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Preparation and antimicrobial activity of poly (vinyl chloride)/gelatin/montmorillonite biocomposite films

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Abstract The aim of this study was using a novel antimicrobial thermoplastic plasticizer based on aliphatic anhydride derivative dodecenyl succinic anhydride (DSA) for blending poly (vinyl chloride), PVC, with gelatin in presence of montmorillonite (MMT) using Brabender via polymer melting technique. This anhydride-based plasticizer blended the membrane ingredients homogenously under melting process. The used plasticizer exhibited high performance antimicrobial potency for some biomedical and industrial applications. The prepared biocomposite films were evaluated for antimicrobial activity using agar disc diffusion method against gram-positive and gram-negative bacteria such as: *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumonia* (*K. pneumonia*), *Bacillus cereus* (*B. cereus*), *Bacillus subtilis* (*B. subtilis*) and *Escherichia coli* (*E. coli*). The majority of these biocomposites, except the plasticized PVC with DOP, have shown inhibitory effect at different concentrations (1.0–20) mg/ml against all above mentioned bacteria. However, *C. albicans* and *A. niger* were the most resistant strains.

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1 Introduction

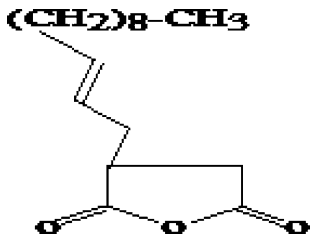
In the past several decades, the growing global concern of emerging infectious diseases has greatly simulated research activities on polymeric biocides [1–4]. Antimicrobial polymers have been used as coating in many areas such as food processing, biomedical devices, filters and as additives for antifouling paints [5]. The use of antimicrobial polymers offered promising results for enhancing the effectiveness of some existing antimicrobial agents and in minimizing the environmental problems accompanying conventional antimicrobial agents by reducing the residual toxicity of the agents, increasing their efficiency and selectivity and prolonging the lifetime [6]. PVC played an important role in the plastic industry and was one of the most versatile thermoplastic, but it must be combined with a number of additives before processing. Low molecular weight plasticizer was one of the major additives used in PVC compounding. The addition of plasticizers to PVC formulation decreased some mechanical properties of the PVC product (hardness, tensile strength and modulus). However, low temperature flexibility, elongation and ease of processing were all improved. The most widely used low molecular weight plasticizer was di-*iso*-octyl phthalate (DOP) which known to lower the glass transition temperature of PVC. These types of blends presented serious problems of high migration and consequent loss of blend properties [7]. It was common practice to blend plasticizers with certain polymer; the plasticizer here served two purposes: lowered the melt viscosity and changed physical properties of the polymer such as increased softness and flexibility [8, 9]. Moreover, the plasticizer should be non-volatile and should interact with the polymer. Pharmacology studies have revealed that MMT adsorbed bacteria such as *E. coli*, *S. aureus* and immobilized cell toxins.

Some researchers found that natural clay minerals showed no antibacterial effect, but could adsorb and kill bacteria when materials with antimicrobial activity were intercalated [10–12]. Also, Haroun et al. found that MMT had improved cytocompatibility between the osteoblasts and the novel prepared scaffolds based on gelatin [13]. On the other hand, some studies have considered the incorporation of aqueous plant extracts and essential oils into the formulation of gelatin and plastic edible films had enhanced their antimicrobial activities, respectively [14, 15]. It is well known that gelatin derives from the hydro-thermal degradation of collagen with average molecular weights from 65.000 to 300.000 g/mol, depending on the degree of hydrolysis. Gelatin contains mainly glycine, proline and 4-hydroxy proline [16]. Gelatin is currently used in pharmaceuticals, wound dressing and adhesives in clinics due to its good cell viability and lack of antigenicity [17]. However, gelatin films do not have ideal mechanical properties and water vapor barrier, as most protein films limited its applications [18]. Consequently, gelatin can be blended with synthetic polymers such as low density polyethylene to enhance its mechanical properties [19, 20]. The primary objective of this study was to investigate the effect of the novel plasticizer DSA on compatibility and homogeneity of PVC blended with gelatin in presence of MMT. Moreover, the antimicrobial activity of the prepared biocomposite films was evaluated.

2 Experimental

2.1 Materials

Industrial gelatin (98%) and poly (vinyl chloride), PVC suspension grade with K-value of 76, were obtained from El-Gomhuria Chemicals Co and Petrochemicals Alex Co, Egypt, respectively. Montmorillonite 99%, (MMT, K_{10}, pH 2.5–3.5, and particle size around 40 μm) and dodecenyl succinic anhydride 96% (DSA, density 20°/4°, Scheme 1) were obtained from Fluka. Glucose, yeast extract, peptone, meat extract and potato dextrose agar media were obtained from Merck. Agar was purchased from Sisco Research Laboratories. All pathogens were isolated and produced by Chemistry of Natural and Microbial Products Department, National Research Center, Egypt. All chemicals and other reagents were used as received without further purification.



