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The efficiency of solvent-free catalyst systems in the synthesis of tritium-labelled biologically active compounds

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Modern theories about the processes that take place inside solvent-free catalyst systems are considered and the various factors that have an effect on the successful introduction of tritium into organic compounds are summarized. Obtaining labelled compounds using solvent-free catalyst systems, liquid-phase methods, and isotopic exchange with tritiated water are all regarded as various manifestations of the same set of occurrences in the presence of tritium, a substrate and a catalyst. The use of various isotopic exchange methods has made possible the introduction of tritium into practically any type of biologically active compound. This will enable scientists to carry out more detailed studies of the processes inside living organisms.

Keywords: solid-phase reactions; isotopic exchange; tritium

Introduction

Hydrogenation (the addition of hydrogen to organic compounds) and hydrogenolysis (the cleavage of bonds resulting from a reaction with hydrogen) are the principal reactions of tritium incorporation in organic compounds. The isotopic exchange reaction can also be considered as a special case of a hydrogenolysis reaction.[1–4]

Methods involving the use of solvent-free catalyst systems have been extensively used to prepare isotopically labelled compounds.[5] This paper presents an overview of recent achievements in this field.

Solid-phase reactions have proved to be extremely efficient for the tritium labelling of organic compounds. Hence, optimization of such reactions is vital. This is especially important for selective labelling methods. Solid-phase reactions afford highly labelled chemicals via selective isotopic exchange, selective hydrogenation of unsaturated carbon–carbon and carbon–heteroatom bonds, as well as selective dehalogenation of a halogen atom in the presence of unsaturated labile fragments in an organic compound.

In addition to the recently verified possibility of solid-phase selective hydrogenation and dehalogenation,[5] the regioselectivity (an uneven distribution of the isotopic label across different positions of the substrate molecule) of tritium introduction by means of this method should be taken into account in the optimization of reaction conditions. The main feature [6] of these reactions is the increased mobility of hydrogen on inert supports (the support is defined as inert if no noticeable isotopic exchange occurs between the tritium donor and the labelling substrate in the absence of the supported metal-catalyst). The flowing over of active hydrogen particles from one phase to another is called hydrogen spillover (interphase diffusion of adsorbed hydrogen). In other words, unlike topochemical reactions in which the movement of the reacting particles must be kept to a minimum, solid-phase chemical reactions involving hydrogen will be governed by totally different laws.[7,8] Many of the well-known labelling reactions exhibit new advantages and peculiarities when they occur in the solid phase.

The mathematical model of spillover has been described.[9–12] It assumes that the reaction takes place on a metal that emits hydrogen particles. The support transports these active particles that interact with the metal through direct and reverse spillover. The construction of one-, two- and three-dimensional diffusion kinetic models has been described for the hydrogenolysis of an organic substrate placed on a support,[9,10] in which the concentration gradient of spillover hydrogen and the catalyst deterioration during the reaction have been taken into account. According to the models, a hydrogenolysis reaction must be localized in the reaction zones around the crystallites of the metal catalyst, and, consequently, there must be an optimum catalyst-support/substrate ratio for this reaction. For example, it can be inferred from the ^{3}H-NMR spectra of sodium acetate labelled by the solid-phase method (5% Pd/C, 200°C, 15 min) that the samples comprise 74% triply-substituted, 24% doubly-substituted and 2% singly-substituted isotopomers, i.e. the specific radioactivity must be about 80 Ci/mmol. However, in reality, the specific radioactivity of $[^3H]CH_3COONa$ is 30–35 Ci/mmol. A simple calculation demonstrates that the discrepancy of the results obtained by ^{3}H-NMR and the measurements of the specific radioactivity is caused by the fact that 60% of the

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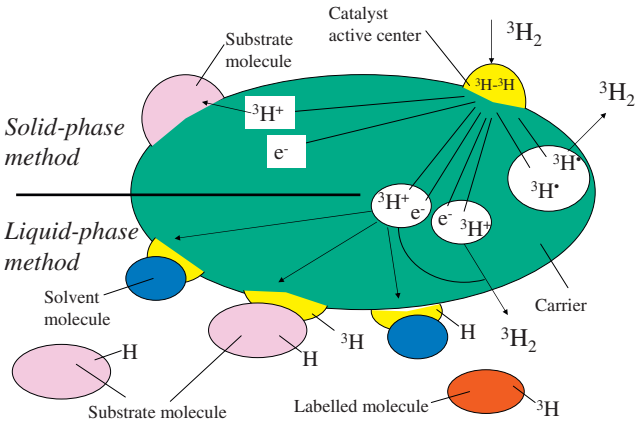


Figure 1. Methods of isotopic exchange with tritium gas.

sodium acetate does not get involved in the isotopic exchange reaction, i.e. activated tritium particles do not interact with these sodium acetate molecules.[13] Later publications report, however, that diffusion of activated tritium particles on the support surface occurs at a distance of up to 90–200 nm from the Pt particles.[6] A schematic representation of the isotopic exchange process with tritium gas is shown in Figure 1.

Unlike a reaction in a solution, where the inclusion of tritium occurs in the active centres of the heterogeneous catalysts, in solid-state reactions the label is introduced into the organic molecules primarily on the support. This happens because the surface of the support is far larger than the zones containing the active centres of the catalyst. Therefore, when the solid-phase method is used, activated tritium molecules interact not with isolated molecules of the organic compound but with the pool of such molecules that gets adsorbed on the surface of the support, i.e. the reaction proceeds in a system that is formed when a layer of the organic compound on the support becomes saturated with tritium particles.

Conditions for tritium introduction in solid-phase reactions

To perform solid-phase reactions, a solid mixture of the organic compound and the catalyst is treated in a tritium atmosphere. The catalyst is usually a metal, normally of the platinum group, deposited over the support. Other transition metals may also be used. The support is usually an inert inorganic compound, such as carbon, barium sulphate, calcium carbonate, aluminium oxide, etc. The process is promoted by heating the reaction mixture[1].

It is interesting to note which reaction conditions are optimal for various substances of different classes (see Table 1). It can be concluded that these conditions depend to a large extent on the nature of the organic compound. A large spread in the yield of labelled substances is characteristic of both aliphatic and aromatic compounds.

