Preparation of 99mTc-PLGA and its distribution studies

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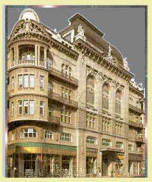

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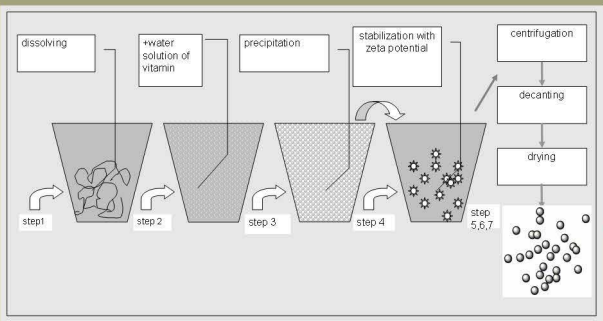
Introduction

Poly(DL-lactide-co-glycolide) (PLGA or DLPLG) is a strong candidate as drug carrier for a drug delivery system because of its biocompatibility and biodegradability. Nanoparticles act as potential carries for several classes of drugs such as anticancer agents, antihypertensive agents, immunomodulators, hormones, vitamins, etc. The encapsulated ascorbic acid within the polymeric matrix should have significantly increased efficiency.

The investigation of distribution and pharmacokinetics of degraded products of PLGA is crucial for effective prediction of host responses to PLGA in particular applications. Thus we present a method of labeling PLGA with 99mTc, which binds outside, leaving the cage intact. This enables quick and convenient investigation of pharmacological behavior and metabolism of PLGA. The samples were characterized by X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and Ultraviolet Spectroscopy (UV). For the determination of radiochemical purity of all 99mTc-labelled samples standard paper (Whatman No1) and instant thin layer chromatography (ITLC-SG) with two solvents (acetone and saline) were used.

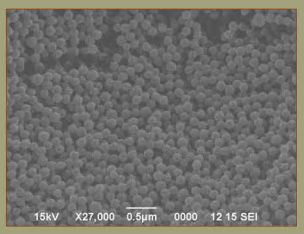
Technetium-99m is still the radionuclide of choice because of his ideal physical properties (T1/2=6.02h, Eγ=141keV) for many applications in nuclear medicine. For radiopharmaceuticals preparation it was often used like technetium pertechnetate (TcO4-), which have to be reduced in lower oxidation state. The results of the conditions and possibilities investigation of poly(DL-lactide-co-glycolide) (PLGA) labelled with 99mTcO4- by stannous chloride method were presented. The radiochemical purity, pharmacokinetics and biodistribution of labeled compounds were investigated.

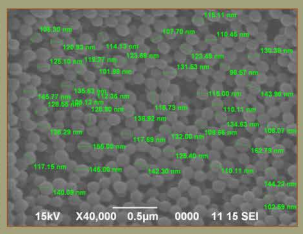
Experimental

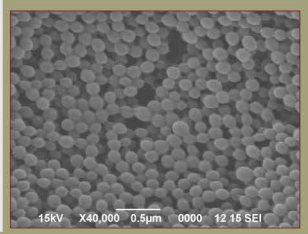


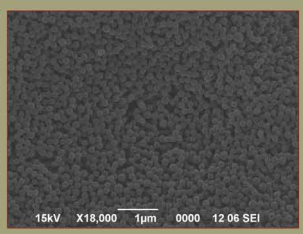
Schematics for obtaining of the PLGA/ascorbic acid nanoparticles

PLGA powder is produced using physicochemical method with solvent/non-solvent systems where obtained solutions were centrifuged. The obtained PLGA powder is non-agglomerated, uniform and with particles sizes in the nanometer scale. The encapsulation of ascorbic acid in the polymer matrix is performed by homogenization of water and organic phases.







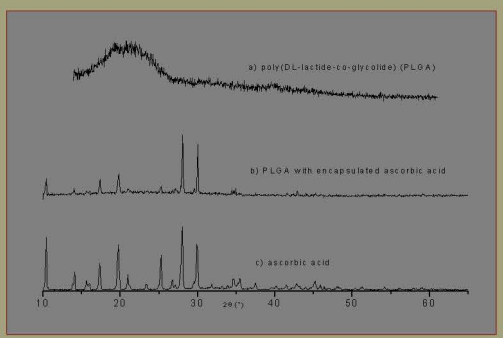


SEM images of PLGA particles

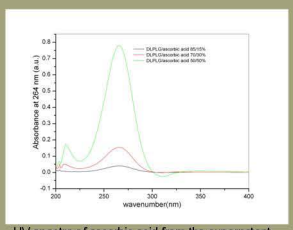
Results

In the nanoparticles of PLGA copolymer, different concentration of ascorbic acid have been encapsulated and yield in preparation was calculated.

