added substances are:

- Phthalate: They are added to impart flexibility and durability to plastic. They are not covalently bonded and leach when in contact. They may also be released into environment during production and use (Staples. *et al.*, 1997; Kumar, 1999).
- Antioxidants: They are added for durability and asthetic quality. In a research study, nonyl phenol (NP) was found in water from HDPE and PVC containers (Rosales, 2004)
- Acetaldehyde: When it leaches and diffuses in the product, it causes an off taste in bottled water (10-20 parts/ billion) (Wikipedia, 2006).

Freezing water bottles may cause plastic to leach into water (Delicious organic, 2005).

Devices made of polypropylene could be autoclaved for sterilization (Remington's, 2005) Results of the present work confirmed this fact. Table 11 shows that there is no change in physical appearance of this type of polymer. Others were found not stable to autoclaving process and are non autoclavable hence they showed physical changes (tables 8-11). PVC has softening point's 70-180°C (Remington's, 2005).

A total of 19 migrations from (PET) bottles have been identified by gas chromatography/ mass spectrometry analysis (Linssen. *et al.*, 1995).

Migration of ethylene glycol from PET stored at 32°C for 6 month into food stimulant(3.0% acetic acid) was studied by gas chromatography at a level of 94µg/ bottle (Kashtock and Breeder, 1980).

Results of analysis of water kept in different types of plastic containers at different storage conditions for ninety days (Tables1-5) revealed possible leaching of materials from plastic into water. This judgment was based on the observation of appearance of UV absorption bands(240-260nm) for PET drug & water bottles and PE sacks, and (320-330nm) for PVC peritoneal dialysis bags and PP intravenous infusion bottles. On the other hand monograph analysis of water was conforming with specifications for all types of plastics at all conditions with only few exceptions .

Study of the effect of plastic containers on pH of water stored at different conditions for ninety days (Table 6) revealed little changes. However, it appears that water stored under sun light had a change of pH which was to lower values in case of PET drug bottles, PVC and PP and to a higher value for PE.

Analysis of plastic containers of PET (drug & water bottles), PVC and PP , was carried out by reusing the containers kept at different storage conditions. They were refilled with deionized water and sterilized by autoclaving at 121˚± 2 for thirty minutes. Tests conducted included acidity and alkalinity, reducing substances, transparency, physical appearance and UV absorbance of the water contained(230-360nm). Freshly water filled and sterilized containers were taken as control. The results obtained (Tables 8-11) showed that all containers used did not affect the water transparency, acidity or alkalinity. There were some variations in reducing substances content (compared to control) of water kept in different types of plastic containers. Apparent increase was noted with PET drug bottles stored in freezer and sun light and PVC stored in stability chamber , freezer and refrigerator . A decrease was noted with PP stored in stability chamber . All containers except PP showed change in physical appearance. The test for UV absorbance of solution S was found to comply with specification in all plastic containers at all conditions of storage except for PET water bottles kept in the freezer.

Lead concentrations in water kept in different plastic containers at different conditions for ninety days was determined. The standard limit of lead according to EPA standards is 15.0µg/L (Judith. et al, 2007). The concentration of lead in water at day zero (control) was 29.0µg/L which was greater than standard limit. All standards(water in borosilicate glass) in different conditions showed greater values than the standard limit, except those kept in the refrigerator (Table 7) . High values of lead were detected in water kept in PE and stored for ninety days in refrigerator or under sun light . Relatively high values of lead were also noted with storage in PET drug bottles in stability chamber and PET water bottles in refrigerator.

4.1 PET:

4.1.1 PET drug bottles:

From the results obtained all samples of water kept in PET (drug bottles) at different conditions: freezer, stability chamber, refrigerator, room temperature, and sun light were found to pass the tests in the monograph of water analysis. However, water in containers in sun light became acidic at day ninety. The UV spectra showed small peaks at different wavelengths (Table1). The pH of water was found to be lower in most conditions and it became acidic in case of samples kept in sun light. The lead concentrations decreased in water from containers stored in freezer, room temperature and sun light. This might be due to adsorption or deposition in the inner wall of the container. Lead concentration increased in water stored in stability chamber. It was noted that lead concentration in water (29.0µg/L) was higher than the standard limit (15.0µg/L).

