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Review Article

NEEDLE FREE MONITORING OF BLOOD GLUCOSE THROUGH REVERSE IONTOPHORESIS

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ABSTRACT

The frequent blood glucose monitoring is highly critical in order to understand the progression of diabetes mellitus and to minimize the probabilities of associated complications. This focuses to the fabrication of a new device for individual blood glucose monitoring. However, this technology turned out to be painful owing to its finger-stick technique, resulting in compromised patient compliance and inconsistent results. A paradigm shift from invasive to non-invasive technique to combat the above-stated limitations became an attractive tool for the researchers. The evolution of reverse iontophoresis to extract the essential plasma biomarkers as well as blood glucose through the skin was the result of the extensive efforts of the researchers as a means to monitor the blood glucose levels. The technology utilises delivery of small amount of electric current through the skin to extract the target molecules. This concept has received tremendous attention in the past decade; however, the technology still needs stringent validation for widespread implementation. Thus, in the present review, we aimed to elaborate the underlying mechanism of the reverse iontophoresis technique in the evaluation of blood glucose levels through skin, its unique features and its advancements towards commercialisation, the challenges faced, additional applications and the future prospects. The review also updates about the other non-invasive glucose monitoring techniques in comparison to reverse iontophoresis.

Keywords: Reverse Iontophoresis, Skin, Extraction mechanism, Electro migration, Electro osmosis, Glucose monitoring, Future approach

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INTRODUCTION

Diabetes mellitus (DM), has undoubtedly become a major health concern in the present era. This deadly disease has become a part of every family and with its continuous trend of gaining higher surge. The major complications associated with DM includes diabetic retinopathy, heart diseases, neuropathy and birth defects. The side effects of antidiabetic drugs cannot be ignored as well, especially hypoglycaemia followed by brain atrophy [1]. Thus, in order to avoid the risk of hypoglycaemia in diabetic patients or a sudden shoot up in glucose level, a continuous monitoring of the blood glucose level is highly crucial. The conventional method used by the patients for measuring blood glucose level is the finger-stick method by a portable analyser. However, this invasive method is painful and at times, may develop into severe wounds in the pricking area, if not handled efficiently. Additionally, this method is not applicable for continuous evaluation, especially when the patient is sleeping or driving, or doing exercises [2]. Experts throughout the world are trying their best to cultivate a blood-free method for monitoring of blood glucose. Thus, the process of iontophoresis was developed that involved small amount of electric current to augment the transportation of ionized drug through the skin [3].

The central mechanism of iontophoresis was reported that, reversely charged ions attract each other whereas the same charged ions turn down. Using this mechanism, the positively charged molecules and negatively charged molecules move through the anode and cathode, respectively under the influence of small current (0.5 mA/cm^2), and ultimately penetrate through the tissues. The highly polar, and frequently charged, nature of these compounds has provoked considerable new research into the mechanism and application of electrically-controlled drug delivery through the skin [4]. These molecules are highly polar and recurrent charged in nature as a result they incite a new area of research in to this non-invasive platform with the application of electric-monitored drug delivery [5].