DLPLG/ascorbic acid (%)	Yield (%)
100/0	51.04
85/15	52.10
70/30	56.41
50/50	52.80
30/70	53.23



XRD patterns of a) PLGA, b) PLGA with encapsulated ascorbic acid and c) ascorbic acid



UV spectra of ascorbic acid from the supernatant

Table Loading efficiency of PLGA/ascorbic acid particles

	supernatant absorbance (264nm)	amount of ascorbic 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%	0.77581	3.1000	93.8

Table The radiochemical purity results of 99mTc-PLGA in percentages, twenty minutes after labeling (Mean values ± SD)

sample	0.9% saline		acetone	
	^{99m}Tc-PLGA	TcO_4^-	^{99m}Tc-PLGA	TcO_4^-
1[a]	65.1±0.1	34.9±0.1	64.7±0.3	35.3±0.4
2[b]	87.1±0.1	12.9±0.2	88.0±0.1	12.0±0.2
3[c]	95.2±0.1	4.8±0.1	93.0±0.1	7.0±0.1

a PLGA:Sn(II)=5ml:0.006ml (C-SnCl2x2H2O=0.01M)
b PLGA:Sn(II)=5ml:0.010ml
c PLGA:Sn(II)=5ml:0.020ml

Radiochemical purity

The biodistributions studies of 99mTc-labeling PLGA were carried out on health white Wistar rats (male). The animals (n=3 animals) were sacrificed thirty minuts after application of 0.1 ml of 99mTc-labelled compound (74kBq). The radioactivity per whole organ of interest (or gram) was measured in a NaI (Tl) _-detector and the percentage of radioactivity related to administrated dose was determined.

Organ distribution data in Wistar rats for 99mTc-PLGA

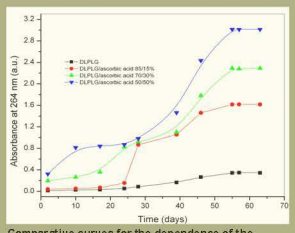
Tissue	Heart	Lung	Liver	Spleen	Kidney	Stomach	Intestine	Blood[a]	Bone[b]
%ID/organ (mean value±SD)	0.07±0.01	0.8±0.3	98.4±0.1	1.9±0.1	1.2±0.5	0.14±0.1	1.81±0.05	0.26±0.01	0.37±0.01

%ID/organ (a percentage of administrated doses per organ of animal)
a-%ID/ml
b-%ID/g

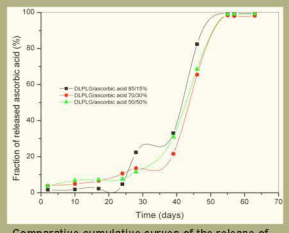
In vivo

In vitro

The degradation of the nanospheres of PLGA, PLGA/ascorbic acid nanoparticles and release rate of the vitamin were studied *in vitro* in PBS as degradation medium.



Comparative curves for the dependence of the absorbance maximum from the time of the degradation for PLGA without and with ascorbic acid



Comparative cumulative curves of the release of the ascorbic acid in percentages over the period of time of the degradation in PBS as degradation medium

Conclusion

The results of the examining of poly(DL-lactide-co-glycolide) (PLGA) labeled with 99mTcO4- by stannous chloride method are presented. The radiochemical study results have shown that content of free 99mTcO4- in the samples is dependent on PLGA-stannous chloride ratio and is increased with ratio. The preliminary biodistribution results for PLGA labeled with 99mTcO4- by this method were: for the sample with ratio PLGA:Sn (II)=250:1, a lot of radioactivity in liver (>98 %) may suggest these samples showed higher affinity for liver due to its hydrophobic character or it may be an indication that in this labeling condition of samples the content of hydrolysed reduced 99mTc (99mTcO2) as radiochemical impurity was high. The next step in the research is examining the biodistribution of 99mTc-labeled PLGA particles after the different periods of time after their instillation into the rats, as well examining of the biodistribution of labeled PLGA particles with encapsulated ascorbic acid.

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