Results of testing plastic type are shown in (Table 8). It was evident that all containers showed physical changes i.e. they are not autoclavable. They all passed the tests of transparency, acidity or alkalinity, for reducing substances test all samples passed the test except samples stored in refrigerator and sun light. All samples tested showed UV absorbance at about 243nm which was less than the limit (0.2).

4.1.2 PET water bottles:

All samples of water kept in PET (water bottles) at different conditions passed the tests of water monograph analysis. However, water in containers under sun light became acidic after ninety days. The UV spectra showed small peaks at different wavelengths ”250-

260nm" (Table 2). UV absorbing materials detected in water from PET (water bottles) might decompose on standing at room temperature and in sun light to give products which do not absorb UV (Table 2). The pH of water stored in PET water bottles was not affected upon storage at different conditions. However, water became acidic at day ninety under sunlight. The lead concentration was reduced in all conditions, possibly due to adsorption in the inner side of the plastic wall and/ or deposition. Water kept in refrigerator showed increased lead content compared to control.

Results of testing plastic type are shown in (Table 9). It was obvious that all containers are not autoclavable. They all passed the tests of transparency, acidity or alkalinity and reducing substances. All samples tested showed UV absorbance at about 243nm which was less than the limit (0.2).

4.2 PVC:

From the obtained results we could notice that all samples of water kept in PVC at different conditions passed the tests in the monograph of water analysis . However water in containers in sun light became acidic and had oxidisable substances starting from day sixty. This indicates the possible leaching of materials from PVC when subjected to sun light. It appears that the leaching material is acidic in nature. The UV spectra showed small peaks at different wavelengths generally around 328nm (Table 3). There was no UV absorption at day ninety. UV absorbing materials leaching from PVC might decompose on standing at different conditions to give products which do not absorb UV (Table 3). The pH was found to be low in water stored under sun light and normal in other conditions. Water became acidic with oxidisable substances deteced. It is therefore clear that storage of material in PVC under sun

light is not recommended. The lead concentration was reduced in water stored in sun light, room temperature and freezer and increased in samples kept in stability chamber and refrigerator. Results of testing the plastic type in (Table 10) showed that all containers are not autoclavable. They all passed the tests of transparency, acidity or alkalinity. In case of reducing substances test the control samples, samples stored at room temperature and in sun light didn't pass the test. All samples showed UV absorbance at about 324nm which was less than the limit (0.2).

4.3 PP:

From the results obtained all samples of water kept in PP at different conditions passed the tests in the monograph of water analysis. However, water in containers under sun light became acidic at day ninety. The UV spectra showed small peaks at different wavelengths around 327nm. Which disappear on standing in freezer, refrigerator, and at room temperature. (Table 4). The water pH was found to be acidic (with detection of ammonium) in samples stored under sun light and normal in other conditions. The lead concentration was decreased in samples stored in stability chamber and increased in water kept in freezer and room temperature conditions. Results of testing the plastic type in (Table 11) showed that all containers are autoclavable. They all passed the tests of transparency, acidity or alkalinity. For reducing substances test all sample passed the test except samples stored in stability chamber. All samples showed UV absorbance around 316-332nm which was less than the limit (0.2).

4.4 PE:

The results showed that all samples of water kept in PE at different conditions passed the tests in the monograph of water analysis. The UV spectra showed small peaks at different wavelengths at 250-255nm(Table 5). UV absorbing materials appear to decompose on standing for ninety days in stability chamber, refrigerator, and at room temperature conditions to give products that do not absorb UV. The pH of water stored in PE increased in all storage conditions. However it decreased in samples stored in refrigerator. The lead concentrations in all conditions was found to be higher specially in samples stored under sun light.

Because of its low melting point(110-130°C) the container monograph test wasn't done for this type of containers.

The results of the present study revealed that some materials might leach out of certain types of plastics into stored water. This process is enhanced by sun light and was observed to be more clear in water stored in PE sacks.

