



Synthesis, Characterization of Phosphate Glass Containing Silver and Antibacterial Studies of Textile Coated with Above Glass using Simple Techniques

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Silver is known to kill more than 650 types of microorganisms. In order to lead a hygiene lifestyle, several attempts were made for incorporating nano particles of silver on various substrates, especially on fabric by different research groups. Nano silver with particle size less than 5 nm, invariably exist in dark yellow colour. Coating of this nano silver on fabric, impart a dirty yellow colour, which is difficult to remove, even after rigorous washing. Silver incorporated in water degradable phosphate glass matrix can be the best solution for the above said problem. In this study, the phosphate glass with varying composition with general formula $(\text{CaO})_x(\text{P}_2\text{O}_5)_y(\text{Ag}_2\text{O})_z; \text{SiO}_2, \text{B}_2\text{O}_3$ were prepared, by mixing corresponding raw materials and melting between 900 °C and 1000 °C. The glass samples were characterized using XRD, FTIR and SEM techniques. The influence of silica and borate contents on solubility of the glass is studied in detail. Different coating techniques were adopted for coating silver on the textile and antibacterial activity studies were carried out for gram positive and gram negative bacteria, using ASTM-E-2149 procedure. Unlike nano colloidal silver, the silver-coated textile using present glass sample retained its original colour.

Key Words: Water degradable glass, Nano silver, Antibacterial, Textile coating.

INTRODUCTION

Silver in various forms has been used for centuries as an antimicrobial agent in curative and preventive health care. With the advent of sulphamide drugs and heavier antibiotics, the use of silver decreased. The evolution of resistant bacterial strains towards these antibiotics and bactericides in recent years triggered interest in finding biocidal materials. Silver has broad antimicrobial activity while exhibiting low toxicity towards mammalian cells¹⁻³. Silver interferes with DNA replication of microorganisms, inhibit electron transport and/or causes conformational changes on the cell membrane⁴. Silver salts leads to uncontrolled release of silver while the bulk metal is sluggish and leads to inefficient releasing. Nanosilver colloids have broader size distribution and diffuse inefficiently. Most of the surfactants used in their preparation of the same are toxic. The promising innovation to overcome the above drawbacks is to obtain silver incorporated phosphate based glass matrix. These are water soluble materials that can act as a unique system for controlled release of silver⁵. One of the important advantages for selecting phosphate glass matrix is their biocompatibility⁶. The target application of the present study is to coat silver released from phosphate glass on textiles, for common/medicinal applications including hospitals, linens and wound dressing. Coating nanosilver prepared by conventional

methods on fabrics imparts a dirty yellow colour, which is difficult to remove even after rigorous washings and hence affects aesthetic value of the fabrics. Therefore, the aims of this study were to (i) produce silver incorporated phosphate based glasses of varying compositions (ii) study the water-degradable property of these glasses and (iii) coating on the fabric with silver, released from the above glasses by adopting domestically used conventional coating techniques (iv) evaluation of antibacterial activity of the above silver-coated fabric.

EXPERIMENTAL

Preparation of phosphate glass: Phosphate glasses were prepared by mixing required quantities of phosphate source, glass former and additives. The role of additives in the glass matrix is to alter the solubility of glass in water and to influence the controlled release of silver for optimum antibacterial activity.

Weighed quantities of calcium carbonate, quartz were mixed. Silver nitrate solution was added to the above mixture to form paste and the contents were blended for 15 min. Phosphoric acid was added in drops to the above paste under vigorous stirring while exothermic reaction with effervescence takes place. The contents were dried at 110 °C for 2 h. The

dried mixture was transferred into alumina crucible and heated between 900 °C and 1000 °C, in a muffle furnace to form molten mass, which was transferred into molds to form beads. The phosphate based water degradable glasses were made with varying $P_2O_5:CaO$ mole ratio, with constant SiO_2/B_2O_3 contents. Another set of samples were prepared by varying the additive content with same $P_2O_5:CaO$ mole ratio. The details of samples and their identification are given in Table-1.

