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Research Article

Effects of different kinds of stabilizers on thermal stability, biocompatibility and blood compatibility of PVC film sterilized by ethylene oxide for blood bag

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Abstract:

Background and objectives: The main purpose of this research was to find a proper Poly Vinyl Chloride (PVC) stabilizer system for medical purpose. It was sterilized by ethylene oxide gas, constant ratio of metallic soap stabilizers (calcium-zinc stearate) as well as stabilizers based on organotin (octyle sn-mercaptide, which is commercially known as 17mok901) and soybean oil were studied.

Methods: PVC S-6058 with value=60 from Iran Petrochemical Commercial Company, di-2 ethyl hexil phethalate plasticizer from Iran Azarshimi Company, epoxide soya bean oil (from South Africa's MBT Company), calcium-zinc stearate (from India's Shital Company), octyl tin mercaptide, commercially known as 17® MOK 901, (from India's Shital Company) and BHT (2,6-di-tert-butyl-4-methyl phenol) anti-oxidant (commercially known as Lanxess from Germany's Volkanux Company) were provided and utilized during the research. PVC mixtures prepared by the aforementioned stabilizers were tested under mechanical testing, thermal gravimetric analysis (weight measurement under heating) (TGA) and Fourier transform infra-red (FTIR) (determination of functional groups in the stabilized PVC) before and after sterilization by ethylene oxide gas.

Results: Assessment of blood compatibility of samples was done by Hemolysis test, according to ISO 10993 standard part 4. Results of the tensile test demonstrated that calcium-zinc stearate was the best PVC stabilizer. In order to improve its tensile properties, with 2.67 strain rate, sterilization improved its mechanical properties while preserving strength.

Conclusion: The results from Fourier transform infra-red (FTIR) on chemical structures of samples suggested that sterilization by ethylene oxide gas had no negative effect on the structure of the stabilized PVC. Furthermore, the mixture made from PVC had stabilized on organotin and was of higher thermal stability as well as had better blood-compatibility properties with respect to other stabilizers. This confirms acceptable efficiency of stabilizers utilized for hygienic and medical purposes.

Keywords: thermal stabilizer, PVC film, sterilization, blood compatibility, blood bag

Background and objectives

PVC is the second largest produced polymer in terms of volume and is the second best among poly olefins**.** Based on market evaluation, PVC comprises nearly 25% of the total plastic production among medical products[1]. Soft PVC products are widely utilized in the manufacture of a broad range of medical devices such as bags for intravenous liquids, tubes, cannula, plasma, blood bags, gloves, oxygen masks, secondary pack, bags, IV sets, catheters, and dialysis instruments. Soft PVC was in medical use for more than 40 years due to its competitive characteristics such as flexibility, transparency, mechanical efficiency, vapour-sterilization ability and cost efficiency[2,3].

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One of its constrictive applications is in high temperature conditions as it tends to plasticize under heating and thermal degradation. Thermal degradation also occurs in lower temperatures when mechanical break-up develops. Thermal effects should be monitored during production and utilization. Moulding temperature during manufacturing should be higher than during its final use. Therefore, stabilizers are utilized during their production in order to prevent their thermal degradation[4].

Classifications of major thermal stabilizers, which are currently utilized, are as follows: Lead-based systems, metallic soaps and organotin materials[5]. Recent studies on thermal stabilization of PVC formulations and different stabilization systems such as organotin carboxylates (Ca) and organotin mercaptides demonstrated feasible performance against degradation. The major reason for using non-toxic stabilizers such as calcium-zinc stearate among other compounds lies in their environmental limitations as they contain heavy metals such as lead[6]. Metallic soap and organo-tin mercaptide stabilizers are safer than stabilizers containing lead-based systems, but their stabilizing effects are generally less than lead-based systems. In fact, calcium-zinc soap and some soya bean oils were investigated and it was proved that they cause higher PVC thermal stability[1,5]. Epoxide soya bean oil was the second thermal stabilizer and plasticizer used in PVC plasticized systems[7].

Heat stabilizers act by stopping oxidation, or by attacking the decomposed products of oxidation. Heat stabilizers affect the process of stabilization and stability properties, as well as resistance to thermal degradation of components under mechanical stress or load, colour and transparency[8].

It is well known that unless it is stabilized, PVC decomposes at processing temperatures, producing Hydrogen chloride (HCl); its decomposition is usually accompanied by material discolouration. PVC thermal degradation is the result of a process called zipper dehydrochlorination, which generates conjugated double bonds in polymer chains. It is generally accepted that visually noticeable colour changes occur when conjugated polyene sequences containing 6 or more double bonds (long polyenes) are formed[9].

