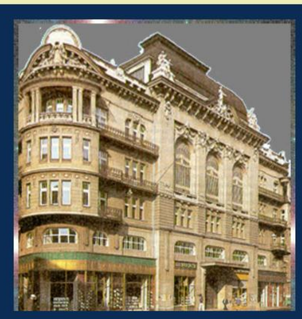
Water-soluble vitamins loaded in biodegradable nanoparticles



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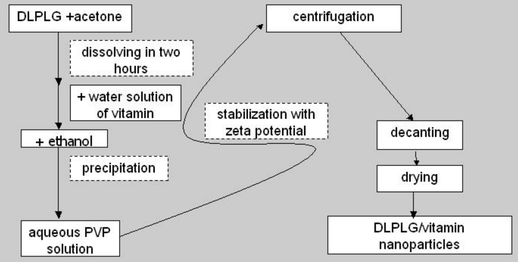
INTRODUCTION:

Poly(DL-lactide-co-glycolide) (DLPLG or PLGA) nanoparticles act as potential carries for several classes of drugs such as anticancer agents, antihypertensive agents, immunomodulators, hormones; vitamins, etc. DLPLG based micro- and nanoparticles offer various advantages compared to other controlled drug delivery systems, including: the possibility to accurately control the resulting drug release kinetics over periods of days to months, complete biodegradability, good biocompatibility, easy administration into the body, etc. The morphological characteristics of the particles, like size, size distribution and shape, are extremely important for the controlled drug delivery, and are particularly influencing the adhesion and interaction with cells (intracellular uptake). Dynamics of the release (rate and concentration) depends on the morphology, i.e. structure of the copolymer.

Ascorbic acid acts as a reductant for many free radicals, thereby minimizing the damage caused by oxidative stress which is a root cause of, or at least associated with, many diseases. Nevertheless, ascorbic acid is very unstable to air, light, heat, moisture, oxygen, and base, and it easily decomposes into biologically inactive compounds such as 2,3-diketo-L-gulonic acid, oxalic acid, L-threonic acid, L-xylonic acid and L-lyxonic acid. In order to overcome the chemical instability of ascorbic acid numerous researches have been staged toward its encapsulation or immobilization. Ascorbic acid introduced in the body in the greater portion is isolated from the body. On the other hand, the encapsulated ascorbic acid within the polymeric matrix should have significantly increased efficiency.

Folic acid (pteroyl-L-glutamic acid, vitamin B9) is also water soluble vitamin and is necessary for the production and maintenance of new cells. Both adults and children need folic acid to make normal red blood cells and prevent anemia. Folic acid also helps prevent changes to DNA that may lead to cancer. With this method we have followed the concept which we have already been applied successfully in the encapsulation of ascorbic acid in DLPLG nanoparticles.

EXPERIMENTAL:

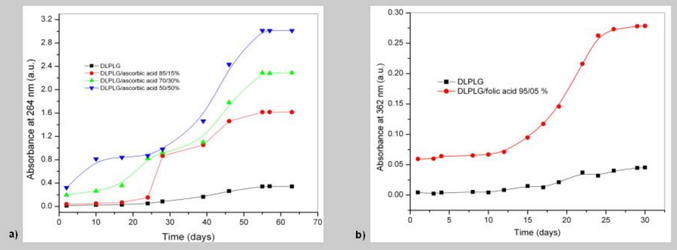


Schematics for obtaining of the DLPLG/ascorbic acid (or folic acid) nanoparticles

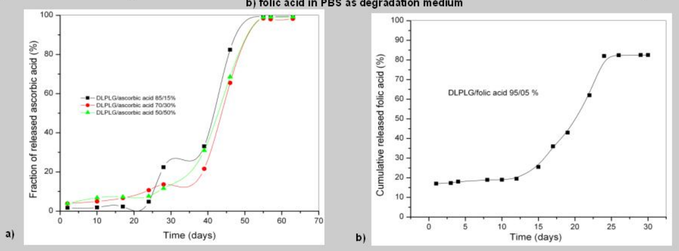
DLPLG powder is produced using physicochemical method with solvent/non-solvent systems where obtained solutions were centrifuged. The obtained DLPLG powder is non-agglomerated, unformatted and with particles sizes in the nanometer scale.

RESULTS:

The degradation of the nanospheres of DLPLG, DLPLG/vitamin nanoparticles and release rate of the vitamin were studied *in vitro* in PBS as degradation medium. The samples were characterized by Ultraviolet Spectroscopy (UV) and Scanning Electron Microscopy (SEM).

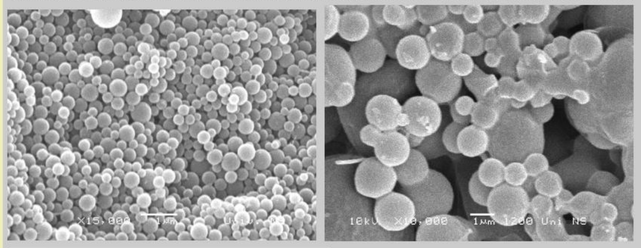


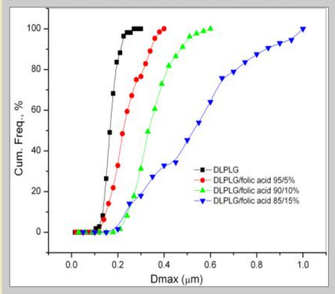
Comparative curves for the dependence of the absorbance maximum from the time of the degradation for DLPLG without and with a) ascorbic acid or b) folic acid in PBS as degradation medium