TABLE-1
COMPOSITION OF PHOSPHATE GLASS SAMPLES

Sample	$P_2O_5 : Ca : Si/B_2O_3 / Na_2O : Ag_2O$	P_2O_5 (%)	CaO (%)	SiO_2/B_2O_3 (%)	Ag_2O (%)
CaP-Si-1	1 : 1.25 : 0.10 : 0.01	64.8	31.9	2.77	0.53
CaP-Si-3	1 : 1:00 : 0.10 : 0.01	69.2	27.3	2.94	0.56
CaP-Si-5	1 : 0.75 : 0.10 : 0.01	74.3	22.0	3.10	0.60
CaP-Si-2	1 : 1:00 : 0.05 : 0.01	70.2	27.7	1.52	0.58
CaP-Si-4	1 : 1.00 : 0.15 : 0.01	68.2	26.9	4.35	0.55
CaP-B-1	1 : 1:00 : 0.05 : 0.01	70.1	27.6	1.73	0.57
CaP-B-2	1 : 1:00 : 0.10 : 0.01	68.4	27.7	3.34	0.56
CaP-B-3	1 : 1.00 : 0.15 : 0.01	67.7	26.7	5.05	0.55
CaP	1 : 1.00 : 0.00 : 0.00	72.0	28.0	0.00	0.00
CaP-Si	1 : 1:00 : 0.10 : 0.00	69.6	27.5	2.94	0.00
CaP-Ag	1 : 1:00 : 0.00 : 0.01	70.9	28.0	0.00	1.10

Physical characterization techniques: Powder diffraction pattern of the glass powder was recorded, after grinding down the particle size of the powder below 45 μ , using Rigaku miniflex (desktop model). Since, the glass powder was opaque in nature; FTIR was recorded in diffuse reflectance mode using, Bruker (Tensor 27) spectrometer. The morphology of the glass powder was analyzed using Hitachi Tabletop SEM (TM-3000) microscope.

Solubility analysis: The glass beads were analyzed for their degradable property in aqueous medium by suspending accurately around 20 g in 1 L beaker containing 600 mL distilled water. The contents were stirred at 600 rpm for 3 h in ambient temperature. The solubility was estimated by calculating decrease in weight of glass beads and reported in terms of mg/g/h.

Coating techniques

Dip-coating technique: The glass beads were dissolved in distilled water to form glass solution with known concentration of silver. Pure cotton cloth of 5 cm \times 5 cm dimension was immersed in 25 mL of the above solution for 5 min. It was then removed using sterilized forceps and dried under shade in dust free atmosphere. The silver coated fabric was sealed and labeled.

Spin-washing technique: Another method of coating silver over the fabric was carried out by dipping the cotton cloth of 5 cm \times 5 cm dimension in 1 L beaker containing 250 mL distilled water. The glass beads were sealed in net-pouch and suspended in the beaker. The contents were stirred at 50 rpm. The silver released from the glass matrix deposits on the surface of the fabric. The cloth was removed with forceps and dried in dust free environment. The silver coated fabric was sealed and labeled.

Steam-pressing technique: The deposition of silver can also be effected by steam-pressing technique. In this technique, the silver glass solution was added in chamber provided to

produce steam in hot-pressing machine. The cotton cloth of dimension 5 cm \times 5 cm was steam-pressed with the machine. The silver dissolved in the solution was transferred on the surface of the cloth along with steam drops. The silver-coated cloth was sealed and labeled.

Antibacterial analysis

Silver containing phosphate glass beads: Antibacterial analysis of silver phosphate glass samples were carried out using ASTM-E-2149 procedure. The bacterial strains selected for the study were gram positive (*S. aureus*, ATCC No. 6568 P) and gram negative (*E. coli*, ATCC No: 25922). The reduction in number of colony forming units (cfu) in the sample in comparison with blank was due to bactericidal property of the glass and reported as "cfu reduction %". The experiment was duplicated for *E. coli* and *S. aureus*.

