

# Transport of Molecular Iodine From Antiseptic Iodophors across Hydrophilic–lipophilic Interfaces: Influence of Phospholipids

Maggie Engert, Christopher Moses, Jack Kessler, and Ilker S. Bayer\*



Cite This: <https://doi.org/10.1021/acsomega.5c06724>



Read Online

ACCESS |



Metrics & More

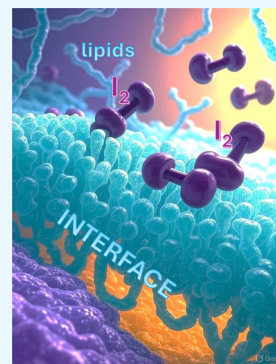


Article Recommendations



Supporting Information

**ABSTRACT:** The release and transport of molecular iodine ( $I_2$ ) from antiseptic iodophors across hydrophilic–lipophilic interfaces were studied to understand the impact of carrier polymers and phospholipids on release and transport of molecular iodine across biologically relevant interfaces. Five commercial povidone-iodine formulations and one modified dextrin-iodine complex, each standardized to 1% thiosulfate titratable iodine, were tested using a heptane–water interface model. Iodine release was quantified by both UV–vis spectroscopy and thiosulfate titration with and without phospholipid-rich lipophilic phase. Dilution significantly increased  $I_2$  release, with dextrin-iodine achieving near-complete transfer at higher dilutions due to reduced polymer-iodine binding. Phospholipids enhanced iodine transport via charge-transfer interactions, with release kinetics well-fitted by Weibull and Michaelis–Menten models ( $R^2 > 0.988$ ). For aqueous iodophors, a dilution ratio of 8–10 fold is optimal, enabling dextrin-iodine to attain rapid 100% iodine release while povidone-iodine formulations released 40.3–68.1% of their iodine content in the presence of phospholipids. The active biocide in iodophors is molecular iodine and these findings highlight the critical role of iodophor carrier/composition and lipid interactions in maximizing iodine delivery, offering insight for developing next-generation antiseptic formulations.



## 1. INTRODUCTION

Molecular iodine ( $I_2$ ) is a highly effective antimicrobial agent, widely used in antiseptics due to its broad-spectrum and rapid activity.<sup>1–6</sup> Its potency stems from reacting with various molecular targets, including aromatic groups in tyrosine and imidazole, double bonds in lipids, cytosine and uracil iodination, and sulphydryl group oxidation in proteins.<sup>7–10</sup> This multitarget action disrupts pathogens' cellular machinery, enabling rapid microbial death across bacteria, viruses, fungi, and resistant spores.  $I_2$ 's fast action, seconds to minutes, and ability to prevent resistant bacteria formation make it a reliable antiseptic.<sup>11–14</sup> It has been widely assumed that  $I_2$  is the primary agent responsible for staining and cytotoxicity observed with aqueous iodine products such as PVPI and Lugol's Solution.<sup>6</sup> Recent observations indicated that  $I_2$  may not be the direct cause of skin staining or irritation, which could indicate that other ingredients present in topical iodine disinfectants might contribute to these adverse characteristics. These observations warrant further investigation to better understand the mechanisms underlying the negative effects associated with iodine-based antiseptic formulations.<sup>12,13</sup>

The active biocide in all iodophors is unbound  $I_2$  which is in equilibrium with other iodine species, e.g. iodide and triiodide and carrier polymers.<sup>23,24</sup> A key challenge in iodine-based formulations has been the limited water solubility of  $I_2$  (~0.03 wt % at room temperature), which historically restricted its therapeutic use in aqueous solutions.<sup>16,17</sup> Early attempts to improve solubility relied on alcohol-based solvents or high iodide concentrations, but these approaches often exacerbated

irritation, limiting their applicability.<sup>18–22</sup> The Food and Drug Administration (FDA) monograph on consumer antiseptic wash ingredients lists iodophors by their carrier polymer, e.g., ammonium lauryl ether sulfate, polyoxyethylene compounds, poloxomers, and polyvinylpyrrolidone.<sup>25,26</sup> The FDA concludes that different iodophor formulations exhibit distinct clinical efficacy profiles based on their evaluation of published clinical data. While complexation of  $I_2$  with carrier polymers enhances iodine solubility, this approach may potentially compromise the release or bioavailability of biocidal molecular iodine, as such formulations appear to result in substantially reduced free  $I_2$  concentrations (0.5–6 ppm).<sup>15</sup> The relationship between polymer complexation and the maintenance of effective antimicrobial iodine levels merits further study.

Iodophors mitigate certain limitations of topical iodine antiseptics using carrier polymers. However, this approach introduces potential performance challenges,<sup>28,29</sup> as their antimicrobial activity depends exclusively on unbound iodine ( $I_2$ ), which is maintained at a low concentration to ensure stability. Once the initial  $I_2$  is depleted, additional iodine must dissociate from the carrier polymer, resulting in a delayed

**Received:** July 10, 2025

**Revised:** November 6, 2025

**Accepted:** November 13, 2025

**Table 1. List of Commercially Available Antiseptics and Their Properties Including Inactive Ingredients**

| product                       | manufacturer    | % PVPI | % iodine | % titrated iodine | pH   | buffer | inactive ingredients                                                                                                     | state  | sample |
|-------------------------------|-----------------|--------|----------|-------------------|------|--------|--------------------------------------------------------------------------------------------------------------------------|--------|--------|
| skin prep solution            | McKesson        | 10.0   | 1.0      | 0.96              | 4.54 | Yes    | citric acid, disodium phosphate, glycerin, water, sodium citrate, Tween 80                                               | liquid | 1      |
| povidone–iodine prep solution | Dynarex         | 10.0   | 1.0      | 0.97              | 4.00 | Yes    | disodium phosphate, glycerin, citric acid, sodium citrate, Tween 80, water                                               | liquid | 2      |
| povidone–iodine swabsticks    | Dukal           | 10.0   | 1.0      | 1.2               | 3.64 | Yes    | alkyl glucoside, citric acid, glycerin, hydroxy ethyl cellulose, nonoxynol-10, potassium iodide, water, sodium hydroxide | liquid | 3      |
| Povidone–iodine swabsticks    | PDI             | 10.0   | 1.0      | 1.1               | 2.80 | ?      | water, sodium hydroxide                                                                                                  | liquid | 4      |
| iodosorb cadexomer-iodine     | Smith & Nephew  | n/a    | 0.9      | 0.86              | 2.88 | No     | none                                                                                                                     | powder | 5      |
| povadyne antiseptic           | Millipore Sigma | 10.0   | 1.0      | 0.97              | 2.90 | No     | none                                                                                                                     | powder | 6      |
| dilute Lugol's solution       | Thermo Fisher   | n/a    | 1.0      | 0.97              | 3.5  | No     | potassium iodide                                                                                                         | liquid | 7      |

release mechanism comparable to that of time-release pharmaceuticals.<sup>29</sup> In 10% povidone-iodine (widely used antiseptic), ~60% of iodine is triiodide or complexed with polyvinylpyrrolidone, ~40% is iodide ions, and only ~0.1% is free molecular iodine, the active antimicrobial agent.<sup>28,29</sup> Thus, more than 99% of the iodine in povidone-iodine (PVPI) exists in an inactive, complexed form. The sole analytical data typically provided on iodophor labels is a thiosulfate titration, which quantifies the combined levels of free iodine ( $I_2$ ), hypiodous acid (HOI), and triiodide ( $I_3^-$ ). Crucially, no information is available regarding the concentration of unbound  $I_2$  or the kinetic and thermodynamic release profile of  $I_2$  during clinical application.<sup>27</sup> This limitation applies universally to all commercial iodophor formulations.

Recent developments have sought to address this fundamental limitation through innovative approaches.<sup>30</sup> Researchers have developed advanced iodophor formulations designed to increase free iodine availability.<sup>31</sup> One notable advancement is modified dextrin-iodine, a polysaccharide-based iodophor that offers improved iodine release characteristics compared to traditional povidone-iodine formulations.<sup>32,33</sup> Another approach involves the strategic dilution of standard 1% available iodine povidone-iodine solutions immediately before use.<sup>34,35</sup> These “on-site dilutions” can increase free molecular iodine concentrations depending on the dilution ratio employed.<sup>35</sup> While original concentrated solutions typically contain only 1–5 ppm of free molecular iodine, properly diluted versions can achieve levels as high as 25 ppm.<sup>34,35</sup> These diluted preparations come with important practical constraints.<sup>36,37</sup> The enhanced formulations are inherently unstable and must be used rapidly after preparation. This instability necessitates their preparation and application during surgical procedures rather than allowing for prepreparation and storage. The term “on-site dilutions” reflects this requirement for immediate use following dilution to maintain their enhanced antimicrobial effectiveness.<sup>36,37</sup>

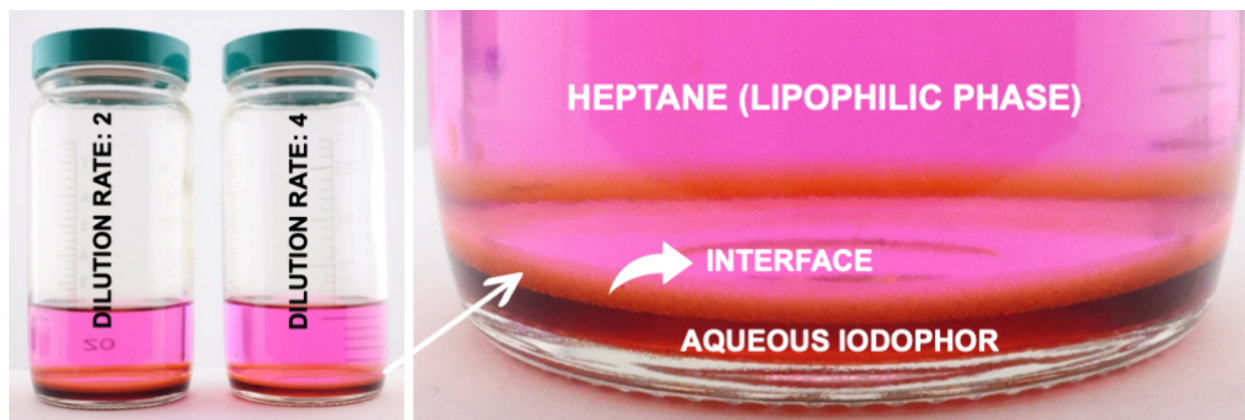
Lipophilic drug transport (such as molecular iodine) across hydrophilic–lipophilic interfaces, such as cell membranes, is predominantly governed by passive diffusion driven by the concentration gradient. This process is directly influenced by a drug's lipophilicity; a higher lipophilicity means a higher propensity for the drug to partition into and traverse the lipid bilayer. Across such interfaces, other forms of transport can

also be possible such as carrier-mediated transport in which specialized vehicles can facilitate the transport of molecules across the membrane, or active transport in which drugs can be transported against their concentration gradient and finally, facilitated diffusion, which is a passive process that uses a carrier but does not require energy. Additionally, a process known as membrane retention can lead to highly lipophilic drugs to get trapped or retained within the lipid bilayer, which can slow down their overall transport and affect their bioavailability. Although more studies are needed it is believed that lipophilic molecular iodine can transport through hydrophilic–lipophilic interfaces through concentration gradients and the extent of this transport may be controlled by the polymer-complexation states in the hydrophilic phases.

