DOUBLE AND TRIPLE NANOCOMPOSITIONS IN THE SYSTEM: HYDROPHILIC POLYMER / INORGANIC CARRIER – SILVER NANOPARTICLES – EUMELANIN

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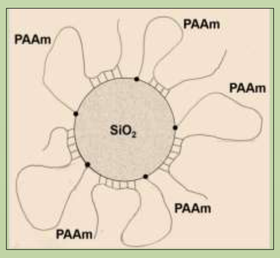
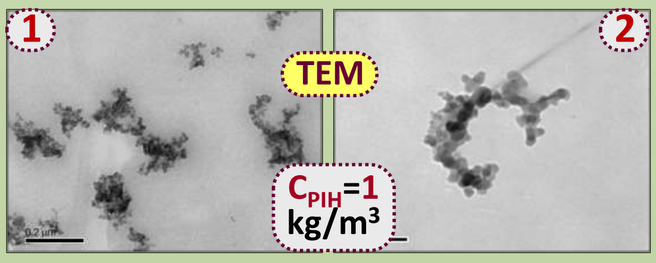
The creation of hydrophilic nanocarriers for the delivery of poorly soluble and toxic drugs and the development of methods for monitoring the rate and direction of drug distribution in living organisms are important tasks of modern biomedicine. The latter problem has so far been solved by the introduction into the carrier or drug by covalent binding or physical encapsulation of various luminescent compounds: organic substances or metal salts/oxides. However, an equally promising way can be the production of nanoparticles of zero-valent metals in carriers or in drugs themselves, which have surface plasmon resonance bands (SPRB) in the UV-Vis spectra and whose integral intensity is proportional to the concentration of nanoparticles. This report discusses an attempt to create effective nanocarriers for the natural sparingly soluble anticancer drug eumelanin (EMel) with a possible control of its pharmacokinetics in living organisms using silver and gold nanoparticles. A hydrophilic polymer/inorganic hybrid (PIH) containing silica nanoparticles and grafted polyacrylamide chains, SiO_2/PAAm, was synthesized, purified, well characterized, and used as nanocarrier for both silver nanoparticles (AgNPs) and EMel preparation in an aqueous medium.

Polymer / inorganic hybrid SiO_2 / PAAm

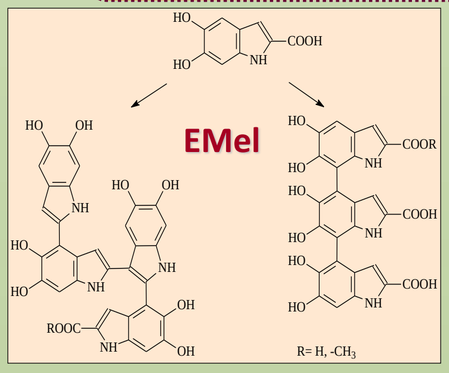
Sample	R_{SiO2}, nm	M_{PAAm}, kDa	N
PIH	7.7	2824	8

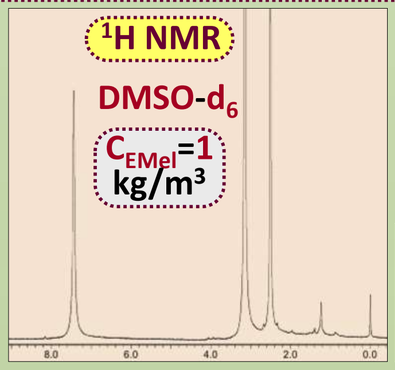


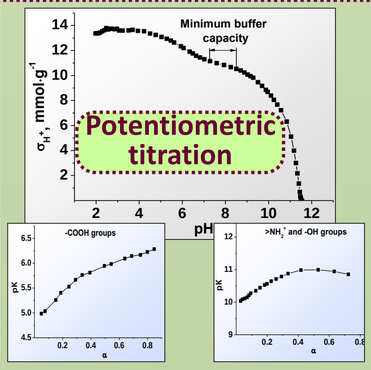


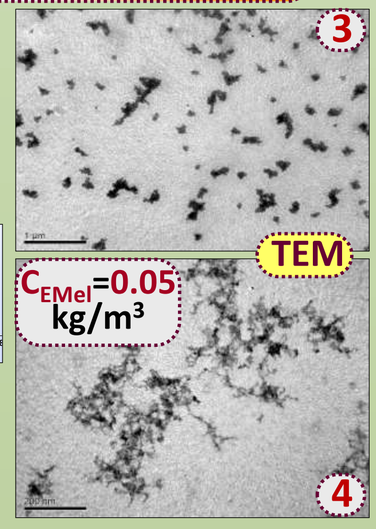
The PIH was synthesized by free-radical polymerization of acrylamide "from" the surface of a silica sol and its main parameters were determined (Table). The actual hybrid structure in the aqueous solution is shown in TEM images 1 and 2. One can see both individual spherical diffuse hybrid particles with d~10–20 nm and their fractal aggregates. The aggregation of PIH particles developed due to the interaction of their "coronas" through hydrogen bonds.