Silver coated cloth: The above procedure was repeated with the silver coated cloth of 5 cm \times 5 cm dimension, obtained from different coating techniques, instead of glass beads. The antibacterial activity exhibited by the cloth was reported as "cfu reduction %".

RESULTS AND DISCUSSION

The source for phosphate was selected initially as P_2O_5 . The disadvantage of using P_2O_5 was that the composition of P_2O_5 could not be maintained accurately in the final product and the reaction between P_2O_5 and calcium source, especially in large batches was explosively exothermic. Hence, phosphoric acid was selected as phosphorous source. The calcium oxide was used as calcium source initially. But the temperature while mixing with phosphoric acid raised upto 300 °C. The purity level of calcium oxide was low. Absorption of moisture from atmosphere made calcium hydroxide unsuitable. Hence, calcium carbonate was used as the source of calcium in the preparation of phosphate glass.

The glass samples were made with varying ratio of P_2O_5 , CaO and SiO_2 . The sample with $P_2O_5 : CaO$ ratio of 1:1.5 formed opaque glass on heating to very high temperature (1400 °C). On the other hand, when the ratio was less than 1:0.5, the unreacted phosphoric acid produced corrosive fumes. Hence, the ratio of $P_2O_5 : CaO$ was varied between 0.75 and 1.25.

The XRD pattern of CaP-Si-3 shows amorphous nature of the glass and is given in Fig. 1. A small peak corresponding to cubic silver was observed⁷.

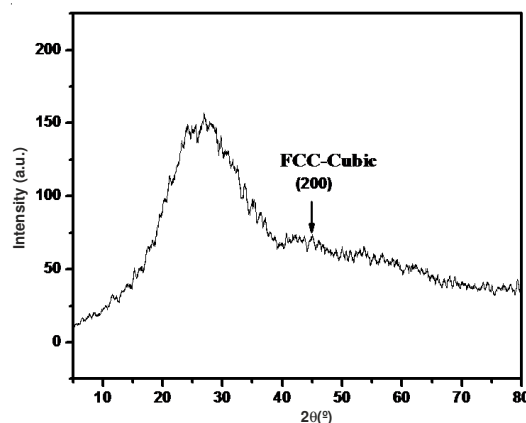


Fig. 1. XRD pattern of CaP-Si glass powder

The FTIR spectra of glass samples, *viz.*, CaP, CaP-Si and CaP-Ag are given in Fig. 2. Si-O vibration is observed at around 530 cm^{-1} for CaP-Si⁸. This vibration is absent in the other two samples. Vibration due to P-O bending was observed in $600\text{--}580\text{ cm}^{-1}$ region for all the 3 samples⁹. Similarly, P-O-P stretching vibration was observed at around $720\text{--}675\text{ cm}^{-1}$. The intensity of P-O-P stretching mode of vibration in CaP-Ag sample was high compared to other two samples. This is due to the presence of short phosphate units, formed by silver through chain termination¹⁰. In case of CaP-Ag, an additional absorption at around 650 cm^{-1} was observed, with corresponding decrease in intensity of O-P-O stretching. This additional absorption at lower frequency is due to stretching vibrations of P-O-Ag unit, when compared to P-O-P stretching frequency¹¹.

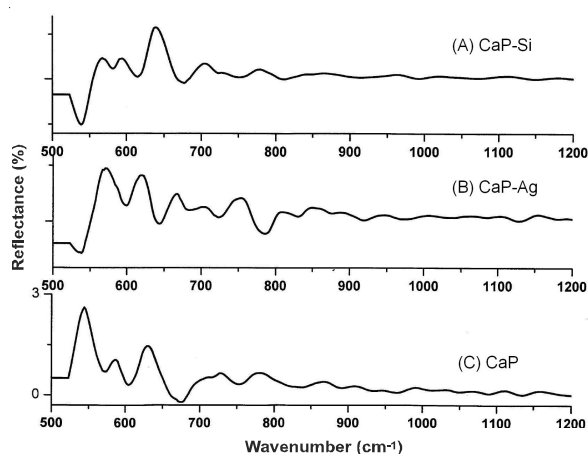


Fig. 2. FTIR reflectance spectra of (A) CaP-Si, (B) CaP-Ag and (C) CaP

The SEM image of the glass powder showed irregular shape of the particles with size in range of $0.5\ \mu\text{m}$ to $10\ \mu\text{m}$ as shown in Fig. 3.