To obtain highly labelled substances in an acceptable yield, it is often necessary to utilize modified tritium introduction methods that make use of additional inert supports. There are obviously many variable parameters that need to be taken into account in solid-phase reactions (e.g. pressure, temperature, reaction time, compound/catalyst ratio, metal content in the deposited catalyst, specific area of the support, dispersion of the metal, etc.). It is therefore practically impossible to determine the correct balance for each specific reaction (i.e. the correct specific radioactivity, yield, label distribution, etc.) by an iterative experimental optimization sequence. Therefore, studying the mechanism of solid-phase reactions and building a model for the labelling process are relevant, not only from a purely theoretical point of view but also in terms of the practical application of such solid-phase reactions.

Features of solid-phase tritium hydrogenolysis reactions

In a previous overview it was reported that solid-phase reactions are subjected to different laws and have a different mechanism than reactions in solution.[5] A 'capacitor model' of solid-phase reactions has been described that explains the basic rules of introducing a tritium label into organic compounds using solvent-free catalyst systems.[22] The main idea behind this concept is as follows. After molecular tritium interacts with the catalyst metal, activated tritium particles ($^3H^•$ and $^3H^+$) emerge. Atomic tritium easily recombines into molecular tritium, while tritium positive ions (tritons) and electrons flow down onto the inert support. This concept is based on the premise that at temperatures between 50 and 200°C the activated particles of hydrogen spillover are primarily tritons. This model explains the experimental fact that the lower the electrophilic substitution energy of a specific carbon atom, the greater the degree of label inclusion in that specific location of the molecule.[23] In this regard, it is obvious that in a molecule comprising a phenolic fragment and a benzoic acid fragment the isotopic exchange will be more effective in the phenolic ring.

In the case of saturated organic compounds, solid-phase isotopic exchange is more effective in non-polar molecules (i.e. fatty acids and alcohols, steroids, etc.) rather than in polar (i.e. saccharides, polyols, etc.), Table 1. Probably, in polar organic compounds, nitrogen and oxygen atoms can be protonated with tritium cations and this might hinder further isotopic exchange.

To further develop these ideas, we used the concepts of primary and secondary hydrogen spillover.[6] When the resultant activated hydrogen isotopes flow from the catalyst metal onto the support this is referred to as primary hydrogen spillover. If the substrate is deposited on a support and the resultant solid is mechanically mixed with a supported metal catalyst, then the molecules of this substance will only be able to interact with activated hydrogen particles on condition that there is secondary hydrogen spillover. Apparently, the characteristics of the activated hydrogen particles that participate in the primary and secondary spillovers may differ significantly.[24]

In primary hydrogen spillover, a dynamic equilibrium is established between the atomic and proton forms of hydrogen.[25,26] The ratio between these two forms of activated

[1] *A typical method of solid-phase isotopic exchange with the tritium gas.* First, the substance is deposited onto the catalyst. For this purpose, a solution of the original compound is mixed with the catalyst and lyophilized (or rotary evaporated, with any traces of solvent then being removed by vacuum drying). The catalyst with the adsorbed substance is then placed inside a reaction vial. The reaction vial is evacuated at room temperature and filled with gaseous tritium until the inside pressure reaches 300–400 hPa. The reaction is usually carried out at a temperature of up to 300°C over a period of 5–180 min. Excess tritium is evacuated. The content of the vial is dissolved in a solvent containing methanol or water, the catalyst is filtered off, and the labile tritium is removed by multiple evaporations of the reaction products with protic solvents. The labelled substance is separated and purified using chromatographic methods. If an additional support needs to be used, then the substance is deposited on it in a similar manner to its deposition on the catalyst and mechanically mixed with the catalyst. Subsequent steps are identical to those described above.

Table 1. Optimized conditions for the tritiation of some typical organic substrates

Compound	Reaction conditions	Specific radioactivity, Ci/mmol	Yield, %	Ref.
2-(Imidazol-1-yl)-1-hydroxyethane-1, 1-diphosponium acid	5% PdO/$BaSO_4$, 190°C, 10 min	20	50	4
Olanzapine	5% Pd/$BaSO_4$, 200°C, 10 min	12	10	14
Maraviroc	Lindlar catalyst, 160°C, 15 min	109	80	15
α-Hederin	Lindlar catalyst, 235°C, 10 min	9.2	29	16
Win 55,212	5% Pd/$CaCO_3$, 130°C, 15 min, $CaCO_3$[a]	55	70	17
CP 55940	5% Pd/$BaSO_4$, 180°C, 15 min	70	25	17
PTC 124	5% Pd/BaSO4, 260°C, 5 min, Al_2O_3[a]	6.5	25	18
Estrone	5% Rh/Al_2O_3, 180°C, 15 min	158	32	19
trans-Zeatin	5% Pd/$BaSO_4$, 155°C, 30 min	35.1	10	20
Triterpenoid glycoside from Sea Cucumbers *Cucumaria japonica*	5% Pd/C, 25°C, 6 h	22	26	21
α-Methyl-glucoside of *N*-acetyl-neuraminic acid	5% Pd/$CaCO_3$, 200°C, 15 min	12.5	25	2
6-Keto-palmitic acid	5% Pt/C, 160°C, 15 min	750.6	32	2
Hexadecane	5% Pt/C, 140°C, 15 min	472.5	80	2

[a]Additional carrier for the substrate.

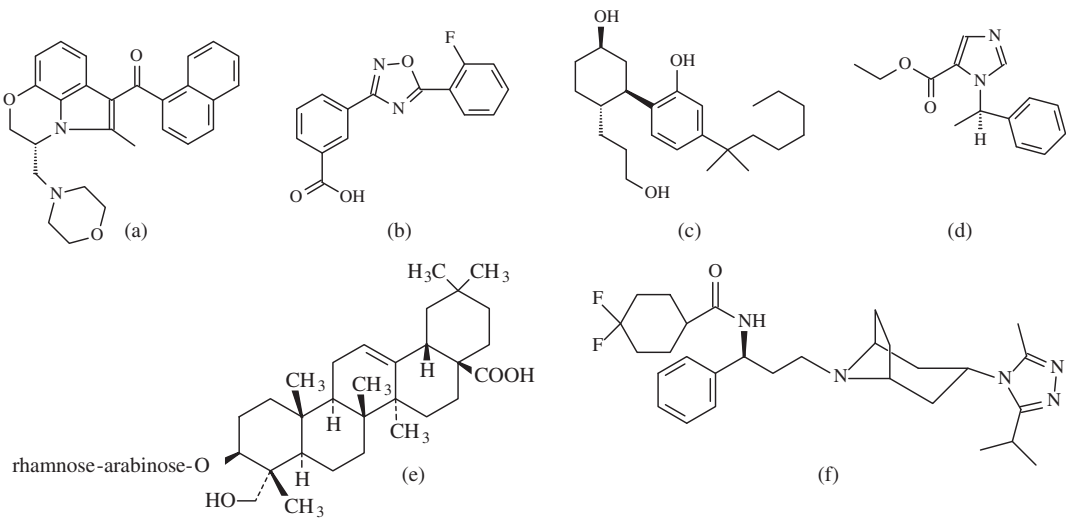


Figure 2. Structures of Win 55.212 (a), PTC 124 (b), CP 55940 (c), etomidate (d), α-hederin (e), and maraviroc (f).