In this study, we experimentally investigated the transport of molecular iodine across a hydrophilic–lipophilic liquid interface from five povidone-iodine and one polysaccharide-based iodophor. The oil–water interface was established by combining excess heptane with various commercial aqueous povidone-iodine solutions and a commercial cadexomer-iodine at different dilution ratios. Additionally, the lipophilic phase was modified by dissolving up to 50% of soy lecithin, a phospholipid, and its impact on iodine transport across the interface was examined. We observed that the release and transport of molecular iodine across the hydrophilic–lipophilic interface is influenced by iodophor chemistry (carrier polymer) and dilution ratios. Higher release and transport of unbound molecular iodine correlate with enhanced antibacterial efficacy of the formulation. The incorporation of phospholipids augmented iodine transport, with molecular iodine immediately reacting with the lipids.<sup>38,39</sup> As a simplified model, these experiments suggest that specific dilution levels of povidone-iodine facilitate significantly greater transport of free molecular iodine across such interfaces compared to standard 10% solutions with 1% titratable iodine levels, potentially enhancing the antibacterial efficacy via disruption of the interface lipid morphology and chemistry.

## 2. MATERIALS AND METHODS

**2.1. Materials.** Seven commercial products were evaluated in this investigation. Six formulations were iodophor-based antiseptics, while one was a dilute Lugol's iodine solution (1%



**Figure 1.** Left: two sealed glass jars containing 5 g of the sample 1 iodophor (10% PVPI) with dilution ratios of 2 and 4 (dark orange bottom phase) and 50 g of heptane (purple top oil phase) and the formation of interfaces. Right: a magnified photograph of the interface formed between the diluted iodophor and heptane. The interfacial film is clearly visible.

w/v), all maintaining equivalent titratable iodine concentrations of 1%. All solutions were obtained directly from their respective manufacturers and utilized without further modification. Serial dilutions were prepared using deionized water. The commercial products examined in this study, including their inactive ingredients and buffering systems where applicable, are detailed in Table 1. Reagent grade heptane and 0.1, 0.01, and 0.001 N sodium thiosulfate solutions were purchased from Thermo Fisher Scientific, USA and used as received. Soybean lecithin mainly comprising phosphatidylcholine and phosphatidylethanolamine was purchased from Spectrum, USA and used as received. Starch indicator solution 1%, w/v aqueous solution (for iodometric titrations), was purchased from Thermo Fisher Scientific, USA and used as received.

Among the iodophor formulations, skin preparation and surgical scrub solutions (samples 1 and 2) contained 10% aqueous povidone-iodine with one percent titratable iodine. The Povadyne antiseptic (sample 6) consisted of PVPI powder that was dissolved in deionized water to achieve a 10% solution and subsequently titrated with 0.1 N thiosulfate solution to confirm 1% titratable iodine content.

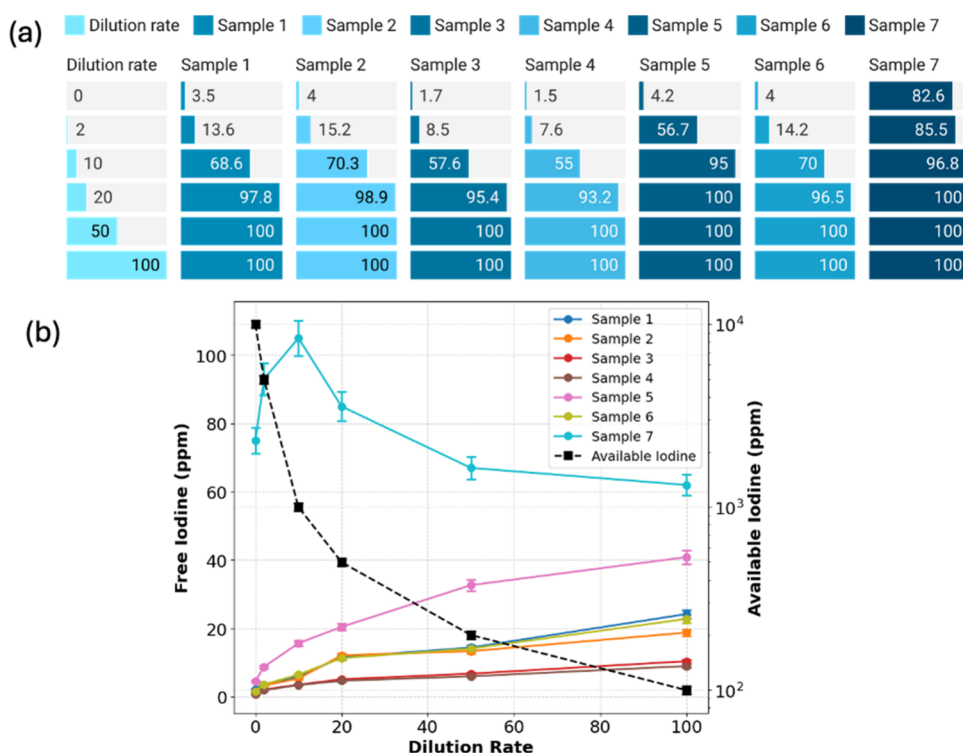
This solution was prepared to eliminate potential interference from buffering agents or other inactive ingredients in commercial products that could affect drug release dynamics. Two commercial swabstick products saturated with 10% povidone-iodine solution were also evaluated as samples 4 and 5 in Table 1. These consisted of cotton swab applicators immersed in PVPI solutions and contained within sealed, medical-grade pouches. Sample 5 was a powder dextran-based iodine, comprising 0.9% iodine bound to a water-soluble polysaccharide complex, developed as wound dressing for exudate absorption. In contrast to polyvinylpyrrolidone polymer, this compound exhibited limited water solubility. To maintain homogeneity in the prepared solutions containing sample 5, continuous stirring was required throughout the measurement process. Finally, a dilute Lugol's solution containing 1% titratable iodine in water, stabilized with potassium iodide, served as the control solution. This formulation contained no iodophors.

**2.2. Methods.** A hydrophilic–lipophilic interface was created by mixing 5 g of aqueous iodophor solution with 50 g of heptane, with the heptane (oil phase) deliberately used in excess to serve as a large reservoir for molecular iodine

diffusion across the interface. The sink condition was also tested by replacing iodine dissolved heptane solutions with a fresh medium after equilibrium to ensure that no sink conditions play a role in molecular iodine transport. To investigate the impact of PVPI concentration on iodine release and transport, at least five dilution ratios (1:2, 1:10, 1:20, 1:50, and 1:100) were prepared using deionized water. The iodine released into the heptane was quantified by sampling 2–3 mL of the solution and measuring its absorbance at 520 nm using UV–vis spectroscopy. Figure 1 illustrates an example of the combined phases for two different dilution ratios of sample 1, as well as the creation of the interface that allows molecular iodine to move into the lipophilic phase, resulting in a distinct purple color. Additionally, the aqueous phase was sampled to determine its titratable and free iodine content. The impact of mixing on the rate and final equilibrium concentration of iodine released at the interface was also investigated utilizing a Roto-Shake Genie, USA rotator/rocker operating at rotation speeds ranging from 5 to 35 rpm (see Supporting Information).

**2.3. Thiosulfate (Available Iodine) and Potentiometric (Free Iodine) Titrations.** PVPI solutions were prepared at varying dilution ratios as mentioned earlier. Each sample was prepared to a final volume of 10.00 mL in a clean volumetric flask. The sample solutions were titrated immediately with standardized sodium thiosulfate solution. For concentrated samples ( $\geq 25\%$  dilution), 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$  was used, while for dilute samples ( $< 25\%$ ), 0.01 or 0.001 N  $\text{Na}_2\text{S}_2\text{O}_3$  was employed. Titration was conducted with continuous swirling until the solution became pale yellow. At this point, 1–2 mL of freshly prepared starch indicator was added resulting in a deep blue coloration. Titration was continued dropwise until the blue color disappeared completely, indicating the end point. All titrations were performed in triplicate. A reagent blank (containing all reagents except PVPI) was also titrated and its value subtracted from sample readings where necessary.

The free molecular iodine concentration of the iodophors was assessed using a potentiometric method based on the protocol described by Gottardi and Nagl<sup>40</sup> and will not be repeated here for brevity. All measurements were conducted at room temperature using a Hanna Instruments, USA, pH/mV/ISE meter. A three-electrode potentiometric system was employed, comprising a platinum redox electrode, an iodide-selective electrode, and an Ag/AgCl electrode as the reference.



**Figure 2.** (a) Equilibrium partitioning of iodine into the oil phase: the mean ( $N = 6$ ) percentage of thiosulfate titratable iodine released into the oil phase at equilibrium is shown as a function of dilution ratio. (b) Free molecular iodine ( $I_2$ ) dynamics: changes in the concentration of free  $I_2$  in the oil phase are plotted against dilution ratio. Note that concentration is expressed in parts per million (ppm) which is equivalent to milligrams per liter (mg/L).

Electrodes were calibrated prior to use with standard iodide and iodine solutions to ensure accuracy. Aliquots of commercial iodophor solutions were diluted appropriately with deionized water to bring the analyte concentrations within the optimal detection range of the electrodes. Care was taken to avoid exposure to light and heat during preparation to minimize iodine degradation. Each sample was subjected to two sequential potentiometric measurements. The potential difference between the platinum electrode and the reference electrode was recorded to determine the redox-active iodine species (primarily  $I_2$ ). Electrode potentials were converted to free iodine ( $I_2$ ) concentrations using the Nernst equation and calibration data.