Scheme 1 The chemical structure of 2-dodecenyl succinic anhydride (DSA)

2.2 Preparation of the biocomposite films

Different blends were prepared using Brabender Plastograph at temperature range 160–170°C and mixed for 7 min. The content of gelatin in the blends PVC/DSA was varied between 0 and 60 wt% based on the weight of PVC. The symbols of the composites indicated the composition variations in DSA and gelatin, respectively (Table 1). The resulting materials were inserted between the plates of the hydraulic press for compression molding heated around 140–170°C then kept for 5 min to allow complete film molding.

2.3 Characterization

To measure the change of the gallery distance of MMT before and after intercalation, 2D-XRD patterns were recorded with 0.5° oscillation over 5 min on Rigaku Micro Max 007 microfocus rotating anode X-ray generator (Cu Ka) with Axco PX70 capillary optic and Rigaku RAxis (IV++) image-plate detector. Calorimetric measurements were performed using Perkin-Elmer Diamond Differential Scanning Calorimeter (DSC) equipped with a model PII intercooler. Temperature and enthalpy calibration was performed using high purity standards (benzene and indium). The measurements were carried out on known amounts of the biocomposite films (3–4 mg of dried sample), which had been stored in a mixed of water/ethanol in the ratio 2:3 for 72 h. The wet samples were wiped with filter paper to remove excess liquid and hermetically sealed in aluminum pans (to prevent any loss of liquid during measurements). Heating was carried out at 10°C min^{-1} in the temperature range from 15 to 300°C. The structural features of the prepared biocomposite films were investigated at different magnification (X220 and X750) using

Table 1 Chemical composition of the prepared biocomposite films

Biocomposite film	Compositions (w/w) g			
	PVC	Gel	DSA	MMT
PVC	100	0	0	0
PVC/DSA-I	100	0	45	0
PVC/DSA-II	100	0	60	0
PVC/DSA-III	100	0	45	1
PVC/DSA-IV	90	10	45	1
PVC/DSA-V	80	20	45	1
PVC/DSA-VI	40	60	45	1

JEOL-Scanning Electron Microscope (SEM). Before observation, the fractured surfaces were coated with Au using SEM coating device. Three micrographs were taken from different zones of each surface film under investigation. The mechanical properties of the prepared biocomposite films were recorded using an electronic Zwick tensile testing machine model 1425. Dog-bone shaped samples, stamp cut from the prepared films, were tested to assess the effect of film composition on the tensile properties and E-modulus according to the standard method ASTM-D 412-98a, at 10 mm/min. The average value for each test was taken for three samples to confirm the results.

2.3.1 The biodegradation test

The biodegradation study of the biocomposites was carried out in vitro by incubating in phosphate buffer at pH 7.4 and 37°C for different periods (1, 3, 9, 14 and 18 days). At various intervals, the composites were taken from the medium, washed with distilled water and dried at 60°C overnight. The biodegradable percentage (D) was examined by the weight loss from the following Eq. [21]:

$$D = [W_o - W_t / W_o]100 \quad (1)$$

where, W_0 and W_t are the original weight and the weight at time t of the samples. Each value was the average of three separate experiments.

2.3.2 Antimicrobial activity

2.3.2.1 Antimicrobial culture preparation Nutrient media was prepared according to manufacture instructions (10 g/l glucose, 6 g/l peptone, 3 g/l yeast, 1.5 g/l meat extract and 28 g/l agar) sterilized for 20 min at 120°C. The following pathogens were used as follows:

I) Gram positive (+) bacteria such as: *Bacillus subtilis* (1), *Bacillus cereus* (2) and *Staphylococcus aureus* (3).
II) Gram negative (−) bacteria such as: *Escherichia coli* (4) and *Klebsilla peneumonia* (5).
III) Fungus such as: *Aspergillus niger* (6).
IV) Yeast such as: *Candida albicans* (7).

2.3.2.2 Inhibition zone technique [22] These mentioned bacteria were cultured overnight at 37°C for 24 h in the nutrient media, while both fungus and yeast were cultured at 27°C for 4 days in potato dextrose agar media (PDA). Each culture media was diluted with sterile 0.9% saline solution (1:1000) to give a suspension of about 5×10^9 cells per ml.

2.3.3 Minimum inhibition concentration (MIC)

Different concentrations of the prepared biocomposite films (0.5, 1, 1.5, 2, 4, 7, 10 and 20) mg/ml were prepared in THF solvent, then 10 µl of each concentration was used to saturate 6 mm filter paper discs. The discs were placed on PDA and nutrient agar media that were previously inoculated with the tested microorganisms. The petri dishes were incubated at 37 and 28°C for tested bacteria and fungi, respectively. MIC was defined as the lowest concentration that inhibits the visible growth of the microorganism after the incubation period [23].