hydrogen particles depends to a large extent on the nature of the support.[27] For instance, careful analysis of the form and shift in the ^{1}H-NMR spectrum revealed that hydrogen migrates over the surface of SiO_2 in the form of $H^{\bullet}$ radical.[28] On the other hand, unlike protons, hydrogen radicals bonding with the model graphite-like surface is thermodynamically disadvantageous.[29] In other words, quantum chemical calculations of the model for the hydrogen spillover on a carbon surface show that tritium migration in the form of a triton ($^3H^+$) provides a more accurate description of the hydrogen spillover phenomenon.

Under the conditions of primary hydrogen spillover, it is possible to introduce a tritium label in which either electrophilic or radical substitution is prevalent. In the first case, tritium was introduced into CP 55940 (Figure 2(c)), camptothecin, tiazofurin, ribavirin, isopropyl *N*-(3-acetylphenyl)carbamate, propamocarb and maraviroc (Figure 2(f)). The specific radioactivity of the labelled substances was 16–109 Ci/mmol.[4,15,17] In the second case, tritium was introduced into sulfobromophthalein and α-hederin (Figure 2(e)). Samples with a specific radioactivity of 3–16 Ci/mmol were obtained.[4,16]

Under the conditions of secondary hydrogen spillover, i.e. when an additional support is used with the organic compound being deposited onto it beforehand, tritium label can be introduced even into substances that are unstable to hydrogenation. It was in this manner that successful tritiations were conducted on the oxadiazole ring-containing PTC 124 (Table 1, Figure 2(b), where the additional support was alumina) [18] and the tetrasubstituted double bond-containing Win 55.212 (Figure 2(a), with $CaCO_3$ as an additional support).[17]

Especially, interesting results were obtained when carbon nanotubes (CNT) with an ordered nanostructure were used as the additional support. CNT comprise very narrow graphite layers, each 30–500 Å wide, arranged in highly ordered structures.[30] It is for this reason that this material adsorbs hydrogen to a far greater extent than does ordinary carbon. Furthermore, significant differences were observed in the

quantity of hydrogen adsorbed and desorbed from CNT at room temperature, which suggests the chemisorption of hydrogen. It is no wonder that these properties of CNT afford it certain advantages over other supports. The unique properties of CNT allowed us to increase the specific radioactivity for various classes of compounds. For instance, the specific radioactivity of phenylalanine increased almost threefold to 30 Ci/mmol, and that of diazepam increased almost sixfold to 22 Ci/mmol when CNT were used as additional support.[31]

Using CNT made it possible to introduce a tritium label into etomidate (Figure 2(d)). It is known that an etomidate molecule contains the fragment $RR'NCH(CH_3)C_6H_5$, which can be easily reduced to RR′NÍ and $C_2H_5C_6H_5$ when treated with gaseous tritium on the catalyst in solution.[32] The increased stability of this type of compound during solid-phase reactions can be explained by participation of $^3H^+$ activated tritium particles.[32] These particles cause the protonation of the $RR'NCH(CH_3)C_6H_5$ fragment and the subsequent formation of $RR'^3HN^+CH(CH_3)C_6H_5$, and tritons must overcome positively changed groups in order hydrogenolysis of the N-$CH(CH_3)C_6H_5$ link takes place.

The nature of the activated particles obviously affects not only the efficiency of labelling and the stability of compounds but also defines label distribution between different fragments of organic compounds. In other words, a prevalent form of activated particles (tritons or atoms) can be determined by studying label distribution in reaction products (Table 2).

As can be seen from the data obtained, at temperatures below 200°C the isotopic exchange mechanism is dominated by electrophilic substitution, i.e. the activated hydrogen particles are positive ions. Hence, in the case of Win 55.212, CP 55940, and maraviroc tritium label is included mainly in fragments with substituents that promote electrophilic substitution (Table 2). At temperatures above 200°C, the isotopic exchange mechanism changes and electrophilic substitution gives way to homolytic substitution. The mass spectroscopy data that we obtained on the distribution of deuterium in the PTC 124 molecule indicated that at a temperature of 270°C deuterium was included primarily in the carboxyphenyl fragment.[18] Such distribution can be explained if the activated deuterium particles are primarily deuterium atoms. In this case, the stability of carboxyphenyl intemediate radical is to be higher then fluorophenyl radical.[33]

Table 2. Deuterium site distribution[a] in labelled compounds[15–18]

Compound	T (°C)[b]	Fragment of the molecule	Deuterium content[c] (%)
α-Hederin	235	Disaccharidic	74
		Triterpenoid	26
PTC 124	270	Carboxyphenyl	95
		Fluorophenyl	5
Win 55.212	130	Naphthalenic	36
		Phenolic	64
CP 55940	180	Phenolic	92
		Aliphatic	8
Maraviroc	160	Benzylic	42
		Fluorine-containing	14
		Triazolic	39
		Aliphatic	5

[a]Mass spectra were obtained using LCQ Advantage MAX (Thermo Electron Corp., USA) with electrospray ion source, by direct syringe infusion of the μg/ml sample solution, at 35 eV of collision energy.
[b]Labelling conditions except for temperature are the same as in the Table 1.
[c]Total quantity of deuterium incorporated into a compound is taken as 100%.

Table 2 illustrates how the label distribution between different substrate fragments depends on their chemical structure. Two extreme cases can be considered for such compounds. In the first case, all parts of the compound molecule interact with positive hydrogen ions independently from each other. In the second case, some moiety seizes a flow of positively charged activated hydrogen particles and suppresses interaction between them and other sections of the substrate molecule.

