JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS Vol. 9, No. 11, November 2007, p. 3364 - 3367

Study of a polymer-clay nanocomposite material interaction with a VERO cell line for cytotoxic evaluation

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A new polymer-clay nanocomposite material based on poly(vinyl acetate), poly(vinyl pyrrolidone) and a layered silicate (sodium montmorillonite) as nanofiller, is deposed in different concentration on a VERO cell line in order to evaluate the cytotoxic behaviour. The polymeric system without nanofiller was also investigated. For both investigated materials the nanocomposite and the unfilled polymeric system the results showed no sign of cytotoxic effect on the cells. The materials interaction with the cell media showed different morphologies depending on the dilution level.

(Received March 23, 2007; accepted November 1, 2007)

Keywords: Poly(vinyl acetate), Poly(vinyl pyrrolidone), Layered silicate, Vero cell line, Cytotoxicity

1. Introduction

Polymer nanocomposites based on layered silicates (LS), showed in the last years an increased interest in research area because of their properties including the bio applications field [1-2]. Coatings based on polyvinyl acetate are already used for drugs controlled release [2].

Using an adequate material such as clays (natural LS) as filler for the polymer matrix, the barrier properties can be increased in order to achieve a better control of the release mechanism. The barrier effect brought by the presence of the LS in the polymeric nanocomposites is very well known and is already described in the literature [1-4]. Non-toxic biodegradable polymers (such as polyvinyl alcohol, polyvinylpyrrolidone, polyvinyl acetate, polylactic acid, etc.) are used for nanocomposites synthesis.

These kinds of polymers are also used in different drug encapsulation formulas. Using natural LS in nanocomposites synthesis may be good valuable way to enhance properties linked both to the barrier effect induced by the inorganic species and the adsorbtion-desorbtion equilibrium of the drug form, which can be controlled on the silicates surfaces. The negatively charged surface of the LS can offer a variety of complexation chemical reactions by the intergalleries cation exchange.

Polymer LS nancomposites obtained from water borne emulsions have great advantages because of the solvent free usage. We already reported different polyvinyl acetate (PVAc) nanocomposites obtained by emulsion polymerization [5-9]. Another advantage of the PVAc - LS nanocomposites obtained by one step emulsion polymerization in presence of polyvinylpyrrolidone (PVP) involves the possibilities of structure design of the LS in the composite system from intercalated to exfoliated. The degree of exfoliation can be controlled by the compositional parameters. The morphology can be also designed from porous bulk materials to hybrid nanoparticles with different sizes and shapes [6]. For future applications in drug delivery systems an accurate investigation involving the interaction with living organisms is required.

Y. Yasumura and Y. Kawakita from Chiba University in Chiba, Japan established the Vero epithelial cell line in 1962. The line was isolated from a tissue obtained from healthy, adult African green monkey kidney. Vero is widely used in transfections and vaccine production, but also for the detection of verotoxins, a group of interrelated toxins produced by some strains of Escherichia coli that are a key cause of hemorrhagic colitic and hemolytic uremic syndrome in humans [10-12].

The purpose of this study is to investigate the cytotoxic activity on the Vero line, of a new polymeric nanocomposite material based on polyvinyl acetate, polyvinylpyrrolidone and natural unmodified LS.

2. Experimental

Vero line was provided by ECACC, PHLS, Centre for Applied Microbiology and Research, Porton, Salisbury Wilts SP40JG-UK. Distemper virus CDV-WHO 134 was provided by Cornell University – ITCHA USA. The nanocomposite material based on PVAc-LS and PVP and PVAc-PVP were synthesised by a specific recipe by emulsion polymerisation [6].

The polymerization of vinyl acetate in presence of PVP was done in water by emulsion polymerization. The process was driven to high conversions degree in order to achieve monomer systems. For the nanocomposite system the polymerization was driven also in emulsion polymerization and the macro-molecular propagation was done by the in situ method in the interlayer spaces of the LS. The LS was used in the sodium unmodified form. By choosing a specific concentration of the partners involved in the polymerization process, different parameters such as monomer conversion, reaction rate ratio, hybrid nanoparticles dimension, polydispersity, latex stability, LS exfoliation, solid state material morphology, thermal

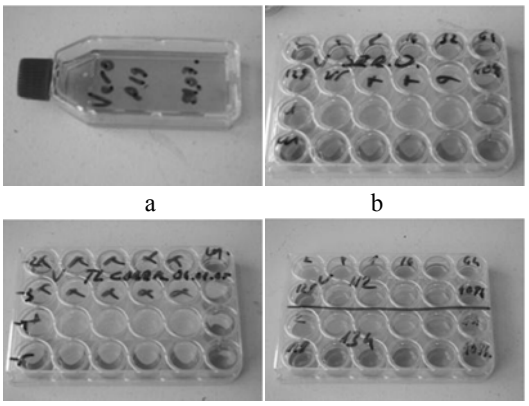
stability, etc. can be designed as wanted for a specific application.

