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Adsorption and release of gemcitabine hydrochloride and oxaliplatin by hydroxyapatite

Maria Betsiou [a], Georgios Bantsis [a], Ioanna Zoi [b], Constantinos Sikalidis [a,*]

[a] Department of Chemical Engineering, Aristotle University of Thessaloniki, Greece
[b] Department of Chemistry, Aristotle University of Thessaloniki, Greece

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Abstract

The aim of this study was to investigate the adsorption and the release profile of two anticancer drugs, gemcitabine hydrochloride (dFdU.HCl) and oxaliplatin (DACH-Pt), from hydroxyapatite (HAP) in order to evaluate HAP as local drug delivery system (DDS). Various initial concentrations of drug aqueous solutions were used in order to determine the maximum adsorption capacity of HAP after 48 h shaking. The maximum adsorption capacity of dFdU.HCl (400 mg/g HAP) was achieved after 40 h while the maximum adsorption capacity of DACH-Pt (49.1 mg/g HAP) was accomplished after 20 h. Adsorption processes for both drugs were found to fit the Freundlich equation. The release processes were studied by soaking the samples of loaded HAP in simulation body fluids (SBF). After only 1 h 65% of dFdU.HCl was released while the release of DACH-Pt from the HAP was more gradual since 55% of DACH-Pt was released in the first 24 h. Finally, in an attempt to understand the molecular basis of the drug action, the chemical interactions involved in the complex processes of drug delivery were studied theoretically.

Keywords: C. Diffusion; D. Apatite; E. Biomedical applications

1. Introduction

The need for safe, therapeutically effective and patient-compliant treatments continuously leads researchers to design novel tools and strategies regarding drugs' delivery. Various diseases, including cancer, a highly invasive disease, that are caused due to both environmental and genetic factors are treated with therapeutic strategies that carry high costs and relatively low effectiveness since the drug delivery mechanisms used do not exhibit high accuracy in targeting. An effective method for the treatment of such diseases is the use of controlled drug release from special delivery systems using novel materials such as ceramics and polymers. The major purpose is to achieve a controlled and low release rate of the drug in order to ensure a constant in vivo drug concentration for a longer period of time while preventing harmful side-effects [1–9].

Many types of ceramic (aluminum–calcium–phosphorous ceramic compounds) drug delivery systems have been used to carry various kinds of drugs such as proteins [10–13], hormones [14,15], antibiotics [16–18], anti-inflammatory [19], polypeptides [20] and anti-cancer drugs [4,21–25]. One of the most commonly used ceramic form is HAP [$Ca_{10}(OH)_2(PO_4)_6$] because of its biocompatibility and osteoconductivity since it is the essentially hard, inorganic component of human bones. Furthermore, physical and chemical properties of HAP such as chemical composition, structure, porosity, particle size, surface area and ionic composition of the equilibrating solution are important determinants regarding drugs' binding and release [3,4,26–29].

The purpose of the present work was to study the adsorption of two anticancer drugs, dFdU.HCl and DACH-Pt, by HAP, as well as their release profiles. Theoretical calculations were also performed to further investigate the adsorption profile of the above anticancer drugs.

dFdU.HCl is a pyrimidine nucleoside (Fig. 1a) that belongs to a general group of chemotherapy drugs known as antimetabolites. It inhibits cells from making DNA and RNA by replacing one of the building blocks of nucleic acids, in this case cytidine, during DNA replication. This stops the growth of cancer cells by triggering cellular ''suicide'' (apoptosis). It is used to treat various types of cancer such as pancreatic, breast

* Corresponding author at: PO Box 1683, 54006 Thessaloniki, Greece. Tel.: +30 2310 996185; fax: +30 2310 996230.
E-mail addresses: sikalidi@auth.gr, sikalidi@eng.auth.gr (C. Sikalidis).