Plasticized PVC formulations have traditionally been used in the production of medical devices, in research of Balakrishnan presented degradation of PVC plasticized with non-phthalate plasticizers under sterilization conditions[10]. Also, in researches of Burgos et al, and maitz et al the mechanical and chemical properties of the materials in medicine applications and their thermal properties have been reviewed. Polymer is the class of biomaterials which is most sensitive to sterilization procedures. There is a large variation, however, among polymers with regard to their sensitivity to radiation, heat and humidity[11,12]. Damage to polymers ranges from some oxidation to cross-linking, complete distortion and melting. Moreover, polymers absorb EO, so that EO can leave a significant quantity of toxic residues[13].

Ethylene oxide (ETO) sterilization imposes no or very little effect on the physical properties of PVC[14]. Sterilization by using ethylene oxide gas has a broad use due to its ability to sterilize at lower temperatures and higher evaporation when the gas is absorbed. Sterilization by ETO is used for products which are sensitive to vapour and temperature sterilization or degraded against sterilization by irradiation[7].

The present research deals with practical investigation of physical characteristics, mechanical properties as well as PVC film production in order to investigate their application in blood bag production and their use in medical mixtures. The purpose of the present research is to investigate PVC's physical characteristics and blood-compatibility in various conditions, including utilizing (Di-2 ethyl hexil phethalate) as plasticizers as well as epoxide soya bean oil, calcium-zinc stearate and organotin mercaptide (commercially known as 17mok901) as thermal stabilizers. Furthermore, ETO effects on mechanical, physical, chemical, and biological characteristics of PVC were investigated.

Methods

Ten kind of the PVC S-6058 with value=60 from Iran Petrochemical Commercial Company, di-2 ethyl hexil phethalate plasticizer from Iran Azarshimi Company, epoxide soya bean oil (from South Africa's MBT Company), calcium-zinc stearate (from India's Shital Company), octyl tin mercaptide, commercially known as 17® MOK 901, (from India's Shital Company) and BHT (2,6-di-tert-butyl-4-methyl phenol) anti-oxidant (commercially known as Lanxess from Germany's Volkanux Company) were provided and utilized during the research.

phases

Sample preparation

First, the fraction of each component was determined in 100 units (phr) when the stabilizer percentage was measured. Then, the weight of each sample considered was 60 grams. The amount of each component brought (in table 1) included PVC, stabilizer, anti-oxidant, plasticizer and relevant calculations were made.

Table 1. Components of 60 gr samfigples

sample	Additives	PVC		Plasticizer(DEHP)		Stabilizer		Antioxidant (BHT)	
		gr	phr	gr	phr	gr	phr	gr	phr
1	Ca-Zn stearate	38.33	100	21.08	55	0.76	2	0.19	0.5
2	soybean oil	38.33	100	21.08	55	0.76	2	0.19	0.5
3	17 mok 901	38.33	100	21.08	55	0.76	2	0.19	0.5
4	Control sample(without stabilizer)	38.33	100	21.08	55	٠	٠	0.19	0.5

In order to prepare a mixture and its homogenization, the component of each sample was non-instrumentally mixed followed by the internal mixture set in order to get a homogenous mixture. The set is installed at a plastic laboratory at the Iranian Polymer and Colour Research Institute. The temperature was set at 160°C, the rotation at 80 rpm and moments at 60 Nm. Each sample was mixed for 2 minutes.

Sterilization

The sterilization procedure by ethylene oxide gas consisted of three stages: pre-vacuum stage in order to attain desired pressure and moisture (less than 100 K Pascal) under 50°C, the second stage when contact with ethylene oxide gas of 90% purity for 15 minutes was provided and the third stage which was ventilation for 12 hours, according to medical devices sterilization standard ISO-11135 at the sterilization equipment in Fakher Baspar Sanaat manufacturing company.

Mechanical properties

In order to evaluate mechanical characteristics of the samples, tensile test had been done where dumb-bell films of 1 mm thickness were prepared. The experiment was conducted on samples of 4±0.2 mm and 10 mm width by GT-TCS2000 Universal Testing made in Taiwan, according to ISO 527 standard on both sterilized and non-sterilized samples individually. The length of the internal spacing between grips was 50 mm and the spacing between the grips was set at 115 mm at 50 N force and constant velocity of 50mm/minutes. The elongation percentage up to break-up point was measured by the software of the instrument during pull-apart. Five parallel measurements were made and the average values were reported.

Thermal analysis

In order to do this, Swiss-made METTLER TOLEDOTGA/DSC was utilized. The temperature programme of the instrument was set from 0°C to 450°C at a rate of 10°C/minute as fro-back-fro. The gas in the unit selected was liquid nitrogen at 30 ml/minute. 10–13 mg of each sample was separated and put in the unit.

Fourier transform infra-red spectrometry

In order to recognize the present functional groups at the sample surfaces, FRONTIER FTIR spectrometer manufactured by US PekinElmer Company was utilized after sterilization by ethylene oxide gas for different stabilizers. All spectrometric measurements were conducted in the range of 400–4000^{-1}.