Sudan is known to have plenty of sun light almost all the year round. People, nowadays, extensively use plastic particularly PE for storage of food and water. It is a common practice to purchase from shops materials like milk, edible oil, soft drinks etc in PE containers. Soft drinks and mineral water, which is only recently introduced to Sudan, are packed in plastic containers. They are transported and stored under adverse conditions usually under direct sun light. The health hazards which might be caused by materials leaching from plastics into such human consumables should be taken seriously.

5- Conclusions& Recommendations

5.1- Conclusions:

Water stored in different types of plastic containers at different storage conditions was found to exhibit some UV absorption at different wavelength. This was taken to indicate leaching of materials from these containers.

Sun light seems to affect the leaching process from plastic. The pH of water stored in different types of plastic containers was found to be affected by both the type of plastic and storage condition.

Water stored in certain types of plastics under sun light were found to have acidic pH with oxidisable substances and ammonium.

High concentration of lead was detected in water stored in PE plastic containers in the refrigerator and under sun light.

Reducing substances were detected to be out of the limit in some types and at different conditions.

All containers except PP showed change in physical appearance(non autoclavable).

These results are considered preliminary results which need further confirmation; however these primary results could point out or could be considered an alarm for possible hazards that can occur from uncontrolled use of plastic containers or exposure to sunlight in Sudan.

5.2-Recommendations:

- Materials stored in plastic containers should be kept away from direct sun light specially PVC and PE types.
- Care should be taken in selecting containers to store pharmaceutical preparations, food and water.
- The use of PE as a container for food or water should be avoided.
- Further work should be carried out to study the nature of materials leaching from different types of plastics and the presence of such materials in stored pharmaceuticals, food, soft drinks, juices etc.
- Presence of other elements such as antimony, cadmium and mercury in water stored in plastic containers should be investigated.
- A study involving a longer storage time of 6 months to one year is recommended.

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Appendix A: Statistics of pH determination for PET drug bottles

Oneway

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
reading	1.190	5	24	.343

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
reading	Between Groups	.523	5	.105	29.821	.000
	Within Groups	8.416E-02	24	3.507E-03		
	Total	.607	29			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: reading

Dunnett (2-sided)[a]

(I) process	(J) process	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
freezer	control day zero	-.1200*	.037	.016	-.2209	-1.91E-02
stability-chamber	control day zero	-.2080*	.037	.000	-.3089	-.1071
refrigerator	control day zero	-5.00E-02	.037	.547	-.1509	5.095E-02
room-temperature	control day zero	-.1020*	.037	.047	-.2029	-1.05E-03
sun light	control day zero	-.4060*	.037	.000	-.5069	-.3051

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Appendix A : Statistics of pH determination for PET water bottles
Oneway

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
reading	4.362	5	24	.006

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
reading	Between Groups	.503	5	.101	4.593	.004
	Within Groups	.526	24	2.190E-02		
	Total	1.029	29			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: reading
Dunnett (2-sided)[a]

(I) process	(J) process	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
freezer	control day zero	-5.00E-02	.094	.976	-.3023	.2023
stability-chamber	control day zero	-.2240	.094	.094	-.4763	2.828E-02
refrigerator	control day zero	.1100	.094	.657	-.1423	.3623
room-temperature	control day zero	-1.40E-02	.094	1.000	-.2663	.2383
sun light	control day zero	-.2600*	.094	.042	-.5123	-7.72E-03

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Appendix A : Statistics of pH determination for PVC peritoneal bags Oneway

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
reading	30.605	5	24	.000

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
reading	Between Groups	8.956	5	1.791	55.442	.000
	Within Groups	.775	24	3.231E-02		
	Total	9.731	29			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: reading

Dunnett (2-sided)[a]

(I) process	(J) process	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
freezer	control day zero	-2.60E-02	.114	1.000	-.3324	.2804
stability-chamber	control day zero	-3.00E-02	.114	.999	-.3364	.2764
refrigerator	control day zero	.3040	.114	.052	-2.40E-03	.6104
room-temperature	control day zero	-.1100	.114	.795	-.4164	.1964
sun light	control day zero	-1.3960*	.114	.000	-1.7024	-1.0896

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Appendix A: Statistics of pH determination for PP I.V infusion bottles