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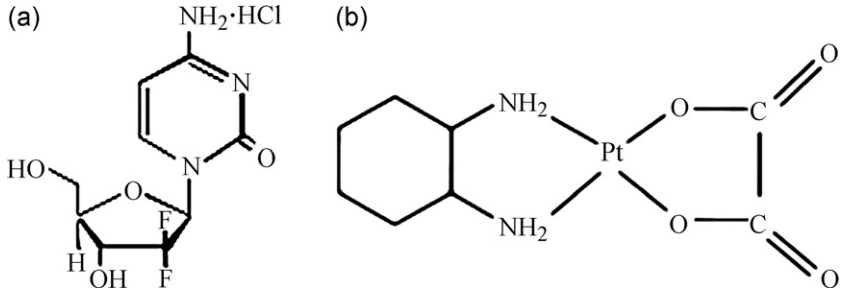

Fig. 1. Molecular structure of (a) dFdU.HCl (2′-deoxy-2′,2′-difluorocytidine monohydrochloride (β-isomer)) and (b) DACH-Pt (cis-[(1R,2R)-1,2-cyclohexane diamine-N,N′]) [31,35].

(along with paclitaxel) and lung cancer (along with cisplatin), while it can be used for the treatment of other cancers as well [30,31].

DACH-Pt is an antineoplastic agent, a member of a new class of platinum based compounds in which the platinum atom is complexed with 1,2-diaminocyclohexane (DACH) and an oxalate group (Fig. 1b). DACH-Pt acts as an alkylating cytotoxic agent, inhibiting DNA replication by forming adducts between two adjacent guanine plus adenine. It is the main drug for the treatment of colorectal cancer and it, also, provides antitumor activity in the treatments of pancreatic, gastric, ovarian, bladder, breast, small and non-small cell lung, head and neck cancer [32–35].

2. Materials and methods

2.1. Materials

HAP was purchased from Riedel – de Haen in the form of tri-calcium phosphate extra pure [$Ca_5(PO_4)_3OH$]. dFdU.HCl was used in the formulation of Gemzar (Eli Lilly and Company, USA). DACH-Pt was used in the formulation of Eloxatin (Sanofi – Aventis U.S. LLC). The drugs were kindly provided by Simeonideio Research Center of Theageneion Cancer Hospital of Thessaloniki, Greece. Platinum atomic absorption standard solution (1 mg/mL Pt in 10% HCl) was bought from Acros Organics. All the materials were stored at room temperature.

2.2. Methods

2.2.1. Adsorption of drugs by HAP

For the determination of the maximum amount of dFdU.HCl that can be adsorbed by HAP, Gemzar was dissolved in double deionized water (10 mL) by rapid mixing in glass flasks in order to produce solutions of various concentrations. 50 mg HAP were dispersed in each one of these solutions. The samples were shacked in a shaker for 48 h and filtered. In the filtrate, dFdU.HCl was determined. For the determination of the maximum amount of DACH-Pt that can be adsorbed by HAP, samples were prepared in the same way as above dissolving Eloxatin in double deionized water [36]. In the filtrate, Pt was determined.

For studding the kinetics of the adsorption, samples were prepared as described previously using as the initial concentration of dFdU.HCl or DACH-Pt, respectively, the one that corresponded the maximum adsorbed amount, determined [36]. Afterwards the samples were shacked and every eight or ten hours one of them was filtered. The dFdU.HCl or Pt, respectively was determined in the filtrate.

2.2.2. Release of drugs by HAP

The release profile was obtained by soaking the samples of loaded HAP in a 50-mL flask containing a solution simulating the inorganic composition of the body fluid (SBF) with the molar composition (mM) shown in Table 1 [37]. A ratio of 1 mL SBF per mg of adsorbed dFdU.HCl or DACH-Pt, respectively was used while the temperature was kept at 37 °C. After soaking at predetermined times, the SBF was collected by centrifugation and replaced with fresh one. The concentration of the released drug was determined in the supernatant obtained from each centrifugation.

2.2.3. Determination of drug concentration

The concentration of dFdU.HCl in the solutions was determined by high performance liquid chromatography using Thermo Electron Corporation Spectra System. The chromatographic separation was accomplished on an Econosphere C18 column (255 mm × 4.6 mm, *m* particle size). The mobile phase used was ammonium acetate (40 mM, pH 5.5)/acetonitrile (97.5:2.5). The detection wavelength was set at 268 nm and the flow rate was 1.5 mL/min. Data acquisition and processing was performed with ChromQuest 4.2 software.

The concentration of DACH-Pt in solutions was determined indirectly through the determination of the concentration of Pt

Table 1
Nominal ion concentrations of SBF in comparison with those in human blood plasma [37].