**2.4. Measurement of Released Iodine.** Iodine dissolved in heptane exhibits characteristic UV–vis absorption features due to its electronic transitions. A strong absorption band in the visible region ( $\sim 500$ – $600$  nm), typically appearing violet or purple, due to the charge-transfer transitions involving the  $I_2$  molecule. In nonpolar solvents like heptane, iodine ( $I_2$ ) exists as discrete molecules, leading to well-defined absorption bands without significant solvent shifts. The visible absorption band is typically centered around  $\sim 520$ – $540$  nm (molar absorptivity  $\epsilon \approx \sim 1000$  L mol $^{-1}$  cm $^{-1}$ ).<sup>41–43</sup> The strength of the absorption band is directly related to the concentration of dissolved  $I_2$  in heptane. A calibration curve was created by dissolving iodine at different concentrations (10–100 ppm) and measuring their absorption intensity at 520 nm, which facilitated the determination of the quantity of iodine released from each aqueous solution at the interface.

To investigate the impact of phospholipid on the rate of iodine diffusion, heptane was saturated with lecithin by creating a 50% by weight solution of lecithin in heptane.

The solutions were prepared by simply blending and dispersing the lecithin powder in heptane under continuous mixing, and within half an hour, the solids were completely dissolved. Unfortunately, the characteristic purple color of iodine was no longer visible, and the only method to assess iodine transport was to titrate the aqueous phase using thiosulfate at a concentration of 0.001 N by withdrawing aliquots with a HandyStep S repetitive pipet. In certain experiments, specifically those involving diffusion into pure heptane, measurements were periodically conducted to corroborate spectroscopic data, thereby ensuring result accuracy. Additionally, a high-sensitivity iodine colorimeter (Hanna Instruments, USA) was utilized to quantify iodine concentrations in aqueous iodophor solutions at dilution ratios greater than 20. The colorimeter employs the DPD colorimetric method, which involves the reaction of iodine with N,N-diethyl-p-phenylenediamine (DPD) to produce a pink coloration. The intensity of this color, directly proportional to the total iodine concentration, is measured at a wavelength of approximately 530 nm. This method is suitable for iodine concentration range of 0–12.5 mg/L (ppm). The iodine released into the lipid-rich oil phase was calculated as the difference between the initial titratable iodine concentration and the residual aqueous-phase iodine concentration measured at the specified time interval.

**2.5. Drug Release Measurements.** The diffusion of free molecular iodine from the aqueous polymer phase into the lipophilic phase was quantitatively assessed at various dilution levels, with measurements conducted at distinct time intervals. This involved extracting 2–3 mL aliquots from the lipophilic phase and promptly measuring their absorbance at 530 nm in heptane. For experiments involving a phospholipid-saturated lipophilic phase, 1 mL aliquots were withdrawn from the

aqueous phase using a HandyStep S repetitive pipet and titrated with a 0.001 N sodium thiosulfate solution. Each measurement was replicated three times to ensure precision. The percentage of iodine released was determined by comparing the iodine diffused or lost from the iodophor solution against the initial titratable iodine concentration, with results graphically represented as a function of time.

To characterize the drug release kinetics, *in vitro* drug release data, expressed as cumulative percentage release over time, were fitted to mathematical models. The following kinetic models were evaluated: Weibull, Michaelis–Menten and Hill which were identified based on Goodness-of-fit  $R^2$  values and RMSE (Root Mean Squared Error). Model fitting was performed using nonlinear regression in Python (version 3.9) with the SciPy library to minimize the residual sum of squares between experimental and predicted values. [Supporting Information](#) displays all three experiments and data as well as models fitted to each set separately along with standard deviation statistics.

### 3. RESULTS AND DISCUSSION

Iodine penetrates the cell wall, reacting with proteins, lipids, and nucleic acids, leading to denaturation and loss of membrane permeability. In Gram-positive bacteria, iodine targets the thick peptidoglycan layer, while in Gram-negative bacteria, it affects both the outer membrane and thinner peptidoglycan layer, causing leakage and cell death. This broad-spectrum activity makes iodine effective against various pathogens.<sup>44–47</sup> The diffusion of molecular iodine into human skin and subsequent outgassing for hours has been contemplated to result from the partitioning of  $I_2$  into lipid-containing adipocyte cells.<sup>48</sup> Given that iodophors are widely used in many different health care settings,<sup>49</sup> it is essential to understand the percentage of available iodine that can release from various iodophor formulations and penetrate hydrophilic–lipophilic interfaces, since this directly determines antiseptic effectiveness.

**3.1. Effect of Dilution Rate Without Lipids.** [Figure 2a](#) presents the percentage of iodine released from various commercial products (listed in [Table 1](#)), including Lugol's solution (sample 7 a noniodophor), as a function of dilution ratio following establishment of an interface between the aqueous iodophor formulations and the lipophilic phase (heptane). Equilibrium levels were reached within 5 h of interface establishment. At zero dilution rate, all iodophors released approximately 4% of their available or titratable iodine, whereas Lugol's solution released more than 80%. Lugol's solution is based on the formation of the triiodide ion ( $I_3^-$ ) through the equilibrium reaction  $I_2 + I^- \rightarrow I_3^-$ , where iodide ions act as electron pair donors to form coordination bonds with elemental iodine molecules.<sup>50–52</sup> The data indicates that the iodophor polymer complexes iodine strongly in water compared to aqueous iodine solutions stabilized with iodides such as Lugol's solution.

Coordination bonds are generally weaker than typical covalent bonds (where two atoms share electrons) and ionic bonds (which involve the complete transfer of electrons from one atom to another), since both atoms contribute electrons, leading to a more stable, stronger bond due to the effective overlap of atomic orbitals.<sup>53,54</sup> This complexation significantly enhances iodine's solubility from its naturally low aqueous solubility ( $\sim 0.3$  mM) to therapeutically effective levels, while potentially allowing iodine to more easily transfer to the

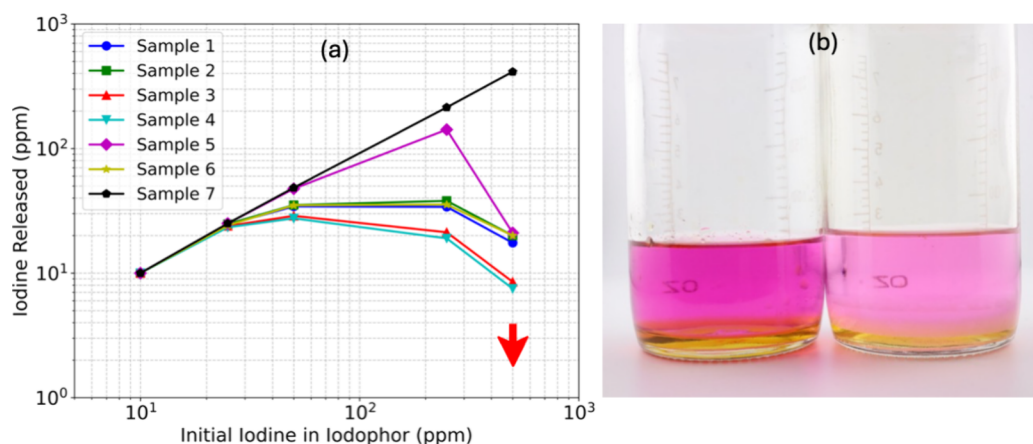
lipophilic phase.<sup>55,56</sup> Unlike simple ionic compounds like table salt (NaCl), iodophors such as Povidone-iodine form a complex involving ionic interactions between charged species (protonated PVP and triiodide anions), covalent bonds within the molecules, and hydrogen bonds between polymer units and surrounding water.<sup>57</sup> The ionically bound triiodide is essential for the controlled release of active iodine, but its stronger bond compared to coordination bonding in Lugol's solution prevents a rapid release of molecular iodine, as observed in Sample 7 in [Figure 2a](#).

Titratable iodine levels in iodophor solutions decrease with increasing dilution as expected; however, the percentage of  $I_2$  released rises significantly ([Figure 2a](#)) with dilution. This effect is more pronounced in the polysaccharide-based iodophor (Sample 5) compared to the polyvinylpyrrolidone iodophors. The dextrin based cadexomer polymer (cross-linked dextrin) iodine complex is significantly different than the povidone-iodine complex. The dextrin-iodine is an iodophor where iodine is physically encapsulated within a polysaccharide matrix. Its structure consists of a helical polysaccharide backbone that traps linear polyiodide chains within its hollow, hydrophobic core. This is the same basic inclusion complexation mechanism seen in the starch-iodine reactions. Note that, in aqueous solution, molecular iodine forms only a very weak donor–acceptor interaction with water through an  $O \rightarrow I$  charge-transfer mechanism, which accounts for its limited solubility. In contrast, dextrin provides an electron-rich environment composed of glycosidic oxygen atoms and hydroxyl groups that can stabilize iodine and polyiodide species through donor–acceptor and polarization effects, in addition to hydrophobic inclusion within the helical cavities. Upon exposure to water or wound exudate, the polymer swells and releases physically trapped iodine. Iodine in PVP forms a charge-transfer or halogen-bonding complex with the carbonyl groups of the polymer. These interactions involve partial charge transfer and molecular orbital overlap.

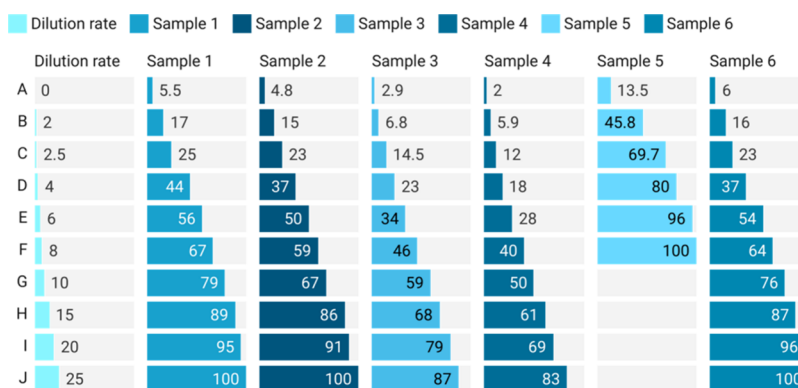
Specifically, at dilution ratios above 10, nearly all available iodine transfers to the lipophilic phase across the interface. This observation may be attributed to significant changes in bonding rearrangement in the aqueous phase. As polymer concentration decreases due to higher water percentages upon dilution, the strong intermolecular hydrogen bonding capacity of water may disrupt the ionic and hydrogen bonds between the iodide-polymer and polymer–polymer species, facilitating greater iodine mobility to diffuse across the interface.

