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In vivo behavior of carboxymethylcellulose based microgels containing ^{67}Cu

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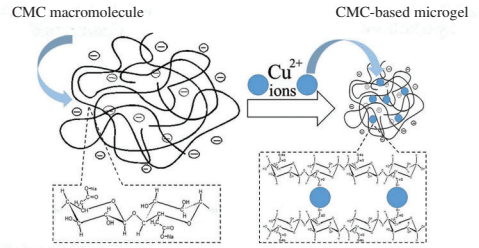
Using a mouse model, an organ distribution for microgels of carboxymethyl cellulose cross-linked with $^{67}Cu^{2+}$ ions was investigated and compared with the distribution of free $^{67}Cu^{2+}$ ions from $^{67}CuCl_2$. The clearance of the microgels through both liver and kidneys was demonstrated. An additional examination of distribution for [^{3}H]CMC microparticles and [^{3}H]CMC–Cu microgels revealed no copper release from the microgels *in vivo*.



Keywords: carboxymethyl cellulose, microgel, ^{67}Cu, tritium, mouse model, organ distribution, radiation stability.

Radiopharmaceuticals with ^{64}Cu (β^+, $t_{1/2}$ = 12.7 h) and ^{67}Cu (β^-, $t_{1/2}$ = 61.83 h) radionuclides are effectively employed due to the diagnostic and therapeutic capabilities, respectively.[1] For their delivery to tumors, various chelators are used including the 2-nitroimidazole, metronidazole and semicarbazone derivatives,[2] the recently introduced hybrid benzoazacrown ligands[3] as well as chelators coupled with antibodies.[4] Complexes based on the cyclam and cyclen[5,6] macrocycles demonstrate remarkable kinetic stability and are used as conjugates with monoclonal antibodies. Thus, the biodistribution of a ^{67}Cu–bifunctional chelator complex conjugated with ChCE7 neuroblastoma-specific antibody is described.[7] Today, in a design of radiopharmaceuticals, a carrier or chelator is needed that stably binds a radionuclide,[8] while the delivery of the carrier is directed by a monoclonal antibody or, alternatively, the carrier itself has a biological target in an organism. The carrier can possess an antitumor effect or be biocompatible and ultimately biodegradable or excretable within a reasonable time. In this regard, carboxymethyl cellulose (CMC) seems promising as a biocompatible material strongly binding metal ions.[9] Besides, CMC is soluble in water, which facilitates *in vivo* administration of the related drug. The use of ^{99m}Tc–CMC as a nondigestible marker of solid gastric content is described.[10] Multifunctional nano- and microgels including the polymeric ones represent emerging materials for bioimaging and targeted drug delivery, they are considered in detail in a review.[11] As an example, CMC is employed in pH-sensitive nanoparticles with the ^{68}Ga radionuclide for imaging of leukocytes to localize inflammations.[12]

In this work, to understand the behavior of CMC–^{67}Cu microgels *in vivo*, we investigated their organ distribution *vs.* time using a mouse model and compared the results with free $^{67}Cu^{2+}$ ions originated from a $^{67}CuCl_2$ administration, which made it possible to assess the binding strength between the radionuclide and CMC in the organism. Since it was initially unknown whether the radionuclide was capable of leaving the microgel due to possible degradation of CMC *in vivo*, we explored the distribution of [^{3}H]CMC particles and the [^{3}H]CMC–Cu microgel. Comparison of the results obtained for the distribution of [^{3}H]CMC–Cu and CMC–^{67}Cu microgels as well as [^{3}H]CMC revealed a complete pattern of the microgel behavior *in vivo*. Moreover, hypothetical loss of the radionuclide by CMC *in vivo* was estimated with a possible resulting additional dose load. Details of separation of the copper radionuclides,[13] TLC, preparation of the CMC–Cu^{2+} complexes[14] and CMC–$^{67}Cu^{2+}$ microgels are given in Online Supplementary Materials.[†]

Using a mouse model, the *in vivo* accumulation and clearance data for two different CMC–$^{67}Cu^{2+}$ microgels with CMC/Cu ratio of 15 : 1 and 7 : 1 in comparison with $^{67}CuCl_2$ were obtained.