Ion	Ion concentrations (mM)	
	Blood plasma	SBF
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	103.0	147.8
HCO_3^-	27.0	4.2
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5
pH	7.2–7.4	7.4

in solutions by atomic absorption spectrometry with flame technique, using a Perkin-Elmer 503 spectrophotometer. Pt standards for calibration were prepared by dilution of stock solution.

2.2.4. Computational details

The optimized geometries of dFdU.HCl and DACH-Pt were obtained using B88PW91/DZVP and B3LYP/LANl2DZ, respectively, within the framework of DFT [37]. From the computed molecular wave functions the molecular electrostatic potential for each drug was calculated, whereas their respective interactions with HAP were determined through molecular docking techniques [38,39].

3. Results

The amounts of adsorbed dFdU.HCl and DACH-Pt by HAP as a function of the residual concentration at equilibrium are presented in Fig. 2. The adsorbed drug increases as its initial concentration increases until a maximum amount of 400 mg dFdU.HCl/g HAP and 49.1 mg DACH-Pt/g HAP is reached. In order to explain the adsorption's mechanism and the relationship between the adsorbed amount of drug (q, mg/g) and its equilibrium concentration (C_{eq}, mg/L) the collected data from our adsorption profile studies were evaluated using two different isotherms namely Langmuir and Freundlich. Using the pertinent equations (Table 2) we drew the relevant plots depicted in Fig. 2. Next, using the derived plots in combination with the correlation coefficients from the Langmuir and Freundlich equations, we observed that the adsorption of dFdU.HCl by HAP is described mathematically by the Freundlich isotherm (Fig. 2a). The adsorption isotherm plots of DACH-Pt by HAP (Fig. 2b) overlay each other inhibiting the accurate determination of an adsorption's mechanism model. Nevertheless, when the isotherm plots are converted to their linear form equivalents, the adsorption of DACH-Pt by HAP is shown to be described more accurately by the Freundlich isotherm (Fig. 3).

The adsorption kinetic of dFdU.HCl and DACH-Pt by HAP is presented in Fig. 4. The maximum adsorption value of DACH-Pt was achieved sooner compared to that of dFdU.HCl (20 h and 40 h, respectively).

We also performed DFT calculations for both cases of anticancer compounds' adsorption profiles, in order to gain

Table 2
Isotherms' equations and linear forms [40].

Isotherm	Relationship	Linear form
Langmuir	$q = \frac{QbC_{eq}}{1+bC_{eq}}$	$\frac{1}{q} = \frac{1}{Qb} \cdot \frac{1}{C_{eq}} + \frac{1}{Q}$
Freundlich	$q = K_F C_{eq}^{1/n}$	$\ln q = \ln K_F + 1/n\ C_{eq}$

q: amount adsorbed.
C_{eq}: final concentration after 48 h equilibration.
b, K_F, $1/n$: characteristics constants.