The results presented in [Figure 2a](#) represent averages from six measurements, incorporating mechanical mixing (5 to 35 rpm) to ensure that equilibrium outcomes are unaffected by interface disruption due to stirring/mixing. Detailed data tables and statistical significance, determined using the two-way ANOVA method, are provided in the [Supporting Information](#). The two-way ANOVA confirms that dilution ratio significantly affected iodine release across the samples ( $p < 0.001$ ), with higher dilutions leading to greater release. Mixing speed (rpm) had no significant effect ( $p > 0.47$ ), and there is no significant interaction ( $p > 0.96$ ), indicating that mechanical mixing ensures equilibrium without influencing results.

[Figure 2b](#) shows the free molecular iodine levels in each solution as a function of dilution levels. As the dilution ratio of povidone-iodine (PVPI) solutions increases (from 0 to 100-fold, reducing available iodine from 10,000 ppm to 100 ppm), the free molecular iodine ( $I_2$ ) concentration generally increases for most samples (1–6), peaking at the highest dilution (100



**Figure 3.** (a) Mean ( $N = 6$ ) amount of iodine released in mg/L or ppm from each iodophor sample as a function of total titratable iodine in each sample. The red arrow indicates the amount of available (titratable) iodine in each sample before dilution. (b) Equilibrium iodine levels in the lipophilic phase. The bottle on the left depicts lower amount of iodine release from samples 3 and 4 compared to other PVP iodophors (excluding sample 5).



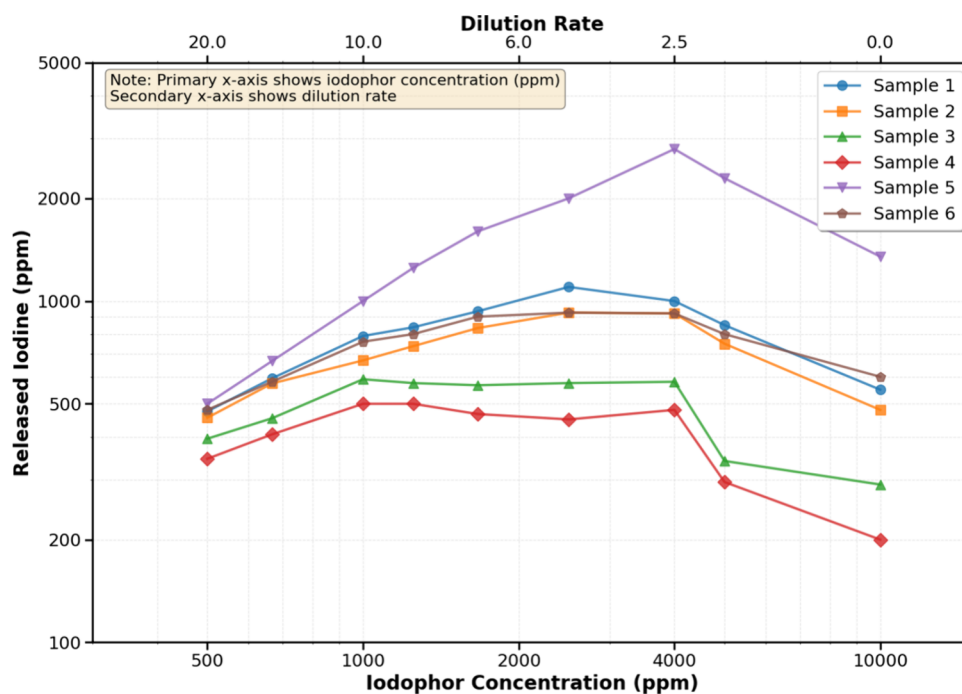
**Figure 4.** Effect of dissolved phospholipids in heptane (lipophilic phase) on molecular iodine transport from iodophors across the interface as a function of dilution ratio. The Lugol's solution is not shown since it is not an iodophor. Percent iodine released from each sample. Initial iodine concentration is 10,000 ppm in each sample.

ppm available iodine) with values ranging from 8.98 to 40.87 ppm. Sample 7 (Lugol's solution, not an iodophor), however, shows a peak free  $I_2$  of 105 ppm at 1000 ppm available iodine (10-fold dilution), decreasing to 62 ppm at dilution ratio of 100. This indicates that free  $I_2$  initially rises with dilution due to reduced povidone binding, but in Sample 7, it declines at higher dilutions due to limited total iodine or conversion of iodine to other iodide species.

Figure 3a shows amount of iodine released at equilibrium with respect to the available iodine at each dilution level. For instance, in the absence of dilution, a 5 g iodophor sample (i.e., Sample 1) contains 0.05 g of available iodine (500 ppm, denoted by the red arrow), with 4% (0.002 g or 20 ppm) partitioning into heptane. At a dilution factor of 10, the available iodine decreases to 0.005 g, yet approximately 69% (0.00345 g or 34.5 ppm) transfers to the heptane phase. Thus, although dilution reduces the total available iodine in the iodophor, it significantly enhances the transfer of free iodine to the lipophilic phase across all tested samples. Notably, with the exception of Lugol's solution, all povidone-iodine samples exhibit increased iodine release at dilution ratios of 2, 10, and 50. In contrast, modified dextrin-iodine complex appears to rapidly release molecular iodine at a dilution ratio of 2, which may not facilitate the controlled iodine release that could be achieved through successive dilutions, potentially requiring

replacement for further controlled release. Figure 3b presents photographic evidence comparing Sample 3 (right) and Sample 2 (left) in their respective containers. The visual comparison clearly demonstrates that Samples 3 and 4 exhibit significantly reduced free iodine release across the hydrophilic–lipophilic interface at equilibrium conditions. This lower release behavior persists consistently across all tested dilution ratios (0–50), indicating fundamental differences in their interfacial transport properties compared to the other samples. Dilution of polyvinylpyrrolidone-iodine (PVP- $I_2$ ) solutions can weaken the complexation between the polymer and molecular iodine, facilitating the transport of iodine through a lipidic membrane or interface. While more research is needed, it is understood that other factors can hinder this process, including the polymer's molecular weight, pH buffering conditions, and interactions between iodine and inactive ingredients that vary significantly across the board in commercial products. This can result in some complexed iodine molecules remaining in the hydrophilic aqueous phase, as shown in this study.

**3.2. Effect of Dilution Rate with Phospholipids.** Figure 4 presents the equilibrium levels of iodine released from each sample into the lipophilic phase, saturated with 50% lecithin. The presence of phospholipids was found to significantly



**Figure 5.** Amount of iodine released in mg/L or ppm from each iodophor sample as a function total titratable iodine in each sample when heptane (the lipophilic phase) is saturated with 50 wt % soybean lecithin. The Lugol's solution was not tested herein since it is not an iodophor. See Section 2.4 for measurement details.

enhance the transport of free molecular iodine from the iodophors, limiting the study to dilution ratios up to 25.

Figure 5, analogous to Figure 3, depicts the impact of dilution on iodine release from iodophor samples, expressed as a function of decreasing iodine content. The cadexomer-iodine complex demonstrates the highest iodine release profile with diminishing titratable iodine levels in the iodophors. At dilution factors of 2.5 and 4, corresponding to iodophor iodine concentrations of 200–250 ppm, the iodine release reaches a maximum before gradually decreasing with further dilution, consistent with the pattern observed in Figure 3 in the absence of phospholipids.

The presence of phospholipids in a lipophilic solvent like heptane significantly enhances the diffusion of free iodine from iodophors at lower dilution ratios. This effect is likely due to the interaction between molecular iodine and lipid molecules. As iodine reacts with these lipids, the concentration of dissolved iodine decreases, inducing greater iodine release from the aqueous iodophor phase. Evidence suggests the existence of a charge-transfer mechanism between iodine and phospholipids. Notably, when iodine was introduced to aqueous media containing lipid bilayers (such as egg lecithin or oxidized cholesterol), the conductivity at the bilayer interface increased by a factor exceeding  $10^4$ , further supporting strong interactions between lipids and iodine.<sup>58</sup> Recent molecular simulations examining the complexation and release dynamics of iodine within PVPI systems have also validated that certain synthetic polymers, biological proteins, and phospholipids possess the ability to extract iodine molecules from PVPI solutions.<sup>59</sup> Bacteria easily adhere to iodine molecules due to the presence of abundant proteins and phospholipids in their cell walls.<sup>59,60</sup> The work by Xu and Guan showed<sup>57</sup> that gelatin and lecithin exhibited a significant capacity to bind iodine molecules. When a bacterial cell wall is attached to a substantial quantity of iodine molecules, its

hydrophilicity and permeability are likely to diminish, potentially impacting the bacteria's normal metabolic processes. Results presented in Figures 3 and 5 indicate that diluting povidone-iodine formulations to achieve titratable iodine concentrations of 500–3500 ppm optimizes the release of free molecular iodine at hydrophilic–lipophilic biological interfaces. Furthermore, in the presence of phospholipid structures, such as those found in bacterial cell colonies, iodine transport to these structures is enhanced, potentially leading to disruption of the cell walls.