3 Results

3.1 Characterization of the biocomposite films

Figure 1 shows the two dimension X-ray diffraction patterns (2D-XRD) of the prepared biocomposite films. It was observed that the shape of the scattering pattern of external plasticized PVC did not change in comparison with the plasticized PVC with DOP, Fig. 2 and Table 2 shows the DSC of the prepared biocomposite films. It has been observed that the plasticized PVC with DOP has T_g of about 50°C. Furthermore, the incorporation of the novel plasticizer DSA in PVC increases the homogeneity and therefore the glass transition temperature of the PVC blend from 50 to 65°C. The external plasticization can also lead to an increase of elastic modulus in the glassy state just below T_g. Also, the thermal degradation of the plasticized PVC was recorded at 288°C this may be due to strongly releasing of HCl. However, the mechanical properties of the prepared biocomposite films were relatively increased

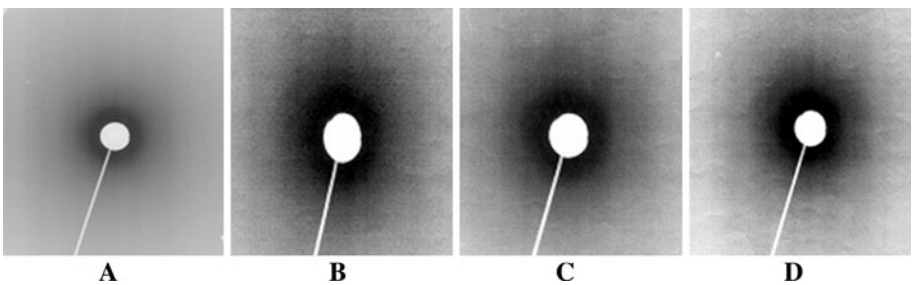


Fig. 1 2D-XRD of the prepared biocomposite films. **a** Plasticized PVC with DOP, **b** PVC/DSA-I, **c** PVC/DSA-III and **d** PVC/DSA-IV

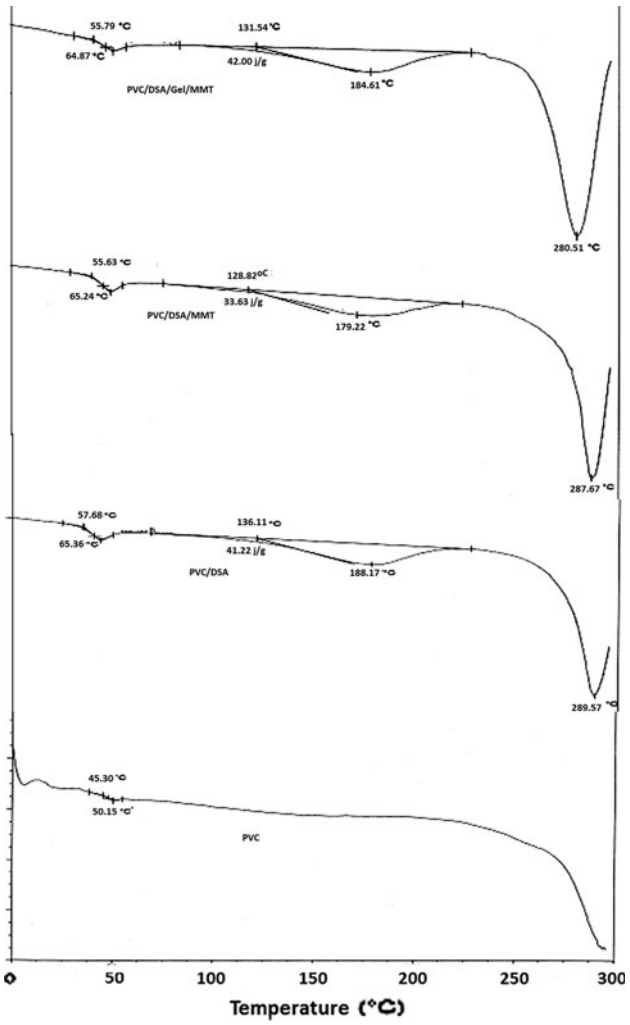


Fig. 2 DSC of plasticized PVC with DOP, PVC/DSA, PVC/DSA/MMT and PVC/DSA/Gel/MMT biocomposite films

Table 2 DSC data of the prepared biocomposite films

Biocomposite film	DSC data		
	T_g (°C)	T_m (°C)	ΔH (j/g)
PVC	50.15	188	41
PVC/DSA	65.36	188.17	41.22
PVC/DSA/MMT	65.24	179.22	33.63
PVC/DSA/Gel/MMT	64.87	184.61	42

to that of the plasticized PVC with DOP, as shown in Table 3; Fig. 3. Figure 3 shows SEM micrographs of the prepared biocomposite films. It was observed that the plasticized PVC with DSA has smooth surface in comparison with that in case of DOP plasticizer (Fig. 3.1). While MMT was agglomerated at the surface of the composite film (Fig. 3.3 and 3.4).