EMel chemical structure, electrochemical properties and morphology



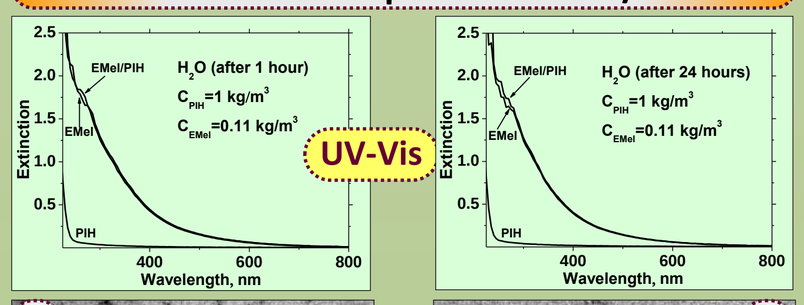


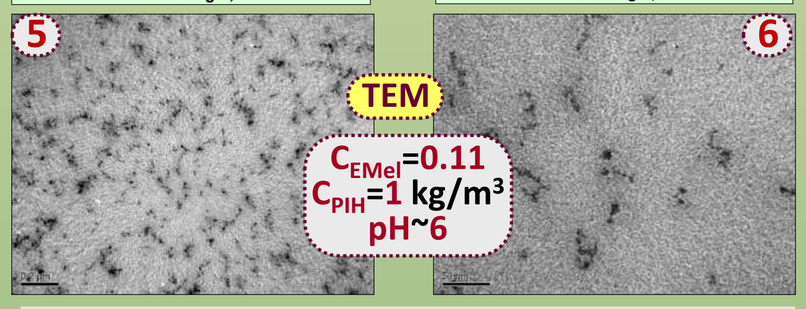




The EMel sample (as a vital product of black yeast "*Nadsoniella nigra sp. X-1*") was characterized in accordance with [1]. Its polyampholyte nature with a significant excess (3.66 times) of the basic phenol and secondary amine groups over acidic carboxyl ones (10.84 against 2.96 mmol·g^{-1}) was confirmed by potentiometric titration. The pK_0 values for the acidic and basic groups were 4.84 and 9.94, respectively. In aqueous solutions, linear and branched molecules of EMel was highly aggregated and partially cross-linked. The size of its loose aggregates containing small dark nanoparticles (TEM images 3, 4) could reach ~1 μm even in a diluted solution.

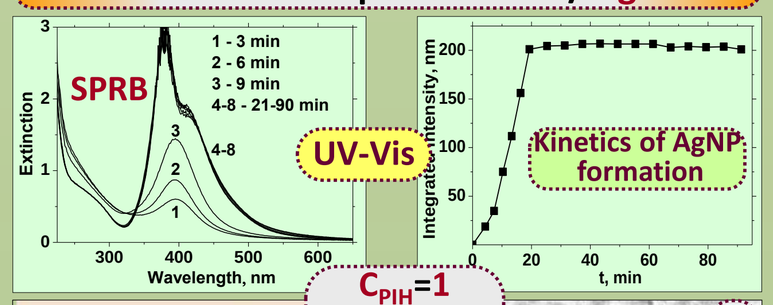
Double nanocomposition EMel / PIH

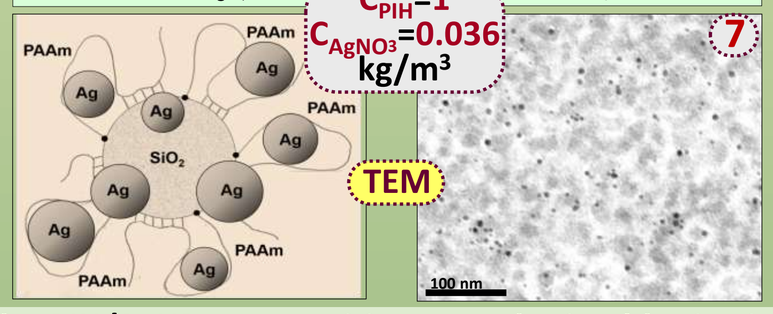




Composition EMel/PIH in an aqueous medium with a ratio of φ=0.05 base-mol$_{EMel}$/base-mol$_{PAAm}$ was homogeneous, almost transparent, and stable over time. According to FTIR data, the PIH and EMel interacted due to hydrogen bonds between the amide and phenol hydroxyl groups. The formation of the composition (TEM images 5 and 6) led to significant destruction of the PIH and EMel aggregates. Thus, the solubility and stability of EMel in water was improved.