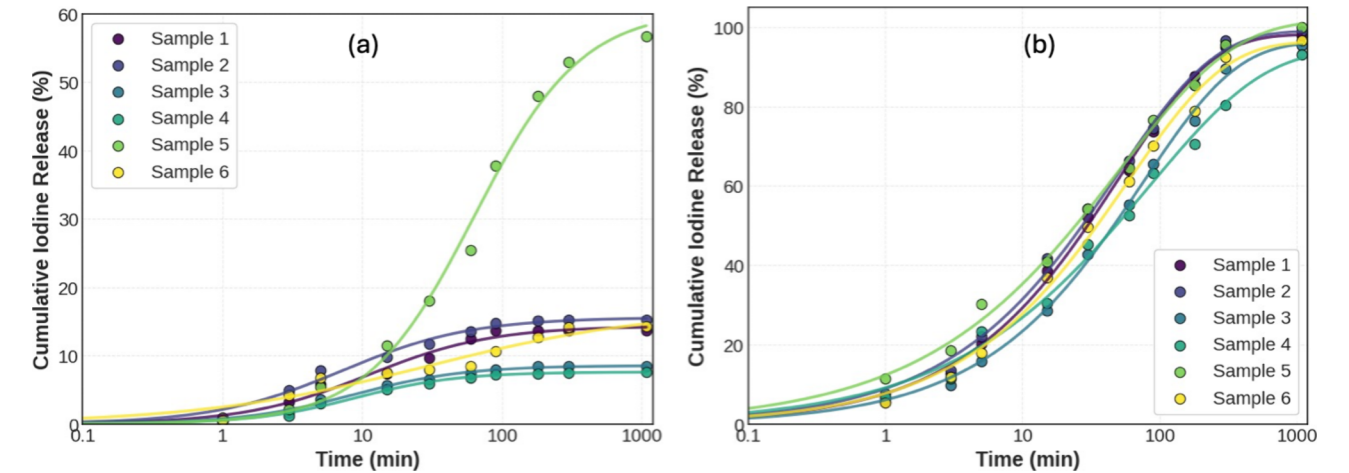
**3.3. Transient Iodine Release.** Several nonlinear mathematical models were evaluated to characterize the kinetics of iodine release from various iodophors into a lipophilic phase.<sup>61–65</sup> Among these, only the Weibull, Michaelis–Menten (M-M) and Hill (modified M-M) models effectively described the observed iodine release behavior. The Weibull model for drug release is commonly expressed as

$$Q(t) = Q_{\max} \left( 1 - \exp \left( - \left( \frac{t}{\beta} \right)^\eta \right) \right) \quad (1)$$

where:  $Q(t)$ : cumulative amount of drug released at time  $t$ .  $Q_{\max}$ : maximum amount of drug released (asymptotic maximum).  $\beta$ : scale parameter (related to the characteristic time of release).  $\eta$ : shape parameter (describes the release profile's shape).

The Michaelis–Menten (M-M) model (eq 2), typically used for enzyme kinetics, can be adapted to model drug release in certain contexts where the release rate follows a saturable process. The model describes the rate of drug release as a function of time, often expressed as

$$\frac{dQ(t)}{dt} = \frac{V_{\max} \cdot t}{K_m + t} \quad (2)$$



**Figure 6.** (a) M-M model fit to iodine release from all six samples at a dilution ratio of 2. (b) Weibull fit of iodine release from all six samples at a dilution rate of 20.

**Table 2. Summary of Model Fit Parameters and Statistical Fit Results for Dilution Ratio of 2**

| sample | Weibull model  |              |         |       |          | M-M model      |             |            |       |          |
|--------|----------------|--------------|---------|-------|----------|----------------|-------------|------------|-------|----------|
|        | $Q_{\max}$ (%) | $\eta$ (min) | $\beta$ | $R^2$ | RMSE (%) | $V_{\max}$ (%) | $K_m$ (min) | $n$ (Hill) | $R^2$ | RMSE (%) |
| 1      | 13.7           | 25.4         | 0.38    | 0.987 | 0.42     | 13.8           | 18.2        | 1.52       | 0.991 | 0.38     |
| 2      | 15.3           | 32.1         | 0.45    | 0.991 | 0.51     | 15.4           | 22.7        | 1.48       | 0.993 | 0.42     |
| 3      | 8.6            | 112.5        | 0.29    | 0.982 | 0.38     | 8.7            | 95.4        | 1.62       | 0.984 | 0.35     |
| 4      | 7.7            | 89.7         | 0.31    | 0.979 | 0.41     | 7.8            | 78.3        | 1.55       | 0.981 | 0.39     |
| 5      | 57.1           | 68.3         | 0.72    | 0.998 | 1.12     | 57.5           | 45.6        | 1.28       | 0.997 | 1.05     |
| 6      | 14.4           | 45.2         | 0.41    | 0.985 | 0.47     | 14.5           | 32.9        | 1.44       | 0.988 | 0.41     |

**Table 3. Summary of Model Fit Parameters and Statistical Fit Results for Dilution Ratio of 20**

| sample | Weibull model  |              |         |       | M-M model |                |             |            |
|--------|----------------|--------------|---------|-------|-----------|----------------|-------------|------------|
|        | $Q_{\max}$ (%) | $\eta$ (min) | $\beta$ | $R^2$ | RMSE (%)  | $V_{\max}$ (%) | $K_m$ (min) | $n$ (Hill) |
| 1      | 98.5           | 52.1         | 0.62    | 0.89  | 98.7      | 45.2           | 1.32        | 1.56       |
| 2      | 100.8          | 49.3         | 0.67    | 1.12  | 101.0     | 42.7           | 1.28        | 1.72       |
| 3      | 96.2           | 68.7         | 0.58    | 1.34  | 96.5      | 58.3           | 1.41        | 2.01       |
| 4      | 93.5           | 75.4         | 0.54    | 1.67  | 94.0      | 67.8           | 1.35        | 2.34       |
| 5      | 100.1          | 43.2         | 0.71    | 0.75  | 100.3     | 38.5           | 1.22        | 1.38       |
| 6      | 97.6           | 55.9         | 0.64    | 0.92  | 97.9      | 49.6           | 1.30        | 1.61       |

where:  $Q(t)$ : cumulative amount of drug released at time  $t$ .  $V_{\max}$ : maximum release rate (units/time).  $K_m$ : time at which the release rate is half of  $V_{\max}$  (analogous to the Michaelis constant, in units of time).

To model cumulative release, we integrate the rate equation to obtain

$$Q(t) = V_{\max} \cdot \frac{t}{K_m + t} \tag{3}$$

where  $V_{\max}$  is the maximum cumulative release (analogous to the total substrate converted in enzyme kinetics). However, this form assumes instantaneous saturation, which may not fully capture the sigmoidal or plateau behavior seen in the present data. A more general form used in drug release studies is (Hill model):

$$Q(t) = V_{\max} \cdot \frac{t^n}{K_m^n + t^n} \tag{4}$$

where  $n$  is a shape parameter (similar to the Hill coefficient) that adjusts the steepness of the release curve, making it more flexible for fitting drug release data. Figure 6 illustrates the

fitting of Weibull and M-M models to transient release data derived from dilution ratios of 2 and 20. Table 2 presents the extracted parameters from the fitted models applied to a dilution ratio of 2, including the Hill equation coefficients (eq 4), along with goodness-of-fit metrics ( $R^2$ ). The table additionally reports the Root-Mean-Square Error (RMSE), which quantifies the average magnitude of differences between model predictions and observed values. Elevated RMSE values indicate greater prediction errors, reflecting poorer model performance in describing the experimental data. The comparative analysis of these metrics enables objective evaluation of each model's predictive accuracy.

Figure 6a illustrates the Michaelis–Menten model fit to iodine release data for all studied samples at a dilution rate of 2, while Figure 6b depicts the Weibull model fit to the same data at a dilution ratio of 20. Concurrently, Tables 2 and 3 compile the fitted model parameters and statistical fit results derived from the nonlinear curve fitting process for both models, providing an overview of the release kinetics and model performance across the samples.

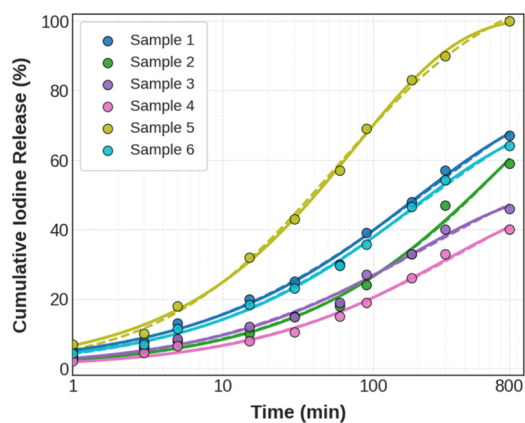
The Weibull and M-M models exhibit excellent parameter consistency, with  $Q_{\max}$  and  $V_{\max}$  values differing by  $\leq 0.4\%$  across all samples at a dilution rate of 2 (no lipids case), confirming model reliability. Sample 5 stands out with a significantly higher release capacity ( $\sim 57\%$ ), distinguishing it from others. Time parameters show that  $K_m$  values (M-M) are consistently lower than  $\eta$  values (Weibull), with Sample 3 exhibiting the longest release times ( $\eta = 112.5$  min,  $K_m = 95.4$  min). The Weibull shape parameter ( $\beta$ ) ranges from 0.29 to 0.72, and the Hill coefficient ( $\eta$ ) ranges from 1.28 to 1.62, indicating cooperative binding (potential multiple binding sites of iodine in the polymer matrix).

For Samples 1–4 and 6, low  $\beta$  values (0.29–0.45) confirm diffusion-controlled release, while high  $\eta$  values (1.44–1.62) suggest cooperative binding at the molecular level, possibly due to excipient-iodine interactions. Sample 5 displays atypical release with a higher  $\beta$  (0.72), indicating partial burst release, and a moderate  $\eta$  (1.28), reflecting reduced cooperativity. Samples 3 and 4 show formulation challenges with minimal release ( $Q_{\max} < 9\%$ ) and extended-release times, likely due to hydrophobic interactions. Sample 6 achieves an optimal balance with  $\beta = 0.41$  and  $\eta = 1.44$ , indicating moderate diffusion and binding kinetics.

Both the Weibull and M-M models demonstrate excellent fit quality for iodine release at a dilution rate of 20 (no lipids case), with Weibull RMSE ranging from 0.75 to 1.67% and Michaelis–Menten RMSE from 1.38 to 2.34%, with Sample 5 showing the best fits (Weibull RMSE = 0.75%, M-M RMSE = 1.38%). All samples achieved over 93% release capacity ( $Q_{\max}/V_{\max}$ ). The Weibull shape parameter ( $\beta$ ) ranges from 0.54 to 0.71, confirming diffusion-dominated release, with Sample 4 showing the strongest diffusion limitation ( $\beta = 0.54$ ) and Sample 5 approaching ideal release ( $\beta = 0.71$ ). Hill coefficients (1.22–1.41) indicate cooperative binding across all samples. Cooperative binding describes a phenomenon where the binding or release of drug molecules becomes easier as more molecules are released from the delivery system. It suggests that the system undergoes structural or chemical changes that facilitate further release over time.

Samples with higher time parameters ( $\eta/K_m$ ), such as Samples 3 and 4, exhibit higher RMSE values, while the fastest-releasing Sample 5 has the lowest RMSE in both models. Sample 5 represents an optimal formulation with a near-ideal  $\beta$  (0.71), low  $K_m$  (38.5 min), minimal cooperativity ( $\eta = 1.22$ ), and the best model fits. In contrast, Sample 4 faces formulation challenges, with the highest  $\eta$  (75.4 min),  $K_m$  (67.8 min), and RMSE values (Weibull: 1.67%, M-M: 2.34%), suggesting a need for iodophor structure or solution modification. The consistent RMSE below 3% across all samples confirms the reliability of both models for describing these release systems, though slower-releasing formulations like Sample 3 and 4 show slightly poorer fits, likely due to other polymer based inactive ingredients in these commercial formulations (see Table 1).