In a specific area, the prospects of iontophoresis to control the transport rates through the transdermal route has a genuine advantage, and there is strong proof that the present profile is likely to be fabricated to differ to obtain from all kinetics and extent of drug absorption. The coherence of iontophoresis illustrates that electric current passage brings different ions and molecules to transport in both directions under the influence of both electrodes [6]. As a result, with a specific level of assay sensitivity, iontophoresis technology is applied to sample analyte transportability through the skin within the body, and at the end it gives the potentiality so that it remains an important particulate of a true closed-loop system. In normal condition due to the negative charge in human skin, a well-known fact is that during the reverse iontophoresis there is a continuous movement of molecules through the electro-osmosis process in the anode-to-cathode direction [7]. In the present era, this technology has innumerable applications towards targeted drug delivery with additional advantages of usage flexibility, and minimum systemic toxicity [8]. Keeping the same line of thought, there has been a reformation in the technique of iontophoresis, in order to widen its application towards investigation of several diagnostic biomarkers, glucose monitoring and therapeutic drug monitoring of different classes of drugs. The concept is termed as "reverse iontophoresis". This technology is channelized as a comparatively new blood-free method along with the application of a low level of electric current it can extract the charged particulates through the skin, encompassing two basic mechanisms: electro-migration [9] and electro-osmosis [10]. In electro-migration, the small molecules move through the skin with the direct impact of a small applied current. Due to the continuous application of current ionic fluxes are formed with the alteration of electron fluxes. In the other end the uncharged molecules and cations with heavy molecular weight are transported by electro-osmosis mechanism. An electro-osmotic solvent flow which can transport neutral molecules from anode to cathode, is instigated by the counter ions [11]. This innovative technique provides filtered samples free from all unwanted elements along with the non-invasive sampling. Thus, skin plays a major role towards the accomplishment of the reverse iontophoresis since it offers a unique gateway for sampling the objects or the macromolecules [12]. As already mentioned, this non-invasive technology provides an excellent tool to evaluate blood glucose level through the epithelial tissues. The first reference of the commercial application of reverse iontophoresis was *Glucowatch*, which further inspired a lot of research in this field. *Glucowatch* was actually the replica of reverse iontophoresis where the same small amount of electric current was used and a needle-free extraction of both charged and uncharged molecules from interstitial fluid beyond the epithelial tissues. This technique provides an excellent rate which is higher than passive permeabilities so that it gives a blood free diagnosis as well as diabetes monitoring to the patients [13]. Calibration of the *GlucoWatch*® was executed with conventional finger-stick model for monitoring of blood glucose thereby which the technology was efficient enough to provide results after every 20 min up to 12 h. This revolutionary application not only improved the patient compliance in monitoring their blood glucose levels, it also became popular amongst the geriatrics due to its ease of handling. The *GlucoWatch*® was accepted for monitoring of blood glucose in adult as well as children. It was indicated as a parallel therapy to traditional glucose monitoring in blood [14]. Studies on reverse iontophoresis have been exploited towards neurodegenerative disorders owing to its feasibility in linear extraction of phynylalanine (anti-epileptic drugs) through instruments like biosensors. Reports highlighted the efficiency of this technology in diagnosis as well as continuous monitoring of the disease [15]. The present review aims to showcase the best implementation of this blood free technology towards blood glucose monitoring with an additional insight of its unique features, detailed extraction mechanism, other applications, advantages and limitations.

Features of transdermal reverse iontophoresis

To understand the in-depth mechanism of extraction of target molecule through reverse iontophoresis, it is highly crucial to understand the key component of this system-the skin [fig. 1], there are three layers which composed the skin. Epidermis (outer most layer), dermis (central one), and subcutaneous (the deepest layer). These three layers have equal importance in this technology. The epidermis is further subdivided into five major layers, namely stratum corneum, stratum luciderm, stratum granulosum, stratum spinosum, and stratum basale, arranged according to the location from the superficial region to the deep region of the skin [16]. Stratum luciderm is present only on hard skin like in the hand palm or feet. Stratum corneum is actually comprised of dead cell. The blood vessels are absent in the epidermis. Dermis, which is rich in blood vessels provides all the nutritional support to epidermis. Papillary dermis and reticular dermis are the two parts of dermis where the blood vessels are majorly found in the papillary layer [17]. On the other hand, reticular dermis provides all the necessities to different glands and hair follicle. The epithelial cells are very tightly structured so that only a few selected molecules like glucose, lithium, phenytoin can pass through it. The second important factor for the transdermal reverse iontophoresis is relied on pH of the skin that ranges from 4-6. [18]. Reverse iontophoresis a non-invasive technology where an electric filed is created by placing the suitable electrode closer to skin. Different molecules or drugs in this region come closer to the cathode or anode chamber due to influence of direct current. Different charged and uncharged molecules transport according to their destination and at last are extracted by the receiver area in to the receiving fluid [19]. The other factors controlling the mechanism of reverse iontophoresis are factors like skin thickness, fat; humidity which can also influence the extraction mechanism. Biologically human body contains both positive and negative ions and it makes the body as a conductor of electricity. Normally in the subdermal layer the movement of different ions are arbitrary. But after application of the low-level direct current the movement of charged particles are significantly increased and it transports to some specific chamber like cathode or anode. In this specification reverse iontophoresis get more appreciation because most of the commercially available molecules are either weak base or acid [20]. The ionization of the molecule has taken place and formulates positive and negative charge because of the impact of physiological pH (pH 7.4). So, by responsible monitoring of the different intensity of the applied electricity, the extraction ratio can be accelerated for a sufficient quantification of molecules [21]. Skin is negatively charged because the external layer of the skin is stratum corneum contains corneocytes which contains the net negative charge in physiological pH solution. Main gateway of the acidic or basic molecules through the skin adjunct like hair follicle, sweat etc [22]. Still this transport is challenging from the deep area to subdermal region and ultimate to the receiving fluid. Two basic simple mechanisms are responsible to perform a reverse iontophoresis extraction of molecules. i) electromigration and ii) electro-osmosis. According to these mechanisms the neutral compound such as glucose is extracted to the cathode whereas, the anionic substances are extracted mostly towards anode [23].