Quality evaluation of sterilization procedure

The purpose of the present test is evaluation of the sterilization guarantee level of the sterilized samples based on the Iranian National Standard registered 3001–1 and 3001–2.

1. Cultivation medium: In order to do the experiment, two types of cultivation medium are required: Fluid thioglycolate medium (FTG) and Tryptic soy broth (TSB). 30 grams of cultivation medium powder was dissolved in 1000 cc distilled

water and put on fire up to boiling point. The solution was kept boiling for one minute. Then, the solution (as much as 80 cc) was poured in special glasses. The samples were preserved in autoclave for 15 minutes at 123°C and 1.1 bar pressure (of 7.3±0.2 cultivation medium pH).

2. The samples were washed by 250 cc 0.9% NaCl washing solution serum. The resulting sample was passed through 0.45 µm filter using vacuum pump. If there was any micro-organism, it was left on the filter. One filter was set in thioglycolate medium and the other in TSB medium.
3. The glass containing FTG was preserved for 12 days at 37°C while the glass containing TSB for 14 days at 25°C.

Hemolysis assay

Hemolysis due to exposure of red blood cells with membrane destabilization agents is used to show the endosomal escape of a system (Plank et al., 1994). In the present study, Hemolysis assay was used to investigate the extract's capability to disrupt the membrane. Briefly, whole human blood was collected in EDTA-containing vacutainers and red blood cells (RBCs) were separated from the plasma by a 2-minute centrifugation in 10,000 rpm. RBCs were washed three times with phosphate buffer saline (PBS). After final washing, the RBCs were resuspended and diluted in PBS at pH 7.4 which yielded a solution with 10^{10} RBCs. This solution adjusted to 1 ml with PBS and extract's samples and then incubated for 3 hours at 37 ◦C. The tubes were centrifuged for 5 minutes at 10,000 rpm and the absorbance of the supernatant was measured at 540 nm. 1% TritonX-100 was used as the positive control and buffer alone was used as negative control.

The test investigated lysis rate of PVC plasticizer-stabilizer on red blood cells. The released haemoglobin released from sterilized samples was measured by Bio wave II WPA spectrophotometer. Hemolysis was calculated by the relationship one as following:

$$\text{Hemolysis percentage} = \frac{\text{absorbtion of the given sample}}{\text{absorbtion of the positive control}} \quad (1)$$

Results

Thermal gravity analysis (TGA)

Results of the present investigation show that degradation occurred in two stages beginning at 0–450°C temperature range. The first stage of dehydrochlorination starts from weak bonds. This led to the development of double bonds, when these bonds break down in the second stage. As illustrated in Figure 1, other stabilizers in improving PVC thermal stability. PVC stabilization mechanism by organotin compounds involves entrapment of hydrochloric acid, active chlorine reaction and replacement by carboxylic group.

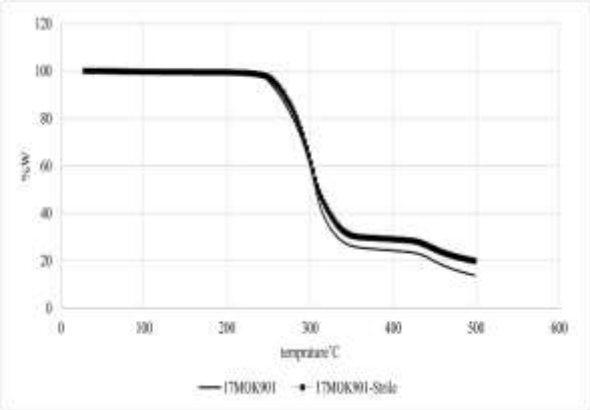

Fig. 1. TGA results of sterilized 17mok901

As shown as, in Figure. 2 and 3, calcium-zinc stearates as well as soybean oil stabilizers had nearly similar degradation temperatures which was 242°C on average.

