2. REVIEW OF LITERATURE

Plastics are polymeric products that have remarkably transformed and alleviated our daily lives by replacing many expensive manufacturing raw materials. Their economical and resilient use in automobile and aerospace industry, electronic gadgets, furniture, kitchen wares, packages, agricultural and construction materials, telecommunication, health and medical products and many other household and industrial products has increased over the years.\(^1\) The demand and extensive use of such polymeric materials is also due to their unique properties such as ease in fabrication, putrefaction and corrosion resistance and incredible stable nature. The ever growing consumer demand for both commercial and domestic plastic materials is mainly due to their ease in availability, affordable cost, user friendly nature and wide range of colored and desired shapes. However these plastics may take years to degrade and thus have both environmental and health hazards. Environmental factors such as light, heat, moisture, chemical and biological processes may bring about physical and chemical changes in the polymers causing bond scission and structural deformations.\(^2,3\) Such polymer degradation causes cracking, erosion, discoloration and delaminating etc.\(^4\) In order to enhance the quality of the commercial polymeric materials many chemicals as additives of various types are consciously added to obtain the desired product. These include stabilizers, fillers, plasticizers, pigments, antioxidants and flame retardants etc.\(^5, 6\) The common use of various inorganic elements such as Al, As, Ba, Br, Ca, Cd, Co, Cr, Cu, Fe, Hg, Ni, Pb, Sb, Sn, Se, Ti and Zn etc. as these additives in plastics is also of concern
from human exposure and pollution point of view as many of these elements are perilous for human health. Many adverse effects can be caused by the materials and different additives causing contamination and breakdown of the products that may result in harmful effects. To ensure safety and to validate that the polymer materials will not critically change with its short or long term usage it is mandatory that these new and degraded plastic containers should be analyzed for their metal contents from health safety and environmental impact point of view.

2.1 Plastics in the society

Production of plastic materials started on an industrial scale in the 1940s and 1950s. In the last 15 years the global annual production of plastics has doubled, reaching 245 million tons in 2008. The annual plastic material consumption per capita in Western Europe, Canada, USA and Mexico, was 100 kg in 2005, which was ten times as much as African countries and five times as much as Asian countries, excluding Japan. This means that there is a large potential for further increased consumption.

Recently (17 February 2011), the European Commission announced a ban on use of six substances which is to be effective within three to five years, unless an authorization has been granted to individual companies for their use. Four of them are used in plastics, i.e. the phthalate plasticizers DEHP, BBP and DBP, mainly used in PVC, and 4, 4’-methyleneedianiline (MDA) used as a curing agent for epoxy resins.

2.2 Plastic composition and hazardous chemicals

Plastic products are made from plastic polymers to which additives are added to enable processing and/or to give certain desired properties for a specific application. The polymers are made by polymerizing monomers
into macromolecular chains. These monomers are almost exclusively derived from non-renewable crude oil. Approximately 4% of world oil demand is used as raw materials for plastic production.\textsuperscript{10}