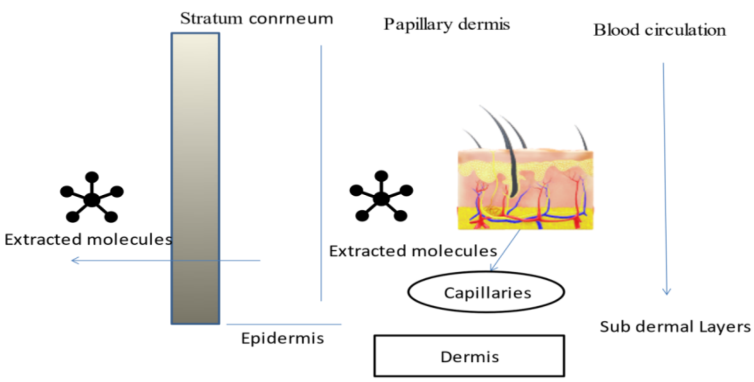

Fig. 1: Extraction of molecules through skins

Extraction mechanism through skin layers

The largest organ in the body is the skin with a surface area $2m^2$. It plays a major role in extraction of molecules. Stratum corneum is the outermost part of the skin that is comparatively strong and robust in nature. It contains different dead and flat cells and behaves as a strong barrier in the extraction of drugs. Some of the physical and chemical method was developed to extract different molecules through the skin. One of the common and widely accepted techniques is iontophoresis [24]. This technique is comparatively new and it is applicable for non-invasive drug delivery, especially for large molecules [25]. In first phase it was taken a long time to evolve as a technique which can increase the transdermal passage through the application of s mall current. A very small electric direct current (0.1-0.5 mA/cm^2) was used in iontophoretic drug delivery for increasing the epithelial tissue passage to transport the large molecules [26]. But the research was continuing to find a new way to extract different molecules or drugs through the skin passage. This mechanism is actually just the opposite of iontophoresis and it is called reverse iontophoresis [27]. Reverse iontophoresis follows the mechanism called extraction that helps to extract molecules from the sub-dermal region of the skin. This method is non-invasive and completely blood-free. It turns out different charged molecules throughout the skin by using a small electric charge which creates a strong repulsive electromotive force [28]. Drug molecules in different categories can be transported through this mechanism from the skin layer to electrode without any needling or pricking. Molecules are extracted from interstitial fluid with the implementation of small current through the layers of skin with two skin electrodes made with either silver or platinum [29]. It involves a simple mechanism to extract charged and neutral molecules also [30]. This technique is working on two mechanisms i) electro migration ii) electro-osmosis. It is responsible for successful extraction of different molecules one of them is glucose. This glucose monitoring by reverse iontophoresis is not a new one it hogged much lime light when the first device came through this technology and it was *Glucowatch* [31]. With the application of low-level current and an auto sensor this device was applied for the measurement of glucose through skin. Skin holds this biogenic *glucowatch* with a hydrogel patch, biosensor and bio adhesive in perfect manner. The hydrogel patch is correlated with auto sensor which helps to monitor glucose in blood up to 12h. Hydrogel patch itself contains glucose oxidase enzyme for extracting glucose from seb-dermal region of skin [32]. Biosensors have two responsibilities first one is to monitor the current when electrons pass through the electrodes and the second one is to monitor the signal processing system which can transport the signal in a readable form. This system works same as that of wristing as a watch. It can generate a small direct current mostly (300-500μA) that affects the sub-dermal region molecules. Under this mechanism or the electrical forces, the both charged molecule can transport to cathode as well as anode [33-35]. As glucose is a neutral molecule so it should be extracted in cathode because of electro-osmosis flow. Hydrogel disk carried the extracted glucose molecules and reacted with glucose oxidase enzyme. That reaction formed hydrogen peroxide [36].

The reaction carried out

Glucose+Oxygen Glucose–Oxidase Gluconolactone+Hydrogen peroxide.