[†] To introduce ^{3}H into CMC and CMC–Cu, the thermal tritium activation technique was employed.[15–17]

The OLINDA/EXM software was used to calculate absorbed dose for complete decay of the ^{67}Cu radionuclide with an activity of 5 mCi, which represented a real activity for the use in nuclear medicine.[18] For the calculation it was assumed, that the microgel was a liquid with a density of 1 g ml^{-1} in the form of an ideal sphere with a volume of 1 ml. The calculated absorbed dose after the complete decay of ^{67}Cu was 750 Gy.

In vivo experiments were approved by the Bioethics Commission of M. V. Lomonosov Moscow State University, meeting no. 126-d, 28.01.2021, protocol no. 124-a. All the experiments with animals and their housing complied with the rules of laboratory practice stated in the European Convention for the Protection of Vertebrate Animals Used for Experimental or Other Scientific Purposes (European Treaty Series no. 123, Strasbourg, 1986), the Order of the Ministry of Health of the Russian Federation no. 199n of 01.04.2016 'On Approval of the Rules for Good Laboratory Practice (GLP)' and in compliance with the international recommendations of the European Convention for the Protection of Vertebrate Animals Used in Experimental Research (1997).

Different functional groups in CMC macromolecules may bind Cu^{2+} ions.[19] In this work, the interaction of Cu^{2+} ions and the deionized carboxyl groups of CMC was expected to occur through an ion exchange and chelating. Seemingly, the multivalent Cu^{2+} cationic solutions are able to form intra- and intermolecular complexes with the carboxylate groups in microgels.[20] In the work of our group,[14] the interaction between CMC macromolecules and Cu^{2+} ions was confirmed by quantitative determination of copper in the composition of complexes as well as using dynamic light scattering and laser microelectrophoresis. It was established, that experimentally determined content of copper in the complexes corresponded to the theoretically calculated one based on the assumption of complete binding of Cu^{2+} ions. Besides, a decrease in electrophoretic mobility, which characterized the charge of the obtained complexes, with an increase in the content of Cu^{2+} ions indicated a significant role of carboxyl groups in the interaction between CMC and Cu^{2+} **(**Figure S2, Online Supplementary Materials).

Distribution of ^{67}Cu from the microgels *in vivo* in comparison with that of free $^{67}Cu^{2+}$ ions from $^{67}CuCl_2$ determined using the activity of ^{67}Cu is shown in Figure 1. In general, the accumulation and excretion of free $^{67}Cu^{2+}$ proceeds faster than CMC–^{67}Cu and the time of maximum accumulation for most organs falls to the range of 0.25–1 h after injection. The time of maximum accumulation for CMC–^{67}Cu was ~1.5 h for heart, lungs and kidneys as well as ~3 h for liver and spleen. The difference between the accumulation of microgels with CMC/Cu ratio of 15 : 1 and 7 : 1 is noticeable for kidneys apparently due to some difference in the size of microgel particles, which decreases with the content of copper atoms. This reflects the importance of particle size for passage through urinary system. Therefore, the accumulation time for CMC–^{67}Cu in the kidneys is in the following order: the 15 : 1 microgel > the 7 : 1 microgel > free $^{67}Cu^{2+}$ ions. At 24 h post injection, a certain amount of free $^{67}Cu^{2+}$ ions was still present in the kidneys, while the amount of microgels was close to zero. In the spleen, a similar pattern was observed, *i.e.*, the maximum accumulation time of CMC–^{67}Cu depended on the CMC/Cu ratio as 15 : 1 > 7 : 1, but for the 7 : 1 microgel the removal was much faster than for the 15 : 1 one. Moreover, at 24 h post injection, the 15 : 1 sample remained in spleen, while in other organs the content of microgels and free copper ions tended to zero.

Accumulation and clearance of the [^{3}H]CMC–Cu^{2+} microgel with CMC/Cu ratio of 10 : 1 and [^{3}H]CMC particles *in vivo* were also explored. The time of maximum accumulation for the microgels was 1.5–5 h depending on an organ (Figure 2). The fastest clearance occurred in the blood (Figure 3). In the tail vein as an injection site, 10–25% of the administered activity remained after 1.5 h and 5–10% after 24 h (see Figure 3). In the urine, blood, lungs and heart no fundamental difference in accumulation or excretion between [^{3}H]CMC and [^{3}H]CMC–Cu was found and at 24 h after injection the residual activity here tended to zero. In the pancreas (see Figure 2) the [^{3}H]CMC–Cu microgel excreted slower, while the [^{3}H]CMC particles were not completely eliminated after 24 h. The longest clearance seemed to occur in the spleen, although the corresponding amount of absorbed activity was small.