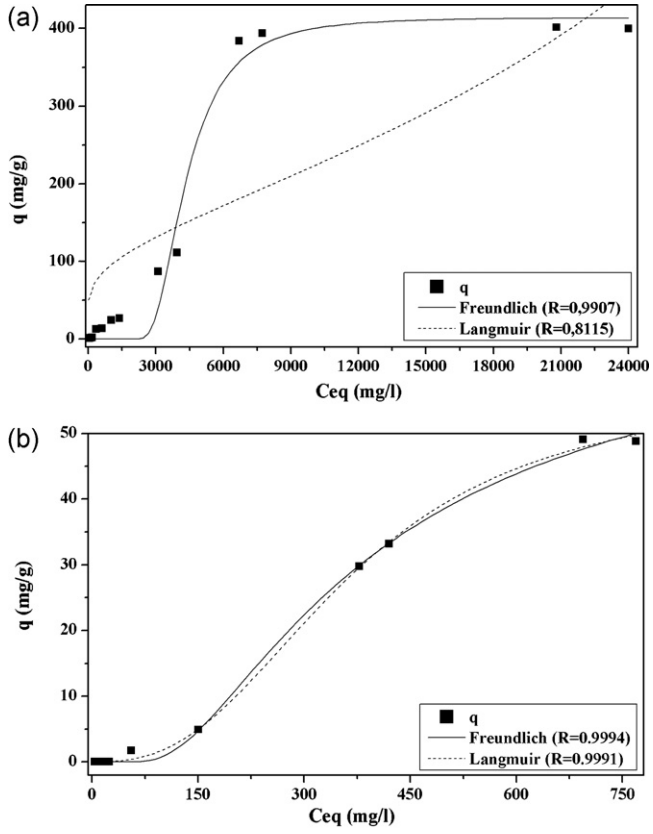

Fig. 2. Freundlich and Langmuir adsorption isotherms of (a) dFdU.HCl and (b) DACH-Pt by HAP (contact time: 48 h).

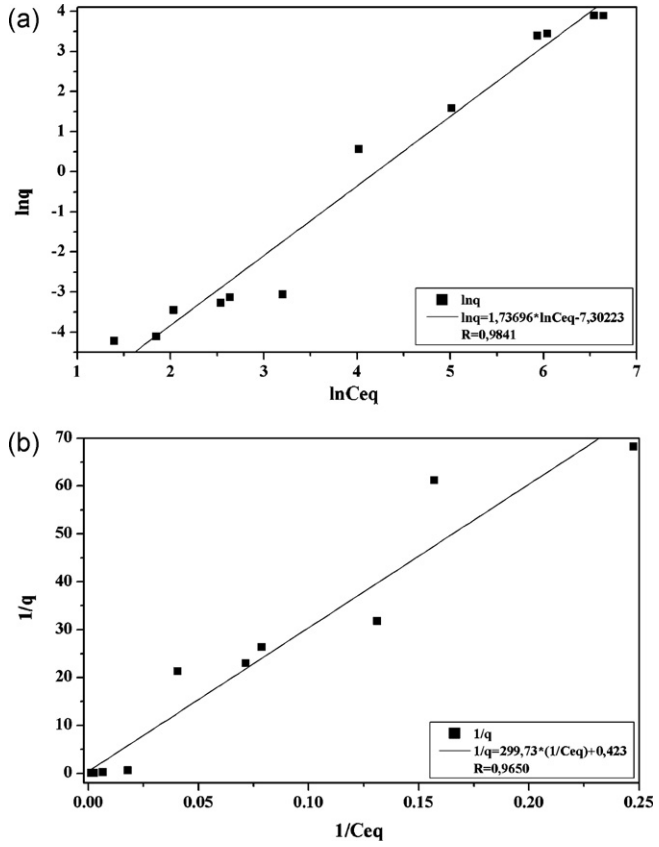

Fig. 3. Linear form of (a) Freundlich and (b) Langmuir adsorption isotherms of DACH-Pt by HAP (contact time: 48 h).

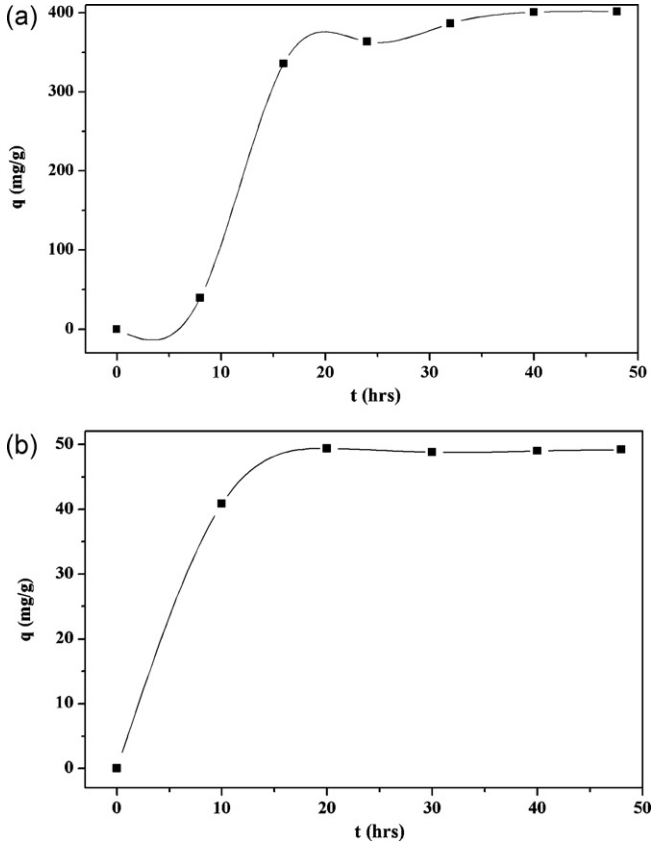

Fig. 4. Kinetic of adsorption of (a) dFdU.HCl and (b) DACH-Pt by HAP.