At platinum or sliver/sliver chloride electrode hydrogen peroxide was reduced.

This electrode assures the total quantity of electrons that was transported through the mechanism is actually proportional to the quantity of glucose molecule which was extracted in cathode. Though this was a novel invention but somehow it was not continued in the market because of the complex calibration procedure and changing of the pad after every 12h. The patient's compliance also poor after developing some skin reaction. Sometime in the hot and humid zone due to the excessive sweating the device was not working properly. After some time that device was taken off from the market in 2007 permanently. But the concept remains to the formulation scientists. The research is going on continuously for the monitoring of blood glucose in non-invasive method. This method is already applied for extraction of different molecules other than blood glucose from sub dermal region of skin example Amio acid [37], Urea [38] etc. So that a successful approach and a novel non-invasive technique is now a market demand to monitor blood glucose non-invasively. The further part of this article will process regarding the details of non-invasive monitoring of blood glucose.

The reverse iontophoresis mechanism is based on the principles of charges (i.e., like charges repel each other) and is quite akin to iontophoresis. An electrode plays a crucial role in this whole mechanism where similar charged particle repels and oppositely charged particles attracted each other. In short both the mechanism electro-osmosis and electro migration are responsible for the transportation of both charged, uncharged and neutral molecules through the skin [39].

Electro osmosis

It is the primary iontophoretic transport mechanism of electro neutral molecules as well as heavy molecular weight positively charged ions like glucose etc [fig. 2]. It is discussed before also that the skin carries negative charge at physiological pH [40], and behaves a perm selective layer to cations. That preferential pathway of counter ions influences an electroosmotic flow which transports neutral molecule towards anode to cathode. An electric field created the potential gradient in this mechanism and that is forecasted become proportional to the volume flow jv (volume per unit time per unit area) [41]. The number of moles per unit time per unit area, an element "j" available at concentration cj is then imitated as

$$J_j = J_v * c_j \text{ .... [1]}$$

Glucose is a neutral and polar substance. The extraction of glucose through reverse iontophoresis is initiated by electroosmosis mechanism. Additionally, this transport of fluids through a porous membrane strengthens the transfer of cations while perform in opposition to that anion. As a result, wrenching of cations will simpler rather than an anion with same physicochemical activity.

Electro migration

Direct effect of electric field through the skin is the root cause of movement of the small ions. This phenomenon is called electro migration. Where ionic fluxes are established due to the transformation of electron fluxes through the reaction of electrodes but electroneutrality is maintained due to the proceeds of ionic transport through the skin. Electric field strength and duration of application plays an important role for transport the total charge. Ionic transport mechanism (inward/outward) is correlated through Faraday's law. It inculcates the whole process like the application of low-level electric current through skin with specific time and the ionic charge [42]. In iontophoresis the major part that to be noticed, the entire ions introduced are actually competing each other for transportation of charge at skins. Due to the direct intervention of DC current (Direct current) drug flux (Ji-mol/s) is given by,

$$J_i = (t_i * I)/(F * z_i) \text{ .... [2]}$$

J_i represents the flux of the i^{th} ion, ti represents the transport number, valence is represented by z_i, faraday's constant is represented by F and total current flowing is displayed by I. In this biophysical system all the charged and mobile ions have an effect on transport number according to their concentration and relative mobility.