The new nanocomposite material was investigated and characterized by: DLS, FTIR, XRD, TGA, DTG, DSC, TEM and SEM analyses. The nanocomposite material involved this study was chousen in order to have a high degree of exfolited LS structure as found by XRD and TEM analyses. Both the polymeric system and the LS filled one were used as obtained from the synthesis process in the latex form: polymer nanoparticles (about 180 nm by DLS) and polymer-LS hybrid nanoparticles (about 320 nm by DLS).

Both latexes had a 30 % of solid content and have good water swelling capacity even if not all the components are water soluble; that's why the particles aggregates when the water volume is drastically increased.

A typical procedure was followed for evaluation. First, the activity of a typically virus (DISTEMPER-Carré's TL/1/2005 CDV – $10^{4.6}$ T.C.I.D.) was observed in order to bring forward (for further modiffications of the VERO line) the Vero line modifications. Then the two investigated materials, one based on PVAc-PVP (P1) and the other P1 nanocomposite (with natural LS) (P2) were disposed on the Vero line. The Vero line was disposed as monolayer on three plates each with 24 wells. In order to evaluate the cytotoxicity, were used different concentration between 10^{-1} and 10^{-5} M.

The media was prepared in PBS 7.5 pH. For the quantitative effect determination several dilutions (½, ¼, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048, 1/4096) were prepared. Before using the materials (P1 and P2), a wet sterilisation at 120^0C was performed for 1 hour and then UV sterilization for 24 hours. The platelets (Nunc) for analyses from non-toxic plastic are specially treated for cell culture (Fig.1).



a b c d

Fig. 1. Vero line (a), "dog blood serum"(b), TL Caree efect (c), P1(112) and P2(134) wells (d).

The microscopy measurements were performed with a Zeiss Kpe W Microscope with light transmission at a 2400X magnification. The lines were evaluated after 6 days.

3. Results and discussion

The Vero line was disposed as monolayer and had a uniform disposal as a pavement. On the layer can also be observed some detached dead cells that are rising from the monolayer (Fig. 2).

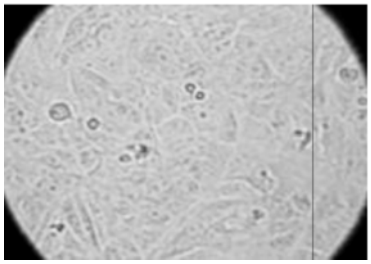


Fig. 2. Vero line normal monolayer.

The virus action was positive for almost all concentration (Fig.3).

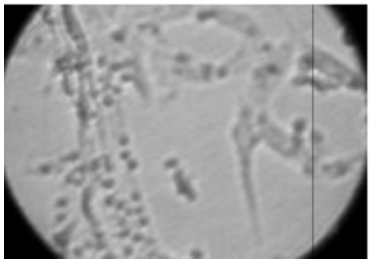
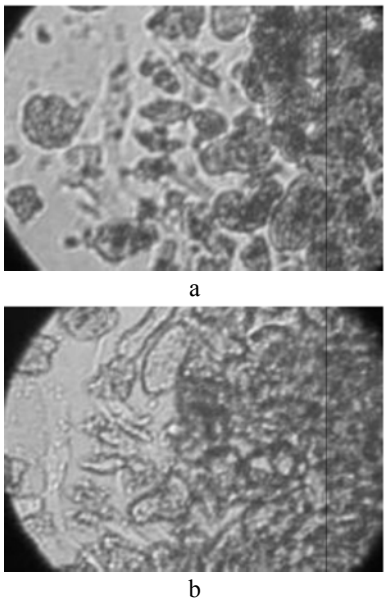


Fig. 3. Distemper cytopatic effect on VERO line.



a b

Fig. 4. P1 at 1/64(a) and 1/128(b) dilution.

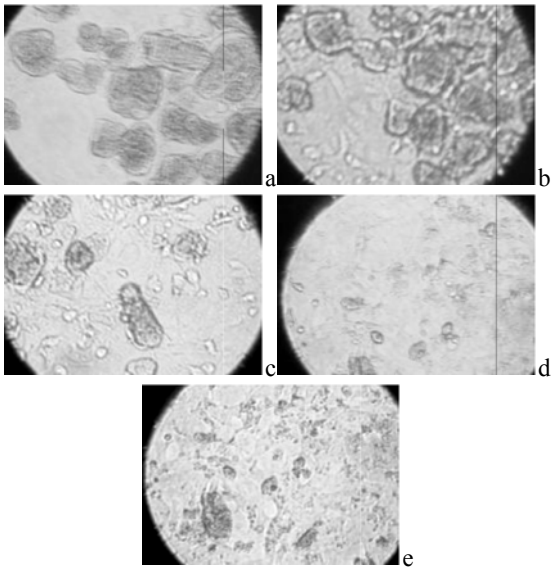


Fig. 5: P1 at 1/256 (a), 1/512(b), 1/1024(c), 1/2048(d) and 1/4096(e) dilution.

For sample P1 at low dilution the polymer aggregates in spherical zones and covers the cells, but as the dilution increases the monolayer can be seen unaffected (Fig.4, 5).