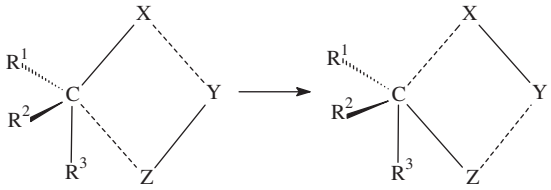
In the first case, isotopic exchange occurs primarily through positive tritium or deuterium ions (usually below 200°C),[1,2,5] and therefore the label usually gets included in the fragment that is more liable to electrophilic substitution. Mass spectrometry results confirmed that the bulk of the deuterium (over 80%) in the maraviroc molecule is included in the benzyl section and in the substituents of the triazole section (Table 2).

In the second case (at temperatures above 200°C),[1,2,5] isotopic exchange is performed by deuterium and tritium atoms formed from positive hydrogen ions and electrons after their desolvation. Apparently hydrogen atoms react with those sections of the substance molecules where they are formed, i.e. the inclusion of hydrogen isotopes into the most easily protonated sections of the molecule must be dominant. This actually happens for the carboxyphenyl fragment of PTC 124 (95%) and the disaccharide fragment of α-hederin (74% of the label) (Table 2).

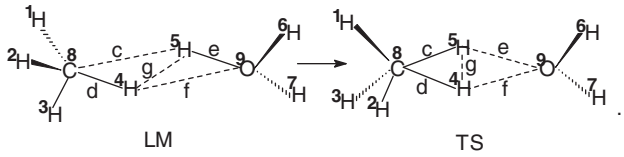
Label distribution in α-hederin requires a more detailed discussion. The fact that the double bond in the triterpenoid section of α-hederin is not hydrogenated even at such a high temperature, and on average less than half of the hydrogen isotope atom gets included in the α-hederin molecule, is evidence that the polar sections of the α-hederin molecule protonated by positive hydrogen ions prevent charged hydrogen particles from spilling over into the triterpenoid section of α-hederin. Therefore, at temperatures above 200°C, atomic hydrogen is formed primarily in the disaccharide section of the α-hederin molecule, and this is where isotopic exchange takes place. Twenty-six per cent of the label found in the triterpenoid section of α-hederin is probably caused by the presence of carboxyl and hydroxyl groups in its molecule, in whose vicinity the hydrogen isotope gets included.

In our work, we intentionally chose not to study the mechanism by which activated tritium particles are included in the molecules of organic compounds, but instead we used existing sources to explain experimental data on how labelled samples were obtained. Existing sources list the following electrophilic substitution mechanisms: bimolecular (S_E2), monomolecular (S_E1) and intramolecular (S_Ei).[33] Thus, an important property of solid-phase isotope exchange is that it preserves the original structure of the compounds, including their chirality.[5] Of particular interest are data on the patterns of the S_Ei electrophilic substitution mechanism that preserves the stereochemical configuration of the original compound (Scheme 1).

The possibility of introducing hydrogen isotopes by using isotopic exchange based on the S_Ei mechanism was confirmed by quantum chemical calculations. In this case, the water



Scheme 1. Transient state for the intramolecular electrophilic substitution mechanism.



Scheme 2. *Ab initio* quantum-mechanical calculation for hydrogen exchange between methane and hydroxonium. Transient state (TS) and intermolecular complex (LM).

molecule was considered as ZY. To simplify calculations, the transient state of the hydrogen exchange reaction between CH_4 and a single H_3H^+ molecule was studied (Scheme 2).[34]

Results of calculations showed that the reaction between CH_4 and H_3O^+ forms a strong molecular complex (LM). This complex is more stable than the original compounds, by 11.4 kcal/mol.[34] A calculation of changes in the bond lengths and total energy of the reaction for single-centre isotope exchange between methane and hydronium may be presented in the following manner (Scheme 3). The calculated activation energy of the reaction is about 0.044 atomic units (28 kcal/mol).

It follows from the data obtained through calculations that hydrogen is exchanged synchronously, creating a transient state with a five-coordinated carbon. It can also be concluded from the data that the additional interaction between hydrogen atoms reduces the amount of energy that goes into the activation of the isotopic exchange, i.e. the transient state of this reaction obtains additional stability from the formation of links between the hydrogen atoms that are being exchanged. When tritium is introduced into alanine, the process can be represented as in Scheme 4.

Thus, the study of solid-state reactions provides us with information not only about the nature of activated tritium and the mechanism by which it can be introduced into the molecules of organic compounds (including mechanisms that preserve the original configuration of the compounds) but also about the possible role of water molecules in this process.

Isotopic exchange with tritium gas and with tritiated water: common and different features

There are several methods proposed for isotopic exchange with tritiated water.[35] When heterogeneous catalysts are used, a solution containing tritiated water and the sample is stirred at various temperatures for various periods of time. As a rule, the effectiveness of isotopic exchange in this case is rather limited, even when 100% tritiated water is used, and the specific radioactivity of the samples remains within several Curie per millimole, or even less. A probable reason for this is that the catalyst's role is limited to weakening the R–H link in the transient state, which facilitates subsequent exchange with HTO molecule (Scheme 5).[36]

For example, trichostatin (Figure 3) has a specific radioactivity of 1.8 Ci/mmol, even when heated to a temperature of 140°C, while pargyline (which is unstable at temperatures above ambient) has a specific radioactivity of only 0.54 Ci/mmol.[4,37]

The most successful method of isotopic exchange with tritiated water, in our opinion, is as follows.[2] In this case, the specific radioactivity of the labelled product was an order of magnitude higher than that obtained with non-activated catalysts: it reached dozens of Ci/mmol (Table 3).[3,36–38]

As can be seen from Table 3, a labelled deltamethrin was obtained with a specific radioactivity of about 9 Ci/mmol. However, in the case of permethrin, which has a similar structure (Figure 3), no tritium inclusion occurred when the reaction was conducted in the same vial with deltamethrin, under the same conditions.