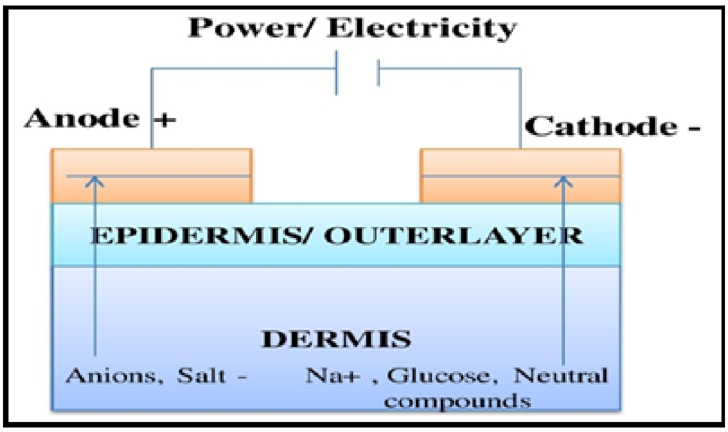

Fig. 2: Details mechanism of reverse iontophoresis

Glucose monitoring through reverse iontophoresis

Diabetes is, nowadays a common household problem. Though a good number of drugs are available in the market for treating but they can only control the disease not cure it. A good number of potent molecules are in the thrust area of different formulation scientists which are aimed to target the pathogenesis of the disease. However, a close and continuous monitoring of the blood glucose levels is equally important in order to prevent the sudden onset of hypoglycaemia due to the effects of drugs or combat the complications that may develop as a subsequent effect of the disease itself. At present, the diagnosis of diabetes or blood glucose level is carried out invasively. It is not a needle-free technique. It needs a better individualistic approach such as expert hand to carry out the venepuncture; else it might cause injury at the site of puncture of the skin as well as wounds and even death to the patients. In modern era scientists are interested to modify this invasive technique to non-invasive. They are applying different technology like sensors and reverse iontophoresis to develop a proper non-invasive model [43]. One of the results of non-invasive glucose monitoring already discussed in the first phase of this article was the biogenic *glucowatch*. However, due to some financial issues and report of negative patient compliance, the product was withdrawn from the market in 2007 [44]. Continuous researches are still on the way to develop a product using reverse iontophoresis technology. Reports on some model devices are in the clinical stage for example Temporary Tattoo, Sensor array patch coupled with induced sweating [45-46]. It has already been mentioned that skin is the main role player in this total event. Cells surrounded by interstitial fluid in skin transports nutrients by diffusion mechanism. This fact provides a healthy correlation between blood and glucose in interstitial fluid [47]. This monitoring of glucose through reverse iontophoresis actually works on the electro osmosis mechanism. Glucose being an uncharged polar molecule is extracted towards the cathode and the extracted amount of glucose through reverse iontophoresis is proportional to subdermal concentration [48]. More concisely, reverse iontophoresis is carried out by applying a very small amount of current (300-500 µm) through the previously worn electrode of either platinum or silver/silver chloride. Skin is negatively charged. As a result, the positively charged sodium ions initiate a remarkable electro-osmotic flow towards the cathode. Hence the neutral molecules glucose is also transported to the cathodal regions [fig. 3] [49]. The whole configuration with entire details can be worn like watch. The extracted amount of glucose is comparatively more dilute than the blood glucose level. A strong sensor is required to accurate calculation of extracted glucose. After implementation of small current for 3 min (approx.) the glucose extracted in cathodal region and then the biosensors are remarkably turned on and the current amplified in biosensor then it was integrated approx. 6-7 min at iontophoretic cathode [50-52]. A reverse process was applied for the polarity of the iontophoretic current and it was repeated again. Then initiate the summed up current to signal processing algorithm [53]. But in this method after every 3h, calibration is required and that can be done by one time finger stick method. So that, the calibration factor is important to measure the blood glucose in every 20 min for long 12 h. Parameter should be checked specifically fluctuation temperature in temperature, excessive sweating or any such of electrical and mechanical faults. If any one of this is present in the time of monitoring of glucose for the time being monitoring may be stopped [54]. That device was commercially launched but with drawn due to some reported problem [55]. To address the entire problem and prepare a newly modified non-invasive system formulation scientists are working on it. Another development of non-invasive glucose monitoring has done by wang *et al.* The mechanism is more or less same that is reverse iontophoresis only it is a tattoo-based system more easily applicable to skin [56]. In this system the amperometric glucose is detected by reverse iontophoretic method and place to suitable substance. Here on a tattoo-based system contains the electrodes and the sensor in miniature form, so that it is easier to worn and patients' compliance will be better respected to *glucowatch*. This flexible device is designed for single use glucose detection. This tattoo-based system [fig. 4] prevent the extra application of current in extraction of glucose by using GOx-generated hydrogen peroxide with a low voltage cathodal detection at a special type of electrode transducer ex Prussian Blue [57]. The result of fasting and after-meal was analysed in a clinical stage with the performance of healthy human volunteer and recorded all the detected value. That value was compared with other sets of commercially available finger stick method. Control study was analysed with the same enzyme of the glucose sensor. There was no update to glucose sensor for confirm the mechanism. Though the tattoo-based system was designed for disposable purpose and single use but its effectiveness and sensor accurateness is remarkably good. The patients' compliance also better in all aspects. Future work is going on in this non-invasive as well as compatible sensor technology to formulate a long-term usable device for a large population for healthy and as well as diabetic patients [58].