Table 3 The mechanical properties of the prepared biocomposite films

Biocomposite films	Mechanical properties		
	Tensile strength (Mpa)	Elongation at break (%)	E-Modulus
PVC	15.5	34.6	16.2
PVC/DSA-I	38	32.4	47.1
PVC/DSA-II	44.6	41.2	72
PVC/DSA-III	34.8	26.6	74.2
PVC/DSA-IV	15.9	24.7	54
PVC/DSA-V	10.4	17.5	44.6
PVC/DSA-VI	8.6	14.1	42.4

3.1.1 Biodegradation

The biodegradation behavior of the prepared biocomposite films in phosphate buffer (pH 7.4) was intensively investigated. The weight loss (%) of biocomposites as a function of degradation time was illustrated in Fig. 4. As expected, the plasticized PVC with DOP has no biodegradation behavior relative to the other biocomposite films.

3.2 Antimicrobial assessment

The capability of the prepared biocomposite films for the growth inhibition of tested microorganisms on solid media in comparison with oxytetracycline (antibiotic) was shown in Figs. 5 and 6. The diameter of the inhibition zone (mm) was recorded and found to be varied according to the active ingredients within the biocomposite films and the examined microorganisms.

3.2.1 The minimum inhibition concentration (MIC)

Figures 7 and 8 illustrated that *B. subtilis* was sensitive to the biocomposite IV at very low concentration (1.5 mg/ml), while at 2 mg/ml of the biocomposites III and VI the growth of the bacteria was reduced. On the other hand, *B. cereus* was inhibited by biocomposite I and II at high concentration (10 mg/ml). While, in case of the biocomposite II, the minimum inhibition concentration that inhibited *S. aureus* and *E. coli*, respectively was (0.5 mg/ml). On the other hand, *K. pneumonia* growth was inhibited at 7 and 10 mg/ml biocomposites IV and V, respectively.

4 Discussion

4.1 Characterization of the biocomposite films

The external plasticized PVC obtained by physical mixture of the synthetic polymer (PVC) and the novel antimicrobial