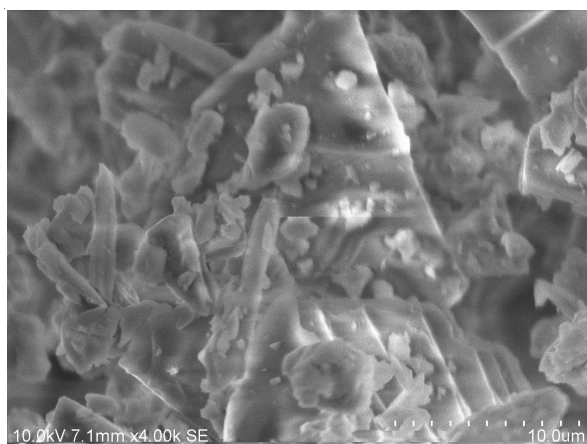
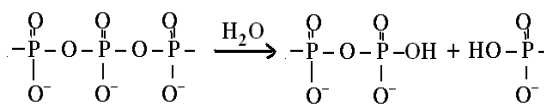


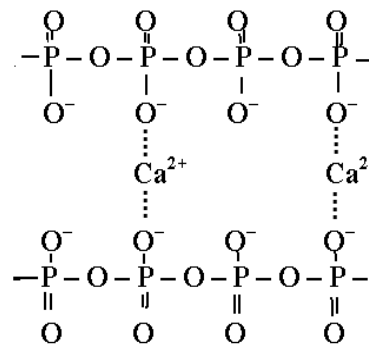
Fig. 3. SEM image of CaP-Si-3

The structure of phosphate glass was discussed by Bunker *et al.*¹². The solubility of the phosphate glass depends on the ease with which the P-O-P bond breaks. The dissolution of the phosphate glass is given in **Scheme-I**.

The role of Ca^{2+} on the formation of glass matrix is effected by cross-linking the two phosphate chains as shown in the **Scheme-II**. Hence, increase in CaO content decreases the solubility of the phosphate glass (Fig. 4).



Scheme-I: Degradation (hydrolysis) of Phosphate glass



Scheme-II: Cross-Linking by Ca^{2+}

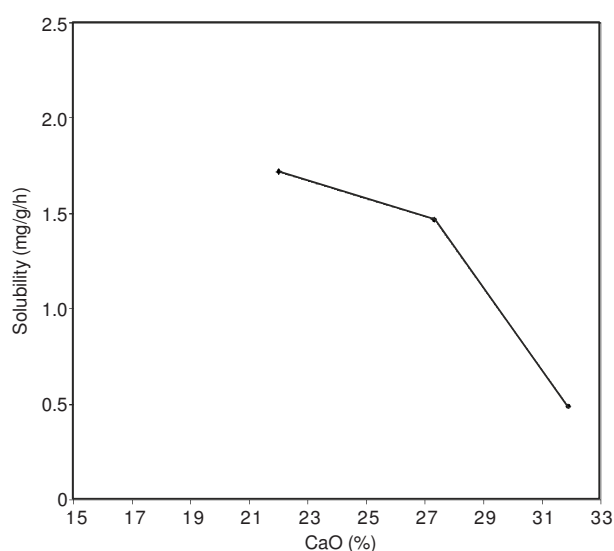


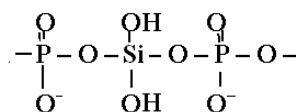
Fig. 4. Influence of CaO content on solubility of CaP-Si glass in water