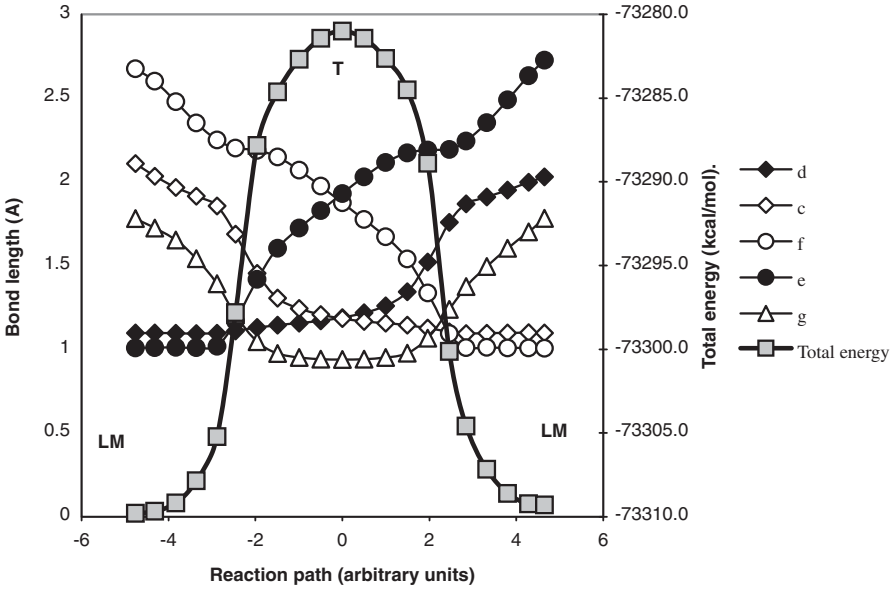
When deltamethrin and permethrin were treated separately, the overall picture remained the same, i.e. label was included in deltamethrin but not in permethrin. It was therefore not the competition for the active catalytic centre by deltamethrin and permethrin molecules that prevented the inclusion of the label into permethrin, but rather it was the inability of the permethrin molecules to displace water molecules and become adsorbed in the active centres of the catalyst (Figure 4).

It is known that when a catalyst treated with gaseous tritium is evacuated, reverse hydrogen spillover (RHS) from the support to the active centres of the metal catalyst is triggered.[6] Apparently, the increased effectiveness of isotopic exchange with tritiated water results from the fact that once gaseous tritium is removed, RHS on the active centres of the metal catalyst causes the formation of protonated water clusters $^3H^+(^3H_2O)_n$ (where *n* is the number of water molecules in the cluster).

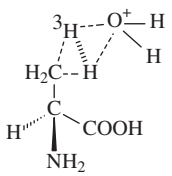
The increased effectiveness of isotopic exchange with tritiated water on acid centres brought about by activated tritium particles is apparently related to the fact that the tritium exchange in the solid-phase method (Scheme 4) has much in common with the same process with tritiated water (Scheme 6). In both cases, hydrogen is exchanged synchronously, creating a transient state with a five-coordinated carbon that obtains additional stabilization from the formation of links between the exchanging hydrogen atoms. The only difference is that solid-phase isotopic exchange with gaseous tritium occurs on the surface of the support, while isotopic exchange with tritiated water occurs on the active centres of the catalyst.

Isotopic exchange of tritiated water with 5-chloro-8-hydroxy-quinoline and 5-chloro-8-hydroxy-7-iodo-quinoline on an activated catalyst confirms the electrophilic substitution mechanism. It emerged that the label was successfully included in 5-chloro-8 hydyroxy-quinoline but failed to enter 5-chloro-8-hydroxy-7-iodo-quinoline (Table 3). When the preparation of 5-chloro-8-hydroxy-7-iodo-quinoline was attempted by iodinating the tritium-labelled 5-chloro-8-hydroxy-quinoline, tritium-free 5-chloro-8-hydroxy-7-iodo-quinoline resulted. This offers an evidence that tritium was included in the phenol part of 5-chloro-8-hydroxy-quinoline in the *ortho* position exclusively. Thus, as was the case with solid-phase reactions, isotopic

[2] *A typical method of isotopic exchange with the tritiated water on activated catalyst.* A mixture of PdO and a supported Pd catalyst are treated with tritium gas to obtain 100% tritiated water and a heterogeneous catalyst saturated with activated tritium. After removal of the tritium gas (by pumping it out), a solution of the organic compound in a dioxane/triethylamine mixture is placed in the vial with tritiated water and the activated catalyst. The vial is then sealed and the reaction conducted at a temperature of 150–200°C.



Scheme 3. Reaction path for exchange between CH_4 and H_3O^+. Change in bond lengths (c, d, e, f, g) and total energy of the reaction calculated by the MP2/6-31G* method.



Scheme 4. Tritium incorporation into the alanine molecule.

exchange with tritiated water on activated catalysts occurs via interaction with tritium cations.

The effectiveness of the adsorption of an organic compound on the catalyst's active centre apparently depends on the number of water molecules in this active centre (Figure 4). This claim was confirmed by quantum chemical calculations.[39] All quantum chemical calculations assumed water clusters to contain between one and three water molecules. Calculations demonstrated that when the size of a water cluster increases, then the energy needed to separate a proton also increases significantly: $n = 1$ (163.1 kcal/mol), $n = 2$ (196.6 kcal/mol), $n = 3$ (220.7 kcal/mol). As a result, the acidity of the protonated water clusters and their complexation energy with a substrate decreases as the number of water molecules in the cluster increases. This obviously predicts the existence of the optimum tritiated water concentration in the solution for the best isotope exchange.

The observed regularities allow for the development of a set of requirements for successful labelling using isotopic exchange with tritiated water. First, catalysts treated with gaseous tritium are preferred to create conditions for the formation of protonated water clusters on the catalyst surface (Table 3). Second, the catalyst must be thoroughly dried before the reaction. This not only reduces the isotopic dilution of tritiated water but also reduces the number of water molecules on the surface of the catalyst, which in turn increases the reactivity of the protonated water clusters, and, consequently, increases the effectiveness of the isotopic exchange. Third, the adsorption properties of the substrate's molecules must be taken into account, because the efficiency of isotopic exchange with tritiated water depends on the ability of substrate molecules to compete with water for the active centres of the catalyst (Table 3).

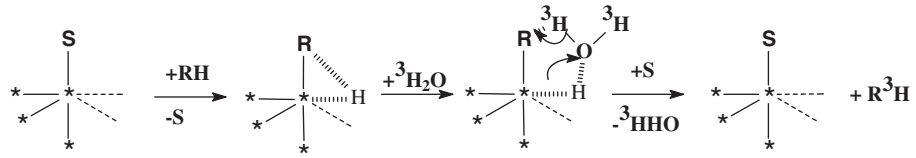
Thus, data on the processes taking place on the catalyst surface in isotopic exchange reactions serve not only to explain the influence of various factors on the synthesis of tritium-labelled compounds but also to enable us to develop recommendations with regard to optimizing the labelling process.