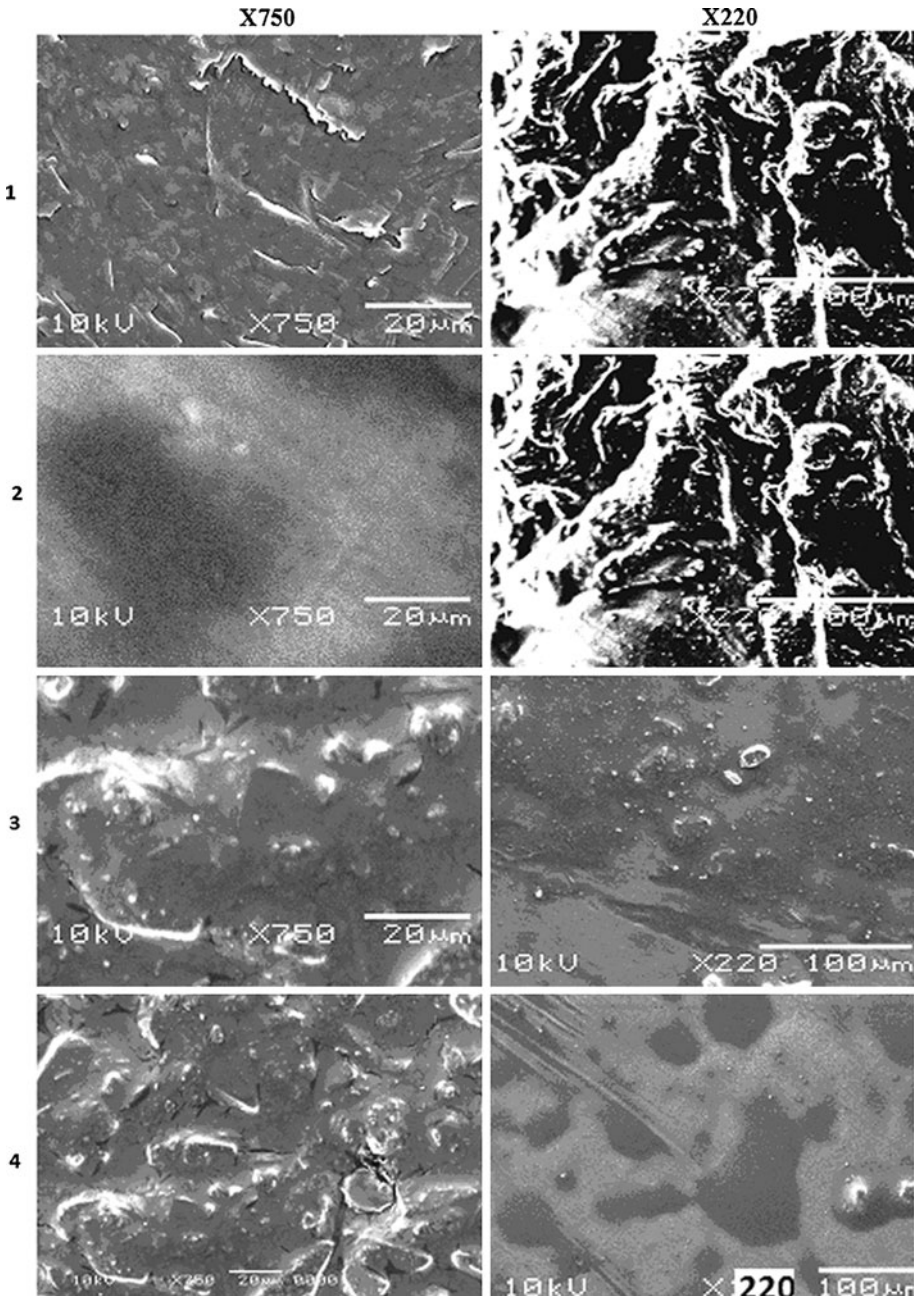


Fig. 3 SEM-Micrographs of the prepared biocomposite films. *1* Plasticized PVC with DOP, *2* PVC/DSA-I, *3* PVC/DSA-III and *4* PVC/DSA-IV

plasticizing agent (DSA) that was chosen for the following reasons: unknown antibiotic, nonvolatile and the possibility of reducing the local release of this material from products compared with the conventional antibiotics. The diffraction signal is slightly different for blended plasticized PVC with gelatin. Furthermore, this fact could be interpreted in terms of the spatial broadening of interchain structural correlation in the case of the last samples. It seemed that at least qualitative results corresponded to the shape of the relaxation function could be related to that corresponding to the static structure factor (Fig. 1). Moreover, the plasticization process takes place within the temperature range of 150–200°C according to Semsarzadeh et al. [24]. As expected, the addition of the plasticizer to PVC formulation decreased some mechanical properties of PVC products (hardness, tensile strength and modulus) according to Pita et al. [7]. The mechanical property profile of the prepared biocomposite films showed outstanding features of the mechanical behavior for the two types of materials (DSA and MMT) that were blended with PVC. The replacement of the traditional plasticizer DOP with DSA promoted rapid modification of the PVC-based composite film characteristics. Data showed that the addition of DSA during PVC blending with gelatin in presence of MMT had exhibited significant change in the mechanical properties. This behavior could be explained by considering the

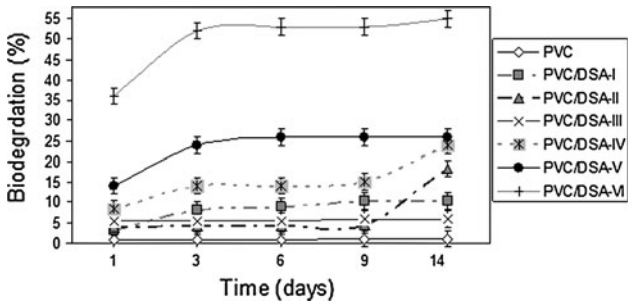


Fig. 4 Biodegradation (%) of the prepared biocomposite films

concentration of the second component on the blends. At low concentration, the second component (gelatin) formed the dispersed phase in the predominantly PVC continuous matrix. It is known that the mechanical properties of PVC were dependent on the degree of the dipolar interaction; any disruption of this interaction would be harmful to the material property. Due to the strong interaction between DOP and PVC, the addition of DOP caused disruptions to the PVC interchain attraction by acting as specific barriers (lubrication) between PVC chains. This disruption caused lowering of the mechanical properties. On the other hand,