Solubility of calcium phosphate glass samples was found to be very low (Table-2). In order to increase the solubility, borate was added as one of the additives in the glass samples. Phosphate glasses with different borate contents were prepared as given in Table-1. The solubility of these samples were evaluated and reported in Table-2. The glass forming temperature increases on addition of borate. When, the borate content was around 16 %, the glass became opaque and it required $1300\text{ }^\circ\text{C}$ to form transparent glass. It is found from the solubility studies that the addition of borate arrests the solubility of the glass and the thermal shock resistance of the same was low. Also, increase in borate content decreases the transparency of the glass beads, but becomes transparent on melting at high temperature (Table-2). The hydrolysis of the P-O-P bond is influenced by (i) the content of glass former (CaO), (ii) introduction of hydroxyl groups in P-O-P chain¹². The reduction in solubility of glass on addition of borate can be attributed to the reduction in hydroxyl group attached with P-O-P linkage. Hence, increase in solubility will be effected by incorporating additives which introduces hydroxyl groups in P-O-P chain.

TABLE-2
COMPARISON OF PHOSPHATE GLASS WITH SILICA, BORATE ADDITIVES AND THEIR SOLUBILITIES

Contents	CaP glass (blank)	CaP-B-1	CaP-B-2	CaP-B-3	CaP-B-4	CaP-Si-2	CaP-Si-3	CaP-Si-4	CaP-Si-6	CaP-Si-B-1
P ₂ O ₅ (%)	71.7	70.1	68.4	67.7	57.4	70.2	69.2	68.2	67.3	65.3
CaO (%)	28.3	27.6	27.0	26.7	22.6	27.7	27.3	26.9	26.5	25.7
B ₂ O ₃ (%)	-	1.7	3.4	5.0	16.8	-	-	-	-	3.2
SiO ₂ (%)	-	-	-	-	-	1.48	2.9	4.3	2.8	2.76
Ag ₂ O (%)	-	0.57	0.56	0.55	3.11	0.58	0.56	0.55	3.3	1.84
P ₂ O ₅ /CaO	1	1	1	1	1	1	1	1	1	1
Melting Temp. (°C)	970	990	990	1000	1300	975	975	975	975	1000
Solubility (mg/g/h)	0.13	Nil	Nil	Nil	Nil	1.01	1.47	2.2	1.79	0.67

Silica is known to introduce terminal -OH groups, when added in small quantities¹³ as given in **Scheme-III**. Hence, silica as an additive to phosphate glass is expected to increase the solubility.



Scheme-III: Si in phosphate glass matrix

The solubility analysis results of the glass samples in water with silica as the additive are given Table-2. The influence of silica content on solubility of the glass is given in Fig. 5. It is clear from the graph that on increasing the silica content, the solubility increases. The possible position of silica in phosphate glass may be explained in the **Scheme-III**. Thus, the P-O-P chain containing silica can be easily hydrolyzed.

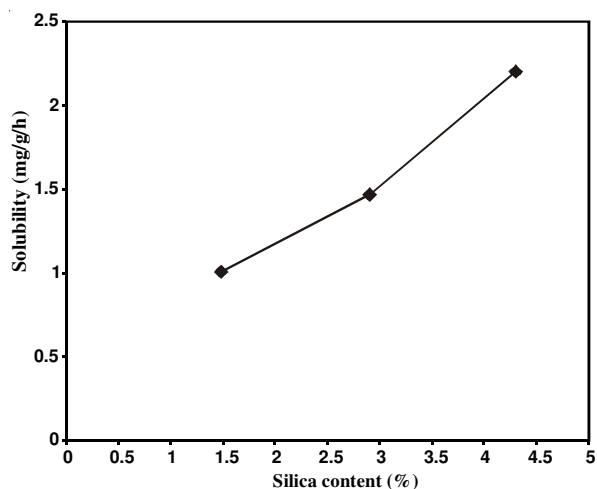
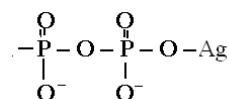


Fig. 5. Influence of solubility of CaP glass varying SiO₂ content

From the above solubility studies, CaP-Si-3, sample was selected for coating of silver over the fabric and for spin-washing method of coating; CaP-Si-6 sample was used. These silver-coated fabrics were evaluated for antibacterial property and compared.