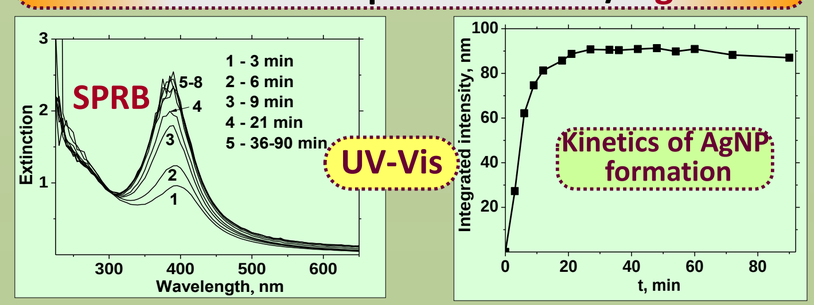
Double nanocomposition PIH / AgNPs

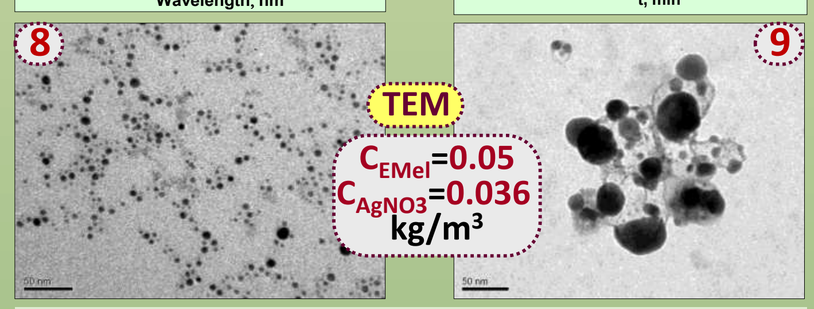




The PIH/AgNPs composition was obtained by *in situ* reduction of $AgNO_3$ with $NaBH_4$ in an aqueous PIH solution. The reaction proceeded for 20 min at a high rate and a yield of about 100 %. Swollen PIH particles with a size of ~10-40 nm firmly held AgNPs with a size of 3-9 nm in their "coronas" (image 7).

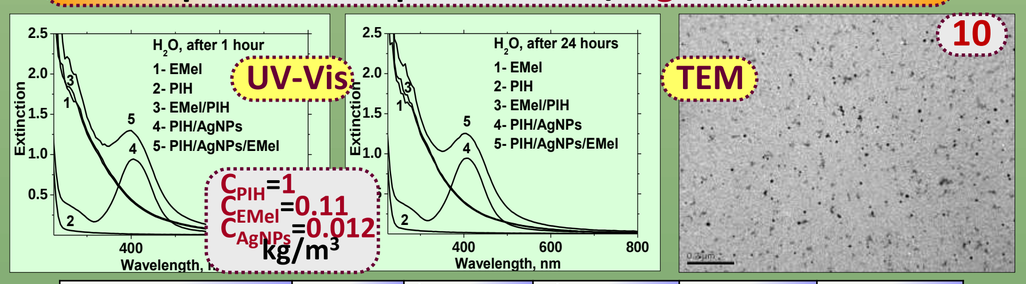
Double nanocomposition EMel / AgNPs





The stable composition EMel/AgNPs was formed by a similar borohydride reduction of $AgNO_3$ in an EMel solution. The vast majority of particles were 1.5-12 nm in size (TEM image 8), but in some large EMel aggregates they could reach ~50 nm (TEM image 9).