Other substances (besides monomers) are often needed for polymerization to occur, for instance initiators, catalysts, and depending on manufacturing process, solvents may also be used. The resulting plastic polymer can be blended with different additives, for instance plasticizers, flame retardants, heat stabilizers, antioxidants, light stabilizers, lubricants, acid scavengers, antimicrobial agents, anti-static agents, pigments, blowing agents and fillers, and is finally processed into a plastic product. There are many different plastic polymers and several thousand different additives, which results in an extremely large variation in chemical composition of plastic products.\textsuperscript{11} Plastic polymers are not particularly reactive and their large size limit transport across biological membranes.\textsuperscript{12} They are, therefore, not considered as toxic. In the polymeric material, however, non-polymeric components such as residual monomers, oligomers, low molecular weight fragments, catalyst remnants, polymerization solvents and a wide range of additives can be present.\textsuperscript{13} Several of these are hazardous to human health and the environment, for instance carcinogenic, mutagenic, toxic for reproduction, sensitizing and hazardous to the human health. Since the non-polymeric compounds usually are of low molecular weight and are either weakly bound or not bound at all to the polymeric macromolecules, they, or their degradation products, can be emitted from the plastic product\textsuperscript{9,13} to air, water or other contact media (e.g. food). The content of non-polymeric substances varies between different plastic polymers and products. The residual monomer content depends on polymer type, polymerization
technique and techniques for reducing residual monomer content.\textsuperscript{14} The contents presented for various polymers, in a review by \textsuperscript{14}, was shown to vary from no or very low levels (100 ppm) to up to 40,000 ppm (i.e. 0.0001-4\%). Also the amount of additives used is highly variable. PVC is the plastic type that requires by far the most additives. Of the world production of additives PVC alone accounts for 73\% by volume, polypropylene and polyethylene account for 10 \%, and styrenes account for 5\%. Many additives are hazardous for human health and the environment. Some are especially hazardous, for instance brominated flame retardants used to retard ignition and prevent fire from spreading; some phthalate plasticizers mainly used to make PVC flexible; and lead heat stabilizers used to prevent degradation of PVC during processing.\textsuperscript{15} Several polybrominated flame retardants are very persistent, very bioaccumulating and toxic, and are listed in the Stockholm Convention on Persistent Organic Pollutants (POPs).\textsuperscript{16} Among the phthalate plasticizers the most hazardous ones, i.e. BBP, DEHP and DBP, are classified as toxic for reproduction (category 1B). BBP is also very toxic to aquatic organisms with long lasting effects.\textsuperscript{17,18} In addition, these phthalates, as well as DEP (diethyl phthalate) and DCHP (dicyclohexyl phthalate), are being evaluated for endocrine disrupting properties.\textsuperscript{19,20} The lead compounds used in heat stabilizers are classified as toxic for reproduction (category 1A), very toxic to the aquatic environment with long lasting effects (both acute and chronic), and may cause damage to organs.\textsuperscript{17} Release of hazardous substances from plastic products to air, extraction fluids, water, food, food simulants, saliva and sweat have been shown by chemical analysis. Examples of substances studied and released from various plastic products include phthalates,\textsuperscript{21,22} brominated flame
retardants, bisphenol A, bisphenol-A dimethacrylate, lead, tin and cadmium, formaldehyde and acetaldehyde, 4-nonylphenol, MTBE (methyl tert-butyl ether), benzene and many other volatile organic carbons. In several of the mentioned studies the released concentrations are low (e.g. compared to guideline values), but in others they are considerably higher. The size and type of emissions from plastic products are controlled by many factors. The content of non-polymeric substances controls what can be released, while other factors control the potential of release into a surrounding medium, i.e. the migration potential.

Migration is generally favoured if the polymer matrix is permeable; if the size of gaps between polymer molecules is larger than the size of migrant; if the migrant is small, has a similar solubility parameter as the polymer and is volatile; if the temperature is high; and if the surrounding medium is water for water soluble migrants, fat containing for hydrophobic migrant and acidic for metals.

2.3 Degradation products

The degradation products formed during degradation will vary depending on polymer type. The type and quantity of degradation products formed may also be influenced by degradation mechanisms, presence of polymerization impurities, and surrounding factors, e.g. temperature and oxygen. During thermal degradation nitrogen-containing plastics (e.g. nylons, polyacrylonitrile, and polyurethanes) release hydrogen cyanide; chlorine-containing materials (e.g. PVC) release hydrogen chloride; and fluorine-containing polymers (e.g. polyvinylidene fluoride and PTFE) release hydrogen fluoride by a chain stripping mechanism. Polymers capable of depolymerization by chain scission include polymethyl
methacrylate, polytetrafluoroethylene, and polyoxymethylene, which can depolymerize completely into their initial monomers. Also polystyrene, polyesters (e.g. PET and polycarbonate), nyons and polyurethanes can depolymerise to some extent into their monomers.\textsuperscript{38, 39, 41, 42} Chemicals used in plastics have been detected in humans. Mainly presence of phthalates and bisphenol A,\textsuperscript{43} and brominated flame retardants\textsuperscript{44} have been studied.