Antibacterial analysis: Silver is associated in glass matrix at terminal P-O-P chain¹⁰ as shown in the **Scheme-IV**. In fact, addition of silver increases the solubility of phosphate glass to a small extent¹⁴. When the glass degrades in water, the silver ions, along with phosphate unit dissolves out to form glass solution. This moiety along with silver, deposits on the surface of the fabric, to impart antibacterial property. The antibacterial activity of silver containing glass was nearly 100 %, when tested with ASTM-E-2149 procedure.



Scheme-IV: Silver in phosphate glass matrix

Fabric was coated with different concentrations of silver, using dip-coating and steam-press coating techniques from CaP-Si-3 solution. The antibacterial analysis was carried out for the above fabric and the results are presented in Table-3. The cfu reduction % increased from 1 ppm to 10 ppm, beyond which it showed nearly plateau (Fig. 6a). The fabric dip-coated with 50 ppm solution showed 100 % cfu reduction. The steam-press coated sample showed nearly the same trend but with less cfu reduction (Fig. 6b). The fabric was coated with silver by spin-washing technique, with 10, 30 and 50 g of the CaP-Si-6 glass sample, respectively. There was a drastic increase in cfu reduction from 10 g to 50 g. The colony growth in uncoated fabric and cfu reduction in spin-wash coated fabric is given in Fig. 7. Based on the solubility data of CaP-Si-6 from Table-2, the concentration of silver deposited was calculated and plotted in Fig. 6c. The enhanced antibacterial activity by spin-washing technique can be attributed to (i) the strong adhesion of the silver on the fabric (ii) almost all silver released from the glass got coated on the fabric, while coating of silver from glass solution in dip-coating technique was partial. It is clear that coating by spin-washing technique shows high antibacterial activity.

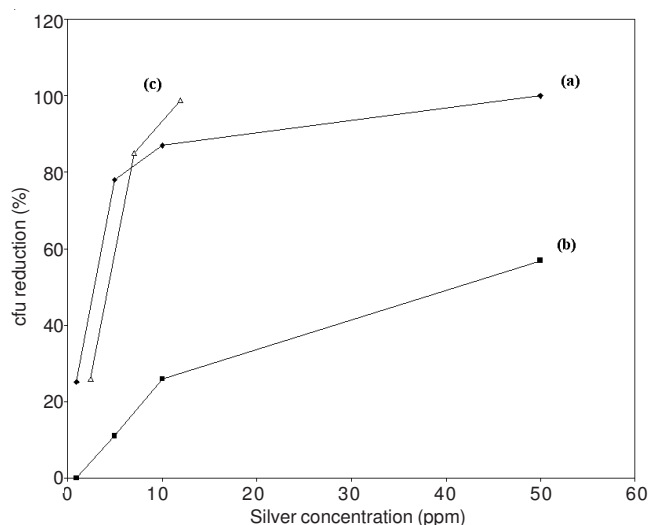


Fig. 6. Antibacterial activity studies of fabric coated with different concentrations of silver by (a) Dip-coating (b) Steam-pressing and (c) Spin-washing techniques

TABLE-3
ANTIBACTERIAL ANALYSIS OF SILVER-COATED FABRIC USING DIFFERENT COATING TECHNIQUES

Coating technique	Concentration of silver (ppm)							
	1		5		10		50	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
Dip-coating (cfu reduc. %→)	25	11	55	62	82	89	96	98
Steam-Pressing (cfu reduc. % →)	0	0	11	3	26	27	57	52
Spin-washing (cfu reduc. % →)	10 g (2.5 ppm)*		30 g (7 ppm)*		50 g (12 ppm)*			
	26	19	85	81	96	99		

*Silver concentration in ppm (given inside parenthesis) is calculated based on the solubility data of CaP-Si-6 from Table-2