Oneway

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
reading	6.526	5	24	.001

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
reading	Between Groups	6.867	5	1.373	78.141	.000
	Within Groups	.422	24	1.758E-02		
	Total	7.289	29			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: reading

Dunnett (2-sided)[a]

(I) process	(J) process	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
freezer	control day zero	-.1820	.084	.145	-.4080	4.400E-02
stability-chamber	control day zero	.1620	.084	.223	-6.40E-02	.3880
refrigerator	control day zero	.1580	.084	.242	-6.80E-02	.3840
room-temperature	control day zero	1.776E-15	.084	1.000	-.2260	.2260
sun light	control day zero	-1.2180*	.084	.000	-1.4440	-.9920

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Appendix A: Statistics of pH determination for PE sacks Oneway

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
reading	18.510	5	24	.000

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
reading	Between Groups	1.676	5	.335	1.972	.119
	Within Groups	4.079	24	.170		
	Total	5.755	29			

Post Hoc Tests

Multiple Comparisons

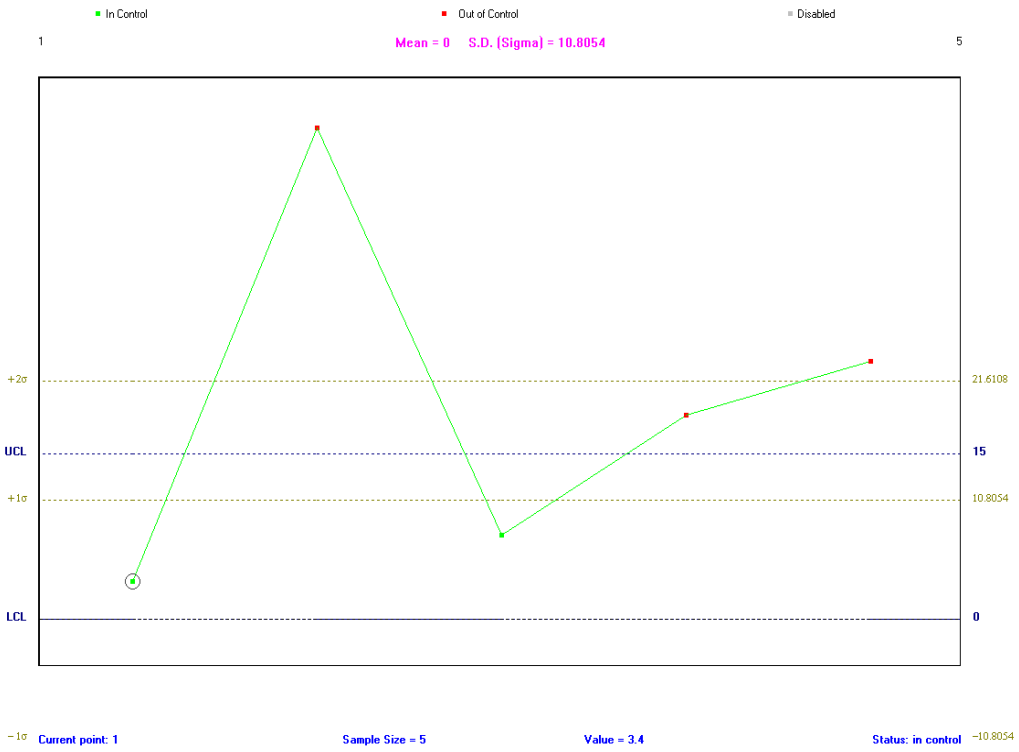
Dependent Variable: reading

Dunnett (2-sided)[a]

(I) process	(J) process	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
freezer	control day zero	.2720	.261	.746	-.4308	.9748
stability-chamber	control day zero	.3700	.261	.492	-.3328	1.0728
refrigerator	control day zero	-.3160	.261	.632	-1.0188	.3868
room-temperature	control day zero	.1240	.261	.985	-.5788	.8268
sun light	control day zero	.3340	.261	.584	-.3688	1.0368

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Appendix B: Lead concentration in water stored in PET type(drug bottles) of plastic containers at different Conditions for ninety days.