theoretical insight to the phenomenon. Their optimized geometries are shown in Fig. 5. The calculated energies are −1014.38 and −750.19 Hartrees for dFdU.HCl and DACH-Pt, respectively. In Fig. 5 is also depicted the calculated electrostatic potential on the molecular surface of the above compounds in kcal mol^{-1}. The values associated with the contours correspond to the interaction energies of a positive point charge with the charge distribution of the molecule. A strongly negative region (V_{min} in red) is characteristically related to electronegative atoms. (For interpretation of the references to color in text, the reader is referred to the web version of the article.) Thus, in dFdU.HCl the potential reaches its most negative value (−44.32 kcal mol^{-1}) near the nitrogen

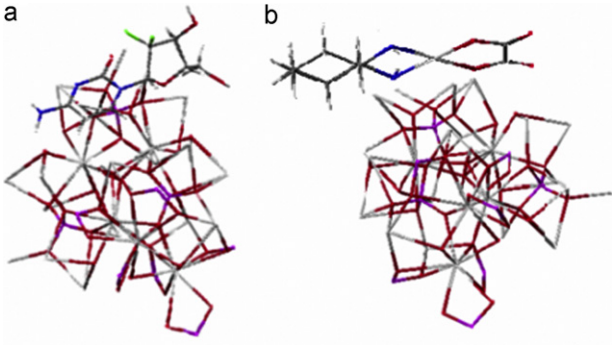

Fig. 6. Molecular long-range interactions of HAP with (a) dFdU.HCl and (b) DACH-Pt.

heteroatom and the carbonylic oxygen, whereas the most negative value (−105.11 kcal mol^{-1}) for DACH-Pt is located near oxalic oxygens. These values indicate the most favorable paths of approach of an electrophile to the molecules.

Hence, as shown in Fig. 6, the most electronegative part of the approaching molecules will seek to dock to an electropositive region of HAP, e.g. the Ca^{2+} ions.

The kinetics of the release of dFdU.HCl and DACH-Pt from the HAP in SBF are illustrated in Fig. 7. Note that the drugs are released from the HAP in different ways. After only 1 h 65% of dFdU.HCl was released in SBF. The rate of this release is decreased at 0.37%/8 h. Reversely, the release of DACH-Pt from the HAP is more gradual since 55% of DACH-Pt is released in the first 24 h. The rate of this release is shaded and abuts to 0.9%/8 h.

4. Discussion

Adsorption experiments showed that HAP adsorbed much more dFdU.HCl (400 mg/g) than DACH-Pt (49.1 mg/g). According to the isotherm models, the interactions of the drugs with HAP follow the Freundlich isotherm which describes the adsorption of solution components of dilute solutions to solid surfaces. Consequently, it is postulated that concerns multilayered adsorptions.

We performed theoretical calculations using the docking technique to examine if the drugs can or cannot be encapsulated inside the porous of HAP. The results showed that the

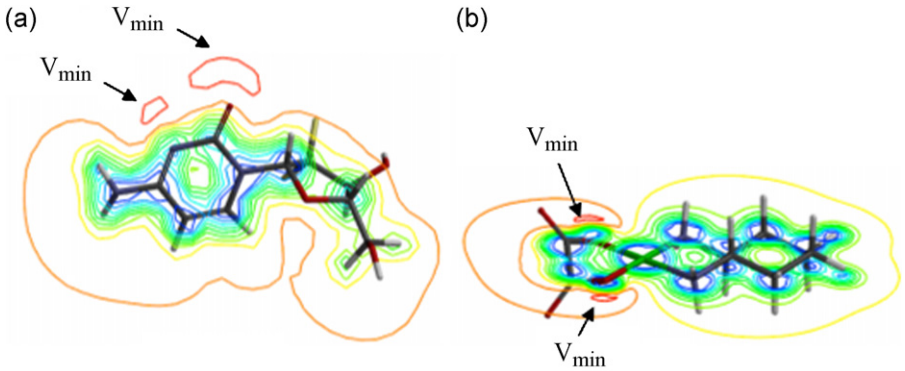

Fig. 5. Calculated electrostatic potential for the optimized structure of (a) dFdU.HCl and (b) DACH-Pt.