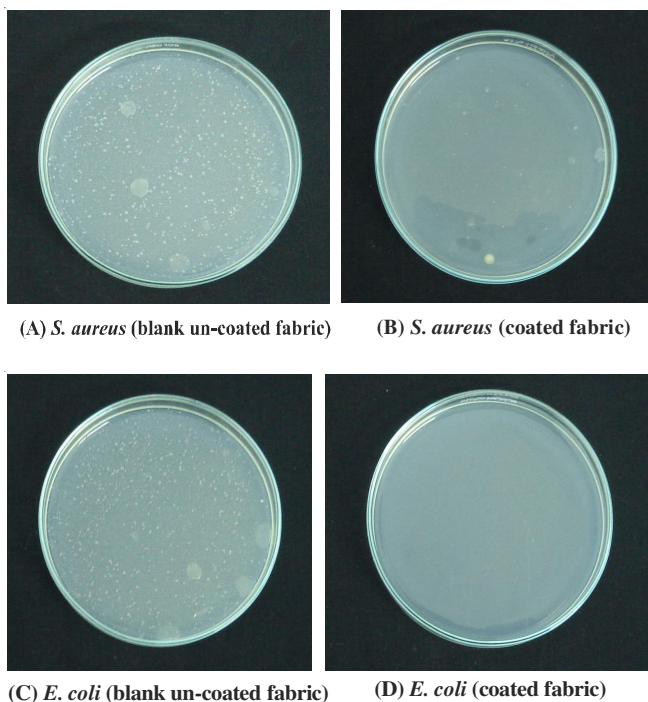


Fig. 7. Illustration of antibacterial activity exhibited by silver-coated fabric using spin-washing technique (Sample: CaP-Si-06)

The adherence nature of silver on the surface of the coated-fabric was tested by subjecting repeated washing cycle studies. The silver-coated fabric from the above studies (dip-coated fabric - 50ppm; steam-press coated fabric - 50ppm; spin-wash coated fabric-12 ppm) were subjected to conventional domestic washing procedure, dried under shade dust free environment and analyzed for antibacterial activity. The results are reported as cycle-II (without coating silver for second time). The same fabric from cycle-II, were again subjected to conventional washing procedure and analyzed for antibacterial activities and are reported as cycle-III (without coating silver for third time). The dip-coated fabric showed less cfu reduction in cycle-II (Fig. 8), which dropped to nearly zero activity in cycle-III. This reveals poor adherence of silver over the coated-fabric. The steam-pressed fabric showed zero activity in cycle-II itself. On the other hand, the spin-wash coated fabric retained its antibacterial activity to an appreciable extent upto cycle-III. The activity was completely lost after cycle-V.

Conclusion

Calcium phosphate glasses with varying compositions were prepared. The solubility of the glass can be altered by varying the additive concentrations. The silver released from

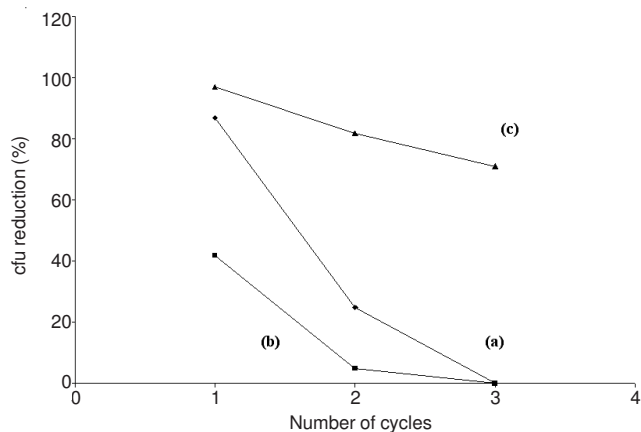


Fig. 8. Antibacterial activity studies exhibited by silver-coated fabric in washing cycles using (a) Dip-coating (b) Steam-pressing and (c) Spin-washing techniques

the glass in aqueous medium was coated over fabric using simple techniques and antibacterial activity of the same was analyzed. Coating of silver by spin-washing technique was found to exhibit good bactericidal property which was retained upto 3rd washing cycle. The original colour of the fabric after coating was retained, while that coated by nano colloidal silver imparted dirty yellow colour.

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