Discussion

Tritium can be introduced into organic compounds through isotopic exchange using liquid-phase, solid-phase, or tritiated water exchange methods. Liquid-phase methods are carried out in the presence of the catalyst in solutions of the substance precursor to be labelled, and tritium gas as isotopic source.[3,40–44]

The main ideas relating to these processes are summarized in the overview in Scheme 7.[43] Further researches confirmed the conclusions presented in the overview. It was demonstrated that the activity of metallic catalysts is determined by the proportion of catalyst active centres, where the metal atoms are located in the corners and edges of the cluster.[45] Theoretical results of computer models simulating the changes in the state of the catalyst in the course of the reaction have been reported.[46–53] The deformation of metal clusters on the surface of the support, bringing about the formation of several types of active centres on the support's surface, which differ from each other in the degree of coordination unsaturation, has also been reported.[52,53] These concepts correlate with the results reported in a paper that presents a model of the interaction between the catalyst and hydrogen isotopes, and between substrate molecules and active centres of the catalyst.[43]

In their theoretical calculations of the processes that occur on metal clusters, the authors of the above papers have assumed the number of atoms in a cluster to be between two and seven,[52,53] which agrees well with the theory that four to six metal atoms can form effectively functioning catalyst centres.[43]



Scheme 5. Isotopic exchange with tritiated water. S–solvent molecule, RH–substrate molecule, R^3H–labelled product molecule.

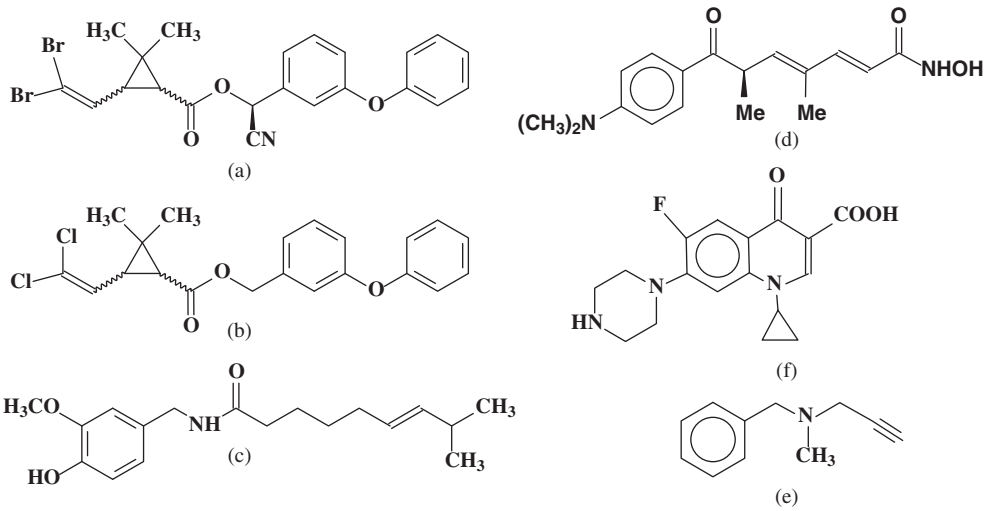


Figure 3. Structures of deltamethrin (a), permethrin (b), capsaicin (c), trichostatin A (d), pargyline (e), and ciprofloxacin (f).

Table 3. Tritium labelling by heterogeneous isotopic exchange with tritiated water[a]

Compounds	*T* (°C)	Specific activity (Ci/mmol)	Catalyst
1,3-Dibenzyloxy acetone	115	59.0	5% PdO/Al_2O_3
Ciprofloxacin	150	35,1	5% PdO/Al_2O_3
Capsaicin	145	7.0	5% $PdO/BaSO_4$
Deltamethrin	140	9,3	5% $PdO/BaSO_4$
Permethrin	140	—	5% $PdO/BaSO_4$
5-chloro-8-hydroxy-quinoline	140	3.8	5% $PdO/BaSO_4$
5-chloro-8-hydroxy-7-iodo-quinoline	140	—	5% $PdO/BaSO_4$

[a]Reaction was carried out in dioxane–triethylamine mixture. Tritiated water was prepared by PdO reduction with tritium gas.

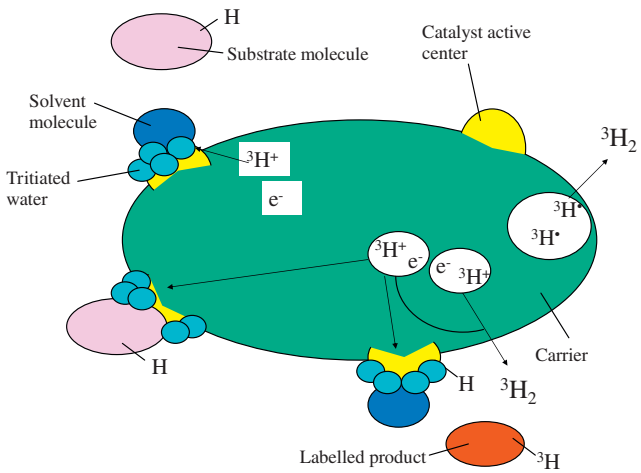
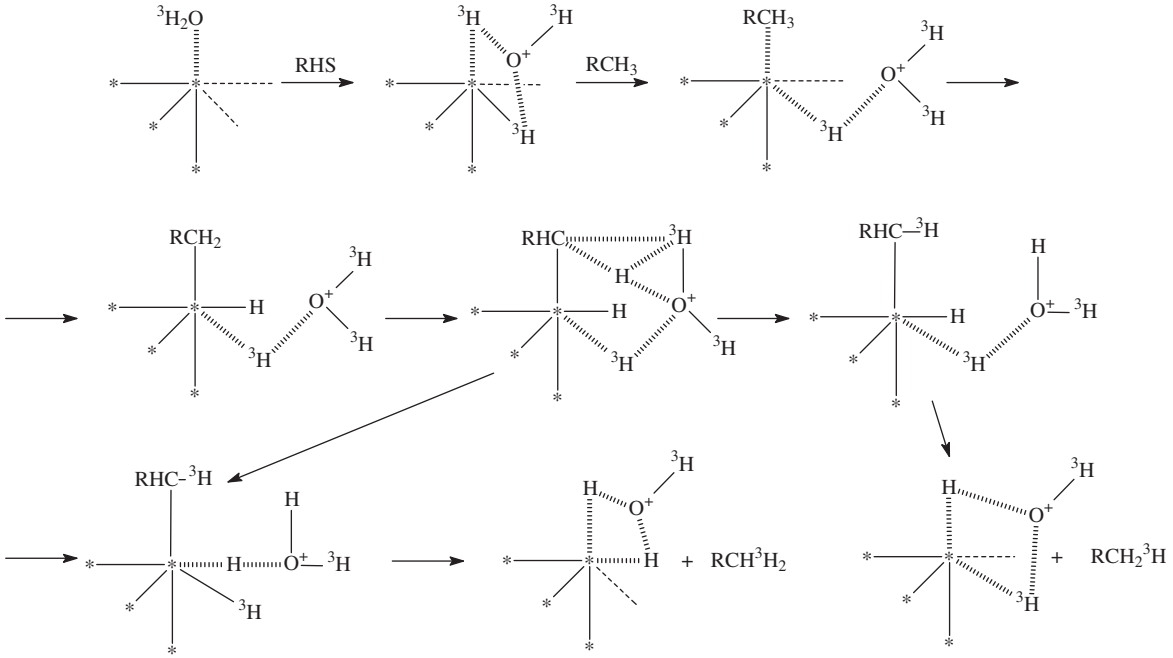