Plastic is a relatively cheap, durable and versatile material. Plastic products have brought benefits to society in terms of economic activity, jobs and quality of life. Plastics have wide applications in agriculture, automobiles, space, vehicles, building materials, cosmetics and medicine. The finished plastics are generally considered to be safe provided they are manufactured standard conditions using permitted chemicals recommended by national and international regulatory agencies and used properly.\textsuperscript{45–51} However, several workers have reported health disorders from use of plastics due to migration of unreacted monomers, copolymers, phthalate plasticizer, organotin stabilizer, colorants, UV-absorbing materials, antioxidants, under the influence of physicochemical factors such as sunlight, temperature, type of solvent and pH of the stored commodity.\textsuperscript{52–58}

2.4 Biocompatibility

Biocompatibility is defined as the ability of a material to perform with an appropriate host response in a specific application.\textsuperscript{59} Medical devices may have harmful effects, so they need testing for biocompatibility before the contact with the human body. Now harmonized standards, drafted by the international bodies for standardization (ISO), give the assurance that the essential requisites of medical devices, including biocompatibility, are verified. In order to limit the use of animals in biocompatibility testing,
some considerations and recommendations arose from the 17th Workshop ECVAM held in 1995.60

2.5 Cytotoxicity

Cytotoxicity tests are recommended for all medical devices as (i) they allow for a rapid evaluation, (ii) standardized protocols are employed, (iii) quantitative and comparable data are produced and (iv) due to their sensitivity, they allow for discarding toxic materials prior to animal testing. Experimental studies have demonstrated the good correlation between in vitro and in vivo tests, thus confirming the usefulness of in vitro tests as systems to select the materials.

Cells in vitro are generally more sensitive to toxic materials than in vivo tissues. Therefore, a material toxic in vitro may result not particularly toxic for the tissues in vivo, while a material harmless to the cells, even in long-lasting assays, is likely to be inert also in vivo. A different picture is seen when a material releases in vivo wear debris or corrosion particles; reactive processes can be triggered, with the risk of loosening the implant and failure of surgery. In cytotoxicity testing, the use of the same type of cells which will face the implant in vivo permit analysis of the specific responses. Therefore, at least in a second-step testing, orthopedic materials should be tested on osteoblasts or osteoblast like cells, materials suspected of eliciting an immune reaction should be applied onto mononuclear cells, and cardiovascular materials should be assayed using endothelial cells.

To evaluate cytotoxicity the standard ISO 10993-Part 5 “Biological Testing of Medical Devices-Part 5: Tests for Cytotoxicity in vitro methods” has been adopted. The crucial parameters for cytotoxicity testing are addressed but not specified, “in order to leave the research lab to decide steps to be
taken with regard to cell type, duration of the exposure and method of evaluation."\textsuperscript{61}

Many types of cells have been proposed for use in cytotoxicity testing of materials. The use of cells freshly derived from explants, namely primary cells or established cell lines, has been debated since the introduction of cell culture systems in biocompatibility testing and not yet resolved. With cells derived from tissue explants a better simulation of the clinical situation is obtained.\textsuperscript{62} Established cell lines ensure the reproducibility of intralaboratory results and allow for the comparison of interlaboratory results.\textsuperscript{63} The duration of the exposure of cells to materials/devices is usually dictated by the method of assay, but the in vivo persistence of the material/device may also be considered. The 24-hr period is the endpoint commonly used for cytotoxicity testing.

The cell culture is put in contact with the extract. In the first instance, both chemical toxicity (due to leachable) and geometry of the device are tested. When the material/device is assayed as extract, only the chemical toxicity is tested. In the evaluation of medical devices, cytocompatibility tests are used both for screening and for the study of toxicity mechanisms. The screening tests are conceived to decide whether a material/device is cytotoxic. These tests are fast and simple, have general validity and do not depend on the final use for which the device is intended. For a more detailed study of the mechanisms of toxicity, supplementary tests are used. These tests take into consideration the final application of the device and allow for a compatibility grading of different materials/devices: this can be used in the evaluation of the risk/benefit ratio.
Thus, in order to assess the magnitude of leaching, types of leachates and their cytotoxic potential, the present investigation are proposed with following major objectives:

✓ To identify and quantify the leachates from various grades of plastic biomedical devices using simulating conditions as per BIS and international guidelines.

✓ To study the possible migration and quantification of the additives from the plastic biomedical devices under specific simulating conditions like, temperature, pH and simulating solvents etc.

✓ To study the biosafety (cytotoxicity and pyrogenecity potential) of these leachates under in vitro environment using L929, a mouse fibroblast cell line (approved by ISO 10993).