The [^{3}H]CMC–Cu microgels were almost completely eliminated from kidneys within 24 h (see Figure 2) and the maximum accumulation time was less than 1.5 h. However, [^{3}H]CMC particles uncompressed by cross-linking by metal ions were constantly at the same, albeit low, amount in this organ for 24 h. Probably, this effect is also associated with the cross-links by copper ions and the resulting change in the size and 'elasticity' of the particles. For kidneys, the particle size is important because of the need to pass through the excretion system.[21] It should be noted, that a recent work[22] points to the possibility of significant reduction in the size of microgels under the influence of UV and local heating. A similar process would occur under β-irradiation inside the microgel if the radiation dose is significant.

The largest difference between the two tritium-labeled microgels was observed in the liver (see Figure 2), where the behavior of [^{3}H]CMC–Cu was similar to other organs with a maximum accumulation time of ~5 h and almost complete

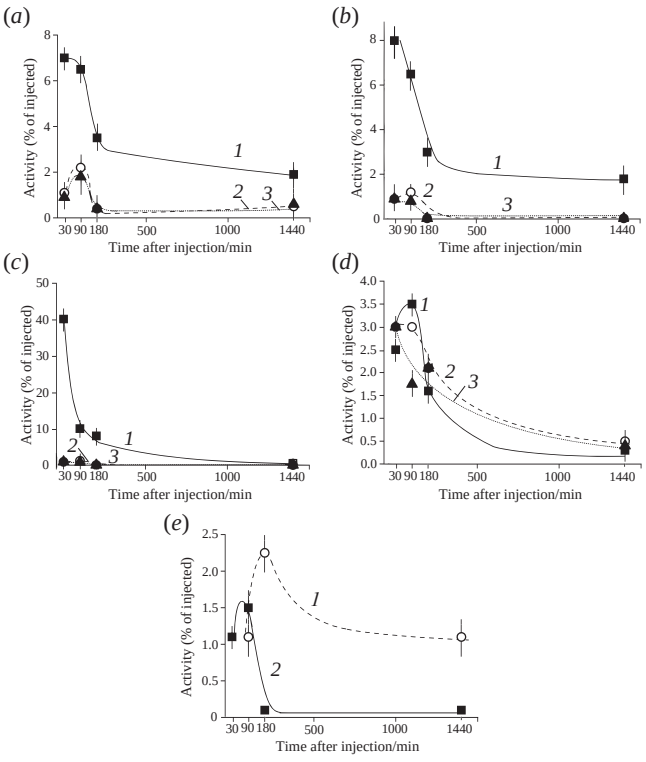


Figure 1 Distribution *vs.* time for (*1*) $^{67}CuCl_2$ as well as CMC–^{67}Cu microgels with CMC/Cu ratio of (*2*) 7 : 1 and (*3*) 15 : 1 in a mouse model: (*a*) lungs (*b*) heart, (*c*) liver, (*d*) kidneys and (*e*) spleen {^{67}Cu–CMC: 1/7 (*1*) and 1/15 (*2*)}.