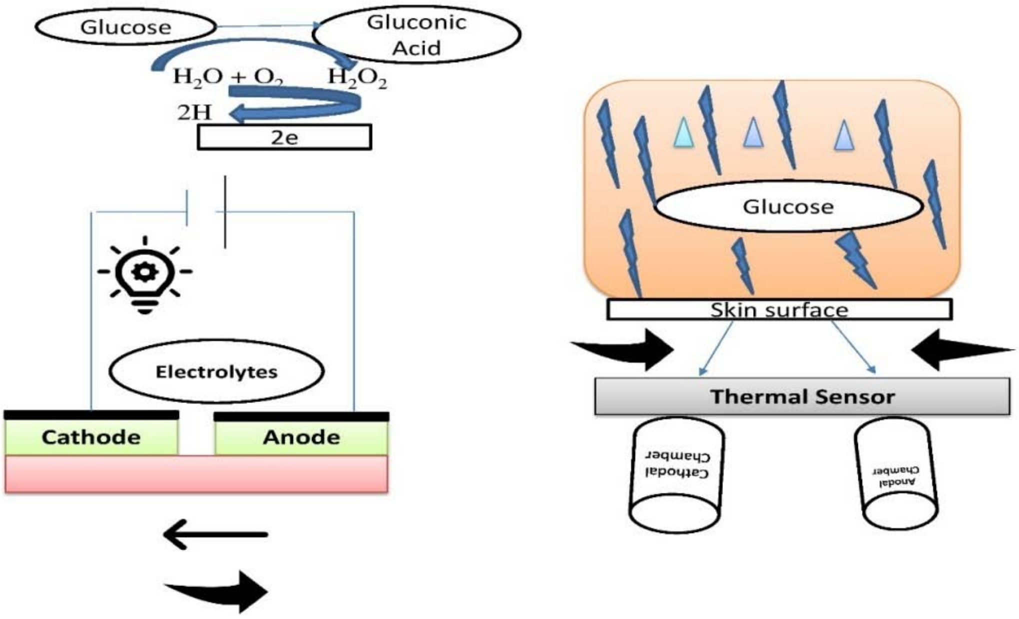

Fig. 3-4: Reverse iontophoresis technique for needle-free glucose monitoring

Non-invasive monitoring of glucose by other techniques

Other than reverse iontophoresis a very attractive non-invasive technology available for glucose monitoring [table 1]. Glucose monitoring through sweat is another alternative of reverse iontophoresis. It is well known that sweat is one of the most important biological fluids for non-invasive monitoring of glucose because there are some marked advantages like number of sampling site is more and broad, collection attachment and detachment of device is easy and most importantly, the composition of sweat holds an extra advantage for experiments [59]. A specific amount of sweat can be taken as a sample through the skin with the help of some small analyte like–glucose that quickly diffused into a sweat and then the total fresh sample can be taken in a specific time interval with best collection procedure. As a result, it gives the required information regarding the monitoring of glucose [60]. But there are also some constrains which can affect the monitoring of glucose like temperature fluctuation, variation of pH and any contamination to the skin, wrong sampling technique, mixing of old sample with new sweat. The concentration of sweat has shown that there is a positive correlation between simultaneous blood glucose and specific sweat concentration [61]. But though there is good correlation in between blood glucose and sweat concentration, it is also observed that this method is very challenging due to the low concentration of glucose. So, it needs a potent sensitive system or better to develop a proper sensor particularly if a patient is suffering from hypoglycaemia [62]. Several models were proposed and execute by scientists mostly that are patch type which can attach to the skin very easily. A group of scientists have already developed an upgraded version of this with using a update sensor and a stretchable fabricated device which are more accurate to capture the glucose signalling [63-65].

Table 1: List of approved devices for non-invasive glucose monitoring

Name of the glucose sensor	Technique used	Exiting features	Development perspective	Reference
Temporary tattoo	Reverse Iontophoresis	i. Skin irritation is nil ii. Low cost and maintenance ii. Easy access.	i. One time use ii. Stability is an issue for continuous operation	[66, 67]
Gluco watch	Reverse Iontophoresis	i. First commercialized non-invasive product for glucose monitoring ii. FDA Approve product iii. Non-invasive continuous monitoring of glucose	i. Skin irritation should be minimized ii. Calibration procedure should be pain less iii. Sweat generation required for interface	[68, 69]
Sensor array patch coupled with induced sweating	Iontophoresis	i. Initiate swat generation with iontophoretic glucose sensing ii. Alliance of wireless electronics	i. Goll to on–body monitoring	[70, 71]
Wearable patch, multimodal glucose sensor	Exercise	i. Sweat extraction is controlled ii. Accurate calibrated value of glucose through strong sensor a well correlation with pH of sweat	i. Requirement of continuous monitoring	[72, 73]