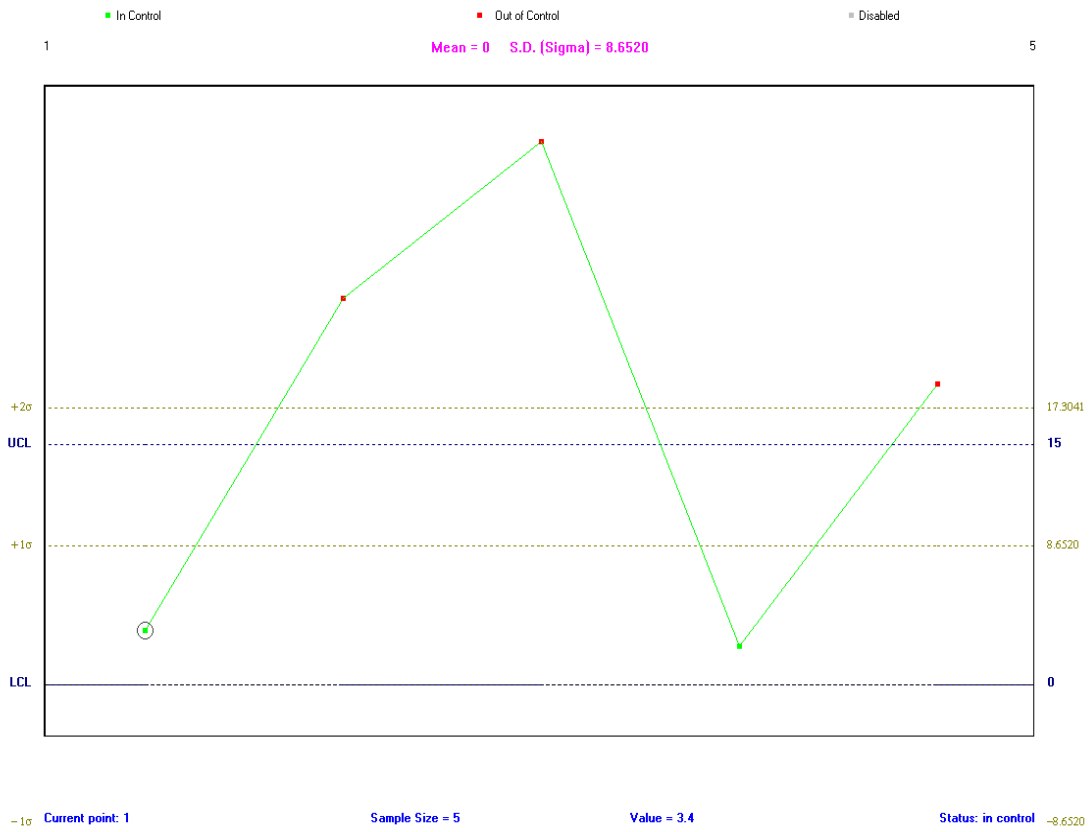


- In control
- Out of control

Starting from left to right:

Freezer, stability chamber, Refrigerator, room temperature and sun light.

Appendix B: Lead concentration in water stored in PET type (water bottles) of plastic containers at different Conditions for ninety days.