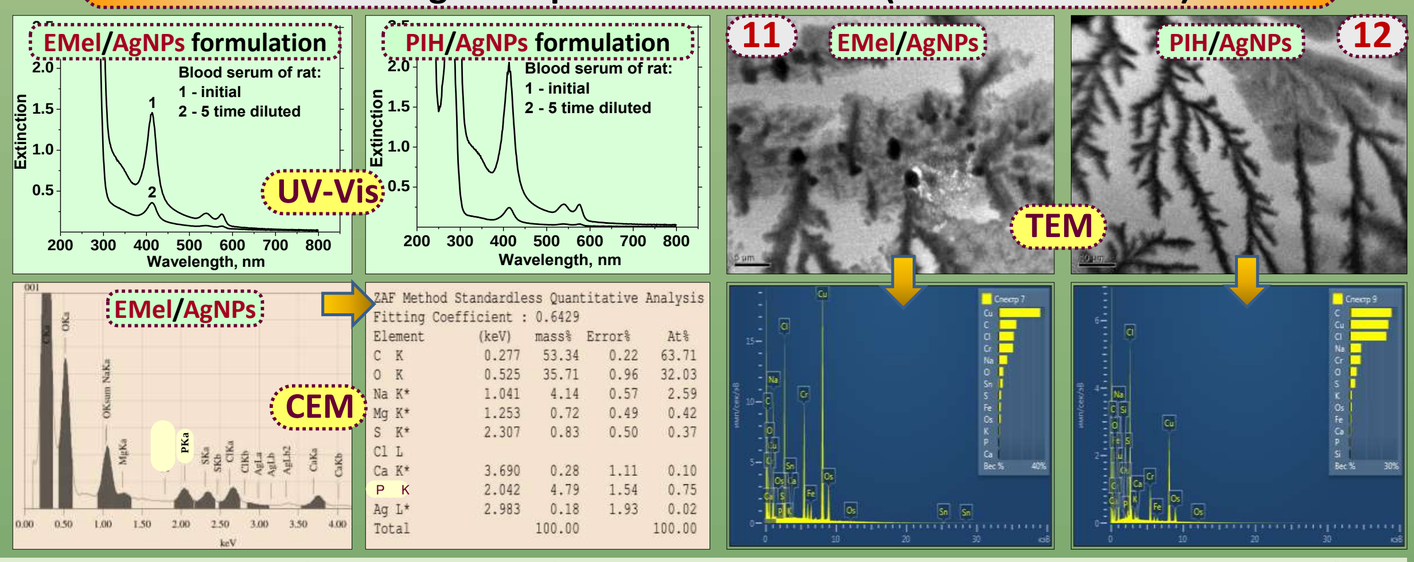
Triple nanocomposition PIH / AgNPs / EMel



Composition	C_{AgNO3}, kg/m^3	λ_{max}, nm (1 hour)	λ_{max}, nm (24 hours)	S_{SPRB}, nm (1 hour)	S_{SPRB}, nm (24 hours)
PIH/AgNPs	0.018	405	405	82.5	73.1
	0.009	405	405	43.0	31.5
PIH/AgNPs/EMel	0.018	400	405	38.8	37.5
	0.009	400	405	18.7	16.9

A triple composition was prepared by adding an EMel solution with pH=11.5 to the PIH/AgNPs composition to achieve φ=0.05 base-mol$_{EMel}$/base-mol$_{PAAm}$. The resulting formulation was completely homogeneous (TEM image 10) and stable in aqueous and "physiological" solutions. The integral intensity (S) of SPRB of AgNPs was constant after 24 hours and regularly decreased by 2 times with a twofold dilution of the system (Table).

In vivo biological experiments with rats (oral formulations)



Two formulations, EMel/AgNPs and PIH/AgNPs, were orally administrated to rats (0.3 mg/kg). After 1 hour, blood serum samples and aqueous extracts of finely ground rat organs: brain, heart, liver and kidneys were prepared and studied using UV-Vis spectroscopy, as well as TEM and SEM with X-ray microanalyzers of elements. Due to the presence in all samples of erythrocytes (images 11, 12) contained hemoglobin with an absorption band ~400 nm, it was impossible to correct determine the integrated SPRB intensity and AgNP content by the UV-Vis method. The detection of AgNPs using an X-ray element microanalysis within the framework of the TEM method also turned out to be impossible due to the small amount of every sample. However, X-ray element analysis of thick films (~1 μm) during SEM studies made it possible to determine the AgNP content. Thus, after 1 hour, the EMel/AgNPs composition was detected only in the blood serum and liver of rats.

[1] N.M. Permyakova, T.B. Zheltonozhskaya, T.V. Beregova, D.O. Klymchuk, T.M. Falalyeyeva, L.N. Grishchenko. Micellar nanocarriers for anticancer drug melanin. Mol. Cryst. Liq. Cryst. 2016, *640,* 122-133.

Conclusion. PIH is a bioavailable, biodegradable, non-toxic and effective carrier for EMel and AgNPs. The control of biodistribution of the PIH/EMel composition by means of AgNPs is possible using X-ray microanalysis within the SEM method. But the UV-Vis control method is promising in the case of gold nanoparticles, since they have isolated SPRB with λ_{max}~520-600 nm.