Limitation of reverse iontophoresis

Though this technique is non-invasive but there is requirement of blood sample for every calibration time. In this biophysical system specific analyte concentration in the prescribed electrode cell is not as same as finger stick method. It is very complex to assume the dilution factor due to the dependency on the transport number of an analyte (a fragment of whole charge is transported through skin by ion of interest) or the charge present on the surface of the skin which governs the electroosmotic flow quantitatively, whereas there is also possibility that the dilution factor also depends on previously known volume of acceptor section. These two parameters are not available at all in a blood sample when it is compared and calibrated with the effectiveness of reverse iontophoretic extraction. In modern days application of internal standard has been proposed for calibration [74, 75]. The focused area is that another compound an internal standard which is available with a target analyte (A) in subdermal layer of skin with a specific concentration, it is also quantified after the extraction trough reverse iontophoresis process. If situation comes like those two molecules are extracted together, the representation of extraction fluxes can be acted as.

$$\frac{J_A}{J_{IS}} = K\frac{[A]}{[IS]} \dots [3]$$

Transdermal fluxes (mol/cm^{2}h) can be represented by J_A and J_{IS} of the internal standard respectively. Whereas the molar blood concentration of the two molecules can be represented as [A] and [IS], and K remains constant in this process. It was assumed that the concentration of blood in internal standard is already understood and continue in a group, as a result the electron fluxes are measured and the unknown concentration of the analyte can be calculated.

But there are other factors which can influence the reverse iontophoretic operation like the skin thickness, gender, electrical parameters, ionic strength etc. In general, the issue is more complex when the skin is thicker because a very lesser number of molecules can pass through. This skin thickness varies in different ages, sex, races and also in different disease conditions [76]. Whenever the reverse iontophoretic patch need to develop the care should be taken on these matters. Though there is limitation but this technology is accepted widely for extraction of different drugs other than the blood glucose monitoring from the year 2005 to continue [table 2].

Future prospects and modern approach

In iontophoresis or reverse iontophoresis mechanism, different methods of penetration enhancers are utilized including application of chemical enhancer, ultrasound, electroporation, micro needles etc. for increasing transdermal transport of drugs as well as extraction through skin. The combined therapy (reverse iontophoresis with chemical enhancers) has got importance especially in transdermal drug delivery system for better extraction of analyte through skin layers.

Combination of iontophoresis with chemical enhancers

Chemical enhancers play an important as well as a crucial role in iontophoretic drug delivery and reverse iontophoretic extraction. It enhances the drug delivery through skin and also influence the extraction of drug molecules through the subdermal layer of skin. A chemical enhancer can be applied combinedly with iontophoresis mechanism for better penetration of drug as well as better extraction of analyte through the skin, when the reverse process is applied. This combined therapy with electrical assurance also give a better patients compliance, different chemical enhancers were used in different extraction as well as drug delivery it was reported that propylene glycol and oleic acid increases the TDDS transport of AZT when combined with iontophoresis method [85].