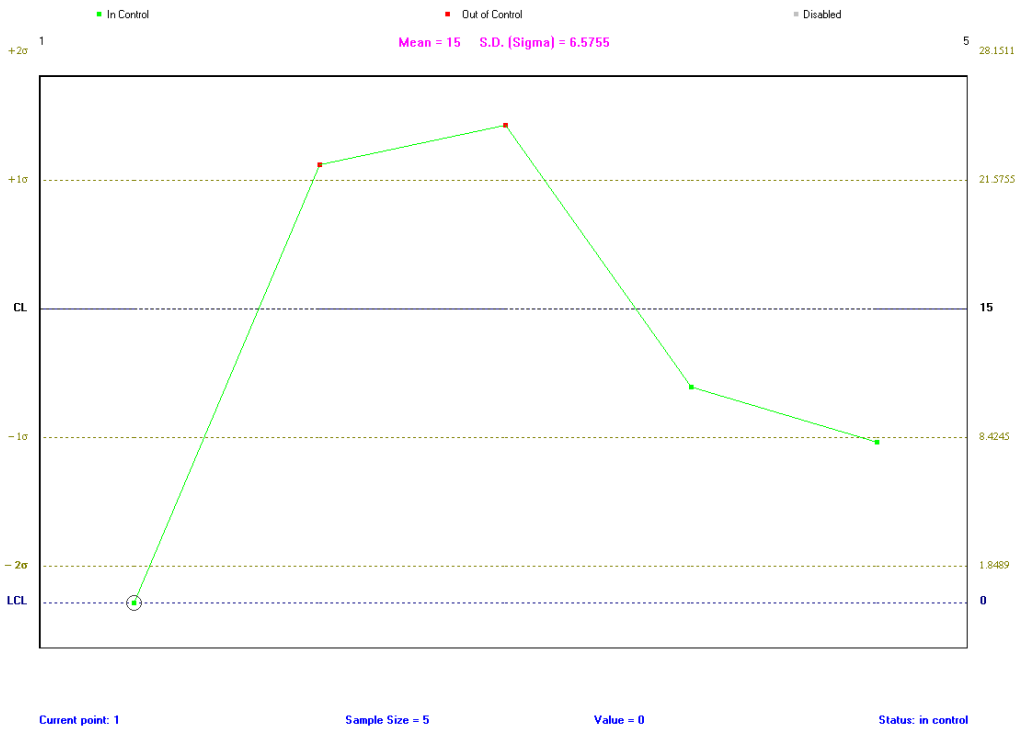


- In control
- Out of control

Starting from left to right:

Freezer, stability chamber, Refrigerator, room temperature and sun light.

Appendix B: Lead concentration in water stored in PVC type (peritoneal dialysis bags) of plastic containers at different Conditions for ninety days.

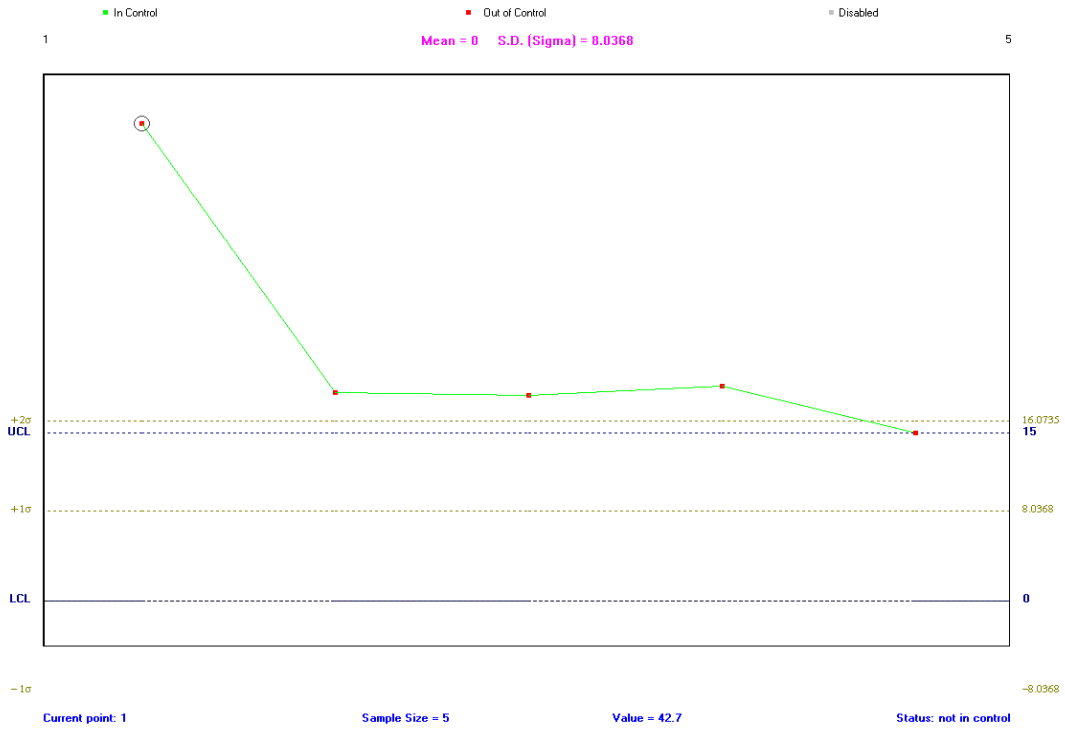


- In control
- Out of control

Starting from left to right:

Freezer, stability chamber, Refrigerator, room temperature and sun light.