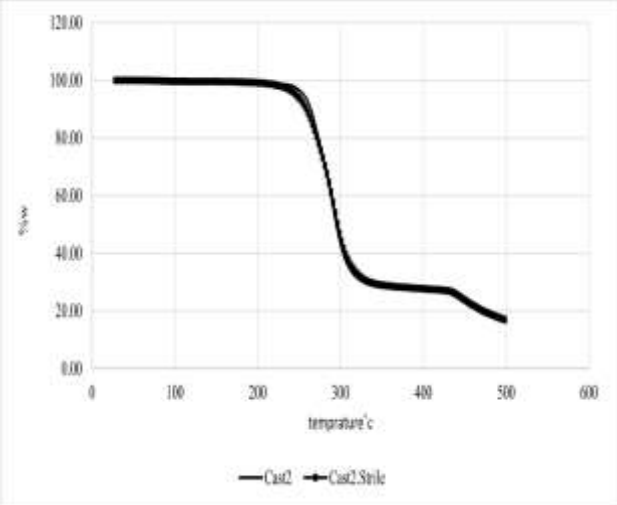

Fig. 2. TGA results of sterilized calcium-zinc stearate

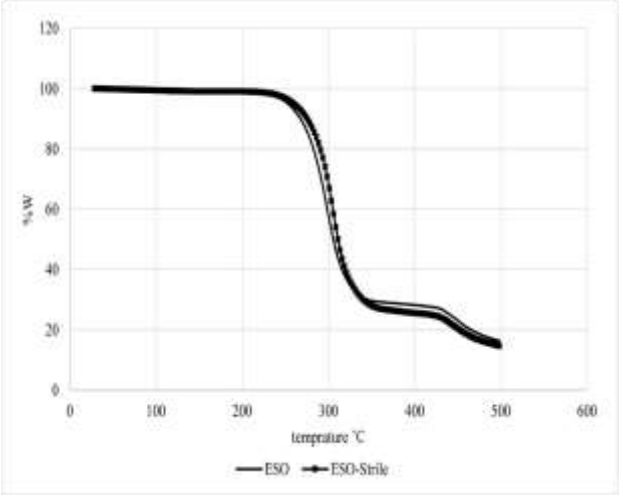

Fig. 3. TGA results of sterilized soy bean

The results of the analysis demonstrated that the sterilization process had no effect on the minimum and maximum degradation temperature of the reference sample and the samples containing stabilizers. Maximum degradation and mass loss of the samples occurred at the first stage of PVC degradation. Mass losses of the samples at the first and second stages were 72.32% and 11% on an average, respectively.

As illustrated in figure 3, degradation temperature in the samples stabilized by 17mok901 was higher before and after sterilization, which was 254°C on an average. It can then be concluded that this stabilizer had a better performance than any other stabilizer in improving PVC thermal stability. As shown in figure 4and5, calcium-zinc stearate as well as soy bean oil had fairly similar degradation temperatures which were 242°C both before and after sterilization. It was observed in figure 4 that the degradation temperature was 240°C for samples without stabilizer which was less than all other samples.

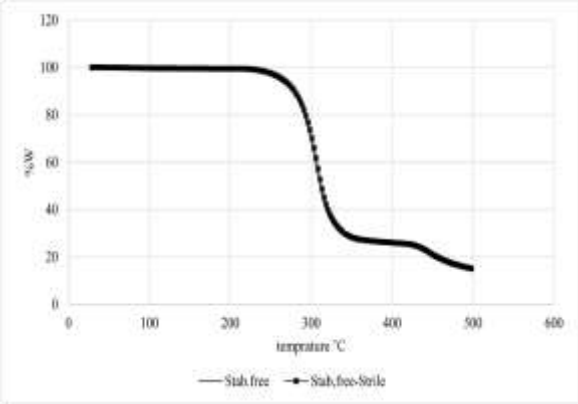

Fig. 4. TGA results of non-stabilized PVC after sterilization

Differential thermo gravimetry (DTG) analysis

The results determined values for the maximum weight loss temperature (T_{max}), initial temperature (Te) and final temperature (T_f). The three aforementioned temperatures changed with changing test conditions. DTG peak temperature indicated mass change velocity.

As observed in fig 5, 6, 7, and 8, PVC loses its mass during the degradation processes. As was illustrated in figure 8, and by comparing thermal stability of each stabilizer utilized, it can be concluded that the initial degradation temperature is higher in the sample stabilized by 17mok901 (nearly 248^{o}C) compared to samples stabilized by other components, both before and after sterilization. Maximum weight loss temperature and the final weight loss temperature were 306^{o}C and 356^{o}C, respectively. Mass variation velocity was 1.4×10^{-2} at T_{max} which is lower than that of other stabilizers. The results suggested better performance of 17mok901 stabilizer compared to other stabilizers. The remaining mass of the sample containing the aforementioned stabilizer at the end of the test was more than that of samples with other stabilizers which was nearly 1.9515 mg.