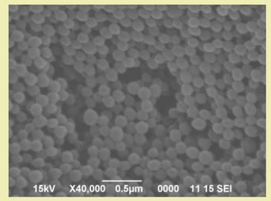
Comparative cumulative curves of the release of the a) ascorbic acid or b) folic acid in percentages over the period of time of the degradation in PBS as degradation medium

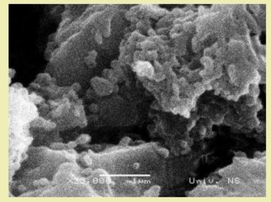
SEM images of particles with different ratio of DLPLG and folic acid a) DLPLG/folic acid 95/5 %; b) DLPLG/folic acid 85/15 %;





Comparative results of the stereological examining of a) DLPLG nanospheres and nanoparticles with different ratio of DLPLG and folic acid b) DLPLG/folic acid 95/5 %; c) DLPLG/folic acid 90/10 %; d) DLPLG/folic acid 85/15 %; based on maximal diameter of the particle Dmax





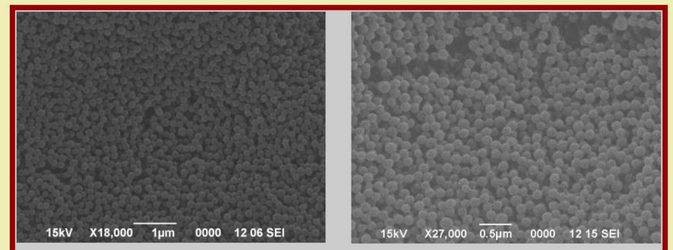
SEM images of DLPLG nanoparticles before (a) and after 28 days (b) of the degradation

Table Loading efficiency of DLPLG/ascorbic acid particles

	supernatant absorbance (254nm)	amount of ascobic acid in supernatant	loading efficiency (%)
DLPLG/ascorbic acid 85/15%	0.03942	0.1581	98.2
DLPLG/ascorbic acid 70/30%	0.24937	0.6214	97.1
DLPLG/ascorbic acid 50/50%	0.77681	3.1000	93.8

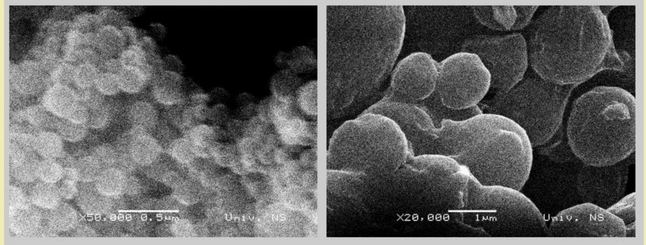
Table Loading efficiency of DLPLG/folic acid particles

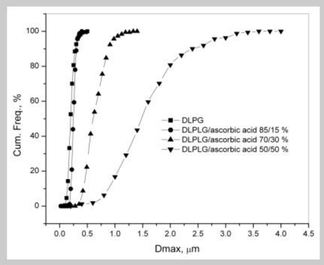
DLPLG/folic acid %	supernatant absorbance (362nm)	amount of folic acid in supernatant (mg)	loading efficiency (%)
95/5	0.11952	2.7849	78.9
90/10	0.26407	6.3936	77.0
85/15	0.43524	10.9368	75.2
80/20	0.57048	14.3750	77.0



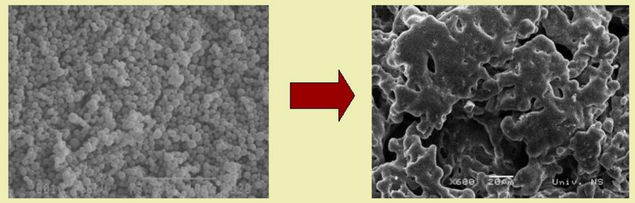
SEM images of DLPLG particles without vitamins and before degradation

SEM images of particles with different ratio of DLPLG and ascorbic acid a) DLPLG/ascorbic acid 85/15 %; b) DLPLG/ascorbic acid 50/50 %;





Comparative results of the stereological examining of a) DLPLG particles and particles with different ratio of DLPLG and ascorbic acid b) DLPLG/ascorbic acid 85/15 %; c) DLPLG/ascorbic acid 70/30 %; d) DLPLG/ascorbic acid 50/50 %; based on maximal diameter of the particle Dmax



SEM images of DLPLG/ascorbic acid 85/15 % nanoparticles before (a) and after 28 days (b) of the degradation

CONCLUSION:

It is possible to encapsulate water-soluble vitamins into DLPLG particles in various concentrations thus producing particles with different morphological characteristics. The particles of DLPLG/ascorbic acid (or folic acid) with lesser ratio of ascorbic acid (or folic acid) have higher uniformity, lower level of agglomeration and their sizes are smaller. The yields in preparation for various DLPLG/vitamin ratios were similar and they were greater than 50% and the loading efficiency was greater than 75%. The nanoparticles of DLPLG/ascorbic acid 85/15% have spherical shapes and their mean sizes are from 130 to 200 nm depending on the stereological perimeter taken in consideration (feret X, feret Y or Dmax). In the current research we are encapsulating folic acid in lesser ratios because the molecular weight of folic acid (441.1396g/mol) is lesser than the weight of ascorbic acid (176.13g/mol). DLPLG completely degrades in phosphate buffered saline within period of 63 days fully releasing all encapsulated ascorbic acid.

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