Table 2: A List of reported drug molecules extracted by reverse iontophoresis

Year	Name of the drug	Cathodal or anodal extraction of drug	Result	Reference
2012	Amikacin	Cathode	Reverse iontophoresis extraction process is applied for extraction of Amikacin, extraction was taken place at the cathodal chamber at pH 4.0 and 8.0 both. In this whole process the electromigration mechanism was involved. Though there is also evidence that electroosmosis mechanism is also implemented when acetaminophen was used as a marker.	[77, 78]
2012	Glucose and potassium	Glucose at cathode	This technique is remarkably better for non-invasive extraction of molecules through transdermal application and extraction of analyte and its correlation with the analyte present in blood. The use of the internal standard in this process also improved the correlation of extracted molecules (glucose) with the blood analytes. But the calibration procedure Is highly required for the clinical approaches further.	[79]
2008	Amikacin	Cathode and anode	In this process it was reported that a good number of analytes like glucose, lactate, urea was extracted in the appearance of skin reservoir. But the practical point of view the non-invasive monitoring of amino acid level, this suggests that a pre-iontophoretic method may be required to reduce the level of analyte to the skin prior the extraction flux would reflect same in the sub dermal layer of skin.	[80]
2008	Amikacin	Cathode and anode	All the extracted amino acids were detected at both electrodes after application of reverse iontophoresis. Highest fluxes were observed in the preliminary phase of current passage (Approaches in the primary periodical gap) before reduction of values after specific time of current application in RI process.	[81]
2008	Urea	Cathode	Urea monitoring and extraction through reverse iontophoresis is non-invasive and it is observed in this process that *in vivo* feasibility is possible and it is applicable in diagnostic as well as the theragnostic purpose of renal impairment. However this is possible but there are different challenges also influence the whole process when fabrication of the devices is developed. At the outer barrier of skin, the presence of urea reservoir suggests the natural emollient effects to skin. This system systematically tracks the urea level non-invasively.	[82]
2007	Glucose and lactate	Both anode and cathode	The movement of glucose and lactate across the skin due to the application of both bipolar current waveforms and pulsed bipolar current waveforms. The duration of application of pulse current is responsible for the extraction of glucose and lactate.	[83]
2007	Lactate	Both anode and cathode	The extraction of lactate through reverse iontophoresis method was fine and irritation on skin was also minimized during the application of small current in combination with electrode polarity reversal with 15 min interval.	[84]

Sonophoresis

Collaboration between sonophoresis with iontophoretic drug delivery is getting importance due to its excellent preformation in transdermal drug delivery as well as transdermal extraction through reverse iontophoresis. Using of ultrasound therapy can introduce a better passage by breaking the lipid bilayer of the skin which can open the pore channel and increase the penetration level or extraction level. This method to misstructure of the lipid layer of skin can be applied in next iontophoretic method to enhance the capability of permeation of molecules for a greater span. This coupled method can give a better individualistic transdermal transport than any other therapy in iontophoresis. Sonophoresis combination with iontophoretic method was applied in transdermal transport of sodium nonivamide acetate. It was also reported that the increasement of heparin flux was enhanced around 56-fold after applying the combination of sonophoresis with iontophoresis. The increased fold was remarkably higher than the total of those iontophoretic method and sonication method alone [86-88].

Combination of iontophoresis with electroporation

A passage or pathway is created by the electroporation method and it helps to enhance the pore channel of skin through the direct current. Different protein, peptides, macromolecules are delivered through the electroporation mechanism. The transport mechanism is identical to sonophoresis. *Chang et al.* illustrates the mechanism of iontophoresis and electroporation with salmon calcitonin and parathyroid hormone through the transdermal delivery. The penetration level was found to be higher and time consumption was found remarkably less in combination of electroporation and iontophoresis [89].

Iontophoresis in combination with microneedles

This technique is not so popular, but a group of studies have found some possibilities that the amalgamation of iontophoresis with the microneedles technologies it helps in drug delivery non-invasively through the human epidermis with sharp electronic balance [90].

Iontophoresis with ion-exchange materials

In this technique first the drug solution was prepared with the standard method and then the ion exchange substances are dipped in to the drug reservoir solution for a specific time (3 h) to overnight. For *in vitro* and *in vivo* test, the drug loaded that previously drug loaded device was removed to the donor part of the diffusion cell. A group of scientists studied the the agnostic efficacy of tacrine successfully *in vivo* delivery [91].

CONCLUSION

A considerable amount of research has been dedicated towards non-invasive technology and its application towards diabetic management. Having said that, the ideation of reverse iontophoresis has a breakthrough implication towards pain free and convenient alternative to monitor the glucose levels especially to those patients who have high probability to develop complications. Apart from this, this technology has also paved its way in the therapeutic drug monitoring of several drugs and plasma biomarkers. Nevertheless, to gauge the ideal application of this technology in the practical scenario stringent validation with consistent results is highly necessary. Previously, we have put some light on the commercialized product that lacked proper calibration and thus had to be retrieved from the market. Yet, to strategize the management of diabetic complications, continuous efforts to optimise the reverse iontophoresis is primarily essential that would clearly elucidate the performance and patient compliance.

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AUTHORS CONTRIBUTIONS

All the listed authors have contributed equally.

CONFLICT OF INTERESTS

The authors of the review article ensure that there is no conflict of interest by any means and the work is not funded by any organization.

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