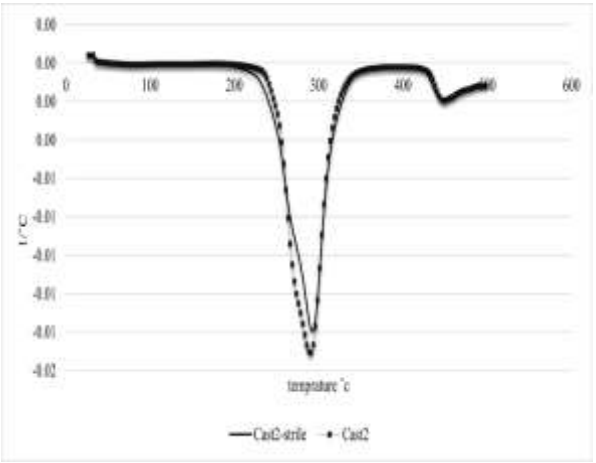

Fig.5. DTG results of sterilized calcium-zinc stearate

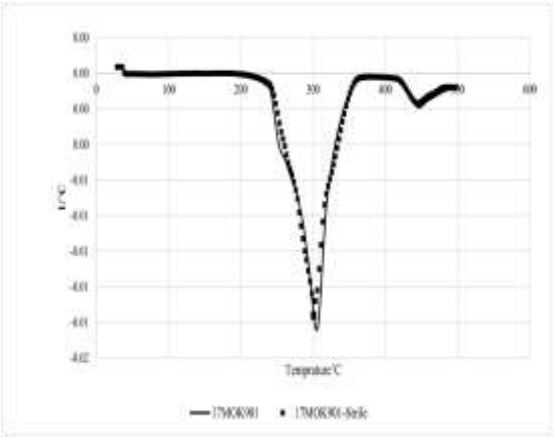

Fig.6. DTG results of sterilized 17mok901

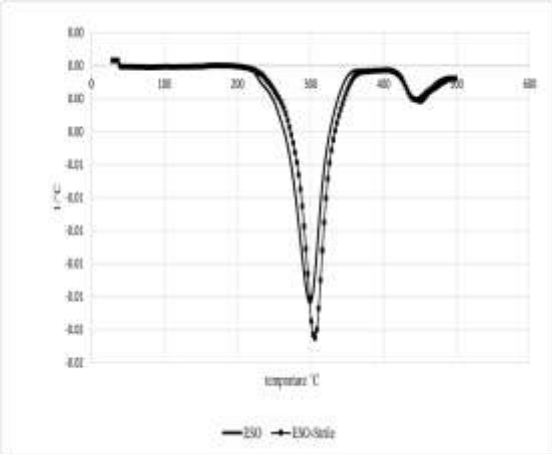

Fig.7. DTG results of sterilized soy bean oil

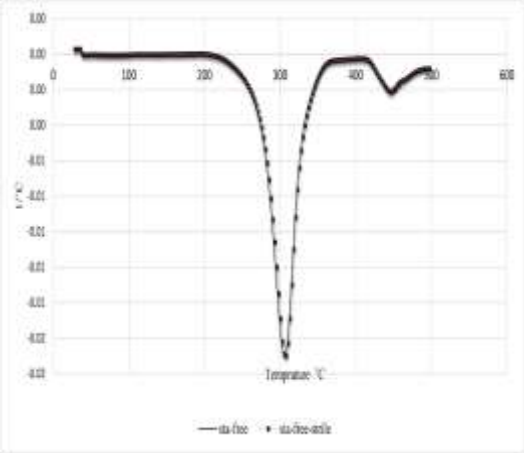

Fig.8. DTG results of non-stabilized PVC after sterilization

Fourier transform infra-red (FTIR) spectroscopy

The sterilized samples were developed as a result of sterilization by ethylene oxide gas and types of stabilizers as well as structure of the processed PVC were compared to the newly-developed peaks or their eliminations as well as their chemical structure.

The major peaks related to carbonyl group were nearly 1700 cm^{-1} with tensional vibrations of 2800–3000 in C–H and 600–900 in C–Cl in the major PVC structure.

As was illustrated in figures 9, 10, 11, and 12, the FTIR results demonstrated that all index peaks obtained from FTIR of samples stabilized by calcium-zinc (Ca-Zn) stearate as well as17mok901 and soy bean oil overlapped the spectra of the sample without stabilizer.

It was observed that in figure 9, 11 and 12, of the PVC samples, two bands were observed within 1500–1600 cm^{-1}range which were ascribed to symmetric and asymmetric tensional vibration of carboxylate group.

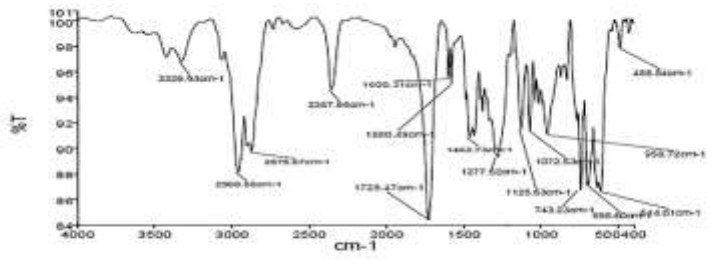

Fig. 9. FTIR spectra of PVC stabilized by calcium-zinc stearate after sterilization