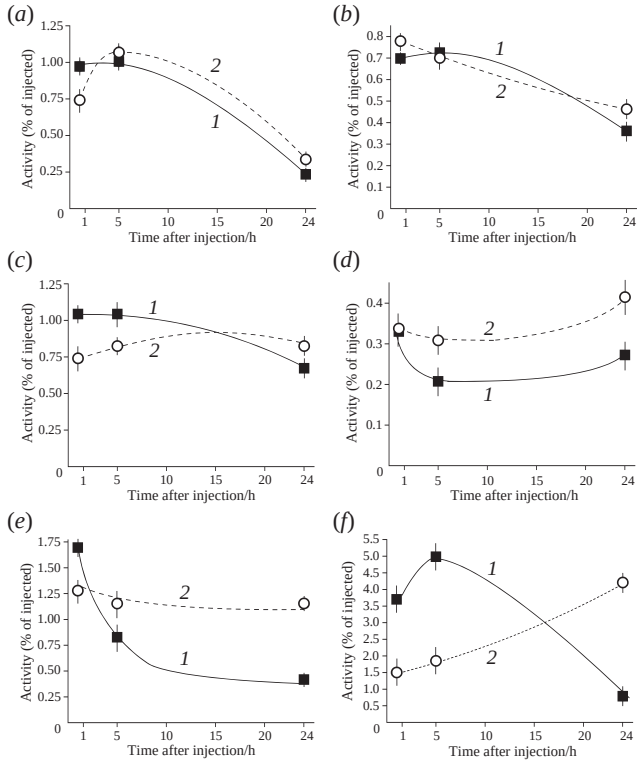


Figure 2 Distribution *vs.* time for (*1*) [^{3}H]CMC–Cu^{2+} and (*2*) [^{3}H]CMC microgels in a mouse model: (*a*) lungs, (*b*) heart, (*c*) pancreas, (*d*) spleen, (*e*) kidneys and (*f*) liver.

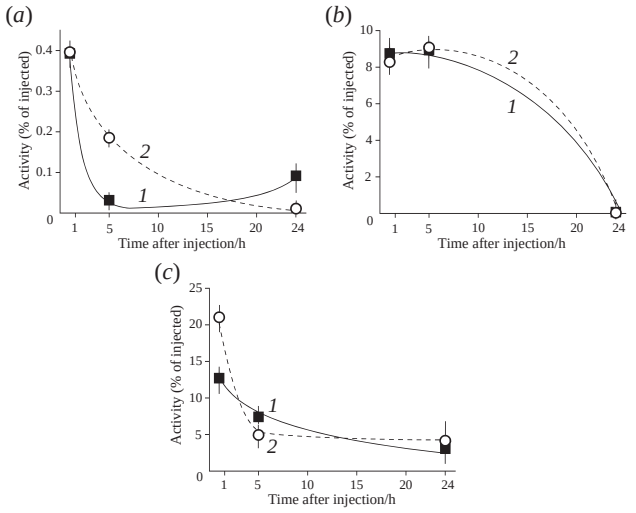


Figure 3 Distribution *vs.* time for (*1*) [^{3}H]CMC–Cu^{2+} and (*2*) [^{3}H]CMC microgels in a mouse model: (*a*) blood, (*b*) urine and (*c*) tail vein as an injection site.

elimination after 24 h. However, accumulation of the [^{3}H]CMC particles increased in this organ by 24 h. Given the great variety of processes occurring in the liver and the presence of different transporters, it can be assumed that the [^{3}H]CMC particles have binding sites that are not involved in interaction with Cu^{2+} ions and are used by biological moieties of the liver, which contribute to their retention.

Comparison of behavior for the different CMC microgels and free $^{67}Cu^{2+}$ ions *in vivo* demonstrates stability of CMC–^{67}Cu complexes in the organism and difference in the rate of accumulation–elimination processes, which are much faster for $^{67}CuCl_2$. However, after 24 h the removal of microgels carrying copper ions from most organs is almost complete regardless of the label type. The clearance of CMC particles is slower, they can linger in some organs, especially in liver.

To complete the picture of the behavior of CMC–Cu^{2+} microgel particles in the organism, it was necessary to consider their radiolysis and assess the extent of 'falling out' of the copper radionuclide, which could increase the dose load on healthy organs and systems. The radiation stability of the complexes was tested using external γ-irradiation.‡ At a dose of 750–1000 Gy, no release of copper from the microgels was observed, which confirmed their radiation stability sufficient for the use *in vivo*.

Thus, CMC represents a promising carrier for radionuclides, in particular ^{67}Cu as well as other transition metals. Moreover, CMC can serve as a platform for multimodal effect on a tumor, since it binds not only a radionuclide, but also an antitumor agent, as we demonstrated using Zn^{2+} ions and *N*-(5,6-dihydro-4*H*-1,3-thiazin-2-yl)benzamide hydrobromide.[9]

‡ Determination of radiation stability of the complexes was carried out using external irradiation with a γ-400 ^{137}Cs source (TU I-150-71, Russia, energy 661.7 keV, power rate of dose 2 Gy min^{-1}).

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Online Supplementary Materials

Supplementary data assosiated with this article can be found in the online version at doi: 10.1016/j.mencom.2022.09.030.

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