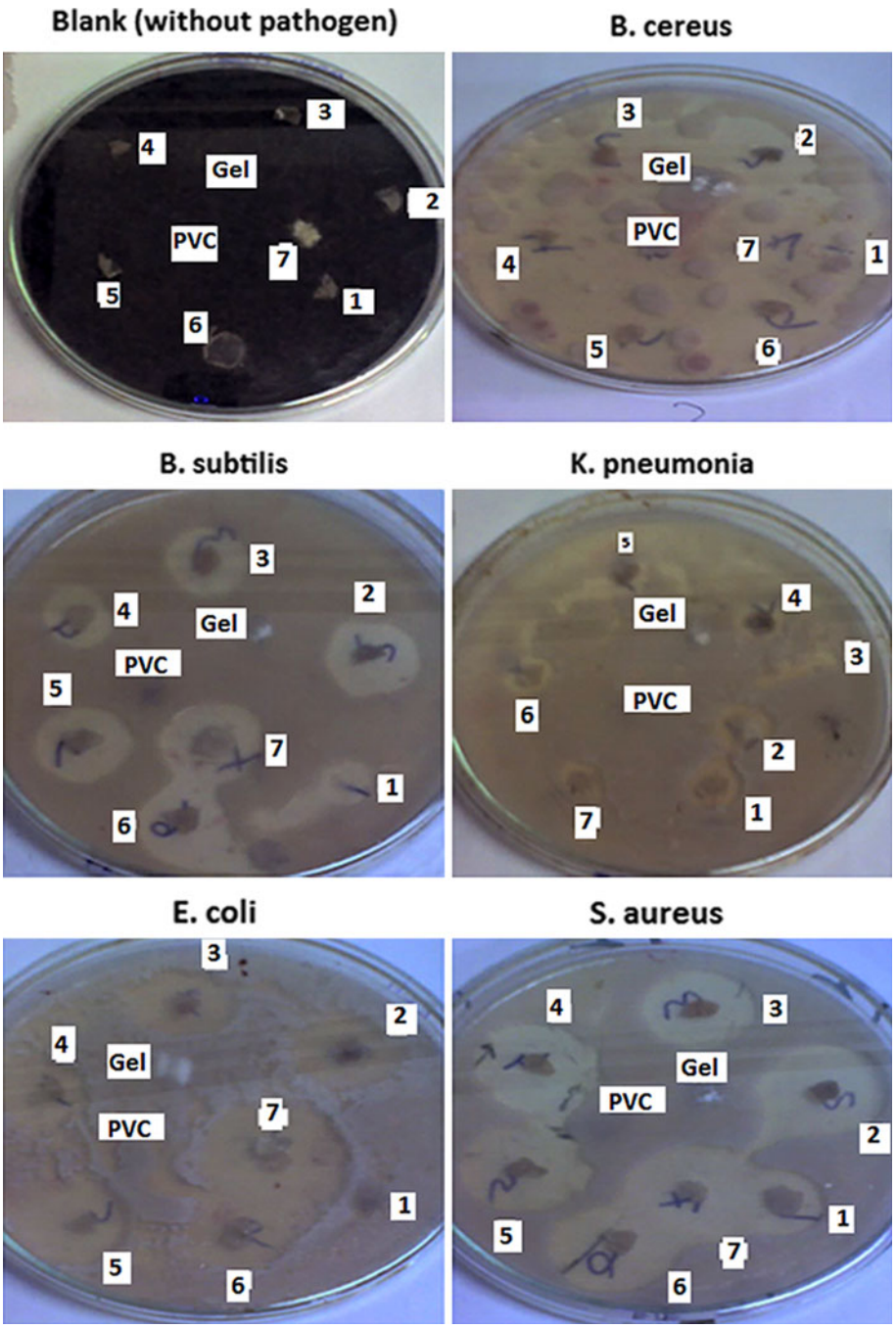


Fig. 5 Photos of antimicrobial activity of the prepared biocomposite films *1* Plasticized PVC with DOP, *2* PVC/DSA-I, *3* PVC/DSA-II, *4* PVC/DSA-III, *5* PVC/DSA-IV, *6* PVC/DSA-V, *7* PVC/DSA-VI, Gel (powder) and PVC (powder)

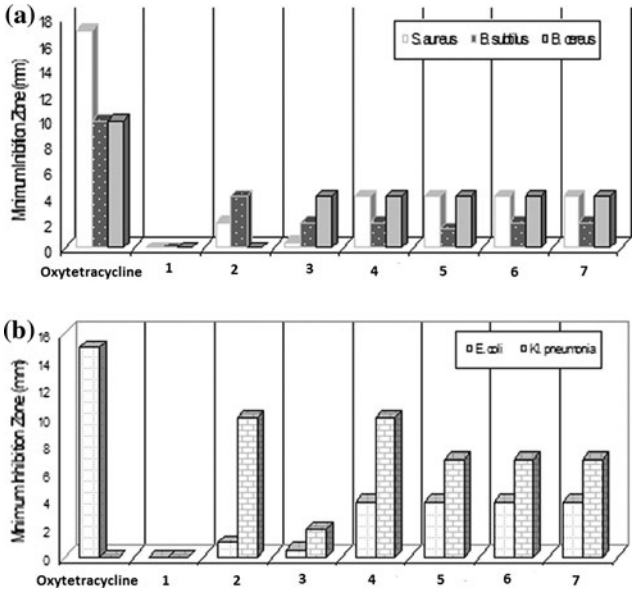


Fig. 6 Comparison study between antimicrobial activities of the standard antibiotic oxytetracycline and the prepared biocomposite films against **a** gram positive bacteria and **b** gram negative bacteria. *1* Plasticized PVC with DOP, *2* PVC/DSA-I, *3* PVC/DSA-II, *4* PVC/DSA-III, *5* PVC/DSA-IV, *6* PVC/DSA-V, *7* PVC/DSA-VI, Gel (powder) and PVC (powder)