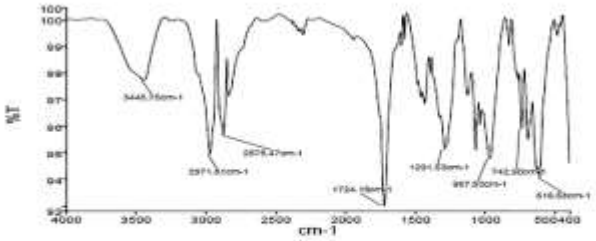

Fig.10. FTIR spectra of PVC stabilized by 17mok901 after sterilization

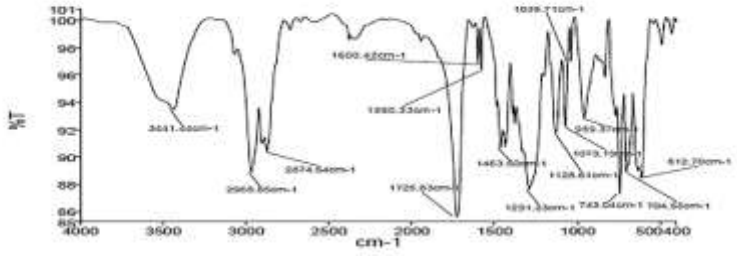

Fig. 11. FTIR spectra of PVC stabilized by soy bean oil after sterilization

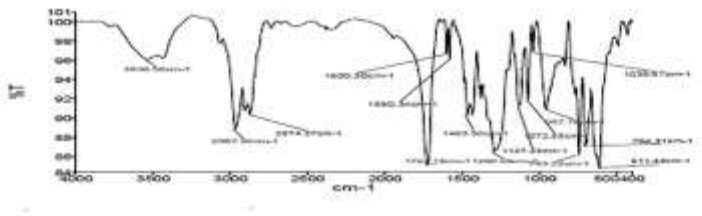

Fig. 12. FTIR spectra of non-stabilized PVC after sterilization

Absorption within 1580–1650 cm^{-1} in the degraded PVC is usually ascribed to olefin groups.

1728.47 peak is of higher intensity than other peaks which is related to tensional vibration of carbonyl group –C=O of an ester bonded to a polymer chain within 1723–1728 cm^{-1}.

743.23 cm^{-1}, 695.60 cm^{-1} and 614.01 cm^{-1} all are ascribed to tensional vibration of C-Cl.

2875.67–2874 cm^{-1} peak was ascribed to C–H asymmetric tensional vibration and 2960.68 cm^{-1} peak was ascribed to C–H symmetric tensional vibration. 1580 cm^{-1} was ascribed to C=C tensional vibration while 3326.93 peak suggested polyene formation and aromatic structures [7].

Absorption was detected within 3400–3500^{-1} range which was related to unreacted fatty acids ascribed to metallic soap (Ca-Zn stearate) structure in the formulation of stabilized PVC.

Regarding the results of the test and study on chemical structure of the samples, it can be concluded that sterilization by ethylene oxide gas has no adverse effect on structure of the stabilized PVC.

Discussion

Results of the mechanical test indicated that all the samples demonstrated flexible behaviour. In other words, they increased in length with increasing force exerted on them.

As illustrated in Figure 13, strain rate of the sterilized samples stabilized by Ca-Zn stearate and soy bean oil increased. The data confirmed that sterilization by ethylene oxide gas promoted tensile strength. The tensile strength increment in the samples stabilized by Ca-Zn stearate was 1.06% while it was higher in ones stabilized by soy bean oil (1.39%). Sterilization did not involve tensile strength of the samples stabilized by 17mok901. The test generally demonstrated that Ca-Zn stearate was the most suitable PVC stabilizer for promoting tensile strain of 2.67%. Sterilization promoted strain rate of 1.06% while preserving its strength.

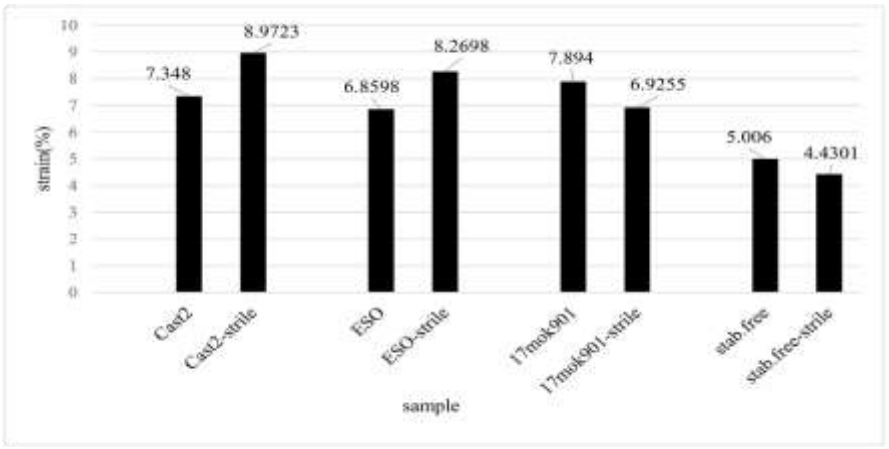

Fig. 13. Comparison of maximum strain rate in samples No.1 to 8.