**3.4. Effect of Phospholipids.** Figure 7 illustrates the iodine release kinetics from iodophor samples across a hydrophilic-phospholipid-rich lipophilic interface at a dilution rate of 8. This dilution rate was selected based on the complete iodine release (100%) observed for Sample 5 under these conditions. Both the Weibull and Michaelis–Menten (M-M) models demonstrate excellent fits to the experimental data, as evidenced by high coefficients of determination ( $R^2 > 0.988$ ) and low root-mean-square errors (RMSE  $< 1.30$ ). The derived model parameters and corresponding statistical metrics are



**Figure 7.** Iodine release profiles and model fit curves for the all the samples across a hydrophilic-phospholipid-rich lipophilic interface at dilution ratio of 8.

summarized in Table 4. The iodine release kinetics from six iodophor samples were effectively modeled using both Weibull and M-M (with Hill coefficient) approaches, demonstrating excellent fits across all samples ( $R^2 = 0.988$ –0.999). The Weibull model generally provided slightly better fits (lower RMSE values of 0.75–1.25) compared to the M-M model (RMSE = 0.80–1.30), suggesting time-dependent release mechanisms dominate the process. Cadexomer-iodine complex (Sample 5) exhibited the most rapid release characteristics, as evidenced by its high Weibull shape factor ( $\beta = 1.20$ ) and Hill coefficient ( $n = 1.5$ ), reaching complete 100% iodine release, while Sample 4 showed the slowest kinetics ( $\beta = 0.90$ ,  $n = 0.9$ ).

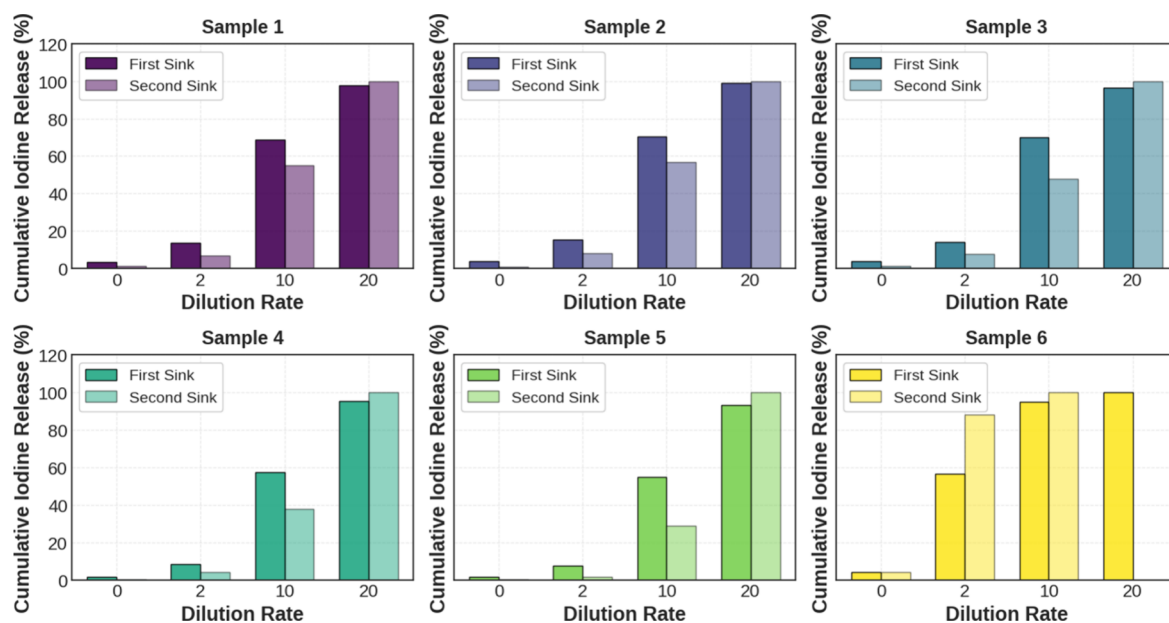
The derived parameters ( $Q_{\max}$ ,  $\eta$ ,  $\beta$ ,  $V_{\max}$ ,  $K_m$ ,  $n$ ) revealed significant variations in release rates and saturation behaviors among samples, with Samples 1, 5, and 6 demonstrating faster release profiles suitable for immediate applications, and Samples 2–4 showing more gradual release patterns potentially advantageous for sustained delivery systems. These modeling results provide quantitative insights to achieve desired release kinetics to optimize iodophor formulations for specific clinical characteristics e.g.,  $I_2$  diffusions across biological barriers like the mucus and cell membranes.

**3.5. Sink Conditions.** Sink conditions refer to an experimental setup where the concentration of the drug in the release medium remains well below its solubility limit, typically less than 10–20% of the saturation concentration, ensuring that dissolution or diffusion is not limited by solubility constraints.<sup>66</sup> In the context of iodine release from iodophor formulations across a hydrophilic–lipophilic interface, as investigated in this study, sink conditions are critical to accurately measure the intrinsic release kinetics of molecular iodine. The use of excess heptane (50 g) as the lipophilic phase compared to the aqueous iodophor solution (5 g) establishes a large reservoir that maintains low iodine concentrations in the lipophilic phase, mimicking sink conditions.

This setup, described in the Section 2.2, ensures that the transfer of iodine across the interface is driven primarily by the dissociation of iodine from the iodophor complex rather than by saturation effects in the receiving phase, allowing for reliable quantification of release rates and equilibrium concentrations. Figure 2 illustrates that, with the exception of the dextrin-iodine complex (Sample 5), significant amounts of iodine remain bound to the polyvinylpyrrolidone (PVP) polymer at dilution ratios below 20. This observation indicates that the

Table 4. Summary of Model Fit Parameters and Statistical Fit Results for Dilution Rate 8, with Phospholipids

| sample | Weibull    |        |         |       |      | Michaelis–Menten |       |            |       |      |
|--------|------------|--------|---------|-------|------|------------------|-------|------------|-------|------|
|        | $Q_{\max}$ | $\eta$ | $\beta$ | $R^2$ | RMSE | $V_{\max}$       | $K_m$ | $n$ (Hill) | $R^2$ | RMSE |
| 1      | 68.1       | 45.2   | 1.12    | 0.997 | 1.08 | 67.8             | 85.3  | 1.3        | 0.996 | 1.15 |
| 2      | 59.5       | 55.7   | 1.05    | 0.994 | 0.85 | 59.0             | 120.5 | 1.1        | 0.993 | 0.90 |
| 3      | 46.8       | 49.8   | 0.98    | 0.991 | 0.92 | 46.5             | 95.7  | 1.0        | 0.990 | 0.95 |
| 4      | 40.3       | 66.4   | 0.90    | 0.989 | 0.75 | 40.0             | 150.2 | 0.9        | 0.988 | 0.80 |
| 5      | 101.2      | 39.5   | 1.20    | 0.999 | 1.25 | 100.5            | 50.1  | 1.5        | 0.998 | 1.30 |
| 6      | 64.7       | 47.3   | 1.10    | 0.996 | 1.02 | 64.2             | 75.6  | 1.2        | 0.995 | 1.10 |



**Figure 8.** Cumulative iodine release (%) from six iodophor formulations (Samples 1–6) at dilution ratios of 0, 2, 10, and 20, comparing the first lipophilic sink (heptane, solid bars) and second lipophilic sink (fresh medium, semitransparent bars). No detectable iodine was found in the second sink for Sample 6 at dilution rate of 20.

polymer-iodine complex retains a substantial fraction of iodine under these conditions. To further elucidate the release dynamics, it is critical to determine whether replacing the lipophilic sink solvent (heptane) with fresh medium (after equilibrium) would enhance the dissociation and release of iodine from these iodophors at lower dilution ratios, potentially increasing the availability of free molecular iodine for antimicrobial applications.

As Figure 8 shows, replacing the first sink with a fresh lipophilic medium (second sink) generally enhanced iodine release at lower dilution ratios (0–10), indicating that sink conditions play a critical role in driving further dissociation of iodine from iodophor complexes. At dilution ratios of 0, the second sink yielded modest additional release (0.3–4.2%), with Sample 6 yielding release (4.2%) equivalent to the first sink, suggesting limited residual iodine availability in undiluted conditions. At a dilution ratio of 2, the second sink significantly increased release for Sample 6 (88.0% vs 56.7% in the first sink), while other samples showed moderate increases (e.g., Sample 1: 7.0% vs 13.6%; Sample 4: 4.0% vs 8.5%). At a dilution ratio of 10, the second sink sustained high release for Samples 1–3 (48.0–57.0%) but reduced release for Samples 4 and 5 (38.0 and 29.0%, respectively), suggesting that these formulations may exhaust available iodine in the first sink. At a dilution ratio of 20, all samples exhibited 100% release in the second sink, while sample 6 had already depleted its iodine in

the first sink (or not detectable levels in the second sink). These results underscore that fresh sink conditions can extract additional iodine at lower dilutions, particularly for formulations with strong polymer binding (e.g., Samples 3 and 4).

The antibacterial efficacy of PVPI (povidone-iodine) is directly related to the release of free iodine ( $I_2$ ). The PVPI complex acts as a carrier or reservoir for iodine, slowly releasing it in a controlled manner. It is this small amount of free iodine, not the bound iodine, that is the active antimicrobial agent. Molecular iodine has a potent, nonspecific antimicrobial action. It rapidly penetrates the cell walls and membranes of microorganisms, including bacteria (Gram-positive and Gram-negative), viruses, fungi, and spores. More research is needed to study certain biological interface conditions in which the effectiveness and efficacy of molecular iodine release from such complexes can be tuned or improved.<sup>67</sup>

#### 4. CONCLUSIONS

Iodophors are often classified as pharmaceutical agents, and their iodine release profiles can affect clinical utility. Specifically, iodophors such as povidone-iodine complex hydrophobic molecular iodine, enabling its release to biologically relevant hydrophilic–lipophilic interfaces. To the best of our knowledge, this work provides the first evidence of

molecular iodine release from these iodophors across hydrophilic–lipophilic interfaces.