Appendix B: Lead concentration in water stored in PP type (IV infusion bottles) of plastic containers at different Conditions for ninety days.



- In control
- Out of control

Starting from left to right:

Freezer, stability chamber, Refrigerator, room temperature and sun light.

Appendix B: Lead concentration in water stored in PE type (sacks) of plastic containers at different Conditions for ninety days.

- In control
- Out of control

Starting from left to right:

Freezer, stability chamber, Refrigerator, room temperature and sun light.

Appendix C: Statistics of reducing substances (T-Test) Blank Vs controls at day zero

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
PET drug bottles	5	17.740	.862	.385
PET water bottles	5	18.760	.336	.150
PVC	4	17.675	1.668	.834
PP	5	18.640	.666	.298

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
PET drug bottles	46.020	4	.000	17.740	16.670	18.810
PET water bottles	124.790	4	.000	18.760	18.343	19.177
PVC	21.192	3	.000	17.675	15.021	20.329
PP	62.622	4	.000	18.640	17.814	19.466

Appendix C: Statistics of reducing substances for PET drug bottles Oneway

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
reading	1.431	5	23	.251

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
reading	Between Groups	13.619	5	2.724	2.533	.057
	Within Groups	24.734	23	1.075		
	Total	38.353	28			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: reading

Dunnett (2-sided)[a]

(I) process	(J) process	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
freezer	control day zero	1.520	.656	.111	-.256	3.296
stability-chamber	control day zero	-.160	.656	.999	-1.936	1.616
refrigerator	control day zero	6.000E-02	.656	1.000	-1.716	1.836
room-temperature	control day zero	.710	.696	.765	-1.174	2.594
sun light	control day zero	1.400	.656	.158	-.376	3.176

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Appendix C: Statistics of reducing substances for PET water bottles Oneway

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
reading	1.100	5	24	.386

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
reading	Between Groups	7.242	5	1.448	8.214	.000
	Within Groups	4.232	24	.176		
	Total	11.474	29			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: reading

Dunnett (2-sided)[a]

(I) process	(J) process	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
freezer	control day zero	.420	.266	.392	-.296	1.136
stability-chamber	control day zero	.540	.266	.187	-.176	1.256
refrigerator	control day zero	-1.00E-01	.266	.995	-.816	.616
room-temperature	control day zero	.100	.266	.995	-.616	.816
sun light	control day zero	-.980*	.266	.005	-1.696	-.264

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Appendix C: Statistics of reducing substances for PVC peritonial bags

Oneway

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
reading	2.931	5	22	.036

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
reading	Between Groups	14.495	5	2.899	1.580	.207
	Within Groups	40.363	22	1.835		
	Total	54.859	27			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: reading

Dunnett (2-sided)[a]

(I) process	(J) process	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
freezer	control day zero	1.285	.909	.481	-1.165	3.735
stability-chamber	control day zero	1.185	.909	.553	-1.265	3.635
refrigerator	control day zero	1.025	.958	.713	-1.558	3.608
room-temperature	control day zero	.365	.909	.992	-2.085	2.815
sun light	control day zero	-.675	.909	.904	-3.125	1.775

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Appendix C: Statistics of reducing substances for PP I.V infusion bottles

Oneway

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
reading	2.734	5	24	.043

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
reading	Between Groups	9.630	5	1.926	1.871	.137
	Within Groups	24.704	24	1.029		
	Total	34.334	29			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: reading

Dunnett (2-sided)[a]

(I) process	(J) process	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
freezer	control day zero	.220	.642	.997	-1.509	1.949
stability-chamber	control day zero	-1.320	.642	.179	-3.049	.409
refrigerator	control day zero	2.000E-02	.642	1.000	-1.709	1.749
room-temperature	control day zero	.400	.642	.955	-1.329	2.129
sun light	control day zero	-.420	.642	.946	-2.149	1.309

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.