Inhibition in mechanical characteristics was observed in the reference sample at 0.15% and the results were similar as the sample was stabilized by 17mok901. It can simply be concluded that this stabilizer had no effect in boosting tensional properties.

As shown in figure 14, maximum stress at rupture point increased 1.63% and 1.41% after sterilization for calcium-zinc stearate and soya oil respectively. Maximum strain respectively increased by 1% and 1.4%. So, it may be concluded that calcium-zinc stearate and soya oil preserve tensile strength of PVC better at higher stress rate.

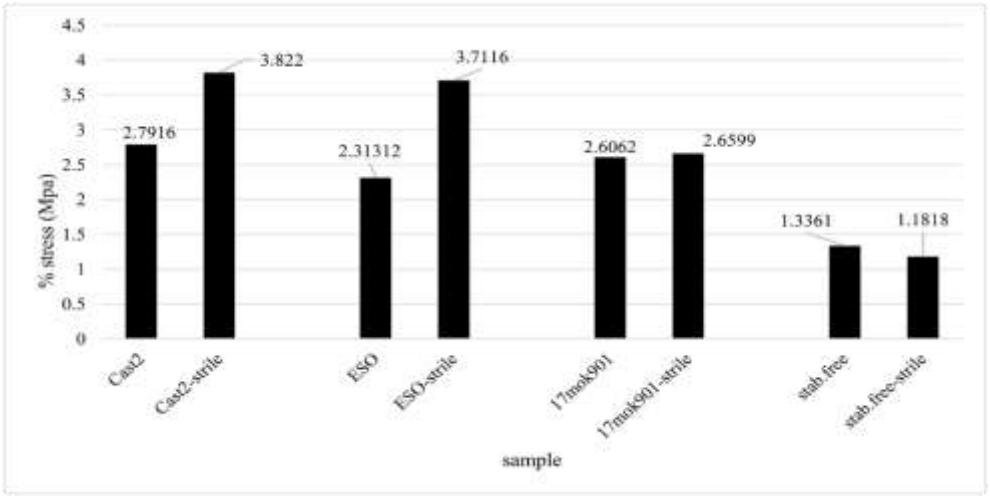

Fig. 14. Comparison of maximum stress rate in samples No.1 to 8.

Tensile test results demonstrated that PVC flexibility can be rather improved using suitable stabilizers. The general test results suggested that calcium-zinc stearate is the best stabilizer to improve PVC tensile strength. Sterilization involved its strength, while promoting its tensile strength.

Quality evaluation of the sterilization process

As illustrated in Figure 15, both the cultivation media proved to be bacteria free and can be considered sterilized.

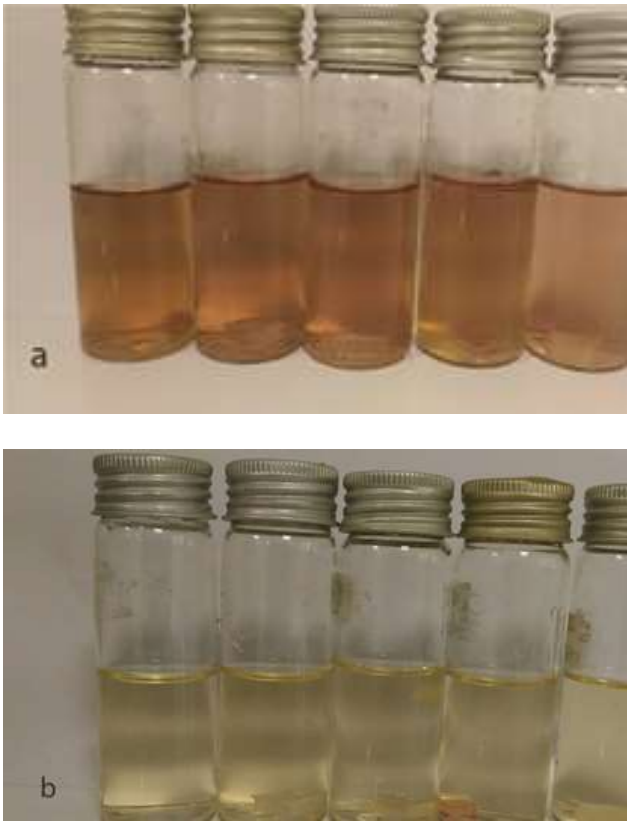

Fig. 15. Fluid thioglycolate medium (FTG) (a) and tryptic soy broth medium (TSB) (b)

Hemolysis test result

Hemolysis percentage of sample 1,2,3 and 4 after sterilization after sterilization is illustrated in Fig16. Samples with hemolysis less than 2% had optimal blood compatibility. Positive control indicated 100% Hemolysis whereas negative control demonstrated 0%.