Dilution significantly enhances the release of free molecular iodine from iodophor formulations across a hydrophilic–lipophilic interface, as evidenced by comparing release profiles at dilution ratios of 2 and 20. At a dilution ratio of 2, the cumulative iodine release ranged from 7.7 to 57.1% across samples, with dextrin-iodine (Sample 5) attaining fastest release (Table 2). In contrast, at a dilution ratio of 20, all samples exhibited near-complete release, with maximum release capacities ( $Q_{\max}/V_{\max}$ ) exceeding 93.5% (Table 3). This substantial increase is attributed to the disruption of polymer-iodine interactions at higher water content, which reduces intermolecular hydrogen bonding and facilitates iodine diffusion into the lipophilic phase, consistent with previous findings on dilution effects (Figure 2). Furthermore, release times, characterized by the Weibull scale parameter ( $\eta$ ) and Michaelis–Menten half-time ( $K_m$ ), decreased from 25.4–112.5 min and 18.2–95.4 min at dilution ratios of 2 to 43.2–75.4 min and 38.5–67.8 min at a dilution ratio 20, respectively, indicating faster diffusion kinetics at higher dilution.

The presence of phospholipids in the lipophilic phase markedly augments iodine release from iodophor formulations, as observed at a dilution ratio of 8 with 50 wt % soybean lecithin in heptane (Table 4). Compared to the same samples at dilution ratio 2 without phospholipids (Table 2), Samples 1, 2, 5, and 6 exhibited increased maximum release capacities, with Sample 5 (cadexomer-iodine) achieving complete release ( $Q_{\max} = 101.2\%$ ) compared to 57.1% without lipids. This enhancement is likely driven by charge-transfer interactions between molecular iodine and phospholipids, which reduce iodine concentration in the lipophilic phase and promote further release, as supported by prior studies. The Weibull shape parameter ( $\beta$ ) increased from 0.29–0.72 (Table 2) to 0.90–1.20 (Table 4), indicating a shift toward burst-like release profiles in the presence of lipids. However, the Michaelis–Menten half-time ( $K_m$ ) was notably higher (50.1–150.2 min) with phospholipids, suggesting that lipid-iodine complexation may delay saturation, particularly for slower-releasing formulations like Sample 4.

Significant variations in iodine release kinetics were observed among the iodophor formulations, with cadexomer-iodine (Sample 5) consistently outperforming other samples. At dilution ratios of 2, 20, and 8, Sample 5 achieved the highest release capacities (57.1, 100.1, and 101.2%, respectively) and the fastest kinetics, with Weibull  $\eta$  values of 68.3, 43.2, and 39.5 min, respectively (Tables 2–4). Its polysaccharide-based structure likely facilitates rapid iodine dissociation, making it ideal for immediate antimicrobial applications. In contrast, swabstick formulations (Samples 3 and 4) exhibited the lowest release (7.7–8.6% at a dilution ratio of 2, 40.3–46.8%) and slowest kinetics ( $\eta$  up to 112.5 min,  $K_m$  up to 150.2 min), potentially due to hydrophobic inactive ingredients such as alkyl glucoside and nonoxynol-10 (Table 1). Samples 1, 2, and 6 displayed moderate release (13.7–15.3%) at a dilution ratio of 2; 59.5–68.1% at a dilution ratio of 8; and 97.6–100.8% at a dilution ratio of 20, suggesting versatility for both immediate and sustained release applications. These findings suggest that iodophors can potentially be formulated to release molecular iodine at biologically relevant interfaces in different manners: either through limited and controlled release via successive dilutions or via more rapid, burst-like release patterns at a low level of dilution. Finally, it was concluded that among inactive

ingredients, buffering agents and surfactants did not influence the free molecular iodine release; however, other polymeric agents such as cellulose derivatives may significantly affect molecular iodine release.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.5c06724>.

ANOVA section includes Table S1, presenting the raw iodine release percentages for Samples 1, 4, and 6 across six dilution rates (0, 2, 10, 20, 50, 100) and six mixing speeds (5, 10, 15, 20, 25, 30 rpm), followed by the statistical results for each sample: Table S2 (Sample 1), Table S3 (Sample 4), and Table S4 (Sample 6), all detailing the Sum of Squares, degrees of freedom (df),  $F$ -value, and  $p$ -value for the main effects (dilution rate and RPM) and their interaction; raw data for kinetic model fitting, categorized by dilution ratio: the dilution ratio of 2 raw data points section includes Table S5 (Weibull model fit parameters) and Table S6 (Michaelis–Menten (M-M) model fit parameters); the dilution ratio of 20 raw data points section includes Table S7 (Weibull model fit parameters) and Table S8 (M-M model fit parameters); and the dilution rate 8, with phospholipids - raw data points section includes Table S9 (Weibull model fit parameters) and Table S10 (M-M model fit parameters), with all kinetic model tables presenting the raw data and mean  $\pm$  standard deviation for three experimental replicates (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Author

Ilker S. Bayer – I2Pure Corporation, Ashburn, Virginia 20147, United States; [orcid.org/0000-0002-7951-8851](https://orcid.org/0000-0002-7951-8851); Email: [ilker@i2pure.com](mailto:ilker@i2pure.com)

### Authors

Maggie Engert – I2Pure Corporation, Ashburn, Virginia 20147, United States

Christopher Moses – I2Pure Corporation, Ashburn, Virginia 20147, United States

Jack Kessler – I2Pure Corporation, Ashburn, Virginia 20147, United States

Complete contact information is available at:

<https://pubs.acs.org/doi/10.1021/acsomega.5c06724>

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

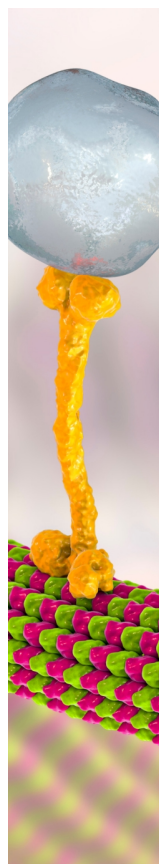
The authors gratefully acknowledge the financial support from I2Pure Corporation.

## ■ REFERENCES

- (1) Gottardi, W. *Iodine Chemistry and Applications; Iodine as Disinfectant*; Wiley, 2014; 375–410.
- (2) Apostolov, K. The effect of iodine on the biological activities of myxoviruses. *Epidemiol. Infect.* **1980**, 84 (4), 381–388.
- (3) Carroll, B. The related germicidal activity of triiodide and diatomic iodine. *J. Bacteriol.* **1955**, 69 (4), 413–417.