the effect of the DSA was quite different; its addition did not disrupt the PVC interchain because these interactions were much stronger than that of PVC-DSA. Obviously, the DSA did not only form barriers between PVC chains, but also maintained other kinds of links between the chains, such as cross linking and/or grafting due to the double bonds. Accordingly, the mechanical properties were improved. In other word, the increase in gelatin content in PVC composite film formulations led to a decrease in their mechanical properties. Moreover, gelatin has a smooth effect on the initial PVC properties, but did not affect the plasticization process. In addition to its good plasticizing effect, these facts suggested that DSA acted as cross linker and/or grafting agent during the blending process.

4.1.1 Biodegradation

The rate of biodegradation behavior of PVC/DSA I, II and III were significantly slower than that of PVC/DSA IV, V and VI at all periods proving the stability of these biocomposites that contained only the hydrophobic plasticizer DSA in their structure. This likely was due to the high attachment of PVC polymer chains to DSA particles. Furthermore, the presence of the hydrophilic gelatin molecules inside the matrix of PVC/DSA IV, V and VI accelerated the penetration and the adsorption of the water molecules to the biocomposite chains, making them more degradable in the aqueous phosphate buffer (Fig. 4).

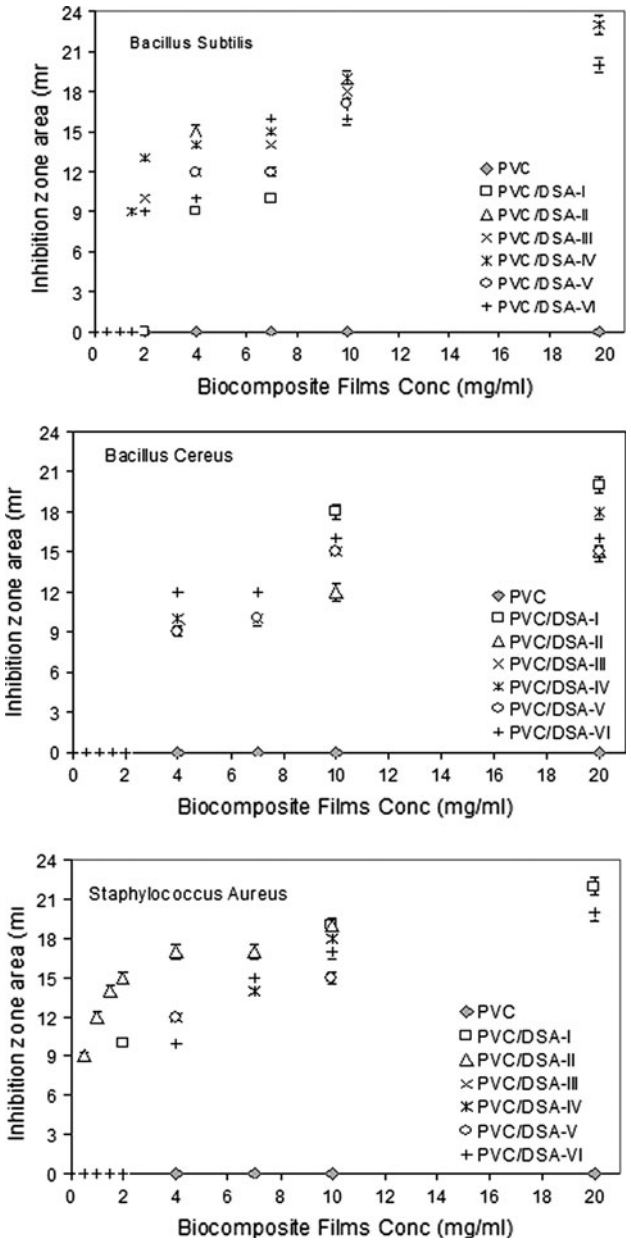


Fig. 7 Minimum inhibition concentration (MIC) of the prepared biocomposite films against gram positive bacteria

4.2 Antimicrobial assessment

To the best of our knowledge, the biocomposite films in this study have not been reported in the literature and this study was the first to evaluate their antimicrobial activities. The selected microorganisms have a major risk to human health. They were used to test the antimicrobial activity of the studied biocomposite films. *S. aureus* was a major food contaminant producing bacterial toxins. *B. Subtilis*, *E. coli* and *K. peneumonia* were also used to evaluate the antibacterial activity of the prepared biocomposite films. On the other hand, the chosen fungal microorganisms were