The same results were obtained for the sample P2. We could not see the cellular layer for dilution under 1/8 because of the P2 aggregation.

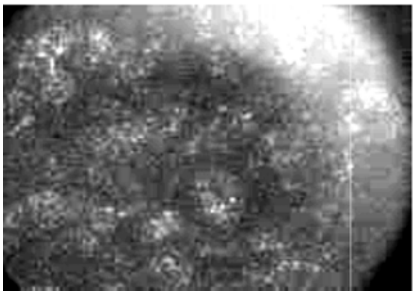


Fig. 6 P2 sample at 1/8 dilution.

Over 1/8 at 1/16 P2 dilution the monolayer can be seen unaffected but, the visibility is also reduced due the high density of aggregated material.

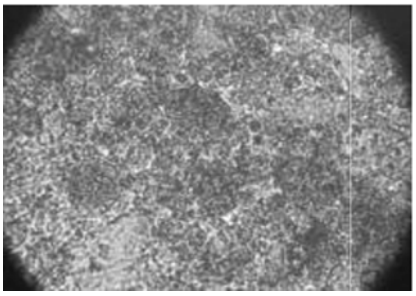


Fig. 7 P2 sample at 1/16 dilution.

For the 1/32 P2 dilution, the aggregation effect decreases and the sample has a different morphology in comparison with P1 sample (without nanofiller).

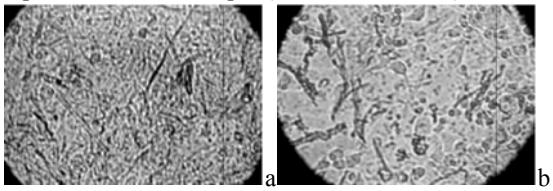


Fig. 8. P2 sample at 1/32 (a) and 1/64 (b) dilution.

As the dilution is increased the morphology of the agregates changes and the unaffected monolayer is more evident.

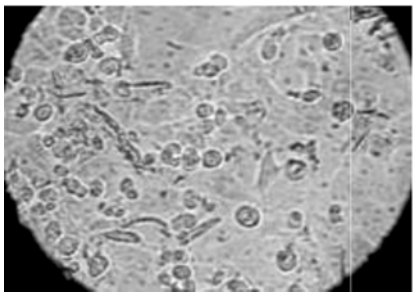


Fig. 9. P2 sample at 1/128 dilution.

For P2 samples at higher dilution the ageing effect of the Vero layer can be seen (as described in Fig. 2), near the agregates, but no cytopatic effect was present.

Over 1/1024 the monolayer is easily seen and the cells are unaffected.

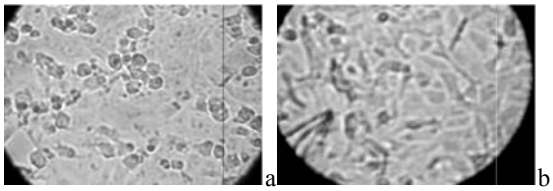


Fig. 10. P2 at 1/256 (a) and 1/512 (b) dilution.

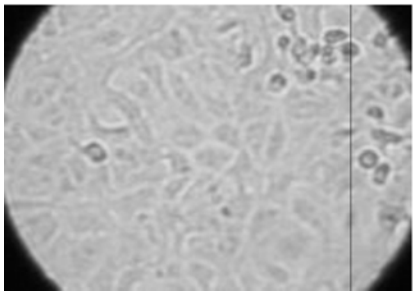


Fig. 11. P2 at 1/1024 dilution.

4. Conclusions

The materials (P1 and P2) showed different morphologies related to the dilution degree and the material nature. This behaviour is normal and reported in previous papers [5,6]. Even if the materials are not totally soluble in water, the insoluble phase has a pronounced water swelling capacity, which can offer the indirect contact between hetorophases by the aqueous media.

To better understand how the cells denaturation occurs the distemper cytopatic effect was observed. The cytopatic effect is characterized by sincitial areas containing large polynuclear cells because of cellular membrane lysis (Fig. 3).

The colour of the pictures is not relevant for samples comparison, because it depends of the different exposure times chosen automatically by the digital camera implied by the amount of light passed through the objective.

All samples showed a weak ageing effect proved by the presence of a small amount of detached cells. This phenomenon is due the time past until the examination (as seen Fig. 2), and not because of any cytopatic effect. The test was especially chosen to be made at the life limit. The sample present in Fig. 2 was on purpose left for exemplification of this effect.

The cytotoxic evaluation of P1 and P2 showed negative results on the VERO line. Samples showed a small ageing effect of the cells, a normal behaviour regarding the time (6 days) passed before analysing. Materials P1 and P2 showed no toxic effects involving the monolayer and they proved to have, at least in the preliminary testing procedures, a good biotolerence, for future bioaplications. Since the material can be designed [5,6]. in different forms from water borne latex nanoparticles with different solid content to solid bulk material future investigation for drug formulation are also required.

Acknowledgements

The Research Department from ROMVAC Company is kindly acknowledged for the technical support.

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