Figure 4. Isotopic exchange with tritiated water on a catalyst pre-activated with tritium gas.

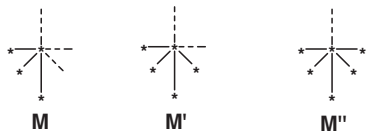
In liquid-phase isotopic exchange with gaseous tritium, it was possible to introduce tritium into both saturated and unsaturated compounds (Table 4).[4,35,43,44] However, the efficiency of liquid-phase isotopic exchange with heterogeneous catalysts by dissociative mechanism of adsorption is very limited, the specific radioactivity rarely exceeds 1–2 Ci/mmol (Scheme 8).

Liquid-phase methods have advantages in the hydrogenation and dehalogenation of thermally labile bioactive compounds only, especially when a high degree of selectivity is required.[54–59] The compounds labelled using these methods have a high specific radioactivity (40–55 Ci/mmol per one reduced carbon–carbon double bond) are obtained in good yield and contain label in preselected positions only.

As a rule, when isotopic exchange with tritiated water on non-activated catalysts and exchange of an organic compound in solution with gaseous tritium are carried out, the specific radioactivity of the product is low. In our point of view, a dissociative reaction mechanism is probable in both the cases



Scheme 6. Isotopic exchange with tritiated water cluster ($^3H^+(^3H_2O)_n$ when n = 1).



M (atom in the corner of a cluster), **M'** (atom in the edge of a cluster), and **M''** (atom in the surface of a cluster)

Scheme 7. Types of catalyst active centre (∗–atoms of a transition metals).

(Schemes 5 and 8). In contrast, when tritiated water on activated catalysts is used, the specific radioactivity may reach dozens of Ci/mmol (Table 3). In the case of unsaturated compounds, isotopic exchange with tritiated water proved to be more promising than isotopic exchange with gaseous tritium in solution. With regard to saturated, aromatic and even some unsaturated compounds, the best results were achieved in solid-phase reactions (Table 5).

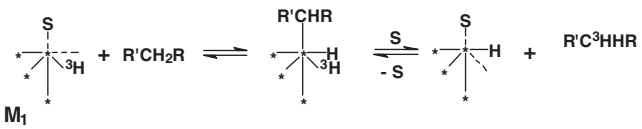
It should be noted that activated tritium particles are usually believed to be tritium atoms,[6] and insufficient attention has been paid to studying the spillover of positive tritium ions and their pairs with electrons over the support, and interactions between electrons and gaseous hydrogen or the substrate. In this respect, the inert supports, which can provide conductivity of mobile hydrogen ions, can be considered as solid electrolytes, and the process could be interpreted as a special case of electrochemical reactions.[2]

As positive tritium ions and electrons transfer from the metal cluster onto the support or from one support onto another, and when they spill over on the support, zones with opposite charges arise (the 'capacitor model'). Depending on the experimental conditions, the tritons and electrons can interact with a certain probability to give tritium atoms that are active in radical substitution reactions (Figure 5(b)).[22] Quantum mechanical calculations confirm that such microsystems may be stable. It has been found that a homogeneous magnetic field applied perpendicular to the direction of the hydrogen spillover flow

Table 4. Tritium labelling of saturated and unsaturated compounds by heterogeneous catalytic isotope exchange with tritium gas in a solution[a]

Compound	Reaction conditions	Specific activity (Ci/mmol)
Methyl stearate	10% $Pd/BaSO_4$, dioxan, 5 h	0.97
Methyl palmitate	10% $Pd/BaSO_4$, dioxan, 5 h	0.32
Methyl laurate	10% $Pd/BaSO_4$, dioxan, 5 h	0.22
Methyl oleate[b]	Lindlar catalyst, dioxan, 1 h	3.94
Prostaglandin E_2[b]	Lindlar catalyst, dioxan, 1 h	1.76
Prostaglandin $F_{1\alpha}$[b]	Lindlar catalyst, dioxan, 3 h	1.89
Polyprenyl phosphate[b]	5% PdO/Al_2O_3, dioxan, 1.5 h	1.35
Epigid	5% $Pd/BaSO_4$,chloroform, 3 h	0.35
8-Methoxypsoralen[b]	5% $Pd/BaSO_4$, dioxan -Et_3N (3:1),3 h	0.43

[a]All reactions were carried out at room temperature.
[b]Partial reduction occurred, saturated by-products were removed by chromatographic methods.



Scheme 8. Label incorporation by liquid-phase exchange with tritium gas.

retards its diffusion over the surface in two-component zeolite Pt/NaY-HNaY samples.[61] The fact that a magnetic field influences hydrogen spillover is proof that there are charged

particles that participate in hydrogen spillover (positive hydrogen ions and electrons move across the surface of the support). In other words, the degree of tritium incorporation into organic compounds via isotopic exchange can be significant if conditions for effective spillover of both positive tritium ions and electrons solvated on the support are created (Figure 5(a)).

It is therefore apparent that the nature of the support plays an important role in the creation of conditions that are optimum for primary and secondary hydrogen spillovers.