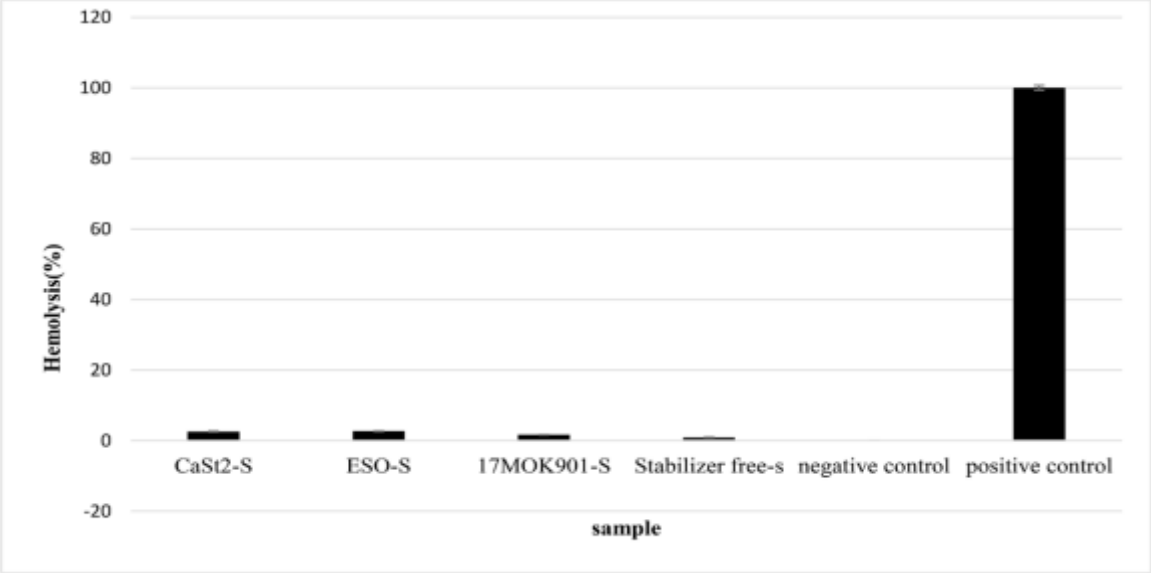

Fig. 16. Hemolysis percentage of samples 1,2,3,4 after sterilization

According to the standards set for medical devices, samples with more than 2% Hemolysis were considered as rather hemolysing. The results suggested that samples had negligible Hemolysis. PVC stabilized by 17mok901 with 1.686% Hemolysis and non-stabilized PVC with 9.079% hemolysis had the least hemolysis and the most blood compatibility. Furthermore, sample stabilized by soy bean oil with 2.7% followed by the one stabilized by Ca-Zn stearate and 2.59% were considered rather hemolysing.

Conclusion

Tensile test results demonstrated that utilizing proper stabilizer improved PVC plasticity. The general results of the test illustrated that calcium-zinc stearate is the suitable stabilizer for PVC which increased its tensile properties with 2.67% strain. Sterilization improved its tensile rate by 1.06% while maintaining its strength.

TGA results demonstrated that the degradation temperature was higher in the sample stabilized by 17mok901 (254^{o}C on average) compared to other stabilizers both after and before sterilization. It can be concluded that 17mok901 had a better performance in promoting PVC thermal stability compared to other stabilizers. PVC stabilization mechanism in organotin stabilizers was based on hydrochloric acid entrapment followed by reaction and replacement of active Cl by carboxyl groups. Calcium-zinc stearate as well as soy bean oil had similar degradation temperature both before and after sterilization which was 242^{o}C on an average.

DTG results indicated that mass loss initial and final temperatures as well as maximum mass loss temperature in all

samples with the same stabilizer were the same both before and after sterilization. It can be concluded that sterilization by ethylene oxide gas had negligible effect on PVC thermal stability.

FTIR, which was done just after TGA tests, suggested proper evidences based on the presence of tensile vibrations of ester carbonyl group –C=O bonded to polymer chain in the range of 1723–1728^{-1}. Regarding results of the test and studying chemical structure of the samples, it can be concluded that the sterilization process by ethylene oxide gas had no adverse impact on the structure surface of the stabilized PVC.

Sterilization confirmation of the samples sterilized by ethylene oxide gas indicated that the stabilizer, in addition to PVC, decreased blood compatibility of the samples so that the sample without stabilizer was the least homolysing. The sample stabilized by organotin stabilizer was the least homolysing (1.68%) compared to other stabilizers.

Competing Interests

The authors declare no competing interests.

Authors' Contributions

The authors contributed equally to the writing of the article

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