Due to the ever increasing use of the plastics, manufactures are providing newer formulations and newer ingredients in plastics.

During the course of their manufacturing, storage, and administration, pharmaceutical drug products come in contact with materials, components, and systems. Such contact may result in an interaction between the drug product and these entities. One such interaction is the migration of substances from these entities and into the drug product, which is of concern due to the potential toxicity of the migrating substances. A thin-layer chromatographic method was used to highlight the leaching into drug preparations of several constituents of elastomeric closures. Polymeric materials are commonly used in medical devices such as syringes. The plastic materials may interact with drug products contained within the device, potentially affecting the quality of the drug products.
These interactions may include leaching, which is the migration of entities out of the material and into the drug product, and binding, which is the migration of substances out of the drug product and into the material.  

The accumulation of organic compounds associated with plastic materials into pharmaceutical products and their associated solutions has important suitability for use consequences for those pharmaceutical solutions, most notably in terms of safety and efficacy. 

The predominant organic extractable associated with the test material were bis (2, 2, 6, 6-tetramethyl-4-piperidyl) sebacate (Tinuvin 770), several Tinuvin-related substances, fatty acids, and antioxidant-related compounds. Based on their potential product safety impact, Tinuvin and one of its related substances were chosen as target leachables. In order to establish the accumulation behavior of these target leachables under conditions that simulate the desired application, monobags (100 mL fill volume) and multichambered bags (1000 mL fill volume) were constructed with injection sites made from the test material, filled with water, and subjected to accelerated aging including multiple sterilization cycles and long-term storage at 40°C. Even under the worse-case contact conditions, the accumulation levels of the target leachables were much less than their total available pool in the injection sites. 

Tinuvin 770 is a light stabilizer present in numerous polymers utilized in medical or pharmaceutical applications (e.g., manufacturing, packaging, delivery systems and devices). Under conditions of use, Tinuvin 770 and its related substances may leach from the polymers and accumulate in pharmaceutical products that are administered to subjects to produce a therapeutic benefit.
Plastic materials are widely used in medical items, such as solution containers, transfusion sets, transfer tubing, and devices. An emerging trend in the biotechnology industry is the utilization of plastic containers to prepare, transport, and store an assortment of solutions including buffers, media, and in-process and finished product. The direct contact of such containers with the product at one or more points in its lifetime raises the possibility that container leachables may accumulate in the finished product.  

A multi-layered, laminated plastic material, similar to those used in flexible intravenous solution containers, was loaded to contain known amounts of 15 model compounds whose partitioning behaviors were previously established. The loaded material was extracted with eight different extracting solutions including water (ambient pH); pH 3, 4, 5, and 7 aqueous buffers; and 20%, 40%, and 60% (v/v) ethanol/water mixtures (pH 3). The accumulation of model compounds in the extracting solutions was measured and related to compound and solution properties.  

The levels of targeted leachables extracted from the tubing materials under simulated use (flow) conditions was much smaller than the total amount of these leachables in the tubing.  

The accumulation level of the leachables increased with increasing solution polarity. To ensure the safety for biomedical applications, rapid and reliable methods need to be developed and validated. Several worker have attempted to develop different types of in vitro toxicity assay systems, using cell culture for screening of chemicals with the aim to reduce the use of animals and time, economic and technological improvements for toxicity studies, which is now well accepted by scientific and regulatory
These in vitro systems for cytotoxicity studies have been developed by using non differentiated cells in culture. In vitro systems with various cytotoxic end points have been shown to be suitable for structural activity relationship studies, which might permit toxicity prediction solely for physiological properties. Different types of biocompatibility tests for biomedical devices have already been suggested in CSD 10993, part I, and FDR’s Blue Book Memorandum G 95-1 (Center for Devices and Radiological Health, 1985). In vitro cytotoxicity assessments using human and animal cells were found to exhibit a good correlation with in vivo studies for about 80% of randomly selected chemicals. In vitro culture using L929 and HeLa cell lines has been considered to be a suitable model to assess the toxicological potential of various chemicals, plasticizers, plastic biomedical products. British Standards and United States Pharmacopoeia reference standards have also recognized the use of L929, a mouse fibroblast cell line for the cytotoxicity evaluation of plastic devices.
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