Table 5. Tritium labelling by the solid-phase method [4,60]

Compound	*T* (°C)	Specific activity (Ci/mmol)
Saxitoxin	160	25
Methyl stearate	210	1026
Camptothecin	190	26
Vitamin K_1	130	62
Ribavirin	160	30
Cetyl alcohol	210	135

Conclusions

1. The possibility of introducing hydrogen isotopes into organic compounds using solvent-free catalyst systems was considered. It was demonstrated that the use of primary or secondary hydrogen spillover affects the yield and specific radioactivity of labelled samples.

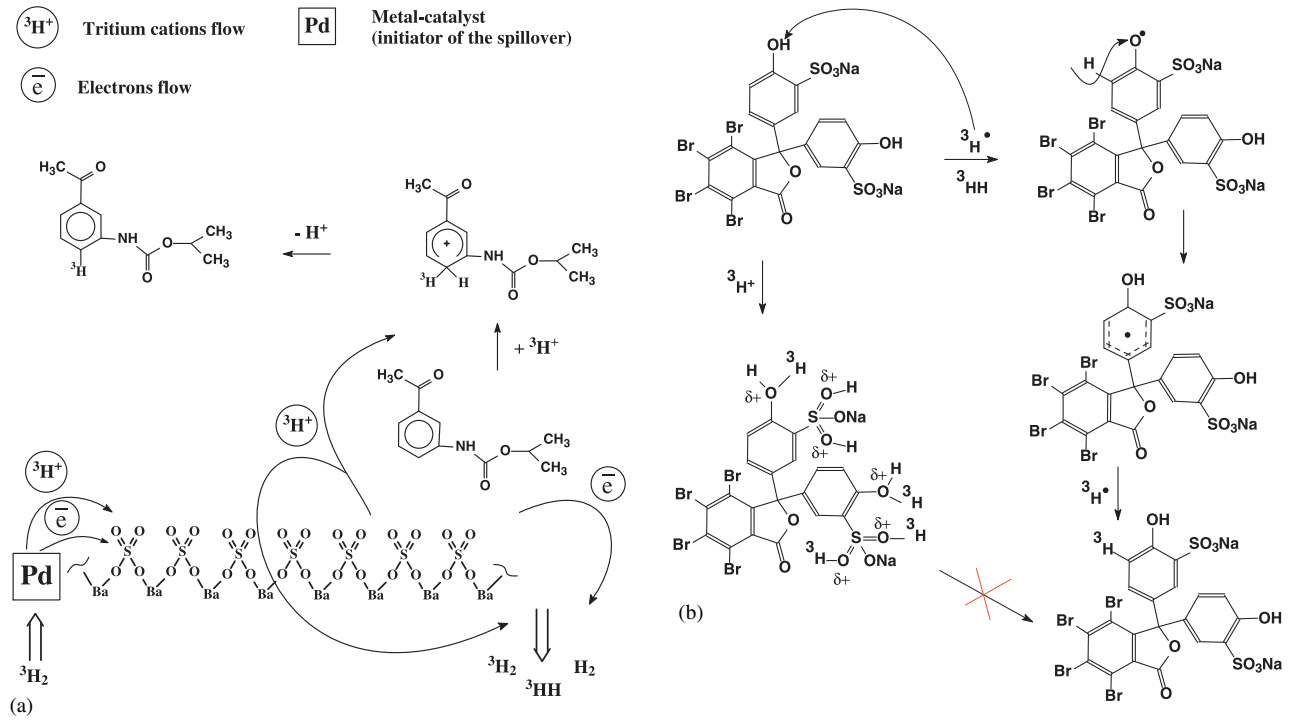


Figure 5. (a) Tritium incorporation in isopropyl *N*-(3-acetylphenyl)carbamate (proposed mechanism of electrophilic substitution) and (b) Tritium incorporation in sulfobromophthalein (proposed mechanism of radical substitution).

Table 6. Efficiency of heterogeneous isotopic exchange for different labelling methods

Compound	Specific activity (Ci/mmol)	Exchange method	Catalyst
Tiazofurin	18.0	Solid-phase[a]	5% $Pd/BaSO_4$
	8.0	HTO[b]	5% PdO/Al_2O_3
	0.5	Liquid-phase[c]	5% $Pd/BaSO_4$
Alprazolam	4.0	Solid-phase	5% $Pd/BaSO_4$
	27.0	HTO	5% PdO/Al_2O_3
trans-Zeatin	35.1	Solid-phase	5% $Pd/BaSO_4$
	18.4	HTO	Pd black
Zaleplon	4.6	Solid-phase	5% $Pd/BaSO_4$
	18.4	HTO	5% PdO/Al_2O_3

[a]Solid-phase isotopic exchange with tritium gas at an elevated temperature.
[b]Isotopic exchange with tritiated water in solution in the presence of a heterogeneous catalyst. Tritiated water was prepared by PdO reduction with tritium gas.
[c]Isotopic exchange with tritium gas in a solution in the presence of a heterogeneous catalyst.

2. The advantages of using CNTs as an additional support have been demonstrated.
3. New data were obtained, which can be reliably explained using the 'capacitor model'.[5,22] If substrate molecule consists of several fragments label distribution between these sections is very different (Table 2) and can be explained by interaction between them. In the first case, the label usually gets included into a fragment that is more liable to electrophilic substitution, and in the second case, it included into the most easily protonated section of the substrate molecule.
4. It was demonstrated that isotopic exchange with tritiated water is more effective on acid centres that form under the influence of activated tritium particles.
5. On the basis of available data, we compared the efficiency of isotopic exchange using various methods for labelling of biologically active compounds (Table 6).[20,35,62] The data show that solid-phase methods are better for compounds resistant to hydrogenolysis. Labile compounds that cannot withstand the conditions of solid-phase methods can be labelled by isotopic exchange with tritiated water.
6. We considered various manifestations of the same set of events occurring in the presence of tritium, substrate and catalyst, and resulting in the labelling of compounds using solvent-free catalyst systems, liquid-phase methods, and tritiated water isotopic exchange. Utilizing data on the processes occurring on the surface of the catalyst enables the optimization of the conditions of solid- and liquid-phase reactions, and demonstrates that there are hidden links between the various methods of labelling organic compounds using isotopic exchange.
7. The use of liquid-phase methods, solvent-free catalyst systems, and tritiated water isotopic exchange makes it possible to introduce tritium into practically any biologically active compound.[63–73]

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