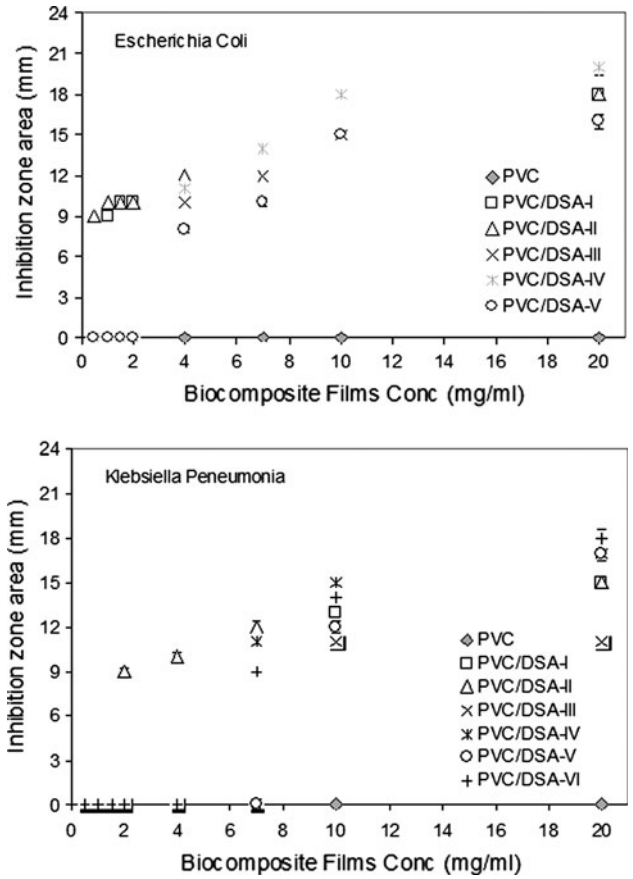


Fig. 8 Minimum inhibition concentration (MIC) of the prepared biocomposite films against gram negative bacteria

C. albicans and *A. niger*. Previously the microrugosity of biomaterials has been reported to affect microbial adherence. For example the direct relationship between microrugosity and adherence of Staphylococcus to continuous ambulatory peritoneal catheters has been reported [25]. Similarly, Jones et al. [26, 27] have reported decreased adherence of *Candida* spp. and bacteria (*S. aureus* and *P. aeruginosa*) to PVC whenever the microrugosity of the biomaterial was decreased by the presence of adsorbed biomolecules. Furthermore, the role of the surface hydrophobicity has been highlighted as a primary determinant of microbial adherence to the biomaterials [28, 29]. Due to the hydrophobicity of the films under investigation, there was no direct relationship between the water wettability and bacterial adherence. In other words, the presence of DSA and MMT inside the biocomposite matrices enhanced the growth inhibition of the tested bacteria on the solid agar media, except in case of *C. albicans* and *A. niger* which were the most resistant strains relative to the plasticized PVC with DOP. This result indicated that the presence of the plasticizer DSA and MMT together have better antimicrobial effect compared to that of the plasticized PVC with DOP and DSA, respectively. The inhibition can be arranged according to the descending order:

B. cereus > *S. aureus* > *B. subtilis*

Generally, the potency of inhibition was varied according to the plasticizer DSA, MMT and the test strain.

4.2.1 The minimum inhibition concentration (MIC)

The minimum concentration that inhibits the growth of the pathogenic microorganism (MIC) was determined using inhibition zone method. Inhibition of growth was judged by comparison the growth in the prepared control plates (without test materials), as well as in the test plates. Minimum inhibition concentration varied according to the kind and the concentration of the biocomposite and the sensitivity of the pathogen.

5 Conclusions

Empiric combination antimicrobial therapy is usually applied to expand antibacterial spectrum and reduce the selection of resistant mutants during treatment. Consequently, this Novel biocomposite films with synergistic antimicrobial activity were prepared. The antimicrobial activity of DSA was maintained when it was incorporated with PVC/gelatin/MMT biocomposite films. Some differences were observed with regard to water solubility which could determine the release of DSA into the media and affect the antimicrobial activity of the films. This could be advantageous because DSA could then be released more slowly so as to maintain sufficient concentration over a longer period of time. 2DXRD demonstrated that the prepared biocomposite films have amorphous structure. As expected, the addition of DSA led to a decrease of the glass transition temperature of PVC; however, the mechanical properties were improved. Due to increasing resistance of the current and old antibiotics, it may be suggested that the use of DSA as antimicrobial plasticizing agent during blending of PVC in presence of MMT may overcome the resistance developed by microorganisms in the future. However, further research is needed in vivo as well as in vitro to get better conclusion.

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