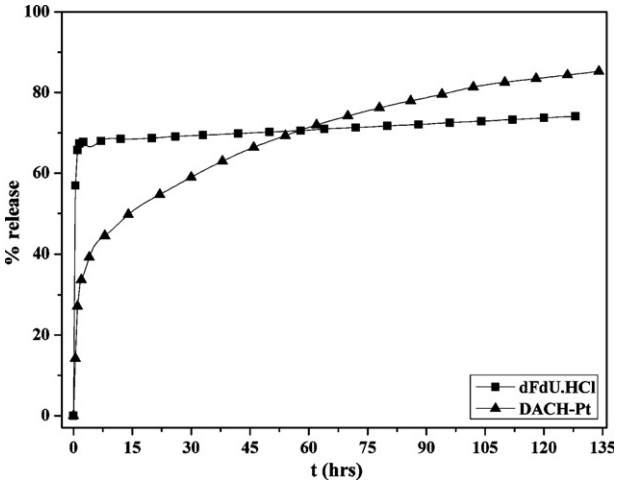

Fig. 7. In vitro release profile of dFdU.HCl and DACH-Pt: amount of drug delivered by HAP at pH 7.4 in SBF.

interactions take place on the surface, explaining the similar adsorption profiles. The different release profiles can be explained by the assumption that DACH-Pt's potential is more negative than dFdU.HCl's, so the attraction is stronger and DACH-Pt is being more normally released.

The release of dFdU.HCl follows a biphasic pattern with a burst phase, since approximately 65% is rapidly released in 1 h, followed by a diffusive slower phase. It is suggested that the high initial burst is due to the fact that dFdU.HCl is adsorbed on the surface of HAP facilitating its release. The release of DACH-Pt follows a more gradual and constant way, but also biphasic, with a much less dramatic burst phase. Indeed, within the first hour 27% of DACH-Pt is released followed by decreased inchmeal release rate that abuts to 0.9%/8 h. However, the entire quantity of DACH-Pt is expected to be released faster (265 h or ∼11 d) than that of dFdU.HCl (688 h or ∼28.5 d). The extra dFdU.HCl amount adsorbed justifies the longer release time.

The advised dose of each drug is 1000 mg dFdU.HCl/m^2 of body-surface area (BSA) every week for 3 weeks [41] and 85 mg DACH-Pt/m^2 BSA every 2 weeks for up to 7 weeks [34]. Drugs are administrated intravenously and dispersed throughout the entire body. Consequently, only a small amount of the active ingredient is expected to reach to the tumor, while the rest causes serious side effects on the human organism. Such side effects could be limited by the topical administration of the drug using a suitable DDS. The proper dose and release rate for the local drug's administration and the mode of this administration have to be determined by further research including in vivo experiments. The purpose of the current study was to investigate in vitro the adsorption and release profile of dFdU.HCl and DACH-Pt anticancer drugs by HAP. Deriving a correlation between the intravenously administered doses and the data from the in vitro experiments is rather challenging without data validation from in vivo experiments because the quantity and efficacy of the active ingredient that actually reaches to the tumor have not been determined accurately. However, the results from our studies presented here could inform the much needed further research on the development of an effective DDS. Additionally, there is a considerable lack of agreement and significant variation in the literature on estimates regarding the percentage of the anti-cancer drug administered that reaches the tumor.

5. Conclusions

The HAP form studied found to be able to be load and release dFdU.HCl and DACH-Pt in a way that could be possible used to develop DDSs'. In vivo experiments are needed for the determination of the suitable dose that ensures that such a DDS will not be toxic in various applications (implants, paste, etc.). Final target is to develop a HAP based porous implant, impregnated with dFdU.HCl or DACH-Pt, which will act also as a DDS.

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