- (4) Gottardi, W. The uptake and release of molecular iodine by the skin: chemical and bactericidal evidence of residual effects caused by povidone-iodine. *J. Hosp. Infect.* **1995**, *29* (1), 9–18.
- (5) Greenwald, M. B. Y.; Zlotkin, M. F.; Soroka, Y.; Sassoon, S. B.; Peled, H. B.; Kohen, R. A novel role of topical iodine in skin: Activation of the Nrf2 pathway. *Free Radic. Biol. Med.* **2017**, *104*, 238–248.
- (6) Cooper, R. A. Iodine revisited. *Int. Wound J.* **2007**, *4* (2), 124–137.
- (7) Klebanoff, S. J. Iodination of bacteria: a bactericidal mechanism. *J. Exp. Med.* **1967**, *126* (6), 1063–1078.
- (8) Li, Q.; Korza, G.; Setlow, P. Killing the spores of *Bacillus* species by molecular iodine. *J. Appl. Microbiol.* **2017**, *122* (1), 54–64.
- (9) Leaper, D. J.; Durani, P. Topical antimicrobial therapy of chronic wounds healing by secondary intention using iodine products. *Int. Wound J.* **2008**, *5* (2), 361–368.
- (10) McDonnell, G.; Russell, A. D. Antiseptics and disinfectants: activity, action, and resistance. *Clin. Microbiol. Rev.* **1999**, *12* (1), 147–179.
- (11) Brandrick, A. M.; Newton, J. M.; Henderson, G.; Vickers, J. A. An investigation into the interaction between iodine and bacteria. *J. Appl. Bacteriol.* **1967**, *30* (3), 484–487.
- (12) Viswanathan, K.; Babu, D. B.; Jayakumar, G.; Dhinakar Raj, G. Anti-microbial and skin wound dressing application of molecular iodine nanoparticles. *Mater. Res. Express* **2017**, *4* (10), 104003.
- (13) Houang, E. T.; Gilmore, O. J.; Reid, C. L. A. R. E.; Shaw, E. J. Absence of bacterial resistance to povidone iodine. *J. Clin. Pathol.* **1976**, *29* (8), 752–755.
- (14) Reva, O. N.; et al. The effect of iodine-containing nanomicelles, FS-1, on antibiotic resistance, gene expression and epigenetic modifications in the genome of multidrug resistant MRSA strain *Staphylococcus aureus* ATCC BAA-39. *Front. Microbiol.* **2020**, *11*, No. 581660.
- (15) Ramette, R. W.; Sandford, R. W., Jr Thermodynamics of iodine solubility and triiodide ion formation in water and in deuterium oxide. *J. Am. Chem. Soc.* **1965**, *87* (22), 5001–5005.
- (16) Makhayeva, D. N.; Irmukhametova, G. S.; Khutoryanskiy, V. V. Advances in antimicrobial polymeric iodophors. *Eur. Polym. J.* **2023**, *201*, No. 112573.
- (17) Soda, T.; Hildebrand, J. H. Solubility of iodine in dimethyl sulfoxide. *J. Phys. Chem.* **1967**, *71* (13), 4561–4563.
- (18) Osol, A.; Pines, C. C. Solubility of iodine in glycol-water solutions. *J. Am. Pharm. Assoc. (Sci. Ed.)* **1952**, *41* (12), 634–637.
- (19) Khan, A. Theoretical studies of the complexes of iodine with methanol, ethanol, and acetone. *J. Chem. Phys.* **1992**, *96* (2), 1194–1198.
- (20) Varlamova, T. M.; Rubtsova, E. M.; Mushtakova, S. P. Solubility diagrams of the potassium iodide-water-ethanol and iodine-water-ethanol ternary systems. *Russ. J. Phys. Chem. A* **2009**, *83* (11), 1896–1899.
- (21) Mikhailov, V. A.; Grigor'eva, É. F.; Semina, I. I. Solubility of iodine in water—Alcohol mixtures comparison with a ternary-solution model. *J. Struct. Chem.* **1969**, *9* (6), 855–860.
- (22) Hildebrand, J. H.; et al. Solubility of iodine in ethyl alcohol, ethyl ether, mesitylene, p-xylene, 2, 2-dimethylbutane, cyclohexane and perfluoro-n-heptane. *J. Am. Chem. Soc.* **1950**, *72* (2), 1017–1020.
- (23) Zamora, J. L. Chemical and Microbiologic Characteristics and Toxicity of Povidone-Iodine Solutions. *Am. J. Surg.* **1986**, *151* (3), 400–406.
- (24) Shelanski, H. A.; Shelanski, M. V. PVP-Iodine: History, Toxicity, and Therapeutic Uses. *J. Int. Coll. Surg.* **1956**, *25* (6), 727–734.
- (25) Gottardi, W. The influence of the chemical behaviour of iodine on the germicidal action of disinfectant solutions containing iodine. *J. Hosp. Infect.* **1985**, *6*, 1–11.
- (26) Food and Drug Administration. Safety and Effectiveness of Consumer Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use. *Fed. Regist.* **2016**, *81* (172), 61106–61130.
- (27) Berkelman, R. L.; Holland, B. W.; Anderson, R. L. Increased Bactericidal Activity of Dilute Preparations of Povidone-Iodine Solutions. *J. Clin. Microbiol.* **1982**, *15* (4), 635–639.
- (28) Gottardi, W.; Koller, W. The concentration of free iodine in aqueous povidone-iodine containing systems and its variation with temperature. *Monatsh. Chem.* **1986**, *117*, 1011–1020.
- (29) Schreier, H.; et al. Molecular Effects of Povidone-Iodine on Relevant Microorganisms: An Electron-Microscopic and Biochemical Study. *Chemotherapy* **1997**, *43* (2), 121–127.
- (30) Makhayeva, D. N.; Irmukhametova, G. S.; Khutoryanskiy, V. V. Polymeric Iodophors: Preparation, Properties, and Biomedical Applications. *Rev. J. Chem.* **2020**, *10* (1–2), 40–57.
- (31) Dattilo, S.; et al. Solid-State Preparation and Characterization of 2-Hydroxypropylcyclodextrins-Iodine Complexes as Stable Iodophors. *Biomolecules* **2023**, *13* (3), 474.
- (32) Malone, M.; et al. Efficacy of Topical Cadexomer Iodine Treatment in Chronic Wounds: Systematic Review and Meta-Analysis of Comparative Clinical Trials. *Int. Wound J.* **2021**, *18* (4), 557–565.
- (33) Brett, D. W. Cadexomer Iodine: A Fresh Look at an Old Gem. *WPA&R J.* **2019**, *27* (1), 42–48.
- (34) Meehan, J. P.; Caylor, M. Dilute Povidone-Iodine Irrigation: The Science of Molecular Iodine (I2) Kinetics and Its Antimicrobial Activity. *J. Am. Acad. Orthop. Surg.* **2025**, *33* (2), 49–55.
- (35) Atemnkeng, M. A.; Plaizier-Vercammen, J.; Schuermans, A. Comparison of free and bound iodine and iodide species as a function of the dilution of three commercial povidone-iodine formulations and their microbicidal activity. *Int. J. Pharm.* **2006**, *317* (2), 161–166.
- (36) Thakur, S. S.; et al. Ex vivo evaluation of the influence of pH on the ophthalmic safety, antibacterial efficacy and storage stability of povidone-iodine. *Clin. Exp. Optom.* **2021**, *104* (2), 162–166.
- (37) Nuckolls, C. Chemical Considerations Related to The Dilution of Commercial 10% Povidone-Iodine For Use In The COVID-19 Pandemic. *OSF Preprints* **2020**
- (38) Chatterjee, I.; Nandy, P.; Bhowmik, B. B. Nature of interaction of phospholipid liposome with iodine. *Chem. Phys. Lipids* **1988**, *49* (1–2), 57–63.
- (39) Chatterjee, I.; Bhowmik, B. B.; Nandy, P. Surface catalytic effect of liposome formation on lecithin-iodine interaction. *A kinetic study. Langmuir* **1989**, *5* (4), 1035–1037.
- (40) Gottardi, W.; Nagl, M. Potentiometric characterization of iodine-based disinfecting preparations. *J. Pharm. Biomed. Anal.* **2019**, *165*, 90–93.
- (41) Tellinghuisen, J. Analysis of the visible absorption spectrum of I2 in inert solvents using a physical model. *J. Phys. Chem. A* **2012**, *116* (1), 391–398.
- (42) Stokes, R. H.; Dunlop, P. J.; Hall, J. R. The diffusion of iodine in some organic solvents. *Trans. Faraday Soc.* **1953**, *49*, 886–890.
- (43) De Maine, P. A. D. Iodine Complexes in Inert Solvents. I. I2–I2 in Carbon Tetrachloride and in n-Heptane. *J. Chem. Phys.* **1956**, *24* (5), 1091–1093.
- (44) Lepelletier, D.; et al. Povidone iodine: properties, mechanisms of action, and role in infection control and *Staphylococcus aureus* decolonization. *Antimicrob. Agents Chemother.* **2020**, *64* (9), No. e00781-20.
- (45) Williamson, D. A.; Carter, G. P.; Howden, B. P. Current and emerging topical antibacterials and antiseptics: agents, action, and resistance patterns. *Clin. Microbiol. Rev.* **2017**, *30* (3), 827–860.
- (46) Lachapelle, J. M.; et al. Antiseptics in the era of bacterial resistance: a focus on povidone iodine. *Clin. Pract.* **2013**, *10* (5), 579.
- (47) Sriwilaijaroen, N.; et al. Mechanisms of the action of povidone-iodine against human and avian influenza A viruses: its effects on hemagglutination and sialidase activities. *Virology* **2009**, *6*, 124.
- (48) Freeman, C.; Duan, E.; Kessler, J. Molecular iodine is not responsible for cytotoxicity in iodophors. *J. Hosp. Infect.* **2022**, *122*, 194–202.
- (49) Leaper, D. J. Povidone-iodine: an update. *J. Hosp. Infect.* **1998**, *40* (Suppl1), S13–S18.

- (50) Calissendorff, J.; Falhammar, H. Lugol's solution and other iodide preparations: perspectives and research directions in Graves' disease. *Endocrine* **2017**, *58* (3), 467–475.
- (51) Erbil, Y.; et al. Effect of Lugol solution on thyroid gland blood flow and microvessel density in the patients with Graves' disease. *J. Clin. Endocrinol. Metab.* **2007**, *92* (6), 2182–2189.
- (52) Palmer, D. A.; Ramette, R. W.; Mesmer, R. E. Triiodide ion formation equilibrium and activity coefficients in aqueous solution. *J. Solution Chem.* **1984**, *13* (9), 673–683.
- (53) Lo, R.; et al. The stability of covalent dative bond significantly increases with increasing solvent polarity. *Nat. Commun.* **2022**, *13* (1), 2107.
- (54) Fiorillo, A. A.; Galbraith, J. M. A valence bond description of coordinate covalent bonding. *J. Phys. Chem. A* **2004**, *108* (23), 5126–5130.
- (55) Sunder, S.; et al. Polyiodine and polyiodide species in an aqueous solution of iodine + KI: theoretical and experimental studies. *J. Phys. Chem. A* **1999**, *103* (50), 10923–10931.
- (56) Calabrese, V. T.; Khan, A. Polyiodine and polyiodide species in an aqueous solution of iodine+ KI: Theoretical and experimental studies. *J. Phys. Chem. A* **2000**, *104* (6), 1287–1292.
- (57) Xu, X.; Guan, Y. Investigating the complexation and release behaviors of iodine in Poly (vinylpyrrolidone)-Iodine systems through experimental and computational approaches. *Ind. Eng. Chem. Res.* **2020**, *59* (52), 22667–22676.
- (58) Bhowmik, B.; Jendrasik, G. L.; Rosenberg, B. Charge transfer complexes of lipids with iodine. *Nature* **1967**, *215* (5103), 842–843.
- (59) Bayer, G.; et al. Antiseptic povidone-iodine encapsulating edible phospholipid gels. *Colloids Surf., A* **2021**, *619*, No. 126537.
- (60) Dhopeswarkar, G. A.; Mandlik, M. Y.; Atmaram, R. H.; Mead, J. F. Role of phospholipids in iodine binding. *Proc. Soc. Exp. Biol. Med.* **1968**, *129* (2), 571–577.
- (61) Costa, I. F.; et al. Kinetic modeling to explain the release of medicine from drug delivery systems. *Pharm. Nanotechnol.* **2024**, *12* (2), 158–171.
- (62) Heredia, N. S.; Vizuete, K.; Flores-Calero, M.; Pazmiño, V. K.; Pilaquinga, F.; Kumar, B.; Debut, A.; Annamalai, P. K.; et al. Comparative statistical analysis of the release kinetics for nanoprecipitated drug delivery systems based on poly(lactic-co-glycolic acid). *PLoS One* **2022**, *17* (3), No. e0264825.
- (63) Kovalenko, I. G.; Garanina, A. S. Drug carriers: A review on the most used mathematical models for drug release. *Pharmaceutics* **2024**, *16* (9), 1133.
- (64) Schoenmaker, L.; et al. Pharmacodynamic models: Parameterizing the Hill equation, Michaelis-Menten, the logistic curve, and relationships among these models. *J. Pharmacokinet. Pharmacodyn.* **2021**, *48* (3), 287–310.
- (65) Elmas, A.; Akyüz, G.; Bergal, A.; Andaç, M.; Andaç, Ö. Mathematical modelling of drug release. *Res. Eng. Struct. Mater.* **2020**, *6* (4), 327–350.
- (66) Hate, S. S.; Thompson, S. A.; Singaraju, A. B. Impact of sink conditions on drug release behavior of controlled-release formulations. *J. Pharm. Sci.* **2025**, *114* (1), 520–529.
- (67) Reimer, K.; Wichelhaus, T. A.; Schäfer, V.; Rudolph, P.; Kramer, A.; Wutzler, P.; Ganzer, D.; Fleischer, W. Antimicrobial Effectiveness of Povidone-Iodine and Consequences for New Application Areas. *Dermatology* **2002**, *204* (Suppl1), 114–120.



CAS BIOFINDER DISCOVERY PLATFORM™

## BRIDGE BIOLOGY AND CHEMISTRY FOR FASTER ANSWERS

Analyze target relationships,  
compound effects, and disease  
pathways

Explore the platform

**CAS**  
A Division of the  
American Chemical Society